BODY COMPOSITION ANALYSIS IN THE
ASSESSMENT OF CANCER CACHEXIA
TREATMENT OUTCOMES

by

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for the degree of PhD within the Department of Surgery, Faculty of Medicine

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I wish to dedicate this Thesis to my parents and sister to thank them for their love, patience, continual support and encouragement without which this Thesis would not have been possible.

I also wish to dedicate this Thesis to the memory of my paternal uncle who passed away in 2007.
AKNOWLEGEMENTS

In a letter to Robert Hooke, Sir Isaac Newton wrote: “If I have seen further it is by standing on the shoulders of giants”. Here, I will attempt to thank and acknowledge some of my “giants” on whose shoulder I have been standing. There are, however, many people not listed below to whom some acknowledgment is due. To them I offer my thanks and apologies for their omission.

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This thesis describes research undertaken in the Department of Surgery, Faculty of Medicine (University of Sydney) under the supervision of Prof Ross C Smith and Prof Barry J Allen. The work presented in this thesis is, to the best of my knowledge and belief, original, except as specified and acknowledged in the text, and has not been submitted, either in whole or in part, for a degree at this or any other university.

Alireza Aslani

August 2008
ETHICS CLEARANCE

All studies described in this thesis were approved by the Medical Research Ethics Committee and the Radiation Ethics Committee of the Royal North Shore Hospital, Sydney. All subjects gave written, informed consent for the studies.
ABSTRACT

Introduction
Cachexia is characterised by a marked weight loss and the presence of anorexia, anaemia, and asthenia. Although cachexia is often associated with the presence and growth of tumour and observed in solid tumours of the upper gastrointestinal tract, its presence is not unique to cancer and is often also present in most chronic, end-stage diseases processes. The loss of body fat, altered lipid metabolism, increase in the resting energy expenditure, and the increased loss of body protein the degree of which is associated with poor survival, are all hallmarks of this detrimental disease. The clinical aspects and consequences of cachexia can simply be summarised as morbidity, debilitating conditions, and mortality. The conditions such as loss of muscle mass, impaired muscle function, fatigue, reduced activity and functional capacity by themselves are enough to severely and significantly affect the patients’ QL.

Although different interventional procedures and therapies are available for the treatment of cachexia and its symptoms, effective methods to evaluate their benefits and outcomes have not been tested or investigated. It was, therefore, the aim of this project to use body composition analysis as a clinical tool and evaluate the effectiveness and outcome of interventional and therapeutic procedures in three groups of patients with cancer.

Methods
Three patient groups were investigated: 1) patients with pancreatic cancer undergoing Whipple’s Procedure, 2) patients with pancreatic cancer undergoing cancer chemotherapy and receiving either EPA or placebo, and 3) patients with malignant mesothelioma undergoing cancer chemotherapy plus thalidomide or thalidomide alone. Body composition analysis
techniques were used to assess the changes in TBN, TBF, TBK, and TBW. In addition, the body composition parameters together with clinical measures were also used to determine parameters influencing survival.

The malignant mesothelioma patients were randomised into patients who received gemcitabine / cisplatin plus thalidomide and those who received thalidomide alone.

The pancreatic cancer patients undergoing chemotherapy were randomised into the group who were receiving EPA and those who were receiving placebo. In addition, these patients were also investigated on the basis of their disease extent where they were separated into two groups of metastatic and locally advanced.

Unpaired T-Test and ANOVA were used to determine differences between groups. Kaplan-Meier analysis and Cox’s Regression were used to assess survival in all three patient cohorts.

The Whipple’s Procedure patients were separated into those who received a Clear Margin and those who received an Unclear Margin during their resection.

Results

1) In the pancreatic cancer patients undergoing Whipple’s Procedure, compared to the baseline, there were highly significant changes in Weight (p=0.006), BMI (p=0.005), and FM (p=0.007) followed by significant changes in %BFat (p=0.016), TBK/Ht (p=0.021), LBM (By TBK) (p=0.023), LBM (Van Loan) (p=0.034), and LBM (Segal) (p=0.038) at the 14 week time-point. At the 26 weeks post-operative time point, the only significant changes were in the FM (p=0.012), %BFat (p=0.003), and BMI (p=0.027) parameters. There was also a deviation between the two groups in their TBN, LBM and TBW content observable in a long-term setting and fat content in the relatively shorter-term. Although the Unclear Margin group had lower body composition values, both groups seem to begin to gradually “equalise” around the 14 weeks post-operative time-point. The survival analysis results for the Whipple’s
ABSTRACT

Procedure patients demonstrated that Margin Status (p=0.001), Fat Mass (p=0.003) and Age (p=0.081) were significant and could influence survival.

2) When the second cohort pancreatic cancer patients undergoing chemotherapy were analysed, they were initially separated according to the extent of their disease. The results of the analyses of body composition changes between measurement time-points for the each group separately, suggested that the patients with locally advanced disease maintain their Weight, FM, and TBN but are more likely to have a lower TBW by the end of the four month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but are more likely to have a decreased fat compartment and a higher FFM. The QL analysis showed that the metastatic group are performing “worse” than the locally advanced group especially in term of their Dyspnoea, Nausea & Vomiting, and Sexuality. In addition, the Karnofsky score showed that the metastatic group are not performing as well as the locally advanced group. Furthermore, for the metastatic group there was an increase in the patients’ pain with a decline in mood and general performance as well as increase in gastrointestinal symptoms. Pain Card scores also showed a general increase for the metastatic group and a general decrease for the locally advanced group.

When the pancreatic cancer patients undergoing chemotherapy were separated according to whether they received EPA or placebo, the results demonstrated that firstly, due to the fact that the patients were well randomised, the two groups commenced the trial with similar and statistically non-significantly different body composition parameters. Secondly, the two groups were also found to be statistically not different at their corresponding measurement time-points. And thirdly, the patients receiving placebo compared to those receiving EPA lost more Weight, and FM but less TBW throughout the trial. The TBK/Ht (p=0.044), TBK (p=0.042), and LBM (By TBK) (p=0.042), however, showed statistically significant differences where in all three parameters the EPA showed an increase compared to the base-
ABSTRACT

line (pre-chemotherapy). Results of the survival analysis demonstrated that the use of EPA in this group of pancreatic cancer patients did not provide any benefit. In fact, as it was shown in the Kaplan-Meier plot, the group of patients receiving the EPA had a “worse” survival than the group receiving the placebo. The QL results showed that placebo group improved in their functional scales, but increased their Altered Bowel Habit scores with an increase in the perception of pain and decrease in relief from pain. The EPA group, however, showed a decrease in the Loss of Appetite, Dyspnoea, Pain, Pancreatic Pain, and Fatigue, and improvements in Role Functioning and Sexuality.

3) Results of the malignant mesothelioma patients demonstrated that both study arms show similar weight changes. In addition, body composition measurements indicated that the gemcitabine / cisplatin chemotherapy plus thalidomide group had a greater TBN loss and a greater TBW gain than the thalidomide-alone group. This loss of TBN and gain in TBW looked to be “concealed” in the weight. The results of the survival analysis carried out on the mesothelioma patient group suggested that haemoglobin levels (p=0.001), Age (p=0.007), and NI (p=0.008) are the parameters that can influence the survival of patients with malignant mesothelioma undergoing chemotherapy.

Conclusions

1) The trend in body composition changes in the Whipple’s Procedure group showed that, although both groups may start with non-significantly different body composition, they tended to grow closer around the 14 week point indicating that the Clear Margin group may lose more than Unclear Margin group.

The implications of these findings, therefore, were that once the most appropriate surgical procedure is performed, an adjuvant therapy regimen (such as chemotherapy) at around 14 weeks may have the most impact on the patient’s overall treatment outcome.
2) When the pancreatic cancer patients were separated by the extent of their disease, the results lead to the conclusion that the patients with locally advanced disease maintain their Weight, FM, and TBN but are more likely to have a lower TBW by the end of the four month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but are more likely to have a decreased fat compartment and a higher FFM. The QL analysis concluded that the results may point to a worsening and/or progressing disease which is consistent with classic metastatic disease aetiology.

From the results of the pancreatic cancer patients undergoing cancer chemotherapy it was concluded that the use of EPA in this group of pancreatic cancer patients undergoing cancer chemotherapy with gemcitabine results in a non-significant reduction in weight loss, FM loss, and TBW gain with a statistically significant increase in FFM. The results of the survival analysis was, however, contradictory suggesting that patients receiving EPA may have a worse survival than the placebo group. The QL analysis here concluded that that EPA does improve the QL of this group of pancreatic cancer patients.

3) From the malignant mesothelioma group it was concluded that provided that the overall anti-cancer potential of gemcitabine / cisplatin plus thalidomide is comparable with that of thalidomide-alone, then by looking purely from the body composition angle one may be able to suggest the use of thalidomide alone in the treatment of malignant mesothelioma in this group of patients. From the results of the survival analysis, the fact that the Study Arm parameter did not reach statistical significance could indicate that survival in these patients is not affected by the presence or absence of chemotherapy with gemcitabine and cisplatin.

The body composition techniques were used here as a tool to monitor changes in various body composition parameters to assess the outcomes, including survival, of the administration of different therapies and interventional procedures in these three groups of cancer patients. For these purposes, these techniques were demonstrated to be an effective and invaluable tool.
# TABLE OF CONTENTS

1. **HYPOTHESES AND AIMS**
   - 1.1 **BACKGROUND AND HYPOTHESES**
   - 1.2 **AIMS**
   - 1.3 **STRUCTURE AND STAGES OF THE PROJECT**

2. **CANCER CACHEXIA**
   - 2.1 **AETIOLOGY OF CACHEXIA**
   - 2.2 **MEDIATORS INVOLVED IN CACHEXIA**
     - 2.2.1 **INTERLEUKINS**
     - 2.2.2 **INTERFERON AND TUMOUR NECROSIS FACTORS**
     - 2.2.3 **UBIQUITIN-PROTEASOME PATHWAY**
     - 2.2.4 **NEUROENDOCRINE STRESS RESPONSE AND NON-CYTOKINE FACTORS**
       - 2.2.4.1 Proteolysis-Inducing Factor
       - 2.2.4.2 Transcriptional Factors
       - 2.2.4.3 Lipid Mobilising Factor
       - 2.2.4.4 Anaemia Inducing Factor
       - 2.2.4.5 Leptin
     - 2.2.5 **OTHER CYTOKINES AND FACTORS**
     - 2.2.6 **PROTEIN DEGRADATION PATHWAYS**
   - 2.3 **STARVATION, ANOREXIA, AND CACHEXIA**
     - 2.3.1 **WEIGHT LOSS IN STARVATION AND CACHEXIA**
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.2</td>
<td>Anorexia in cachexia</td>
<td>51</td>
</tr>
<tr>
<td>2.4</td>
<td>Altered metabolism in cachexia</td>
<td>53</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Basal metabolic rate and hypermetabolism</td>
<td>54</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Carbohydrate metabolism</td>
<td>55</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Fat and lipid metabolism</td>
<td>58</td>
</tr>
<tr>
<td>2.4.4</td>
<td>Protein metabolism</td>
<td>59</td>
</tr>
<tr>
<td>2.5</td>
<td>Fatigue in cachexia</td>
<td>64</td>
</tr>
<tr>
<td>2.6</td>
<td>Symptoms and diagnosis of cachexia</td>
<td>67</td>
</tr>
<tr>
<td>2.6.1</td>
<td>Weight loss and anorexia</td>
<td>67</td>
</tr>
<tr>
<td>2.6.2</td>
<td>Altered food intake</td>
<td>68</td>
</tr>
<tr>
<td>2.6.3</td>
<td>Haematological changes</td>
<td>69</td>
</tr>
<tr>
<td>2.6.4</td>
<td>Psychological changes</td>
<td>70</td>
</tr>
<tr>
<td>2.6.5</td>
<td>Hormonal changes</td>
<td>70</td>
</tr>
<tr>
<td>2.7</td>
<td>Body composition changes in cachexia</td>
<td>71</td>
</tr>
<tr>
<td>2.8</td>
<td>Clinical consequences of cachexia</td>
<td>74</td>
</tr>
<tr>
<td>2.9</td>
<td>Treatment and management of cachexia</td>
<td>76</td>
</tr>
<tr>
<td>2.9.1</td>
<td>Symptomatic treatment of nausea and vomiting</td>
<td>77</td>
</tr>
<tr>
<td>2.9.2</td>
<td>Symptomatic treatment of reduced food intake</td>
<td>78</td>
</tr>
<tr>
<td>2.9.3</td>
<td>Symptomatic treatment of fatigue</td>
<td>81</td>
</tr>
<tr>
<td>2.9.4</td>
<td>Metabolic and systemic management</td>
<td>82</td>
</tr>
<tr>
<td>3.1</td>
<td>The pancreas</td>
<td>86</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Anatomy of the pancreas</td>
<td>86</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Functions of the pancreas</td>
<td>87</td>
</tr>
<tr>
<td>3.2</td>
<td>Pancreatic cancer</td>
<td>89</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Aetiology of pancreatic cancer</td>
<td>90</td>
</tr>
<tr>
<td>3.2.1.1</td>
<td>Chronic pancreatitis</td>
<td>90</td>
</tr>
<tr>
<td>3.2.1.2</td>
<td>Inherited and hereditary factors</td>
<td>91</td>
</tr>
<tr>
<td>3.2.1.3</td>
<td>Race and gender</td>
<td>93</td>
</tr>
<tr>
<td>3.2.1.4</td>
<td>Smoking as a risk factor</td>
<td>93</td>
</tr>
<tr>
<td>3.2.1.5</td>
<td>Diabetes</td>
<td>95</td>
</tr>
<tr>
<td>3.2.1.6</td>
<td>Dietary factors</td>
<td>95</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Symptoms of pancreatic cancer</td>
<td>98</td>
</tr>
<tr>
<td>3.2.2.1</td>
<td>Presenting symptoms</td>
<td>98</td>
</tr>
<tr>
<td>3.2.2.2</td>
<td>Psychological symptoms</td>
<td>100</td>
</tr>
<tr>
<td>3.2.2.3</td>
<td>Symptoms presented on physical examination</td>
<td>102</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Prognosis of pancreatic cancer</td>
<td>102</td>
</tr>
<tr>
<td>3.3</td>
<td>Diagnostic tests</td>
<td>109</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Laboratory tests</td>
<td>109</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Imaging modalities</td>
<td>111</td>
</tr>
<tr>
<td>3.3.2.1</td>
<td>Computerised tomography</td>
<td>111</td>
</tr>
<tr>
<td>3.3.2.2</td>
<td>Magnetic resonance imaging</td>
<td>111</td>
</tr>
<tr>
<td>3.3.2.3</td>
<td>Ultrasonography</td>
<td>112</td>
</tr>
<tr>
<td>3.3.2.4</td>
<td>Endoscopic retrograde cholangio-pancreatography</td>
<td>112</td>
</tr>
<tr>
<td>3.3.2.5</td>
<td>Tissue diagnosis</td>
<td>113</td>
</tr>
<tr>
<td>3.3.2.6</td>
<td>Nuclear medicine imaging</td>
<td>113</td>
</tr>
<tr>
<td>3.3.2.7</td>
<td>Positron emission tomography</td>
<td>113</td>
</tr>
<tr>
<td>3.3.2.8</td>
<td>Miscellaneous imaging and visualisation modalities</td>
<td>114</td>
</tr>
<tr>
<td>3.4</td>
<td>Surgical treatment of pancreatic cancer</td>
<td>115</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Surgical methods in pancreatic cancer</td>
<td>115</td>
</tr>
<tr>
<td>3.4.2</td>
<td>The Whipple’s procedure</td>
<td>117</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Whipple’s procedure survival rate</td>
<td>119</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>CHEMOTHERAPY OF Pancreatic Cancer</td>
<td>123</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Gemcitabine Therapy</td>
<td>124</td>
</tr>
<tr>
<td>3.5.1.1</td>
<td>Pharmacology And Pharmacokinetics</td>
<td>126</td>
</tr>
<tr>
<td>3.5.1.2</td>
<td>Metabolism</td>
<td>128</td>
</tr>
<tr>
<td>3.5.1.3</td>
<td>Side Effects</td>
<td>128</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Gemcitabine Combination Chemotherapy</td>
<td>129</td>
</tr>
<tr>
<td>3.6</td>
<td>Palliation Of Symptoms Of Pancreatic Cancer</td>
<td>132</td>
</tr>
<tr>
<td>3.6.1</td>
<td>Biliary And Duodenal Obstructions</td>
<td>132</td>
</tr>
<tr>
<td>3.6.2</td>
<td>Pain</td>
<td>134</td>
</tr>
<tr>
<td>3.6.3</td>
<td>Pancreatic Insufficiency</td>
<td>136</td>
</tr>
<tr>
<td>4.</td>
<td>MALIGNANT MESOTHELIOMA</td>
<td>137</td>
</tr>
<tr>
<td>4.1</td>
<td>Aetiology And Pathogenesis Of Malignant Mesothelioma</td>
<td>137</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Pathogenesis Of Malignant Mesothelioma</td>
<td>138</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Clinical Presentation Of Malignant Mesothelioma</td>
<td>140</td>
</tr>
<tr>
<td>4.2</td>
<td>Diagnosis Of Malignant Mesothelioma</td>
<td>142</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Cytology And Histopathology</td>
<td>142</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Imaging Modalities In Malignant Mesothelioma</td>
<td>143</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Serum Markers In Malignant Mesothelioma</td>
<td>143</td>
</tr>
<tr>
<td>4.2.4</td>
<td>Staging Of Malignant Mesothelioma</td>
<td>144</td>
</tr>
<tr>
<td>4.3</td>
<td>Prognosis And Treatment Of Malignant Mesothelioma</td>
<td>146</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Prognosis Of Malignant Mesothelioma</td>
<td>146</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Treatment Of Malignant Mesothelioma</td>
<td>149</td>
</tr>
<tr>
<td>4.3.2.1</td>
<td>Localised Treatment Approaches</td>
<td>149</td>
</tr>
<tr>
<td>4.3.2.2</td>
<td>Systemic Treatment Approaches</td>
<td>151</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Palliation For Malignant Mesothelioma</td>
<td>155</td>
</tr>
<tr>
<td>5.</td>
<td>Eicosapentaenoic Acid And Omega-3 Fatty Acids</td>
<td>157</td>
</tr>
<tr>
<td>5.1</td>
<td>Fatty Acids</td>
<td>157</td>
</tr>
<tr>
<td>5.2</td>
<td>Omega-3 And Omega-6 Fatty Acids</td>
<td>158</td>
</tr>
<tr>
<td>5.3</td>
<td>Eicosapentaenoic Acid</td>
<td>159</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Pharmacology And Mechanism Of Action</td>
<td>160</td>
</tr>
<tr>
<td>5.3.1.1</td>
<td>Mechanisms Of Action</td>
<td>161</td>
</tr>
<tr>
<td>5.3.1.2</td>
<td>Pharmacology</td>
<td>165</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Therapeutic Uses</td>
<td>172</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Side Effects</td>
<td>184</td>
</tr>
<tr>
<td>6.</td>
<td>Thalidomide In Cancer Therapy</td>
<td>186</td>
</tr>
<tr>
<td>6.1</td>
<td>Pharmacology And Mechanism Of Action</td>
<td>186</td>
</tr>
<tr>
<td>6.2</td>
<td>Uses Of Thalidomide</td>
<td>189</td>
</tr>
<tr>
<td>6.3</td>
<td>Toxicity And Side Effects Of Thalidomide</td>
<td>190</td>
</tr>
<tr>
<td>7.</td>
<td>In Vivo Body Composition</td>
<td>192</td>
</tr>
<tr>
<td>7.1</td>
<td>Total Body Nitrogen (TBN)</td>
<td>194</td>
</tr>
<tr>
<td>7.1.1</td>
<td>Measurements Of TBN</td>
<td>194</td>
</tr>
<tr>
<td>7.1.2</td>
<td>IVNCA Technique</td>
<td>196</td>
</tr>
<tr>
<td>7.1.3</td>
<td>Physics Of Neutron Activation</td>
<td>199</td>
</tr>
</tbody>
</table>
## TABLE OF CONTENTS

### 7.2 TOTAL BODY WATER (TBW)
- 7.2.1 DEUTERIUM OXIDE ISOTOPE DILUTION
  - 7.2.1.1 Preparation Phase
  - 7.2.1.2 Analysis Phase
- 7.2.2 DEUTERIUM OXIDE DOSE AND TOXICITY
- 7.2.3 FTIR ANALYSIS TECHNIQUE
- 7.2.4 BIOELECTRICAL IMPEDANCE ANALYSIS (BIA) METHODS
- 7.2.5 OTHER TOTAL BODY WATER MEASUREMENT METHODS

### 7.3 BODY FAT MEASUREMENTS
- 7.3.1 GOLD STANDARD
- 7.3.2 BIOELECTRICAL IMPEDANCE ANALYSIS (BIA) METHODS
- 7.3.3 NUCLEAR TECHNIQUES
- 7.3.4 SKINFOLD THICKNESS
- 7.3.5 SIGNIFICANCE OFLEAN BODY MASS

### 7.4 FAT FREE MASS (FFM) MEASUREMENTS

### 7.5 QUALITY OF LIFE MEASUREMENTS
- 7.5.1 THE EORTC QUALITY OF LIFE ASSESSMENTS

### 8. METHODS: BODY COMPOSITION AND QUALITY OF LIFE MEASUREMENTS

#### 8.1 BODY FAT, SKINFOLD THICKNESS, AND ANTHROPOMETRICS
- 8.1.1 PERCENTAGE BODY FAT AND LEAN BODY MASS
- 8.1.2 LEAN BODY MASS BY BIOELECTRICAL IMPEDANCE ANALYSIS

#### 8.2 TOTAL BODY NITROGEN MEASUREMENTS
- 8.2.1 IVNCA INSTRUMENTATION
  - 8.2.1.1 The IVNCA Measurement Area
  - 8.2.1.2 The Analogue-to-Digital Converter System
  - 8.2.1.3 Multi-channel Analyser (MCA) Card
- 8.2.2 CALIBRATION WITH PHANTOMS
- 8.2.3 IVNCA SPECTRA
- 8.2.4 RADIATION AND DOSIMETRY
  - 8.2.4.1 Body Habitus Corrections
  - 8.2.4.2 Annual Calibration Experiments
  - 8.2.4.3 Calibration Experiments For Americium-241 Source
  - 8.2.4.4 Reproducibility of the System
  - 8.2.4.5 Errors in Nitrogen Measurements

#### 8.3 TOTAL BODY WATER MEASUREMENTS
- 8.3.1 ISOTOPE DILUTION TECHNIQUE
  - 8.3.1.1 Equipment And Consumables Required
- 8.3.2 BIOELECTRICAL IMPEDANCE ANALYSIS TECHNIQUE

#### 8.4 TOTAL BODY POTASSIUM MEASUREMENTS
- 8.4.1.1 The Whole Body Counter Measurement Chamber
- 8.4.1.2 The Analogue-To-Digital Converter System
- 8.4.1.3 The Multi Channel Analyser (MCA) System
- 8.4.1.4 Additional Equipments
- 8.4.2 CALIBRATION WITH PHANTOMS
- 8.4.2.1 Reproducibility Of The System

#### 8.5 QUALITY OF LIFE MEASUREMENTS
- 8.5.1 EPA CLINICAL TRIAL

#### 8.6 CLINICAL TRIAL SAMPLE SIZE DETERMINATION
- 8.6.1 THE 15% WEIGHT LOSS SCENARIO
- 8.6.2 THE 5% WEIGHT LOSS SCENARIO
- 8.6.3 THE 2.5% WEIGHT LOSS SCENARIO
TABLE OF CONTENTS

9. WHIPPLE’S PROCEDURE PATIENT RESULTS 271

9.1 PATIENT DEMOGRAPHICS 271
9.2 COMPLETION RATES 272
9.3 BODY COMPOSITION RESULTS 274
9.3.1 DIFFERENCES IN BODY COMPOSITION BETWEEN CLEAR AND UNCLEAR MARGINS 274
9.3.2 BODY COMPOSITION CHANGES BETWEEN TIME-POINTS 284
9.3.3 CHANGES BETWEEN GROUPS AT SPECIFIC TIME-POINTS 286
9.3.4 CHANGES BETWEEN GROUPS AT SPECIFIC TIME-POINTS COMPARED TO BASE-LINE 288
9.3.5 CHANGES WITHIN EACH GROUP BETWEEN DIFFERENT TIME-POINTS 294
9.4 CONCLUSIONS 298

10. WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS 301

10.1 BACKGROUND 301
10.2 KAPLAN-MEIER ANALYSIS: METHODOLOGY AND RESULTS 303
10.2.1 EFFECTS OF MARGINS 303
10.2.2 EFFECTS OF NITROGEN INDEX 304
10.2.3 EFFECT OF GENDER 307
10.3 COX’S REGRESSION ANALYSIS: METHODOLOGY AND RESULTS 308
10.4 SUMMARY AND CONCLUSIONS 314

11. MESOTHELIOMA PATIENT RESULTS 316

11.1 PATIENT DEMOGRAPHICS 316
11.2 COMPLETION RATES 317
11.3 BODY COMPOSITION RESULTS 319
11.3.1 DIFFERENCES IN BODY COMPOSITION ARM A AND ARM B 319
11.3.2 BODY COMPOSITION CHANGES BETWEEN TIME-POINTS 327
11.3.3 CHANGES BETWEEN GROUPS AT SPECIFIC TIME-POINTS 330
11.3.4 CHANGES BETWEEN GROUPS AT SPECIFIC TREATMENT CYCLE COMPARED TO BASE-LINE 331
11.4 CONCLUSIONS 339

12. MESOTHELIOMA SURVIVAL ANALYSIS RESULTS 341

12.1 BACKGROUND 341
12.2 KAPLAN-MEIER ANALYSIS: METHODOLOGY AND RESULTS 342
12.2.1 EFFECTS OF TREATMENT 342
12.2.2 EFFECTS OF NITROGEN INDEX 343
12.2.3 EFFECT OF GENDER 346
12.2.4 EFFECT OF PREVIOUS CHEMOTHERAPY 347
12.2.5 EFFECT OF PREVIOUS RADIOTHERAPY 348
12.3 COX’S REGRESSION ANALYSIS: METHODOLOGY AND RESULTS 350
12.4 SUMMARY AND CONCLUSIONS 354

13. EPA CLINICAL TRIAL PATIENT RESULTS 355

13.1 PATIENT DEMOGRAPHICS 355
13.2 COMPLETION RATES 357
13.3 BODY COMPOSITION RESULTS: METASTATIC VERSUS LOCALLY ADVANCED DISEASE 359
13.3.1 Differences in Body Composition Between Metastatic and Locally Advanced 359
13.3.2 Body Composition Changes Between Time-Points 371
13.3.3 Changes Between Groups at Specific Time-Points 376
13.3.4 Changes Between Groups at Specific Time-Points Compared to Base-Line 377
13.3.5 Summary and Conclusions: Metastatic Versus Locally Advanced 382
13.4 Body Composition Results: EPA Versus Placebo 383
13.4.1 Differences in Body Composition Between EPA and Placebo Administration 383
13.4.2 Body Composition Changes Between Time-Points 392
13.4.3 Changes Between Groups at Specific Time-Points 397
13.4.4 Changes Between Groups at Specific Time-Points Compared to Base-Line 398
13.4.5 Summary and Conclusions: EPA Versus Placebo 403

14. EPA Clinical Trial Patient Quality of Life Results 405
14.1 Patient Demographics 405
14.2 Scoring Quality of Life Questionnaires 405
14.3 Quality of Life Results: Metastatic Versus Locally Advanced Disease 407
14.3.1 Differences in Quality of Life Between Metastatic and Locally Advanced 407
14.3.2 Quality of Life Changes Between Time-Points 414
14.3.3 Changes Between Groups at Specific Time-Points Compared to Base-Line 419
14.3.4 Summary and Conclusions: Metastatic Versus Locally Advanced 425
14.4 Quality of Life Results: EPA Versus Placebo 426
14.4.1 Differences in Quality of Life Between EPA and Placebo Administration 426
14.4.2 Quality of Life Changes Between Time-Points 432
14.4.3 Changes Between Groups at Specific Time-Points Compared to Base-Line 438
14.4.4 Summary and Conclusions: EPA Versus Placebo 441

15. EPA Clinical Trial Survival Analysis Results 443
15.1 Background 443
15.2 Kaplan-Meier Analysis: Methodology and Results 443
15.2.1 Effects of Treatment 444
15.2.2 Effects of Nitrogen Index 445
15.2.3 Effect of Gender 448
15.2.4 Effect of Disease Extent 449
15.3 Cox’s Regression Analysis: Methodology and Results 451
15.4 Summary and Conclusions 455

16. Discussion and Conclusions 456
16.1 Discussion 456
16.2 Summary and Conclusions 466
16.3 Future Directions 470

REFERENCES 473

APPENDIX A: QUALITY OF LIFE QUESTIONNAIRE 517

APPENDIX B: PAIN CARD 521
APPENDIX C: PUBLICATIONS AND PRESENTATIONS 524

APPENDIX D: TOTAL BODY WATER VALIDATION ARTICLES 531

APPENDIX E: SELECTION, RECRUITMENT AND MEASUREMENT PROTOCOLS 541

1.1 BODY COMPOSITION MEASUREMENT PROTOCOLS 541
1.1.1 TOTAL BODY NITROGEN MEASUREMENT PROTOCOL 541
1.1.2 TOTAL BODY WATER MEASUREMENT BY D2O DILUTION PROTOCOLS 544
1.1.2.1 D2O Dosing 544
1.1.2.2 Patient Sample Collection 545
1.1.2.3 Patient and Standard Sample Processing 546
1.1.2.4 Standard Concentrations Preparation 548
1.1.2.5 Sample Analysis 549
1.1.3 TOTAL BODY WATER MEASUREMENTS BY BIA PROTOCOLS 550
1.1.3.1 Pre-Measurement Preparations 550
1.1.3.2 Electrode Attachments And Data Acquisition 551
1.1.4 TOTAL BODY POTASSIUM MEASUREMENT PROTOCOL 552
1.1.4.1 Pre-Measurement Preparations 553
1.1.4.2 Patient Measurements 554
1.1.4.3 Background Measurements 555
1.2 PRE- & POST-OPERATIVE SURGICAL (“WATER”) VALIDATION STUDY 557
1.2.1 ETHICAL APPROVAL 558
1.2.2 RECRUITMENT AND FOLLOW-UP PROCESS 558
1.2.3 INCLUSION CRITERIA 560
1.2.4 EXCLUSION CRITERIA 561
1.2.5 BODY COMPOSITION MEASUREMENTS 562
1.2.5.1 Anthropometric And Total Body Fat Measurements 563
1.2.5.2 Total Body Nitrogen And Total Body Protein Measurements 563
1.2.5.3 Total Body Water Measurements 563
1.3 PANCREATIC CANCER SURGERY FOLLOW-UP STUDY 565
1.3.1 ETHICAL APPROVAL 566
1.3.2 RECRUITMENT AND FOLLOW-UP PROCESS 566
1.3.3 INCLUSION AND EXCLUSION CRITERIA 567
1.3.4 BODY COMPOSITION MEASUREMENTS 568
1.3.4.1 Anthropometric And Total Body Fat Measurements 568
1.3.4.2 Total Body Nitrogen And Total Body Protein Measurements 568
1.3.4.3 Total Body Water Measurements 568
1.3.4.4 Total Body Potassium Measurements 568
1.4 THALIDOMIDE IN MESOTHELIOMA CLINICAL TRIAL 570
1.4.1 ETHICAL APPROVAL 572
1.4.2 RECRUITMENT AND FOLLOW-UP PROCESS 572
1.4.3 STUDY DESIGN 573
1.4.3.1 Patient Selection 574
1.4.4 INCLUSION CRITERIA 575
1.4.4.1 Common Inclusion Criteria 575
1.4.4.2 Inclusion Criteria Specific For Arm A 576
1.4.4.3 Inclusion Criteria Specific For Arm B 576
1.4.5 EXCLUSION CRITERIA 576
1.4.5.1 Common Exclusion Criteria 576
1.4.5.2 Exclusion Criteria Specific For Arm A 577
1.4.5.3 Patient Restrictions 578
1.4.6 MATERIALS AND SUPPLIES 578
1.4.7 BODY COMPOSITION MEASUREMENTS 579
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4.7.1</td>
<td>Anthropometric And Total Body Fat Measurements</td>
<td>579</td>
</tr>
<tr>
<td>1.4.7.2</td>
<td>Total Body Nitrogen And Total Body Protein Measurements</td>
<td>579</td>
</tr>
<tr>
<td>1.4.7.3</td>
<td>Total Body Water Measurements</td>
<td>579</td>
</tr>
<tr>
<td>1.4.8</td>
<td>STUDY DRUG ADMINISTRATION</td>
<td>579</td>
</tr>
<tr>
<td>1.4.8.1</td>
<td>Dose Reductions And Modifications</td>
<td>580</td>
</tr>
<tr>
<td>1.4.9</td>
<td>SERIOUS ADVERSE EVENTS</td>
<td>581</td>
</tr>
<tr>
<td>1.5</td>
<td>EPA CLINICAL TRIAL PROTOCOLS</td>
<td>582</td>
</tr>
<tr>
<td>1.5.1</td>
<td>ETHICAL APPROVAL AND CLINICAL TRIAL REGISTRATION</td>
<td>583</td>
</tr>
<tr>
<td>1.5.2</td>
<td>RECRUITMENT AND FOLLOW-UP PROCESS</td>
<td>583</td>
</tr>
<tr>
<td>1.5.3</td>
<td>RANDOMISATION PROCESS</td>
<td>585</td>
</tr>
<tr>
<td>1.5.4</td>
<td>INCLUSION CRITERIA</td>
<td>586</td>
</tr>
<tr>
<td>1.5.5</td>
<td>EXCLUSION CRITERIA</td>
<td>587</td>
</tr>
<tr>
<td>1.5.6</td>
<td>TERMINATION CRITERIA</td>
<td>588</td>
</tr>
<tr>
<td>1.5.7</td>
<td>STUDY DISCONTINUATION</td>
<td>588</td>
</tr>
<tr>
<td>1.5.8</td>
<td>CHEMOTHERAPY AND EPA</td>
<td>589</td>
</tr>
<tr>
<td>1.5.8.1</td>
<td>EPA And Placebo</td>
<td>589</td>
</tr>
<tr>
<td>1.5.8.2</td>
<td>Treatment And Placebo Arm Regimens</td>
<td>590</td>
</tr>
<tr>
<td>1.5.9</td>
<td>BODY COMPOSITION AND QUALITY OF LIFE</td>
<td>591</td>
</tr>
<tr>
<td>1.5.9.1</td>
<td>Anthropometric And Total Body Fat Measurements</td>
<td>591</td>
</tr>
<tr>
<td>1.5.9.2</td>
<td>Total Body Nitrogen And Total Body Protein Measurements</td>
<td>591</td>
</tr>
<tr>
<td>1.5.9.3</td>
<td>Total Body Water Measurements</td>
<td>592</td>
</tr>
<tr>
<td>1.5.9.4</td>
<td>Total Body Potassium Measurements</td>
<td>592</td>
</tr>
<tr>
<td>1.5.9.5</td>
<td>Quality Of Life Measurements</td>
<td>592</td>
</tr>
<tr>
<td>1.5.10</td>
<td>BLOOD TESTS AND VITAL SIGNS</td>
<td>592</td>
</tr>
</tbody>
</table>

APPENDIX F: EPA & PLACEBO DATA SHEETS 594
LIST OF FIGURES

Figure 1 Metabolic pathways for protein, carbohydrates and fats & lipids ........................................ 53
Figure 2. Diagram to show the Cori Cycle ....................................................................................... 57
Figure 3. Islets of Langerhans .......................................................................................................... 88
Figure 4. Organs involved in the Whipple’s Procedure .................................................................... 118
Figure 5. Gemcitabine ...................................................................................................................... 127
Figure 6. Butyric acid ...................................................................................................................... 157
Figure 7. Example of an omega-3 fatty acid .................................................................................... 159
Figure 8. Example of an omega-6 fatty acid .................................................................................... 159
Figure 9. Structure of eicosapentaenoic acid (EPA) ...................................................................... 160
Figure 10. Structure of docosahexaenoic acid (DHA) .................................................................... 160
Figure 11. Mechanism of action of EPA in the macrophage cell .................................................... 171
Figure 12. The axial view of the IVNCA measurement area (not to scale) ....................................... 238
Figure 13. The side view of the IVNCA measurement area (not to scale) ....................................... 239
Figure 14. The IVNCA’s gamma spectroscopy electronic system ..................................................... 242
Figure 15. Graph of Nitrogen Counts against Width in water filled phantoms ............................... 250
Figure 16. Graph of N/H Ratios against Widths .............................................................................. 252
Figure 17. Variations in ABNOH and KNH values during a calendar year ..................................... 254
Figure 18. Percentage CV for CNOH and CMN against Time for a calendar year. The CMN curve lies just above the one for CNOH .......................................................... 255
Figure 19. Graph to show the Net potassium calibration counts for a typical calendar year ........... 266
Figure 20. Mean (±SE) changes in Weight (Kg), %BFat (%), and BMI (Kg/Ht^2) with time in patients with Clear and Unclear Margins ................................................................. 275
Figure 21. Graph of mean (±SE) TBN against time (measurement time-point) for Clear and Unclear Margin groups ........................................................................................................ 276
Figure 22. Graph of mean (±SE) NI and TBK/Ht against time for the Clear Margin and Unclear Margin groups .............................................................................................................. 277
Figure 23. Graph of mean (±SE) LBM measurements using BIA technique against time for Clear Margin and Unclear Margin groups ........................................................................... 279
Figure 24. Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for Clear Margin and Unclear Margin groups .................................................. 280
Figure 25. Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for Clear Margin and Unclear Margin groups .................................................. 281
Figure 26. Graph of mean (±SE) TBK measurements against time for Clear Margin and Unclear Margin groups .............................................................................................................. 283
Figure 27. Graph of mean (±SE) TBW measurements against time for Clear Margin and Unclear Margin groups using the Fredrix, Pullicino, and Kushner equations .................................. 284
Figure 28. Changes in Weight with time in Whipple’s Procedure patients with Clear and Unclear Margins ...................................................................................................................... 289
Figure 29. Changes in %BFat with time in Whipple’s Procedure patients with Clear and Unclear Margins ...................................................................................................................... 290
Figure 30. Changes in BMI with time in Whipple’s Procedure patients with Clear and Unclear Margins ...................................................................................................................... 291
LIST OF FIGURES

Figure 31. Changes in BMI with time in Whipple’s Procedure patients with Clear and Unclear Margins................................................................. 292
Figure 32. Curve for the effect of margins (Clear/Unclear) on survival................................................................. 304
Figure 33. Survival curve for the effect of NI cut-off values of 0.85 on survival ..................................................... 306
Figure 34. Survival curve for the effect of NI cut-off values of 1.00 on survival ...... 306
Figure 35. Survival curve for the effect of gender on survival ............................................................................. 307
Figure 36. Box plot of Fat Mass against Margin status ......................................................................................... 312
Figure 37. Box plot of Age against Margin Status ................................................................................................. 312
Figure 38. Mean (±SE) changes in Weight (Kg), %BFat (%), and BMI (Kg/Ht²) with time in Arm A and Arm B patients .........................................................................................320
Figure 39. Graph of mean (±SE) TBN against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 321
Figure 40. Graph of mean (±SE) TBN against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 322
Figure 41. Graph of mean (±SE) LBM against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 322
Figure 42. Graph of mean (±SE) BIA-derived FM against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 323
Figure 43. Graph of mean (±SE) FM against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 324
Figure 44. Graph of mean (±SE) TBW against time (measurement time-point) for Arm A and Arm B patient groups ........................................................................................................ 325
Figure 45. Graph of mean FM (By Van Loan) against time for the Arm A and Arm B groups ................................................................................................................................................ 327
Figure 46. Changes in Weight compared to base-line with time in patients with mesothelioma .......................................................................................................................... 333
Figure 47. Changes in %BFat compared to base-line with time in patients with mesothelioma .......................................................................................... 334
Figure 48. Changes in Fat Mass compared to base-line with time in patients with mesothelioma ................................................................................................................................................ 335
Figure 49. Changes in LBM (Lukaski) compared to base-line with time in patients with mesothelioma ................................................................................................................................................ 336
Figure 50. Changes in TBN compared to base-line with time in patients with mesothelioma ................................................................................................................................................ 337
Figure 51. Changes in TBW compared to base-line with time in patients with mesothelioma ................................................................................................................................................ 338
Figure 52. Curve for the effect of treatment (Arm A/Arm B) on survival................................................................. 343
Figure 53. Survival curve for the effect of NI cut-off values of 0.85 on survival in mesothelioma patients ................................................................................................................................................ 345
Figure 54. Survival curve for the effect of NI cut-off values of 1.00 on survival in mesothelioma patients ................................................................................................................................................ 345
Figure 55. Survival curve for the effect of gender on survival of patients with malignant mesothelioma ................................................................................................................................................ 347
Figure 56. Survival curve for the effect of previous chemotherapy on survival of patients with malignant mesothelioma ................................................................................................................................................ 348
Figure 57. Survival curve for the effect of previous chemotherapy on survival of patients with malignant mesothelioma ................................................................................................................................................ 349
Figure 58. Flowchart demonstrating the number of patient “drop-outs” and the stage of the event ................................................................................................................................................ 358
Figure 59. Mean (±SE) changes in Weight (Kg), %BFat (%), and BMI (Kg/Ht^2) with time in patients with metastatic and locally advanced disease........................................................... 360
Figure 60. Graph of mean (±SE) TBN against time (measurement time-point) in patients with metastatic and locally advanced disease. ................................................................. 361
Figure 61. Graph of mean (±SE) NI and TBK/Ht against time for the patients with metastatic and locally advanced disease................................................................. 362
Figure 62. Graph of mean (±SE) LBM measurements using BIA technique against time for the patients with metastatic and locally advanced disease................................................................. 363
Figure 63. Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for the patients with metastatic and locally advanced disease................................................................. 364
Figure 64. Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for the patients with metastatic and locally advanced disease................................................................. 365
Figure 65. Graph of mean (±SE) TBK measurements against time for the patients with metastatic and locally advanced disease................................................................. 366
Figure 66. Graph of mean (±SE) TBW measurements against time for the patients with metastatic and locally advanced disease................................................................. 367
Figure 67. Changes in Weight with time in pancreatic cancer patients with metastatic and locally advanced disease. ....................................................................................................... 378
Figure 68. Changes in Fat Mass with time in pancreatic cancer patients with metastatic and locally advanced disease. ....................................................................................................... 379
Figure 69. Changes in LBM (By Lukaski) with time in pancreatic cancer patients with metastatic and locally advanced disease. ....................................................................................................... 380
Figure 70. Changes in TBW with time in pancreatic cancer patients with metastatic and locally advanced disease. ....................................................................................................... 381
Figure 71. Mean (±SE) Weight (Kg), %BFat (%), and BMI (Kg/Ht^2) with time in patients receiving EPA or Placebo. ........................................................................................................ 384
Figure 72. Graph of mean (±SE) TBN against time (measurement time-point) in patients receiving EPA or Placebo. ........................................................................................................ 385
Figure 73/ Graph of mean (±SE) NI and TBK/Ht against time for the patients receiving EPA or Placebo............................................................................................................................... 386
Figure 74. Graph of mean (±SE) LBM measurements using BIA technique against time for the patients receiving EPA or Placebo. ........................................................................................................ 387
Figure 75. Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for the patients receiving EPA or Placebo. ........................................................................................................ 388
Figure 76. Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for the patients receiving EPA or Placebo. ........................................................................................................ 389
Figure 77. Graph of mean (±SE) TBK measurements against time for the patients receiving EPA or Placebo. ........................................................................................................ 390
Figure 78. Graph of mean (±SE) TBW measurements against time for the EPA and Placebo disease patient groups using the Fredrix, Pullicino, and Kushner equations. ........................................................................................................ 391
Figure 79. Changes in Weight with time in pancreatic cancer patients receiving EPA or Placebo. ........................................................................................................ 392
Figure 80. Changes in FM with time in pancreatic cancer patients receiving EPA or Placebo. ........................................................................................................ 393
Figure 81. Changes in TBN with time in pancreatic cancer patients receiving EPA or Placebo. ........................................................................................................ 394
Figure 82. Changes in TBW with time in pancreatic cancer patients receiving EPA or Placebo. ........................................................................................................ 395
Figure 83. Changes in TBK and LBM (by TBK) with time in pancreatic cancer patients receiving EPA or Placebo. ........................................................................................................ 396
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>Changes in TBK/Ht with time in pancreatic cancer patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>85</td>
<td>Graph of mean (±SE) Dyspnoea scores against time (measurement time-point) in patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>86</td>
<td>Graph of mean (±SE) Nausea &amp; Vomiting scores against time (measurement time-point) in patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>87</td>
<td>Graph of mean (±SE) Sexuality scores against time (measurement time-point) in patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>88</td>
<td>Graph of mean (±SE) Karnofsky Performance Status scores against time (measurement time-point) in patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>89</td>
<td>Changes in Dyspnoea scores with time in pancreatic cancer patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>90</td>
<td>Changes in Nausea &amp; Vomiting scores with time in pancreatic cancer patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>91</td>
<td>Changes in Sexuality scores with time in pancreatic cancer patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>92</td>
<td>Changes in Pain Card Q1 scores with time in pancreatic cancer patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>93</td>
<td>Changes in Karnofsky Performance Status scores with time in pancreatic cancer patients with metastatic and locally advanced disease.</td>
</tr>
<tr>
<td>94</td>
<td>Graph of mean (±SE) Healthcare Satisfaction scores against time (measurement time-point) in patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>95</td>
<td>Graph of mean (±SE) Hepatic Symptoms scores against time (measurement time-point) in patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>96</td>
<td>Graph of mean (±SE) EORTC’s Sexuality scores against time (measurement time-point) in patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>97</td>
<td>Graphs of mean Pain Card Q3, EORTC Cognitive Functioning, Hepatic Symptoms, and Altered Bowel Symptoms scores against time for EPA and Placebo groups.</td>
</tr>
<tr>
<td>98</td>
<td>Mean Pain Card scores against time (Therapy Cycle) for patients receiving Placebo.</td>
</tr>
<tr>
<td>99</td>
<td>Mean Pain Card scores against time (Therapy Cycle) for patients receiving EPA.</td>
</tr>
<tr>
<td>100</td>
<td>Changes in Altered Bowel Habit scores with time in pancreatic cancer patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>101</td>
<td>Changes in Dyspnoea scores with time in pancreatic cancer patients receiving EPA or Placebo.</td>
</tr>
<tr>
<td>102</td>
<td>Curve for the effect of Study Arm (EPA or Placebo) on survival.</td>
</tr>
<tr>
<td>103</td>
<td>Survival curve for the effect of NI cut-off values of 0.90 on survival.</td>
</tr>
<tr>
<td>104</td>
<td>Survival curve for the effect of NI cut-off values of 1.00 on survival.</td>
</tr>
<tr>
<td>105</td>
<td>Survival curve for the effect of gender on survival.</td>
</tr>
<tr>
<td>106</td>
<td>Survival curve for the effect of the extent of disease on survival.</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

Table 1. Regions of interest (ROI) for the sodium iodide detectors .......................................................... 246
Table 2. Regions of interest (ROI) for the bismuth germanium oxide detector......................................... 247
Table 3. Annual nitrogen background experiment results ........................................................................ 250
Table 4. Annual nitrogen to hydrogen ratio width experiment results .................................................... 251
Table 5. Reproducibility of the system during one calendar year ............................................................ 254
Table 6. Summary of statistical results (p-Values) for comparison between Clear Margins and Unclear Margins group. Results sorted by parameters ................................................................. 286
Table 7. Statistical comparison of body composition parameters of Clear Margin and Unclear Margin groups at corresponding time-points. Results (p-Values) sorted by parameter ................................ 288
Table 8. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-operative) values between the Clear Margin and Unclear Margin groups. Results (p-Values) sorted by parameter ................................................................. 293
Table 9. Summary of the comparison (paired T-Test) of body composition parameters between different measurement time-points for the patients with Unclear Margins. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 297
Table 10. Summary of the comparison (paired T-Test) of body composition parameters between different measurement time-points for the patients with Clear Margins. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 298
Table 11. Kaplan-Meier analysis results for different NI cut-off values .................................................. 305
Table 12. Cox’s Regression univariate analysis (sorted by p-value) .......................................................... 309
Table 13. Backward Stepwise Likelihood Ratio Multivariate Cox’s Regression of the parameters with Univariate Cox’s Regression \( p \leq 0.25 \) ................................................................................................................................. 310
Table 14. Multivariate Cox’s Regression adjusted for Fat Mass, Margin Status, and Age ......................... 311
Table 15. Summary of statistical results for comparison between Arm A and Arm B groups using AUC and slopes. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 326
Table 16. Comparison of body composition changes between different chemotherapy cycles in Arm A and Arm B. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 329
Table 17. Comparison of different body composition parameters between the Arm A and Arm B at the corresponding chemotherapy cycle. Results (p-Values) sorted by parameter ................. 331
Table 18. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the Arm A and Arm B groups. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 332
Table 19. Kaplan-Meier analysis results for different NI cut-off values in patents with mesothelioma. ......................................................................................................................................................................................... 346
Table 20. Cox’s Regression univariate analysis (sorted by p-Value) .......................................................... 351
Table 21. Backward Stepwise Likelihood Ratio Multivariate Cox’s Regression of the parameters with Univariate Cox’s Regression \( p \leq 0.25 \) ................................................................................................................................. 352
Table 22. Multivariate Cox’s Regression adjusted for Haemoglobin, Age, and NI .................................. 353
Table 23. Summary of statistical results for comparison between metastatic and locally advanced groups using AUC and slopes. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 370
Table 24. Comparison of body composition changes between different chemotherapy cycles in metastatic patients. Results (p-Values) sorted by parameter ......................................................................................................................................................................................... 373
Table 25. Comparison of body composition changes between different chemotherapy cycles in locally advanced patients. Results (p-Values) sorted by parameter.................................................................375
Table 26. Statistical comparison of body composition parameters of metastatic and locally advanced groups at the corresponding time-points. Results (p-Values) sorted by parameter. ................................................................................................................................................376
Table 27. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the metastatic and locally advanced groups. Results (p-Values) sorted by parameter. .................................................................381
Table 28. Summary of statistical results for comparison between EPA and Placebo groups using AUC and slopes. Results (p-Values) sorted by parameter. ................................................................................................................................................392
Table 29. Comparison of body composition changes between different chemotherapy cycles in patients receiving Placebo. Results (p-Values) sorted by parameter. ................................................................................................................................................394
Table 30. Comparison of body composition changes between different chemotherapy cycles in patients receiving EPA. Results (p-Values) sorted by parameter. ................................................................................................................................................396
Table 31. Statistical comparison of body composition parameters of the EPA and placebo groups at the corresponding time-points. Results (p-Values) sorted by parameter. ................................................................................................................................................403
Table 32. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the EPA and Placebo groups. Results (p-Values) sorted by parameter. ................................................................................................................................................408
Table 33. Statistical comparison of QL parameters of metastatic and locally advanced groups at the corresponding time-points. Results (p-Values) sorted by parameter. ................................................................................................................................................413
Table 34. Summary of statistical results for comparison between metastatic and locally advanced groups using AUC and slopes. Results (p-Values) sorted by parameter. ................................................................................................................................................416
Table 35. Comparison of QL changes between different chemotherapy cycles in metastatic patients. Results (p-Values) sorted by parameter................................................................................................................................................419
Table 36. Comparison of QL changes between different chemotherapy cycles in locally advanced patients. Results (p-Values) sorted by parameter................................................................................................................................................420
Table 37. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the metastatic and locally advanced groups. Results (p-Values) sorted by parameter. ................................................................................................................................................427
Table 38. Statistical comparison of QL parameters of EPA and Placebo groups at the corresponding time-points. Results (p-Values) sorted by parameter. ................................................................................................................................................431
Table 39. Summary of statistical results for comparison between EPA and Placebo groups using AUC and slopes. Results (p-Values) sorted by parameter. ................................................................................................................................................434
Table 40. Comparison of QL changes between different chemotherapy cycles in patients receiving the Placebo. Results (p-Values) sorted by parameter. ................................................................................................................................................437
Table 41. Comparison of QL changes between different chemotherapy cycles in patients receiving the EPA. Results (p-Values) sorted by parameter. ................................................................................................................................................439
Table 42. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the EPA and Placebo groups. Results (p-Values) sorted by parameter. ................................................................................................................................................446
Table 43. Kaplan-Meier analysis results for different NI cut-off values. ................................................................................................................................................452
Table 44. Cox’s Regression univariate analysis (sorted by p-values)................................................................................................................................................453
Table 45. Backward Stepwise Likelihood Ratio Multivariate Cox’s Regression of the parameters with Univariate Cox’s Regression p≤0.25 ................................................................................................................................................454
Table 46. Multivariate Cox’s Regression adjusted for Disease Extent, %BFat, and Age. .................................454
LIST OF EQUATIONS

Equation 1. Vartsky’s equation ........................................................................................................ 199
Equation 2. Nuclear conversion of $^{14}$N to $^{15}$N ........................................................................ 200
Equation 3. Alternative equation for the nuclear conversion of $^{14}$N to $^{15}$N. ......................... 200
Equation 4. Resolution of Fourier Transform spectrometers ......................................................... 207
Equation 5. Kushner equation for TBW estimation for males ....................................................... 209
Equation 6. Kushner equation for TBW estimation for females .................................................... 210
Equation 7. Pullicino equation for TBW estimation ....................................................................... 210
Equation 8. Fredrix [753] equation for TBW estimation ............................................................... 210
Equation 10. Watson [725] equation for TBW estimation for females ......................................... 210
Equation 11. Siri’s equation ........................................................................................................... 219
Equation 12. Regression equation for the prediction of body density in men ................................ 220
Equation 13. Regression equation for the prediction of body density in women ......................... 220
Equation 14. Wang’s equation for the estimation of SSM ............................................................. 225
Equation 15. Estimation of FFM from a four compartmental model .............................................. 225
Equation 16. Calculation of LBM from %body fat and body weight ............................................ 236
Equation 17. Polynomial fit for Nitrogen Count variations with Widths ...................................... 250
Equation 18. Polynomial fit equation for the N/H Ratios against Widths plot ............................. 252
Equation 19. Linear fit equation for the N/H Ratios against Widths plot ..................................... 252
Equation 20. ABNOH calculations ............................................................................................... 253
Equation 21. Determining sample size ......................................................................................... 269
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{[18F]}$FDG</td>
<td>F-18-2-Fluoro-2-Deoxy-Glucose</td>
</tr>
<tr>
<td>$^{241}$AmBe</td>
<td>Americium-241/Beryllium</td>
</tr>
<tr>
<td>$^{252}$Cf</td>
<td>Californium-252</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Amino Transferase</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine 5'-Monophosphate</td>
</tr>
<tr>
<td>ANC</td>
<td>Absolute Neutrophil Count</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Amino Transferase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine 5'-Triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Are Under the Curve</td>
</tr>
<tr>
<td>BCM</td>
<td>Body Cell Mass</td>
</tr>
<tr>
<td>BFat</td>
<td>Body Fat</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic Fibroblast Growth Factor</td>
</tr>
<tr>
<td>BGO</td>
<td>Bismuth Germanium Oxide</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Analysis</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CA 19.9</td>
<td>Carbohydrate Antigen 19.9</td>
</tr>
<tr>
<td>CALGB</td>
<td>Cancer and Leukaemia Group B</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine 5'-Monophosphate</td>
</tr>
<tr>
<td>CEA</td>
<td>Carcino-Embryonic Antigen</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Meaning</td>
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</tr>
<tr>
<td>CIVBC</td>
<td>Centre for In Vivo Body Composition</td>
</tr>
<tr>
<td>CM</td>
<td>Clear Margins</td>
</tr>
<tr>
<td>CMF</td>
<td>Cyclophosphamide, Methotrexate, 5-Fluorouracil</td>
</tr>
<tr>
<td>CNTF</td>
<td>Ciliary Neurotrophic Factor</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CTC</td>
<td>Common Toxicity Criteria</td>
</tr>
<tr>
<td>dATP</td>
<td>deoxy-Adenosine Triphosphate</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic Acid</td>
</tr>
<tr>
<td>DVT</td>
<td>Deep Vein Thrombosis</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EGRF</td>
<td>Epithelial Growth Factor Receptor</td>
</tr>
<tr>
<td>EORTC</td>
<td>European Organisation for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic Acid</td>
</tr>
<tr>
<td>ERCP</td>
<td>Endoscopic Retrograde Cholangio-Pancreatography</td>
</tr>
<tr>
<td>ESPAC</td>
<td>European Study Group for Pancreatic Cancer</td>
</tr>
<tr>
<td>EUS</td>
<td>Endoluminal Ultrasonography</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat Free Mass</td>
</tr>
<tr>
<td>FM</td>
<td>Fat Mass</td>
</tr>
<tr>
<td>FNA</td>
<td>Fine Needle Aspiration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Meaning</td>
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<td>--------------</td>
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<tr>
<td>GITSG</td>
<td>Gastrointestinal Study Group</td>
</tr>
<tr>
<td>HAD</td>
<td>Hospital Anxiety and Depression</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>Ht</td>
<td>Height</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>IL-</td>
<td>Interleukin-</td>
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<tr>
<td>INF-γ</td>
<td>Interferon-γ</td>
</tr>
<tr>
<td>IVBC</td>
<td>In Vivo Body Composition</td>
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<tr>
<td>IkB</td>
<td>Inhibitor Kappa B</td>
</tr>
<tr>
<td>K-ras</td>
<td>Kirsten ras sarcoma</td>
</tr>
<tr>
<td>LASA</td>
<td>Linear Analogue Self Assessment</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean Body Mass</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukaemia Inhibitory Factor</td>
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<tr>
<td>LMF</td>
<td>Lipid Mobilising Factor</td>
</tr>
<tr>
<td>MCA</td>
<td>Multi Channel Analyser</td>
</tr>
<tr>
<td>MIBG</td>
<td>Metaiodobenzylguanidine</td>
</tr>
<tr>
<td>MPH</td>
<td>Medium Phantom</td>
</tr>
<tr>
<td>MRCP</td>
<td>Magnetic Resonance Cholangio-Pancreatography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>MW</td>
<td>Medium Water</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>NaI</td>
<td>Sodium Iodide</td>
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<td>Abbreviation</td>
<td>Meaning</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear Factor- kappa B</td>
</tr>
<tr>
<td>NI</td>
<td>Nitrogen Index</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drugs</td>
</tr>
<tr>
<td>PEF-G</td>
<td>Cisplatin, Epirubicin, 5-Fluorouracil, Gemcitabine</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PIF</td>
<td>Proteolysis-Inducing Factor</td>
</tr>
<tr>
<td>QL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>SMM</td>
<td>Skeletal Muscle Mass</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computerised Tomography</td>
</tr>
<tr>
<td>TBCa</td>
<td>Total Body Calcium</td>
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<tr>
<td>TBCl</td>
<td>Total Body Chlorine</td>
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<tr>
<td>TBF</td>
<td>Total Body Fat</td>
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<tr>
<td>TBH</td>
<td>Total Body Hydrogen</td>
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<tr>
<td>TBK</td>
<td>Total Body Potassium</td>
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<tr>
<td>TBN</td>
<td>Total Body Nitrogen</td>
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<tr>
<td>TBW</td>
<td>Total Body Water</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor-α</td>
</tr>
<tr>
<td>TPN</td>
<td>Total Parenteral Nutrition</td>
</tr>
<tr>
<td>UCM</td>
<td>Unclear Margins</td>
</tr>
<tr>
<td>UF</td>
<td>Ultrafiltration</td>
</tr>
<tr>
<td>ULRR</td>
<td>Upper Limit of Reference Range</td>
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**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>VS</td>
<td>Vacuum Sublimation</td>
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Chapter 1: HYPOTHESES AND AIMS

1. HYPOTHESES AND AIMS

1.1 Background And Hypotheses

Cachexia, or the cachectic syndrome, is a self perpetuating condition which is characterised by a marked weight loss and the presence of anorexia, anaemia, and asthenia. It is often associated with the presence and growth of tumour. Although cachexia is often observed in solid tumours of the upper gastrointestinal tract, its presence is not unique to cancer and is often also present in most chronic, end-stage diseases processes.

It has been reported [1] that there is a direct link between the loss of body weight and an increase in morbidity and mortality from many diseases as well as the fact that patients with gastrointestinal malignancies and weight loss have a reduced survival and QL. The evidence for these effects has been reported and is available in the literature. There are a number of different treatment procedures currently available which have been used to treat cachexia. In fact, the treatment of cachexia is considered to be a multidisciplinary approach. Two of the recent therapeutic agents that have been suggested to be effective in the treatment of cachexia have been EPA and thalidomide.

Although both agents have been tested in a number of different disease conditions, their effects on patients’ body composition using compartmental body composition analysis and under chemotherapy conditions have not been fully investigated. Therefore, we hypothesise that there are significant changes in the body composition of patients receiving these therapeutic agents and that these changes affect the survival of these patients.

The long-term survival of cancer patients undergoing major surgery can also depend on the pre-operative body composition status as well as the maintenance of an adequate body
composition and nourished status. In addition, the tumour burden affects the body compartment levels which will, in turn, affect the long-term survival. We, therefore, hypothesise that the long-term survival of this group of patients depends on the success and effectiveness of surgery in the complete removal of the tumour as well as the maintenance of adequate body composition levels.

1.2 Aims

Based on the above background and hypotheses, the aims of this project were, therefore, to achieve and carry out the following:

1. Investigate and assess the long-term changes in body composition of pancreatic cancer patients undergoing Whipple’s Procedure.
2. Investigate and assess the long-term survival of pancreatic cancer patients undergoing Whipple’s Procedure.
3. Investigate and assess the changes in body composition of patients with malignant mesothelioma receiving thalidomide.
4. Investigate and assess the survival of patients with malignant mesothelioma receiving thalidomide.
5. Investigate and assess the changes in body composition of pancreatic cancer patients undergoing cancer chemotherapy and receiving EPA compared with placebo.
6. Investigate and assess the survival of pancreatic cancer patients undergoing cancer chemotherapy and receiving EPA compared with placebo.
7. Investigate the use and effectiveness of body composition techniques in assessing the outcomes of cancer cachexia treatments.
1.3 Structure and Stages of the Project

The project was designed to investigate the effects of two different cancers in two different patient populations on body composition. In addition, the effect of different treatment and/or intervention methods on the body composition and survival of these patients groups were also investigated.

The initial step of the project was to try and test some of the methods and to stream-line these procedures to reduce time and cost. Therefore, the initial step was to develop, validate and test a new TBW measurement technique that would enable a faster through-put of patient samples. The results of these validations and testings were subsequently published in peer-reviewed journals and included in the Appendix section.

The first group of patients investigated were pancreatic cancer patients undergoing surgery. These patients had long-term follow-up to assess their survival and changes in body composition over time. The interventional procedure used on these patients was, therefore, major surgery.

The second group of patients studied were patients with malignant mesothelioma. These patients were randomised to receive standard chemotherapy with thalidomide or thalidomide alone. This phase of the project provided valuable information on the effect of mesothelioma on body composition and survival as well as the efficacy of the new treatment schedule.

The third group of patients investigated were, again, patients with pancreatic cancer who were receiving standard chemotherapy. As these patients were at a high risk of cachexia, they were entered into a double-blind, placebo-controlled, randomised trial to receive EPA as a part of their standard chemotherapy. The body composition, survival and QL were measured and followed for four months.
2. CANCER CACHEXIA

“...the shoulders, clavicles, chest and thighs melt away. This illness is fatal...”

Hippocrates (460-370 BC)

The word cachexia comes from the Greek word “kakos” meaning “bad” and “hexis” meaning “condition” [2]. Cachexia, or the cachectic syndrome, is a self perpetuating condition [3] characterised by a marked weight loss and the presence of anorexia, anaemia, and asthenia. It is often associated with the presence and growth of tumour. Although cachexia is often observed in solid tumours of the upper gastrointestinal tract, its presence is not unique to cancer and is often also present in most chronic, end-stage diseases processes such as heart failure, Human Immunodeficiency Disease [4], chronic renal failure [5], chronic obstructive pulmonary disease [7, 6], etc. This also includes non-disease conditions such as aging [7]. In fact it has been demonstrated that in patients with chronic heart failure, it is an independent risk factor for mortality [8]. Compared to other types of cancers, pancreatic cancer has the highest rate of cachexia reaching as much as 80% at the time of diagnosis [9, 11, 10].

The pattern of weight loss seen in cachexia is different to simple starvation. In cachexia there is a loss of LBM as well as fat mass. However, in starvation there is primarily a loss of fat mass at the initial stages of weight loss. The other major difference between the two is the non-reversibility of weight loss in cachexia regardless of increase in food intake [13, 12].

There is a direct link between the loss of weight and an increase in the rate of morbidity and mortality in diseases such as cancer, AIDS, sepsis, diabetes, renal failure, denervation atrophy, congestive heart failure, etc. In all these cases the loss of fat-free mass involves the loss of only skeletal muscle mass and not the visceral protein where protein synthesis often increases. The skeletal muscle atrophy itself is characterised by a reduction in protein content,
muscle fibre diameter, force production, and fatigue resistance. Studies of Windsor et al \cite{14} have shown that with patients in these states, death usually occurs when the total body weight loss reaches 30% and that this appears to be as a result of respiratory failure through the erosion of the diaphragm causing hypostatic pneumonia.

\section*{2.1 Aetiology Of Cachexia}

In general, the most overt catabolism is seen in patients admitted to an intensive care unit after major trauma, surgery, or sepsis \cite{15}. Although the pathophysiology of cancer cachexia has been extensively reviewed in the literature \cite{7, 17, 18, 19, 20, 16, 21}, the actual cause of cancer cachexia is often not clear until the tumour itself interferes with food/energy intake or causes their obstruction \cite{7, 21}. Other problems such as infections (resulting from immunodeficiency caused by chemotherapy) affect the energy requirements of the host. It also occurs in children and has similar aetiology, prognosis, and consequences with surprising frequencies \cite{22}. The degree of cachexia is inversely correlated with survival time and often implies a poor prognosis \cite{17, 18}.

As cancer chemotherapy and radiotherapy are not confined exclusively to tumour cell populations, their effect on normal host tissues also contribute to specific nutritional problems. The loss of visceral protein in LBM has a greater negative prognostic effect than the loss of adipose tissue, and the greater the loss the poorer the prognosis \cite{23}. Studies of Lundholm et al \cite{24} in the mid 1970’s showed that there is decreased muscle protein synthesis in cancer cachexia. The types of skeletal muscle lost during cancer cachexia have been long known to be largely the white or “phasic” muscles rather than the red or tonic muscles \cite{25}. The regulation of muscle mass in cancer cachexia has been suggested to be carried out by changes in the rate of protein synthesis with the changes in protein degradation being mainly
a secondary effect [25]. Studies of Smith and Tisdale [26] in the early 1990’s have also
demonstrated this increased skeletal muscle protein degradation and decreased protein
synthesis during cancer cachexia.

Amongst other metabolic disturbances that have been reported and described in the literature
are changes in lipid metabolism, skeletal muscle proteolysis, apoptosis, and acute-phase
protein synthesis [27].

The underlying mechanisms of chronic catabolism in patients with cancer cachexia are similar
to those seen in cases of acute injury or sepsis [28]. Under normal, non-cachectic conditions
tissue and muscle repair occur in four separate steps or phases of degeneration followed by
inflammation, regeneration and fibrosis [29]. The “healing” process is often considered to be
the continuous process of inflammation and repair where different immune cells are involved
[30]. One of the major functions of these inflammatory cells which invade the area is the
phagocytosis and removal of the remaining cellular debris [29]. These inflammatory cells,
when activated, secrete a variety of different cytokines which act to modulate vascular
permeability, blood flow, and speed-up the inflammatory response. These cytokines include
TNF-α, IL-1β, IL-6, and IL-8. In the case of muscle injury, these cytokines may be produced
either intrinsically by the muscle or by inflammatory cells such as neutrophils, macrophages,
fibroblasts, vascular smooth muscle, or the vascular endothelial cells [31]. In disease
pathogenesis, there is a shift in balance in favour of the cytokine environment which is
persistent and results in host’s muscle wastage [28]. An example of such a condition is cancer
cachexia.

One of the main aetiological factors that can lead to cachexia is the presence of malnutrition
particularly under cancer settings [3]. The diagnosis of malnutrition is usually complicated due
to the fact that various nutritional alterations can involve macronutrients or micronutrients as
well as the fact that their depletion may be stable or progressive. It is accepted that the cancer-
associated malnutrition itself occurs as a result of an imbalance between the nutritional needs of the host, the nutritional demands of the tumour, and the availability of the appropriate nutrients in the body \[^3\]. It is very important to diagnose and treat the cancer-associated malnutrition as early as possible to delay or stop the progression of malnutrition \[^32\]. Unfortunately, malnutrition occurs before clinical signs are evident and the consequences of a poor nutritional state may not be apparent making its early diagnosis difficult. However, if left untreated, the cancer-associated malnutrition can lead to cancer cachexia which further exasperates the malnutrition, predisposing the patient to increased complication rate and a more severe disease promoting a further decline in the nutritional status and, thus, entering a vicious cycle of cancer cachexia \[^3\]. One possible method that has been suggested in the literature to diagnose cancer-associated malnutrition at an early stage would be the assessment of the resting energy expenditure (or the basal metabolic rate) of the patient \[^34,33\].
2.2 Mediators Involved in Cachexia

It has long been suspected that, since cachexia can also occur in the absence of anorexia, some catabolic mediators are produced by the tumour, host, or both and are involved in the overall process of cancer cachexia\textsuperscript{[37, 17, 36, 18, 35]}. The mediators involved in cancer cachexia are reported to be the pro-inflammatory cytokines\textsuperscript{[37, 38, 17, 18]} and are reported to play a pivotal role in the aetiology of cancer cachexia\textsuperscript{[36, 39]}.

Cytokines are cellular proteins produced by inflammatory cells that function as paracrine intracellular mediators\textsuperscript{[7]}. Most studies reported in the literature on these pro-inflammatory cytokines have been carried out on animal models with only a few on human subjects. The major cytokines involved include the TNF-\(\alpha\), IL-1, IL-6, and INF-\(\gamma\)\textsuperscript{[37, 38, 17, 18]}\textsuperscript{[37, 38, 17, 18]}. It should be noted that there are overlapping activities of the cytokines TNF-\(\alpha\), IL-1, IL-6, and INF-\(\gamma\) and the exact contribution of each cytokine to the process of cancer cachexia remains unclear. In addition, cytokines have even been suggested to support the host’s tumour growth by acting like growth factors\textsuperscript{[20]}.

2.2.1 Interleukins

Earlier studies of Strassman et al\textsuperscript{[40]} on murine colon adenocarcinoma-bearing mice have shown that anti-mouse IL-6 antibody can successfully reduce cachexia symptoms in these mice indicating that the IL-6 may be involved in cachexia. However, other studies have reported that IL-6 is not involved in cachexia or on muscle proteolysis\textsuperscript{[17, 18]}.

The actions of IL-1 and TNF-\(\alpha\) have been thought to be involved in the induction of anorexia in patients with cancer cachexia\textsuperscript{[18]}. This mechanism of action is thought to be by elevating
the levels of corticotrophin-releasing hormone in the brain which suppresses food intake as well as modulating the functions of glucose-sensitive neurons\[18\].

The IL-1 has been shown to induce anorexia in patients with cancer cachexia by affecting gastric motility and has been shown to block the actions of neuropeptide Y\[41\].

### 2.2.2 Interferon And Tumour Necrosis Factors

Amongst other potential pro-inflammatory cytokines thought to be involved in cachexia are the INF-\(\gamma\) and TNF-\(\alpha\). INF-\(\gamma\) is produced by activated T-cells and natural killer cells and have similar activities to TNF-\(\alpha\) in regards to cachexia. INF-\(\gamma\)’s role in cachexia has long been established, confirmed and reported in the literature and demonstrated in animal models\[42\]. However, there seems to be less investigation and research being conducted and reported in relation to cachexia in the literature on INF-\(\gamma\) than TNF-\(\alpha\).

TNF-\(\alpha\), however, is a pleiotropic cytokine and is an important simulator of the “second wave” cytokines including IL-6 and chemokines\[43\]. It has also long been known that chronic TNF-\(\alpha\) exposure results in sustained muscle wastage\[44\] consistent with the pathology of cachexia. When injected in the brains of laboratory animals, TNF-\(\alpha\) have been shown to produced an increase in basal metabolic rate without an increase in the metabolic activity\[17, 18\]. This increase has been mainly due to an increase in blood flow and thermogenic activity of brown adipose tissue which is also seen in both human and laboratory animals during the cachectic state\[17, 18\].

TNF-\(\alpha\) has also been shown to promote muscle wasting\[44\]. Studies both in human\[45\] and animal models\[46\] have shown that the administration of TNF-\(\alpha\) produces an increase in muscle proteolysis which is associated with an increase in gene expression and higher levels
Chapter 2: CANCER CACHEXIA

of free and conjugated ubiquitin. The actions of ubiquitin has been shown to be down-regulated by EPA \(^ {48, 47}\).

In summary, TNF-\(\alpha\) may be responsible for the induction of events associated with the hypermetabolism state, such as the ones related to the brown adipose tissues and the uncoupling protein \(^ {36}\). In addition, TNF-\(\alpha\), alone or in combination with other proteins seem to mediate most of the changes seen in cachexia which is related to nitrogen metabolism and skeletal muscle protein break down \(^ {36, 49, 50}\).

2.2.3 Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome pathway is considered to be the major pathway by which the proteins in the muscle tissue are degraded during cancer cachexia \(^ {51}\). The ubiquitin-proteasome pathway and its use as a therapeutic target in cachexia has been recently reviewed by Tisdale \(^ {52}\).

Ubiquitin itself is a 76 amino acid protein which is found in most eukaryotic cells. It becomes covalently attached to certain residues of other proteins \(^ {51}\). The attachment of a chain of ubiquitins tags a protein for intracellular proteolytic destruction. Here, several ubiquitin molecules attach themselves to the condemned protein (also called polyubiquitination) forming multimeric polyubiquitin chains, and then move to a proteasome, a barrel-shaped structure where the proteolysis occurs \(^ {53}\). Ubiquitin can also mark transmembrane proteins (such as receptors) for removal from the membrane.

The ubiquitin gene has been shown to be activated by cytokines such as IL-1 and INF-\(\gamma\) indicating that the TNF-\(\alpha\) acting alone or in combination with these cytokines mediates changes in nitrogen metabolism during cachexia \(^ {49}\). In addition to massive protein loss, muscle DNA is decreased during cancer cachexia causing DNA fragmentation and apoptosis.
This action is mimicked by TNF-α. The ubiquitin-proteasome system also indirectly modulates protein synthesis through degradation of inhibitory κB protein-NFκB gene regulation. The production of and TNF-α–mediated metabolic changes have been shown to be counteracted by Thalidomide (α-N-phthalimidoglutaramide) and EPA. In addition, thalidomide have been investigated as a possible agent to counteract the detrimental effects of TNF-α in chronic heart failure.

The process of apoptosis, or cell death, is the primary mechanism by which muscle degradation occurs in cachexia. Apoptosis itself is a complex processes involving a cascade mechanism that employs many proteins. The key enzymes in this process are the Caspases. Caspases are a family of cysteine proteases that control and mediate the apoptotic response and are present in all animal cells. They are mainly present as inactive zymogens that cannot carry out apoptosis. There are various different triggers that can lead to their activation, which usually occurs through the proteolytic processing of the zymogen at conserved aspartic acid residues. Their activation and suicidal function is, however, highly regulated. Once activated caspases act as cysteine proteases, using a cysteine side chain as a catalysing peptide bond cleavage at aspartyl residues in their substrates. The name “caspase” denotes their function: Cysteine-dependent ASPartyl-specific proteASE. Caspases have been shown to be involved in disease-related muscle degradation. The caspase activities have recently been shown to be inhibited by cyclooxygenase inhibitors suggesting another possible anti-cachectic mechanism of EPA.

It has been shown that there are interaction between ubiquitin-proteasomal mechanisms and caspases where caspases execute cell death in response to cytokines such as TNF-α. These processes are carried out by the controlled destruction of the cell’s own repair mechanisms. Studies of Denecker et al have shown that the caspase-2, caspase-8, caspase-9, and caspase-10, also known as the apoptotic initiator caspases, lie upstream of the
Chapter 2: CANCER CACHEXIA

procaspase-3, procaspase-6, and procaspase-7 which themselves exist in a latent form until activated by their initiators through a process of cleavage, oligomerisation, and auto-activation.

Under normal, non-disease conditions the function of the ubiquitin-proteasome proteolytic pathway is to remove damaged proteins. Normally damaged proteins are produced as a result of genetic alterations, thermal stress, or oxidative stress and the resulting proteins tend to aggregate. The slowing and stopping of the aggregation process is carried out by molecular chaperons which is also present the aberrant proteins for proteolysis, the majority of which is performed by the proteasome. The other function of the ubiquitin-proteasome proteolytic pathway under normal, non-disease conditions, is to ubiquinitate and degrade newly synthesised, but defective proteins. These are proteins which are created as a result of errors in translation or post-translational processing and are generally defective ribosomal products which are incapable of attaining their native protein structures.

Under disease conditions, such as cancer cachexia, diabetes, sepsis, metabolic acidosis, etc, the expression of the ubiquitin-proteasome proteolytic pathway in skeletal muscle is increased leading to a muscle wasting syndrome condition or state \[70, 69\]. Unfortunately, as the normal function of the ubiquitin-proteasome proteolytic pathway is independent of the amount of protein consumed, simple nutritional supplementation will not slow down the rate of muscle catabolism \[52\].

The functions of the ubiquitin-proteasome proteolytic pathway have been shown to be regulated by a number of different factors. One of the main factors that is involved in its regulation, as mentioned above, are the cytokines. These are primarily the TNF-\(\alpha\), IL-6 and IFN-\(\gamma\). In addition, the cytokines have been shown to act synergistically to produce myofibrillar protein loss. As an example, recent studies of Acharyya et al \[72, 71\] have shown
that neither TNF-α or IFN-γ alone can induce myosin heavy chain loss in murine myotubules. However, in combination, they are strongly capable of myosin expression. One factor that is capable of influencing the changes in proteasome activity and, therefore, to reduce the increased muscle protein breakdown has been insulin. Studies of Bennet et al. have shown that insulin can directly inhibit proteasome activity. It has also been suggested that possible mechanisms of proteasome inhibition could be the generation of inhibitory fragments of insulin, or by the displacement of an insulin-degrading enzyme from the proteasome.

2.2.4 Neuroendocrine Stress Response And Non-Cytokine Factors

During cachexia the neuroendocrine stress response is also activated causing an increase in the cortisol levels with an increase in the renin-angiotensin as well as the adrenergic systems. The increased release of cortisol and catecholamines from the adrenal glands is triggered by the pro-inflammatory cytokines. This increase in cortisol release further exasperates the activity of the ubiquitin-proteasome system causing an increase in the resting energy expenditure of the patients. As a result of this activation there is also a reduction in the circulating insulin levels.

One of the other actions of cytokines affecting the endocrine system is the increase in the production of low density lipoprotein synthesis and a decrease in the lipoprotein lipase activity. This reduces the clearance of triacylglycerol and results in a state of hypertriglyceridemia. The net result is a state of negative energy balance and weight loss.
2.2.4.1 Proteolysis-Inducing Factor

As previously mentioned, the tumour itself also releases cachectic factors which act to worsen the cachexia. A proteolysis-inducing factor (or PIF) was isolated by Todorov et al. [78, 79, 80] in the mid to late 1990s. It is a sulphated glycoprotein produced by cachexia-inducing murine and human tumours. This factor has been found to be a 24 kDa proteoglycan which has also been detected in the urine of pancreatic cancer patients with cachexia [81] and promotes both increased protein degradation as well as decreased protein synthesis [83, 82]. PIF is released by the tumour itself [18]. The method of action of the PIF is thought to be by the activation of protein degradation through the stimulation of adenosine triphosphate and the ubiquitin proteasome-dependent pathway [78, 79, 80]. PIF is released by the tumour and is often detected in the urine of patients with cachexia. PIF causes an increase in muscle catabolism and a decrease in muscle anabolism [81, 83, 82]. The actions of PIF, particularly the proteolytic actions, is blocked by EPA [84, 85].

The disruption of protein metabolism is caused by two factors: a) activation and increase in proteolysis; and b) inhibition or reduction in proteosynthesis. The net result is the continuous loss of skeletal muscle mass. These effects are thought to be due to PIF [81, 79]. Protein degradation then releases amino acids which are then used by the liver for gluconeogenesis. The usage of these amino acids in the gluconeogenesis pathway will, in turn, waste LBM. This is to an extent that the daily glucose production for use by the brain cannot be met. Glucose catabolism through anaerobic metabolism requires more energy than through oxidative metabolism, which is another factor in causing the increased daily energy loss [9].

Studies of Smith et al. [86] have also demonstrated that PIF is able to increase the NF-κB expression in muscle cells in vitro and have suggested that this may be linked to the proteolytic actions of PIF in cachexia. This has been confirmed by the studies of Cai et al. [87] in animal models. As with TNF-α, the induction of the ubiquitin-proteasome pathway by PIF
is also associated with a transient decrease in the cytosolic concentration of the IκB as well as an increase in NF-κB nuclear migration \[88\].

### 2.2.4.2 Transcriptional Factors

There are a few transcriptional factors that can be possible candidates for inducing, causing, or promoting cachexia in humans and animals. The actions of these factors have been reviewed in the literature \[17, 18\]. One of the most investigated of these transcriptional factors is the NF-κB. The NF-κB proteins comprise a family of structurally-related eukaryotic transcription factors that are involved in the control of a large number of normal cellular processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. In addition, these transcription factors are persistently active in a number of disease states, including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease. Although not demonstrated in vivo, PIF has been shown to increase the expression of NF-κB \[86\]. The actions of NF-κB proteins have been shown to be inhibited by EPA \[89\] and thalidomide \[90, 91\].

### 2.2.4.3 Lipid Mobilising Factor

An LMF has been described by Khan and Tisdale \[92\] in the late 1990’s which was shown to be able to stimulate adenylate cyclase activity in order to induce lipolysis in adipose tissue. Although Khan and Tisdale \[92\] had purified, described and characterised this factor, the LMF was first detected by Hirai et al \[93\] in the urine of cancer patients suggesting that it would cause lipid mobilisation and catabolism in patients with cancer cachexia. The release of LMF, which is also found in the urine of patients with cachexia, contributes to the breakdown of adipose tissue \[93\].
The LMF is an \( \alpha_2 \)-glycoprotein which acts by activating the cyclic-AMP in the adipocytes causing the release of free fatty acids and glycerol into the blood circulation \[94\]. The LMF is thought to be released by the tumour \[18\].

### 2.2.4.4 Anaemia Inducing Factor

Other factors that cause cachexia have also been isolated, but they have been mostly in laboratory animals. Ishiko et al \[95\] in the late 1990s isolated an anaemia-inducing factor in rabbits. This factor was shown to be a 50kDa protein which is secreted by tumours \[18\]. It has the ability to depress erythrocyte and immunocompetent cell functions, reduce food intake, promote the loss of body fat and weight, all classical symptoms of cachexia \[95\].

### 2.2.4.5 Leptin

Leptin is a hormone released by the adipose tissue and it is well known that it plays a central role in the homeostasis of body weight as well as regulating the resting energy expenditure. High levels of leptin in the brain reduce the activity of the hypothalamic orexigenic mediators \[9\]. These mediators include ghrelin, neuropeptide Y, agoutin, orexin and melanocortin-releasing hormone. High levels of leptin in the brain also increase anorexigenic signals which include cholecystokinin, glucagon-like peptide, pro-opiomelanocortin, thyroid-releasing hormone, corticotrophin-releasing hormone, and oxytocin \[9\]. As a result, when the leptin levels are increased, the equilibrium that exists in body weight and energy expenditure becomes severely disrupted. The suppression of appetite and an increase in energy expenditure further exasperate the weight loss \[96\].
2.2.5 Other Cytokines And Factors

Cytokines such as the LIF and Transforming Growth Factor-β (also known as IL-1) have been suggested as possible mediators involved in cancer cachexia \[39\]. The levels of these protein have been shown to be elevated in patients with malignancies \[36, 39\].

Involvement of another factor, the CNTF, has also been reported in the literature \[36, 39\]. CNTF is a member of a family of cytokines which include IL-6 and LIF. CNTF is primarily produced by the glial cells of the peripheral nervous system. The cachectic effects of CNTF as well as its function as an inducer of acute-phase proteins, was demonstrated by Henderson et al \[97\] in the mid-1990s in animal models. CNTF is also expressed in the skeletal muscles \[36\].

2.2.6 Protein Degradation Pathways

Although there are a number of different pathways involved in protein degradation, there are three main pathways that are known to cause cellular protein degradation. These pathways or protein degradation systems work synergistically and in harmony, rather than individually, to cause muscle proteolysis. The actions of these pathways have been detailed in relation to other factors involved in cancer cachexia in the relevant sections of this chapter.

The lysosomal proteases (cathepsins) usually do not degrade cytosolic proteins. Instead, their major role is to breakdown the cellular membrane proteins such as the receptors and ion channels.

The second system that is involved in protein degradation is the calcium-activated proteases (calpains). The calpains are unable to breakdown actin and myosin and their major role in protein degradation seems to be in myofibrillar disassembly. The disassembled myofibrils are released and degraded by the ubiquitin-proteasome proteolytic pathway \[98\].
The third and the most important pathway in protein degradation is the ATP-dependent ubiquitin-proteasome proteolytic pathway. This is probably the major pathway involved in the breakdown of the skeletal muscle proteins in cachexia. This pathway has been shown to work together with the cathepsins to breakdown specific substrates \cite{99, 100}. It has also been shown that the ATP-dependent ubiquitin-proteasome proteolytic pathway is responsible for the myofibrillar protein degradation seen during muscle atrophy seen in diseases such as sepsis, diabetes, cancer cachexia, burn injuries, and renal tubular defects \cite{70, 69}. The mechanism of action of ATP-dependent ubiquitin-proteasome proteolytic pathway is detailed in section 2.2.3.
2.3 Starvation, Anorexia, And Cachexia

There are fundamental differences between the weight loss and body composition disturbances observed in cachexia to that observed in simple starvation. It is probably for this reason that most attempts to reduce weight loss and/or to correct body composition disturbances through aggressive or traditional feeding and re-feeding techniques often fail.

A concise overview of how anorexia is produced in patients with cancer cachexia is as follows: In cancer cachexia, there is an increased production of corticotrophin-releasing factor triggered by the excess production of cytokines. The corticotrophin-releasing factor, itself a very potent anorectic agent, together with the circulating prostaglandins, suppress the production of neuropeptide Y which is an orexigenic agent \[101, 102, 103, 104\]. In addition to the suppression of this feeding stimulus, proteolysis also occurs which is stimulated through the production of the proteasome system and the transcription factor NF-\(\kappa B\) \[86\]. Reduction in gastric emptying reduction in serum albumin and an enhanced lipolysis adds to this detrimental state \[105, 106\]. An LMF also activates the cyclic-AMP in the adipocytes causing the release of free fatty acids and glycerol in to the blood circulation \[94\]. Excessive production of lactate through anaerobic processes of the tumour increases the overall energy wasting by inducing the Cori cycle in the liver and the extra-hepatic tissues \[107\].

2.3.1 Weight Loss In Starvation And Cachexia

The weight loss seen in starvation is different to the weight loss seen in cachexia with the main observable difference being its irreversibility, even with feeding, in cachexia.
In the initial stages of starvation, the requirements of the brain and erythrocytes for glucose will cause the depletion of the liver and muscle glycogen as well as an increased production by the liver. This utilises the gluconeogenic amino acids derived from the catabolism of muscle. In long term starvation, this early phase is then replaced by the use of body fat as fuel where the free fatty acids released from the adipose tissue are converted into ketone bodies. These ketone bodies are then used by peripheral tissues and, to a larger extent, by the brain for energy. The net result in long term starvation would, therefore, be the conservation of muscle mass. In anorexia nervosa where there is a long term starvation, over 75% of the weight lost is the fat with a loss of a small amount of muscle [2, 108]. Also, it has long been know that in normal subjects, a forced reduction in caloric intake leads to a reduction in caloric expenditure, but in cancer cachexia patients this natural adaptation is blocked or severely attenuated [109].

In cancer cachexia, unlike long term starvation, there is an equal loss of fat and muscle mass. For the same percentage of weight loss there would, therefore, be more loss of muscle mass in patients with cachexia than patients with anorexia nervosa [108]. Although up to 40% of patients with cachexia have anorexia at first presentation [110], the changes in body composition suggest that the anorexia alone may not be responsible for the cachexia. It has long been known that in these malnourished patients, the measured amount of food intake does not correspond to the degree of weigh loss and malnutrition [111, 112]. In addition, the loss of muscle and adipose tissue commences well before the observed reduction in food intake [111, 112]. Studies carried out in the 1980s have demonstrated that in contrast to long term starvation, attempts to increase the food and energy intake to reverse weight loss in patients with cachexia will fail [113]. Also, attempts to reduce or reverse weigh loss and improve survival using TPN have also met with failure [114] with any gain in weight being only an increase in water retention [116, 115] rather than the much needed LBM.
2.3.2 Anorexia In Cachexia

Although cachexia can occur in the absence of anorexia, anorexia seems to be a component of cachexia and has both neurohumoral mechanisms as well as a broad range of clinical causes \cite{117}. It also seems to play an important role in the malnutrition observed in cachectic patients. Anorexia itself results from the failure of the usual appetite signals whereas cachexia is the actual debilitating state of involuntary weight loss \cite{9}.

Psychologically, the anorexia observed in cachexia has been reported to be a very distressing syndrome. Studies carried out in the mid-1980s have reported that the loss of appetite as well as their inability to eat has a large impact on the patients’ physical and psychological aspects of QL \cite{118, 119}.

The syndrome where anorexia and cachexia is present in cancer patients is referred to in the literature as the Cancer Anorexia-Cachexia Syndrome \cite{107, 120}. The cancer anorexia-cachexia syndrome, like simple cachexia, is often accompanied by weight loss, anorexia, malnutrition, and reduced food intake.

Pro-inflammatory cytokines may play a role in cancer-induced anorexia since they have been shown to control gastric motility and gastric emptying directly in the gastrointestinal system or through the brain pathways by affecting the efferent signals that regulate satiety \cite{41}. One of these pro-inflammatory cytokines is the IL-1 \cite{41} which acts by blocking the actions of neuropeptide-Y, a feeding simulating peptide \cite{101, 102, 103, 104}.

Both the IL-1 and TNF-\(\alpha\) have been suggested as possible mediators of cancer-induced anorexia \cite{17, 18}, which are thought to act by increasing the levels of corticotrophin-releasing hormone. The corticotrophin-releasing hormone acts centrally to suppress food intake and
reduce the firing of glucose-sensitive neurons with the net effect of reducing food intake and early satiety.

Although Leptin is a mediator that provides an “adiposity signal” to the brain it has been shown not to lay any role in cancer-induced anorexia in animal models \cite{122, 121} as well as human subjects \cite{124, 123}. In fact, leptin levels have been reported to be inversely proportional to the intensity of the systemic inflammatory response \cite{125}.

Earlier studies of Cangiano et al \cite{126, 127} and Rossi-Fanelli et al \cite{128} have also suggested tryptophan as an agent causing anorexia in patients with cachexia. The increased serotonergic activity within the brain is secondary to the enhanced availability of tryptophan to the brain.

As the uptake of tryptophan into the brain is competitive with that of branched chain amino acids (leucine, isoleucine, and valine), one way to reduce the uptake of tryptophan is to increase the levels of branched chain amino acids. This has been reported in the literature, but did not seem to be very successful in attenuating anorexia \cite{126, 127}. One of the other changes in protein metabolism during cachexia is the change in the turnover of these branched chain amino acids \cite{129, 130}.
2.4 Altered Metabolism In Cachexia

Altered metabolism is one of the hallmarks of cancer cachexia. It can occur to different extents depending on the host and type of tumour. The general areas where changes can occur include the basal metabolic rate, carbohydrate metabolism, lipid metabolism, and protein metabolism. If left untreated, all these changes will invariably and eventually lead to weight loss and the complications associated with weight loss.

The normal metabolic pathways are shown in the diagram below:

*Figure 1 Metabolic pathways for protein, carbohydrates and fats & lipids.*
2.4.1 Basal Metabolic Rate And Hypermetabolism

Metabolic changes in cachexia are also different from the metabolic changes in starvation. Changes in basal metabolic rate are one of the major metabolic changes that occur in cancer cachexia. In long term and chronic starvation a low-protein and low-calorie environment is created. In order to maintain adipose and muscle tissues, the basal metabolic rate is reduced by the body. Studies of Hyltander et al \cite{131} in the early 1990s have demonstrated that, in patients with cancer, the basal metabolic rate is increased and that this increase occurs even before the onset of weight loss. This would probably mean that the increased basal metabolic rate may be the cause rather than the consequence of the tumour \cite{131}.

The increased metabolic rate has been demonstrated in patient with pancreatic cancer and has been shown to be higher in pancreatic cancer patient who have an acute-phase response \cite{132}. The reason(s) for the elevated basal metabolic rate in patients with cancer cachexia, however, remains unclear.

In cancer cachexia, due to the induced hypermetabolic state, the host is more energy inefficient than a non-tumour bearing host. This increase in energy expenditure plus a decreased food intake plays an important and central role in the development of cancer cachexia. These energy inefficiencies are caused by two main “futile” cycles and processes: a) the Cori cycle; and b) Na\(^+\)-K\(^+\)-ATPase system \cite{39}.

It has long been known that there is an increase in brown adipose tissue thermogenesis during cachexia \cite{133}. This cytokine-induced non-shivering thermogenesis by the brown adipose tissues may be another factor contributing to the hypermetabolism and inefficient energy production frequently seen in patients with cancer cachexia \cite{37, 39}. An increase in the resting energy expenditure is also due to the uncoupling of oxidative phosphorylation which mainly occurs in the brown adipose tissue \cite{134}. Here, the uncoupling protein-2 and uncoupling
protein-3 channel energy away from oxidative phosphorylation into heat rather than ATP production\cite{134}.

Generally, uncoupling proteins are a family of mitochondrial membrane proteins which can increase thermogenesis and energy expenditure. For example, uncoupling protein-1 generates heat by uncoupling the oxidation of fatty acids from the generation of ATP and is only found in the brown adipose tissue. Interestingly, brown adipose tissue itself is not usually present in adults, but it is present in more than 80% of patients with cancer cachexia as compared to 13% of controls\cite{135}. The uncoupling protein-2 and uncoupling protein-3 are present in the skeletal muscle and play a significant role in energy balance and lipid metabolism.

### 2.4.2 Carbohydrate Metabolism

Generally, carbohydrate metabolism in cancer and cancer cachexia involves the metabolism of glucose. Most solid tumours have long been known to use anaerobic metabolism of glucose to obtain their energy requirements. In fact an important aspect of cachexia is the maintenance of gluconeogenesis and the use of glucose as the preferred fuel source that promotes the loss of body’s muscle and fat. In order to do this, they convert the glucose into lactate\cite{136}. This is the process of glycolysis which is the initial process of most carbohydrate catabolism. Glycolysis, in general, is the process by which a six-carbon glucose molecule is oxidised to pyruvic acid. Unlike oxidative phosphorylation, this process (i.e. glycolysis) is not an efficient method of energy production from glucose. As a result, larger amounts of glucose will be consumed by the tumour resulting in an accelerated energy loss by the host. This is due to an increase in the Cori cycle activity\cite{138,137}. At the same time, glucose production by the host has been long known to be less sensitive to suppression by insulin\cite{139}.  


In glycolysis, glucose and glycerol are metabolized to pyruvate via the glycolytic pathway. In most organisms this process occurs in the cytosol. Glycolysis generates a net two molecules of ATP through substrate phosphorylation catalysed by the two enzymes: phosphoglycerate kinase (a transferase enzyme) and pyruvate kinase. Here, two molecules of NADH are also produced, which can be oxidised via the Electron Transport Chain and will then result in the generation of an additional ATP by the ATP synthase enzyme. The pyruvate generated as an end-product of glycolysis is a substrate for the Citric Acid or Krebs cycle (named after German Scientist Hans Adolf Krebs, 1953).

Pyruvate itself is an important chemical compound in biochemistry, mainly being the output product of the metabolism of glucose (glycolysis). One molecule of glucose breaks down into two molecules of pyruvic acid, which are then used to provide further energy, in one of two ways:

- Provided that sufficient oxygen is available, pyruvic acid (2-oxopropanoic acid) is converted into acetyl-coenzyme A, which is the main input for a series of reactions known as the Krebs cycle. Pyruvate is also converted to oxaloacetate by an anaplerotic reaction and then further broken down to carbon dioxide. This is the aerobic process.

- If insufficient oxygen is available, the acid is broken down anaerobically, creating lactic acid in animals and ethanol in plants. Pyruvate from the process of glycolysis is converted by anaerobic respiration to lactate using the enzyme lactate dehydrogenase and the coenzyme NADH in lactate fermentation, or to acetaldehyde and then to ethanol in alcoholic fermentation. This is the anaerobic process, where unlike aerobic processes requires an electron acceptor to replace oxygen.

When muscles require energy for short duration or strenuous movements, muscle cells default to anaerobic glycolysis to quickly produce abundant amounts of ATP. The by-product of anaerobic glycolysis is lactate. Lactate then diffuses into the blood and is taken up by the
liver, where it is converted back into pyruvate by the enzyme lactate dehydrogenase. Pyruvate is then converted back into glucose via the process of gluconeogenesis. The newly formed glucose is then released into the blood to be used once again for energy by the red blood cells, muscles, etc. This process is referred to as the Cori Cycle (named after Carl Cori and Gert Cori). As mentioned previously, this process is energy inefficient and consumes four net ATP molecules per cycle. The Cori cycle, in essence is the cooperation between two processes of glycolysis and gluconeogenesis and it involves the muscle and the liver. It is an important factor in the aetiology and prognosis of cancer cachexia.

Figure 2. Diagram to show the Cori Cycle.

The Cori cycle normally accounts for approximately 20% of all glucose turnover. However, earlier studies of Holroyde et al \cite{138, 137, 140} in patients with cancer cachexia have shown that this glucose turnover to be 50% with the disposal of 60% of the lactate produced.
2.4.3 Fat And Lipid Metabolism

One of the other characteristics of cancer cachexia is the loss of body fat and an altered lipid metabolism with the main two cytokines implicated being the TNF-α and IL-1 \cite{37, 39, 141}. Here, there is a large and significant loss of white adipose tissue. This is primarily due to a decrease in the lipoprotein lipase activity and an increase in the activity of the hormone-sensitive lipase \cite{37, 39}. In addition, there is an inhibition of glucose and de novo lipogenesis in the tissues which has been shown to be due to the cytokines TNF-α and IL-1 \cite{37, 39}.

In non-cachectic individuals, body fat accounts for approximately 90% of fuel or energy reserves. During cachexia, there is an increase in the rate of lipolysis. The evidence for this lipolysis comes from the early studies of Drott et al \cite{142, 140} where the fasting plasma levels of glycerol were found to be higher in weight-losing patients as compared to normal individuals. The loss of fat in patients with cancer cachexia is due mainly to the fatty acids and glycerol from the host’s adipose tissue being mobilised rather than a decrease in its production or synthesis \cite{143}. This is most likely due to the release of LMF by the tumour to supply lipids to the tumour \cite{111}. In addition, in patients with cancer cachexia there is an increased rate of oxidation of fatty acids. This, together with the reduced food and calorie intake, results in the depletion of fat stores which is often seen in patients with cancer cachexia.

One of the hallmarks of cancer cachexia is the presence of an increased level of circulating lipids which is due to the increase in the levels of circulating triacylglycerols and cholesterol \cite{37, 39}. The hypertriglyceridemia itself is due to a reduction in lipoprotein lipase activity. This, in turn causes a decrease in the plasma clearance of endogenous as well as exogenous triacylglycerols. In addition to the presence of hypertriglyceridemia, there is also a marked hypercholesterolemia \cite{141, 144, 145}.
In cancer cachexia patients there is a reduction in lipoprotein lipase activity. Lipoprotein lipase is an endothelial-anchored enzyme primarily responsible for hydrolysis of chylomicron and very-low-density lipoprotein triglycerides, especially in muscle and adipose tissue. In other words it is responsible for breaking down fat in the body. The diminished activity of lipoprotein lipase seen in cancer cachexia has long been known not to be related to hyperinsulinaemia in these patients [146].

In summary, under normal non-cachectic conditions the fatty acid oxidation is suppressed by glucose administration. In patient with cachexia this suppressive effect of glucose is reduced which is similar to conditions such as sepsis. In addition, the loss of body fat seen in cachexia is due to the enhanced lipolysis which results primarily from the activation of the hormone-sensitive lipase.

2.4.4 Protein Metabolism

Unlike carbohydrates and fat, the main roles of proteins are not to provide energy. Proteins perform either structural or functional roles in the body. Structural roles include muscles and connective tissues, whereas functional roles include enzymatic, antibodies, and hormonal functions. However, under conditions where carbohydrates and fat are not available or cannot be utilised (such as disease conditions), proteins (mainly the skeletal muscles) are used as energy sources.

Probably the most destructive and detrimental aspect of cancer cachexia is the increased loss of body protein the degree of which is associated with poor survival [18]. It is another cardinal feature of cancer cachexia which cannot be reversed by an increase in food and calorie intake or through parenteral nutritional intervention [107]. In cancer cachexia the net protein loss is
Chapter 2: CANCER CACHEXIA

carried by a combination of increased protein degradation and a decrease in protein synthesis [147, 148].
The breakdown of protein in the muscle tissues is carried out through one of the three or a combination of the three main proteolytic pathways:

- The lysosomal pathway: In this pathway the extracellular proteins and cell surface receptors are degraded.
- Calcium activated protease pathway: Initially the calpains mediate the release of actin and myosin from the sarcomere in this pathway. The actin and myosin are then degraded by the proteasome complex [149, 150].
- Ubiquitin-proteasome pathway: This pathway is considered to be the main pathway involved in protein breakdown and is the primary pathway for protein and muscle degradation in cancer cachexia [52, 51].

As one would expect, the major site of protein loss is the muscles [151]. The reason why skeletal muscle is an ideal site for apoptosis and degradation during cachexia is that the skeletal muscle cells are multinucleated, contain a variable mitochondrial content, which is dependent on the fibre type and activity, as well as containing two morphologically and biochemically distinct mitochondrial pool [152, 153]. This loss of body protein together with a reduction in protein synthesis [24] results in a net and continuous loss of body protein. This loss of body protein and general muscle wasting also leads to asthenia or lack of strength. Asthenia is also considered to be one of the main characteristics of cancer cachexia.

It has long been known that the abnormalities in protein metabolism in cancer patients include the flow of amino acids into the tumour for protein synthesis, reduction of synthesis of some host proteins, and an increase in the synthesis of other host proteins [16]. In addition, the syntheses of muscle protein are reduced [16]. The pathophysiology of these changes in muscle metabolism has been explained by changes occurring in the muscle enzyme levels with a
decrease in the key anabolic enzymes and an increase in the activity of the catabolic enzymes such as cathepsin D \cite{16}. The amino acids released from the muscle catabolic processes are then used by the tumour for gluconeogenesis to produce glucose for tumour metabolism.

The mechanism by which the muscle proteins are broken down in cancer cachexia is through the ATP-ubiquitin-dependent proteolytic system. Here, the proteins are conjugated with ubiquitin. These will then be degraded by an ATP-dependent proteasome. The ubiquitin system and the cytokines involved during cancer cachexia have also been described in detail in the section on Interferon And Tumour Necrosis Factors (Chapter 2.2.2).

Of the other changes in protein metabolism during cachexia is the change in the turnover of branched chain amino acids such as leucine, isoleucine, and valine \cite{129, 130}. Studies of Garcia-Martinez et al in the mid-1990s have demonstrated that the oxidation of the branched chain amino acid, leucine, is increased in conditions such as sepsis and that the mediators triggering this increase in oxidation are the cytokines TNF-\textit{\alpha} and IL-1 \cite{154}. It is well known that there is a great demand for leucine by the tumour. As a result there is a large movement of amino acid from the host’s muscle to the tumour resulting in a general muscle wastage \cite{129, 130}. Since there is only a minor demand by then tumour for leucine, it has been suggested by Argiles et al \cite{129, 130} that the major contribution to branched chain amino acid oxidation is made by the skeletal muscles. The fact that branched chain amino acids are the only amino acids being degraded in the muscles have also long been known and proven in the literature \cite{155, 156}. Therefore, during cancer cachexia there is an altered response of muscle turnover to branched chain amino acids.

Another aspect of protein metabolism that is altered during cancer cachexia is the Acute-Phase Response. In general, the body’s response to injury is for the local inflammatory cells (such as the neutrophils, granulocytes, and macrophages) to secrete a number of different cytokines into the bloodstream. These cytokines include the IL-1, IL-6, and IL-8, as well as
the TNF-α. As such, the Acute Phase Response is a systemic reaction to tissue injury which is characterised by a series of hepatocyte-derived plasma proteins, the Acute Phase Reactants, and by a reduced synthesis of transferrin and albumin. These reactants include CRP, serum amyloid A, α1-antitrypsin, fibrinogen, complement factor B, and complement factor C3. The proteins released as a result of this response, are also known as the Acute Phase Proteins.

With respect to pancreatic cancer patients, the proportions of these patients exhibiting the acute-phase response have been shown to be increasing with disease progression [2, 132]. In addition, the presence of acute-phase response has long been known and demonstrated to be related to the increase in weight loss seen in these patients [158, 160, 159, 157]. Earlier studies of O’Gorman et al [162, 161] have also shown that the QL is significantly is also reduced under these settings. Furthermore, as one would expect when there is weight loss and acute-phase response, the survival will also be reduced [157].

Although the process of apoptosis, or cell death, is the primary mechanism by which muscle degradation occurs in cachexia [28], studies of Pollack et al [163] and Dirk and Leeuwenburgh [164] have shown that the process of aging can also be viewed as a chronic and gradual process of skeletal muscle loss and that there may be similarities between aging and cachexia. It has been long known that during the process of aging, oxidants damage the cell mitochondria releasing cytochrome c into the cytosol [165]. This release of cytochrome c initiates the first steps of cell death. This causes the formation of an apoptosis-initiating complex with procaspase-9 apoptosis protease activating factor-1 and dATP [166]. This complex is also known as the apoptosome which enables the cleavage and activation of procapsase-9. The capsase-9 enzyme then cleaves and activates effector capsases such as procapsase-3, which then activates a cascade of capsases [28]. The capsase activation then destroys the cell by causing the reorganisation of the cytoskeleton, shutting down of the DNA replication and
repair, destruction of the DNA, disruption of the nuclear structure, and the disintegration of the cell into apoptotic bodies[167].

There are also other pathways by which the same overall effect of cell death can occur. These pathways involve an alternate upstream activator to initiate the caspase cascade. An example would be the binding of TNF-α to its receptor[28]. Here the TNF-α binds to the TNF surface receptor (the TNFR1) containing an analogous cytoplasmic domain called the TNFR1 Associated Death Domain[28]. The TNFR1 Associated Death Domain then binds to the Fas Associated Death Domain mediating apoptosis. When procaspase-8 binds to the Fas Associated Death Domain, it is cleaved, and is activated to caspase-8. The caspase-8 then, in turn, causes the conversion of procaspase-3 into the effector caspase-3 required for the process of apoptosis[28].
2.5 Fatigue In Cachexia

Patients with cancer often report of fatigue and the lack of energy. Although reported in the literature, the cause and aetiology of cancer-induced fatigue seem to be multifactorial and unclear with potential etiological factors co-existing \([38]\). These physiological, pathological, and psychological factors all play a part and can act synergistically to promote fatigue. These factors, most associated with the presence of cancer include anaemia, therapy, depression, anxiety, insomnia, hormonal factors, and even the presence of the tumour itself \([168]\).

Fatigue is one of the most common symptoms seen in patients requiring palliative care including cancer \([169, 170]\). It is often reported by cancer patients with cachexia and is considered to be the longest lasting and most disruptive of the symptoms \([171, 172]\). Frequencies rates of as much as 96\% for fatigue have been reported by cancer patients undergoing chemotherapy and/or radiotherapy \([173]\). In cancer survivors, fatigue has been reported as a disruptive symptoms months or even years after the completion of their cancer therapy \([174, 175]\).

Multiple aetiological factors may be present and can act alone or synergistically to induce fatigue. The most common aetiological factors causing fatigue are the presence of the tumour itself, various different modes and modalities of therapies used to combat the cancer, and the pro-inflammatory cytokines \([176]\). Fatigue can be caused by the cancer, cachexia, cancer therapy or a combination of all of them. The possible etiological factors and their respective treatments have been reviewed in detail by Barnes and Bruera \([177]\) and Del Fabbro et al \([176]\).

Although cachexia and malnutrition have recognised as one of the major causes of fatigue seen in cancer patients, other pre-existing co-morbidities also seem to play important roles in inducing fatigue. Co-morbidities such as congestive heart failure, endocrine disorders, or various paraneoplastic neurologic disorders have been implicated in fatigue \([176, 177]\).
Cytokines produced by the host and the tumour itself can act as autocrine or paracrine tumour growth factors and can cause conditions such as anaemia, cachexia, fever, and depression \(^{[178]}\). The anaemia seen in patients with cancer and cancer cachexia can also be caused by reasons other than the cytokines. It is frequently observed to be as a result of bleeding, chemotherapy-induced toxicity (myelosuppression), and malnutrition with a haemoglobin of less than 8 g/dl causing fatigue \(^{[176, 177]}\). Studies of Cella et al \(^{[179, 180, 182, 181]}\) has shown this cut-off value to be as high as 12 g/dl. The major treatment modality that has been suggested in the literature has been the administration of epoetin alpha \(^{[183, 184]}\).

Both cancer and cancer-induced cachexia are devastating conditions which also has a significant impact on the psychological and emotional state of the patient. These mood disturbances are common and have been suggested to lead to fatigue \(^{[176, 177, 185]}\).

As a part of the cancer and cancer cachexia therapies, other medications may also be used to treat co-morbidities and other disease conditions that often are present. Drugs such as opioid analgesics, antihistamines, antiemetics, anxiolytics, antidepressants, etc have been know to cause fatigue \(^{[176, 177]}\).

The assessment of fatigue is important in the determination of the application of the correct treatment. Like most diseases, this involves the characterisation of the severity, temporal features, exacerbating factors, relieving factors, distress, and the general impact on the QL \(^{[169]}\). Guidelines have been developed by Cella et al \(^{[186, 182, 181]}\) and Portenoy et al \(^{[169]}\) to classify and assess the fatigue in cancer patients.

Once the assessment of fatigue has been carried out, its treatment will involve the use of an appropriate modality to treat its potential contributors. The recommended primary approach is to utilise non-pharmacological methods, such as education and counselling \(^{[176, 177]}\). However, if fatigue persists, pharmacological approaches for the treatment of specific causes may be necessary \(^{[176, 177]}\). For example:
• Severe pain may be relieved using opioid analgesics and, in less severe pain, over the counter analgesics, such as NSAIDs and paracetamol may be sufficient;

• Anaemia can be reduced and in most cases eliminated with blood transfusions or erythropoietic agents;

• Endocrine-related disorders can be reversed with the replacement of the appropriate hormone, e.g., thyroid hormones, or the administration of hormone stabilisers;

• Antidepressants, anxiolytics, and counselling are generally used for psychological and mood disorders often seen in cancer cachexia patients.

Failing non-pharmacological approaches as well as pharmacological approaches for the treatment of specific causes, symptomatic pharmacological treatment has been recommended [176, 177]. These include psychostimulants, cholinesterase inhibitors, corticosteroids, progestational agents, etc [176, 177].
2.6 Symptoms And Diagnosis Of Cachexia

The steady progressive loss of muscle mass is the most prominent phenotypic feature of cancer cachexia. The pathogenesis of cancer cachexia is multifactorial and not completely understood. The usual manner by which cachexia is discovered or diagnosed in most patients is through history and examination. In patients with cancer, the most common symptoms presented are weight loss, anorexia, and fatigue. In fact all published studies, reports, and reviews in the literature describe cachexia as “a syndrome characterised by a marked weight loss, anorexia, asthenia, and anaemia” which is usually associated with the growth of the underlying tumour and leads to malnutrition \cite{17, 18}. Asthenia itself is one of the most pragmatic characteristics of cachexia which seems to be the most prevalent symptom of patients with advanced cancer. The muscle degradation can be confirmed by measuring the levels of 3-methylhistidinewhich has long been known to be a marker for myofibrillar protein degradation \cite{187}. The major cytokines implicated in the increased protein and muscle degradation has been reported to be the TNF-\(\alpha\) and IL-1 \cite{37, 39}.

2.6.1 Weight Loss And Anorexia

The weight loss seen in cancer is common and is observed in the majority of patients with lung, gastric, pancreatic, prostate, and colonic cancers \cite{188}. Earlier report of Dewys et al \cite{188} who reanalysed the results of 12 different clinical trials in cancer (3047 patients) showed that the patients who reported losing as little as 6% body weight in the preceding six months has a shorter life span from the time of diagnosis as compared with the patients who had maintained their body weight \cite{189}. This prognostic effect occurred independently of tumour type, tumour
stage, and patient performance status \[189\]. The main and most disturbing symptom of cancer cachexia is the weight loss. The weight loss seen in cachexia involves the depletion of host adipose tissue and skeletal muscle mass. It is generally considered that weight loss of greater than 5% of pre-disease weight is one of the indicators of a developing cachectic state. A weight loss of greater than 15% of pre-disease weight is considered to be an advanced state of cachexia. The perception of weight loss or weight gain is often masked by the presence of ascites, oedema, and fluid retention. It is precisely for this reason that there is a necessity of regular full body composition measurements in these patients to assess the components of weight loss or weight gain.

Anorexia seems to be the effect rather than the cause of the weight loss seen in cancer cachexia \[39\]. Generally, cachexia perpetuates and worsens itself through a mechanism involving anorexia, in turn, through a “positive feedback” loop that would eventually lead to the death of the host \[39\]. The main body of evidence for this comes from the fact that traditional approaches such as enteral and parenteral nutrition in these patients have not demonstrated any benefit in symptom control, maintenance of LBM or survival \[107\]. In fact, attempts to reduce or reverse weight loss and improve survival using TPN have also met with failure \[114\] with any gain in weight being only an increase in water retention \[116, 115\] rather than the much needed LBM. Additional evidence comes from earlier studies of Lowry \[190\] who has reported that the metabolic changes that has resulted as a consequence of tumour burden resemble more like sepsis or injury than reduced food and calorie intake or starvation.

### 2.6.2 Altered Food Intake

Generally there are signs of reduction in food intake which may be as a result of loss of appetite, and early satiety. Other signs, which can also be due to cancer chemotherapy,
include altered taste and smell \cite{192, 191}, and nausea and vomiting. These will, in turn, increase the weight loss and promote malnutrition. Altered smell and taste \cite{192, 191} observed in pancreatic cancer patients \cite{9} has been suggested to be a contributing factor in the increasing anorexia observed in these patients.

Particularly in the pancreatic patients, the loss of appetite may also be due to the presence of abdominal pain, restricted dietary intake or maldigestion due to exocrine pancreatic insufficiency \cite{9}.

An obvious cause of reduced food intake is the presence of a mechanical obstruction in the gastrointestinal tract. This can induce both pain as well as early satiety which can lead to an overall reduced food intake.

Brain serotonin and its precursor, plasma tryptophan, have been reported to be involved in the pathogenic mechanisms leading to cancer-related anorexia. Here, increased brain concentrations of available tryptophan during cancer results in an increase in brain serotonin synthesis and, as a result, in an increase in the brain’s serotonergic activity causing a reduction in food intake \cite{128, 193}.

### 2.6.3 Haematological Changes

The addition of routine blood test to the above can further confirm the presence of cachexia. A reduction in plasma albumin levels together with an increase in CRP and erythrocyte sedimentation rate is an indicator of a systemic inflammatory response. This is often present in a cancer cachexia setting and contributes to the weight loss \cite{158}. 

69
2.6.4 Psychological Changes

The main psychological change that has been observed in patients with cancer cachexia has been depression.

2.6.5 Hormonal Changes

The major hormonal change that is often observed in patients with cancer cachexia is the alterations in insulin functions. Patients with cancer cachexia have been shown to have increased glucose production as well as glucose intolerance \([195, 194]\). In addition, there is also insulin resistance which involves the liver, adipose tissue and the skeletal muscle. The increased hepatic glucose production is partly due to the lack of inhibition of gluconeogenesis by insulin \([39]\). Similarly, the reduction in glucose utilisation by the skeletal muscles is also due to insulin resistance \([39]\). This insulin resistance seen and its involvement in patients with cancer cachexia is thought to be mediated by TNF-\(\alpha\), parallels of which with diabetic patients have been noticed since mid-1990s \([197, 196]\).

It is well known that the administration of hydrocortisone, cortisol, adrenaline, or glucagon to healthy humans will result in protein loss, acute-phase protein response, increased energy expenditure, and glucose intolerance. In other words, the administration of hydrocortisone, cortisol, adrenaline, or glucagon mimics many of the cachectic effects. Indeed, elevated levels of both glucagon as well as cortisol have been detected in weight losing cancer patients \([138]\). Such changes have been suggested to be able to amplify the acute-phase protein response \([198]\).
Chapter 2: CANCER CACHEXIA

2.7 Body Composition Changes In Cachexia

The body of a normal human individual is composed of three distinct compartments of adipose tissue, extracellular fluid, and the body cell mass \[^{[199]}\]. The body cell mass is considered to be the metabolic or the activity functioning and protein-rich tissue. In individuals between the ages of 20 and 60 years (i.e. not including the elderly) the body weight is divided (approximately equally) between these three compartments \[^{[199]}\]. However, the size of these compartments is highly dependent on age, sex, and nutritional status \[^{[200]}\]. The body composition as well as its rate of change in the elderly is well established to be different to the younger individuals \[^{[201]}\]. During catabolic illnesses, the relative sizes of these compartments are rapidly changed. The most important of these alterations is the increase in the extracellular fluid component which is accompanied by sodium retention and weight gain \[^{[199]}\]. The other two compartments (i.e. the adipose tissue and body cell mass) gradually shrink, resulting in weight loss which is due to the loss of TBF and body cell mass \[^{[200]}\]. Body fat is a highly efficient form of storage fuel which can be used during stress. However, unlike body fat, body protein is not stored and actually makes up the functional and structural tissue (e.g. muscles). Loss of protein will, therefore, cause loss of function. Recent studies of Monk et al \[^{[202]}\] has confirmed and quantified the previous findings that major trauma is associated with hypermetabolic conditions, lipolysis, proteolysis, and gain in extra-cellular water and shown that these effects of major trauma lasts longer than previously expected.

Studies of MacFie and Burkinshaw \[^{[203]}\], found that there is no evidence for an increase in the TBW content of cancer patients, but a significant decrease in TBW with weight loss. They also found that there was no difference between patients with benign and malignant disease in the parameters (TBW, TBF, TBP, and minerals) measured. Other similar studies \[^{[204]}\] has..
shown that weight loss occurs in patients with solid tumours of the gastrointestinal tract and this weight loss is associated with the loss of muscle protein and (to a lesser extent) fat. These finding were consistent with the findings of MacFie and Burkinshaw [203]. An interesting early study of Warnold et al [205] showed that although the cancer patients studied were found to be below normal for their weight and body cell mass, but only the females had a significant reduction in TBF.

Changes in body composition following stress, induced by major surgery have been demonstrated in our in vivo body composition facility in patients undergoing aortic reconstruction [206]. Stress-induced protein losses under other clinical conditions have also been investigated and demonstrated using the same techniques [207].

The systemic metabolic response following conditions such as trauma, surgery, infections, etc, have also been reported to correlate with other physiological responses [208]. It was found that metabolic measurements correlate significantly with the cardiopulmonary measurements in that the physiological responses reflect the metabolic responses and the metabolic responses are consistent with a peripheral energy (or fuel) deficit [208].

The importance of LBM and the fact that it is a vital body compartment has been summarised earlier by Moore et al [209], especially in its function as an important element in the energy exchange mechanisms. The report of Moore et al [209] clearly indicated that the LBM holds an extensive infrastructure for mechanisms such as the cellular metabolism and blood circulation as well as a variety of different activities which are critical to sustaining life. It has been suggested that if as much as 40% of the LBM is lost, it might translate into the death of the patient [189] and has in fact been shown that loss of body cell mass is directly associated with survival [210]. It is, therefore, for this reason that it is important to measure and monitor the changes in body composition and specifically the LBM (or body protein) in disease conditions that have been known to or can lead to the loss of body weight and/or cachexia. In
fact the recent study of Davidson et al \[211\] demonstrated that amongst their unresectable pancreatic cancer patient population, the patients whose weight was stabilised survived longer from baseline, reported higher QL scores, and had a greater mean energy intake than those who continued to lose weight. Davidson et al \[211\] then concluded that by just stabilising the weight in weight-losing patients over an eight week period can improve survival and the QL.
2.8 Clinical Consequences Of Cachexia

Cancer cachexia is preceded by a state of cancer-associated malnutrition \[3\]. This in itself is associated with a range of morbidities and debilitating conditions such as loss of muscle mass, impaired muscle function, fatigue, reduced activity and functional capacity \[212, 213\]. Due to the presence of illness and the associated fatigue the patients are less likely to carry out much physical activities. This reduction in or lower levels of physical activity leads to more muscle weakness and muscle wastage \[3\]. In addition, this low level physical activity reduces appetite and increases early satiety leading to a reduction of nutrition intake \[3\] resulting in the inability to maintain a balanced diet. There are numerous reports from as far back as the early-1980s demonstrating a correlation between malnutrition and poor performance status \[213, 214, 188\] as well as shorter survival \[213, 214\] and increased mortality especially in cancer patients \[215\].

One of the other consequences of malnutrition and cachexia in cancer patients is the poor and impaired response to therapy. As with conditioned mentioned above, there has also been numerous reports from as far back as the early-1980s demonstrating a correlation between malnutrition and impaired response to therapy \[214, 188\]. In particular, weight loss has been correlated with reduced response to chemotherapy, increased relapse, and reduced survival in cancer patients \[188\]. Earlier studies of Andreyev et al \[214\] has suggested that the shorter chemotherapy received by malnourished patients was due to their malnourished condition rather than inherit non-responsiveness.

Malnutrition also has long been known to affect the post-operative complications. It has been demonstrated that there are more sever and higher frequency of post-operative complications in malnourished patients than their nourished counter parts \[215, 217, 216\]. These post-operative complications include infections and pressure ulcers \[217\]. The increased occurrence and
severity of post-operative complications also has a financial impact on the hospital and healthcare system \cite{32, 218, 219, 220}. These complications often lead to extra treatment and management costs resulting from an increase in the duration of hospitalisation and rehabilitation, additional therapy requirements and costs, and increase in the frequency of medical specialist consultation \cite{32, 218, 219, 220}.

In addition to the extra financial burden on the healthcare system, cancer-associated malnutrition and cachexia also affects the patient, both physically as well as financially. The financial burden comes from reduced number of days at work, general fatigue, and the inability to perform normal activities independently, this requiring extra assistance \cite{3}. The physical burdens include the reduced QL associated with the increased frequency of complications, hospitalisations, and therapies \cite{215, 214}.
2.9 Treatment And Management Of Cachexia

Cachexia is a chronic condition. As such, it requires regular evaluation and management by a multidisciplinary team. As with most similar diseases, it is best to take prophylactic measures before the condition or symptoms enter an advanced state, i.e. it is better to commence treatment(s) early than late. Although there have been numerous studies on the aetiology and pathogenesis of cachexia, there has been relatively few clinical studies addressed to investigate new and efficacious therapies. Also, there seems to be relatively few clinical trials targeting and aiming to improve the QL of advanced and terminally ill cancer patients.

Why is it important to manage and treat weight loss and cachexia? As recently noted by Tisdale \cite{1}, there is a direct link between the loss of body weight and an increase in morbidity and mortality from many diseases as well as the fact that patients with gastrointestinal malignancies and weight loss have a reduced survival and QL. The evidence for these effects have been reported and are available in the literature. As an example, the study of Viganò et al \cite{221} demonstrated that in a group of patients with terminal cancers of gastrointestinal, lung, and breast shorter survival was independently associated with a weight loss of greater than eight kilograms in the preceding six months. Similarly, study of Anker et al \cite{222} on patients with chronic heart failure and cachexia demonstrated that patients who had cachexia had an 18 month mortality of 50% whereas the chronic heart failure patients without cachexia had an 18 month mortality of only 17%. Also, study of Kotler et al \cite{223} in the early 1990s demonstrated that HIV patients with 34% loss of ideal body weight were at the risk of imminent death.

Treatment and management of cachexia is essential for the treatment and survival of the patient. Cancer cachexia represents the result of a complex interaction between the tumour...
and the host response with a multifactorial pathogenesis. Presence of cachexia is often associated with poor response to chemotherapy and an increase in chemotherapy-induced toxicity.  

One of the effects of cancer and disease in general is the lack of exercise of the patients. This is probably due to the fatigue, lack of motivation, and the effects of the illness itself. Physical activity is a key influence on the maintenance of skeletal muscle mass. The loss of skeletal muscle mass associate with various types of diseases has been suggested to be partly due to prolonged periods of inactivity. A review by Ferrando has suggested that a loss of postural and locomotive muscle mass is evident within seven days of inactivity, such as bed rest, and that this loss in muscle mass continues for in excess of one month without stabilising. It was also indicated that there is no long term mechanism to induce conservation of muscle mass.

### 2.9.1 Symptomatic Treatment Of Nausea And Vomiting

One of the major complaints of patients with cancer cachexia is the presence of nausea and vomiting. This can be caused by the presence of tumour, cachexia, as well as the chemotherapy and cancer related therapies. Generally, nausea and vomiting are controlled by the use of antiemetics or, if caused by gastrointestinal obstruction, by appropriate surgical procedures. An antiemetic that has been used in patients with terminal cancer is thalidomide. In addition to its mildly antiemetic effect, thalidomide has also been shown to be anti-cachectic, analgesic, and have sedative effects.
2.9.2 Symptomatic Treatment Of Reduced Food Intake

As noted by Barber [19], it remains difficult to persuade patients with cancer cachexia to consume significant amounts of nutritional supplements in addition to their normal diet and it is the dietician and clinician that have the important role of advocacy in this regard. There have been a large number of reports in the literature investigating conventional as well as aggressive nutritional support. However, they all seem to fail in producing weight gain or improve QL and survival in patients with cancer cachexia [227]. Traditional approaches such as enteral and parenteral nutrition in these patients have not demonstrated any benefit in symptom control, maintenance of LBM or survival [107]. Here, attempts to reduce or reverse weigh loss and improve survival using TPN have also met with failure [114] with any gain in weight being only an increase in water retention [116, 115] rather than the much needed LBM. Early satiety is observed in patients with cancer and cachexia. This condition contributes to the process of weight loss and the reduction of QL. Early satiety reduces food intake which can lead to malnutrition. Usually appetite stimulants are used to control early satiety conditions. Drugs such as corticosteroids, cyprohexidine, and progestational agents have been developed in the 1980s and 1990s and used to stimulate appetite and increase food intake in patients with cachexia [176, 228, 13, 229, 230]. These compounds were unfortunately developed without reference to the biology of cachexia [19]. Although these agents increase weight in weight-losing cachectic patients, the weight gain has been reported to be as a result of water retention rather than the required LBM or protein [228, 13, 229, 230]. A recent report by Tisdale [1] suggests that the ideal drug therapy would in fact be a combination of an appetite stimulant plus an agent which promotes protein synthesis. This, however, was to some extent in contrast to the study of Jatoi et al [231] who concluded that EPA plus megestrol acetate is not any better than megestrol acetate alone in improving weight loss or appetite in patients with cancer-associate wasting.
Chapter 2: CANCER CACHEXIA

Malnutrition alone affects up to 85% of patients with pancreatic cancer [3]. Although nutritional supplements have been tested in cancer patients with cancer-associated malnutrition, it has been reported that the malnutrition can be reversed more effectively by the administration of nutritional supplements containing anti-inflammatory agents such as EPA [232, 3, 233].

Anorexia, although present in most cases of cachexia, it is not necessarily a component of cancer cachexia. If left untreated, it can lead to weight loss and malnutrition. Appetite stimulants have been used in cases of cancer cachexia-induced anorexia. Although they will temporarily relieve symptoms, but drugs such as metoclopramide, megestrol acetate, and medroxyprogesterone have been shown to improve appetite in a significant proportion of patients [234]. Long term treatments with these agents as well as corticosteroid are, however, plagued with negative side effects that can potentially overwhelm their potential benefits [9].

Although its full potential may not yet have been fully realised, thalidomide may be useful in increasing food intake. In addition to its anti-cachectic effects [58], thalidomide has also been shown to improve appetite in terminal cancer patients [226].

A new growth hormone-releasing acylated peptide, Ghrelin, first reported to have been isolated from the stomach in 1999 [235], has been suggested as a possible agent to increase appetite and food intake in cancer cachexia. Ghrelin itself is a 28-amino acid peptide and an endogenous ligand for the growth hormone secretagogue receptor [235]. Its mechanism of action has been suggested to be through the regulation of the body’s metabolic balance [237, 236, 238]. This is done centrally by decreasing fat use through growth hormone-dependent mechanisms and causing potent stimulation of food intake via the activation of neuropeptide Y neurons in the hypothalamic arcuate nucleus [237, 236, 238].

Recent studies of DeBoer et al [239] in animal models have demonstrated that ghrelin is effective in increasing food intake and the maintenance of LBM in cancer cachexia. However,
the use of ghrelin in cancer cachexia to promote food intake has been reported to be limited \[240\]. This limitation has been due to the fact that it is a protein with a relatively short half life and is required to be administered by injection \[240\]. A synthetic ghrelin receptor agonist is, therefore, required which would allow its administration under clinical settings. Recent phase I studies of Garcia et al \[240\] have successfully demonstrated that a novel ghrelin-mimetic and growth hormone secretagogue, RC-1291, can be administered orally in human volunteers. The RC-1291 was reported \[240\] to produce dose-related increases in body weight with no dose-limiting adverse effects. In addition to its positive effects on body weight and food intake, ghrelin has been shown to also have anti-inflammatory effects \[241\]. The administration of ghrelin in animal models have been shown to decrease serum levels of IL-6 \[241\].

With gastrointestinal tract cancers, the presence of malabsorption is relatively common. Malabsorption, if left untreated, can lead to both weight loss and malnutrition. Both these conditions reduce survival and increase therapy-induced toxicities and complications. In the cases of patients with pancreatic cancer, pancreatic enzymes are used to manage the malabsorption.

In order to increase or promote food intake, it is advised that fatty meals which reduce gastric emptying to be avoided as much as possible \[38\]. Although the use of nutritional supplementation has been recommended \[38\], these supplements should not replace normal food. Instead they should be taken as “supplements”. In severe cases of cachexia and malnutrition, invasive and artificial feeding techniques such as TPN may also become necessary.

As a part of the multidisciplinary team, nutritional counselling from qualified dieticians has been shown to be beneficial \[117\]. It should also be noted that a simple increase in weight or a reduction in weight loss is not enough. It is, in fact, the “quality” of the weight gain that is important. An increase in weight may be due to an increase in fluid retention or the fat mass.
What is lost in cachexia is the LBM (or the protein compartment) which is more difficult to replace, maintain, or prevent its loss. As such, regular body composition assessments are required as an integral part of the management of cachexia.

The consequences of malnutrition in patients with cancer and cancer cachexia are detrimental. Recent studies reported in the literature have shown that EPA and EPA-enriched nutritional supplementation are useful and effective in the treatment of malnutrition and weight loss in cancer and cancer cachexia. As an example, the recent study of Baur et al [242] has demonstrated a significant association between improvement in nutritional status through the use of EPA and EPA-enriched nutritional supplementation and improvements in the QL, functional capacity, and maintenance of LBM in patients with cancer cachexia who are receiving chemotherapy. Similarly, the recent study of Isenring et al [243] using the Subjective Global Assessment techniques demonstrated that an improvement in nutritional status is associated with an improvement in QL. In addition, improvements in the nutritional status through the use of EPA and EPA-enriched nutritional supplementation have also been demonstrated to improve the mobility and physical activities of patients with cancer and cancer cachexia [244].

Overall, the majority of studies currently reported in the literature seem to suggest that EPA improves appetite and body weight, or at least stabilise weight loss, if administered for at least three weeks [1].

2.9.3 Symptomatic Treatment Of Fatigue

Treatment of fatigue is probably the most elusive and difficult of all cancer cachexia symptoms to properly diagnose and treat effectively. What makes the treatment and management of fatigue in cancer cachexia difficult is the fact that it is a very subjective
Chapter 2: CANCER CACHEXIA

sensation which appears with varying degrees and dimensions of impaired physical, cognitive, and affective functioning\textsuperscript{245}.

Generally, the management of the cancer cachexia usually improves the fatigue\textsuperscript{38}. Here, when the food intake reverts back to normal and the symptoms of cachexia are reduced, patients’ mood, energy levels, and QL will increase, thus reducing the fatigue.

2.9.4 Metabolic And Systemic Management

It is our opinion, other than curing the cancer, the only other way to treat cancer cachexia is through its systemic and metabolic management. Once the underlying causes of cancer cachexia have been resolved, their symptoms will also resolve themselves.

Various different feeding technique, including appetite stimulant, to increase the food and calorie intake in patients with cancer cachexia have been reviewed in the literature\textsuperscript{37, 19}. Although majority have shown weight gain to different extents, but the cachectic patients that do gain weight by the provision of excess calories, show an increase only in the body fat without a significant increase in their LBM or TBN\textsuperscript{246}.

The metabolic management of cachexia was the integral part of our clinical trial investigating the effects of EPA on body composition in pancreatic cancer patients undergoing chemotherapy. The main metabolic change that occurs in cachexia is the systemic inflammatory response. In cancer cachexia this seems to involve both the host as well as the tumour with the chronic release of pro-inflammatory cytokines. The metabolic management of cancer cachexia must, therefore, target this systemic inflammatory response.

There are reports in the literature that have reviewed the use of non-steroidal anti-inflammatory agents to reduce the systemic inflammatory response in cancer patients\textsuperscript{158}. As an anti-inflammatory agent, eicosapentaenoic acid (EPA) has been suggested as a down-
regulator of the systemic inflammatory response in cancer cachexia [244, 247]. EPA is a natural component of fish oil obtained from oily fish such as salmon. It is present in and often incorporated in high protein and high calorie oral feeds [244, 247]. EPA (and other ω-3 polyunsaturated fatty acids) effects are through their uptake into the cellular substrate pool and their competitive metabolism with arachidonic acid at the cyclooxygenase and 5-lipooxygenase levels. The resulting metabolites have less inflammatory and immunosuppressant potency than the substrates derived from arachidonic acid [248].

Studies of Wigmore et al [249, 250] and Barber et al [251, 253, 252] demonstrated that treatment with daily dietary oral supplementation containing ω-3 fatty acid (EPA and DHA) produced positive changes in weight together with a significant reduction in the acute-phase protein production, stabilisation of the increased resting energy expenditure, and improvements in appetite and performance status. Using the ω-3 fatty acid oral supplement, significant reduction in the production of IL-6, increase in serum insulin concentrations, reduction in the insulin to cortisol ratios, and reduction in PIF excretion in urine were also observed [251].

Studies of Fearon et al [247] on ω-3 fatty acid oral supplementations demonstrated that only the protein dense and energy rich oral supplements containing high doses of ω-3 fatty acids are capable of inducing weight gain, increase in LBM, and an improvement in the QL of weight losing patients. From all the available compounds for the treatment of cancer cachexia, EPA has the least adverse effects [176].

This compound was used in our clinical trial on pancreatic cancer patients undergoing chemotherapy to investigate cachexia and the components of weight change in cachexia.

It should be pointed out that due to the involvement of pro-inflammatory cytokines in cancer-induced anorexia [17, 18], systemic therapy with anti-inflammatory agents to down-regulate the pro-inflammatory cytokines and the systemic inflammatory response may also reduce the cancer-induced anorexia.
Chapter 2: CANCER CACHEXIA

Another anti-cachectic agent that has been used is Thalidomide (α-N-phthalimidoglutaramide). Thalidomide has been demonstrated in randomised, placebo controlled trials to be well tolerated and effective at reducing weight loss and the loss of LBM in pancreatic cancer \[58\] and oesophageal cancer patients \[56\]. The attenuation of weight loss is thought to result from the modulation of the inflammatory response \[58\]. In addition to its anti-cachectic effects, thalidomide has also been shown to have antiemetic, analgesic, and sedative effects, proving effective in the palliation of intractable symptoms \[226\].

Treatments with the cyclooxygenase pathway inhibitors, such as the non-steroidal anti-inflammatory drugs, have also been reported in the literature \[254\]. These treatments also target the systemic inflammatory response and aims to decrease cytokine levels and hence the weight loss and cachexia in cancer patients \[255, 256\].

Inefficient energy production triggered by the anaerobic processes of the tumour has also been the target of anti-cancer cachexia treatments. Inhibitors of the Cori cycle, such as hydrazine, have not been useful or successful \[107\].

The use of growth hormone in the treatment of cachexia in Human Immunodeficiency Disease has also been suggested. Earlier studies of Schambelan et al \[257\] carried out in the mid-1990s have shown an increase in the LBM, but the cost may be prohibitive. No successful trial of growth hormone in cancer cachexia has been reported in the literature.

Another hormone being investigated for the treatment of cachexia is Melatonin, (5-methoxy-N-acetyltryptamine. It is a hormone produced by pinealocytes in the pineal gland and has been linked to the regulation of circadian rhythms. Studies of Lissoni \[258\] have suggested that this hormone may inhibit cancer cell growth as well as improve survival. The frequency of cachexia, asthenia, and thrombocytopenia has been reported to be lower in patients who have been administered melatonin at night \[258\].
Studies involving amino acids have also long been reported in the literature\cite{261, 262, 260, 259}. However, these studies have mostly been carried out on animal models with only a handful on human subjects. Examples of amino acid studies include arginine and glycine \cite{259}, glutamine \cite{260}, β-hydroxy-β-methylbutyrate \cite{261}, and a combination of arginine plus glutamine plus β-hydroxy-β-methylbutyrate \cite{262}.
3. THE PANCREAS AND PANCREATIC CANCER

3.1 The Pancreas

The pancreas is an oblong flattened gland located deep in the abdomen. It is sandwiched between the stomach and the spine. It lies partially behind the stomach. The other part is nestled in the curve of the duodenum. Because of the pancreas' deep location, tumours are rarely palpable. It also explains why many symptoms of pancreatic cancer often do not appear until the tumour grows large enough to interfere with the function of nearby structures such as the stomach, duodenum, liver, or gallbladder.

3.1.1 Anatomy of the Pancreas

The pancreas is made up of glandular tissue and a system of ducts. The main duct is the pancreatic duct which runs the length of the pancreas. It drains the pancreatic fluid from the gland and carries it to the duodenum. The main duct is about one-sixteenth of an inch in diameter and has many small side branches. The pancreatic duct merges with the bile duct to form the ampulla of Vater (a widening of the duct just before it enters the duodenum).

The pancreas can be divided into five main sections of body, head, neck, tail, and uncinate process:
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

a) Body: The body is the middle part of gland between the neck and the tail. The superior mesenteric blood vessels run behind this part of the gland.

b) Head: The head is the widest part of the gland. It is found in the right part of abdomen, nestled in the curve of the duodenum which forms an impression in the side of the gland.

c) Neck: The neck is the thin section between the head and the body of the gland.

d) Tail: The tail is the thin tip of gland in the left part of abdomen in close proximity with the spleen.

e) Uncinate Process: The uncinate process is the part of the gland that bends backwards and underneath the body of the pancreas. Two very important blood vessels, the superior mesenteric artery and vein cross in front of the uncinate process.

3.1.2 Functions of the Pancreas

The pancreas is an integral part of the digestive system. Once the food enters the stomach acids that break down the food are produced. From the stomach, the food then flows directly into the duodenum. It is here that bile and pancreatic fluids enter the digestive system. Bile is produced by cells in the liver and travels down through the bile ducts which merge with the cystic duct to form the common bile duct. The cystic duct then runs to the gallbladder located underneath the liver and serves as a storage pouch for the bile. The point of entry for the common bile duct into the pancreas is the head of the pancreas and joins the pancreatic duct (which runs the length of the pancreas) to form the ampulla of Vater which then empties into the duodenum.

The food, bile and pancreatic fluid travels through many more feet of continuous intestine including the rest of the duodenum, jejunum and ileum which comprise the small intestine, then through the caecum, large intestine, rectum, and anal canal.
At the cellular level, the pancreas has two functional components of endocrine and exocrine, both having different “normal” functions. Tumours can appear in both of these sections. Depending on within which section the tumour occurs, the symptom(s) will, therefore, be different.

The endocrine cells of the pancreas are generally considered to be the Islets of Langerhans and the exocrine cells to be the Acinar Cells. The Islets of Langerhans (Figure 3) release hormones into the blood vessels whereas the Acinar Cells secrete pancreatic enzymes into the pancreatic duct. The hormones release by the Islets of Langerhans include insulin and glucagon, which work together to maintain the proper levels of sugar in the blood.

Figure 3. Islets of Langerhans.
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

3.2 Pancreatic Cancer

Pancreatic cancer is one of the deadliest cancers affecting 10 in 100,000 individuals in the Western Europe [263], and probably affecting similar numbers in Australia. It is the fourth leading cause of cancer death in the United States [264], the sixth leading cause of cancer death in the European Union [265], the fifth most common cause of cancer-related deaths in Australia [266], and the eight commonest cause of cancer mortality in the world [267]. Cancer of the pancreas is not one disease. Probably as many as twenty different tumours have been classified under the umbrella term "Pancreatic Cancer." As one would expect, each of these tumours has a different pathology, aetiology, and appearance under the microscope, some requiring different treatments, and each carrying its own unique prognosis. However, the pancreatic cancers can be broadly separated into the Primary and Metastatic cancers. In the clinical trial section of this project, one of the randomisation factors was the fact that whether the patient’s tumour was primary or metastatic.

Generally in the literature, and in most instances, the term “pancreatic cancer” refers to the primary cancers of the pancreas. The primary cancers can be further separated broadly into the subgroups of the ones that show endocrine effects and the ones that don’t show endocrine effects.

Locally advanced pancreatic cancer is defined as the pancreatic cancer whose tumour encases a vascular structure, such as the superior mesenteric artery, coeliac axis, or superior mesenteric vein-portal vein confluence [268]. Tumours associated with bulky peri-pancreatic lymphadenopathy are also considered to be unresectable [268]. The metastatic pancreatic cancer is both progressive and debilitating and is, generally, characterised by pain, asthenia, anorexia, and cachexia. These patients are likely to have sudden clinical changes with continuing problems with pain, thromboembolic events, intestinal dysmotility, intractable
ascites, and biliary and gastric obstructions. The survival of patients with metastatic pancreatic cancer is dependent on tumour burden and performance status at presentation and chemotherapy is never curative [268].

3.2.1 Aetiology Of Pancreatic Cancer

The aetiology of pancreatic cancer remains, largely, elusive. From the patients diagnosed with pancreatic cancer, the majority have been reported to be men [269]. A significant percentage of patients diagnosed with pancreatic cancer report having a family history of pancreatic cancer [270] with those having rare familial cancer syndromes or hereditary pancreatitis have even a higher risk of pancreatic cancer [271].

One of the most frequently asked question by patients diagnosed with pancreatic cancer is: “What causes pancreatic cancer?”. By far the most common and biggest aetiological factors for pancreatic cancer that have been reported in the literature are the increasing age [269, 272], BMI [273, 274] and obesity [275], smoking [276], new onset diabetes mellitus [278, 277], chronic and past history of pancreatitis [280, 281, 279] including hereditary pancreatitis [282] and inherited predisposition to pancreatic cancer [283, 284, 270]. For example, age itself was shown, in a study of 1552 patients [272] with chronic pancreatitis, to produce an independent increase in pancreatic cancer such that the relative risk for the development of pancreatic cancer was more than three times greater for a patient over the age of 60 years compared with younger patients.

3.2.1.1 Chronic Pancreatitis

Chronic pancreatitis is a progressive inflammatory disease which is characterised by irreversible histological transformation. Chronic pancreatitis is now a recognised aetiological
factor for pancreatic cancer \(^{[284, 280, 281, 279]}\). Results of studies in the literature have reported an increased risk of 15 up to 25 fold in these patients \(^{[284, 280, 281, 279]}\). The time line for these cases has been at least 20 years before the diagnosis of pancreatic cancers. In these patients, a higher rate of complications, increased pancreatic calcification, and a more severe disease have been noted.

Pancreatitis itself can be caused by alcohol consumption and choledocholithiasis which account for 70% of all acute cases \(^{[285]}\). Other causes of recurrent acute pancreatitis have been suggested to include toxic-metabolic, mechanical, and genetic. Studies of Parenti et al \(^{[286]}\) carried out in the mid-1990s have suggested that infections with virus, bacteria, fungi, and parasites can also lead to and cause pancreatitis. Examples of virus that have been implicated are mumps, Coxsackie’s, hepatitis B, cytomegalovirus, varicella-zoster virus, and the herpes simplex virus \(^{[286]}\). Examples of bacteria suggested by Parenti et al \(^{[286]}\) that cause infections which can lead to pancreatitis are Mycoplasma, Legionella, Leptospira, and Salmonella. Parasites and fungi that cause infections and are thought to be associated with pancreatitis are Toxoplasma, Cryptosporidium, Ascaris and Aspergillus \(^{[286]}\).

The mechanism(s) causing pancreatic cancer as a result of chronic pancreatitis remains unclear. However, studies of Apple et al \(^{[287]}\) in the late 1990s have suggested that ductal epithelial hyperplasia, metaplasia and dysplasia, and K-ras gene mutations might be involved in causing pancreatic cancer. It has also long been known and recognised \(^{[289, 288]}\) that chronic inflammation plays an important role in the genesis of pancreatic cancer. This inflammation can be due to many factors including genetic as well as environmental factors.

### 3.2.1.2 Inherited And Hereditary Factors

There are several different inherited cancer syndromes which have been associated with pancreatic cancer and have been reviewed in the literature \(^{[290, 291]}\) and their relative effects in
increasing the risk of pancreatic cancer has been investigated. It is interesting to note that of these inherited cancer syndromes, the one that has been reported to have the highest risk of pancreatic cancer is the Peutz-Jeghers syndrome with a 120-fold lifetime risk and a 36% cumulative lifetime risk \(^\text{[292]}\). The Peutz-Jeghers syndrome itself is an autosomal-dominant disorder which is characterised by hamartomatous polyps of the gastrointestinal tract and by pigmented macules of the lips, buccal mucosa, and digits \(^\text{[292]}\). Studies of Su et al \(^\text{[292]}\) have concluded that germ-line and somatic genetic alterations of a specific gene causes carcinogenesis and that the same gene contributes to the development of both sporadic and familial forms of cancer.

In addition to chronic pancreatitis having a strong influence on the risk of developing pancreatic cancer, hereditary pancreatitis has an even higher risk for developing pancreatic cancer. This has been reported to be of the order of 70 fold increase in risk \(^\text{[282, 281]}\). However, hereditary pancreatitis is a relatively rare condition which accounts for approximately 1% of all cases of pancreatitis \(^\text{[284]}\). It is an autosomal dominant disease with 80% penetrance and is characterised by the onset of recurrent attacks of acute pancreatitis in childhood with frequent progression to chronic pancreatitis \(^\text{[284]}\).

The familial pancreatic cancer has also been suggested to be an autosomal dominant condition \(^\text{[293]}\). A recent prospective, registry-based study of approximately 5200 subjects demonstrated that the risk of pancreatic cancer is significantly increased in familial pancreatic cancer family members which increase even higher with increasing number of affected first degree relatives \(^\text{[270]}\). Although the actual gene and the causative mutation has not yet been identified and remains unclear, 20% of the families where familial pancreatic cancer is present have been shown to have the BRCA2 mutations \(^\text{[283]}\). BRCA2, or the Breast Cancer-2 Early Onset, gene belongs to a class of genes known as tumour suppressor genes. Like many other tumour suppressors, the protein produced from the BRCA2 gene helps prevent cells from growing
and dividing too rapidly or in an uncontrolled way, i.e. tumour production and cause cancer. Recent studies of Pogue-Geile et al. [294] have also identified another candidate gene whose mutation has been shown to cause familial pancreatic cancer. These investigators suggested that the presence of a mutation of this gene, the Palladin gene, in familial pancreatic cancer and the over expression of the Palladin protein in sporadic pancreatic cancer causes cytoskeletal changes in pancreatic cancer and may be responsible for or contribute to the pancreatic tumour's invasive and migratory abilities [294].

3.2.1.3 Race And Gender

As there inherited and hereditary factors affecting the incidence and risk factors of pancreatic cancer, one can also expect there to be links between race and gender-specific factors and pancreatic cancer. The incidence of pancreatic cancer has been reported [269, 296, 295] to be slightly higher in males than females with black males having between 30% to 40% higher incidence of pancreatic cancer. Although one would expect that other factors, such as environmental and occupational factors, also affect the predisposition to pancreatic cancer, this does not seem to be the case. For example, it has been reported by Greenlee et al. [297] that despite similar smoking statistics between black and white males, the incidence of pancreatic cancer is quite different. It has been reported by Boyle et al [295] that the incidence of pancreatic cancer in males is highest amongst the Maori, native Hawaiians, and black Americans, whereas Indians and Nigerians have the lowest incidence of pancreatic cancer.

3.2.1.4 Smoking As A Risk Factor

Tobacco smoking has been associated with a two-fold increase in the risk of pancreatic cancer [298] and earlier studies in the literature [299] have strong causal relationship between cigarette smoking and risk of pancreatic cancer, and have indicate that cessation of smoking is likely to
prove a rapidly effective preventive measure for this major type of cancer. Because of its prevalence, it may account for around 30% of all cases with pancreatic ductal adenocarcinoma \[290\] and quarter of all pancreatic cancer \[269\]. The majority of the gender-specific as well as country or region-specific differences seen in pancreatic cancer rates have been reported \[269\] to be due to the differential smoking rates seen between different genders and countries or regions.

Although smoking has been associated with a two-fold increase in the risk of pancreatic cancer, recent studies of Alguacil and Silverman \[300\] have suggested that non-cigarette smoking, i.e. using tobacco products such as cigars and chewing tobacco, moderately increases the risk of pancreatic cancer and pipe smoking does not increase the risk of pancreatic cancer.

The mechanism by which cigarette smoking increases the risk of pancreatic cancer does not seem to be very clear. Possible mechanisms that have been suggested in the literature are the inactivation of the body’s natural detoxification and defence systems through mutations \[291\]. Studies of Duell et al \[301, 298\] and Ockenga et al \[302\] suggest that the possible mutations occur in the cytochrome system enzymes and the n-acetyltransferase enzymes.

The duration of exposure to and the cessation of smoking have also been explored. Studies of Buena de Mesquita et al \[303\] have reported that although smoking cigarettes increases the risk of pancreatic cancer, but cessation of smoking for at least 15 years reduces the risk to levels similar to the non-smokers. This would probably indicate that within this period body’s own repair and defence mechanisms would rectify damages and mutations caused by cigarette smoking.
3.2.1.5 Diabetes

A link between diabetes and pancreatic cancer has long been known \[^{304}\]. The meta-analysis of Everhart and Wright \[^{305}\] carried out in the mid-1990s has demonstrated that 80% of all patients with pancreatic cancer have an impaired glucose metabolism, impaired glucose tolerance, or diabetes mellitus. However from the available literature, it is not clear whether diabetes causes pancreatic cancer or that diabetes is caused by pancreatic cancer. Recent studies of Senior \[^{306}\] has shown that there is a significantly increased frequency of patients with type II diabetes developing pancreatic cancer several years after their diagnosis of type II diabetes \[^{306}\]. A large ten year prospective study of approximately 1.3 million men and women was carried out by Jee et al \[^{307}\] between 1995 and 2005 to investigate the relationship between fasting blood glucose levels, diabetes and risk of different cancers. The results of this study concluded that high fasting blood glucose levels and diagnosis of diabetes are independent risk factors for several different types of cancer. These researchers \[^{307}\] also concluded that the relationship is strongest with pancreatic cancer and that the risk increases with increasing fasting blood glucose levels. In addition, the death rates from cancer were higher in patients with higher fasting blood glucose levels \[^{307}\].

The actual mechanism by which diabetes and increased fasting blood glucose levels causes or promotes pancreatic cancer is also not clear. However, it has been suggested that since type II diabetes is associated with hyperinsulinaemia, the mitotic effects of insulin may result in an increased risk of cancer \[^{308}\].

3.2.1.6 Dietary Factors

Dietary factors are one of the possible reasons that there are different pancreatic cancer incidence rates in different countries and probably races. Although there are numerous studies
reported in the literature investigating the link between diet and pancreatic cancer, the majority suffer from recall bias and are contradictory or controversial.

Reports in the literature on the increased risk of pancreatic cancer and high meat and fat consumption have also been contradictory and inconclusive \(^{309}\). The study of Michaud et al \(^{309}\) did not support the findings of others that there is any relationship between high meat and fat intake and the increased risk of pancreatic cancer. However, the studies of Nothlings et al \(^{310}\) concluded that red and processed meat consumption were linked to an increased risk of pancreatic cancer with fat and saturated fat not being likely contributors to the underlying carcinogenic mechanism. With the processed meat, carcinogenic substances related to the actual meat processing methods were thought to be the possible factors for the positive association with cancer \(^{310}\).

As a part of dietary intake, the effects of obesity and abdominal obesity on the risk of pancreatic cancer have also been reported. It has been shown that obesity has negative effect of the risk of pancreatic cancer \(^{311}\) as well as other different types of cancers \(^{312}\). This has been confirmed by a recent meta-analysis of Larsson et al \(^{274}\) in pancreatic cancer patients where a positive link between high BMI and the risk of pancreatic cancer in both men and women was demonstrated. Here it was demonstrated that a 5 kg/m\(^2\) increase in BMI was associated with a 12% increased risk of pancreatic cancer \(^{274}\). Such effects of excessive weight and obesity pose a serious threat and it has been estimated \(^{312}\) that being overweight and obese accounts for one in seven of cancer deaths in men and one in five cancer deaths in women in the United States.

The mechanism by which obesity and abdominal obesity increases the risk of pancreatic cancer is probably due to the fact that obesity, particularly abdominal or central obesity, is shown to be associated with the glucose intolerance and insulin resistance and the development of type II diabetes \(^{313}\). Recent meta-analysis of 36 studies \(^{314}\) has shown that
long standing type II diabetes is associated with an increased risk of pancreatic cancer. A positive causal association between obesity, particularly abdominal or central obesity, or BMI and increased risk of pancreatic cancer is, therefore, possible. It is interesting to note that in a recent multi-ethnic cohort study \cite{273} obesity, defined as a BMI of $\geq 30$ kg/m$^2$, was associated with an increased risk of pancreatic cancer in men but a reduced risk in women. The increased risk in men was reported \cite{273} to be higher in men who had never smoked than in the current or former smokers.

The relationship between other components of diet and the risk of pancreatic cancer has also been investigated and reported in the literature. These include the long-term consumption of alcohol, tea, and coffee. As with the other dietary factors mentioned above, reports seem to also be contradictory and controversial. Earlier reports of Bueno de Mesquita et al \cite{315} confirmed the evidence against a positive association between consumption of alcohol, tea or coffee and the development of pancreatic cancer. Other studies have indicated that the excess risk of pancreatic cancer among alcoholics is small and could be confounded by other factors, such as smoking \cite{316}. However, there is a link between chronic pancreatitis and the risk of pancreatic cancer. Also, it is known that the most common cause of pancreatitis in the Western countries is heavy alcohol consumption \cite{291}. The term “heavy alcohol consumption” has been referred to six or more drinks per day for a period of 20 years, whereas “moderate alcohol consumption” referred to approximately 20 units of alcohol per week \cite{291}. Therefore, one may probably conclude that heavy alcohol consumption can eventually, in turn, increase the risk of pancreatic cancer.

Epidemiological studies \cite{269} have concluded and reported that for pancreatic cancer diet alone is not as a strong risk factor as is other factors, such as smoking. In addition, these studies \cite{269} have recommended that a diet which has ample fruits and vegetables, moderate fat content,
and without excess calorie to avoid obesity and diabetes may reduce the risk of pancreatic
cancer.

### 3.2.2 Symptoms Of Pancreatic Cancer

Pancreatic cancer rarely occurs in persons aged less than 50 years and the risk increases with
increasing age. Majority of all pancreatic cancers are adenocarcinomas of the ductal
epithelium[^264]. From these adenocarcinomas, approximately two thirds occur in the head of
the pancreas[^264].

#### 3.2.2.1 Presenting Symptoms

As one would expect, majority of symptoms of pancreatic cancer is highly dependent on its
type, size and location as well as whether there are metastases present. Majority of all
pancreatic cancers are adenocarcinomas of the ductal epithelium and symptoms are caused by
the effects of the tumour mass rather than the disruption of the endocrine or exocrine
functions[^264]. As such, the classical presenting symptoms of pancreatic cancer often include
pain, jaundice, loss of appetite abdominal discomfort, and weight loss, all of which can be
vague and non-specific. However, a predominant feature of pancreatic cancer is the weight
loss and cachexia[^317]. As majority of symptoms are mainly non-specific, majority of cases of
pancreatic cancer often lead to diagnosis delays and are, therefore, advanced on diagnosis.

The adenocarcinomas of the head of the pancreas are, generally, presented as steadily
increasing jaundice caused by biliary duct obstruction. Here, the painless obstructive jaundice
is associated with surgically resectable cancers[^318]. When the bile duct is obstructed, the
patient is presented with jaundice and a disproportionately increased blood level of
conjugated bilirubin and alkaline phosphatase. The urine in these patients will be dark due to
the presence of high levels of conjugated bilirubin and the absence of urobinogen. Patient’s
stool is usually pale due to the lack of stercobilinogen in the bowel. The rising blood levels of bilirubin also cause severe pruritus. In addition, as the hepatic functions become compromised, patients will begin to feel fatigued, anorexic, and easily bruised due to the loss of clotting factors in the blood. The obstruction of the pancreatic duct also causes steatorrhoea, increased weight loss and malnutrition [264].

When the tumour occurs in the body and tail of the pancreas, the patients mainly have non-specific pain and non-specific weight loss. As one would expect, these tumours do not cause obstructive signs and symptoms. The pains are generally localised in the epigastrium and/or the back. The extent of the pains, ranging from a dull ache to a severe pain, increases in severity by eating or lying flat. The symptoms usually do not appear until the tumour is relatively large. It is probably for this reason that most pancreatic cancer patients with tumours in the body and tail of the pancreas present with locally advanced disease extending to the peritoneum and spleen [264]. As with pancreatic cancer patients with tumours in the head of the pancreas, these patients also have weight loss, anorexia, early satiety, and diarrhoea.

Apart from the predominant weight loss and cachexia seen in patients with pancreatic cancer, patients are also presented with the gastrointestinal symptoms, both at diagnosis as well as throughout the course of the disease. These symptoms include indigestion, early satiety, and pancreatic exocrine insufficiency, inevitably requiring dietary changes. Of the other distressing gastrointestinal symptoms are the changed bowel habits which can cause flatulence and steatorrhoea.

Pain is perhaps one of the main presenting symptoms of pancreatic cancer. It is a well-reported problem with estimates of up to 80% of patients presenting with pain [317]. The difficulty for both the patients as well as the medical staff is that the actual perception of pain can be compounded by other symptoms, such as gastrointestinal obstructions, vomiting, and anxiety, becoming severe with persistent disease progression (also see section 3.6.2).
Fatigue is probably one of the most common and least understood symptoms associated with pancreatic cancer. It is a subjective experience and has a detrimental effect on the patient’s QL, and the diminishing physical, emotional, work, and social relationships [319]. Although almost 90% of patients’ relatives and 76% of oncologists report fatigue in their patients, the knowledge of the causes of fatigue related to cancer remains extremely limited [320]. However, there has not been much advances in the field of fatigue treatment and, as such, palliative treatments in pancreatic cancer have mostly focused on the symptoms that are treatable and correctable.

3.2.2.2 Psychological Symptoms

On diagnosis and throughout the course of the disease, majority of patients with pancreatic cancer are presented with emotional as well as social consequences of this disease and are faced with coping with the inevitable challenges of a potentially deadly disease. The fact that there is little change or hope for a cure or long term survival also compounds the emotional symptoms.

One of the main psychological symptoms that is usually present in patients with pancreatic cancer is depression. It has been long known that depression is more common in patients with pancreatic cancer than patients with other types of cancer [321]. It has also long been known that patients with pancreatic cancer already had psychological symptoms up to 43 months before any of the physical symptoms appeared [322]. It has also been reported that compared to other abdominal cancers, pancreatic cancer patients have shown more symptoms of anxiety, depression, and loss of ambition [322]. This was confirmed by laster studies of Holland et al [323] carried out in the mid-1980s which demonstrated higher self-ratings of depression, tension-anxiety, fatigue, and total mood disturbances in pancreatic cancer patients as compared with patients with other abdominal cancers.
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

The actual cause of depression in pancreatic cancer remains unclear and there have not been many reports in the literature investigating the cause(s). It is also not clear whether depression is caused by pancreatic cancer or is pancreatic cancer caused by depression. However, earlier studies of Jacobsson et al.\[324\] and Brown et al.\[325\] have suggested that pancreatic tumours secrete antibodies that either block the central nervous system serotonin receptors or reduce the synaptic availability of serotonin. Other mechanisms that have also been suggested\[326, 323\] are that the gastrointestinal tract is rich in neuropeptides and that the malignancies developing in this region may secrete biogenic amines that can alter mood. There have also been reports in the literature indicating that there might be a link between pain and depression. Studies of Kelsen et al.\[327\] carried out in the mid 1990s demonstrated that there is a strong correlation between increasing pain and depressive symptoms among pancreatic cancer patients with pain.

One of the most plausible mechanisms or causes of depression that has recently been reported is the excessive production of pro-inflammatory cytokines\[330, 328, 329\]. This, which is also known as the “cytokine hypothesis of depression” suggests that the pro-inflammatory cytokines act as neuromodulators and are the major factors in centrally mediating the behavioural, neuroendocrine, and neurochemical features of depressive disorders\[330\]. Other recent reports also suggest that the arachidonic acid cascade and prostaglandins may be involved in unipolar disorders\[331\] and that monotherapy with EPA, which is considered to be an anti-inflammatory agent, can successfully treat depression\[332\].

It is interesting to note that there are reports in the literature that link depression with the incidence of cancer. Report of Mathe\[333\] suggests that depression may predispose the body to development of malignancies. Depression has been shown to cause immune dysfunction through different mechanisms which include reduced cellular activity, increased cortisol levels that impair healing, and defects in the DNA repair of cells\[334, 335, 336\]. In addition,
psychological stress has been shown to have measurable effects on the pro-inflammatory cytokine production in the local wound environment\textsuperscript{[335]}.

3.2.2.3 Symptoms Presented On Physical Examination

Following the presenting symptoms, the next stage of diagnosis would be the physical examination of the patient. Although most physical examination findings may be normal, the Courvoisier’s Sign have been reported \textsuperscript{[264]} to be up to 90% specific and up to 55% sensitive for the malignant obstruction of the bile duct indicating that its absence does not rule out the presence of a malignancy. Other symptoms that might be present during physical examination is the presence of a tender and enlarged liver which may be accompanied by ascites, palmar erythema, and spider angioma. Supraclavicular lymphadenopathy and recurring superficial thrombophlebitis may also be present in patients with advanced pancreatic cancer \textsuperscript{[264]}.

The usual physical findings that are found on first physical examination in patients with pancreatic cancer include abdominal mass, ascites, and non-tender palpable gallbladder. In patients with jaundice, the gallbladder is usually felt at the right costal margins. Patients with widespread and advanced disease generally have left supraclavicular lymphadenopathy or a palpable rectal shelf.

3.2.3 Prognosis Of Pancreatic Cancer

Pancreatic cancer has the poorest prognosis of any common gastrointestinal malignancy with a five year survival of less than 5% \textsuperscript{[337]}. Recent reports \textsuperscript{[338]} indicate that the overall survival of patients with pancreatic cancer is three to five months with a 12 month survival rate of 10% and a five year survival rate less than 5%. The poor survival and prognosis of pancreatic cancer is mainly due to the fact that, in the majority of cases, the tumour is at an advance
stage on diagnosis. Pancreatic cancer patients who have the most favourable outcome are the ones that have an ECOG performance status of zero or one, early lesions, no evidence of pre-operative lymph node involvement, or tumour encasement of the superior mesenteric vessels and portal vein \cite{337}. However, patients with a poorer outcome are the ones on whom there have been attempts of curative surgery, but there are gross or microscopic residual of disease still present \cite{337}. This also includes the patients with major vessel invasion as well as perineural and lymphatic invasion. In fact one of the main negative prognostic factors for the overall survival in pancreatic cancer is metastases, specifically to local lymph nodes and distant metastases.

One of the rare tumours of the pancreas is the neuroendocrine tumours which affect one in 100,000 of the population and compared to the adenocarcinomas of the pancreas, it has a better prognosis. The overall five year and ten year survival has been reported to be 54% and 28%, respectively \cite{339}. It has also been reported that the completely resected pancreatic neuroendocrine tumours have a median survival period of seven years with the unresected ones, in the absence of widely metastatic disease, having a five year survival period \cite{340}. The pancreatic neuroendocrine tumours are classified as being either functioning or non-functioning islet cell tumour. These patients are generally presented with synchronous liver metastases which makes the decision on treatment approach difficult \cite{341}.

The actual diagnosis of pancreatic neuroendocrine tumours relies on imaging technique and histopathological features \cite{342}. The imaging technique used here is the multi-detector CT of the abdomen which allows the assessment of tumour burden as well as the determination of systemic or regional metastases \cite{342}. Other modalities that can also be used here are the MRI and Indium-111 Octreotide (OctreoScan® 111) which is often carried out in conjunction with CT or EUS to allow delineation of the primary tumour and presence of metastatic disease \cite{342}. Studies of Chu et al. \cite{341} have demonstrated that the best method to treat the neuroendocrine
tumours of the pancreas is to follow an aggressive approach with surgery still being the cornerstone of treatment. In their study, these authors suggested that the predictors of long term survival in these patients are surgical resection of the primary tumour, absence of liver metastases, metachronous liver metastases, and aggressive treatment of any liver metastases present with age, sex, and the location of tumour having no effect on survival. Due to its better survival rate \cite{339} the neuroendocrine tumours of the pancreas can potentially be cured, but requires life-long monitoring as liver metastases can still occur.

Another rare but serious type of pancreatic tumour is the intraductal papillary mucinous tumours. These tumours are characterised by an adenomatous proliferation of the pancreatic duct epithelium that may involve the main pancreatic duct or branched ducts and is often diagnosed as chronic pancreatitis \cite{343}. The prognosis of the malignant intraductal papillary mucinous tumours which have been resected in situ/invasive stage I malignant intraductal papillary mucinous tumours have been shown to be excellent, in contrast to the locally advanced forms (stage II and III) which are as poor as in patients with pancreatic ductal adenocarcinoma \cite{344}. The study of Maire et al \cite{344} demonstrated that the only prognostic survival factor in patients with malignant intraductal papillary mucinous tumours undergoing resection was lymph node involvement. These authors also showed a five year survival rate of 48% post-operatively in these patients \cite{344}.

For the long-term survival of patients with pancreatic ductal adenocarcinoma, complete excision of the tumour has been suggested to be the most important therapeutic option \cite{345}. The fact that on first presentation majority of these patients have advanced unresectable disease, even after radical resection only a few of the patients will survive up to five years \cite{345}. The single institution survival rates have been reported to be 16% for the five year survival and 12 months for the median survival time \cite{345}. Recent study of Moon et al \cite{345} reported that the adequacy of resection (R0) with a rate of 73% was the most significant factor
for predicting long-term survival. Other studies investigating the effects of resection in tumours of the head of pancreas reported that negative resection margin or a complete resection status were the important predictors of outcome [351, 348, 349, 347, 350, 352, 346]. For example, the study of Benassai et al [348] concluded that the presence of unclear resection margins was the strongest independent predictor of decreased survival with the lymph node metastasis, tumour size greater than three centimetres, and poor histologic differentiation were also independent predictors of poor survival. These authors [348] reported a five year survival rate of almost 53% in their most favourable subset of patients. Similarly, a large population study reported that following a multivariate analysis of the results of their peri-ampullary adenocarcinoma patients who had Whipple’s Procedure, the most powerful independent predictors favouring long-term survival included a pathologic diagnosis of duodenal adenocarcinoma, tumour diameter less than three centimetres, negative resection margins, absence of lymph node metastases, well-differentiated histology, and no re-operation [350]. However, the studies of Magisterelli et al [353] reported that T and nodal stage are the strongest independent prognostic factors for survival with limited intraoperative transfusion, reduced operative time, and clear margins have a lesser effect and may play a role. Although several studies have demonstrated the lymph node status being a prognostic factor for the survival rates [350, 346], other studies following multivariate analysis have suggested that it may not be a significant prognostic factor [345, 347, 354]. Some studies have also reported that tumour involvement of surgical margins and the histological grade of the tumour are not associated with survival where instead adjuvant therapy with radiation and chemotherapy to be the strongest prognostic factors for survival [355]. A more recent study [356] on the prognostic factors in the operative and palliative treatment of pancreatic cancer also investigated and included the presenting symptoms in their analysis. This study [356] concluded that presenting symptoms, such as jaundice, diabetes, high aminotransferase levels, alkaline phosphatase,
aspartate, and a history of cholecystectomy did not have a significant impact on survival, whereas the operative treatment and tumour size of less than three centimetres were the only significant independent prognostic factors for improved survival [356].

The post-resection prognostic factors in patients with pancreatic cancer are of great interest to most clinicians. The prognostic factors that have been shown and are known to increase the long-term survival rates include tumour size, lymph node metastases, cellular differentiation, and tumour involvement of the resection margin [357, 346]. As such, some studies reported in the literature recommend that surgical resection to be even performed on patients with advanced disease. Recent study of Han et al [358] demonstrated that surgical resection should be performed on patients who even have a more advanced disease, such as a large tumour size and/or lymph node metastases, as they still have a chance for cure through resection. This has been confirmed by recent cases studies of Spinelli et al [338]. The importance of resection and having a negative or clear margin with no post-operative complications in achieving a long-term survival in pancreatic cancer was confirmed by the recent study of Howard et al [359].

The landmark ESPAC-1 randomised controlled trial published in 2001 investigated and assessed the influence of resection margins on survival in patients who had received adjuvant chemoradiation and/or chemotherapy [360]. This study concluded that the patients who have a resection margin-positive pancreatic cancer represent a biologically more aggressive cancer and, as such, will benefit from surgical resection and adjuvant chemotherapy but not chemoradiation [360]. In addition, it was suggested that the benefit of chemotherapy for patients with pancreatic cancer is reduced if they have an unclear margin as compared to the patients with a clear resection margin [360].

There have not been many trials or studies investigating the prognostic power of performance status in pancreatic cancer reported in the literature. One recent study [361] that looked at this prognostic factor, investigated the performance status, as measured by the ECOG scale, of
patients with pancreatic cancer who were receiving gemcitabine chemotherapy. These researchers using univariate analysis demonstrated that worse results were found in patients with a performance status score of 2 and in patients with primary tumour located in the body or tail of the pancreas. They subsequently concluded that a low performance status is a negative predictive factor for advanced pancreatic cancer patients receiving gemcitabine chemotherapy \cite{361}. Similar results have also been obtained when using other performance indexes, such as the Karnofsky Performance Index \cite{362}. Study of Brasiunas et al \cite{363} on pancreatic cancer patients demonstrated that the patients diagnosed with locally advanced pancreatic cancer and patients with Karnofsky Performance Index higher than 70 at diagnosis had a longer survival period compared to patients diagnosed with metastatic disease or having a Karnofsky Performance Index of 70 or lower at diagnosis.

Another factor that has been utilised as a prognostic factor (as well as a diagnostic factor) is the cancer antigen, CA19.9 (also see section 3.3.1). Studies reported in the literature investigating the prognostic values of CA19.9 in pancreatic cancer patients receiving gemcitabine chemotherapy indicate that in patients with an increase in the CA19.9 levels or a decrease of less than 20% in the levels of CA19.9, prognosis is extremely poor and further gemcitabine chemotherapy is not indicated unless there is a significant improvement in the clinical benefits response \cite{364}. These measurements have been suggested to be performed together with clinical benefits response at the optimal time point of eight weeks post-chemotherapy \cite{364}.

In summary, the prognosis for patients who do undergo pancreatic resection is determined by the both the pathologic as well as the molecular characteristics of the resected tumour specimen \cite{360}. Long-term survival occurs only in patients who have their tumour completely resected and is influenced by prognostic factors that can be classified into being either tumour-related, surgery-related, or treatment related \cite{359,351,352}. These factors include tumour
classification, tumour differentiation, resection margin status, blood loss during surgery, and systemic adjuvant/neoadjuvant therapy \cite{359, 351, 352}. 
3.3 Diagnostic Tests

Following the presenting symptoms and initial physical examination, appropriate diagnostic tests are performed to locate and stage the pancreatic cancer. Locating as well as staging of the disease is crucial as the correct and appropriate planning and strategy for therapy is dependent on it. Once the therapy has been planned and commenced, the diagnostic tests are carried out at regular interval to monitor the progress of the therapy and make adjustments as required.

3.3.1 Laboratory Tests

One of first diagnostic laboratory test usually performed on the potential and symptomatic pancreatic cancer patients is the Carbohydrate antigen 19.9 (or Ca 19.9) $^{[365]}$. It is a sialylated Lewis blood group antigen targeted by the monoclonal antibody 111NS 19.9 $^{[365]}$. Ca 19.9 is a tumour marker which was initially found in colorectal cancer patients. It was subsequently also identified in patients with pancreatic, stomach, and bile duct cancer. Ca 19.9 itself is a cell surface glycoprotein expressed on the surface of pancreatic cancer cells as well as by normal human pancreatic and biliary duct cells, and gastric, colonic, endometrial and salivary epithelia $^{[291]}$. The blood levels of Ca 19.9 is usually increased above 100 U/ml in hepatocellular carcinoma, ovarian carcinoma, bronchial cancer, colonic cancer, gastric cancer, and pancreatic cancer $^{[291]}$. In patients with pancreatic cancer, higher levels of Ca 19.9 tend to be associated with more advanced disease. Non-cancer conditions such as gallstones, pancreatitis, cirrhosis of the liver, and cholecystitis, may elevate serum Ca 19.9 levels giving a false positive result. Earlier studies have shown the Ca 19.9 to be a useful tool in confirming the diagnosis in symptomatic patients $^{[366, 367]}$ as well as predicting and monitoring of
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

recurrence \[369, 368\] and is routinely used despite other newer markers being available and shown to be more powerful \[370\]. However, like any test, Ca 19.9 also has some limitations. Recent studies of Kim et al \[371\] demonstrated that despite Ca 19.9’s high specificity and sensitivity it suffers from a low positive predictive. In addition, the Ca 19.9 test cannot be used in patients with negative Lewis blood group antigen (i.e. Lewis a-, b-) as these patients are unable to synthesise Ca 19.9 \[291\].

The Ca 19.9 has also been used and reported in the literature as a prognostic marker. Studies of Katz et al \[372\] in the late 1990s have demonstrated that Ca 19.9 is a useful prognostic indicator in patients treated with radiotherapy. In their study, the use of more traditional prognostic indicators, such as including age, gender, pre-diagnosis weight loss, location of tumour, clinical TNM staging, size of lesion, vascular involvement on angiography, and sequence of radiation with respect to resection, were found not to be significant \[372\]. Similarly, the studies of Ziske et al \[364\] on pancreatic cancer patients with inoperable tumours who were receiving gemcitabine chemotherapy demonstrated that kinetics of CA19.9 serum concentration does serve as an early prognostic indicator of response to gemcitabine chemotherapy where tumour regression can be difficult to determine due to massive desmoplastic tissue. In addition, base-line measurements of CA19.9 have been shown to be an important independent prognostic factor in patients with inoperable pancreatic cancer \[365\]. Maisey et al \[365\] concluded that base-line concentrations of CA19.9 above or below the median value of 958 U/ml was an independent prognostic factor for overall survival of 19% and one year survival of 46%.

In our clinical trial on EPA and pancreatic cancer, the CRP, CEA, and Ca 19.9 markers were used as a part of the routine monthly blood tests carried out.
3.3.2 Imaging Modalities

Majority of the imaging modalities are relatively non-invasive and, as such, widely used in pancreatic cancer as an initial tool for diagnostic purposes.

3.3.2.1 Computerised Tomography

CT is probably one of the most common imaging modalities used in cancer. There are different types of CT imaging modalities which have different advantages in terms of their speed of scanning and image quality and have been used since the early 1970s to image various disease conditions including pancreatic cancer.

3.3.2.2 Magnetic Resonance Imaging

MRI is another imaging modality that has been used in the diagnosis and follow-up of pancreatic cancer. The reports on the usefulness and diagnostic advantages of MRI in pancreatic cancer have been contradictory with some reports indicating no diagnostic advantage over CT \[^{373}\]. The studies of Sheridan et al \[^{374}\] in the late 1990’s, however, have demonstrated that compared to CT, MRI can more accurately predict resectability in patients with pancreatic cancer, suggesting that MRI can potentially save a significant proportion of patients from attempted surgical resection.

Coronal and axial MRCP with single shot fast spin echo is usually used to look at the cystic lesion of the pancreas. However, for the arterial, portal and delayed phases, the fat-suppressed three dimensional spoiled gradient echo sequences (post-gadolinium-DTPA administration) are used \[^{375}\].

To image the ducts, MRCP with breath-hold project in the desired plane is used. Again, no contrast is required with the final image looking very similar to one obtained using ERCP.
However, overlap of the pancreatic ducts and other fluid-containing organs may exist in which case a negative contrast agent is used that eliminates the signal from the superimposed structure [375]. Another method which has been used since the late 1970’s is the administration of Secretin prior to MRCP. Secretin is generally administered at a dose of 1 ml per 10 kg body weight, approximately 10 to 15 minutes prior to MRCP [376].

3.3.2.3 Ultrasonography

In general and under normal circumstances, CT scanning is initially performed. However, if this yields an indeterminate or negative result causing clinical suspicion to remain high, then the use of endoscopic ultrasonographic techniques has been suggested [377, 378] in the literature as the next step in the diagnosis and staging of pancreatic tumours. These are, somewhat invasive and can cause distress. EUS is used to further investigate the local extent of the disease, especially in the cases where CT did not definitely identify a mass [337].

3.3.2.4 Endoscopic Retrograde Cholangio-Pancreatography

The last resort for diagnostic imaging is ERCP and is generally used when all other modalities are considered inconclusive. It has been reported to have a specificity of 88%-94% and a sensitivity of 70%-82% [379]. ERCP itself has been used in the diagnosis of pancreatic diseases since the early 1960s. It allows anatomic visualisation of the hepato-biliary tree as well as providing a mechanism for the collection of pancreatic juices for genetic analyses, brush cytology, and biopsy.
3.3.2.5 Tissue Diagnosis

Tissue diagnosis can be obtained for patients with inoperable pancreatic cancers using fine-needle aspiration biopsy \[^{380,381}\]. FNA has been added to both the EUS \[^{379}\] as well as CT \[^{382}\] and have been shown to be highly accurate in identifying malignancies which are visible to the but not seen on CT. The combination of EUS and FNA in the detection of pancreatic cancer has been reported to be almost 90\% \[^{382}\].

3.3.2.6 Nuclear Medicine Imaging

Compared to other modalities reviewed here, the use of nuclear medicine imaging techniques are less common and less routine in pancreatic cancer. One of the few nuclear medicine imaging techniques used in pancreatic cancer is the Indium-111 Octreotide scan. The Indium-111 Octreotide is commercially available as OctreoScan\textsuperscript{®} 111 (Mallinckrodt Australia Pty Inc, Victoria, Australia) and is currently being used at the Department of Nuclear Medicine, Royal North Shore Hospital. The OctreoScan\textsuperscript{®} 111 works by attaching itself to the somatostatin receptors on the cells surfaces of tissues. The tissues where, as a result of disease, has higher number of these receptors will consequently be visualised as “hot” areas on the final OctreoScan\textsuperscript{®} 111 scan image. The Indium-111 itself has a half-life of approximately 2.8 days. Pharmacologically, octreotide is the octapeptide analogue of somatostatin which is a powerful inhibitor of pancreatic secretions.

3.3.2.7 Positron Emission Tomography

One of the other sensitive imaging modalities used to evaluate suspected pancreatic lesions is the PET. Probably one of the reasons that has led to the development and use of PET has been that the common diagnostic and imaging modalities, such as EUS, CT, and ERCP have
difficulty distinguishing pancreatic cancer from the chronic mass-forming pancreatitis and in
distinguishing viable tumour from post-therapy changes [383].

PET is capable of providing functional information on the tumour which can be correlated with
the stage and development of the lesion. However, PET has a relatively poor anatomic
accuracy. This is now overcome by the fusion of PET and CT scanners thus creating a PET-
CT hybrid to overcome this limitation.

One of the most common tracers used in PET imaging is the F-18-2-fluoro-2-deoxy-glucose
or [\(^{18}\text{F}\)] FDG. This tracer works by being taken-up into the cell which is due to the fact that
protein glucose transport-1 is almost ubiquitously expressed in all cell types. In the malignant
cells, the protein glucose transport-1 is over expressed and results in the accumulation of [\(^{18}\text{F}\)]
FDG which can be viewed using PET techniques.

### 3.3.2.8 Miscellaneous Imaging And Visualisation Modalities

Diagnostic laparoscopy is another modality which enables the surgeon to directly view the
liver and peritoneum. This also enables and assists in the detection of small metastases that
may have escaped detection by other diagnostic tests [337]
3.4 Surgical Treatment of Pancreatic Cancer

It is well known that surgical resection is, potentially, the only curative treatment currently available for patients with pancreatic cancer. Due to the advanced nature of the disease at first presentation, only a relatively small population of these patients are potential candidates for surgical resection. However, today with the improvements in surgical techniques and peri-operative care in pancreatic cancer resection, the degree of resectability has increased and the operative mortality and morbidity has decreased.

3.4.1 Surgical Methods In Pancreatic Cancer

It has been reported in the literature that only 15% to 20% of all pancreatic cancer patients have resectable disease on diagnosis or first presentation [268]. The surgical procedure carried out on these patients is the Whipple’s Procedure, which is detailed in section 3.4.2 below.

In addition to curative purposes, surgery is also performed for palliation. Biliary decompression for palliation of jaundice is often achieved by performing choledochojejunostomy or cholecystojejunostomy. The endoscopic application of expendable wire stents also can achieve biliary decompression for palliation of jaundice. This technique is considered to have a lower risk than surgery [268, 384, 385]. This endoscopic placement of stents have been reported [384, 385] to be able to relieve the obstructive symptoms in 97% of patients. In addition, it has a relatively low mortality rate of 3% and a morbidity rate of 12% [384, 385].

The possible complications for this procedure include bleeding, infections, and pancreatitis [384, 385].

The correct diagnosis and pre-operative staging of pancreatic cancer is essential as the tumour stage usually determines the surgical procedure and further therapies. In general, the tumour
resectability is determined by the extent of loco-regional spread and the tumour features that prevent surgery includes [376]:

- The invasion of the:
  - Portal vein;
  - Superior mesenteric artery and superior mesenteric vein;
  - Common hepatic artery and proper hepatic artery;
- The involvement of the neural plexus around the coeliac axis or the superior mesenteric artery;
- The invasion of contiguous structures, such as the stomach, kidneys, colon, spleen;
- The invasion of the spine;
- The presence of metastases to the liver and/or peritoneum.

For patients with tumours in the head of the pancreas and jaundiced at presentation, the biliary tree may be drained pre-operatively to reduce the surgery-associated morbidity and mortality. These patients are, generally, at a risk of coagulopathy, malabsorption, and malnutrition.

For patients with pancreatic tumour in the head of the pancreas or in the uncinate process of the pancreas, the Whipple’s Procedure is the standard surgical procedure. This procedure is reviewed in the sections on Whipple’s Procedure (3.4.2 and 3.4.3).

One surgical procedure that has been recommended since the early 1970s to be better than the Whipple’s Procedure for patients with pancreatic cancer is Total Pancreatectomy [387, 386]. Total pancreatectomy has also been successfully used in patients with ductal adenocarcinoma of the pancreas [388]. The study of van Heerden et al [388] in the early 1980’s has also suggested that total pancreatectomy will also eliminate the pancreatojejunal anastomosis which has associated lethal complications. However, as one would expect this procedure has some morbidities, such as diabetes and obligate exocrine insufficiencies, which may be no better
than Whipple’s Procedure \cite{388}. In fact some more recent studies \cite{389} have shown that total pancreatectomy has a significantly worse median survival compared to the standard Whipple’s Procedure.

Regional pancreatectomy has also been used in patients with pancreatic cancer. This procedure involves the resection and reconstruction of the superior mesenteric vein-portal vein confluence as well as \textit{en bloc} regional lymph node dissection. Although some studies advocate the use and benefits of this procedure \cite{390}, other studies indicate that its should only be performed on carefully selected patients \cite{391}. Studies of Wagner et al \cite{392} have even reported that regional pancreatectomy and resection with extended lymph node dissection does not show any improvements in long-term survival compared to the standard types of resection.

3.4.2 The Whipple’s Procedure

The Whipple’s Procedure, also called a pancreaticoduodenectomy, is generally the removal of the gallbladder, common bile duct, part of the duodenum, and the head of the pancreas and was the principle surgical procedure performed on the Surgical Follow-up studies as well as the EPA And Pancreatic Cancer patient populations. This operation was first described by the surgeon Dr. Alan Oldfather Whipple (1881-1963) of New York Memorial Hospital (now called Memorial Sloan-Kettering) who devised the procedure in 1935. Originally performed in a two-step process, he refined his technique in 1940 into a one-step operation.
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

Figure 4. Organs involved in the Whipple’s Procedure.

The Whipple’s procedure today is very similar to Dr Whipple’s original procedure of 1940. In short, it consists of removal of the distal half of the stomach (antrectomy), the gall bladder (cholecystectomy), the distal portion of the common bile duct (choledochectomy), the head of the pancreas, duodenum, proximal jejunum, and regional lymph nodes. Reconstruction consists of attaching the pancreas to the jejunum (pancreaticojejunostomy) and attaching the common bile duct to the jejunum (choledochojejunostomy) to allow digestive juices and bile to flow into the gastrointestinal tract and attaching the stomach to the jejunum (gastrojejunostomy) to allow food to pass through.

Studies of Seiler et al \[393\] have demonstrated that the pylorus-preserving Whipple’s procedure offers the same long-term benefits as the classical Whipple’s procedure. These authors indicated that the pylorus-preserving Whipple’s procedure has a shorter operation time, reduced blood loss, and less chance of requiring blood transfusions. However, both surgical techniques are associated with similar risk factors. These risk factors include delayed gastric emptying, pancreatic fistulae, anastomotic leaks, infections, intra-abdominal abscesses, haemorrhage, diabetes, and pancreatic exocrine insufficiency \[393\].
Both the Whipple’s procedure and the pylorus-preserving Whipple’s procedure are performed on patients where the tumour is located in the head of the pancreas. For patients with resectable cancer where the tumour is located in the body or tail of the pancreas, distal pancreatectomy is generally performed. Generally, proximal Whipple’s procedure is not performed and not recommended in patients with neuroendocrine tumours of the pancreas [394].

3.4.3 Whipple’s Procedure Survival Rate

Recent data from the National Cancer Database indicate that Whipple’s procedure is the most commonly performed cancer-directed operation for pancreatic cancer, although it is used in only 9% of patients. In a large national database, the five-year survival rate for patients treated by Whipple’s procedure was reported to be 3% [395, 396].

In contrast to these national figures, specialized centres have reported decreasing operative mortality rates and improving long-term survival rates after Whipple’s procedure for pancreatic cancer. For example, the five-year survival rate for patients treated surgically at Johns Hopkins now exceeds 20%. Many factors are likely to be responsible for the improving safety of pancreaticoduodenal resection, including improvements in intensive and critical care, improved surgical experience with decreases in operative time and less need for blood replacement, and regionalization of patient care to specialized "Centres of Excellence," such as Johns Hopkins [395, 396].

The peri-operative mortality rate of patients undergoing Whipple’s procedure has declined in the recent years with reports in the literature indicating significantly lower mortality rates in more experienced centres (3.8%) than the less experienced ones (7.5% to 17.6%) [395]. Studies of Ahmad et al [355] have demonstrated an overall five year survival rates of 19% and seven
year survival rates of 11% post-Whipple’s Procedure confirming the fact that resection in patients with pancreatic adenocarcinoma can result in relatively long-term survival, especially when combined with adjuvant therapies. These authors [355] also reported that these resections were performed with a low mortality rate of 3%. Other researchers have reported even higher survival rates of 21% [346] and 24% [397] in post-Whipple’s Procedure patients.

The five year survival rate for resectable tumours located in the head of the pancreas is similar to the ones located in the body or tail of the pancreas [398]. The five year survival rates after surgical resection range from approximately 10% to almost 30% [399, 400, 350]. Negative prognostic factors include poorly differentiated histology, positive resection margins, lymph node involvements, and tumours greater than two centimetres [399, 400, 350].

A interesting recent retrospective study carried out by Schmidt et al [401] on 516 patients, majority (57%) of which were periampullary adenocarcinoma, investigated the survival rates of different pancreatic cancer patients who underwent the Whipple’s Procedure. This study revealed that there was a 43% complication rate, although they were minor and not life threatening. In addition, it was demonstrated that in their 516 pancreatic cancer population, the tumour grade was a statistically significant univariate, but not multivariate, predictor of survival [401]. Also, the margin status was reported to be a statistically significant univariate, but not multivariate, predictor of survival [401]. However, blood loss, type of operation, and total bilirubin values were found to be statistically significant multivariate predictors of long-term survival [401].

A recent analysis performed at The Johns Hopkins Hospital has determined the factors which favour long-term survival after Whipple’s procedure [395, 396]. Here, between April 1970 and April 1994, 208 patients underwent a standard Whipple’s procedure for adenocarcinoma of the head of the pancreas at The Johns Hopkins Hospital. The preference of surgeons at Johns Hopkins is to perform partial pancreatectomy whenever possible, leaving the pancreatic body
and tail in place. Distal gastrectomy is typically reserved for tumour involvement of the distal stomach or first portion of the duodenum. Multiple factors were analysed including patient demographics, intraoperative factors, tumour characteristics, and post-operative use of adjuvant therapy. The primary outcome variable analysed was survival. The results of this review, which is the largest single institution experience reported to date, allowed assessment of 201 of the 208 patients. Seven patients had incomplete outcome data and were excluded.

For the group of 201 patients comprising the study population, the overall postoperative in-hospital mortality rate was low, with the current figure being 0.7% (for the last 149 patients). This means that survival data for patients treated at Johns Hopkins reflect an operative mortality rate of less than 1%. The mean age of the patients was 63 years, with a slight male predominance (108 men and 93 women). There were no differences in survival based on age, gender, or race. The actuarial one, three and five-year survival rates for all 201 patients were 57%, 26%, and 21% respectively, with a median survival of 15.5 months. There were 11 five-year survivors, 7 six-year survivors, and only one fifteen-year survivor. By univariate statistical analysis, a significant improvement in survival has been observed from the decade of the 1970s, through the decade of the 1980s, to the decade of the 1990s. Patients resected in the 1970s had a median survival of 7.5 months and a three-year survival of only 14%, while patients undergoing resection in the 1990s had a median survival of 17.5 months and a three-year survival of 36%.

Studies of Birkmeyer et al [402, 403] and Finlayson et al [404] have demonstrated that the mortality of pancreatic cancer resection is proportional to both the number of pancreatic resections performed by the individual surgeons and the number of patients attending and undergoing major surgical procedures in that particular hospital or institution. The prognostic factors for recurrence after Whipple’s Procedure and extended radical operation has also been investigated and reported in the literature. Studies of Mu et al [405] has demonstrated that
tumours in the head of pancreas which are larger than four centimetres, lymph node involvement, and the presence of interstitial invasion are all high risk factors for recurrence.

In summary, the surgical treatment of adenocarcinoma of the head, neck or uncinate process of the pancreas via Whipple’s procedure has now been associated with falling postoperative morbidity and mortality rates and improving long-term survival. The results from recent single institution experience demonstrate\textsuperscript{[395, 396]} an actuarial five-year survival of 21\% for all patients undergoing pancreaticoduodenal resection for adenocarcinoma of the pancreas. Importantly, the actuarial five-year survival is improved for patients resected with tumours less than 3 cm in diameter (28\%), negative margins (26\%), negative nodal involvement (36\%), or diploid tumour DNA content (39\%). Multivariate analysis indicated that the parameters that serve as the strongest independent predictors of favourable outcome are tumour DNA content, tumour diameter, status of resected lymph nodes, margin status, and decade of resection (resection in the 1990s being most favourable). With the improvement of operative mortality for Whipple’s Procedure, some researchers\textsuperscript{[406]} have even reported concomitant improvements of 30\% in five-year survival of patients with pancreatic cancer. The increasing use of postoperative combined modality chemotherapy and/or radiation therapy appears to be another factor favouring long-term survival\textsuperscript{[395, 396]}. It should be noted that following the landmark results of the first ESPAC trial (ESPAC-1) in 2001\textsuperscript{[360]}, it was demonstrated that there are no survival benefits for adjuvant chemoradiotherapy but there are potential benefits for adjuvant chemotherapy. This, however, may be contradictory and controversial as recent reports\textsuperscript{[407]} indicate that this may not be universally accepted.
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

3.5 Chemotherapy Of Pancreatic Cancer

The aims of treatment of advanced pancreatic cancer are to control tumour progression, to relieve the disease-related symptoms, and to improve and maintain the patient’s QL \[408\]. Statistically around 80% of all pancreatic cancer patients are found to be unresectable at first diagnosis \[268\]. As a result, these patients require primary treatment and management modalities other than surgery. In addition, from those patients who undergo potentially curative surgery, the five year survival rate is approximately 20% \[337\] and, therefore, pancreatic carcinomas cannot be cured by surgery alone. Recent review by Kosuri et al \[409\] and El Kamar et al \[410\] have detailed the clinical trials of different adjuvant therapies as well as different agents currently available for pancreatic cancer chemotherapy.

Earlier randomised, multicentre study of Bakkevold et al \[411\] randomising patients to receive adjuvant chemotherapy or surgery alone demonstrated that the median survival was significantly longer in the adjuvant chemotherapy group (23 months) than the control group (11 months) despite the fact that the five year survival rates were not significantly different.

Non-surgical treatment of pancreatic cancer often involves chemotherapy, radiotherapy, or a combination of chemotherapy and radiotherapy which is also referred to as chemoradiation.

In general, non-surgical treatment of pancreatic cancer is performed as an adjuvant therapy in patients who have already received surgery, as a palliative approach in advanced and non-resectable setting, or in some cases as an attempt to reduce the tumour bulk.

Adjuvant therapies are also used post-operatively to maximise the effect of surgical resection and, therefore, to prolong survival in pancreatic cancer. It is an important approach in pancreatic cancer as, compared with other cancers, pancreatic cancer metastasises at an early stage. This would probably mean that the majority of pancreatic cancer patients may have occult metastases at the time of their surgery, thus requiring systemic therapy.
The randomised trial of the GITSG carried out in the early 1980s \cite{412} demonstrated that the combination of 5-fluorouracil and radiation therapy may double the survival duration in patients with pancreatic cancer. The EORTC multicentre trial results published in 1999 \cite{413} investigated the outcome of adjuvant chemoradiation in pancreatic and periampullary cancer patients. The results of this trial, unlike the GITSG, demonstrated that there were no significant effects in using chemoradiation. Following the landmark results of the first ESPAC trial (ESPAC-1) in 2001 \cite{360}, it was demonstrated that there are no survival benefits for adjuvant chemoradiotherapy but there are potential benefits for adjuvant chemotherapy. However, evidence available in the literature favours the use of adjuvant therapy, but the value of added chemoradiation still is unclear \cite{414}.

The two chemotherapy agents that have been successfully used in the treatment of pancreatic cancer are gemcitabine and 5-fluorouracil. These agents have also been combined with other antineoplastic agents to increase their efficacy.

It should be noted that with all its beneficial effects for palliation and increasing survival, unfortunately chemotherapy has been shown to impose oxidant stress on the patient and, thus, providing an extra boost to the already present inflammatory process \cite{415}. This can further exasperate the cachectic state of the patient, thus decreasing survival (also see section on Cachexia (Chapter 2)).

### 3.5.1 Gemcitabine Therapy

Gemcitabine chemotherapy is now considered to be the golden standard treatment for unresectable pancreatic adenocarcinoma \cite{416}, the treatment of choice for patients with inoperable pancreatic cancer \cite{417}, as well as being indicated for metastatic lesion of the pancreas \cite{264, 268}. This agent has been used in our malignant mesothelioma as well as the
pancreatic cancer study patient population. Recent study of Ishii et al.\textsuperscript{[418]} has demonstrated that the frequency of non-progressive disease, CA19.9 response, and favourable prognosis compared with estimated survival were all significantly higher in pancreatic cancer patients receiving gemcitabine therapy than the ones not receiving gemcitabine (receiving 5-fluorouracil) with the median survival time being 5.7 months versus 2.9 months in patients not receiving gemcitabine.

The pivotal study of Burris et al.\textsuperscript{[419]} carried out in the late 1990’s has demonstrated that, compared with 5-fluorouracil, gemcitabine given intravenously at a dose of 1000 mg/m\textsuperscript{2} infused over 30 minutes provides a small but statistically significant improvement in clinical benefit response as well as survival rates. Here, gemcitabine was shown to produce an objective response rate of 5%, a median survival of 5.7 months, and a six month survival rate of 46%\textsuperscript{[419]}. A recent meta-analysis of 20 phase III trials\textsuperscript{[420]} has also concluded that the single-agent gemcitabine remains the standard of care for patients with advanced pancreatic cancer. In addition, the meta–analysis has also suggested that platinum/gemcitabine combinations appears to improve both the progression free survival rates as well as the overall response rates and can be used in selected patients\textsuperscript{[420]}.

Survival analysis and phase II studies carried out in the mid-1990s show a response rate of 6% to 11% with disease stabilisation in a further 19% to 32% of pancreatic cancer patients for gemcitabine administered as a single agent\textsuperscript{[422, 423, 421]}. These studies also report a medial overall survival of 6.3 months\textsuperscript{[422, 423, 421]}. Gemcitabine chemotherapy-induced toxicities that have been reported in the literature include bone marrow suppression, fatigue and lethargy, flu-like symptoms, nausea, vomiting, and peripheral oedema\textsuperscript{[422]}.

Recent studies have also investigated the anti-tumour activities of gemcitabine in patients with other carcinomas, such as advance-stage biliary tree carcinoma. Studies of Tsavaris et al.\textsuperscript{[424]} have demonstrated that the weekly administration of gemcitabine provides a safe, well-
tolerated, and effective treatment for patients with advanced cholangiocarcinoma, particularly with a gallbladder origin.

Gemcitabine chemotherapy was the chemotherapy that was the chemotherapy agent used in our EPA clinical trial in pancreatic cancer patients and as combination chemotherapy in the mesothelioma trial. The single agent gemcitabine still remains the treatment of choice in patients with metastatic pancreatic cancer [425].

### 3.5.1.1 Pharmacology And Pharmacokinetics

Gemcitabine (2’,2’-difluorodeoxycytidine, Gemzar®) is an antimetabolite (Pyrimidine Antagonist) and an antineoplastic agent used for non-small cell lung cancer, metastatic breast cancer, ovarian cancer, and pancreatic cancer. Gemcitabine is indicated as a single agent (given alone) for the first-line treatment for patients with locally advanced (stage II or stage III when surgery is not an option) or metastatic (stage IV) adenocarcinoma of the pancreas. Gemcitabine is also indicated for patients with 5-fluorouracil-refractory pancreatic cancer [422].

Chemically gemcitabine is a deoxycytidine analogue in which the hydrogen atoms on the 2’ carbons of deoxycytidine are replaced by fluorides. It also has structural and metabolic similarities to cytarabine. Gemcitabine is a clear liquid after being dissolved from a white powder and has an International Union of Pure and Applied Chemistry (IUPAC) name of: 4-amino-1-[3,3-difluoro-4-hydroxy-5- (hydroxymethyl) tetrahydrofuran-2-yl]- 1H-pyrimidin- 2-one (also see Figure 5).
Figure 5. Gemcitabine.

Gemcitabine’s actions are as a result of it being a pyrimidine antimetabolite that inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase, specific for the S-phase of the cell cycle. In order for gemcitabine to have anti-tumour activities, it must first be phosphorylated to its active metabolites of gemcitabine diphosphate and gemcitabine triphosphate. First, gemcitabine is phosphorylated in the cells by deoxycytidine kinase to gemcitabine monophosphate, which in turn is phosphorylated to two active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. Gemcitabine diphosphate itself inhibits DNA synthesis by inhibiting ribonucleotide reductase and depletes intracellular pools of all deoxynucleotide triphosphates which are required for DNA synthesis. The gemcitabine triphosphate, however, incorporates itself into DNA and inhibits DNA polymerase leading to premature chain termination. In addition, the gemcitabine triphosphate can impede normal DNA repair, which may explain gemcitabine’s radio-sensitising properties and synergy with other chemotherapeutic agents [268].
3.5.1.2 Metabolism

Gemcitabine has a low protein binding and is metabolized in the cells by nucleoside kinases to the active difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate (dFdCTP) nucleoside metabolites.

When infused in less than one hour, it has an elimination half-life of 42 to 94 minutes. However, when infused over a longer period of three to four hours, the elimination half-life is between four to 10.5 hours.

Majority of the gemcitabine (92% to 98%) is excreted as an inactive uracil metabolite in the urine.

3.5.1.3 Side Effects

Phase II trials of gemcitabine in pancreatic cancer patients have reported of mild toxicities which mainly consisted of moderate and rapidly reversible myelosuppression [426]. The haematological side effects included leucopenia, thrombocytopenia, and anaemia [426]. The non-haematological side effects that were reported in the phase II trial were nausea, vomiting, skin rashes, peripheral oedema, asthenia, and alopecia [426]. An earlier review of phase I and phase II trials of gemcitabine reported in the literature also confirms the fact that myelosuppression is the main toxic side effect at the commonly used weekly schedules [427].

The uncommon and manageable side effects reported were fever (7.3%), pain (6.8%), asthenia (6%), abdominal pain (5.5%), dyspnoea (5%), vomiting (3.9%), anorexia (3.6%), and deep vein thrombosis (3.2%) [427]. One interesting study has even recently reported that the gemcitabine administration in patients who were post-resection resulted in a more severe leucopenia than in non-resected patients [428].
In general, one of the most common side effects of cancer chemotherapy is the haematological effects. Recent study of Plate et al.\(^429\) have indicated that there is an impact on the absolute number of white blood cells after the first dose of gemcitabine with the cell numbers stabilising during subsequent gemcitabine administrations. Specific changes were noted by Plate et al.\(^429\) on day seven post-therapy where there was significant increases in the percentages of CD4\(^+\), helper T-cells and CD3\(^+\), CD45RO\(^+\) double-positive, memory T-cell subset which then declined after the second gemcitabine treatment. These researchers concluded that the immune cell activation increases during the initial course of gemcitabine therapy with the overall CD45RO\(^+\) memory cells significantly declining, therefore indicating that this mode of chemotherapy is not immunodepleting and its effect on the immune function of patients with pancreatic cancer undergoing gemcitabine therapy is more positive than negative\(^429\).

### 3.5.2 Gemcitabine Combination Chemotherapy

Gemcitabine combination chemotherapy has been studied and investigated to potentiate the effects of gemcitabine and/or the other cytotoxic agents and there has been numerous studies comparing the gemcitabine monotherapy with gemcitabine-based cytotoxic combination regimens\(^430\). Agents that have been studied include 5-fluorouracil, oxaliplatin, cisplatin, docetaxel, and irinotecan. However, not all combination chemotherapies were found to be successful and many combinations, especially the novel agents, are found not to be superior to single agent gemcitabine chemotherapy in pancreatic cancer patients. As a result, despite promising phase II trial results from the gemcitabine combination chemotherapies, the phase III trial results have been less successful in showing overall survival benefits superior to that of gemcitabine monotherapy\(^431\).
Gemcitabine has also been combined with cisplatin as they are thought to act synergistically where gemcitabine is thought to enhance the formation of cisplatin-DNA adducts and cisplatin augments the incorporation of gemcitabine triphosphate into DNA, thus potentiating the anti-tumour activities [432]. Recent phase III trial of Heinemann et al [433] demonstrated that their results support the efficacy and safety of an every-two-weeks treatment with gemcitabine plus cisplatin. Although, the median overall survival and progression-free survival were more favourable in the gemcitabine plus cisplatin arm as compared with gemcitabine alone, the difference was not statistically significant [433]. Phase III trial of Colucci et al [434], however, demonstrated that the addition of cisplatin to gemcitabine significantly improves the median time to disease progression and the overall response rate compared to gemcitabine alone and that the median survival rate is more favourable for the combination therapy despite the fact that the clinical benefit rate were similar for both groups. One of the other anti-tumour agents that have been combined with gemcitabine is docetaxel. The results for this combination chemotherapy seem to be more encouraging. Studies of Jacobs et al [435] suggest that that gemcitabine plus docetaxel could be more effective than either gemcitabine or docetaxel alone in the treatment of pancreatic cancer. A recent phase II trial carried out by the EORTC’s gastrointestinal group [436] also confirmed the safety efficacy, and the activity of the gemcitabine plus docetaxel combination chemotherapy in pancreatic cancer. There, however, does not seem to be any phase III trials of this combination chemotherapy been reported in the literature.

A new platinum anti-tumour agent, oxaliplatin has also been combined with gemcitabine for combination chemotherapy of pancreatic cancer. The trials for this combination chemotherapy has now reached phase III. A recent phase III trial [437] which compared gemcitabine plus oxaliplatin combination with gemcitabine alone reported that following successful phase II trials [438], these phase III results confirm the efficacy and safety of gemcitabine plus
oxaliplatin combination. However, this study failed to demonstrate a statistically significant advantage in terms of the median overall survival compared with gemcitabine alone \[^{437}\]. This study also stated that gemcitabine plus oxaliplatin combination is a promising regimen and the first combined treatment to be superior to gemcitabine alone in terms of its clinical benefit \[^{437}\].

Interestingly, by far the best results in terms of response rates, survival, time to tumour progression, and clinical benefits seems to be from the combination of gemcitabine and a cocktail of other anti-tumour agents. Phase II trials \[^{439}\] of gemcitabine plus 5-fluorouracil, epirubicin, and cisplatin (also known as PEF-G) in stage IV pancreatic cancer demonstrated a median duration of response of 8.5 months, median time to tumour progression of 7.5 months, median survival time of 11 months in the assessable population and 10 months in the intent-to-treat population. Here, a clinical benefit of 78% was also achieved and was concluded that this combination chemotherapy is a well-tolerated and safe regimen with a very high rate of durable response \[^{439}\].
3.6 Palliation Of Symptoms Of Pancreatic Cancer

Generally, palliative treatment in pancreatic cancer is provided for those patients who have recurrent or unresectable pancreatic cancer. These patients may require palliative treatment to relieve their symptoms of conditions such as gastric outlet obstruction, obstructive jaundice, pain, and pancreatic insufficiency. Although a lot of the symptoms are more difficult to palliate, there are a few important ones that can be successfully palliated using pharmacological and/or surgical interventional techniques. There is a distinction between therapy for curative results and therapy for end-of-life care. At the point where there is no benefit in continuing anticancer treatment as it fails to improve functional limitations, prolong life, prolong survival, or reduce the symptoms of the disease, management measures should focus on the palliations of symptoms.\textsuperscript{[319]} Palliative therapies in pancreatic cancer have been shown to improve the QL as well as to relieve the disease-related symptoms, increasing the quality-adjusted survival time \textsuperscript{[440]}.

3.6.1 Biliary And Duodenal Obstructions

Two pancreatic cancer complications that usually require invasive, surgical intervention include cases of established duodenal obstruction and jaundice. Here, surgical bypass of the tumour mass or re-routing of the obstructed biliary or gastric outflow tract often relieves the symptoms. The obstructive jaundice itself can be relieved by choledochojejunostomy whereas the gastric outlet obstruction secondary to tumour compression on the duodenum usually relieves the symptoms \textsuperscript{[441]}. In these patients an additional chemical splanchnicectomy is also recommended for pain relief \textsuperscript{[441]}. 
Biliary obstructions are usually relieved by endoscopic biliary stenting. Stenting is normally considered either when endoscopic decompression of the biliary tract has failed, or the patient has developed clinical evidence of gastroduodenal obstruction \[^{441}\]. Two types of stent are currently available: plastic and metallic. Plastic stents, although cheaper than the metallic stents, have the disadvantage of having a greater propensity to occlusion than the metallic stents \[^{441}\]. Recent studies \[^{442}\] have demonstrated that endoscopic stenting is the method of choice for relieving biliary obstruction in patients with pancreatic cancer.

Obstructive jaundice is one of the most common complications of pancreatic cancers which occur in the head of the pancreas. It has been reported \[^{443}\] to account for up to 80% of patients. The obstructive jaundice itself causes conditions such as pruritus, nausea, anorexia, malnutrition, and cholangitis. Surgical options for the palliation of obstructive jaundice include external biliary diversion and internal biliary bypass. The external biliary diversion is usually not recommended \[^{441}\] due to its undesired effects. Therefore, the remaining options would be the internal biliary bypass. The procedures here that are usually performed include choledochoenterostomy, cholecystoenterostomy, hepaticocholedochojejunostomy, and cholecystogastrostomy \[^{441}\]. From these procedures the most effective and safest procedure has been reported \[^{444}\] to be the cholecystoenterostomy technique. Obstructive jaundice can also be relieved by non-surgical procedures. These procedures include percutaneous transhepatic or endoscopic techniques \[^{441}\]. Endoscopic biliary drainage has been demonstrated to be cheaper and able to provides better QL in patients with biliary obstruction and metastatic pancreatic cancer \[^{445}\].

Duodenal obstruction is another common complication of pancreatic cancer causing symptoms such as nausea and vomiting. Generally, the tumours which are located in the head of pancreas or in the uncinate process are most likely to affect the second and third parts of the duodenum the tumours located in the body or tail of the pancreas are most likely to affect
Chapter 3: THE PANCREAS AND PANCREATIC CANCER

the fourth section of the duodenum \[^{446}\]. The treatment of choice for duodenal obstructions have been reported to be endoscopic stenting \[^{442, 446}\]. This has also been demonstrated to be safe and effective in the long term, the use of which has been justified as repeat procedures are rarely required even in patients who have a long survival \[^{446}\].

3.6.2 Pain

Pain is one of the most common and debilitating symptoms of pancreatic cancer which is often difficult to control. It has long been known that cancer-related pain is a multidimensional entity consisting of sensory, affective, cognitive, and behavioural components \[^{447}\]. The potential causes of pain in pancreatic cancer are not fully understood \[^{448}\] and have been also associated with depression in pancreatic cancer patients \[^{327}\]. The incidence and frequency of pain among patients with pancreatic cancer, as reported in the literature, seems to be relatively high. A prospective study of 1107 patients carried out by Brescia et al \[^{449}\] in the early 1990s demonstrated that almost 44% of their population of pancreatic cancer patients had pain.

Possible causes of pain in patients with pancreatic cancer include the proximity of the organ to a number of other critical structures, such as the duodenum, liver stomach, jejunum, and the transverse colon \[^{319}\]. Pancreas itself is innervated by nerve networks that interact with both the sympathetic as well as the parasympathetic nervous systems producing a variety of different pains. The pain symptoms that are due to the tumour being located in the body of the pancreas appears as midepigastric discomforts, while the pains caused by the tumour being located in the tail of the pancreas appears as being localised in the left epigastrium and the left intercostal space \[^{319}\]. The obstructive symptoms of pancreatic cancer are crams which are poorly localised with a crescendo-decrescendo quality, while the destruction of the pancreas
itself causes further inflammation and discomfort \[319\]. As one would expect, the pancreatic cancer pain is progressive, and its character, quality, and temporal nature, worsens as the disease progresses \[319\].

Other possible causes that have been reported include celiac plexus invasion by tumour infiltration, pancreatic duct obstruction and distension, inflammation and ischemia \[450\]. The palliation of pain in patients with unresectable pancreatic cancer has been suggested \[441\] to be best achieved using a combination of pharmacological and non-pharmacological techniques and procedures. Pharmacological approaches include the use of NSAIDs and narcotic analgesics. Patient can either receive administration of slow-release morphine orally, by intravenous route, or by epidural infusion using portable pumps. These techniques have been reported to be suboptimal methods of pain relief and are associated with significant side effects \[451\] such as constipation, nausea, and drowsiness with morphine, and precipitation of acute renal failure with NSAIDs. Instead, other techniques such as celiac plexus block or celiac plexus neurolysis have been demonstrated to be the most effective means of pain relief by some researchers \[451\] and of limited use by others \[441\]. Available surgical techniques include splanchnicectomy and celiac plexus block.

The celiac plexus block or celiac plexus neurolysis is often performed when the traditional methods of pain control fail and the pain management becomes difficult. Anatomically, the celiac plexus consists of a right and left ganglion which is located anterolaterally to the aorta at the level of the celiac trunk with the crura of the diaphragm and the L1 vertebral body being located posterior to the celiac plexus. The kidneys, adrenals, and the inferior vena cava are located laterally to the celiac plexus with the pancreas covering it anteriorly. The choice of performing celiac plexus block or celiac plexus neurolysis is dependent on whether a temporary or permanent pain relief is required. In order to perform the celiac plexus block a corticosteroid injection is made together with a local anaesthetic, such as bupivacaine, to
provide a more prolonged analgesic effect compared to the local anaesthetic alone and is generally performed in patients with benign pancreatic diseases [450]. Celiac plexus neurolysis causes the ablation of the plexus. Celiac plexus neurolysis is performed by the injection of alcohol or phenol, administered together with a local anaesthetic such as bupivacaine, which is injected first to prevent pain associated with the alcohol injection [450]. Recent reports have suggested that the ideal method of carrying out the celiac plexus block or celiac plexus neurolysis is by EUS [451]. The EUS guided celiac plexus block or EUS guided celiac plexus neurolysis has been also shown not to have the complications of the posterior approach techniques [451].

3.6.3 Pancreatic Insufficiency

One of the often overlooked symptoms of pancreatic cancer is pancreatic insufficiency. Pancreatic insufficiency occurs as a result of the pancreas not functioning properly or that the pancreas has been partially or completely removed.

Symptoms of pancreatic insufficiency include steatorrhoea, weight loss, and diabetes. The administration of pancreatic exocrine supplements often relieves these symptoms. The diabetes which is a symptom of pancreatic endocrine insufficiency is, however, treated as type II diabetes where regular insulin injections would be required.
4. MALIGNANT MESOTHELIOMA

Malignant mesothelioma is an insidious neoplasm that occurs mainly in the mesothelial surfaces of the pleural cavity. It can, less commonly occur in the peritoneal surfaces and rarely from the pericardium or the tunica vaginalis. It has a very poor prognosis with a reported median survival time of between seven to 11 months after diagnosis [452].

4.1 Aetiology And Pathogenesis Of Malignant Mesothelioma

Malignant mesothelioma is considered to be one of the most aggressive tumours which is not only treatment-resistant, but its frequency is on the rise throughout the world. The main risk factor associated with malignant mesothelioma is the exposure to asbestos and is probably the most famous and commonly known cause [453, 454]. However, other risk factors, such as exposure to the simian virus 40 has also recently been suggested to be an important risk factor [455, 456]. In addition to malignant mesotheliomas as well as atypical mesothelial proliferations and superficial non-invasive lesions of the mesothelium, the simian virus 40 DNA sequences have also been shown to be present in the brain tumours, bone tumours, and lymphomas [457]. The simian virus 40 is thought to belong to the polyoma group of viruses, which are small double-stranded DNA viruses, and block the tumour suppressor genes in human and rodent cells, making it a potent oncogenic virus [459, 458].

One of the other risk factors associated with mesothelioma is the exposure to ionising radiation. A recent study [460] of approximately 78,000 patients with non-Hodgkin’s
Lymphoma concluded that there was an increased risk of mesothelioma in the survivors of this population of patients and that this increased risk was limited only to those patients who received radiation therapy. This was confirmed by a later study of Teta et al [461].

Malignant mesothelioma is currently reported to be as common and prevalent as liver, bone, and bladder cancers in Australian and Europe [456, 453]. Recent reports from the World Health Organisation [462, 454] have shown rates of malignant mesothelioma to demonstrate large differences by sex and region or country. These reports have indicated that, in all countries and regions, the rates of males with malignant mesothelioma is higher than that of the females with the industrialised countries showing higher rates than the non-industrial countries [454]. This would probably indicate past production and use of asbestos in the industry.

4.1.1 Pathogenesis of Malignant Mesothelioma

As previously mentioned, currently the main cause of malignant mesothelioma has been associated with exposure to asbestos. This is especially true in Australia [453]. The highest annual crude incidence rates (approximately 30 cases per million) are observed in Australia, Belgium, and Great Britain [463]. In addition, the malignant mesothelioma of all patients recruited for the thalidomide clinical trial was diagnosed to be caused by asbestos. It will be, therefore, appropriate and important to concentrate on the pathogenesis of malignant mesothelioma with asbestos being the primary cause. Also, the mechanism of action of asbestos and pathogenesis of malignant mesothelioma is ultimately important in its diagnosis and treatment.

In the normal healthy lung, the entire surface of the pleura is covered with a single layer of mesothelial cells which help free movement of pleural surfaces during respiration. This process is carried out by enmeshing lubricating glycoproteins. The cells are capable of readily
proliferating in response to injury and growth factors \cite{465, 464}. There are, however, a number of different methods or pathways by which asbestos and asbestos-like fibres can cause malignant mesothelioma.

The simplest and most straightforward mechanism is the irritation of pleural surfaces by these fibres. Earlier studies \cite{466, 468, 467} have demonstrated that the most important physical attribute that determines, when inhaled, how far and deeply the fibres penetrate into the lung is the fibre’s shape and length-to-width ratio. These attributes also determine whether the fibre has the capacity to penetrate the lung epithelium and enter or irritate the pleural space \cite{466, 468, 467}. Needless to say the most dangerous of these fibres are the ones which are long and thin and can easily penetrate the lung, repeatedly scratching the mesothelial surface and, therefore, causing prolonged cycles of damage, repair, and local inflammation. This repeated process can result in the formation of plaques by causing scarring or cause mesothelioma.

Another mechanism by which asbestos fibres can cause mesothelioma is by physically severing or piercing the mitotic spindle and, therefore, disrupting mitosis. This mechanism has been long known to produce aneuploidy and the other forms of chromosomal damage that characterises malignant mesothelioma \cite{469}.

One other mechanism of action of asbestos that has been suggested to cause mesothelioma is the production of toxic oxygen radicals which eventually leads to DNA damage and strand breaks. Earlier studies of Koerten et al \cite{470, 471}, Shatos et al \cite{472, 473}, and Weitzman et al \cite{474} carried out in the 1980s and early 1990s have demonstrated that the actual asbestos-induced cell death is mediated by iron-related reactive oxygen species. These reactive oxygen species then, in turn, produce cell death by damaging and breaking DNA strands within the cell.

Growth factors have also been shown to be involved and play a significant part in malignant mesothelioma. Reports of Manning et al \cite{475, 476} and Zanella et al \cite{478, 477} have suggested that the asbestos fibres phosphorylate the mitogen-activated protein kinases and extracellular
Chapter 4: MALIGNANT MESOTHELIOMA

signal-regulated kinase I and kinase II as well as increasing the expression of early response proto-oncogenes in mesothelial cells. Blocking the transforming growth factor β and platelet-derived growth factor α chain has been shown to stop the growth of mesothelioma [479]. The latency periods between first exposure to asbestos and development of mesothelioma are usually longer than 40 years [463]. This latency period between the initial asbestos exposure and death has been reported by Bianchi et al [480] to be as much as 72 years with a wide variability associated with occupational group, type of asbestos fibres and the intensity of exposure to these asbestos fibres. An inverse relationship exists between intensity of asbestos exposure and length of the latency period. Mesothelioma generally develops after long-time exposures to asbestos [463]. It is interesting to note that reports of the early 1980s indicate that although approximately 80% of patients with malignant mesothelioma have some history of exposure to asbestos, only approximately 10% of these patients acquire mesothelioma [481]. This probably indicates that other factors and/or mediators may be involved, either independently or as co-factors, in the development of this malignancy [482]. One of these factors probably is genetic susceptibility. An interesting epidemiological study carried out in the early 2000s in Turkey using genetic epidemiology maps of two villages tested the hypothesis of whether some villagers were genetically predisposed to mesothelioma [483]. This study analysed six-generation extended pedigree of over 500 individuals and demonstrated that mesothelioma was in fact genetically transmitted, probably in an autosomal dominant way [483].

4.1.2 Clinical Presentation of Malignant Mesothelioma

As one would expect a typical patient with malignant mesothelioma is presented with pleural effusion which is often associated with chest wall pain. Most common signs are often those
typical of a pleural effusion. Other symptoms such as weight loss, fatigue, and cancer-induced cachexia will appear later in the course of the disease. It is also generally considered that the presence on these latter symptoms earlier in disease and at diagnosis is associated with poor prognosis \cite{484}. Some cases of malignant mesothelioma show no symptoms and are discovered and diagnosed during a routine radiological “check-up”. However, these are not very common. The average time between the onset of symptoms and the mesothelioma diagnosis have been reported to be between two to three months \cite{456}.

Peritoneal mesothelioma often produces symptoms such as abdominal distension, pain, and sometimes bowel obstruction \cite{462}. In these patients, the more common signs include ascites, tenderness, and palpable masses which occur later in the course of their disease \cite{462}. Subcutaneous masses are usually seen in patients who have had surgical intervention and are almost always associated with operative procedures or the insertion of intercostal drainage tubes \cite{484}. Subcutaneous masses are, generally, rare in the absence of surgical procedures in these patients.

With the mesothelioma of the pericardium or the tunica vaginalis the presenting symptoms tend to be pericardial effusions or tamponade, or blood-stained hydrocoele, respectively \cite{485,486}.

Metastases do occur in patients with malignant mesothelioma. However, these are generally detected during the post-mortem investigations. The most common metastasis site in patients with malignant mesothelioma are the hilar mediastinal, internal mammary, and supraclavicular lymph nodes \cite{484}. The metastases can also affect major organs such as the bone or miliary spread. Local invasion also involves other contiguous organs such as the spinal cord, pericardium, and the contralateral lung \cite{486}. The local invasion to the spinal cord usually results in back pain and to the pericardium results in pericardial effusions and tamponade.
4.2 Diagnosis Of Malignant Mesothelioma

Mesothelioma is usually diagnosed between the fifth and seventh decade of life and is more predominant in men and, generally, exposure to asbestos is also involved\(^{[487, 489, 488]}\). In the United States, there are approximately 2000 cases of mesothelioma in men and 500 in women reported annually, and the incidence rate is on the increase\(^{[488]}\).

A variety of methods are currently available for the diagnosis of malignant mesothelioma. As with all diseases, an accurate and early diagnosis increases the chance of cure and increased survival. For malignant mesothelioma, the main difficulty in diagnosis is distinguishing mesothelioma from adenocarcinoma, especially when the tumour has invaded the pleura\(^{[490]}\).

4.2.1 Cytology And Histopathology

Generally, fluid from a serous effusion is analysed in the laboratory with electron microscope. For pulmonary, chest wall, and lymph node masses fine needle aspiration cytology is performed. If there substantial pleural-based lesions are present, thin-core biopsy samples are used to aid diagnosis. This method has been known to be well tolerated by the patients as well as providing enough tissue sample for electron microscopy as well as histopathology.

The histopathological techniques are used when the cytological investigations of the pleural effusions fail to distinguish mesothelioma from adenocarcinoma. However, this may also prove to be unsuccessful as mesothelioma cells look similar to adenocarcinoma cells both macroscopically as well as microscopically. However, it has been reported that in experienced hands, cytology alone enables a confident diagnosis to be made in 80% of cases\(^{[456]}\).
Immunohistochemical techniques are useful in finding the origin of the tissue samples obtained from the patients. These techniques can determine whether the tissue is of mesothelial origin and whether it is malignant mesothelioma. Cytokeratin stains have been shown and used to confirm invasion and distinguishing mesothelioma from sarcomas and melanomas [490].

### 4.2.2 Imaging Modalities In Malignant Mesothelioma

Initial chest x-rays carried out on patients with mesothelioma can show a pleural effusion or a pleural-based mass. In addition, it can be used to determine the presence of diffuse lobular masses and calcified plaques. CT scans also show pleural effusions or pleural-based masses with or without thickening of the interlobular septae at presentation [456]. In peritoneal mesothelioma, CT scans only show ascites and scattered mesothelioma masses [456].

The use of PET scanning has been on the increase in the last few years. In malignant mesothelioma PET scanning has been used as an adjunct to diagnosis. PET images of the lungs have demonstrated avid uptake suggesting malignant disease [491]. It has been reported that PET has proven to be useful in the detection and identification of extrathoracic disease especially lymph node involvement. These are especially useful when considering the fact that hypermetabolic lymph node involvement is often evident when the lymph nodes appear normal on a CT scan image [492, 493].

### 4.2.3 Serum Markers In Malignant Mesothelioma

Unfortunately, there have not been many studies or reports in the literature investigating different serum markers that can be used successfully in the diagnosis of mesothelioma.
Similarly, there have not been any reliable serum markers available commercially for the diagnosis of mesothelioma.

However, recent reports suggest that a serum mesothelin-related protein may be utilised as a marker of mesothelioma in its diagnosis [494, 496, 495, 497]. The serum mesothelin-related protein is the circulating product of mesothelin. The mesothelin itself is a surface protein which is thought to be important in the mesothelial cell adhesion and signalling [494, 496, 495, 497]. The studies of Creaney et al [494, 496, 495] have suggested that the measurement of mesothelin concentrations in the pleural and/or peritoneal effusion of patients may aid in the differential diagnosis of mesothelioma in patients presenting with effusions. The fact that the changes in serum mesothelin-related protein levels parallel clinical course and tumour size and serum mesothelin-related protein is elevated in 75% of patients at diagnosis [497], the serum mesothelin-related protein has been suggested to be a useful tool for the monitoring disease progression, and may also prove to be useful for screening asbestos-exposed individuals for early malignant mesothelioma [497]. The serum mesothelin-related protein has been reported [497] to have a sensitivity of 84% and a specificity of close to 100% compared with other lung tumours or pleural diseases and a specificity of greater than 80% compared with individuals who have been exposed to asbestos.

4.2.4 Staging Of Malignant Mesothelioma

Mesothelioma itself can be separated and classified into three types. These are epithelial, sarcomatoid, and mixed which is a combination of the epithelial and sarcomatoid. The epithelial type is probably more prevalent and can be further subdivided into tubular, papillary, large cell, small cell, and myxoid [482]. The sarcomatoid mesothelioma has a poorer prognosis than either the epithelial or the mixed type and is characterised pathologically by
The staging of mesothelioma is carried out using radiographic, surgical, or radiographic plus surgical techniques. The use of surgical techniques, however, provides the most accurate staging in a substantial number of patients with mesothelioma. The radiographic methods generally used to aid diagnosis and staging are mainly confined to CT scanning and/or PET techniques. Pet techniques are ideal for differentiating between benign and malignant mesothelioma. In addition, PET is more sensitive than other radiological modalities, such as CT, for investigating extrathoracic disease and spread. However, PET seems to have a limited sensitivity for locoregional staging and potential resectability. Earlier studies have shown MRI to be significantly better than CT scanning in the detection of diaphragmatic invasion of tumour as well as the invasion of endothoracic fascia or chest wall. Both techniques have been reported to be equally as accurate in diagnosing mediastinal node disease.

A number of different staging systems have been developed and utilised. A staging system was developed in the mid-1990s to provide a framework for the analysis of prospective clinical trials of new and emerging treatment modalities for mesothelioma. This staging system was later, in 2002, adopted by the American Joint Committee on Cancer for their new TNM staging system.
4.3 Prognosis And Treatment Of Malignant Mesothelioma

Prognosis of malignant mesothelioma at the time of diagnosis is usually poor. The median survival of patients with malignant mesothelioma is between six to 18 months depending on the initial stage at diagnosis and various other prognostic factors [452, 504]. Generally, this median survival period is rarely affected by the currently practiced therapeutic methods and techniques and it seems that the only patients that do benefit are the ones receiving aggressive multi-modality treatments [505, 506, 507].

4.3.1 Prognosis Of Malignant Mesothelioma

Probably the most useful prognostic scoring system for malignant mesothelioma has been developed by the CALGB [504] and EORTC [452] in the late 1990s. The CALGB report [504] suggested that in their group of patients tested, the subgroup with the best survival (mean survival time of 13.9 months) included patients with performance status of 0 and age less than 49 years, and patients with performance status of 0, age of greater than 49 years, and a haemoglobin levels of greater than or equal to 14.6. The worst survival (mean survival time of 1.4 months) occurred for patients with performance status of $\frac{1}{2}$ and a white blood count of greater than or equal to 15.6/µL [504].

The EORTC report [452] suggested similar factors to be the determinants of prognosis in malignant mesothelioma. This study reported that poor prognosis was associated with a poor performance status, a high white blood cell count, a probable/possible histologic diagnosis of mesothelioma, male gender, and having sarcomatous tissue as the histologic subtype. The study then took the above five factors into consideration and classified patients into two
groups of good-prognosis group (one-year survival rate of 40%) and a poor-prognosis group 
(one-year survival rate of 12%) \cite{452}.

It is generally accepted that \cite{452, 504, 509, 508} a relatively bad or poor prognosis at the time of 
first presentation or diagnosis is associated with the presence of thrombocytosis, leukocytosis, 
low haemoglobin levels, sarcomas, mixed histology, male gender, advanced age (between 65 
to 75 years of age), poor performance status, and the presence of atypical or persisting fever 
of unknown origin. However, patients who are considered to have a good prognosis are the 
one who, at the time of presentation or diagnosis, have epithelial histology, younger than 65 
years of age, stage I disease, a performance status of 0 or 1, and lack of chest pain \cite{452, 504, 509, 
508}.

As the disease progresses, the resulting morbidity and mortality is primarily due to the 
unstoppable and the inevitable local invasion. Patients with malignant mesothelioma 
generally develop shortness of breath and chest pain, both of which is due to the tumour 
gradually destroying the pleural spaces and replacing the pleural fluid.. The spreading tumour 
causes the blood to be shunted through the encased lung producing the typical symptoms of 
fatigue, dyspnea, and hypoxemia. When there are local invasion of crucial thoracic structures, 
symptoms such as dysphagia, hoarseness, brachial plexopathy, superior vena cava syndrome, 
cord compression, Horner’s syndrome, etc can occur \cite{510, 509, 508}. In addition, symptoms such 
as thrombocytosis, migratory thrombophlebitis, Coombs-positive haemolytic anaemia, 
hypoglycaemia, and disseminated intravascular coagulation as well as other para-neoplastic 
syndromes have been known and described \cite{510}.

In patients with malignant mesothelioma, death generally occurs as a result of local extension 
and respiratory failure \cite{510, 509, 508}. Here, the tumour growth below the diaphragm causes small 
bowel obstruction resulting in death \cite{510, 509, 508}. Tumour invasion of the heart or pericardium 
can also be fatal as a result of arrhythmias, heart failure, or stroke \cite{510, 509, 508}.


Chapter 4: MALIGNANT MESOTHELIOMA

Reports of Edwards et al [511, 512, 513, 514] since the early 2000s have investigated the role of different pathways that can involve the immune system and their effect on the prognosis of malignant mesothelioma. In their earlier report, Edwards et al [513] demonstrated that cyclooxygenase-2 expression is a prognostic factor in malignant mesothelioma and, therefore, it may be a potential therapeutic target in malignant mesothelioma. This is probably due to the fact that cyclooxygenase-2 plays an important role in solid tumour growth, its invasiveness, and angiogenesis, through the synthesis of prostaglandins such as prostaglandin E$_2$ [513].

Angiogenesis itself is a poor prognostic factor in malignant mesothelioma and is independent of other clinico-pathological variables and the EORTC prognostic scoring system [514]. In addition, it was later demonstrated that microscopic tumour necrosis was also correlated with angiogenesis and, therefore, a poor prognostic factor in malignant mesothelioma [512].

The earlier studies of Ohta et al [516, 515] on VEGF as a prognostic marker for malignant mesothelioma indicate an association between VEGF and vessel density suggesting that these factors play an important role in angiogenesis and lymphangiogenesis in this tumour, and assessment of vascularity may be a useful prognostic indicator in these patients. VEGF was also found to be expressed in the airways of patients with lung cancer suggesting a possible role of VEGF as a prognostic factor as well as a therapeutic target [516]. VEGF is also considered to be an autocrine growth factor for mesothelioma not only by promoting tumour angiogenesis but also by directly stimulating tumour growth [517]. In addition, it has long been known that patients with malignant mesothelioma have significantly higher VEGF levels than patients with other types of solid tumours [518].

In malignant mesothelioma, death is usually not as a result of metastatic disease but due to infection or respiratory failure as well as constitutional symptoms associated with progressive and advanced malignancy [482].
4.3.2 Treatment Of Malignant Mesothelioma

Depending on the location and stage of disease, treatment for malignant mesothelioma can be carried out either through localised treatment approaches or systemic treatment approaches. However, there are currently no available treatment procedure that can provide cure or significantly increase survival other than to provide palliation of symptoms of mesothelioma.

4.3.2.1 Localised Treatment Approaches

Majority of patients with malignant mesothelioma are not suitable candidates for radical surgery resection due to the unresectable, locally advanced nature of the disease or the presence of co-morbid medical illness \[482\]. However if patients are suitable, surgery still remains one of the localised treatment approaches that can be used in malignant mesothelioma to give any chance of increased survival. Surgical procedures can also be used for the purposes of palliation, diagnosis, and extremely rarely to cure. Although the majority of surgical procedures tend to be palliative, earlier studies carried out in the mid to late 1990s have reported that aggressive surgical approaches may benefit patients by increasing survival \[521, 520, 519, 522\]. However, less than a quarter of the patients who receive aggressive surgery will be alive at five years and even fewer will be disease-free \[521, 520, 519, 522\].

Other surgical techniques that have been carried out include pleurodesis and have been mainly used for the palliation of symptoms of pleural effusions. Pleurodesis involves the complete drainage ion the pleural effusions and the introduction of an irritative agent into the pleural space. This procedure relieves the persistent dyspnoea that is caused by large, unilateral pleural effusions. Thoracoscopy has also been used as a diagnostic tool to obtain tissue samples as well as for pleurodesis to palliatively treat recurrent or symptomatic pleural effusions.
Surgical procedures, such as pleurectomy and extrapleural pneumonectomy, have been tried in an attempt to treat the primary tumour and cure. However, recent reports of Cameron [523] and Maziak et al [524] suggest that neither of these two surgical procedures is able to increase survival in patients with malignant mesothelioma.

Non-surgical localised treatment approaches include the use of radiation therapy. Mesothelioma is more responsive to radiation therapy than other types of lung cancer, such as small cell lung cancer. The dose of radiation usually used is determined by the fact that adjacent internal organs are sensitive to radiation. These include the heart, lung, oesophagus, liver, and the spinal cord. As a result, the treatment of the entire involved pleura may be difficult. However, it has long been experienced [525] that the radiation therapy, despite its limitations and difficulties, can provide and effective means of palliation in patients with malignant mesothelioma.

In addition to the use of radiation therapy as an effective palliation technique, it can also be used in an adjuvant setting. As such and since mesothelioma has been known to seed along the tracts of biopsies, thoracoscopy trocars, surgical incisions, and chest tubes, adjuvant radiation therapy can be used as an effective means of preventing recurrences in the chest wall.

Other therapeutic techniques that have also been used include multimodality approaches. Earlier studies of Sugarbaker et al [526, 505, 506] have demonstrated that extrapleural pneumonectomy followed by radiation therapy and adjuvant chemotherapy may be an effective treatment approach. From their latest study [526], these authors concluded that multimodality therapy including extrapleural pneumonectomy is a feasible approach in selected patients with malignant pleural mesotheliomas and that by pre-selecting extrapleural nodes one may better and more appropriately select patients for radical therapy [526]. In addition, microscopic resection margins affect long-term survival with patients with
epithelial, margin-negative, extrapleural node-negative resection generally have an extended survival [526].

Recent studies of Weder et al [529, 527, 528] and Opitz et al [530] have demonstrated significant improvements in outcome and survival of up to a median of 23 months in the neoadjuvant chemotherapy followed by surgery and radiation therapy approach. It was also suggested [530] that extrapleural pneumonectomy after neoadjuvant chemotherapy may be performed achieving mortality rates comparable to that of standard pneumonectomies with only frequent but manageable. In addition, the EORTC-score was shown to be a predictor for post-operative complications in their population of patients [530].

The biggest limitation of malignant mesothelioma that prevents the more common use of radiotherapy is the actual diffuse nature of the tumour which covers most of the pleural surfaces as well as the interlobular fissures. Needless to mention that in order to irradiate all the known disease areas as well as areas at high risk, radiation therapy usually requires to be irradiating a prohibitively large field as the entire pleural surface is at risk. Therefore, in addition to the lung parenchyma itself, other thoracic structures and organs are at risk and can be dose-limiting. Earlier retrospective studies carried out in the late 1980s have demonstrated that there may not be a clear survival benefit for extensive radiation therapy [531].

4.3.2.2 Systemic Treatment Approaches

Although there has been no chemotherapy regimens for mesothelioma that have been proven to be curative, majority of the available ones have been shown to be valuable for palliation purposes [456]. Both single agent and combination chemotherapy approaches have been used, but none have proved at any stage of the disease to be any more effective than simple palliation of symptoms.
Single Agent Approaches

As a single agent approach, the anthracyclines have been one of the first agents used for malignant mesothelioma. The earlier retrospective review of Lerner et al [532] in the early 1980s showed that doxorubicin can be considered as a very active single agent for first-line therapy of malignant mesothelioma producing a mean survival time of 30 months in the responding patients.

Platinum agents, such as cisplatin and carboplatin have also been used in the treatment of malignant mesothelioma. Berghmans et al’s [533] meta-analysis of all published phase II chemotherapy trials (up to 2002) for malignant mesothelioma demonstrated and concluded that cisplatin is the most active single agent that can be used for the chemotherapy of malignant mesothelioma. In fact cisplatin was also used in our clinical trial of patients with malignant mesothelioma receiving thalidomide. The meta-analysis also indicated that, although cisplatin is the most active agent, but it may not be superior to some combination chemotherapy regimens available for malignant mesothelioma [533].

Antifolates and antimetabolites have also been used as single agent chemotherapy for malignant mesothelioma. Examples include pemetrexed [535, 534], methotrexate [536], trimetrexate [537], edatrexate [538], raltirexed [539], and gemcitabine [540, 541].

Other single agents, such as vinca alkaloids, have been trialled and reported in the literature. However, they did not show as much promise and be as effective as the agents mentioned above in the treatment of malignant mesothelioma [542].

Combination Chemotherapeutic Approaches

Combination chemotherapy regimens are currently more favoured and show higher promise and effectiveness in the treatment of malignant mesothelioma. This superiority has been demonstrated for the combination of cisplatin plus pemetrexed and cisplatin plus raltitrexed
Premetrexed also has been combined with other platinum-based agents, such as carboplatin. Recent studies of Ceresoli et al. have demonstrated a median survival of 12.7 months and a median time to progression of 6.5 months with this combination regimen.

As combination chemotherapy regimen agents, cisplatin plus doxorubicin combination have shown to have one of the highest response rates. At present the general consensus in the literature, however, seems to be that the regimen of cisplatin / pemetrexed is the medical treatment of choice. The benefits of the combination of cisplatin plus raltitrexed versus cisplatin alone were demonstrated by the recent studies of van Meerbeeck et al. Bottomley et al. in international phase III trials confirming the fact that the combination of cisplatin plus an anti-folate is superior to that of cisplatin alone and has a positive effect on the health-related quality of life of patients with malignant mesothelioma.

Antifolates have also been combined with platinum agents and have been shown to be effective in mesothelioma. Reports of Fizazi et al. demonstrated that the combination of oxaliplatin plus raltitrexed is an active outpatient regimen in malignant mesothelioma with an acceptable tolerability profile. The most common adverse events that were reported were asthenia, nausea and vomiting, and paraesthesia, with no treatment-related deaths. Recent phase II study of Castagneto et al. on the combination of carboplatin and pemetrexed showed an median survival of 14 months. However, these researchers concluded that the combination of carboplatin and pemetrexed is only moderately active in malignant mesothelioma.

Other combinations that have been tested are the combination of gemcitabine plus a platinum agent. These platinum agents include oxaliplatin, cisplatin, and carboplatin. The combination of gemcitabine and cisplatin was used in our study. The oxaliplatin combination was demonstrated in a phase II trial to be active in malignant mesothelioma and to exhibit tolerable toxicity in an outpatient setting. With cisplatin, recently Castagneto et al.
showed that the gemcitabine plus cisplatin combination has an acceptable toxicity profile but it is only moderately active in malignant mesothelioma. The carboplatin plus gemcitabine combination is probably the better option amongst the platinum compounds. Study of Favaretto et al. [552] have demonstrated a median survival of 66 weeks with 53%, 30%, and 20% of patients being alive at 1, 2, and 3 years, respectively. In addition, the median progression-free survival period was demonstrated to be 40 weeks, indicating acceptable toxicity profile, good response rate, and the clinical benefit to patients [552].

There have also been other combinations of the above agents tested and reported in the literature. However, the majority have been shown to exhibit similar efficacy and/or toxicity profiles to the above combinations. There are, however, novel agents that have been suggested and reported in the literature. Amongst these agents are the anti-angiogenic agents. A recent review of Dowell et al. [553] has indicated that anti-angiogenic therapy may be effective in the treatment of malignant mesothelioma. An example of an anti-angiogenic agent is thalidomide which was used in our clinical trial of patients with malignant mesothelioma.

Other agents that have been tested and reported are the growth factors. One of these factors, the EGFR, has been shown to be over expressed in patients with malignant mesothelioma [555, 554]. However, the studies reported in the literature indicate that despite the fact that 97% of patients with mesothelioma had EGFR over expression, neither gefitinib [554] nor single-agent erlotinib [555] was active in malignant mesothelioma. In addition, the EGFR expression was demonstrated not correlate with failure-free survival [554]. However, recent reports of Edwards et al. [511] indicates that although EGFR status is not an independent prognostic factor, the EGFR expression in malignant mesothelioma correlates with epithelioid histology and tumour necrosis.

Gene therapy, immunotherapy, chemo-immunotherapy, new cytostatic agents, vaccines, and other novel agents and treatment approaches have been tested and reported in the literature.
However, these agents are yet to be fully tested in human and to enter the mainstream and/or routine therapeutic regimens. These agents have been shown to have varying degrees of efficacy, effectiveness, and toxicity profiles. A comprehensive review of these agents have been reported by Nowak et al [556].

4.3.3 Palliation For Malignant Mesothelioma

Current reports in the literature suggest that palliation of symptoms is the primary goal of most therapeutic procedures in malignant mesothelioma and that these therapies mainly focus on the two symptoms of chest wall pain and dyspnoea [482]. The general palliation procedures and other needs of patients with malignant mesothelioma are probably similar to the ones of patients with other types of advanced cancer, such as the ones in pancreatic cancer. In malignant mesothelioma, there is a need to address the patients’ psychosocial problems, their physical pain, dyspnoea and breathing problems, and the presence of anorexia, cachexia, and weight loss.

The psychosocial problems are, generally, addressed by appropriate counselling and the involvement of trained clinical psychologists. The psychosocial problems are, generally, in common with that of other advanced cancers, such as pancreatic cancer. These are associated with the presence of the disease and its therapy and include anger, fear and anxiety.

The presence of pain is one of the main hallmarks of malignant mesothelioma and is one of the main causes of fear, stress, and anxiety in this population of patients. The pain in malignant mesothelioma tends to be diffuse visceral pain. In the presence of chest wall invasion and intercostal nerve involvement, there is the additional somatic and neuropathic pain as well. NSAIDs, such as naproxen, are usually used for somatic pains, whereas anticonvulsants, such as carbamazepine or sodium valproate, are used for the neuropathic
pain. Opioid analgesics and regional nerve block are used for higher degrees of pain. There is no upper limit of dose for opioid to control pain in mesothelioma\(^{[456]}\). Radiation therapy procedures with doses of 4000 rad (or above) in 4 weeks have long been known and have been demonstrated to be beneficial in the reduction of pain and symptoms of dyspnoea\(^{[557]}\). The palliation of breathing difficulties and dyspnoea are carried out by addressing the underlying cause. These problems are usually caused by the increasing presence of effusions or the spread of the tumour. In general, effusions are managed by drainage and talc pleuradesis. However, chemotherapy and localised treatment procedures themselves have significant effect on the reduction of dyspnoea and breathing difficulties.

The final issue that requires careful attention in patients with mesothelioma is the presence of anorexia, cachexia, and uncontrolled weight loss. These conditions are treated the same way as with the patients with pancreatic cancer. The procedures and therapies are reviewed in detail in the Cancer Cachexia section (Chapter 2). However, in our malignant mesothelioma patient population thalidomide, which has both anti-cachectic and anti-angiogenic properties, was used as the trial agent to treat the anorexia, cachexia, and uncontrolled weight loss.
Chapter 5: EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

5. EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

A fatty acid is a carboxylic acid often with a long unbranched aliphatic tail, which is either saturated or unsaturated. Carboxylic acids as short as butyric acid are considered to be fatty acids, whereas fatty acids derived from natural fats and oils may be assumed to have at least eight carbon atoms, e.g. caprylic acid (or octanoic acid).

The fatty acids are themselves divided into two groups of saturated fatty acids and unsaturated fatty acids which are described in section 5.1 below. In addition there are also essential fatty acids, free fatty acids, and trans fatty acids.

5.1 Fatty Acids

Fatty acids consist of the elements carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end. Saturated fatty acids have all the hydrogen that the carbon atoms can hold, and therefore, have no double bonds between the carbons. Monounsaturated fatty acids have only one double bond whereas the polyunsaturated fatty acids have more than one double bond. Butyric acid is given as an example in Figure 6 below:

Figure 6. Butyric acid.
Butyric acid (or butanoic acid) is one of the saturated short-chain fatty acids responsible for the characteristic flavour of butter. This image is a detailed structural formula explicitly showing four bonds for every carbon atom and can also be represented as the equivalent line formulas:

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}
\]

or

\[
\text{CH}_3(\text{CH}_2)_2\text{COOH}
\]

The numbers at the beginning of the scientific names usually indicate the locations of the double bonds. By convention, the carbon of the carboxyl group is carbon number one. Greek numeric prefixes such as di-, tri-, tetra-, penta-, hexa-, etc., are then used as multipliers and to describe the length of carbon chains containing more than four atoms.

**5.2 Omega-3 And Omega-6 Fatty Acids**

Our project is mainly concerned with the omega-3 fatty acids (particularly EPA) and their use in cachexia in pancreatic cancer patients undergoing chemotherapy. Omega-3 (ω-3) and omega-6 (ω-6) fatty acids are unsaturated essential fatty acids which are also known as n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids, respectively. These fatty acids use the Greek alphabet (α, β, χ, δ, … ω) to identify the location of the double bonds. The alpha (α) carbon is the carbon closest to the carboxyl group (carbon number two), whereas the omega (ω) is the last carbon of the chain since omega is the last letter of the Greek alphabet. Therefore, linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the omega (ω) carbon. Similarly, alpha-linolenic acid is an omega-3 fatty acid.
because it has a double bond three carbons away from the omega (ω) carbon. These are demonstrated in Figure 7 and Figure 8 below.

In order to obtain their classifications, the highest double-bond located in the scientific name is subtracted from the number of carbons in the fatty acid. As an example, for arachidonic acid we subtract 14 from 20 to obtain six. Therefore, arachidonic acid is an omega-6 fatty acid. This type of terminology is often applied to oleic acid which is an omega-9 fatty acid.

Figure 7. Example of an omega-3 fatty acid.

```
\[ \text{Alpha-Linolenic Acid (omega-3)} \]
```

Figure 8. Example of an omega-6 fatty acid.

```
\[ \text{Linoleic Acid (omega-6)} \]
```

It should be noted that in the above simplified structural formulas of unsaturated fatty acids, each angle represents a carbon atom and that all the double bonds have the Cis configuration.

In summary, the parent fatty acid of the omega-6 fatty acids is the linoleic acid. The linoleic acid is desaturated in vivo to produce arachidonic acid, which itself is an important mediator in intracellular and extracellular signalling. However, the parent fatty acid of the omega-3 fatty acids is the alpha-linolenic acid.

### 5.3 Eicosapentaenoic Acid

Eicosapentaenoic acid (EPA) is an essential omega-3 fatty acid and a cyclooxygenase inhibitor that can be distinguished from other polyunsaturated fatty acids by the presence of a
double bond that is located three carbon atoms from the N-terminal of the molecule\textsuperscript{[558]}. It has the chemical structure CH\textsubscript{3}CH\textsubscript{2}(CH=CHCH\textsubscript{2})\textsubscript{5}(CH\textsubscript{2})\textsubscript{2}COOH (also see Figure 9 below). In general, omega-3 polyunsaturated fatty acids are ingested as a part of normal diet through the consumption of cold water fish, such as salmon, sardines, mackerel, etc with EPA being the major omega-3 fatty acid component. It is also the compound used in our clinical trial investigating changes in body composition and cachexia in pancreatic cancer patients undergoing chemotherapy.

\textbf{Figure 9. Structure of eicosapentaenoic acid (EPA)}

\begin{center}
\includegraphics[width=0.5\textwidth]{Figure9.png}
\end{center}

\textbf{Figure 10. Structure of docosahexaenoic acid (DHA).}

\begin{center}
\includegraphics[width=0.5\textwidth]{Figure10.png}
\end{center}

\textbf{5.3.1 Pharmacology And Mechanism Of Action}

Generally, the synthesis of lipid mediators, such as the prostaglandins, thromboxanes, leukotrienes, lipoxins (i.e. the eicosanoids) is carried out via the cyclooxygenase, lipooxygenase, and cytochrome P450 pathways. These in turn have an impact on the secretion of immune-regulatory cytokines, secondary mediators, or the proteases and autocrine eicosanoid regulation loops. It has been shown that the eicosanoids modulate and control both
the pro-inflammatory as well as the anti-inflammatory effectors that are associated with inflammation [559]. Similarly, it has been shown that mediators such as the prostaglandins and thromboxanes modulate the vasomotor tone and regulate the blood flow in various vessel beds [560]. Therefore, in order to modulate the inflammatory response using the omega-3 fatty acids, the metabolic pathways that use EPA instead of the omega-6 fatty acid arachidonic acid have to be targeted. In humans, the immune response involves lipid mediators which are based on both EPA and arachidonic acid with the ones formed from EPA having a less pro-inflammatory impact on the immune system [561].

### 5.3.1.1 Mechanisms of Action

At the cellular level the mechanism of action of EPA has been suggested to be its incorporation into the cellular membranes [563, 562]. This incorporation into the cellular membranes alters the fatty acid composition as well as the biochemical activity within the cellular membranes which, in turn, leads to changes in the eicosanoid intermediates and the pro-inflammatory cytokines produced by the cell membrane from arachidonic acid [563, 562, 564]. The available evidence also suggests that both in the in vivo [565] [566] and in vitro [563, 562, 564] settings, one mechanism of action of EPA in the reduction of pro-inflammatory cytokines is through the substitution of EPA for arachidonic acid.

The omega-3 and omega-6 polyunsaturated fatty acids alter the protein-lipid-interactions and lipid-based signal transduction pathways [567]. The receptor-operated signalling is based on the cell membrane and second-messenger pathways in cells, many of which are bound to lipids or are sensitive to modulation by omega-3 or omega-6 lipids [567]. Recent studies of Denys et al [568] has demonstrated that there is a phosphatidylinositol-specific phospholipase-C-dependent simultaneous generation of inositol phosphates and diacylglycerol yielding the secondary release of intracellular calcium and activation of protein kinase-C. Examples of important
lipid-sensitive pathways which relate lipids to the generations of oxygen radicals, cell motility, control of organ perfusion, reaction to ischaemia and injury, and apoptosis include the activation of protein kinase B which is downstream of phosphatidylinositol kinase-3-related signalling [567, 569].

Mayer et al [567] have suggested that both EPA and DHA directly as well as indirectly modulate gene expression and that their effects are mediated by their ability to bind to positive or negative regulatory transcription factors. Their indirect effects, however, are mediated through alterations in the generation of intracellular second messengers, such as the diacylglycerol and ceramide [567]. Recent studies of Sweeney et al [570] and Stulnig et al [571] have suggested that the polyunsaturated fatty acids can directly interact with the nuclear receptor proteins, such as the peroxisome proliferators-activated receptor, which when activated mediates the genes responsible for β-oxidation as well as affecting the NF-κB signalling. It has also been shown that there is another mechanism by which the omega-3 polyunsaturated fatty acids can affect the intracellular transduction pathway. Mayer et al [572] have recently reported that the omega-3 polyunsaturated fatty acids can also reduce the generation of platelet-activating factor in leukocytes and endothelial cells which is necessary for the promotion of leukocytes from rolling to firm adhesion. This is in addition to the reduction of leukocyte adhesion to endothelial cells through the reduction of inflammation-induced up-regulation of endothelial adhesion molecules by omega-3 polyunsaturated fatty acids [567].

It has been well established that there are a range of different pro-inflammatory cytokines that are responsible for many of the characteristic features seen in cachexia and that the cytokine axis by itself can activate the neuroendocrine response (see section on Cachexia (Chapter 2). In general, the polyunsaturated fatty acids have long been reported to have a variety of different biological effects at the tissue, cellular, as well as molecular levels [574, 575, 573]. It has
been reported that fatty acids of the omega-3 series are rapidly incorporated into cell membranes and profoundly influence biological responses. These lipids influence membrane stability, membrane fluidity, cell mobility, the formation of receptors, binding of ligands to their receptors, activation of intracellular signalling pathways either directly or through the formation of eicosanoids, gene expression, and cell differentiation \(^{[576]}\). Reports in the literature \(^{[15]}\) indicate that there are three main sites of action of polyunsaturated fatty acids with the cells:

- Effects of polyunsaturated fatty acids on membrane fluidity and composition;
- Interactions of polyunsaturated fatty acids with eicosanoid synthetic pathways; and
- Modulation of signalling pathways by the polyunsaturated fatty acids.

As polyunsaturated fatty acids are lipid-soluble, they can easily penetrate the cell’s plasma membrane and, therefore, alter its fluidity and lipid dynamics. Studies of Escudero et al \(^{[577]}\) carried out in the late 1990s have demonstrated that the dietary intake of omega-3, omega-6, and omega-9 polyunsaturated fatty acids can alter the membrane content and morphology of erythrocytes. Similar effects have also been reported on the endothelial cells’ surface molecules \(^{[578]}\) and the cell surface molecules of the immune system cells \(^{[575]}\) which has led to Ross et al \(^{[15]}\) to speculate that the change in membrane content and morphology may influence the activity of different receptors, transporters, and enzymes embedded in the cell membrane which play important roles in cellular signalling. For example, the stimulation of membrane receptors can activate G-proteins, ion channels, etc. These signals can be amplified through different routes and mechanisms, such as adenylate cyclase or a phospholipase, and the secondary messenger products can subsequently influence the actions of cAMP-dependent protein kinase and protein kinase C \(^{[15]}\). The omega-3 fatty acids have been reported to also influence the effects of adenylate cyclase, cAMP-dependent protein kinase, protein kinase C, and phospholipase A2 \(^{[15, 573]}\). In addition, the omega-3 fatty acids have been reported to be
able to bind to the cytoplasmic glucocorticoid receptor at a site which is different to that of
the hormone binding site causing a marked reduction in the affinity of the receptor for the
hormone [15, 573]. Studies of Kang et al. [579] carried out in the mid-1990s also provide evidence
that the binding of EPA to the membrane voltage-sensitive channels may be able to change
the conductance by directly binding to the channel proteins. This binding of the EPA to this
site on the sodium channel is reported to be reversible and structure-specific [579]. Studies of
Nair et al. [580] on cardiac cells have also demonstrated that, since membrane phospholipids
control the ion transfer across the membrane, then the fatty acid composition of membrane
phospholipids may also influence the properties of the calcium channels.

The second site of action of polyunsaturated fatty acids with the cells is the interactions of
polyunsaturated fatty acids with eicosanoid synthetic pathways. It has been well known and
established that during inflammatory response, inflammatory diseases, and cachexia both
prostaglandins and leukotrienes play important roles in the production of cytokines, including
the catabolic cytokines (see section on Cachexia (Chapter 2)).

The third site of action of polyunsaturated fatty acids with the cells is the modulation of
signalling pathways. Studies of Jolly et al. [581] have demonstrated that low dose, short term
dietary exposure to highly purified EPA or DHA suppresses mitogen-induced T-lymphocyte
proliferation by inhibiting the IL-2 secretion, and these events are accompanied by a
reductions in the production of essential lipid second messengers, diacylglycerol and
ceramide. Also, the studies of Sanderson et al. [582] have demonstrated that fish oil feeding
appears to result in inhibition of one or more tyrosine kinases. The polyunsaturated fatty acids
have also been suggested to be able to modulate transcriptional regulatory mechanisms such
as the peroxisome proliferators-activated receptors, NF-κB, and the sterol regulatory element
binding proteins [15]. Studies of Aoyama et al. [583] carried out in the late-1990s have shown
that the peroxisome proliferators-activated receptor-α is mainly expressed in the tissues where
there are high rates of fatty acid catabolism, such as that of the liver, kidneys, muscles, and the heart. In addition, it has been shown that the peroxisome proliferators-activated receptor-\(\alpha\) induces the breakdown of leukotrienes and, therefore, limits the duration and extent of inflammatory response \([583]\) and that a variety of polyunsaturated fatty acids including EPA increase the activities of the peroxisome proliferators-activated receptor-\(\alpha\) \([584]\). The peroxisome proliferators-activated receptors have also been shown to inhibit the transcriptional activities of the NF-\(\kappa B\) \([585]\) which may provide evidence for the reduction in cytokine production effects of the omega-3 polyunsaturated fatty acids \([573, 576, 586]\). In addition, the NF-\(\kappa B\) itself has been suggested to be a possible target for the polyunsaturated fatty acids since the omega-3 polyunsaturated fatty acids have been shown to have effects on cytokine production and adhesion molecule expression in immune and endothelial cells as well as effects on tumour cell growth \([15]\).

5.3.1.2 Pharmacology

It is known that the major polyunsaturated fatty acids incorporated in human cell membranes are EPA, DHA, arachidonic acid, and oleic acid, with the EPA, DHA, and \(\gamma\)-linolenic acid being the major substrates for eicosanoids \([587]\). It has been reported in the literature that the linoleic acid and the alpha-linolenic acid compete for desaturation and chain elongation and that a proper balance between these two are required to produce an optimum and correct levels of omega-3 and omega-6 content of cell membranes \([588]\). A study of Barber et al \([251]\) on the effects of a fish oil-enriched nutritional supplement on the metabolic mediators of cachexia demonstrated a reversal of weight loss. The study concluded that the circulating concentrations of leptin were not affected by the treatment and that the reversal of weight loss is associated with a significant decrease in IL-6 produced by the peripheral blood
mononuclear cells, an increase in serum insulin concentrations, a decrease in the cortisol to insulin ratios, and a decrease in the proportions on patients excreting proteolysis-inducing factor \[251\]. Considering the fact that proteolysis-inducing factor muscle protein breakdown is inhibited by EPA \[589\], then it can be suggested that EPA will not only inhibit the end-organ effects of proteolysis-inducing factor but also reduce its production \[251\].

It has been known that the levels of both IL-6 and TNF are increased in weight losing patients with advanced pancreatic cancer \[250, 132\] (also see section on Cachexia (Chapter 2)). Further evidence for the interactions of polyunsaturated fatty acids with eicosanoid synthetic pathways comes from the studies of Endres et al \[565\], Meydani et al \[566\], and Wigmore et al \[250\] who all demonstrated, following EPA and fish oil administration, a reduction in the production of IL-1, IL-6, and TNF from the peripheral blood mononuclear cells isolated from healthy volunteers as well as patients with pancreatic cancer.

The 20-carbon polyunsaturated fatty acids are metabolised by cyclooxygenase into prostanoids, prostaglandins, and thromboxanes. Similarly, the 20-carbon polyunsaturated fatty acids can also be metabolised into leukotrienes by 5-lipooxygenase enzyme. The arachidonic acid which is an omega-6 20-carbon polyunsaturated fatty acid is the major precursor for the eicosanoids produces the 2-series prostanoids and the 4-series leukotrienes. Some examples of the 2-series prostanoids include thromboxane A\(_2\), prostaglandin E\(_2\), and prostaglandin I\(_2\). Some examples of the 4-series leukotrienes include leukotriene B\(_4\) and leukotriene C\(_4\). These enzymes also metabolise EPA which is an omega-3 polyunsaturated fatty acid. Here, the cyclooxygenase enzyme metabolises EPA into 3-series prostanoids and the 5-lipooxygenase enzyme into 5-series leukotrienes. The different series of prostaglandins have been known to have various different physiological as well as pathophysiological effects on many immune and non-immune systems \[15\]. The leukotrienes play important roles in modulating the inflammatory response with the 5-series leukotrienes being less active than and competing
with the 4-series leukotrienes for binding site, thus resulting in anti-inflammatory effects \[15\].

From the review of Alexander \[576\] one it can be concluded that one of the mechanisms by which omega-3 polyunsaturated fatty acids modulate catabolism is through the reduction in inflammatory response.

A recent review of Tisdale \[590\] indicated that apoptosis is an important factor in the loss of muscle protein in cachexia. The review also suggests that the induction of the proteasome expression by glucocorticosteroids may be due to the down regulation of the NK-κB activity while the proteolysis-inducing factor acts through 15-hydroxyeicosatetraenoic acid as an intracellular transducer and that the formation of 15-hydroxyeicosatetraenoic acid is inhibited by EPA, which has been shown to reduce the rate of weight loss in patients with pancreatic cancer \[590\]. Since EPA also prevents protein catabolism and activates the ubiquitin-proteasome proteolytic pathway, Tisdale \[590\] also suggested that a similar pathway may be involved and that EPA may, therefore, be effective in the treatment of protein catabolism in disease conditions other than cancer \[590\].

In a recent large multicentre study \[247\] it was demonstrated that there is a linear relationship between change in LBM and the enrichment of plasma phospholipids with EPA suggesting that the higher the intake of omega-3 polyunsaturated fatty acids, the higher the protein accumulation in these patients. In addition, it was shown that there is a synergistic effect when protein is consumed in the presence of omega-3 polyunsaturated fatty acids with only those patients with a higher protein intake in the presence of the omega-3 polyunsaturated fatty acids demonstrating a positive weight change \[247\].

Probably one of the first reports in the literature providing evidence for the anti-catabolic properties of omega-3 polyunsaturated fatty acids comes from the studies of Endres et al \[565\] and Caughey et al \[591\]. Further evidence of benefits of EPA in catabolic diseases comes from other studies reported in the literature which has been reviewed in detail in the section on
EPA’s therapeutic uses (section 5.3.2) below. Studies of Tisdale et al [589] on the transplantable mouse colon adenocarcinoma model has demonstrated that oral EPA administration results in a reduction in tumour growth as well as termination of weight loss and the preservation of body fat and muscle. It has been demonstrated that EPA’s anti-tumour but not anti-cachectic effects were suppressed by linoleic acid administration, suggesting that EPA may have two separate functionalities [589]. This anti-tumour ability of EPA has been confirmed by the study of Wallace et al [592].

The modulation of the acute-phase protein response is another method of action of EPA important cachexia. The study of Barber et al [252] on patients with advanced pancreatic cancer and cachexia receiving 2 g of EPA and 1 g of DHA per day demonstrated a median weight gain of 2.5 kg which was shown not to be caused by simple fluid retention. This study also showed that in the placebo group, not only there was a significant weight loss, but there was also a significant increase in the serum concentration of CRP [252].

It has been shown that in patients with cancer, the hepatic acute-phase response is associated with symptoms of cachexia, such as increase in the metabolic rate and weight loss as well as poor survival [160, 157, 132]. This has been suggested to be due to an increase demand for amino acids [593]. Recent trial of Barber et al [593] has demonstrated that the administration of an oral nutritional supplement containing high concentrations of omega-3 fatty acids (ProSure®) is able to modulate the hepatic export protein synthesis resulting in a net whole-body anabolism in patients with cancer cachexia. This weight gain observed in the previously weight-losing patients was shown to be mainly lean body mass [593].

The primary action of EPA seems to be in the suppression of the well-characterised mediators of cancer-associated wasting [586]. The principal mechanisms of action of omega-3 polyunsaturated fatty acids in preventing LBM wasting in cancer, in short, has been suggested to be: a) suppression of the ubiquitin-proteasome system; b) suppression of the inflammatory
cytokines; and c) the suppression of cancer cachectic factors \cite{189}. It has been shown that the potential of lipopolysaccharide-stimulated peripheral blood mononuclear cell supernatants to stimulate C-reactive protein production by hepatocytes could be attenuated by neutralising anti-IL-6 antibody in control subjects and in patients before, but not after, treatment with EPA suggesting that EPA is capable of down regulating the acute-phase response by suppression of IL-6 production \cite{586}. Studies of Smith et al \cite{85} has demonstrated that EPA suppresses the proteolysis-inducing factor which is a well-described and well know mediator involved in cancer cachexia. In addition, studies of Baracos et al \cite{594} carried out in the mid-1990s, and more recently Whitehouse et al \cite{84}, demonstrated that EPA is also capable of controlling the ubiquitin-proteasome system which is the pathway believed to be responsible for the bulk of muscle wasting in cancer cachexia. The proteasome was shown to become non-functional after EPA treatment \cite{84}.

In a tumour model (MAC16) it has been shown that EPA treatment can significantly reduce the ubiquitin protein ligase E2 and E3 levels of mRNA in the skeletal muscles \cite{84}. EPA has also been shown to have attenuating effects on the protein degradation effects of the proteolysis-inducing factor by preventing the release of arachidonic acid from membrane phospholipids and its subsequent metabolism to eicosanoids \cite{85}. This has been considered to represent a signal transduction pathway resulting in the up-regulation of the ubiquitin-proteasome pathway \cite{85}. Omega-3 fatty acids’ anti-inflammatory effects have been shown to be as a result of the inhibition of the cyclooxygenase-2 enzyme \cite{595}. In addition, studies \cite{60,596,61} reported in the literature have indicated that omega-3 fatty acids can suppress pro-inflammatory cytokine levels, such as that of TNF-\(\alpha\), IL-6 and IL-1, which are produced by activated macrophages. Here, the study of Endres \cite{596} demonstrated that an inhibition of inflammatory cytokine synthesis can also be achieved by increasing the content of n-3 polyunsaturated fatty acids in leukocyte membranes. Also, the recent study of Trebble et al \cite{61}
showed that there was a positive dose-dependent relationship between dietary n-3 polyunsaturated fatty acid intake and EPA and DHA incorporation into plasma phosphatidylcholine and erythrocyte phosphatidylethanolamine, with a tendency towards a plateau at higher levels of intake. It was also shown that the production of TNF-α and IL-6 by peripheral blood mononuclear cells decreased with the increasing n-3 polyunsaturated fatty acid intake [61]. Both responses were shown to be amplified by antioxidant co-supplementation at intermediate levels of supplementary n-3 polyunsaturated fatty acid intakes [61].

Recent studies of Novak et al [89] on NF-κB have demonstrated that omega-3 fatty acids are capable of preventing the translocation of NF-κB into the nucleus. This has been shown to be mediated through the phosphorylation of the I-κB resulting in the inhibition of its dissociation from the NF-κB and the prevention of its subsequent translocation into the nucleus [89] (see Figure 11 below). The I-κB themselves are a family of inhibitory proteins which bind to the rel family of transcription factors and modulate their activity. The transcription factor NF-κB is generally present in an inactive cytoplasmic form, bound to inhibitory I-κB proteins. Cell stimulation causes its dissociation and translocation of active NF-κB to the nucleus. The EPA effect on the decreased NF-κB transcriptional activity is thought to be caused by an alteration in the affinity of the NF-κB for I-κB-α as a direct result of direct phosphorylation of the I-κB complex [597, 562] (see Figure 11 below). In addition, recent studies of Babcock et al [598] and Lo et al [563] have also demonstrated that omega-3 fatty acids are also capable of inhibiting the activation of the activator protein-I transcriptional factor and the mitogen-activated protein kinase signalling pathways thus regulating the amplification of pro-inflammatory cytokine activities.
Latest results reported in the literature also indicate that omega-3 fatty acids have a positive effect on the pancreatic cancer gemcitabine chemoresistance which has been demonstrated to be associated with enhanced NF-κB activation and anti-apoptotic protein synthesis \[^{599}\]. Here, omega-3 fatty acids treatment was shown to be specifically associated with the inhibition of proliferation in pancreatic cell lines irrespective of varied gemcitabine resistance \[^{599}\].

Studies of Barber et al \[^{252}\] carried out in the late 1990’s have demonstrated that administration of EPA also has positive effects on the potential mediators of cachexia. Here, it has been demonstrated that EPA significantly reduces the production of IL-6 and excretion of Proteolysis-Inducing Factor, and increases the insulin concentrations \[^{252}\]. In addition, these studies have also shown that the levels of acute phase proteins will stabilise in patients receiving EPA whereas the levels will continue to increase in the EPA-untreated patients \[^{252}\].
One of the hallmarks of the cachexia syndrome is the increased resting energy expenditure which further exasperates the loss of weight and LBM. Fish oil-enriched nutritional supplements containing 2 g of EPA have also been shown to reduce the resting energy expenditure levels \(^{[600]}\) which was confirmed by the recent study of Moses et al \(^{[244]}\).

### 5.3.2 Therapeutic Uses

One of the main therapeutic uses of EPA seems to lie in the prevention and therapy of cachexia and wasting associated with cancer and other catabolic diseases. However, there are currently reports in the literature \(^{[601]}\) that may suggest that its benefit in cancer cachexia is controversial. It was for this reason that our clinical trial in pancreatic cancer patients undergoing chemotherapy was designed and conducted.

It is very important to note that the anti-tumour and anti-inflammatory effects of fish oils are specific to EPA and not to all omega-3 polyunsaturated fatty acids \(^{[597]}\). This has been demonstrated by the earlier study of Beck et al \(^{[602]}\) who compared EPA with \(\gamma\)-linolenic acid in the mouse tumour model and showed that the EPA and not the \(\gamma\)-linolenic acid inhibits both host weight loss and tumour growth rate in a dose-dependent manner. Similarly, Wallace et al \(^{[592]}\) showed that omega-6 polyunsaturated fatty acid emulsions appear to impede tumoricidal activities as compared to EPA. Since conventional cytotoxic chemotherapy in pancreatic cancer seems to be of limited benefit and effectiveness resulting in only a relatively small increase in survival and low objective response rates for a substantial side effects (see section on pancreatic cancer chemotherapy (Chapter 3.5)), other treatment modalities, such as high dose EPA therapy, may provide some solution \(^{[603]}\). For this purpose high dose high-purity EPA has been tested and reported in the literature and has been demonstrated to be safe and effective \(^{[603]}\).
Chapter 5: EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

Generally, oral administration of high doses of high-purity EPA means ingesting more than 25 capsules, each containing one gram of oil, per day. This can cause problems in some patients, such as patients with pancreatic cancer, who suffer from malabsorption as a result of their disease. Barber et al. [603] have now demonstrated that pancreatic patients can take a 50 mL dose of a liquid fish oil preparation delivering up to 18 g of EPA per day (instead of a minimum of 18 one gram capsules) with easily manageable side effects, thus allowing high doses of high-purity EPA to be administered. Trials of oral protein and energy-dense oral supplements containing omega-3 fatty acids have demonstrated that only oral supplements containing omega-3 fatty acids are capable of providing net gain in weight and lean body mass as well as improvements in quality of life (post hoc analysis) [247]. This trial also indicated that the administration of EPA alone may not be enough as additional oral protein and energy input are required for protein synthesis and maintenance of lean body mass [247]. The study of Barber et al. [253] carried out in the late 1990’s have demonstrated that their EPA-enriched nutritional supplement may reverse cancer-induced cachexia in their group of pancreatic cancer patients who have been losing weight at a median rate of 2.9 kg/month. These authors, using bioelectrical impedance analysis techniques, demonstrated that there is a statistically significant increase in lean body mass with the fat mass remaining stable and no change in the hydration levels expressed as total body water as a percentage of body weight [253]. In addition, an increase in nutritional intake and a fall in resting energy expenditure were achieved in their group of pancreatic cancer patients [253].

One of the metabolic changes that have been demonstrated in weight-losing cancer patients as compared with controls is the disruption of metabolic processes to the extent that in the fasting state these patients have a lower serum insulin concentration, increased energy expenditure per unit weight as well as an increase in fat oxidation rate. In addition, these patients show glucose intolerance after feeding with a reduction in the metabolic cost of
feeding. These metabolic disturbances as well as weight loss have been shown to be normalised following the administration of three weeks of EPA-enriched oral nutritional supplements [600].

The treatment of cancer cachexia and its associated weight loss is of great importance and the best way to treat cancer cachexia is to cure or control the cancer. In cancer and other cachectic patients weight loss is associated with psychologic distress and a lower quality of life. Also, patients with weight loss have been shown to have a relatively shorter survival time and a decrease in response to therapy [604]. Although it has been reported that approximately half of all cancer patients show some degree of weight loss [604], the highest frequency of weight loss has been observed in pancreatic cancer [605]. Studies of Wigmore et al [605] in pancreatic cancer patients showed a median of 14.2% weight loss (as compared with their pre-diagnosis stable weight). This weight loss was reported to have increased to a median of 24.5% just before death. It has been suggested that patients with greater than 15% weight loss, as compared to their pre-illness stable weight, are likely to have substantial total body protein (TBP) loss [2]. At this level, physiological functions, such as respiratory muscle function, are severely impaired [14]. If death is to be prevented in these patients, effective therapy is required and it is required as early as possible after diagnosis.

Unfortunately, an improvement in appetite or increase in calorie intake does not fully reverse the cachectic effects. Although studies of Downer et al [606] on oral medroxyprogesterone acetate showed an increase in appetite of patients, but this did not result in weight gain or any improvement in performance status, energy levels, relief from pain or mood. Studies of Loprinzi et al [607] on megestrol acetate as an appetite stimulant demonstrated weight gain that was as a result of increase in adipose tissue and body fluid with no increase in LBM, which was similar to observations obtained with TPN. Pharmacological agents have also been used as alternative approaches for the treatment of the weight loss. Agents such as hydrazine
sulphate, which are phosphoenolpyruvate carboxykinase inhibitors have shown to influence protein metabolism and maintain and to maintain body weight \cite{608}. Ibuprofen, a non-steroidal anti-inflammatory agent and a cyclooxygenase inhibitor when used in pancreatic cancer has reduced the resting energy expenditure \cite{609}, which may have a role in reducing the weight loss in this group of patients.

The use of EPA as an effective therapeutic agent in the treatment of cancer cachexia has been growing in the past few years. It has been reported that EPA is not only effective in counteracting the weight loss, but also in stabilising the protein and fat stores in pancreatic cancer patients as well as reducing acute phase protein production and stabilising resting energy expenditure \cite{250}. This effect was reported to be specific to EPA since a related polyunsaturated fatty acid (\(\gamma\)-linolenic acid) had no effect when administered to pancreatic cancer patients. It has also been reported \cite{610} that EPA acts by reducing the actions of catabolic agents, such as LMF, in cachexia by preventing the rise in adipocyte cyclic adenosine monophosphate levels. Studies of Beck et al \cite{602} have also demonstrated that the administration of EPA also significantly reduces protein degradation. Similarly, study of Barber et al \cite{251} demonstrated that post-administration of a fish-oil enriched nutritional supplement, the circulating concentrations of leptin were not affected by the treatment and that the resulting reversal of weight loss is associated with a significant decrease in IL-6 produced by the peripheral blood mononuclear cells, an increase in serum insulin concentrations, a decrease in the cortisol to insulin ratios, and a decrease in the proportions on patients excreting proteolysis-inducing factor.

Clinical studies carried out in the past few years have demonstrated the beneficial effects of fish oil-based interventions in cancer cachexia. Interest in fish oils, containing omega-3 fatty acids, and in particular EPA was initiated in the cardiovascular field where their consumption was associated with a reduced tendency to platelet aggregation, blood viscosity, and
improvement in lipid profile [611]. These effects have been suggested to be mediated by an alteration in the balance of eicosanoids to less inflammatory and aggregatory compounds than those produced from ω-6 fatty acids [228]. Fish oil and EPA have been reported to act by suppressing the endotoxin-induced production of pro-inflammatory cytokines, which are potential mediators of cachexia [591, 566, 565]. This effect is evident in pancreatic cancer patients receiving 6g/d EPA [586] reported in the literature. Animal studies [589] have also demonstrated that EPA is also capable of reducing the end organ effects of PIF, which is a 24kDa glycoprotein, found in the urine of weight losing cancer patients.

The doses of EPA used in various clinical studies involving cachexia or weight loss in pancreatic cancer patients reported in the literature ranges from 2g/day [251, 252] and 2.2g/day [251, 250] to 6g/day [249]. The effects and side effects of these dose levels were, somewhat, similar.

In a study of Wigmore et al [250] 1g soft gelatine capsules of fish oil containing EPA 18% w/w and DHA 12%w/w were administered orally to patients with unresectable pancreatic cancer. The initial dose of 2g fish oil was increased daily by 2g to 16g/day. Approximately 25% of patients developed steatorrhoea managed successfully by oral pancreatic enzyme supplementation. The authors stated that: "a number of patients found high purity EPA capsules unpleasant to take because of problems with occasional leakage of contents resulting in unpleasant chemical taste. The patients were requested to ask for replacement capsules if this occurred.". It was also reported here that patients tolerated a median maximum dose of 12g fish oil/day, the equivalent of 2g EPA/day.

In a subsequent study of Wigmore et al [249] dose of 6g/day of 95% pure EPA in capsules was given orally after a 4-week dose escalation. At base-line patients were losing weight at a rate of 2kg/month while after 4 weeks of EPA their weight stabilised and was maintained for more than 3 months. Here, only 5 out of 26 (19%) patients reported EPA-induced side effects.
(nausea and steatorrhoea), which was successfully reversed. These authors reported that no patient developed adverse effects that fell outside the normal pattern of events for patients with advanced pancreatic cancer.

In the studies of Barber et al \cite{251, 253, 252} also on unresectable pancreatic cancer patients 2.2g/day EPA plus 0.96g/day DHA was given orally in two cans of nutritional supplement. These authors reported that patients managed to consume a median of 1.9 cans/day of the fish oil-enriched nutritional supplement. From the 20 patients studied, 2 patients (10%) developed steatorrhoea, which was successfully treated with pancreatic enzyme supplementation.

In our personal communications with Prof Michael J Tisdale, (a leading authority from the United Kingdom on EPA and fish oil administration in pancreatic cancer patients) with regards to the safe and effective dose of EPA to give our cancer patients, it was suggested that: "the minimum effective dose is 2g EPA per day, (not total fatty acids; DHA has no effect) and there is no toxicity up to 6g EPA per day. Patients would require this dose daily throughout the study. It would not be sufficient to administer it at the start of chemotherapy and at 6 and 12 weeks post chemotherapy. We have found that patients can take the EPA themselves at home."

The effects of EPA combined with nutritional support have also been reported in the literature. The study of Barber et al \cite{253} carried out in the late 1990’s investigated the effects of a nutritional supplement containing 2 g of EPA plus 600 kcal administered orally on a daily basis. This study demonstrated that in weight losing patients (2.9 kg/month) this combination therapy resulted in a median weight gain of 1 kg after only three weeks and 2.5 kg after seven weeks of treatment with improvements in appetite, no change in fat mass, and a significant increase in LBM \cite{253}. There was also a significant improvement in the patients’ functional abilities, as indicated by the Karnofsky Performance Index measurements \cite{253} and reduction in the total energy expenditure \cite{244}. The recent studies of Baur et al \cite{242} have also
demonstrated a significant association between improvement in nutritional status through the use of EPA and EPA-enriched nutritional supplementation and improvements in the quality of life, functional capacity, and maintenance of lean body mass in patients with cancer cachexia who are receiving chemotherapy.

There have also been contradictory and controversial reports in the literature that indicate that EPA may not have the weight stabilising effect in cancer cachexia. Preliminary reports of Fearon et al [247] investigated the effect of EPA nutritional supplementation in patients with pancreatic cancer. These reports indicate that, at the four and eight weeks post-treatment time points, there were no statistical differences in weight change between the patients who received EPA nutritional supplementation and the placebo group [247]. Similarly, the study of Bruera et al [612] indicate that fish oil did not significantly influence appetite, tiredness, nausea, well-being, caloric intake, nutritional status, or function after 2 weeks compared with placebo in patients with advanced cancer and loss of both weight and appetite. In a randomised controlled trial [613] carried out in the late 1990s on 60 patients with incurable solid tumours receiving 18 grams of fish oil in capsules per day, it was demonstrated that there was a significant improvement in survival and the T-helper to T-suppressor cell ratio in the treatment group. However, the trial could not demonstrate any difference in weight loss between the placebo and the fish oil-treated groups [613].

One of the hallmarks of cancer cachexia is the increase in the resting energy expenditure which has also has been demonstrated in patients with advanced pancreatic cancer [132]. This has been confirmed by recent studies of Moses et al [244] who indicated that in cachectic patients the resting energy expenditure is increased whereas the total energy expenditure and physical activity is decreased. It has now been shown that the administration of nutritional supplement enriched with EPA is associated with an increase in physical activity and decrease
Chapter 5: EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

in resting energy expenditure whereas energy and protein dense oral supplement on its own does not have an effect \[244\].

Other uses and benefits of EPA that has been reported in the literature include improved post-operative immune function \[614\], improved immune cell function during chemoradiation \[615\], and increased concentrations of effector T-cell in cancer patients. Animal studies have also demonstrated improved nitrogen retention and protein metabolism with EPA administered as a part of TPN therapy \[616\]. Administration of EPA has also been shown to result in the reduction of systemic inflammatory response syndrome which is associated with bone marrow transplantation as well as increasing survival \[617\]. Recent reviews and studies have also reported that EPA may also have direct effects on tumour growth and metastatic spread and, generally, anti-proliferative and anti-cachectic actions in malignancy \[233, 618, 619\]. A recent report of a systemic review of the literature indicated that there may not be an overall trend across different cohorts and categories of omega-3 polyunsaturated fatty acid consumption to suggest that omega-3 polyunsaturated fatty acids reduce the overall cancer risk and that it is unlikely to prevent cancer \[620\].

Post-operative complications can consume significant hospital resources and, therefore, be costly to the patients as well as the hospital. In addition, these complications reduce patients’ quality of life and survival. The costs vary according to the type of complication that occurs. Generally, it has been reported that the most expensive are sepsis, anastomotic leak, wound dehiscence, abdominal abscess, and pancreatic fistulae \[621\]. There have been studies reported in the literature demonstrating the beneficial effects of pre-operative nutritional supplementation and improvement of outcome in gastrointestinal surgery \[622\]. Recent study of Braga et al \[621\] has demonstrated that the pre-operative administration of specialised nutritional supplement containing omega-3 polyunsaturated fatty acids and arginine can significantly improve outcome. This was in line with previously reported studies which
demonstrated significant decrease in post-operative infections by peri-operative administration of nutritional supplementation enriched with arginine and omega-3 polyunsaturated fatty acids [623]. The use of fish oil in the critically ill patients through enteral routes have been shown and proven to be an effective immunonutrition technique as a part of the peri-operative nutritional support. The data and evidence for these have been used in two meta-analyses reported by Heyland et al [624] and Heys et al [625]. These meta-analyses concluded that by using post-operative enteral immunonutrition, infections in surgical patients can be prevented as well as the length of hospital stays reduced [567, 624, 625]. Although there has not been many reports on the use of omega-3 polyunsaturated fatty acids as a part of the parenteral nutrition, earlier studies of Grimminger et al [630, 629, 626, 627, 628] and Breil et al [631] in animal models and isolated organs have demonstrated that omega-3 polyunsaturated fatty acids can potentially reduce pulmonary hypertension, reduce lung oedema, increase the arterial pO2, and promote bacterial killing. Recent study of Mayer et al [632] demonstrated that in patients with sepsis generation of pro-inflammatory cytokines by mononuclear leukocytes is markedly increased during omega-6 polyunsaturated fatty acids administration and was suppressed during omega-3 polyunsaturated fatty acids administration. These authors also suggested that the use of lipid infusions might allow the combination of intravenous alimentation with differential impact on inflammatory events and immunologic functions in patients with sepsis [632].

Another recent review of Mayer et al [567] has also reported of the drawbacks of the oral compared with the intravenous administration of fish oil and omega-3 polyunsaturated fatty acids and suggested that the therapeutic effects have a slower onset and, as such, may not be suitable for patients with acute disease. There are a number of reasons for this slow onset of effect. One of the reasons is that the resorption and transport of nutrients in the severely ill patients is not at the same rate as healthy individuals since the gut is an organ affected by
Chapter 5: EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

multiple organ failure [567]. Another reason is that the administration of fish oil as fish oil capsules restricts the availability of bioactive molecules due to the fact that fatty acids that enter the circulation after crossing the intestinal wall are packed as triglycerides in vesicles and are transported to the liver in a process leading to the loss of free omega-3 fatty acids through lipid remodelling [567]. This would, therefore, limit the participation of free fatty acids in inflammatory pathways in leukocytes as well as endothelial cells [567]. Infusions of lipid emulsions based on omega-3 fatty acids are, therefore, more effective and have been shown to be an order of magnitude greater in generating increases in plasma concentrations of omega-3 fatty acids [633].

Although the parenteral administration of fish oil is still in its early days, it has been demonstrated in the literature that the intravenous administration of fish oil to surgical patients may have beneficial effects. Recent study of Tsekos et al [634] showed that there were significantly reduced need for ventilation, length of hospital stays, as well as mortality in surgical patients who received peri-operative supplemental intravenous fish oil as compared with those patients who received the standard intravenous nutrition. Similarly the prospective, randomised, double-blind clinical trial of Heller et al [248] demonstrated that patients who received fish oil supplementation had better post-operative liver and pancreatic functions which contributed to their faster recovery. In addition, they showed that the surgical patients who received the fish oil supplementation did not show the weight loss which was evident in the placebo group who received soybean oil [248]. It is important to point out that in our EPA clinical trial in pancreatic cancer patients receiving Gemcitabine chemotherapy, we also used soybean oil as the placebo.

With regards to improvements in liver and pancreatic functions, the study of Pscheidl et al [635] demonstrated that with short-term intravenous feeding with fish oil can improve intestinal perfusion and portal blood flow, improve glucose tolerance, and increase lactate clearance in
Chapter 5: EICOSAPENTAENOIC ACID AND OMEGA-3 FATTY ACIDS

the liver. Other studies on the rat model also demonstrated an enhanced hepatic immune competence whereby diets enriched with fish oil were shown to abolish the endotoxin-induced decrease of nutritive blood flow to the gut and ameliorate the bactericidal defence of the splanchnic region and that the lower count of the viable bacteria seen in the subjects treated with fish oil were more related to an improved killing of translocated bacteria than a reduction of the translocation rate [636]. Recent human multi-centre trials have demonstrated that the administration of omega-3 fatty acid may reduce mortality, antibiotic use, and length of hospital stay in different diseases and that the effects and effect sizes related to fish oil doses are diagnosis dependent [637]. Reports of Stehr et al [638] also indicate that short-term post-operative intravenous administration of omega-3 polyunsaturated fatty acids improves liver function without untoward effects on platelet function and coagulation. In addition, it was also indicated that the omega-3 polyunsaturated fatty acid administration helps to maintain the pro-inflammatory and anti-inflammatory cytokine balance and, therefore, prevent hyper-inflammatory complications [638].

An emerging field in the therapeutic use of EPA has been in the induction of apoptosis in human cancer cells. In particular, the recent report of Shirota et al [639] demonstrated that EPA can successfully inhibit the growth of human pancreatic cancer cells by inducing apoptosis in these cells. The possible mechanism was suggested to be the activation of caspase-3 and the suppression of cyclooxygenase-2 expression [639]. Although the cyclooxygenase-2 is induced in response to inflammation, it is not detectable in most normal, non-inflamed tissue, it is over-expressed in human pancreatic cancer [640].

EPA has also been shown to be able to act centrally and have anti-depressant properties. However, the number of studies or clinical trials investigating this potentially useful effect of EPA is very small. Animal studies have demonstrated positive anti-depressant effect [641] and its use in pregnancy has been advocated [642]. A recent meta-analysis [642] reviewing the
double-blind, placebo-controlled trials available in the literature between 1966 and 2006 found that, in general, omega-3 polyunsaturated fatty acids have a significant antidepressant effect. In addition, the omega-3 polyunsaturated fatty acids were found to be able to significantly improve depression in patients who were found to have a clearly defined depression or had a clearly defined bipolar disorder \cite{642}. Interestingly, the actual dosage of EPA was found not to affect the antidepressant efficacy significantly \cite{642}. In a recent eight week double-blind clinical trial of EPA in patients with clinical depression, it was found that EPA (1 g/day) and fluoxetine (20 mg/day) had equal therapeutic effects in major depressive disorder \cite{332}. These researchers also demonstrated that the EPA plus fluoxetine combination was superior to either of EPA or fluoxetine alone \cite{332}. Since depression is one of the common symptoms of pancreatic cancer, the use of EPA as an anti-cachectic agent may, therefore, not only improve cachexia, but also improve the mood and hence the quality of life of patients with pancreatic cancer. The mechanism of action of EPA in reducing depression has been suggested to be related to its anti-inflammatory actions since the excessive production of pro-inflammatory cytokines have been reported to be the cause of depression \cite{330,328,329}. This, which is also referred to as the “cytokine hypothesis of depression” suggests that the pro-inflammatory cytokines act as neuromodulators and are the major factors in centrally mediating the behavioural, neuroendocrine, and neurochemical features of depressive disorders \cite{330}. Recent report of Sublette et al \cite{331} also suggests that arachidonic acid and prostaglandins may be involved in unipolar disorders which may explain the reported anti-depressive effects of EPA as EPA is known to modulate the arachidonic acid cascade and prostaglandins.
5.3.3 Side Effects

Generally, EPA has been shown not to have distressing side effects. Side effects have been reported to be minimal with nausea, steatorrhoea, and fishy burps being the most common\textsuperscript{332, 249, 250}. In our clinical trail, we utilised special enteric-coated capsules and only a handful of incidents of these side effects were observed.

The study of Barber et al\textsuperscript{603} investigating the tolerance of high dose EPA diester emulsion in patients with pancreatic cancer demonstrated that patients were capable of tolerating doses of as high as 18 g per day, with doses between 9 g and 27 g daily being taken for at least one month. The dose-limiting factors reported were, generally, the feelings of fullness, abdominal pain, nausea, and steatorrhoea which were controlled by dose-reduction and pancreatic enzyme administration\textsuperscript{603}. Other studies have shown steatorrhoea being the only adverse effect occurring in only a very small number of patients\textsuperscript{253}.

Earlier study of Clarke et al\textsuperscript{643} carried out in the early 1990s have indicated that there is a clinically significant problem causing prolonged bleeding time, inhibition of platelet function, and increased rate of epistaxis. However, more recent studies have not found such adverse effects, even with higher EPA doses\textsuperscript{603}.

The use of omega-3 fatty acid supplementations have been suggested in two early studies\textsuperscript{644, 645} to be adversely affecting glucose metabolism in otherwise healthy subjects with type II diabetes. However, the placebo-controlled study of Toft et al\textsuperscript{646} carried out in the mid-1990s demonstrated that even after the administration of 4g of fish-oil for 16 weeks showed no change in glucose tolerance testing, insulin release, or insulin sensitivity.

Earlier studies of Meydani et al\textsuperscript{647} have suggested that the administration of high doses of can suppress cytokine production and lymphocyte proliferation thus having an immunosuppressive effect and producing detrimental consequences for the patients, especially in older women. Recent studies\textsuperscript{648} have, however, demonstrated that the
administration of high doses of EPA does not produce a generalised immunosuppression and instead, produces subtle effects in modulating the immune system with no effect on a mimic of antigen-specific responses.
6. THALIDOMIDE IN CANCER THERAPY

A review of the recent literature indicates there have not been any successful procedures that can be curative in malignant mesothelioma and that this disease has been notoriously refractory to all therapeutic procedures. As reviewed in the section on Malignant Mesothelioma (Chapter 4), most chemotherapy regimens and procedures have also proved to be disappointing and unsuccessful. Currently, the best chemotherapy regimen available is the combination of cisplatin plus premetrexed [543].

Angiogenesis is one of the distinct characteristics of malignant mesothelioma [514]. Studies of Edwards et al in the early 2000s have demonstrated that not only angiogenesis, is a poor prognostic factor in malignant mesothelioma, it is also independent of other clinicopathological variables and the EORTC prognostic scoring system [514]. It has also long been known that malignant mesothelioma cells often express the VEGF and the basic Fibroblast Growth Factor [649, 650]. These factors also play an important role in the formation of new blood vessels in embryogenesis, tumour progression, diabetic neuropathy, and wound healing [515].

The general view in the published reports in the literature is that thalidomide itself is considered to be an immunomodulator, anti-angiogenic, anti-cytokines, anti-integrin, anti-cachectic, and anti-neoplastic.

6.1 Pharmacology And Mechanism Of Action

Thalidomide (α-N-phthalimidoglutaramide) is a potent teratogen developed by Chemie Grunenthal in the 1950s. It is a derivative of glutamic acid and is poorly soluble in water. Thalidomide has two enantiomers: R and S. The R-enantiomer has sedative properties
whereas the S enantiomer has teratogenic properties [651, 57, 652]. It has first-order absorption and elimination and its pharmacokinetics have been suggested to be independent of age, sex, and race [57].

Earlier studies of Figg et al [653] on the pharmacokinetics of thalidomide using low doses (200mg) and high doses (200mg with escalation up to 1200mg) demonstrated that the elimination half-lives for the low and high dose are 6.52 and 18.25 hours, respectively. In addition, when multiple dosing of thalidomide was used, the oral clearance and the apparent volume of distribution in their low-dose group were shown to be 6.35 Litres/hour and 64.63 Litres, respectively [653]. However, in their high-dose group the oral clearance and apparent volume of distribution were shown to be 7.73 Litres/hour and 167.85 Litres respectively [653]. Figg et al [653] also demonstrated that the elimination half-lives for the low and high dose were 7.08 and 16.19 hours, respectively concluding that for both the single and multiple dosing of thalidomide, the apparent volume of distribution and half-life were significantly higher in their high-dose group than in their low-dose group.

Other studies reported in the literature indicate that clearance of thalidomide is primarily non-renal with an average terminal half-life of approximately 4.7 hours [654]. Review of Davis et al [655] suggest that the absorption half-life of thalidomide is between 0.09 to 1.7 hours with time to maximum concentration being 2.5 to 5.7 hours. The elimination half-life has been suggested to demonstrate high variability and be around eight hours [655]. It crosses blood-brain barrier and blood-placenta barrier due to its lipid solubility, and it does not undergo hepatic metabolism [655], but is hydrolysed in plasma to multiple metabolites with only 0.7% of the original drug appearing in the urine [57].
As indicated above, thalidomide has multiple different actions including anti-emetic, anti-cancer, anti-cachectic, etc. Of relevance and interest to us here are the anti-cancer and anti-cachectic properties of thalidomide.

The use of thalidomide as an anti-cachectic agent was probably tested and/or noticed when it was used as an agent which was able to reduce the production of TNF-α by increasing the rate of TNF-α mRNA degradation rate [656]. The majority of the reports in the literature on the anti-cancer activities of thalidomide have been limited to myeloma. The anti-cancer properties of thalidomide, in the form anti-angiogenesis, was probably first reported in the literature in the early 1990s [657]. The report indicated that the mechanism of action of thalidomide in preventing angiogenesis is not clear but it is thought to be due to the interruption of the processes which are mediated by the bFGF and/or VEGF [657]. Early studies of Moreira et al [656] have suggested that by the induction of TNF-α mRNA degradation, thalidomide is able to inhibit synthesis, which is one of the major mediators of cancer cachexia (also see section on Cancer Cachexia (Chapter 2)). In fact, the production of and TNF-α–mediated metabolic changes have been shown to be counteracted by thalidomide [58, 56, 57, 4] similar to that of EPA [59, 61, 60]. Another major mediator of cancer cachexia is NF-κB. The actual mechanism of action of NF-κB and its involvement in the induction of Cancer Cachexia is detailed in the section on Cancer Cachexia (Chapter 2) which, in short, is mediated through the IκB kinase activity. The actions of NF-κB proteins have been shown to be inhibited by EPA [89] and thalidomide [90, 91] through the inhibition of this kinase.

Other effects of thalidomide on the inflammatory response that have been shown to affect cachexia include the inhibition of IL-6 and IL-12 reduction as well as that of free radicals that can cause oxidative DNA damage [654]. Similarly, recent studies of Kedar et al [658] in the use of thalidomide in patients with metastatic renal cell cancer demonstrated that thalidomide is able to reduce the serum levels of CRP and IL-6 and, as a result, increase renal cell
carcinoma’s responsiveness to IL-2. This is important as CRP can block the recognition of tumour cells by the host’s cytotoxic cells \cite{658}.

Since anorexia is caused by the actions of IL-1 and serotonin on the ventromedial hypothalamus, and cachexia is caused by inflammatory cytokines (such as TNF-\(\alpha\), IL-6, IL-1, etc) which produce alteration in lipid and protein metabolism \cite{659, 39, 246}, it is understandable that anti-cachectic agents such as thalidomide and EPA can act as anti-cytokines, anti-inflammatory, and immunomodulatory agents \cite{661, 660, 662, 663, 253, 246}. In addition, as IL-6 has been shown to promote tumour growth, agents such as thalidomide and EPA can also be used as anti-tumour agents \cite{57, 253, 246}.

In effect, the anti-cachectic effects of thalidomide are as a result of its immunomodulatory nature \cite{651, 652}. Its ability to decrease the serum levels of TNF-\(\alpha\), IL-6, IL-1, IL-2, IL-4, IL-12, and IFN-\(\gamma\) have been reported in the literature most of which have been implicated in cancer cachexia especially that of TNF-\(\alpha\) which is carried out by the inhibition of transcription \cite{57, 659}. These are in addition to the decrease in NF-\(\kappa\)B expression \cite{659}.

### 6.2 Uses Of Thalidomide

In addition to its mildly antiemetic effect, thalidomide has also been shown to be anti-cachectic, analgesic, and have sedative effects \cite{58, 56, 226}. In addition thalidomide has also been shown to improve appetite in terminal cancer patients, proving effective in the palliation of intractable symptoms \cite{226}.

Thalidomide has also been demonstrated in randomised, placebo controlled trials to be well tolerated and effective at reducing weight loss and the loss of lean body mass in pancreatic cancer \cite{58} and oesophageal cancer patients \cite{56}. The attenuation of weight loss is thought to result from the modulation of the inflammatory response \cite{58}.
Chapter 6: THALIDOMIDE IN CANCER THERAPY

Thalidomide have been reported to have been used in a variety of diseases, such as dermatological diseases, graft-versus-host disease, inflammatory bowels disease, HIV, and cancer, to palliate symptoms [57, 664, 665]. Many of symptoms have been shown to be palliated without a reduction in the TNF-α serum concentrations [664, 665]. As such, thalidomide has been used in many dermatological conditions, such as leprosy and lupus, to relieve inflammation-related symptoms [666].

One of the particularly useful effects of thalidomide that has been reported in the literature has been analgesia. Earlier studies of George et al [667] on animal models have demonstrated benefits of thalidomide and anti-cytokine agents as potential analgesics in neuropathic pain. The mechanism of action suggested by these authors was that since TNF-α produces hypergesia, then its suppression by thalidomide produces analgesia [667]. In addition, thalidomide was shown to increase met-enkephalins in the dorsal horn, resulting in a reduced thermal hypergesia and mechanical allodynia [667].

Thalidomide has also been shown to be effective in the palliation of symptoms such as malignant-associated night sweats [57, 668]. Although the exact mechanism is not clear, but it is thought to be due anti-cytokine and anti-inflammatory actions of thalidomide [57, 668].

6.3 Toxicity And Side Effects Of Thalidomide

The most recognised and probably the most notorious side effect of thalidomide is teratogenesis. Teratogenic properties of thalidomide are not dose-dependent and generally occur when thalidomide is administered in early pregnancy. As such, pregnancy testing as well as pregnancy prevention is highly recommended before, during, and after therapy with thalidomide.
Although lower doses of around 200mg per day are mainly used, the general toxicity of thalidomide administration of doses of 800mg to 1200mg per day has been associated with non-haematological with a grade 3 or 4 neuropathy, sedation, and constipation [669].

One of the major side effects of the use of thalidomide has been its sedative effects. This is not surprising as this drug was initially developed as a sedative. However, it has been indicated that the sedative effects of thalidomide decreases with continued administration at a constant dose [670].

The most serious side effects of thalidomide are DVT and peripheral neuropathy. DVT has been reported to occur in 15% of patients receiving thalidomide and cytotoxic chemotherapy [671]. Peripheral neuropathy is, however, more common and can affect 10% to 50% of patients receiving thalidomide [670]. The neuropathy is dose-dependent and most frequent symptoms include numbness and paresthesia, both of which are reversible on thalidomide cessation [670]. However, toxic neuropathy limits its use [672]. Studies of Chaudry et al [672] on thalidomide-induced neuropathy have demonstrated that thalidomide induces a dose-dependent sensorimotor length-dependent axonal neuropathy and, therefore, should be judiciously used with close neurologic monitoring.

Other reported, but minor side effects of thalidomide include constipation and pruritus. The constipation can often be relieved by the appropriate use of laxatives and adjustment of daily diet and dietary intake. With pruritus and dermatitis, alcohol-based lubricants are indicated which often relieves these symptoms. Studies of Clark et al [673] and Thomas et al [659] have also reported dry mouth, coma, headache, hypertension, bradycardia, dizziness, orthostatic hypotension, altered temperature sensitivity, and lower extremity swelling as possible side effects of thalidomide therapy.
7. IN VIVO BODY COMPOSITION

The idea of in vivo body composition measurements is to accurately and efficiently derive various body compositional parameters non-invasively and use the information gathered for the benefit of the patient. The main advantages of these systems are that they cause no harm or discomfort to the subject (both human and animal).

The chemical composition of the human body the figures was published by three German authors in the mid to late 19th century. The first studies of body composition in humans probably commenced during the early 1950’s when researchers started investigating cadavers of various ages and sex [674]. Today, with the introduction of various techniques and equipment including nuclear techniques, the body composition can be measured in vivo with relative ease and accuracy in normal and disease conditions.

One of the approaches to analysing the body composition of animals or humans is its division into a variable number of different compartments. The two compartmental approach to body composition is probably the simplest and the most basic of the divisions. Here, the body is divided into fat mass and FFM. In FFM (unlike LBM) the fat in cell membranes is excluded and is, therefore, included in the fat compartment. The two compartmental approach, however, obscures the distinct physiologic functions of the various tissues: for example, bone versus muscle, water versus body cell mass, etc. and is, therefore, considered to be unsatisfactory for most purposes of body composition analysis. This approach especially does not allow longitudinal study of body compositional changes under normal and diseased conditions.

A four compartmental approach to body composition involves the separation of the human body into: fat, water, protein, and mineral (or bone). This approach is the one used when longitudinal study of body compositional changes needs to be carried out. In a normal
individual, the levels of these compartments are in equilibrium and in a specific ratio to each other. Under disease conditions, such as cancer, the relative levels of these compartments are altered and are not in equilibrium. The study of the relationships between these compartments under normal and disease conditions is essential for the management of diseased subjects, will help maintain normal equilibrium.
7.1 Total Body Nitrogen (TBN)

Most nitrogen in humans and animals is present as amino acids, which form the fundamental proteins for the body. Nitrogen is also present in important biological molecules (such as the nuclear DNA and RNA) and urea. Because of the predominant amounts of amino acids in protein, TBN reflects the protein compartment of the body. Changes in nitrogen balance reflect changes in the protein content under healthy and disease conditions. Although, most of the protein is present in muscle, it is also present in cell membrane, plasma, and other sites. TBN is, therefore, an extremely useful clinical and research tool for predicting, preventing, and rehabilitating individuals during and after serious medical conditions involving nitrogen metabolism.

7.1.1 Measurements Of TBN

The measurement of TBN is the central component of the research in this project. The facility used to make the TBN measurements is the one at the Body Protein Monitor unit of the Department of Nuclear Medicine, Royal North Shore Hospital. This is one of the three units available in Australia capable of measuring TBN in adults and children. There are approximately ten other similar facilities in New Zealand, Europe, and North America.

The actual technique of estimating the TBN by in vivo gamma-neutron-capture analysis has been used for more than two decades to offer information on protein and nutritional status in adults, children and adolescents with a wide variety of diseases [677, 676, 678, 675, 670]. The first nuclear technique used to indirectly measure TBN was carried out by Nagai et al [680] in the late 1960’s. The first direct in vivo neutron-activation analysis carried out in humans was
reported by Anderson et al [681] in 1964. Historically, TBP (or TBN) has been estimated by indirect techniques. These include mid-upper-arm circumference measurements for estimation of skeletal muscle [682] and plasma protein for non-muscle protein stores [683]. Other techniques developed during early 1970’s up to mid 1980’s for the estimation of body protein included TBK measurements [684], creatinine height index [685]. These measurements were later found to be inaccurate when applied to various disease conditions. For example, no correlation was found between total body potassium and total body protein in patients with renal disease [687, 686, 691, 207, 690, 688] and patients with cystic fibrosis [692]. Measurements of TBK which has been used to estimate FFM, has been suggested by McNeill et al [678] to reflect body protein (i.e. nitrogen and potassium correlate) in normal individuals but not in subjects with diseases associated with wasting. These authors have also indicated that the various anthropometric measurement techniques currently used to estimate FFM in all subjects have been derived from data on young and healthy subjects and may not hold under disease and other conditions.

In the past decade, TBN measurements has been carried-out in a wide variety of patients, both adults and children, with different disease conditions [689, 691, 693, 207, 692]. Examples include: malnourished children under various different disease conditions [695, 694, 699, 697, 696, 698, 700], individuals with anorexia nervosa [701, 702], males infected with human immunodeficiency virus [703, 704], renal dialysis patients [687, 686, 689, 691, 693, 690], and individuals undergoing surgery [206]. The main aim in all these studies has been to monitor malnutrition as malnutrition is associated with poor prognosis. TBN (or TBP) is a valuable clinical measure that can be utilised to estimate the degree of protein depletion or malnutrition and to guide and monitor protein replenishment applied during nutritional rehabilitation programs. It is also provides an invaluable tool for assessing the efficacy of a variety of different nutritional regimens enabling the researchers and clinicians to “tailor” the most effective regimen for the individual patients. Studies of Beddoe et al [705] and Finn et al [706] have shown that in critical
Chapter 7: IN VIVO BODY COMPOSITION

illnesses, the relative proportions of all four body compartments (fat, water, mineral, and protein) are altered and unless these compartmental levels are restored to approximately normal levels, ill patient are unlikely to regain their normal health. Beddoe et al \cite{705} have indicated that “an index of the patient’s state of health” can be obtained by investigating the body composition of ill patients.

At the Royal North Shore Hospital, the use of TBN measurements to assess the nutritional status of renal dialysis patients has been a routine procedure. Dietary intervention is carried out to reduce or even prevent the loss body protein. The reports by Pollock et al \cite{691} and Cooper et al \cite{687, 686} have shown the TBN measurements and its accuracy (as measured at the Body Protein Monitor unit, Royal North Shore Hospital, Sydney) to be effective in the determination of malnutrition in the haemodialysis and CAPD patients. Here, it was found that the probability of death within twelve months in patients with a nitrogen index of 80% or less was 48%. In children, the protein malnutrition has been clinically defined by the occurrence of stunting and wasting \cite{694, 699, 698, 707}. The use of height and weight as a measure of nutritional status has been suggested to be inaccurate \cite{694, 699, 698} especially in the cases of cirrhosis and cystic fibrosis where there are overestimations of patient’s well being due to the malnutrition or derangement in fluid and electrolyte balance produced \cite{708, 709}.

### 7.1.2 IVNCA Technique

The development, use, and the basis of the IVNCA technique has been fully described and reviewed by Allen et al \cite{692}. The IVNCA facility currently at the Royal North Shore Hospital had been previously operated at the Lucas Heights Research Laboratories (Australian Nuclear Science and Technology Organisation (ANSTO)) and was moved to the Royal North Shore Hospital during the early 1990’s. This facility initially utilised a $^{252}$Cf neutron source to
irradiate subjects. However, due to its relatively short half-life and the requirement of lengthy annual calibration processes and adjustments, the $^{252}$Cf source was replaced by $^{241}$AmBe in the late 1998. The decision to replace the $^{252}$Cf was due to the fact that the $^{252}$Cf had significantly reduced in activity. The current $^{241}$AmBe source in this facility is a twin source with an activity of 592 GBq each (approximately 1.2 TBq in total) and a half life of greater than 400 years.

Three main types of sources reported in the literature, $^{238}$PuBe [705, 710, 675, 679], $^{252}$Cf [714, 713, 712, 711], and $^{241}$AmBe [715, 716, 719, 718, 717, 720], have been used in the TBN facilities. The advantages of using sources like the $^{252}$Cf or $^{241}$AmBe in our IVNCA facility are that it has the highest nitrogen yield per unit dose equivalent and the largest yield-to-background ratio when compared to other available neutron sources. Both the $^{252}$Cf and $^{241}$AmBe sources were also found to have a lower initial cost as well as a smaller size with their smaller size contributing to a more effective collimation. Earlier calculations of McGregor and Allen [722, 721] showed that the $^{252}$Cf source in particular, produces approximately 40% more thermal flux per total dose equivalent than a $^{238}$PuBe neutron source of identical neutron flux. The only disadvantage of the $^{252}$Cf source encountered was its relatively shorter half-life of 2.65 years and hence its replacement with the $^{241}$AmBe source.

In the Royal North Shore Hospital’s IVNCA facility, the subject lies on a movable table in both supine and then prone positions which passes over the neutron source in 26cm increments. These increments correspond to the effective width of the collimated beam of neutrons at the movable table’s surface. As a result, the patient is irradiated in sections: the legs and buttocks, the abdomen, and the chest region.

The basis of the IVNCA technique is as follows: The fast neutrons emitted by the neutron source penetrate deep into the subject’s tissue and are slowed down by elastic scattering collisions with the hydrogen atoms in the subject’s body. The nuclei of other atoms in the
body then absorb these slowed or thermal neutrons causing an emission of a gamma ray. The gamma ray emitted has an energy which is characteristic of the atom which had captured the thermal neutron. Therefore, during the irradiation process the nitrogen-14 ($^{14}\text{N}$) is converted or “activated” to nitrogen-15 ($^{15}\text{N}$). When the $^{15}\text{N}$ returns to its ground state, it emits a photon of the characteristic energy of 10.83 MeV. The time for the activated nitrogen ($^{15}\text{N}$) to return to its ground state is between $10^{-15}$ seconds for “prompt” neutron activation to hours or days for “delayed” neutron activation. The “prompt” neutron activation is used in the IVNCA facility at the Royal North Shore Hospital. The gamma-ray emission is then detected and measured by two sodium iodide detectors placed on both sides of the table and perpendicular to the direction of travel of the table and the neutron beam. The total exposure time is approximately ten minutes. The effective dose equivalent delivered to an adult subject using this technique is 0.2 mSv. This is in line with the recommendation of the quality factor for fast neutrons (Q=20) by the International Committee for Radiation Protection [711].

Earlier studies [698] carried out on the very same IVNCA facility demonstrated a precision of 2.5% and an accuracy of 3.0%. The precision of the instrument was calculated using phantoms of varying sizes filled with known concentrations of urea solutions whereas the accuracy of the instrumentation was calculated using Perspex boxes (phantoms) containing either known concentrations of urea or wheat and irradiated (static scan) for 2000 seconds. An internal standard was incorporated into the measurements and calculations in order to calibrate for absolute levels of TBN. This involved simultaneous measurement of TBN and TBH, the TBH having a 2.2 MeV gamma ray emission. This method of nitrogen-to-hydrogen ratios was originally developed by Vartsky et al [710] in the late 1970’s, who found it to be less sensitive to changes in body habitus than nitrogen alone. The equation (Equation 1) used to calculate the mass of nitrogen is as follows:
Equation 1. Vartsky’s equation.

\[ M_N = \left( \frac{Y_N}{Y_H} \right) \times K \times M_H \]

where \( M_N \) is the mass of nitrogen, \( M_H \) is the mass of nitrogen, \( K \) is the calibration factor determined from a phantom containing known concentrations of nitrogen and hydrogen, \( Y_N \) is the nitrogen yield, and \( Y_H \) is the hydrogen yield. Corrections for the gamma attenuation and background attenuations are also made to the \( \frac{Y_N}{Y_H} \). These corrections were found to be dependent on the effective size of the subject \[692\]. As with all studies utilising this IVNCA facility for the measurements of TBN \[691, 207, 690, 693, 692\], all measurements were made relative to standard phantoms (Perspex box) containing known concentrations of hydrogen and nitrogen. Once the mass of nitrogen (TBN) is obtained, the mass of protein is calculated by multiplying the mass of nitrogen by the factor of 6.25 \[719, 696, 207, 678\].

### 7.1.3 Physics of Neutron Activation

In general, “activation” methods involve the irradiation of samples with neutrons or charged particles which results in the sample becoming radioactive and emit radiation. High energy neutrons required for neutron activation can be produced by three main sources:

1. Nuclear reactors;
2. Radio nucleotides; and
3. Accelerators.

The neutrons generated by these sources are usually passed through a moderating material that reduced their energy to a few hundredths of an electron volt by elastic scattering. The nuclei will then reach thermal equilibrium with their surroundings, resulting in thermal
neutrons. Substances that contain a large number of protons or deuterium atoms per unit volume are considered to be good moderators. Examples include paraffin, water, D₂O, etc.

Due to the limitations in terms of price and availability of the above three neutron sources, radioactive neutron sources are much more preferred. These sources are generally transuranium elements that undergo spontaneous fission to produce neutrons. The neutron flux of these sources range from 10⁵ to 10⁸ neutrons/cm²/second. The source currently used in the facility at the Royal North Shore Hospital is an ²⁴¹AmBe neutron source. With this source there is a thermal flux densities of about 4 x 10⁷ neutrons/cm²/second. The previous source at this facility (²⁵²Cf), approximately 3% of its decay involves spontaneous fission, yielding 3.8 neutrons per fission with thermal flux densities of about 3 x 10⁷ neutrons/cm²/second [723].

The most important (and the one used in our study) reaction of neutron activation is neutron capture. During the process of neutron capture, a neutron is captured by the nucleus of the atom of the substance to be analysed. This produces an isotope of the same atomic number but a mass number of one higher than the original atom, eg ¹⁴N to ¹⁵N, in our case. This new nuclide is in a highly excited state because of the release of the binding energy of the neutron which is usually around 8 MeV (or 10.83 MeV for the ¹⁵N). This energy is released by “prompt gamma-ray emission” or the emission of one or more nuclear particles, such as neutrons, protons, or alpha particles [723]. The nuclear reaction for the nitrogen used in our study is as follows:

Equation 2. Nuclear conversion of ¹⁴N to ¹⁵N.

\[ ^{14}\text{N}(n, \gamma)^{15}\text{N} \]

which also can be written as:

Equation 3. Alternative equation for the nuclear conversion of ¹⁴N to ¹⁵N.

\[ ^{14}\text{N} + {}_{0}\text{n} \rightarrow ^{15}\text{N} + \gamma \]
The major disadvantage of the activation methods has been suggested to be their need for large and expensive equipment and facilities \(^{[723]}\). The advantages of this technique have been its wide scope of and sensitivity to elemental detection and its accuracy. The major errors that occur in this technique are the ones caused by self-shielding, unequal neutron flux, counting uncertainties, and other errors which are due to scattering, absorption, and differences in geometry between the standard and sample (in our case the patient). These can, however, be reduced to less than 10\% \(^{[723]}\).
7.2 Total Body Water (TBW)

The water compartment is one of the important compartments of the body that is altered under disease conditions. Its importance lies in the fact that the critically ill patients often retain excess amounts of water as a result of the combined effects of septicaemia and fluid managements. During nutritional assessments, if weight is used in these individuals, a false over-estimation of the state of health is obtained. In these patients, the significant errors resulting in incorrect estimations of BSA (from height and weight) and volumes of distribution, can result in excessive toxicities and side effects, especially in the cases of cytotoxic and anti-cancer agents. Therefore, measurement and understanding of the TBW content (and probably its separation into its intracellular and extracellular components) is invaluable for optimisation of fluid management, nutritional support, and therapeutic drug dosage. The concept of TBW clearly excludes fat mass and includes both intracellular water and extracellular water. This does not include the extracellular connective tissues or the intracellular non-water structures. The TBW, to some extent, overlaps with muscle which contains water, nitrogen, and potassium.

Water disturbances are a recognised consequence of severe illness in surgical patients [706, 724]. This conceals the loss in body protein and, therefore, is the reason why weight is a poor reflection of malnutrition. Available nomograms such as the Watson's Equations [725] and 58%BW do not account for this malnourished state that frequently occurs in the ill surgical patients. Clinical assessment mainly relies on physical signs of oedema and congestive heart failure for fluid retention, and on dehydration for patients suffering fluid loss. Excess fluid is not always apparent until pulmonary oedema occurs which can sometimes be fatal [726].
7.2.1 Deuterium Oxide Isotope Dilution

Isotope dilution techniques have been employed by many researchers in the fields of body composition and nutrition for the estimation of TBW. The basic principles of isotope dilution methods are reviewed by Skoog and Leary. The isotope dilution techniques currently available for use in biological samples use either tritium oxide (T\textsubscript{2}O), oxygen-18 (H\textsubscript{2}\textsuperscript{18}O), or deuterium oxide (D\textsubscript{2}O). These isotopes are then detected in various body fluids, such as plasma, urine, and saliva, using appropriate analytical techniques. In this study, the D\textsubscript{2}O isotope technique has been used. The analysis method of Blagojevic et al was utilised for the determination of D\textsubscript{2}O levels in patient’s plasma. D\textsubscript{2}O was used in our study because of its relatively cheaper price (approximately $585 per kg) as compared with the other readily available isotopes, such as H\textsubscript{2}\textsuperscript{18}O. It also had the advantage of being stable (non-radioactive) and having negligible toxicity in trace doses. These properties especially aided in obtaining Medical Research Ethics Committee approvals and in recruiting subjects for the study. Also, the preparation and detection procedures are relatively simpler and less expensive. With isotopes such as tritium oxide, their radioactive properties make their use inappropriate in children and pregnant women.

For the detection of D\textsubscript{2}O in biological fluids, a number of different techniques have been reported in the literature. These methods were developed during the 1970’s and early 1980’s and although they all had certain advantages, they required specialised and/or expensive equipments making their use as a routine test or as a research tool in a small laboratory, rather difficult. With D\textsubscript{2}O, the suggested technique of Blagojevic et al, Fourier Transform Infra Red (FTIR) analysis, was adopted in this study. The FTIR technique is sufficiently sensitive to measure low levels of D\textsubscript{2}O in biological samples or water.
The techniques and advantages of the use of FTIR are reviewed by Skoog and Leary. The FTIR method involved two phases: a) the preparation phase; and b) the analysis phase.

### 7.2.1.1 Preparation Phase

The preparation of samples is essential as the measurement of untreated samples has been reported to consistently be approximately 7% lower than treated samples. During the preparation phase the biological fluid sample was subjected to vacuum sublimation in order to extract the “pure” water/D$_2$O mixture. As noted by Blagojevic et al, although the vacuum sublimation method has a number of advantages, the procedure is time consuming and can severely limit the throughput of samples. From our experience, a maximum of only 12 samples could be prepared for FTIR analysis in a day. As a result, a new preparation technique was developed and validated (see Chapter 8.3). The new technique involves ultrafiltration of plasma samples and although is more expensive than vacuum sublimation ($5 per sample), it dramatically increases the sample throughput. The number of samples that can be prepared per day is limited by the centrifuge’s bucket size and number.

### 7.2.1.2 Analysis Phase

The analysis phase involves the measurement of D$_2$O concentration in the “prepared” samples. This is carried-out in an FTIR apparatus. The FTIR facility used for this study was the one at the Department of Chemistry, University of Sydney. Problems of “drift” has been pointed out in the literature. Here, the D$_2$O absorption band drifts as its intensity decreases as the sample is heated by the infrared source during
measurement. The facility at the University of Sydney has an appropriate cooling system incorporated in the FTIR facility to maintain a constant temperature and prevent such drifts. The precision of the FTIR analysis technique has been reported \cite{727} to be 1% standard deviation (SD) for 1.0 g/l to 2.6 g/l range and has been suggested that the D$_2$O dose of 1 g/kg body weight can be reduced by 50% without reducing the accuracy \cite{727}. The accuracy of the FTIR analysis technique for the detection of D$_2$O in water is reported in the literature \cite{737} to be between 1.3% to 2.5% depending on the type of instrumentation used.

### 7.2.2 Deuterium Oxide Dose And Toxicity

Deuterium oxide (“heavy water”) is readily absorbed by the gastrointestinal tract and rapidly equilibrates with the body fluids. Pure D$_2$O is not too palatable to rodents, since neither mice or rats drink it readily \cite{738}. Addition of salt or aeration seems to make it more palatable to rodents \cite{738}. Early animal studies carried out on rodents has shown that as the concentration of D$_2$O is increased in the drinking water, the amount of water drunk by the animal is decreased \cite{739,738}. To humans, pure D$_2$O seems merely insipid, tasting like distilled water.

Majority of the research on the effects and toxicity of D$_2$O has been carried out in the 1950’s, 1960’s and early 1970’s. The main adverse effect of D$_2$O is the positional nystagmus and dizziness. To our knowledge, there have been no reports of D$_2$O-induced toxicity at the current dose level (1 gram per kg of LBM) nor were there toxicities observed by us in our study. There have been early studies of D$_2$O-induced toxicities when much higher doses of D$_2$O had been administered to individuals and animals under experimental conditions \cite{740,739,738}.

Reports from the 1950’s \cite{741} and 1970’s \cite{742} indicate that the administration of D$_2$O (100 grams adults and 2 gram per kg body weight children) was a routine clinical and experimental
procedure for the estimation of TBW levels in humans. These reports also show that no reports of nausea, dizziness, or nystagmus were associated with these dose levels.

It has been cited that it is a routine clinical and experimental procedure \cite{742, 741} to administer 100 g of D\textsubscript{2}O to adult patients for the determination of their TBW, or 2 g/kg to children \cite{743}. There have been no reports of nausea, dizziness or nystagmus associated with these techniques \cite{739}.

### 7.2.3 FTIR Analysis Technique

The FTIR instruments are considered to have a “multiplex” design. Most multiplex instruments depend on the Fourier transform for signal decoding and, as a result, are referred to as Fourier Transform instruments. This type of spectroscopy was originally developed by astronomers to enable them to detect and study the infra red spectra of distant stars. The Fourier Transform technique was able to resolve the weak infra red signals received from the distant stars from the environmental background noise. The Fourier Transform Spectroscopy has three main advantages \cite{735} over other techniques (such as the dispersive instruments):

1. The “through-put” or “Jaquinot Advantage” allows the power of radiation (in our case, infra red radiation) reaching the detector to be much greater than the dispersive instruments, resulting in a much greater signal-to-noise ratios. This is true over most of the mid-infra red spectral range;

2. The extremely high wave length accuracy and precision in Fourier Transform instruments also results in good signal-to-noise ratios; and

3. The “Multiplex” or “Fellgett Advantage” allows rapid (often one second or less) acquisition time for an entire spectrum.
Using the Fourier Transform spectrometers for infra red analyses has the added benefit that the interferometer is free from the problem of stray radiation because each infra red frequency is, in effect, modulated at a different frequency.

The resolution of a Fourier Transform spectrometer is simply defined as the difference in wave number between two lines that can be separated by the Fourier Transform instrument \[735, 734\], i.e.:

\[
\Delta \nu = \nu_1 - \nu_2
\]

where \(\nu_1\) and \(\nu_2\) are wave-numbers for a pair of barely resolvable lines.

The procedure for determining transmittance or absorbance with Fourier Transform instruments as described by Skoog and Leary \[735, 734\] was followed in our study to determine the D\(_2\)O concentration in a vacuum sublimed or ultrafiltered sample of plasma which essentially was a D\(_2\)O/H\(_2\)O mixture. The first step in this procedure, as recommended by these authors, is to obtain a reference interferogram by scanning a reference (in our case, air) 20 or 30 times, co-adding the data and storing the results in the instrument’s memory. In the FTIR instrument used for our study, there were 256 scans and the data was converted to, displayed, and stored as a spectrum. The sample to be analysed received the same treatment. The ratio of sample and reference spectral data is then computed to give the transmittance at various frequencies.
7.2.4 Bioelectrical Impedance Analysis (BIA) Methods

BIA technique has been developed to allow rapid bedside measurements of TBW, as few clinically accessible methods are available to evaluate fluid losses during surgery [744]. The reports on the use and acceptance of BIA as a clinical tool to measure TBW and/or its components have, however, been controversial. While some researchers in the early to mid 1990's have found BIA to be a useful and viable tool [746, 748, 745, 747], others have concluded that it is neither useful nor sufficiently accurate for clinical use [746].

The BIA technique has been reported to be able to accurately measure changes in TBW in cancer [749], renal dialysis [686, 750], and surgical [751] patients. Unlike the isotope dilution techniques, BIA is non-invasive, painless, portable, and only requires passive patient involvement. It also has the advantage of being cheaper, having a rapid turn around time of results, and ease of use enabling it to be used at the patient's bedside. In terms of accuracy, the D₂O technique has been reported to be over-estimating TBW by 4 to 5% [729] and the BIA having an uncertainty of 2 to 4% for TBW prediction [746].

BIA is an electrical method of assessing human body composition by quantifying TBW, fluid volumes, BCM, and FFM through measuring tissue conductivity. This measurement has the advantage of assessing different degrees of hydration of lean tissue. BIA works on the basic principle that under stable conditions the conductivity of a body segment is directly proportional to the amount of electrolyte-rich fluid present. It assumes that the body is a cylindrical shaped ionic conductor where the extracellular as well as intracellular non-adipose tissue compartments act as resistors and capacitors, respectively.

In addition, the BIA technique also assumes that electrical conductivity varies through fat and body fluids: the conductivity is higher in the FFM which contains virtually all the water and conducting electrolytes than the fat mass. The accuracy and/or precision of electrical techniques, however, have been reported to be dependent on the subjects’ hydration state [752].
which needs to be taken into consideration when taking measurements. One point to note is that the bioelectrical impedance techniques have been largely validated in normal healthy individuals and reports of its use or validation under disease conditions are relatively few. One study in cancer patients [753] reported in the literature utilised bioelectrical impedance technique for body composition measurements. Here, the authors concluded that bioelectrical impedance techniques are useful and accurate means of body composition estimations in patients with cancer when compared with reference methods. A number of different equations have also been developed to be utilised with the bioelectrical impedance machines. Although, originally there were only a few of these equations, more equations have been produced which are specific for different patient/subject groups as well as being sex-specific [754].

At the Royal North Shore Hospital and for our research projects, clinical trials, as well as routine clinical assessments the three equations of Fredrix [753], Pullicino [755], and Kushner [747] to estimate TBW are used and reported. These equations are shown below.

The Pullicino [755] equation was developed and derived from healthy subjects, whereas the Fredrix [753] equation was developed in patients with cancer. As indicated in the original article, the Watson [725] equations, "… most of the individuals were healthy volunteers, some were patients hospitalised for minor disorders with no clinical evidence of oedema …". The Kushner [747] equation included a wider range of subjects, which included patients with obesity, diabetes, inflammatory bowel syndrome, and on TPN.

In the following equations, H is the height in centimetres, W is the weight in kilograms, A is the age in years, and R is the resistance in ohms.

**Equation 5. Kushner equation for TBW estimation for males.**

\[
TBW = 8.399 + 0.396(H^2/R) + (0.143W)
\]
\[ TBW = 8.315 + 0.382\left(\frac{H^2}{R}\right) + (0.105W) \]

Equation 7. Pullicino equation for TBW estimation.
\[ TBW = 0.585\left(\frac{H^2}{R}\right) + 1.825 \]

Equation 8. Fredrix \[753\] equation for TBW estimation.
\[ TBW = 8.9 + 0.5\left(\frac{H^2}{R}\right) \]

Equation 9. Watson \[725\] equation for TBW estimation for males.
\[ TBW = 2.447 - 0.09516A + 0.1074H + 0.3362W \]

Equation 10. Watson \[725\] equation for TBW estimation for females.
\[ TBW = 0.1069H + 0.2466W - 2.097 \]

Another method which is related to the BIA technique is the total body electrical conductivity technique and it utilised electrical conductivity rather than capacitance. The equipment for this technique is larger and more difficult to use than the ones used for the BIA technique.

7.2.5 Other Total Body Water Measurement Methods

The isotope dilution techniques are considered to be the "reference standards" in TBW determination. The isotope dilution techniques currently available for use in biological samples use either tritium oxide (T\textsubscript{2}O), oxygen-18 (H\textsubscript{2}\textsuperscript{18}O), or D\textsubscript{2}O. Following their administration, these isotopes are then detected in various body fluids, such as plasma, urine, and saliva, using appropriate analytical techniques. In this study, the D\textsubscript{2}O isotope dilution technique was used as it is relatively cheap and is not radioactive. The analytical method \[727\]
used with the addition of the 1.04 correction factor [755], involved the use of FTIR analysis for
the determination of D$_2$O levels in the patient’s plasma.

The technique of TBW estimation by D$_2$O dilution (or isotope dilution in general) and the
FTIR analysis technique, may not be suitable for use when patient or subject numbers are
large, such as in routine clinical practice [750, 727, 751]. This is mainly due to the long preparation
phase required for FTIR analysis and the reliance on full cooperation from patients for D$_2$O
administration as well as fluid balance recordings, and access to complex and expensive
equipment not available in most laboratories and hospitals. There is, therefore, a need for
bedside measurements of TBW for its assessment in individual patients and to study
therapeutic outcome in groups of patients.

Estimation of TBW from LBM is amongst the non-invasive techniques currently available.
Here the body fat is calculated from skinfold thickness using the Durnin and Womersley's [756]
equations and then LBM is, in turn, calculated from the body fat. The error for the
measurement of body fat is around 10%. However, as fat can be 25% to 45% of body mass, it
implies an error of 3% to 5% of body weight. In addition, this measure does not take the
differences in the hydration of LBM into account.

TBW may additionally be estimated using the 58% body weight (58%BW), Watson equations
[725], and from LBM (i.e.. 73.2%LBM) [757].

Our results of comparisons of different methods of TBW analysis has been published in
international peer-reviewed scientific journals [758, 686].
7.3 Body Fat Measurements

The measurement of body fat is more common than the measurements of either TBW or TBN. Majority of fundamental research into the measurement(s) of TBF has been carried out in the 1970’s, 1980’s, and early 1990’s. It has developed, together with the measurements of LBM or FFM, with the increase in sports participation and the prescription of exercise. The measurements of TBF and its quantification is also related to the treatments of obesity [759], used for monitoring of TBF during pregnancy and lactation [760] and as a tool for the assessment of nutritional status under various disease conditions. Other physiological and medical importance of body fat includes its role in affecting the effectiveness of drugs and anaesthetics and affecting the ability of human or mammalian tolerance to cold and starvation. Measurements of body fat has been suggested [761] to be of invaluable psychological value in weight-loss programs as it allows both patient and physician the reinforcement of seeing that the weight lost is from the fat compartment of the body, and at the same time, the measurements of body fat provides a “control” to prevent excess weight loss which can lead to other complications. In our study, it was found that the female breast cancer patients are very conscious of their appearances and generally notice the gain in weight which was associated with chemotherapy. Measurements and reports of body composition in these subjects reassured them that the gain in weight was mainly due to an increase in the TBW and only to a very small extent body fat.

Body fat measurements are highly reviewed and reported in the literature and the efficacy and efficiency of measurement techniques in various subject groups tested and compared. As the measurements of body fat are very common, there are a number of different techniques available for its evaluation. The method(s) used under various conditions depend on the
expertise, need, and the degree of accuracy and/or precision required. The technique (used in our study) which is probably the simplest and has the advantage of low cost and reasonable validity is the estimation of body fat (and body density) from skinfold thickness measurements \cite{763, 759, 762, 764, 765, 766, 767, 756, 768, 772, 773, 769, 771, 770} utilising the Durnin and Womersley equations \cite{774, 756, 772, 770}. Some of the other techniques and equipment currently used to measure body fat (some directly but mainly indirectly) include: body mass index centile charts \cite{775}, body mass index \cite{777, 776}, infra red interactance techniques \cite{778, 752}, total body electrical conductivity \cite{779}, bioelectrical impedance analysis \cite{779, 781, 752, 780, 754}, dual-energy X-ray absorptiometry (DEXA) \cite{782, 783, 784}, whole body counting of potassium-40 (\textsuperscript{40}K) isotope \cite{785}, oxygen-18 (\textsuperscript{18}O) \cite{786}, tritiated water dilution \cite{761}, and in vivo neutron capture analysis (IVNCA) \cite{788, 787}. The simple techniques of estimating TBF, such as anthropometric techniques and height and weight tables, are generally quite satisfactory when the clinician is measuring the degree of obesity in patients, especially the grossly obese. These techniques, however, have limited value when used on subjects who appear only slightly overweight or their overweight state is as a result of athletic conditioning \cite{761}. It is in these subjects that more accurate means of body fat estimations are said to be required \cite{761}.

It is very important to bear in mind that all TBF measurements carried out today on patients and healthy subjects are carried out using indirect methods. This is to say that the techniques used do not involve carcass analysis. Therefore, it is crucial that the most appropriate methods and equipment be utilised so that the TBF estimations are as accurate as possible under the given circumstance(s).
7.3.1 Gold Standard

As one can expect, the measurement techniques mentioned above have their advantages as well as their disadvantages. Although new techniques are rapidly being developed and used for the measurement of body fat, the “gold standard” (or criterion method) is still considered by many to be hydrodensitometry or under water weighing [781, 792, 752, 759, 789, 790, 791]. This technique although accurate and reliable, it requires specialised equipment and expertise. It is also found to be rather uncomfortable for the subject being measured. As this technique requires the subject to be completely immersed in water to be weighed as well as having to completely exhale and hold their breath while immersed, it is rather not suitable for subjects who are ill, elderly, with respiratory problems, or afraid of water. The appropriate calculations and assumptions required for densitometric analysis of body composition has been reviewed in detail by Brozek et al [793] in their 1963 paper. As with any technique and equipment, errors and accuracies associated with hydrodensitometry have also been reported in the literature [794, 790]. One of the problems associated with this technique is temperature variations that may occur during measurements that can lead to errors: Fidanza et al [795] investigated the density of the human and mammalian body fat at various temperatures and found that the density and hence the values obtained from hydrodensitometric measurements vary with temperature.

7.3.2 Bioelectrical Impedance Analysis (BIA) Methods

Electrical methods of estimating body fat have become popular in last decade or so. Reports indicate their close correlation with the “gold standard” techniques such as hydrodensitometry [752]. In terms of their cost, ease of use, and portability, techniques such as bioelectrical impedance and infra red interactance have gained interest and their use in epidemiological studies has been increased. The added advantage of being non-invasive and requiring no
active collaboration of the patient/subject makes them even more attractive for both research and clinical use. The safe nature of these techniques has allowed their use in human infants for rapid and accurate body composition assessments [796]. However, the bioelectrical equipment tend to be much more expensive than the standard skinfold callipers for the estimation of body fat.

At our centre and in these studies, the equations used to calculate the BIA-derived LBM were the ones of Lukaski [797, 798], Segal [799, 754], and Van Loan [800, 780]. Our results of comparisons of different methods of TBW analysis has been published in international peer-reviewed scientific journals [758, 686].

Another method which is related to the BIA technique is the total body electrical conductivity technique and it utilised electrical conductivity rather than capacitance. The equipment for this technique is larger and more difficult to use than the ones used for the BIA technique. Results reported in the literature [780] on the use of total body electrical conductivity technique show more accuracy than densitometry and hydrometry in FFM estimations.

### 7.3.3 Nuclear Techniques

Nuclear methods of estimation of TBF have also been reported in the literature [788]. These involve direct measurements of LBM and then estimating TBF from LBM. Nuclear techniques, in general, tend to require expensive and specialised equipment as well as operator expertise. They, however, tend to be accurate and non-invasive which makes them suitable for ill patients. The three most common techniques used are the in vivo neutron capture analysis, dual energy X-ray absorptiometry, and whole body potassium ($^{40}$K) counting.
In a study by Cohn et al. [788] in vivo neutron capture technique was utilised to measure TBN, TBCI, and TBCa. The TBW was estimated by dilution techniques and total body potassium which is an index of body cell mass was measured using whole body counting method. Here, it was concluded [788] that nuclear techniques are reliable methods for estimating TBF. A similar study carried out by Morgan and Burkinshaw [766] utilising total body potassium and TBN measurements suggested sex differences in the techniques: average values of TBN and total body potassium were greater in men than women with the relationship between total body potassium and TBN being identical in both sexes. Studies of Hill et al. [801] in the late 1970’s showed high correlation between the FFM calculated from simple anthropometric measurements (skinfold thickness) and FFM calculated from TBN measurements (IVNCA) despite the fact that the subjects used had a wide range of nutritional status. They also indicated that FFM may be able to be used as an indicator of TBN. The above mentioned nuclear techniques, although reliable and accurate, have the disadvantage of not being readily available at all centres making it unattractive for the average nutritionist, clinician, or researcher.

The total body potassium measurements is probably one of the simplest of the nuclear techniques and involves the measurement of the naturally occurring radioactive potassium-40 ($^{40}\text{K}$) in the body at a fixed proportion to the total potassium that is assumed to be constant at 0.012% for all potassium in the biosphere. The whole body counting of $^{40}\text{K}$ is, therefore, used to determine total body potassium as a measure of body cell mass. This requires the patient or subject to lie still for approximately 20 minutes in a small steel chamber. The technique can be vulnerable as great care must be taken to isolate the patient or subject as well as the detectors from other sources of radioactive decay. This includes other people nearby. Again, this technique requires specialised equipment and expertise which may not readily be available to many researchers and clinicians.
An alternative method to the $^{40}$K measurement to estimate the body cell mass reported in the literature in the late 1970’s is the exchangeable sodium to potassium ratio $^{[802]}$. This technique may not be very popular with many subjects as it requires the injection of tritium and sodium-22 ($^{22}$Na) both of which are radioactive (2.4 mSv). However, it has the advantage of not requiring the cooperation of the patient or subject for long periods of time making it particularly suitable for the critically ill and uncooperative patients.

### 7.3.4 Skinfold Thickness

Historically, Brozek and Keys were reported to be the first investigators to utilise the relationship between skinfold thickness and body density as a means to estimate TBF content in humans. In our study, TBF was estimated using skinfold callipers as it is one of the most common techniques used today. The %BFat was subsequently estimated using the Durnin and Womersley’s equation $^{[756, 770]}$. As with the bioelectrical impedance techniques, there are a number different equations for the estimations of TBF from skinfold thicknesses. However, due to its longer history, the number of equations is relatively higher: In a review by Lohman $^{[803]}$ in the early 1980’s, it was reported that since the 1950’s, there has been over 40 studies carried out on the prediction of TBF from skinfold thickness measurements. This has resulted in excess of 100 equations.

The use of skinfold thicknesses to estimate TBF comes from the fact that the subcutaneous adipose tissue contains a large proportion of the human body’s total fat content. Its ease of access to this fat as well as being non-invasive has made this technique of TBF estimation quite attractive to researchers and clinicians. As with other techniques, there are certain assumptions made when estimating TBF using skinfold thicknesses. These assumptions as
well as the available evidence have been reviewed in detail by Martin et al [765] in the mid 1980’s.

The first step in the measurement of TBF from skinfold thickness is the measurement of the thickness of the compressed double layer of skin which includes the subcutaneous adipose tissue using skinfold callipers. The assumption made here is the existence of constant compressibility. This, however, is not entirely true: after the initial application of the skinfold callipers, the calliper reading seem to decline. One method often used to overcome this is to time the calliper reading, i.e. to take the reading after a standardised time of, for example ten second, after the skin is compressed. This, however, does not eliminate the effect of variable degree of tissue compressibility. Difference in the skinfold compressibility between sexes has also been observed [756] which has been suggested to account for the altered relationships seen between body density and skinfold thickness in males and females.

The thickness of skin also plays an important part during the measurements of skinfold thicknesses and the assumption often made is that the thickness of skin is either negligible or is of a constant fraction of the skinfold. This effect of the skin has never seriously assessed in the literature.

The actual thickness of the subcutaneous adipose layer poses another problem. The thickness of this layer is not constant all over the body. If this thickness was constant, then measuring subcutaneous adipose layer at one site would provide an accurate estimate of the volume of this tissue. Therefore, the assumption made here is that the thickness of the subcutaneous adipose layer is constant all over the body. High correlations between the directly measured adipose tissue thicknesses and the measured subcutaneous adipose tissue have been found for the lower limb sites [765].

The mass of total subcutaneous adipose tissue and the total mass of the subcutaneous fat is also not constant. In other words, fat is unevenly distributed under the skin and this difference
is exaggerated with increase in body fat \[768\]. Two identical thicknesses of adipose tissue can contain different concentrations of fat. Therefore, it needs to be assumed that there is a constant fat fraction in adipose tissue. Another problem that arises with the use of skinfold thickness measurements to estimate TBF is the relationship between the subcutaneous fat and the internal fat content. The assumption made here is that there is a constant relationship between the subcutaneous fat content and the internal fat content (i.e. they being proportional). Studies of Martin et al \[765\] has shown that there are high correlations between internal to subcutaneous adipose tissue masses in both males and females. The density of fat has been assumed to be fairly constant and equal to 0.90x10\(^3\) kg/m\(^3\) by Durnin and Womersley \[756\] in their 1974 paper investigating the estimations of body fat from skinfold thickness.

The skinfold thickness technique used in our study was adopted from the technique of Durnin and Rahaman \[770\] with the difference that three sites (biceps, triceps and, subscapular) rather than the four was utilised in our study. Reports in the literature \[768\] suggest that at least three skinfolds are necessary to ensure a correct assessment of body fat. The equation used by Durnin and Rahaman for the calculation of TBF was based on Siri’s equation \[804\] (Equation 11) which is as follows:

\[ \text{Equation 11. Siri’s equation.} \]

\[
\text{Percentage fat} = \left[\frac{(4.95/\text{density}) - 4.50}{100}ight]
\]

Durnin and Rahaman \[770\] found that the thickness of the four skinfolds is of the same order for men and boys with the men having slightly higher mean skinfold thickness. The thicknesses of the four skinfolds were also found to be of the same order for women and girls. They reported that the correlations between skinfold thickness and density to be different for boys and young men but the same for girls and young women.
Chapter 7: IN VIVO BODY COMPOSITION

The paper by Durnin and Rahaman [770] also reported regression equations for men, women, boys and girls for the prediction of body density \( y \) from the log of the sum of skinfold thicknesses at all four sites \( x \):

**Equation 12. Regression equation for the prediction of body density in men.**

\[
y = 1.1610 - 0.0632 \log(x)
\]

**Equation 13. Regression equation for the prediction of body density in women.**

\[
y = 1.1581 - 0.0720 \log(x)
\]

The calculation then involved calculating the sum of the skinfold thicknesses, converting them into their logarithmic value and substituting them into the appropriate equation above. This gave a value for the body density which was then substituted in the Siri’s equation [804] (Equation 11). No exact conclusion was drawn for the relationship between TBF content and body density in adolescents and different relationships were suggested that may exist between skinfolds and TBF in the middle-aged and elderly individuals as compared to the young adult. Later, in their paper of 1974, Durnin and Womersley [756] indicated that the relationship that exists between body density and skinfold thickness is not linear neither in males or females especially in the obese individuals where large increments in skinfold thickness are associated with only small changes in body density. The relationship was suggested to be logarithmic or quadratic. Also, they indicated that a given skinfold thickness corresponds to a considerably lower body density in women than in men which could mean that in women a greater fraction of the body fat is internal rather than subcutaneous. The above mentioned sex differences have also been reported by others [768] in the literature.

Age is another factor that can and does alter the relationship between skinfold thickness and body density. Aging affects the density of the FFM primarily when bone mineral content is decreased with increasing age. This decrease was suggested [756] to account for the different
regression lines obtained for men and women as women lose a greater percentage of their bone mineral and at a different rate to that of the men. Durnin and Womersley\textsuperscript{[756]} suggested a density of 1.11x10\textsuperscript{3} kg/m\textsuperscript{3} for the FFM. This was broken down into 72% water of density 1.00x10\textsuperscript{3} kg/m\textsuperscript{3}, 20% protein of density 1.34x10\textsuperscript{3} kg/m\textsuperscript{3} and 7% mineral of density 3.00x10\textsuperscript{3} kg/m\textsuperscript{3}. However, they\textsuperscript{[756]} have concluded that the observed change with age in the relationship between skinfold thickness and body density cannot be accounted for by the changes in the FFM composition but is likely to result from the decrease in the proportion of the subcutaneous TBF.

Among other methods and equations reported in the literature is the use of body surface area for the derivation of equations to estimate TBF from skinfold thickness measurements. The argument put forward by Katch et al\textsuperscript{[767]} for this is that the use of body surface area exploits the anatomical relationship between subcutaneous fat from skinfold thicknesses and body surface area. These authors suggest that the regression equations that are currently used to estimate TBF from body density are mainly population-specific and are restricted to the homogenous samples from which they were originally developed whereas the body surface area-derived equations will more “individualise” the measurements.

Skinfold thickness measurements using the skinfold callipers has the advantage of being cheap and simple. With the exception of bioelectrical impedance method, it is the only suitable technique for field measurements. It, however, has the disadvantage of being sensitive to inter-observer error and requires training and cross-validation of investigators. This disadvantage is dwarfed by the ready availability of this technique as compared to others in the measurement of TBF.
7.3.5 Significance of Lean Body Mass

Reports of TBF, TBW, and TBN (or protein) in the literature talk about the concepts of “Lean Body Mass” (LBM) and “Fat Free Mass” (FFM). In general, the loss of either of these are considered to have a negative impact on the patient’s health, quality of life, and response to therapy. These two concepts are slightly different although they both originate from the same idea of the division of fat mass and non-fat mass. The fat in cell membranes is excluded in FFM but is included in LBM due to the anatomical proximity of this small amount of fat to lean tissue \[805\]. The LBM, however, is made up of body cell mass and the non-fatty intracellular connective tissue, which includes bones (and not fatty bone marrow), basement membranes, tendons, ligaments, etc. Nearly all of the body’s metabolic processes take place within the body cell mass \[805\]. Since the early 1990’s, correlations of LBM and the immune competence as well as functional status have been suggested and reported in the literature \[806\]. LBM has been reported to be a major predictor of functional capacity as well as morbidity and mortality under many circumstances. Therefore, LBM can be considered to be a method of determining, monitoring, and measuring the impact of metabolic changes over time on the body. Generally, conditions which have been known to causing metabolic changes and the loss of LBM include acute and chronic illness, starvation, and aging.

The metabolic changes induced by starvation are the best example of the effects of LBM depletion. Reports in the literature suggest that death occurs when the loss of LBM is approximately 40% \[807\]. Human and animal data \[807\] available suggest that during the progression of malnutrition, muscle cells first shrink (loss of intracellular water) and then are completely lost, and this loss is mainly protein.

Loss of LBM is considered to be a part of normal aging. This is said to be slowly progressive until the age of about 65 years and then a rapid decline \[806\]. The progressive loss of LBM is
then accompanied by the physiological and immunological changes frequently seen in the elderly.

During acute and chronic illness, such as after major surgery, physical trauma, or severe burns, large negative nitrogen balance is often observed for several weeks \cite{808}. In a large (greater than 3000 subjects) group of cancer patients matched for their type, grade, and stage of cancer, loss of body weight was found to be a strong predictor of shortened survival \cite{188}. In subjects with rheumatoid arthritis, increased mortality from infectious diseases has been reported in the literature \cite{806}. Death from infectious diseases is a strong indicator of starvation, so there is strong support for the hypothesis that loss of LBM plays a role in increased mortality in rheumatoid arthritis \cite{806}. Similarly, reduction in human defence system has been suggested to be directly caused by the loss of LBM and protein calorie malnutrition \cite{806, 809, 810}. Loss of LBM is also associated with the loss of other minerals from the body. Negative balances of calcium, potassium, and phosphorous have been found to be associated with the negative nitrogen balance during the loss of LBM \cite{811}. 
7.4 Fat Free Mass (FFM) Measurements

At the Royal North Shore Hospital the method of choice for the estimation of FFM is the Whole Body Potassium Counting which is used to estimate TBK.

Whole Body Counting is referred to the measurement of induced or naturally occurring radiation in the body. In a low radiation setting it is performed in a shielded room or chamber to reduce background radiation. In a higher-than-background setting, shielding is not required and instead, the background radiation is subtracted from the total radiation detected/measured. TBK has been used as a measure of FFM, of muscle mass, or (more accurately) of cell mass. FFM itself refers to all body tissue excluding fat: skeletal muscle, visceral organs, bone and skin, and body water, as well as hair, blood, and lymph. Because of its association with the metabolising, oxygen-consuming portion of the body, a decline in TBK is usually interpreted as a loss of muscle mass due to a catabolic condition [813, 812]. It is, however, assumed that adipose tissue contains negligible potassium, and that the fat-free tissues of the body contain a constant proportion of potassium.

The Whole Body Potassium Counting facility at this centre and used in these projects/clinical trials was calibrated by Hansen and Allen [814] in the mid-1990’s for the estimation of FFM from TBK measurements.

The principle behind the Whole Body Potassium Counting to estimate TBK is relatively simple. The technique works on the principle of the constant ratio of the naturally occurring potassium-40 ($^{40}$K) to potassium in all living matter: $^{39}$K (93.26%), $^{40}$K (0.0117%) and $^{41}$K (6.73%). The average potassium content of an adult male is approximately 140 grams, or about 15 milligrams of $^{40}$K. This translates to a natural signal (1.46 MeV γ-rays) given off by the body of 30,000 γ/minute. In adult females, the level is about 20,000 γ/minute.
TBK is measured by supine geometry NaI counting. The precision and accuracy of this method, expressed as coefficients of variation, are 1.5% and 4.5% respectively \[8^{14}\]. TBK was used to estimate FFM, assuming that the potassium content of FFM is 2.26 g/kg in females and 2.52 g/kg in males \[2^{04}\].

SMM can be estimated from TBN and TBK using the Wang’s equation \[8^{15}\]. The FFM can then be estimated using the four compartmental body composition model. This is a widely-used body composition model which assumes that the body consists of fat, protein, water and mineral compartments \[8^{16}\] \[8^{17}\]. In this model the TBW is estimated using BIA technique (in kg), the total mineral content is calculated from the bone mineral content (in kg as bone mineral content/0.84) \[8^{18}\], and the TBP (in kg) from TBN measurements using the IWNCA technique.

**Equation 14. Wang’s equation for the estimation of SSM.**

\[
SMM = (0.188 \times TBK) + (0.00183 \times TBN)
\]

where TBK and TBN are in g and SMM\textsubscript{KN} in kg.

**Equation 15. Estimation of FFM from a four compartmental model.**

\[
FFM = TBP + TBW + \text{Total Mineral Content}
\]

It should be noted that in these postgraduate projects/clinical trial the Whole Body Potassium Counting was only used to investigate the changes in the FFM and was not utilised to estimate the SMM.
7.5 Quality Of Life Measurements

As this study is concerned with improving body composition and reducing cachexia to reduce therapy-induced complications and increase survival, it is essential that the “quality of life” of the patients be monitored and assessed. In the case of patients with metastatic disease, the chemotherapy is used mainly to provide palliation, and the appropriate end points are measures of the duration of survival and the quality of life during the survival period. The selection of correct and most suitable scale or index to be used to assess the quality of life, although essential, is generally not very easy. This is especially true as the “quality of life” also includes a number of other factors in addition to the general feeling of well-being, i.e. quality of life is multi-factorial. Therefore, a combination of two or three quality of life scales or methods would provide a better assessment of the overall quality of life. There are numerous examples of such studies in the literature [819] including this project.

There are a number of different scales or indices available for patients with malignant diseases [820]. However, in our study, special consideration has to be given to the fact that the questionnaire incorporating the scale or index is to be filled by the patient rather than being administered by the clinician or the researcher. This, together with the fact that the questionnaires have to be short and simple to maintain the patient’s interest, narrowed the list down to three scales or indexes which are reviewed below. A brief scale is generally considered also to be more cost effective.

The question that often arises is that why do people measure the quality of life in patients anyway? Particularly in cancer and terminally ill patients, there are three main reasons that a measure of the quality of life is required which are mainly associated with accurately estimating the length of life remaining to cancer patients:
• The measurement and use of quality of life enables the clinicians to advise the patient and their families on the general prognosis of the disease, and as a result, to enable better planning for the care of the patient.

• From the statistical and epidemiological data currently available, it is very difficult to accurately estimate the need for services for the terminally ill cancer patients. The knowledge of the patient’s quality of life enables a better forecast of the services required and various points in time for an individual patient. Studies of Morris et al\(^{618,619}\) has shown that the mean QL Index scores declines as death approaches indicating the ability of the quality of life measure to reduce prognostic uncertainty in terminal care.

• The knowledge of the patient’s current and future status also helps to ensure that specialist terminal care resources are allocated to the patients who need them most.

However, a more important clinical question that has to be answered by quality of life assessments is whether the survival benefits are worth the adverse effects induced by cancer surgery and chemotherapy on the quality of life. Studies reported in the literature\(^{822,821}\) have indicated that its is quite feasible to carry out quality of life measurements in large multi-centre, international adjuvant clinical trials.
7.5.1 The EORTC Quality Of Life Assessments

The assessment of quality of life has been gaining interest with many researchers [825, 823, 824] and has been considered one of the major end-points in most clinical trials involving cancer patients [317], surgery [826], and pancreatic surgery [827]. The primary benefit in the use of quality of life assessment methods in research as well as under clinical settings is to assess the effect(s) and comparison between different therapies. For example, in pancreatic cancer patients it has been reported to have been used as a decision-making tool in the process of selecting the surgical procedure to provide maximum long-term survival [828, 829]. Also, quality of life questionnaires, completed by the patients themselves, may function as a surrogate marker or indicator to assess the severity of symptoms during disease progression where there may be major psychological and physical impact on the patients’ quality of life.

The actual “quality of life” is an entity which is difficult to define, subjective and comprises of many different factors and parameters. As majority of cases of pancreatic cancer are diagnosed at a late stage, the outcomes of therapies are mostly aimed at symptom palliation and perhaps to extend the survival period. The ultimate goal here is to return patients’ quality of life, as closely as possible, back to their pre-disease levels. The assessment of quality of life in this patient population is, therefore, considered to be of very high importance [317].

There also subtle differences in perception of the patients’ quality of life between the clinicians or medical staff and the patients themselves [830]. Here the perceived level or status of quality of life is not directly affected by the severity of the disease or symptom. In fact there are subtle mediating cognitive processes related to the perceived threat ascribed to each symptom [830]. It is, in fact, for these reasons that the EORTC’s QLQ-PAN26 was developed which incorporated the patients’ viewpoints.
For a good questionnaire that can be successfully used in a clinical trial, especially one carried out in pancreatic cancer patients, it must be psychometrically valid and reliable. Technique to test and confirm the validity and reliability of quality of life questionnaires have been detailed by Streiner and Norman [831, 832].

Although there are questionnaires available which are aimed at a specific patient population [834, 833], there are also a number of different questionnaires have been reported in the literature to be reasonably reliable and valid [836, 835] but are more “general”. These can successfully be used across different patient populations to assess the quality of life but suffer from an inability to assess the changes in specific groups or populations of patients. Questionnaires such as that of the HAD [820, 837] were designed to assess a particular aspect of the quality of life of patients.

The EORTC’s quality of life questionnaires were used for the assessment of quality of life in our EPA Clinical Trial on the group of pancreatic cancer patients undergoing chemotherapy with gemcitabine and receiving EPA or placebo.

The questionnaire used in our clinical trial consisted of the “core questionnaire” (QLQ-C30) and the pancreatic cancer-specific module (QLQ-PAN26). These were obtained directly from the EORTC by contacting the centre (D Fitzsimmons. University of Southampton, University Surgical Unit, Mailpoint 816, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK) and registering our clinical trial. These questionnaires are widely used in patients with cancer as well as other disease conditions [826, 838]. In fact the core questionnaire has been made and tested in nine different languages with the pancreatic module being translated into 16 languages and is currently being used world wide.

The development and use of the QLQ-C30 has been reviewed in the literature by Aaronson et al [839] and Sprangers et al [841, 840] in the 1990’s. The use of the QLQ-C30 plus its pancreatic module, the QLQ-PAN26, has been developed and reviewed in detail by Fitzsimmons et al
in the late 1990’s who also concluded that the combination can successfully be applied to and used in patients with chronic pancreatitis as well as post-pancreatectomy patients. Although these questionnaires have been developed for cancer patient populations, recent studies in the literature have even reported of its successful use to measure and investigate quality of life in other disease conditions, such as patients with Human Immunodeficiency Virus and various gastrointestinal diseases.

The QLQ-C30 core questionnaire has been shown to demonstrate very good reliability as well as validity and that it can be used in multicultural clinical research settings. Its versatility and usefulness probably comes from the fact that it was developed specifically as a core questionnaire to be supplemented by additional questionnaire modules to measure and investigate specific disease symptoms and therapy side effects of different but specific disease conditions. The QLQ-C30 core questionnaire contains questions which cover a range of quality of life questions and issues applicable to a wide range of cancer patients. The disease-specific module would then cover the items (or questions) which measure the quality of life that were not covered by the QLQ-C30 and was considered to be relevant for the target patient population. As an example, the QLQ-C30 core questionnaire may be supplemented with the specific lung module to be used to measure and investigate symptoms and side effects of patients with lung cancer.

The QLQ-PAN26 pancreatic cancer module, which was used in our clinical trial together with the QLQ-C30 core questionnaire, was intended by its designers to be used in pancreatic cancer patients of all disease stages as well as patients who are undergoing cancer curative or palliative therapy. These included chemotherapy, surgery, stents and, radiotherapy. For these reasons, the QLQ-PAN26 module contains questions about the patient’s body image, symptoms relating to their treatment and disease, and their outlook and thoughts on life and the future.
Chapter 7: IN VIVO BODY COMPOSITION

The QLQ-C30 itself contains 30 questions whereas the QLQ-PAN26 contains 26 questions. Although the combination of the two adds up to a total of 56 questions, the time taken to complete the whole questionnaires has been around 15 minutes. The time taken to complete the QLQ-C30 together with its lung cancer module has been reported [840] to be around 11 minutes when tested in 305 patients from 12 different countries.

In summary, from the available evidence in the literature, the combination of the QLQ-C30 core questionnaire and the QLQ-PAN26 pancreatic cancer-specific module produce a quick, reliable and valid questionnaire which has been demonstrated to be capable of successfully assessing the quality of life of post-operative pancreatic cancer patients undergoing chemotherapy.
8. METHODS: BODY COMPOSITION AND QUALITY OF LIFE MEASUREMENTS

The human body can be separated into four main compartments. These compartments are: the fat, water, protein, and bone (or mineral). The least variable of these compartments is the bone. Under normal conditions the relative levels of these compartments are constant. However, under disease conditions this balance is disrupted. Depending on the particular disease condition, different compartments are affected to a different extent. What is common between all diseases is that these levels must be returned to their “normal” relative levels in order for the patient to recover.

In vivo body composition methods allow one to measure and monitor these levels during normal, disease, and recovery states. This allows appropriate nutritional and therapeutic intervention to be individualised and applied at the correct time. The benefit of the in vivo body composition analysis techniques is that they are non-invasive and are safe and are relatively less stressful to the patients.

8.1 Body Fat, Skinfold Thickness, And Anthropometrics

The purpose of the skinfold thickness and anthropometric measurements were to enable the estimation of %BFat as well as measurements of patient dimensions that were essential for the TBN calculations using the IVNCA technique. All anthropometric measurements were carried out on the non-dominant side.
All anthropometric and skinfold thickness measurements were carried out while the patient was wearing minimum clothing. This was achieved by having the patient to strip down to their underwear and wear a standard hospital gown.

Skinfold thickness measurements were carried out on the three sites of triceps, biceps, and subscapular, with the patient in a standing position. The measurements were carried out similar to those of Durnin and Rahaman \cite{770} and Durnin and Womersley \cite{756, 772, 773} In order to carry out skinfold thickness measurements on the upper arm, first the upper arm mid point was identified. This point was the midway between the lateral projection of the acromion process of the scapula and the inferior projection of the olecranon process of the ulna. The skinfolds were picked-up between the thumb and the forefinger and the calliper jaws were applied. Measurements were read 3 seconds after when full pressure of the calliper jaws were applied to the skinfold and the thickness of the skinfold was recorded to 0.1 mm.

- For the \textit{triceps}, the skinfold was picked-up at the back of the arm about one centimetre above the level marked for the upper arm mid point and directly in line with the point of the elbow or olecranon process;

- For the \textit{biceps}, the skinfold was picked-up in front of the arm at the same level as which the triceps was measured; and

- For the \textit{subscapula}, the skinfold was picked-up under the angle of the left scapula with the fold being vertical or slightly pointing downwards and outwards. Slight twisting of the arm to the back was found to be very useful in less lean subjects.

All skinfold measurements were made in triplicates. The average value for each site was used for the estimation of \%BFat. In order to avoid skin fat layer compression, skinfold thicknesses
were measured three times in the order of biceps, triceps, and subscapula. This provided sufficient time for the fat layer to “spring-back” into its original shape.

The circumferences measured here were: chest, waist, mid-thigh, mid-calf, and mid-upper-arm. These measurements were made using standard tape measures in centimetres. As with the skinfold thickness measurements, these measurements were also done in triplicates and their averages were used in the study.

- The chest circumference was measured at the marked point where the fourth rib joins with the sternum and at right angles to the axis of the body at the end of a normal expiration;
- The waist circumference was measured at the narrowest part of the torso as seen from the anterior. This is to say at the minimal abdominal circumference between the lower edge of the rib cage and the iliac crests;
- The mid-thigh circumference was measured by first measuring and marking the mid point between the anterior superior iliac spine and the proximal patella, and then measuring the horizontal circumference around the thigh. For this measurement the subject is required to stand with their legs slightly apart and their weight evenly distributed on both legs;
- The mid-calf circumference was simply measured at the largest point of the calf;
- The mid-upper-arm circumference was measured at the mid-point marked for the biceps and triceps skinfold thickness measurements, i.e. at the midway between the lateral projection of the acromion process of the scapula and the inferior projection of the olecranon process of the ulna.

The three length measurements required were: the shoulder-to-hip, hip-to-knee, and the height. The former two were measured using standard tape measure and in triplicates. The
height was measured using a KabiVitrum™ Stadiometer which was permanently fixed to the
wall at the Body Protein Monitor unit.

- The shoulder-to-hip distance was measured as the length from the acromion to the anterior
  superior iliac spine;
- The hip-to-knee distance was measured as the length from the anterior superior iliac spine
to the proximal patella;
- The height was measured using a KabiVitrum™ Stadiometer, by standing the patient up
  right, bare feet, and with their back to the wall and the back of their heels touching the
  wall.
- The weight was measured using electronic medical scales. The one used at the Body
  Protein Monitor unit was the Wedderburn™ Scales, UWBW-150.

The anthropometric data as well as the skinfold thicknesses measured were then noted down
on the TBN Measurement request form. These were later entered into the computer database
when processing the TBN measurements.

8.1.1 Percentage Body Fat And Lean Body Mass

The %BFat was estimated from the skinfold thickness measurements at the three sites of
biceps, triceps and subscapula. The equations of Durnin and Womersley \(^{756}\) and Durnin and
Rahaman \(^{770}\) were used to estimate the %BFat. These equations had been incorporated in a
computer program (Alex Rose, Department of Radiation Oncology, Prince of Wales Hospital,
Sydney, NSW, Australia) which enabled easy estimation of %BFat as well as body density.
The program required the input of the subject’s age and sex as well as the skinfold thickness
values to be able to estimate the %BFat and body density. This program is not available commercially.

When the system was upgraded to Microsoft FoxPro® (Version 2.5) database, the above program was incorporated into the database so as to calculate the %BFat automatically when the TBN report is generated. However, the original program is being still used as a stand-alone program whenever %BFat estimation is required.

The LBM was calculated as follows:

**Equation 16. Calculation of LBM from %body fat and body weight.**

\[ LBM = Body \; Weight - \left( \frac{\%Body \; Fat \times Body \; Weight}{100} \right) \]

As with the rest of the anthropometrics and skinfold thickness measurements, the LBM and %BFat values were noted on the TBN request form as well as entered in the computer database.

### 8.1.2 Lean Body Mass By Bioelectrical Impedance Analysis

The LBM in all patients testing in our study was also measured using the BIA technique. The protocol used to carry out the required BIA measurements were identical to the one used for TBW and is detailed in section 1.1.3 of Appendix E. The only difference, however, was that the raw reactance and resistance values together with patient’s height, weight, and sex were put into the equations of Lukaski [797, 798], Segal [799, 754], and Van Loan [800, 780] using Microsoft Excel® (version 2003), to obtain the final LBM values.
8.2 Total Body Nitrogen Measurements

The TBN was measured by the In vivo neutron capture analysis or IVNCA technique\(^{[691, 694, 207, 692]}\). This technique enables the measurement of nitrogen and hence the muscle mass in a non-invasive manner. It, however, involves the bombardment of the patient’s body with fast neutrons emitted from an \(^{241}\)AmBe neutron source. The overall radiation received by the patient is 0.2 mSv and is comparable to a standard chest X-ray or approximately 10 hours flying in a jet aircraft at an altitude of 35,000 feet.

8.2.1 IVNCA Instrumentation

As with any elaborate scientific instrument, maintaining an up-to-date instrument log book is essential. This not only helps to track its use, but also is essential in its maintenance, service, and repairs. For this reason a log book for the IVNCA instrumentation was kept and maintained at the Body Protein Monitor Unit. All experiments, scans (both phantom and patient), as well as adjustments to the system was noted in this log book for future reference.

The IVNCA facility or set up can be divided into three physical sections:

- The IVNCA measurement area;
- The analogue-to-digital converter system;
- The multi-channel analyser card.
8.2.1.1 The IVNCA Measurement Area

The measurement area of the instrument is where measurement of patients are physically carried out. This is where the patient receives the neutron dose and the resulting gamma-rays are detected. The diagrams (Figure 12 and Figure 13) below schematically show the arrangement of the movable table, the neutron source, and the two sodium iodide detectors (BGO detector not shown).

Figure 12. The axial view of the IVNCA measurement area (not to scale).
The measurement area can be further divided into five different areas:

- The main body;
- Motorised movable table;
- Neutron source and its shielding;
- Sodium iodide detectors; and
- Bismuth Germanium Oxide (BGO) detector.

**Main Body of the Instrument**

The main body of the instrument had been constructed of wood. The motorised table and the detectors were attached to the table. The electric motor used to drive the table was located on
the underside of the main body. The main body of the instrument, therefore, served as a skeleton on which the other components were placed.

**Motorised Movable Table**

The motorised movable table was also constructed from wood and placed horizontally on the main body of the instrument. Steel cables attached to either end of the table were connected to an electric motor which pulls the table between the two sodium iodide detectors and back, over the BGO detector, and over the neutron source. A set of seven magnetic “gates” controlled the direction and number of table stops. These gates were placed on the side of the main body of the instrument and were triggered off by the magnet attached to the motorised table. The number of table stops as well as the duration of each stops were controlled through an electronic system located on the underside of the main body of the instrument.

**The Neutron Source and Shielding**

Two 592GBq Americium-241 ($^{241}$Am) sources (as Americium-Beryllium ($^{241}$AmBe)) with a source output of $4.0 \times 10^7$ n/s were used as the neutron source. The neutron source was located in a stack and collimator 57.8cm below under the plane of the 2cm thick wooden motorised table within the main body of the instrument. The beam aperture between the two bilateral sodium iodide detectors was 23 x 23cm on which the patient was passes to be irradiated. The stack was made of 23 x 23 x 15cm blocks of borated paraffin (approximately 50% boric acid of 1.1 g/cm$^3$ density). The neutron beam was collimated by a wedge-shaped collimator. Also, lead bricks were placed at the ends of this wedge as well as at the top of the stack to shield the two sodium iodide detectors from gamma-rays derived from the $^{241}$AmBe’s fission products ($^7\text{H}$ (n, $\gamma$) and $^{10}\text{B}$ (n, $\alpha\gamma$)) reactions. The purpose of the collimator was to collimate the
neutrons generated by the source towards the area between the two sodium iodide detectors where the patient lying on the motorised tabled passed.

**Sodium Iodide Detectors**

The two bilateral sodium iodide detectors (20 x 10cm) were placed perpendicular to and on either side of the direction of travel of the table and the neutron beam. The purpose of the two sodium iodide detectors was to detect the gamma rays emitted from the patient or the phantoms being scanned. The two detectors were, therefore, shielded in their longitudinal axis from stray neutrons and gamma rays. The shielding consisted of 5cm annular lead shields and then completely being encased by 5cm thick borated paraffin. Information gathered by these two detectors (in analogue form) were then sent through appropriate leads to the analogue-to-digital converter system.

**The Bismuth Germanium Oxide (BGO) Detector**

The BGO detector was located immediately below the motorised table on the source end of the main body of the instrument. The BGO detector’s purpose was to provide information for the measurement of total body chlorine and, hence, TBW. However, although the detector was operational and collecting data, it required to be fully calibrated. Unfortunately although the calibration was completed, its incorporation in the unit’s Microsoft FoxPro® database could not be made in time, and therefore the BGO information could not be used in this project.

**8.2.1.2 The Analogue-to-Digital Converter System**

The analogue-to-digital converter system, as the name suggests, was an electronics system that converted the analogue signals received from the detectors into digital signals. The signal
had to be in a digital format so the multi-channel analyser card could recognise and use. The electronics system is shown diagrammatically in Figure 14.

Each of the two sodium iodide detectors had a linear amplifier (Ortec® model 410) and an analogue-to-digital converter (Canberra-Packard® ADC model 8075). The BGO detector used a spectroscopy amplifier (Canberra-Packard® Spectroscopy Amplifier model 2020). One mixer/router (Canberra-Packard® Mixer/Router model 8222) was used for all three detectors. A high voltage power supply (Canberra-Packard® H.V Power Supply model 3002) supplied 800 volts to the system. Although in the initial setup, the system was powered using a 650 volt supply, this was increased in 2006 to 800 volts to compensate for the aging NaI detectors and spectral drifts. All the above units were assembled on an appropriate rack allowing easy access to the units. The system was always kept switched on, unless a power cut to the Body Protein Monitor Unit was expected. In the case of a shut-down, an appropriate shut-down and
then start-up protocol was followed which ensured that the system was not damaged as a result of electric spikes, etc.

8.2.1.3 Multi-channel Analyser (MCA) Card

The multi-channel analyser card (Canberra-Packard® MCA S100 System) was installed in an Intel®-based Pentium™ personal computer. The software for the multi-channel analyser card (Canberra-Packard® MCA S100 version 1.7) ran under Microsoft Windows® 95 and was capable of simultaneously gathering and displaying information from up to four detectors. Area-under-curve and integral values of the regions of interest were also calculated and reported by the software.

The area-under-curve and integral values form the two sodium iodide detectors were initially printed out and processed by hand or electronic calculator before entry into the TBN Data Base (Knowledge Manger® version 2, MDBS Inc.) for final calculation by the computer. Soon after the start of the PhD project the TBN database was upgraded and replaced by a Microsoft FoxPro® database. This allowed the information generated by the MCA S100 System card to be sorted, calculated, and then reported in a convenient manner ready to be entered into the TBN data base.

The switching on and off of the MCA S100 System, i.e. its counting period, was controlled by the gate signal generated by the motorised table passing the gate switch. The switching on of the MCA S100 System was made when the motorised table first passed the preset gate switch. Once the scanning was complete, i.e. after 200 second, the motorised table passes the gate switched again, switching off the MCA S100 System.
8.2.2 Calibration With Phantoms

As a part of TBN and TBCI measurements, background and standard phantoms had to be scanned as well on a daily basis using “box phantoms”. The background was measured using a perspex box (22.0 x 45.0 x 17.5cm) filled with water. This phantom was referred to as the MW (Medium Water) box. The second perspex box (22.0 x 45.0 x 17.5cm), which was used as a standard, contained a urea solution of known nitrogen concentration (8.7M urea solution). This was referred to as the MPH (Medium Phantom) box. This concentration of urea is equivalent to 2110 grams of nitrogen, which is within the physiological range for nitrogen content in the adult man. The patient counts were compared to the counts from this standard, after correction for background and attenuation, to estimate the patient nitrogen content. These calculations were carried out by the TBN data base.

Standard and background measurements were carried out daily, once in the morning and once in the afternoon after the last patient has been measured. Each background or standard was scanned (or counted) for 1000 seconds giving a total of 2000 seconds for the MPH and 2000 seconds for the MW phantoms. The spectra from the two standards and the two background scans were processed and analysed in almost an identical manner to that of the patient scans. The appropriate area-under-curve and integral values of the regions of interest, given by the MCA S100 System software, were then entered into the TBN data base at the same time as the patient’s results. The data base software then utilised the background and standard results to accordingly corrected and calculate the patient’s final results. The measurements of standard and background phantoms (MPH and MW, respectively) enabled the system to make corrections to account for any drift or change to the equipment or the environment.

One of the two boxes was always kept on the table between the two sodium iodide detectors in the neutron path. This was done to prevent the spectra shifting out of position. When
spectra shifted out of position it usually required from a few hours to a whole day to get back to its normal position.

Both phantom boxes were originally constructed when the IVNCA machine was itself first constructed. Due to the age of these boxes, always great care was taken in moving them on and off the IVNCA machine. Initially as a safety measure, an identical new MPH box was constructed from its original constituents (see the section on MPH Box Construction (Chapter 7.1)) according to the original detailed “blueprint” to have as a back-up in case the old one becomes damaged. Both boxes were identical except for their contents. However, due to considerable leakages and concerns about the accuracy of the MPH phantom, the new MPH box phantom was tested and brought into use prior to the commencement of this PhD project (see the section on MPH Box Construction (Chapter 7.1)).

An empty perspex box was also available in case the MW phantom required replacement.

8.2.3 IVNCA Spectra

The spectra obtained for the sodium iodide and BGO detectors were simultaneously displayed on the computer screen by the MCA S100 System card and software. The positions of the regions of interest were critical to the accuracy and precision of the overall results. As there is a shift as the day progresses, it was very important to monitor this shift and adjust accordingly using the fine gain adjuster of the linear amplifier.

For the two sodium iodide detectors, the first region of interest, the hydrogen peak, was used as a measure of the degree of spectral shift. Therefore, a “standard” was set and adopted to be followed. This was to keep the first three channels in the hydrogen region of interest in a “U” or “V” shape, i.e. the first and third channels had to have higher counts than the second or middle channel. Ensuring that this is so, automatically ensured correct positions for the rest of
the regions of interest for that sodium iodide detector. All fine gain changes were noted in the instrument’s log book for future reference and analyses.

The Table 1 shows the regions of interest, corresponding channel numbers, and the elements for the two sodium iodide detectors:

![Table 1. Regions of interest (ROI) for the sodium iodide detectors.](image)

Due to the nature and the mechanism of action of the BGO detector, the regions of interest were adjusted after the measurement scan has been made. In addition, regions of interest were adjusted and corrected using energy calibrations (± 0.01 MeV) rather than channel numbers. The regions of interest, their corresponding energy values (in MeV), and elements are shown in the Table 2 below:
Table 2. Regions of interest (ROI) for the bismuth germanium oxide detector.

<table>
<thead>
<tr>
<th>ROI #</th>
<th>RANGE (MeV)</th>
<th>ELEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1.9 to 2.5</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>14</td>
<td>4.1 to 4.8</td>
<td>Carbon</td>
</tr>
<tr>
<td>15</td>
<td>5.0 to 7.0</td>
<td>Chlorine 1</td>
</tr>
<tr>
<td>16</td>
<td>7.0 (+1 channel) to 9.0</td>
<td>Chlorine 2</td>
</tr>
<tr>
<td>17</td>
<td>9.9 to 10.8</td>
<td>Germanium</td>
</tr>
</tbody>
</table>

As all corrections and adjustments to the above ranges are made after the actual scan, the MCA S100 System software is utilised to make the corrections and adjustments rather than the fine gain (as used with the sodium iodide detectors).

### 8.2.4 Radiation And Dosimetry

When dealing with “radioactivity”, there is always concern about the safety of the instrumentation, chronic, and acute effect. As seen during the recruitment process, the immediate concern of majority of the patients was to know how much radiation they will be getting and whether it would affect the effects of chemotherapy and/or radiotherapy. Some cases of refusal were attributed to the fact that the patient “does not want to get more radiation than now getting from radiotherapy…”.

When dealing with the concept of “radiation risk” from a particular source, one has to interpret it in terms of other radiation risks and/or by considering risk factors that estimate the likelihood of inducing fatal malignant disease or substantial genetic defects in live descendants (National Health and Medical Research Council (NH&MRC), 1981). It is well known that the average annual background radiation is 2.5 mSv of which 85% to 90% is from natural sources. A standard chest X-ray is reported to be approximately 0.02 mSv (NRPB-W4, 2002) \(^{[845, 844]}\). When this is compared to the radiation dose received by a adult from a TBN measurement (0.2 mSv), one can see that the chest X-ray is about 10 fold higher. Assuming that the annual back ground radiation in Australia is 2.1 mSv, a typical TBN scan is
equivalent to less than $\frac{1}{10}$th of the annual background radiation. Studies by Allen et al.\cite{711} show that there is approximately a 1 in 100,000 chance in a lifetime of inducing cancer as a result of exposure to neutrons.

Reports of Allen et al.\cite{711} made on a neutron source with $9.5 \times 10^7$ neutrons per second output was found to be in close agreement with the later theoretical determination of McGregor and Allen.\cite{721} The study of Allen et al.\cite{711} utilised water-filled plastic bottle phantoms to simulate body scattering. A calibrated rem-meter placed at the aperture of the IVNCA instrument with its centre at the top of the patient motorised table, was then used to measure the neutron dose equivalent\cite{711}. Gamma ray dose rate here was measured with a gamma dosimeter\cite{711}.

The total radiation dose at the Body Protein Monitor Unit has also been determined. It has been estimated that at a distance of one meter from the source stack, the radiation is less than 0.01 mSv per hour.

### 8.2.4.1 Body Habitus Corrections

The body habitus or antero-posterior and width thickness is a factor that needs to be considered in terms of gamma-ray attenuation which affects the overall hydrogen and nitrogen counts obtained from the IVNCA instrument. The studies of Allen et al.\cite{692} and McGregor and Allen\cite{721} utilised water-filled plastic bottles to determine the nitrogen background. They found that the nitrogen background was independent of the depth of the phantom but dependent on the width of the phantom. Correction factors to adjust the measured nitrogen-to-hydrogen counts ratio for the different attenuations of the hydrogen (2.2 MeV) and nitrogen (10.8 MeV) gamma-rays in body and borated paraffin shield were addressed by McGregor and Allen.\cite{721} They found, both theoretically (by Monte Carlo calculations) and experimentally, that the above are independent of the patient’s thickness and related (linearly) to the patient’s width.
8.2.4.2 Annual Calibration Experiments

As a part of the maintenance of the IVNCA instrumentation, annual “width” experiments were carried out to correct for the ageing of the neutron source, etc.

It should be noted that these experiments were only necessary when the californium-252 were used at this facility. This was due to its relatively low half-life of approximately 2.65 years. However, this source was replaced in 1998 with the $^{241}$AmBe which has a very long half-life of greater than 400 years. As a result, only one such experiment was required just after its installation.

These calibration experiments involved the measurements of plastic bottles (13.5 x 18.0 x 22.0cm) filled with different solutions by placing them on the IVNCA instrument and counting for 1000 seconds. Various arrangements (not exceeding a total length of 56cm) were used to give a number of different widths. The nitrogen and hydrogen backgrounds were among the most important ones. The resulting equations and correction factors were then entered into the TBN data base to be used for patient/phantom corrections from that point onwards.

For the nitrogen background experiments, the plastic bottles were filled with water and arranged to give varying widths (hence the name “width experiments”) along the sodium iodide detector axis. Each arrangement was counted for 1000 seconds. Repeat measurements were carried out to give, statistically, better results. The results obtained here showed and were described as the nitrogen background being a function of the width by a third order polynomial expression.

As an example, a typical result for the experiments carried out for one calendar year is shown below (Table 3). For this experiment n=8.
Chapter 8: METHODS: BODY COMPOSITION AND QUALITY OF LIFE MEASUREMENTS

Table 3. Annual nitrogen background experiment results.

<table>
<thead>
<tr>
<th>Width (cm)</th>
<th>Mean</th>
<th>Variance</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.0</td>
<td>1814.88</td>
<td>2186.98</td>
<td>43.74</td>
<td>2.41</td>
</tr>
<tr>
<td>18.5</td>
<td>1881.63</td>
<td>2581.70</td>
<td>47.53</td>
<td>2.53</td>
</tr>
<tr>
<td>28.0</td>
<td>1983.13</td>
<td>1566.41</td>
<td>37.02</td>
<td>1.87</td>
</tr>
<tr>
<td>32.5</td>
<td>1895.63</td>
<td>3710.84</td>
<td>56.98</td>
<td>3.01</td>
</tr>
<tr>
<td>37.0</td>
<td>1801.13</td>
<td>2276.41</td>
<td>44.63</td>
<td>2.48</td>
</tr>
<tr>
<td>42.0</td>
<td>1635.88</td>
<td>4679.84</td>
<td>63.99</td>
<td>3.91</td>
</tr>
<tr>
<td>46.5</td>
<td>1580.75</td>
<td>1870.21</td>
<td>40.45</td>
<td>2.56</td>
</tr>
</tbody>
</table>

where SD is the standard deviation and %CV is the percentage coefficient of variation.

The mean values of the nitrogen counts were then plotted against various widths of the plastic bottle phantoms (Figure 15) and a third order polynomial fitted (Equation 17). The plot and the third order polynomial is shown in Figure 15 below:

Figure 15. Graph of Nitrogen Counts against Width in water filled phantoms.

![Graph of Nitrogen Counts against Width in water filled phantoms](image)

Mean Nitrogen Counts Against Width In Water Filled Bottle Phantoms

\[
y = 0.0275x^3 - 3.4427x^2 + 118.27x + 738.01 \\
R^2 = 0.9632
\]

The equation of the polynomial fit is also shown below (Equation 17):

Equation 17. Polynomial fit for Nitrogen Count variations with Widths.

\[
y = 0.0275x^3 - 3.4427x^2 + 118.27x + 738.01 \text{ and } R^2 = 0.9632
\]
For the hydrogen background experiments, the plastic bottles were filled with D₂O of 99.9% purity. The same arrangement of bottles as with the nitrogen background experiments were used.

The gamma-ray attenuation was also measured annually as this can be affected by the age of the neutron source. For this experiment, again, the same plastic bottles as above were used. However, for measuring gamma-ray attenuation, these plastic bottles were filled with tissue-equivalent urea-saline solution and were arranged in the same arrangements as above. This solution was composed of 2.60% nitrogen, 0.14% chlorine, and 10.97% hydrogen, by weight.

The results (Table 3) of the water-filled plastic bottle phantoms were used to correct for the background and the resulting nitrogen-to-hydrogen ratios were then plotted against the width of the bottle which gave a linear relationship. As with the above, the gamma-ray attenuation coefficients were then entered in the TBN data base for final patient gamma-ray attenuation corrections for TBN estimations. The results for the experiments carried out for one calendar year are shown in the Table 4 below. For this experiment also n=8.

<table>
<thead>
<tr>
<th>Width (cm)</th>
<th>Mean</th>
<th>Variance</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.0</td>
<td>26.70</td>
<td>3.56</td>
<td>1.89</td>
<td>7.06</td>
</tr>
<tr>
<td>18.5</td>
<td>40.89</td>
<td>1.31</td>
<td>1.14</td>
<td>2.80</td>
</tr>
<tr>
<td>28.0</td>
<td>70.10</td>
<td>5.94</td>
<td>2.44</td>
<td>3.48</td>
</tr>
<tr>
<td>32.5</td>
<td>79.80</td>
<td>10.46</td>
<td>3.23</td>
<td>4.05</td>
</tr>
<tr>
<td>37.0</td>
<td>86.41</td>
<td>4.53</td>
<td>2.13</td>
<td>2.46</td>
</tr>
<tr>
<td>42.0</td>
<td>92.52</td>
<td>5.04</td>
<td>2.24</td>
<td>2.43</td>
</tr>
<tr>
<td>46.5</td>
<td>92.37</td>
<td>11.98</td>
<td>3.46</td>
<td>3.75</td>
</tr>
</tbody>
</table>

where SD is the standard deviation and %CV is the percentage coefficient of variation.

The mean values of the nitrogen to hydrogen ratio counts were then plotted against various widths of the plastic bottle phantoms (Figure 16) and a polynomial line fitted (Equation 18).

The plot and polynomial equation are shown in the Figure 16 and Equation 18below:
The equation of the third order polynomial fit to the above plot was:

**Equation 18. Polynomial fit equation for the N/H Ratios against Widths plot.**

\[
y = -0.0009x^3 + 0.0276x^2 + 3.2114x - 21.355 \text{ and } R^2 = 0.9995
\]

A linear fit of this same curve would produce a line with the equation:

**Equation 19. Linear fit equation for the N/H Ratios against Widths plot.**

\[
y = 2.118x + 3.7157 \text{ and } R^2 = 0.9349
\]

**Figure 16. Graph of N/H Ratios against Widths.**

8.2.4.3 Calibration Experiments For Americium-241 Source

The process of calibrating the system once the old \(^{252}\text{Cf}\) was replaced by the new \(^{241}\text{AmBe}\) was identical to that of the annual calibration experiments (or the “width experiments”) which were performed when the \(^{252}\text{Cf}\) was in use. The only difference would be that once it is carried out and the results and equations incorporated into the Microsoft FoxPro\textsuperscript{®} database, no further annual calibration experiments were required.
8.2.4.4 Reproducibility of the System

The MPH box phantom counts were utilised to assess the reproducibility the IVNCA system. These box phantoms (22.0 x 45.0 x 17.5cm) were measured twice on a daily basis as a part of the routine procedure in TBN measurements to correct for the shift in the spectra as a result of daily changes in the IVNCA instrument’s environment. These values were then reported annually as a part of the Body Protein Monitor Unit annual report which reflected the performance of the IVNCA system for that calendar year.

The values of ABNOH and KNH are monitored for any abnormal changes. ABNOH is calculated using the results obtained from the morning and afternoon MPH and MW scans. It is defined as the “Nitrogen Over Hydrogen” ratio which is the sum of background-corrected morning and afternoon nitrogen counts divided by the sum of morning and afternoon hydrogen counts (Equation 20). KNH is defined as being the MPH calibration factor and is related to ABNOH. ABNOH itself is calculated as follows:

**Equation 20. ABNOH calculations.**

\[
ABNOH = \frac{(ANNN + BNNN)}{(ANHY + BNHY)}
\]

where

\[
ANNN = ANNY - ANNB and BNNN = BNNY - BNNB
\]

[Note: ANNY and BNNY are the MPH nitrogen counts and, ANNB and BNNB are the MW nitrogen counts. The prefixes: "A" and "B" designate the morning and afternoon measurements, respectively.].

The variation of the ABNOH and KNH values over a calendar year is plotted and is shown in Figure 17 below:
Figure 17. Variations in ABNOH and KNH values during a calendar year.

Table 5 below shows the reproducibility statistics for one calendar year.

Table 5. Reproducibility of the system during one calendar year.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>ABNOH</th>
<th>KNH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average</strong></td>
<td>5.320</td>
<td>3.695</td>
</tr>
<tr>
<td><strong>Coefficient Of Variation (%)</strong></td>
<td>1.547</td>
<td>1.534</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>0.007</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>0.082</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>5.119</td>
<td>3.554</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>5.560</td>
<td>3.860</td>
</tr>
</tbody>
</table>

In Table 5, the difference between maximum and minimum values reflects the effect of the decay of the neutron source, in this case the californium-252, and electronic pile-up events in the regions of interest. Pile-up preferentially reduces the hydrogen peak, giving a higher
“Nitrogen Over Hydrogen” (patient net nitrogen/hydrogen yield) value. Thus, “Nitrogen Over Hydrogen” slowly reduces as the source strength decays with time.

Reproducibility throughout a calendar year is, generally, very good with an overall coefficient of variation of approximately 1.5%. It should again be noted that normalisation occurs each day so the observed variations in KNH are all accounted for in the subject data.

Also, calculations and plots of the percentage coefficient of variation (%CV) of the corrected nitrogen to hydrogen ratio (CNOH) and the corrected mass of nitrogen (CMN) against Time are made annually to estimate the statistical error of TBN measurements, as the Californium-252 neutron source decays in strength. These are done to see whether or not it is necessary to increase the table time (i.e., the total exposure time) on the IVNCA instrument to compensate for this decay. Currently the patient CVs are around 5% for the measurement of nitrogen.

Figure 18 below shows these variations with time for a typical calendar year:

Figure 18. Percentage CV for CNOH and CMN against Time for a calendar year. The CMN curve lies just above the one for CNOH.
8.2.4.5 Errors in Nitrogen Measurements

Accuracy of the IVNCA technique for the measurement of TBN has been tested on carcass analysis [677, 705]. Carcass chemical analysis, has been undertaken in cadavers who had their TBN measurements (using IVNCA technique) just prior to death [677, 705]. Carcass analysis involves the measurement of cadavers or carcasses using the IVNCA instrument and then carrying out a destructive chemical analysis. The instrument at the Royal North Shore Hospital, for obvious reasons, has not been tested in this way.

Direct measurement or theoretical estimations of errors in the absolute measurement of nitrogen can, however, be carried out. This is done by estimating the errors for each of the variables in the Vartsky’s equation [710] (see Equation 1).

As a result there would be three main factors which can produce errors in estimating the mass of nitrogen. These factors are as follows:

- The prediction of nitrogen background counts;
- The counting statistics for the total nitrogen counts; and
- The estimation of the mass of hydrogen.

The first two factors mainly concern small subjects, such as children and not adults as used in this study. The third factor particularly affects the obese subjects where the relative contributions of fat mass to the hydrogen mass are much greater than the lean subjects. Studies on the Royal North Shore Hospital’s IVNCA instrument [696, 698, 207] has estimated the cumulative error to be between ±3.5% to ±10% and the error in the estimation of hydrogen mass to be less than 2.5%. This calculated error was then found to be higher than the experimentally determined error which used anthropomorphic phantoms suggesting an over-estimation of cumulative error.
8.3 Total Body Water Measurements

The TBW can be measured using the heavy water or D₂O dilution technique. This method is considered to be the “gold standard” method of estimating human subjects’ TBW content. However, it suffers from being extremely time consuming and requires full operator supervision during its sample preparation phase. It was for these reasons that modifications were made to its sample preparation or processing phase. This significantly reduced and virtually eliminated operator supervision and significantly increased the number of samples that can be processed per unit time.

For this purpose, the BIA technique was validated against the above “gold standard” prior to the commencement of the PhD project and the BIA technique was used through-out for TBW measurements in all patients.

8.3.1 Isotope Dilution Technique

Although different isotopes are currently available, Deuterium Oxide (D₂O) was chosen because of its lower cost as well as being non-radioactive. D₂O (1 g kg⁻¹ of LBM) was given orally, at night, before sleeping. This dose was chosen as it has a safe therapeutic threshold that also resulted in post dose plasma concentrations detectable by the analysis technique. Blood samples were collected, in duplicates, in the morning in heparinised tubes containing 143 USP units of lithium heparin (Vacutainer® PST (Becton Dickinson & Co., USA)). Fluid input and output was measured during the equilibration period for the corrections to the final TBW estimation. The blood samples were centrifuged at 1520g at 4°C for 20 minutes (Beckman® Model TJ-6 centrifuge with a TH-4 rotor at 2700 rpm). Our own published
ultrafiltration technique \cite{846} was used to separate the D$_2$O/H$_2$O mixture from the plasma. The resulting D$_2$O/H$_2$O mixture was then analysed using Fourier Transform Infra-Red (FTIR) analysis \cite{727} to determine the final D$_2$O concentration in the patient’s plasma. A correction factor \cite{755} of 1.04 was also applied to correct for the non-aqueous hydrogen exchange. The accuracy of the isotope dilution techniques has been reported to be 1\% to 4\% \cite{725}. The coefficient of variation was 1\%.

It should be noted that in order to use the ultrafiltration technique mentioned above to significantly speed-up the plasma preparation phase, the technique needed to be scientifically validated. This process of validation is detailed in a peer-reviewed scientific journal \cite{846}.

### 8.3.1.1 Equipment And Consumables Required

The equipment required for the dosing, sample collection, preparation, and analysis phase of TBW measurement by D$_2$O are shown below. It should be noted that the equipment below are for one patient only. When the test was carried out for more than one patient, the numbers were increased accordingly.

**Dosing Of D$_2$O**

- Clean glass bottle (200mL) and cap.
- Clean glass beaker (500mL).
- D$_2$O (at least 99\% pure).
- Identification sticky labels.
- Fruit cordial.
- Access to balance(s) capable of measuring weight up to 350g and accurate to 2 decimal places.
Sample Collection

Although other centres use other body fluids (e.g., urine, saliva, etc.), blood samples were used for the measurement of TBW at the Department of Nuclear Medicine.

- Latex examination gloves.
- Syringe (10mL) with needle.
- Heparinized blood collection tubes (10mL Vacutainer® PST (Becton Dickinson & Co., USA "green top").
- Access to a refrigerated centrifuge capable of at least 1500g and 4°C.
- Glass Pasteur pipettes and the appropriate teat.

Sample Processing

- Latex examination gloves.
- Access to a high speed centrifuge capable of 2500g and available for two sessions on the same day, each for a period of 2 hours.
- Two plastic ultrafiltration tubes (Sartorius Centrisart® I) with a 5000 Dalton membrane.
- Eppendorf® plastic container.

Standard Preparation

- Latex examination gloves.
- Frozen, expired human plasma (7.0L).
- Seven 1.0L volumetric flasks.
- Two litre glass beaker.
- Eight 50mL "yellow screw top" plastic sample containers.
- Eight plastic ultrafiltration tubes (Sartorius Centrisart® I) with a 5000 Dalton membrane.
Access to a high speed centrifuge capable of 2500g and available for two sessions on the same day, each for a period of 2 hours.

- Eppendorf® plastic container.

**Analysis Phase**

- Latex examination gloves.
- Syringe (1.0mL) with needle.
- Access to a Bruker® IFS66v FTIR Analyser (MCT detector) running the Opus® version 2.01 software capable of 4 wave number resolution, 256 scans, at mid infrared with a Calcium Fluoride liquid cell (0.1mm path length).
- Microsoft Excel® or similar spread sheet computer software for TBW calculations.

It should be noted that the analysis phase of the TBW measurement by D₂O dilution was carried out at the School of Chemistry, University of Sydney.

### 8.3.2 Bioelectrical Impedance Analysis Technique

The BIA technique is considered to be an alternative method to isotope dilution for the measurement of TBW. This method has successfully been used in patients with cancer cachexia to estimate TBW.\(^{[847]}\)

For the BIA method, measurements were carried out on the patient in the supine position using two leads on the non-dominant hand and two leads on the ipsilateral foot (tetrapolar arrangement). Leads were attached to the skin using Red Dot™ Ag/AgCl Resting EKG Electrodes (3M Health Care, St Paul, MN 55144-1000). Patient's skin was cleaned with alcohol swab and then dried prior to electrode attachment. Patients were rested in the supine position for at least five minutes, and the measurements were performed with their arms parallel but separate to the trunk, and their legs apart far enough so their thighs were not
touching. The body's resistance was measured in triplicate using a swept frequency bioelectrical impedance meter (SEAC® SFB23, UniQuest Limited, Queensland, Australia) at 50 kHz was used with three different equations of Kushner [747], Pullicino [755] and Fredrix [753] used to calculate the TBW. The actual equations can be found in the TBW measurement section of In Vivo Body Composition (Chapter 7.2).

For the project of comparing different methods of TBW estimations in gastrointestinal surgery patients [758, 848], TBW was additionally estimated using the 58% body weight, Watson equations [725], and TBW from LBM (i.e., 73.2% of LBM) [757]. The latter three techniques did not require BIA measurements. It should also be noted that the three BIA equations used here all used corrected dilution spaces whereas the TBW measurement by Watson’s equation [725] did not. The actual equations can be found in the TBW measurement section of In Vivo Body Composition (Chapter 7.2).

Although the measurement of TBW by isotope dilution technique is considered to be the “gold standard”, the actual process is not only expensive, but also very time-consuming and potentially prone to errors. Even with the introduction of our new ultrafiltration technique [846] to increase the throughput of samples, the process could not be satisfactorily be used under routine clinical setting. As the BIA technique could speed-up the process of TBW measurements under clinical setting, it was decided to be used in this project as the method of TBW measurement instead of the isotope dilution technique. The effectiveness of this technique was tested in our study comparing different methods of TBW measurements in gastrointestinal cancer patients undergoing surgery [758, 848]. This evaluation process is detailed in our article published in a peer-reviewed scientific journal [758]. It is interesting to point out that the results were consistent with the results we obtained in a similar study on renal dialysis patients [686].
8.4 Total Body Potassium Measurements

Measurements of TBK are carried out by supine geometry sodium iodide counting. It is considered to be a non-invasive procedure within minimal distress to the patient. The precision and accuracy of this method, expressed as coefficients of variation, are 1.5% and 4.5% respectively \[818\].

The TBK measurements for our projects were carried out using the Whole Body Counting Potassium technique utilising the Whole Body Counting facility of the Department of Nuclear Medicine, Royal North Shore Hospital. The Royal North Shore Hospital’s Whole Body Counter was originally calibrated by Hansen and Allen \[814\] in the mid-1990’s.

The Whole Body Counter facility or set up can be divided into three physical sections:

- The Whole Body Counter measurement chamber;
- The analogue-to-digital converter system;
- The multi-channel analyser unit.
- Additional equipments.

8.4.1.1 The Whole Body Counter Measurement Chamber

The measurement system of the Whole Body Counter was very similar to that of the IVNCA facility. It consisted of a steel chamber made from 83mm thick pre-World War II battleship steel with an additional 2.8mm thick lead sheet attached to the exterior surfaces of the chamber to reduce background radiation. The reason for using pre-World War II steel was that it does not contain radioactive materials which can interfere and produce excess background radiation.
The measurement chamber area of the Whole Body Counter was quite small. One of the main reasons for patients refusing TBK measurements has been the small and claustrophobic nature of the chamber. Because of space limitations, subjects were positioned with the head against the right wall. The maximum height of patient that could be accommodated was 192 cm.

The natural $\gamma$-rays emitted from the patients were detected by two sodium iodide detectors placed under the static bed in the chamber. The detectors were 150mm in diameter, stainless steel clad and 175mm thick. As the system employed a supine geometry arrangement, both detectors were mounted on a stationary frame beneath the measurement bed (or table). Lead brick collars, 51 mm thick and 94 mm high, surrounded the detectors, such that there was a gap of 25 mm between the collar and detector, with 5 mm of unshielded detector extending above the collar.

8.4.1.2 The Analogue-To-Digital Converter System

Each of the two sodium iodide detectors were connected to a linear amplifier (Canberra-Packard® model 2022) and an analogue-to-digital converter (Canberra-Packard® ADC model 8075). A high voltage power supply (Ortec® H.V Power Supply model 456) supplied 900 volts to the system. All the above units were assembled on an appropriate rack allowing easy access to the units.

8.4.1.3 The Multi Channel Analyser (MCA) System

The signal from the analogue-to-digital converter were then passed onto a Canberra-Packard® Series-40 MCA unit (MCA-40) which carried out the function of the mixer/router as well as displaying the spectra and calculating the peaks’ integrated values. The $^{40}$K region of interest was at 1.29 to 1.62 MeV.
8.4.1.4 Additional Equipments

The additional equipment present at the Whole Body Counter facility and used for the purpose of TBK measurements included the anterior-posterior thickness and transverse thickness measurement rulers and the potassium source for calibrations. As with the IVNCA facility, a logbook was also present for the recording of patient information, peak measurements, and any other event affecting the working and performance of the Whole Body Counter.

The anterior-posterior thickness and transverse thickness measurement rulers were standard large callipers and meter rules, respectively. These were always kept within the chamber and free of any biological or radiological contaminations.

The potassium source for calibration was located in a plastic jar and was always stored outside the Whole Body Counter chamber. The plastic jar contained 500 grams of analytical grade potassium chloride (Sigma Chemical Company and Sigma-Aldrich Pty Ltd) and was sealed to ensure that no moisture enters the jar. The potassium chloride contained large amounts of $^{40}$K which was required and used at the beginning of the measurement day to calibrate the Whole Body Counter for $^{40}$K measurements.

8.4.2 Calibration With Phantoms

As a part of the TBK measurements, background and standard measurements had to be carried out on the measurement day.

The background was measured with the chamber empty, i.e. no patient or calibration source present. Measurements of $\gamma$-radiation were then made for a period of 1000 seconds and the results, peaks’ integral values, were noted in the logbook. It should be noted that the back...
ground measurements were made as close as possible to the time that the patient was measured, preferably as soon as the patient had left the chamber to ensure accurate background estimations.

For standard measurements, the plastic jar containing 500 grams of analytical grade potassium chloride was used. The purpose of the standard measurements was to ensure that the regions of interest were correctly placed on the potassium peaks for each detector as well as to control for any unacceptable drift in the TBK measurement precision.

In order to carry out standard measurements, the thin mattress was rolled back and the bare bed was exposed. On the bed the correct position to place the potassium source jar was clearly marked which corresponded to the exact position between the two sodium iodide detectors. The potassium jar was then placed at this position on the measurement bed and the $\gamma$-radiation was measured for 1000 seconds. Once the 1000 seconds had elapsed, the correct regions of interest were placed on the appropriate peaks and the integrated value for each peak was noted in the logbook. It should be noted that the standard measurements were always carried out before the patient or background measurements to ensure that the regions of interest were placed in the correct position.

8.4.2.1 Reproducibility Of The System

Quality control carried out during the year indicated the stability of the system, with acceptable drift in net counts (i.e. the potassium jar minus the background) obtained from a potassium source jar (500 gram KCl calibrator).

An extensive technical report on the setting-up and calibration of the Whole Body Counter for TBK assessment commenced in 1998 and was published by Hansen and Allen [814]. As a part of ongoing quality control, a daily calibration correction factor was established to maintain the accuracy and precision of the system.
Figure 19. Graph to show the Net potassium calibration counts for a typical calendar year.

### KCl Calibration Counts For Typical Calendar Year
(Solid Line = Mean ± 1SD (Dashed Lines))

- Net Kcal counts vs. Time (Day Number)
8.5 Quality Of Life Measurements

The EPA clinical trial received a questionnaire developed by the EORTC specifically for cancer patients with a pancreatic cancer-specific additional module.

8.5.1 EPA Clinical Trial

The QL of patients in this clinical trial was assessed using the EORTC’s QLQ-C30 core questionnaire instrument together with their pancreatic cancer-specific module, QLQ-PAN26 [841, 840, 839]. The QLQ-C30 core questionnaire consisted of 30 questions and the QLQ-PAN26 pancreatic module consisted of an additional 26 questions.

These questionnaires were developed by the EORTC and were obtained directly from that centre (D Fitzsimmons. University of Southampton, University Surgical Unit, Mailpoint 816, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK) and registering our clinical trial.

8.6 CLINICAL TRIAL SAMPLE SIZE DETERMINATION

Based on the studies of Barber et al [228, 251, 253, 252] and Wigmore et al [249, 605, 250] reported in the literature on pancreatic cancer patients, we are expecting 50% of patients in the placebo arm to have a minimum of 15% weight loss. With the administration of EPA, we are expecting only 10% of patients (i.e. an absolute improvement of 40%) receiving EPA treatment arm to have 15% weight loss. Due to the nature of the disease and the time and
financial limitations, it was decided that a sample size of 30 would be suitable for the purposes of this trial.

### 8.6.1 The 15% Weight Loss Scenario

Assuming a standard pre-chemotherapy weight of 70 kg\(^{[849]}\), a 15% weight loss will equate to a 10.5 kg weight loss. Also assuming that approximately 40% of body weight is skeletal muscle\(^{[849]}\) and that there has been an equal loss from all body compartments during this weight loss, the total loss of skeletal muscle from the body is 4.2 kg, i.e., \(10.5 \times 40 / 100 = 4.2\).

It is known that 17.2% of muscle is protein\(^{[850]}\). Therefore, the total loss of protein during this 15% weight loss would be 722 g.

The ratio of protein to nitrogen is 6.25\(^{[207]}\). Therefore, the total amount of nitrogen lost will be 115.6 g.

Using duplicate measurements of over 30 human subjects obtained over a number of months we have obtained a mean standard deviation (\(\sigma\)) value of 41.7. With phantoms created in Auckland, New Zealand (Prof L Plank) and measured (nine repeats) at our centre consecutively in one day, a mean standard deviation (\(\sigma\)) value of 69 was obtained.

Using the Equation 21 and \(\alpha\) set to 0.05 to estimate the number of subjects needed to show the maximum difference resulting from the 15% weight loss, the total number of patients required in each arm would be 0.84 if standard deviation of 41.7 is used and 1.37 if a standard deviation of 69 is used.

\[ E = z_{\alpha/2} \times \frac{O}{\sqrt{n}} \]

where \( E \) is the maximum difference between the observed sample mean, \( z_{\alpha/2} \) is the critical value (the positive \( z \) value that is at the vertical boundary for the \( \alpha/2 \) in the right tail of the standard normal distribution), \( \sigma \) is the population standard deviation, and \( n \) is the sample size.

**8.6.2 The 5% Weight Loss Scenario**

If one uses the scenario of a 5% weight loss which is considerably more subtle than a 15% weight loss, and assuming a standard pre-chemotherapy weight of 70 kg [849], a 5% weight loss will equate to a 3.5 kg weight loss. Also assuming again that approximately 40% of body weight is skeletal muscle [849] and that there has been an equal loss from all body compartments during this weight loss, the total loss of skeletal muscle from the body is 1.4 kg, i.e. \( 3.5 \times 40 / 100 = 1.4 \).

Using the fact that 17.2% of muscle is protein [850]. Therefore, the total loss of protein during this 5% weight loss scenario would be 241 g. The total amount of nitrogen lost here would, thus, be 38.5 g.

Using the Equation 21 and \( \alpha \) set to 0.05 to estimate the number of subjects needed to show the maximum difference resulting from the 5% weight loss, the total number of patients required in each arm would be 4.5 if standard deviation of 41.7 is used and 12.3 if a standard deviation of 69 is used.

**8.6.3 The 2.5% Weight Loss Scenario**

Using a far lesser weight loss scenario of a 2.5% weight loss which is also considerably more subtle than a 15% weight loss, and assuming a pre-chemotherapy weight of 70 kg [849], a 2.5%
weight loss will equate to a 1.75 kg weight loss. Also assuming again that approximately 40% of body weight is skeletal muscle \[849\] and that there has been an equal loss from all body compartments during this weight loss, the total loss of skeletal muscle from the body is 0.700 kg, i.e. \(1.75 \times 40 / 100 = 0.700\).

Using the fact that 17.2% of muscle is protein \[850\]. Therefore, the total loss of protein during this 5\% weight loss scenario would be 120 g. The total amount of nitrogen lost here would, thus, be 19.3 g.

Using the Equation 21 and \(\alpha\) set to 0.05 to estimate the number of subjects needed to show the maximum difference resulting from the 2.5\% weight loss, the total number of patients required in each arm would be \textbf{18.0} if standard deviation of 41.7 is used and \textbf{49.3} if a standard deviation of 69 is used.
9. WHIPPLE’S PROCEDURE PATIENT RESULTS

9.1 Patient Demographics

A total of 67 patients with upper gastrointestinal cancer undergoing major surgery were recruited for this study and body composition measurements were carried out. This was a heterogeneous patient population requiring different methods of surgery. From this population, 27 patients underwent Whipple’s Procedure (pancreaticoduodenectomy) for pancreatic cancer. All 27 patients underwent at least three body composition measurements, carried out at the pre-operative, two weeks post-operative, and five weeks post-operative time-points. The selection of only Whipple’s Procedure patients was done in order to produce a homogenous patient population in order to more accurately investigate the effect of different body composition parameters and/or malnutrition as well as surgical outcome on the long term survival following a major surgical operation such as Whipple’s Procedure.

These patients were then further separated into patients whose operation resulted in a clear margin or unclear margin. This then enabled the assessment of the influence of margin status on long term outcome and survival. There were a total of 17 patients in the Clear Margin group and 10 in the Unclear Margin group.

In this group of 27 patients, there were a total of 14 males and 13 females with an age range of 42.2 to 81.1 years (Mean: 67.7 years; Median: 69.0 years) at the time of operation. The age range for males was 49.0 to 80.0 years (Mean: 64.7 years; Median: 67.1 years) and for females was 42.2 to 81.1 years (Mean: 70.9 years; Median: 74.8 years) at the time of operation.
Body composition measurements were carried out at five time-points of: pre-operatively, two weeks post-operative, five weeks post-operative, 14 weeks post-operative, and 26 weeks post-operative. All 27 patients completed the first three measurements, with 22 (81.5%) completing four measurements and 16 (59.3%) completing the full five measurements. There were a total of two deaths (7.4%) within the 26 weeks post-operative period. The range for time to end point was 91 to 2143 days (Mean: 822.3 days; Median: 596 days). The range for time to end point in males was 91 to 2106 days (Mean: 776.4 days; Median: 580 days) and in females was 101 to 2143 days (Mean: 873.1 days; Median: 596 days). The reasons for the remaining 11 (35.5%) patients who did not complete the full five measurements was that they were feeling too sick to continue (n=3 (11.1%)), moved away from the Sydney area and therefore unable to attend for body composition measurements (n=3 (11.1%)), or refusal (n=5 (18.5%)) giving no specific reason.

9.2 Completion Rates

There were a total of 10 patients with Unclear Margins who were enrolled in the study and commenced the measurement. Up to the third measurement (five weeks post-operative) time-point all patients were still enrolled. However, at the next measurement time-point (14 weeks post-operative) there were three “drop-outs” reducing the number of Unclear Margin patients to seven. This number was further reduced to two when there was a further five “drop outs” at the time of the 26 weeks post-operative time-point measurements.

With the Clear Margin group, there were 17 patients enrolled at the start of the study which is almost twice as much as the Unclear Margin group. As with the Unclear Margin group, the patients completed all measurements up to and including the third time-point (five weeks post-operative time-point). The number of patients that dropped out of the study by the fourth
time-point was two. However, as a percentage of the group population the total reduction in patients (as compared to the base-line) was only 11.8% as compared to the 30% of the Unclear Margin group. There were a further two patients who dropped out before the 26 weeks post-operative time-point reducing the patient numbers to 13. Again, as compared to the base-line numbers, this was a reduction of 23.5% as compared to the 70% of the Unclear Margin group.


9.3 Body Composition Results

All relevant parameters resulting from or during the TBN, TBK and TBW measurements carried out at the CIVBC were investigated to determine the changes as well as differences in the Clear Margin and Unclear Margin groups of Whipple’s Procedure surgical patients.

Generally tissue margins refer to the edges of a resection or excision during a surgical procedure. The specimen and margins are then examined by a pathologist to determine whether the disease (in this case pancreatic cancer) has been completely removed or not. The terms Clear Margins or Negative Margins refer to the cases where the surgeon’s cut was through healthy tissue and, therefore, the diseased tissue (in this case the pancreatic tumour) was completely removed. The terms Unclear Margins or Positive Margins refer to the cases where the surgeon’s cut was through the diseased tissue (in this case the pancreatic tumour) and some of the diseased tissue was left in the patient, which may increase the likelihood of the disease recurring. Whether the patients receive a clear or unclear margin depends on a number of different factors including the degree of infiltration and the size of the tumour.

9.3.1 Differences In Body Composition Between Clear And Unclear Margins

The initial step in analysing the difference between the Clear Margin and Unclear Margin groups was to see the differences between the average IVBC values in each group. Please note that in the graphs demonstrating these values, for ease of viewing purposes, the body composition parameters with similar range of values were placed and displayed on the same graph.
The statistical significance of the differences between the Clear Margin and Unclear Margin groups at each measurement time-point is investigated and results shown in the statistical analysis sections below.

The trend for changes in the Weight, %BFat, and BMI shows that the average values for the Clear Margin group are higher at all measurement time-points. This is demonstrated in Figure 20.

**Figure 20.** Mean (±SE) changes in Weight (Kg), %BFat (%), and BMI (Kg/\(\text{Ht}^2\)) with time in patients with Clear and Unclear Margins.

There were statistically significant differences in Weight between the Clear Margin and Unclear Margin groups at the pre-operative (p=0.047), two weeks (p=0.041), and five weeks (p=0.049) post-operative measurement time-points. The BMI was also shown to be statistically different between the two groups at the pre-operative (p=0.048) and two weeks (p=0.035) post-operative measurement time-points. However, there were no statistically significant differences in %BFat between the two groups at any measurement time point.
As with the Weight, %BFat, and BMI, the mean TBN values were higher for the Clear Margin group at all measurement time-points. This is demonstrated in Figure 21.

The TBN in the Unclear Margin group is shown to decrease and continue decreasing from the pre-operative time-point with its biggest drop at the final 26 weeks post-operative time-point. There is virtually no change between the five weeks and 14 weeks post-operative time-points. However, there seems to be an increase in TBN for this group of patients at the 14 weeks post-operative time-point which cannot be explained. For the Clear Margin group, the TBN levels seem to be stable after its initial post-operative drop. This indicates that in the Clear Margin group the patients stabilise but do not regain their pre-operative TBN during the 26 weeks post-operative period. The Unclear Margin group, not only do not regain their pre-operative TBN levels, but also will continue to decline in TBN content.

Figure 21. Graph of mean (±SE) TBN against time (measurement time-point) for Clear and Unclear Margin groups.
The difference between the TBN levels in the Clear margin and Unclear Margin groups were found to be statistically significant (p=0.052) at the 26 weeks post-operative time-point.

Similar to NI, the TBK/Ht range is used at our centre as a measure of the patient’s TBK levels compared to the “normal” range for their gender. The NI is the TBN expressed as a percentage of age, sex, and height-matched “normal” and the “normal” range at our centre is generally considered to be from 0.95 to 1.05, i.e. 95% to 105% of normal. The TBK/Ht’s “normal” range is considered to be $0.86 \pm 0.09$ g/cm (or 0.72 to 1.00 g/cm) for males and $0.59 \pm 0.05$ g/cm (or 0.52 to 0.66 g/cm) for females \cite{813, 818, 812, 814}. In our group of patients who had undergone Whipple’s Procedure, both the NI as well as TBK/Ht was shown to be higher for the Clear Margin group. These differences are demonstrated in Figure 22.

**Figure 22.** Graph of mean (±SE) NI and TBK/Ht against time for the Clear Margin and Unclear Margin groups.
Chapter 9: WHIPPLE’S PROCEDURE PATIENT RESULTS

The difference between the NI values in the Clear margin and Unclear Margin groups were found to be statistically significant at the five weeks (p=0.046), 14 weeks (p=0.016), as well as the 26 weeks post-operative time-point (p=0.027). None of the differences between the TBK/Ht values in the Clear margin and Unclear Margin groups were found to be statistically significant.

In Figure 22 it can be seen that patients with Clear Margins had mean NI values which were within the normal range up to 26 weeks post-operatively. However, patients with Unclear Margins had mean NI values which were below the 0.95 cut-off of normal range and, therefore, were not within the normal range.

One of the methods of measuring the LBM is using the BIA technique. The details of this method are given in the section on BIA (Chapters 7.2.4 & 8.1.2 and Appendix E 1.2.5.3). Once the resistance and reactance values are obtained using the BIA instrument, together with the patient’s height, weight, and gender are placed in the appropriate equations to calculate the LBM. The three most common equations used at our centre to calculate the LBM using the BIA technique are the Lukaski [797, 798], Segal [799, 754], and Van Loan [800, 780] equations. Figure 23 shows the LBM measurements for the Clear Margin and Unclear Margin groups at each of the measurement time-points. It demonstrates that at all five measurement points the Clear Margin group had a higher LBM than the Unclear Margin group.

The difference between the LBM levels in the Clear margin and Unclear Margin groups were found to be statistically significant at different measurement time-points depending on which of the three equations were utilised. The Lukaski (p=0.022) and Van Loan (p=0.047) equations demonstrated statistically significant differences only at the 26 weeks post-operative measurement time-point. However, the Segal equation demonstrated almost statistically significant differences at the pre-operative (p=0.054) and five weeks (p=0.052),
and statistically significant differences at 26 weeks (p=0.024) post-operative measurement time-points.

Figure 23. Graph of mean (±SE) LBM measurements using BIA technique against time for Clear Margin and Unclear Margin groups.

The LBM can also be measured by other techniques. The traditional, and perhaps the most common, method of measurement of LBM is to measure skinfold thicknesses and calculate the %BFat using the Durnin and Womersley’s equations \cite{756, 772, 771} and then calculate the LBM from weight and %BFat. Details of skinfold thickness measurement technique and measurements of %BFat are given in the anthropometry section (Chapters 7.3.4 & 8.1 and Appendix E 1.2.5.1). Although the LBM can also be calculated from TBK the actual measurement provides FFM (see sections 7.3, 7.4, and 8.4). However, for the purposes of consistency and to avoid confusion, the LBM (or FFM) measurements resulting from TBK
Whole Body Counting will be referred to as LBM (by TBK). Details of TBK measurement using the Whole Body Counter technique are given in the TBK section (Chapters 7.4 & 8.4 and Appendix E 1.3.4.4).

With these LBM measurements, as with the previous body composition parameters measures, the mean values for the Clear Margin group was higher than the Unclear Margin group. Neither the skinfold-measured LBM nor the TBK-derived LBM were shown to be statistically different between the two surgical groups.

LBM measurement results for the above two techniques for the Clear Margin and Unclear Margin groups are shown in Figure 24.

Figure 24. Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for Clear Margin and Unclear Margin groups.

Mean (±SE) LBM Measurements Using Skinfolds And TBK Techniques For Clear And Unclear Margin Groups
The FM is measured by subtracting the LBM from the total body weight. So, in general, the trends observed in LBM and Weight should be observed with FM as well. Figure 25 demonstrates the differences between the Clear Margin and Unclear Margin groups at different measurement time-points.

In general, the FM content of the Unclear Margin group tends to be lower than the Clear Margin group. The FM levels measured using the skinfold technique seem to decrease and continue decreasing from the pre-operative time-point and stabilise after the 14 weeks post-operative time-point in the Clear Margin group. Interestingly, the FM levels measured by the TBK technique also seem to decrease and continue decreasing, but only after the two weeks post-operative time-point and in the Unclear Margin group. The two weeks post-operative time-point represents the point of discharge from the hospital.

Figure 25. Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for Clear Margin and Unclear Margin groups.
Chapter 9: WHIPPLE’S PROCEDURE PATIENT RESULTS

The differences in the skinfold-derived FM between the Clear Margin and Unclear Margin groups were shown to be statistically significant at the pre-operative (p=0.027), the two weeks (p=0.044), and the five weeks (p=0.051) post-operative measurement time-points. However, there were no statistically significant differences in TBK-derived FM between the two surgical groups.

Measurements of TBK are very important as the TBK levels represent the BCM. The TBK measurements provide an estimate of the FFM on the basis that potassium is mainly present in the FFM. Details of the TBK measurement method are given in the TBK section (Chapters 7.4 & 8.4 and Appendix E 1.3.4.4).

Although the TBK levels in the Clear Margin group were higher at all five measurement time-points, the actual levels were found to be relatively stable following the observed drop (statistically not significant) after the pre-operative measurement. There is a general steady decrease in the TBK levels of the Unclear Margin group with the exception of a non-statistically significant increase at the 14 weeks post-operative measurement time-point which cannot be explained. This trend is consistent with the changes observed with the TBN and NI during the five measurement points which is expected as both NI and TBN also represent the “metabolic” compartment of the body. The TBK changes are demonstrated in Figure 26. There were no statistically significant differences in TBK between the two surgical groups.

The TBW is one of the main four body compartments. It can change as a result of surgery as well as therapy, chemotherapy, etc. At our centre, the TBW is measured using the BIA technique and the equations of Fredrix [753], Pullicino [755], or Kushner & Schoeller [746, 748, 745, 747] are used to calculate the final TBW from the resistance and reactance values obtained.
from the BIA instrument. These were validated at our centre in gastrointestinal cancer patients [758, 851, 846] as well as renal dialysis patients [686].

**Figure 26.** Graph of mean (±SE) TBK measurements against time for Clear Margin and Unclear Margin groups.

Figure 27 demonstrates changes in TBW, using the Fredrix [753], Pullicino [755], or Kushner & Schoeller [746, 748, 745, 747] equations, in the Clear Margin and Unclear Margin groups. As shown here, the Clear Margin group has a lower TBW than the Unclear Margin group. The TBW levels are relatively stable with a rapid drop at the last measurement time-point. This large drop is specific to the Clear Margin group.
The difference between the TBW levels in the Clear margin and Unclear Margin groups were found to be statistically significant only at the 26 weeks post-operative measurement time-point when the Fredrix (p=0.015), Pullicino (0.015), or the Kushner (p=0.015) equations were used.

9.3.2 Body Composition Changes Between Time-Points

For all parameters measured, ANOVA was performed to statistically determine changes within each of the Clear Margin and Unclear Margin groups. This was used to see whether statistically there are any significant differences between body composition measurement time-points in each group. Results for these analyses are summarised and tabulated in Table 6. The results of the ANOVA carried out on the Clear Margin and Unclear Margin groups showed that there are highly significant differences in the Weight, %BFat, and FM with no
statistically significant differences for the TBN, and NI for either the Clear Margin or the Unclear Margin groups. Results of the ANOVA test demonstrated no statistically significant differences in any of the other parameters tested.

One method of determining and statistically analysing differences between the two groups was to look at the AUCs and slopes. In another words, to statistically look at the differences in body composition parameters of the groups as a whole, regardless of the time-points where measurements were carried out. Here, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the AUC for each patient curve is calculated. These AUC values for each group is then compared statistically (Two Sample T-Test (assuming unequal variances)). Similarly, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the slope for each patient curve is measured. This slope values for each group is then compared statistically (Two Sample T-Test (assuming unequal variances)). The results for these measurements are also summarised and tabulated in Table 6 together with the ANOVA results.

With the exception of %BFat By TBK (p=0.07), all AUC results comparing the Clear Margin and Unclear Margin groups were shown to be significant. Interestingly, none of the slope comparisons were found to be significant.
Table 6. Summary of statistical results (p-Values) for comparison between Clear Margins and Unclear Margins group. Results sorted by parameters.

<table>
<thead>
<tr>
<th>Body Composition Parameter</th>
<th>ANOVA Unclear Margins p-Value</th>
<th>ANOVA Clear Margins p-Value</th>
<th>AUC p-Value</th>
<th>Slope p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.012</td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
<td>0.033</td>
</tr>
<tr>
<td>%BFat By TBK</td>
<td>0.349</td>
<td>0.694</td>
<td>0.07</td>
<td>0.457</td>
</tr>
<tr>
<td>BMI</td>
<td>0.143</td>
<td>0.141</td>
<td>0.006</td>
<td>0.460</td>
</tr>
<tr>
<td>FM</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>0.448</td>
</tr>
<tr>
<td>FM By TBK</td>
<td>0.260</td>
<td>0.527</td>
<td>0.009</td>
<td>0.385</td>
</tr>
<tr>
<td>LBM</td>
<td>0.294</td>
<td>0.014</td>
<td>0.005</td>
<td>0.407</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.338</td>
<td>0.946</td>
<td>0.001</td>
<td>0.393</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.567</td>
<td>0.420</td>
<td>0.001</td>
<td>0.377</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.472</td>
<td>0.319</td>
<td>0.001</td>
<td>0.402</td>
</tr>
<tr>
<td>LBM By TBK</td>
<td>0.842</td>
<td>0.104</td>
<td>0.004</td>
<td>0.312</td>
</tr>
<tr>
<td>NI</td>
<td>0.558</td>
<td>0.895</td>
<td>0.001</td>
<td>0.203</td>
</tr>
<tr>
<td>TBK</td>
<td>0.914</td>
<td>0.495</td>
<td>0.004</td>
<td>0.328</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.898</td>
<td>0.179</td>
<td>0.004</td>
<td>0.319</td>
</tr>
<tr>
<td>TBN</td>
<td>0.324</td>
<td>0.626</td>
<td>0.001</td>
<td>0.289</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.529</td>
<td>0.626</td>
<td>0.002</td>
<td>0.225</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.463</td>
<td>0.991</td>
<td>0.005</td>
<td>0.491</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.529</td>
<td>0.626</td>
<td>0.001</td>
<td>0.225</td>
</tr>
<tr>
<td>Weight</td>
<td>0.021</td>
<td>&lt;0.0001</td>
<td>0.002</td>
<td>0.475</td>
</tr>
</tbody>
</table>

9.3.3 Changes Between Groups At Specific Time-Points

In order to investigate whether each of the two Clear Margin and Unclear Margin groups progress at the same or different rates, one has to determine whether there are any differences between these groups at different measurement time-points.

To show this, body composition parameters measured at each time-point from the Clear Margin group was statistically (T-Test) compared with the body composition parameters measured at the same time-point in the Unclear Margin group. As it can be seen in Table 7, the FM (p=0.027) was statistically the most significant parameter that was different between the two groups at the pre-operative base-line time-point. This was closely followed by Weight (p=0.047) and BMI (P=0.048). There were virtually no significant differences in the other
pre-operative (base-line) measured body composition parameters between the Clear Margin and Unclear Margin groups. The same was almost also true for the two weeks post-operative values, with the BMI (p=0.035), weight (p=0.041), and FM (0.044) again being the closest ones to statistical significance. At the five weeks post-operative time-point the BMI, although close to being statistically significant (p=0.058), could no longer be considered as significant. However, the NI (p= 0.046) was the parameter which was most significantly different between the two surgical groups. This was closely followed by Weight (p=0.049), FM (p=0.051), and LBM by Segal (p=0.052). At the 14 weeks post-operative time-point, the only parameter tested that was significantly different between the two surgical groups was NI (p=0.016). None of the parameters tested reached statistical significance. The major differences between the Clear Margin and Unclear Margin groups were seen at the 26 weeks post-operative measurements. Here, all three TBW measurements (all measured using the BIA technique), NI, TBN, and all LBM measurements measured using the BIA technique were found to be significant. Weight was found to be closest with p=0.058 and FM (p=0.217) did not reach statistical significance.
Table 7. Statistical comparison of body composition parameters of Clear Margin and Unclear Margin groups at corresponding time-points. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Body Composition Parameters</th>
<th>Pre-Operative p-Value</th>
<th>2 Weeks Post-Op p-Value</th>
<th>5 Weeks Post-Op p-Value</th>
<th>14 Weeks Post-Op p-Value</th>
<th>26 Weeks Post-Op p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.114</td>
<td>0.193</td>
<td>0.195</td>
<td>0.572</td>
<td>0.891</td>
</tr>
<tr>
<td>%BFat By TBK</td>
<td>0.680</td>
<td>0.646</td>
<td>0.354</td>
<td>0.787</td>
<td>0.675</td>
</tr>
<tr>
<td>BMI</td>
<td>0.048</td>
<td>0.035</td>
<td>0.058</td>
<td>0.198</td>
<td>0.131</td>
</tr>
<tr>
<td>FM</td>
<td>0.027</td>
<td>0.044</td>
<td>0.051</td>
<td>0.185</td>
<td>0.217</td>
</tr>
<tr>
<td>FM By TBK</td>
<td>0.112</td>
<td>0.129</td>
<td>0.078</td>
<td>0.365</td>
<td>0.656</td>
</tr>
<tr>
<td>LBM</td>
<td>0.186</td>
<td>0.137</td>
<td>0.154</td>
<td>0.334</td>
<td>0.106</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.087</td>
<td>0.318</td>
<td>0.084</td>
<td>0.340</td>
<td>0.022</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.055</td>
<td>0.080</td>
<td>0.052</td>
<td>0.187</td>
<td>0.024</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.075</td>
<td>0.106</td>
<td>0.074</td>
<td>0.251</td>
<td>0.047</td>
</tr>
<tr>
<td>LBM By TBK</td>
<td>0.230</td>
<td>0.304</td>
<td>0.373</td>
<td>0.537</td>
<td>0.144</td>
</tr>
<tr>
<td>NI</td>
<td>0.201</td>
<td>0.092</td>
<td>0.046</td>
<td>0.016</td>
<td>0.027</td>
</tr>
<tr>
<td>TBK</td>
<td>0.246</td>
<td>0.319</td>
<td>0.345</td>
<td>0.528</td>
<td>0.148</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.213</td>
<td>0.288</td>
<td>0.358</td>
<td>0.539</td>
<td>0.118</td>
</tr>
<tr>
<td>TBN</td>
<td>0.244</td>
<td>0.240</td>
<td>0.136</td>
<td>0.112</td>
<td>0.052</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.103</td>
<td>0.455</td>
<td>0.104</td>
<td>0.404</td>
<td>0.015</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.131</td>
<td>0.331</td>
<td>0.104</td>
<td>0.401</td>
<td>0.015</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.103</td>
<td>0.455</td>
<td>0.104</td>
<td>0.404</td>
<td>0.015</td>
</tr>
<tr>
<td>Weight</td>
<td>0.047</td>
<td>0.041</td>
<td>0.049</td>
<td>0.137</td>
<td>0.058</td>
</tr>
</tbody>
</table>

An interesting observation that can be made here is that in the first 14 weeks post-surgery, the FM and Weight seem to be the common parameters that were found to be significantly different between the Clear Margin and Unclear Margin groups.

9.3.4 Changes Between Groups At Specific Time-Points

Compared To Base-Line

In order to assess the changes in body composition parameters, for each group the pre-operative measurements were taken as the base-line values. Measurements at the next four time-points were then each compared to this base-line. T-Test was then used to compare the same time-point measurements between the Clear Margin and Unclear Margin groups. For example, the two weeks post-operative measurements in the Clear Margin group were
statistically compared with the two weeks post-operative measurements in the Unclear Margin group. This was then repeated for the five, 14 and 24 weeks post-operative measurements. The results for these measurements are summarised and tabulated in Table 8.

The patterns of change in the body composition parameters with time were also investigated. In order to visually establish how and when these changes occur, the measurements at each of the chemotherapy cycle time-point were subtracted from the base-line values. These were then plotted against time and T-Test was used to compare the Arm A and Arm B patient groups.

The most obvious of all measurements one can expect to change as a result of major abdominal operation is the weight. Figure 28 shows the changes in weight with respect to the pre-operative weight with time.

Figure 28. Changes in Weight with time in Whipple’s Procedure patients with Clear and Unclear Margins.

When the means for each time-point was compared statistically, the changes at 14 weeks (p=0.006) was found to be statistically significant. The changes at 26 weeks were found to be
“almost” significant (p=0.061). A two-tailed T-Test of the Unclear Margin versus Clear Margin group’s Weights also showed an “almost” significant (p=0.061) difference.

The %BFat, which is derived from skin fold thickness measurements and calculated using the Durnin & Womersley’s equations were investigated. These overall mean changes were found to be statistically significant (p=0.054, two-tailed T-Test). The graph of these changes is shown in Figure 29.

Statistically, the overall mean changes in the BMI (p=0.112, two-tailed T-Test), which is calculated from height and weight, were not found to be significant. The changes in BMI are shown in Figure 30. As with the Weight, the changes in BMI at 14 weeks (p=0.006) was found to be statistically significant. In addition, at 26 weeks the changes between the surgical groups were also found to be statistically significant (p=0.027). These similarities are probably expected as Weight is one of the two components of BMI.
As previously indicated, FM has been one of the most common parameters that has been shown to be significantly different between the Unclear Margin and Clear Margin groups at almost all measurement time-points. These changes are shown in Figure 31. When the FM values are compared to the base-line values, the differences between the two surgical groups were found to be statistically significant at the five weeks (p=0.022), 14 weeks (p=0.007), and the 26 weeks (p=0.012) post-operative measurement time-points.
It is interesting to note that when one statistically (T-Test) compares the changes (with respect to base-line) in the body composition parameters of the Clear Margin and Unclear Margin groups, i.e., looking at individual time-points between the two groups, a more detailed picture emerges: There does not seem to be a statistically significant change, in any of the parameters measured, between the base-line and two weeks post-operative or the base-line and five weeks post-operative time-points. The only exception is the FM (p=0.022) between the base-line and five weeks measurement time-point. Generally, statistically significant changes seem to mostly be between the base-line and 14 weeks post-operative (50%) followed by, to a lesser extent, the base-line and 26 weeks (16.7%) post-operative time-points. These results are summarised and tabulated in Table 8.

As can be seen from Table 8, the changes in Weight (p=0.006), BMI (p=0.005), and FM (p=0.007) at the 14 week post-operative time-point compared with base-line were found to be highly significant. At this time-point, the %BFat, TBK/Ht, LBM (By TBK), LBM (Van Loan), TBK, and LBM (Segal) were found to be significantly different to the base-line. Statistically LBM was found to be “almost” significant (p=0.058). All other body composition parameters...
parameters measured were not found to show a statistically significant difference between the 14 weeks post-operative time-point and the base-line measurements.

At the 26 weeks post-operative time-point, only the %BFat (p=0.003), FM (p=0.012), and BMI (p=0.027) parameters were found to show a statistically significant difference as compare to the base-line measurements. The closest significance parameter was the Weight (p=0.062). None of the other body composition measurements measured showed a statistically significant difference.

<table>
<thead>
<tr>
<th>Body Composition Parameters</th>
<th>2 Weeks Post-Operative p-Value</th>
<th>5 Weeks Post-Operative p-Value</th>
<th>14 Weeks Post-Operative p-Value</th>
<th>26 Weeks Post-Operative p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.229</td>
<td>0.143</td>
<td>0.016</td>
<td>0.003</td>
</tr>
<tr>
<td>%BFat By TBK</td>
<td>0.868</td>
<td>0.211</td>
<td>0.687</td>
<td>0.070</td>
</tr>
<tr>
<td>BMI</td>
<td>0.924</td>
<td>0.734</td>
<td>0.005</td>
<td>0.027</td>
</tr>
<tr>
<td>FM</td>
<td>0.110</td>
<td>0.022</td>
<td>0.007</td>
<td>0.012</td>
</tr>
<tr>
<td>FM By TBK</td>
<td>0.971</td>
<td>0.610</td>
<td>0.753</td>
<td>0.084</td>
</tr>
<tr>
<td>LBM</td>
<td>0.781</td>
<td>0.726</td>
<td>0.058</td>
<td>0.521</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.204</td>
<td>0.547</td>
<td>0.071</td>
<td>0.601</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.469</td>
<td>0.340</td>
<td>0.038</td>
<td>0.703</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.434</td>
<td>0.344</td>
<td>0.034</td>
<td>0.603</td>
</tr>
<tr>
<td>LBM By TBK</td>
<td>0.453</td>
<td>0.230</td>
<td>0.023</td>
<td>0.815</td>
</tr>
<tr>
<td>NI</td>
<td>0.750</td>
<td>0.523</td>
<td>0.808</td>
<td>0.982</td>
</tr>
<tr>
<td>TBK</td>
<td>0.625</td>
<td>0.398</td>
<td>0.035</td>
<td>0.747</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.538</td>
<td>0.275</td>
<td>0.021</td>
<td>0.886</td>
</tr>
<tr>
<td>TBN</td>
<td>0.765</td>
<td>0.915</td>
<td>0.919</td>
<td>0.801</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.241</td>
<td>0.530</td>
<td>0.198</td>
<td>0.200</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.296</td>
<td>0.602</td>
<td>0.096</td>
<td>0.469</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.241</td>
<td>0.530</td>
<td>0.198</td>
<td>0.200</td>
</tr>
<tr>
<td>Weight</td>
<td>0.381</td>
<td>0.123</td>
<td>0.006</td>
<td>0.062</td>
</tr>
</tbody>
</table>
9.3.5 Changes Within Each Group Between Different Time-Points

In order to determine statistically whether there were any changes between different measurement time points, both for the Clear Margin as well as the Unclear Margin groups, paired T-Test was performed on different body composition parameters between the time-points. The results for the Unclear Margin group is summarised in Table 9 and the Clear Margin group in Table 10.

**Clear Margins**

Results of the comparisons between different measurement time-points in the Clear Margin group demonstrated that there is a highly significant decrease in Weight between base-line and five weeks post-operative and significant decreases between base-line and 14 weeks post-operative as well as two and five weeks post-operative time-points.

There were highly significant decreases in both % BFat and FM between the pre-operative and two weeks post-operative time points, the pre-operative and five weeks post-operative time points, the pre-operative and 14 weeks post-operative time points, and the pre-operative and 26 weeks post-operative time points. In addition, a significant decrease in FM (p=0.017) was found between the two and five weeks post-operative time points. In effect, compared to the pre-operative status, there was a statistically significant decline in %BFat, and FM up to the 26 weeks post-operative time-point.

Although there were decreases in Weight, % BFat, and FM during the entire 26 week period, the Weight, LBM and FM were the only parameters found to have a statistically significant decline between the two and five weeks post-operative time-points.

The BMI, despite having a weight component, only showed a statistically significant decrease between the pre-operative and 14 weeks post-operative time points and the 26 weeks and five weeks post-operative time points.
The general trend for TBN values was a constant decline. The TBN and NI parameters both showed a statistically significant decrease between the pre-operative and two weeks post-operative time points, two and five weeks post-operative time points, two and 14 weeks post-operative time points, and the five and 14 weeks post-operative time points. The NI, however, did not show the statistical significant decrease between the two and five weeks post-operative time points that TBN did.

The TBK results showed significant decreases between the two and five weeks post-operative time points, two and 26 weeks post-operative time points, five and 14 weeks post-operative time points, and five and 26 weeks post-operative time points. The TBK/Ht which has been used at our centre as an indicator of fat-free mass, as well as the LBM (by TBK) both showed a statistically significant decrease between pre-operative and five weeks post-operative time points, five and 14 weeks post-operative time points, to and 26 weeks post-operative time-points, and five and 26 weeks post-operative time points.

The FM measured by TBK showed statistically significant decreases between the pre-operative and 26 weeks post-operative time-points, two weeks and 14 weeks post-operative time-points, two weeks and 26 weeks post-operative time-points, five weeks and 14 weeks post-operative time-points, and the five weeks and 26 weeks post-operative time-points.

The LBM measured using the skinfold technique showed slight but statistically significant decreases between the two and five weeks post-operative time-points and the five and 26 weeks post-operative time-points only. The LBMs measured using the BIA technique showed statistically significant decreases between pre-operative and five weeks post-operative time points and statistically significant increases between five and 14 weeks, and five and 26 weeks (Lukaski) post-operative time-points. The LBM (Segal) and LBM (Van Loan) showed statistically significant decrease between the five and 26 weeks post-operative time-point.
The TBW measurements using the Fredrix and Pullicino equations both showed statistically significant increases between the pre-operative and 14 weeks post-operative, two and 14 weeks post-operative, five and 14 weeks post-operative, and five and 26 weeks post-operative time-points. The TBW (Kushner), however, showed a statistically significant decrease between pre-operative and 5 weeks post-operative time-points, but an increase between five and 14 weeks post-operative time-points. These results overall may suggest a general, statistically significant increase in TBW compartment.

**Unclear Margins**

Compared with the Clear Margin group, the patients with Unclear Margins generally did not show many statistically significant changes in the body composition parameters measured. Majority of these changes were found to be between the pre-operative and five weeks post-operative time-points.

The Weight, %BFat, FM, LBM, BMI, TBN, and NI showed statistically significant decrease between the pre-operative and five weeks post-operative time-points. The FM additionally showed a significant decrease between the two and five weeks post-operative time points.

As with the Clear Margin group, there were significant increases in TBW. However, only the increases between the pre-operative and 14 weeks post-operative time-points and the pre-operative and 26 weeks post-operative time-points were found to be statistically significant.

Both the TBK and TBK/Ht showed statistically significant increases between the two and 14 weeks post-operative time-points. The LBM measured using TBK also showed a statistically significant increase between the five and 14 weeks post-operative time-points.
Table 9. Summary of the comparison (paired T-Test) of body composition parameters between different measurement time-points for the patients with Unclear Margins. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Body Composition Parameters</th>
<th>Baseline &amp; 2 Wks</th>
<th>Baseline &amp; 5 Wks</th>
<th>Baseline &amp; 14 Wks</th>
<th>Baseline &amp; 26 Wks</th>
<th>2 &amp; 5 Wks</th>
<th>2 &amp; 14 Wks</th>
<th>2 &amp; 26 Wks</th>
<th>5 &amp; 14 Wks</th>
<th>5 &amp; 26 Wks</th>
<th>14 &amp; 26 Wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.099</td>
<td>0.286</td>
<td>0.555</td>
<td>0.146</td>
<td>0.843</td>
<td>0.390</td>
<td>0.767</td>
<td>0.261</td>
<td>0.813</td>
<td></td>
</tr>
<tr>
<td>%BFat By TBK</td>
<td>0.739</td>
<td>0.513</td>
<td>0.062</td>
<td>0.349</td>
<td>0.321</td>
<td>0.820</td>
<td>0.485</td>
<td>0.380</td>
<td>0.990</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.142</td>
<td>0.637</td>
<td>0.388</td>
<td>0.143</td>
<td>0.301</td>
<td>0.177</td>
<td>0.190</td>
<td>0.158</td>
<td>0.256</td>
<td></td>
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<tr>
<td>FM</td>
<td>0.152</td>
<td>0.339</td>
<td>0.560</td>
<td>0.046</td>
<td>0.924</td>
<td>0.263</td>
<td>0.656</td>
<td>0.248</td>
<td>0.753</td>
<td></td>
</tr>
<tr>
<td>FM By TBK</td>
<td>0.777</td>
<td>0.087</td>
<td>0.592</td>
<td>0.260</td>
<td>0.409</td>
<td>0.983</td>
<td>0.558</td>
<td>0.353</td>
<td>0.994</td>
<td></td>
</tr>
<tr>
<td>LBM</td>
<td>0.582</td>
<td>0.221</td>
<td>0.488</td>
<td>0.294</td>
<td>0.196</td>
<td>0.340</td>
<td>0.141</td>
<td>0.377</td>
<td>0.967</td>
<td></td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.702</td>
<td>0.090</td>
<td>0.376</td>
<td>0.338</td>
<td>0.170</td>
<td>0.147</td>
<td>0.193</td>
<td>0.538</td>
<td>0.341</td>
<td></td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.831</td>
<td>0.138</td>
<td>0.940</td>
<td>0.567</td>
<td>0.187</td>
<td>0.066</td>
<td>0.178</td>
<td>0.858</td>
<td>0.296</td>
<td></td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.804</td>
<td>0.171</td>
<td>0.921</td>
<td>0.472</td>
<td>0.207</td>
<td>0.082</td>
<td>0.190</td>
<td>0.875</td>
<td>0.296</td>
<td></td>
</tr>
<tr>
<td>LBM By TBK</td>
<td>0.344</td>
<td>0.108</td>
<td>0.479</td>
<td>0.842</td>
<td>0.017</td>
<td>0.340</td>
<td>0.029</td>
<td>0.789</td>
<td>0.848</td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>0.167</td>
<td>0.120</td>
<td>0.399</td>
<td>0.558</td>
<td>0.546</td>
<td>0.214</td>
<td>0.825</td>
<td>0.383</td>
<td>0.463</td>
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</tr>
<tr>
<td>TBK</td>
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<td>0.479</td>
<td>0.914</td>
<td>0.009</td>
<td>0.252</td>
<td>0.029</td>
<td>0.789</td>
<td>0.848</td>
<td></td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.345</td>
<td>0.081</td>
<td>0.469</td>
<td>0.898</td>
<td>0.014</td>
<td>0.346</td>
<td>0.026</td>
<td>0.802</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>TBN</td>
<td>0.268</td>
<td>0.140</td>
<td>0.594</td>
<td>0.324</td>
<td>0.675</td>
<td>0.172</td>
<td>0.954</td>
<td>0.553</td>
<td>0.395</td>
<td></td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.507</td>
<td>0.051</td>
<td>0.054</td>
<td>0.529</td>
<td>0.170</td>
<td>0.251</td>
<td>0.219</td>
<td>0.234</td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.766</td>
<td>0.527</td>
<td>0.100</td>
<td>0.463</td>
<td>0.225</td>
<td>0.869</td>
<td>0.264</td>
<td>0.190</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.507</td>
<td>0.051</td>
<td>0.054</td>
<td>0.529</td>
<td>0.170</td>
<td>0.251</td>
<td>0.219</td>
<td>0.234</td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.268</td>
<td>0.782</td>
<td>0.481</td>
<td>0.131</td>
<td>0.431</td>
<td>0.214</td>
<td>0.208</td>
<td>0.211</td>
<td>0.483</td>
<td></td>
</tr>
</tbody>
</table>
### Table 10. Summary of the comparison (paired T-Test) of body composition parameters between different measurement time-points for the patients with Clear Margins. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Body Composition Parameters</th>
<th>Base-line &amp; 2 Wks</th>
<th>Base-line &amp; 5 Wks</th>
<th>Base-line &amp; 14 Wks</th>
<th>2 &amp; 5 Wks</th>
<th>2 &amp; 14 Wks</th>
<th>2 &amp; 26 Wks</th>
<th>5 &amp; 14 Wks</th>
<th>5 &amp; 26 Wks</th>
<th>14 &amp; 26 Wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.122</td>
<td>0.139</td>
<td>0.136</td>
<td>0.260</td>
<td>0.240</td>
</tr>
<tr>
<td>%BFat By TBK</td>
<td>0.539</td>
<td>0.247</td>
<td>0.545</td>
<td>0.078</td>
<td>0.694</td>
<td>0.107</td>
<td>0.014</td>
<td>0.061</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI</td>
<td>0.179</td>
<td>0.012</td>
<td>0.007</td>
<td>0.082</td>
<td>0.141</td>
<td>0.118</td>
<td>0.458</td>
<td>0.760</td>
<td>0.471</td>
</tr>
<tr>
<td>FM</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.048</td>
<td>0.527</td>
<td>0.045</td>
<td>0.022</td>
<td>0.053</td>
<td>0.019</td>
</tr>
<tr>
<td>FM By TBK</td>
<td>0.842</td>
<td>0.724</td>
<td>0.075</td>
<td>0.927</td>
<td>0.014</td>
<td>0.240</td>
<td>0.980</td>
<td>0.102</td>
<td>0.010</td>
</tr>
<tr>
<td>LBM</td>
<td>0.954</td>
<td>0.035</td>
<td>0.005</td>
<td>0.239</td>
<td>0.420</td>
<td>0.198</td>
<td>0.471</td>
<td>0.010</td>
<td>0.013</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.090</td>
<td>0.019</td>
<td>0.440</td>
<td>0.708</td>
<td>0.946</td>
<td>0.027</td>
<td>0.187</td>
<td>0.003</td>
<td>0.008</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.113</td>
<td>0.005</td>
<td>0.329</td>
<td>0.230</td>
<td>0.420</td>
<td>0.198</td>
<td>0.471</td>
<td>0.010</td>
<td>0.013</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.090</td>
<td>0.003</td>
<td>0.223</td>
<td>0.148</td>
<td>0.319</td>
<td>0.198</td>
<td>0.467</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>LBM By TBK</td>
<td>0.069</td>
<td>0.010</td>
<td>0.235</td>
<td>0.732</td>
<td>0.104</td>
<td>0.253</td>
<td>0.044</td>
<td>0.026</td>
<td>0.002</td>
</tr>
<tr>
<td>NI</td>
<td>0.037</td>
<td>0.125</td>
<td>0.638</td>
<td>0.627</td>
<td>0.895</td>
<td>0.045</td>
<td>0.273</td>
<td>0.007</td>
<td>0.586</td>
</tr>
<tr>
<td>TBK</td>
<td>0.065</td>
<td>0.039</td>
<td>0.405</td>
<td>0.577</td>
<td>0.495</td>
<td>0.140</td>
<td>0.051</td>
<td>0.054</td>
<td>0.025</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.093</td>
<td>0.016</td>
<td>0.244</td>
<td>0.875</td>
<td>0.179</td>
<td>0.278</td>
<td>0.039</td>
<td>0.038</td>
<td>0.003</td>
</tr>
<tr>
<td>TBN</td>
<td>0.020</td>
<td>0.046</td>
<td>0.848</td>
<td>0.455</td>
<td>0.626</td>
<td>0.040</td>
<td>0.182</td>
<td>0.004</td>
<td>0.382</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.320</td>
<td>0.383</td>
<td>0.023</td>
<td>0.224</td>
<td>0.626</td>
<td>0.018</td>
<td>0.246</td>
<td>0.002</td>
<td>0.045</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.193</td>
<td>0.046</td>
<td>0.848</td>
<td>0.430</td>
<td>0.991</td>
<td>0.240</td>
<td>0.652</td>
<td>0.003</td>
<td>0.116</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.320</td>
<td>0.383</td>
<td>0.023</td>
<td>0.224</td>
<td>0.626</td>
<td>0.018</td>
<td>0.246</td>
<td>0.002</td>
<td>0.045</td>
</tr>
<tr>
<td>Weight</td>
<td>0.071</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.067</td>
<td>0.008</td>
<td>0.122</td>
<td>0.460</td>
<td>0.351</td>
<td>0.084</td>
</tr>
</tbody>
</table>

### 9.4 Conclusions

The main conclusion that can be drawn from the results is that post-operatively, with the exception of FM, there were no significant changes in the measured body composition parameters at the two and five weeks time-points. However, the main time-points where changes did occur were around the 5 and 14 weeks post-operative time-point.

When changes were measured compared to the base-line pre-operative time points and the two groups were compared statistically at the corresponding time points (see Table 8), highly
Chapter 9: WHIPPLE’S PROCEDURE PATIENT RESULTS

significant changes in Weight (p=0.006), BMI (p=0.005), and FM (p=0.007) followed by significant changes in %BFat (p=0.016), TBK/Ht (p=0.021), LBM (By TBK) (p=0.023), LBM (Van Loan) (p=0.034), and LBM (Segal) (p=0.038) at the 14 week time-point. At the 26 weeks post-operative time point, the only significant changes were in the FM (p=0.012), %BFat (p=0.003), and BMI (p=0.027) parameters. There was no significant changes in any of the body composition parameters tested at the two and five weeks post-operative time-points. These suggest that the only body compartment that was affected post-operatively may be the fat compartment. This would, therefore, suggest that in pancreatic cancer patients undergoing Whipple’s Procedure the major long term change in their body composition is in the fat compartment.

When the two Clear Margin and Unclear Margin groups were compared at each corresponding time-point (see Table 7), the statistically significant differences were found in the TBW (Fredrix), TBW (Pullicino), TBW (Kusner), LBM (Lukaski), LBM (Segal), and LBM (Van Loan), TBN, and NI, between the two groups at the 26 weeks post-operative time-point. The NI was also shown to demonstrate additional significant difference at the five and 14 weeks post-operative time-point. Additionally, the FM was shown to be statistically significant at the pre-operative, two weeks, and five weeks post-operative time-points with the BMI being significant at the pre-operative and two weeks post-operative time-points. Therefore, the major statistically difference in body composition between the two groups were in TBN, NI, LBM (measured using BIA techniques), and TBW parameters mainly at the 26 weeks post-operative time-points although the FM and BMI are being affected earlier in the disease. This may, therefore, suggest a deviation between the two groups in their TBN, LBM and TBW content observable in a long term setting and fat content in the relatively shorter term. For this a larger patient population and a 12 or 18 month follow-up is required, which may not be possible due to the nature of this disease.
When the Clear Margin and Unclear Margin groups were compared by statistically analysing the differences between different time-points within each group, the results showed that although the Unclear Margin group has lower body composition values than the Clear Margin group, both groups seem to begin to gradually “equalise” around the 14 weeks post-operative time-point. This may suggest that regardless of whether a curative or a palliative surgery is performed on patients with pancreatic cancer, at least by around 14 weeks, their body composition statuses can become relatively similar. These changes are predominantly in Weight, and the body fat and water compartments. The trend in body composition changes shows that, although both groups may start with non-significantly different body composition, they tend to grow closer around the 14 week point indicating that the Clear Margin group may lose more than Unclear Margin group. The implications of these findings, therefore, are that once the most appropriate surgical procedure is performed, an adjuvant therapy regimen (such as chemotherapy) at around 14 weeks may have the most impact on the patient’s overall treatment outcome.

The reason why some patients’ surgery results in Clear Margins whereas others result in Unclear Margins is multifactorial. The most common reasons include the size, location, grade, and the degree of advancement at the time of surgery. However, the fact that the Unclear Margin group consistently had lower body composition parameters indicates that this group of patients, although technically not malnourished, start major surgery with lower “stores”. In addition, the fact that none of the body composition parameters’ slope values were significantly different between the two groups would indicate that the rate of change of the measured body composition parameters is similar for both the Clear as well as the Unclear surgical margin groups.
Chapter 10: WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

10. WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

All survival analyses carried out on the Whipple’s Procedure surgical patient data were performed using SPSS® Version 14 (SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois, USA).

10.1 Background

The Kaplan-Meier [852] test was performed to report survival outcomes. This test, in general, provides an estimate of the survival function from life-time data. In medical research, it is usually used to measure the fraction of patients living for a certain amount of time after a medical procedure such as surgery. A plot of the Kaplan-Meier estimate of the survival function is a series of horizontal steps of declining magnitude which, when a large enough sample is taken, approaches the true survival function for that population. The value of the survival function between successive distinct sampled observations is assumed to be constant. The Kaplan-Meier [852] curve can take into account "censored" data, i.e., losses from the sample, before the final outcome is observed. This is particularly useful when, for example, a patient drops out of the study/trial before it is completed. On the Kaplan-Meier [852] plot, small vertical tick-marks indicate losses, where patient data has been censored. When no truncation or censoring occurs, the Kaplan-Meier [852] curve is equivalent to the empirical distribution. This scenario often occurs in research and clinical trials involving patients, especially with devastating diseases such as pancreatic cancer and mesothelioma.
Hence in our study, the Kaplan-Meier survival analysis test using the Log Rank test for significance was used for the comparison of survival data between groups.

The Cox’s Regression was carried out to investigate the effect of different body composition parameters on the survival of the surgical patients. Cox’s Regression [853], also referred to as the Proportional Hazards Regression, is generally used to investigate the effect of several variables upon the time a specified event takes to happen. In the context of an outcome such as death this is known as Cox Regression for Survival Analysis. The method does not assume any particular "survival model" but it is not truly non-parametric because it does assume that the effects of the predictor variables upon survival are constant over time and are additive in one scale. The Hazards Ratio [853] resulting from the Cox’s Regression, therefore, provides the “relative death rate”.

Hence in our study the Cox’s proportional hazards model was used to assess survival adjusted for multiple covariates. The hazard ratio is defined as an estimate of the ratio of the hazard rate in the treated versus the control group [854]. For each model, all variables that were considered as known risk factors were included in the initial model. Other variables that had a P value of $\leq 0.25$ on univariate analysis were also included. The analysis was performed as a Stepwise-Backward analysis.

In our study, although body composition measurements were carried out at the appropriate intervals up to 26 weeks, the patients were followed-up for 72 months to determine whether they are dead or alive. For the purposes of survival analysis, the “time to end point” was considered to be either a confirmed death or the last time patient visited clinic within 72 months post Whipple’s Procedure.

The body composition data used for the survival analysis was the patient’s first (i.e. the pre-operative) measurements.
10.2 Kaplan-Meier Analysis: Methodology And Results

The initial step was to perform a Kaplan-Meier test on the body composition parameters measured in the study. This provided a graphical demonstration of the effects of various parameters on the survival of the Whipple’s Procedure surgical patients. The following are the survival curves of these parameters measured in the patient population. In all cases the patient groups compared were the patients with Clear Margins and patients with Unclear Margins.

As the Kaplan-Meier analysis can only be performed on categorical variables and data, the effect of Margins (i.e. clear or unclear), NI cut-off values, and Sex (male or female) were investigated. The NI cut-off values used were: 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00, and 1.05.

10.2.1 Effects of Margins

The initial Kaplan-Meier survival analysis was carried out to investigate the effect of margins on survival.

The actual terms Clear Margins or Negative Margins refer to the cases where the surgeon’s cut was through healthy tissue and, therefore, the diseased tissue (in this case the pancreatic tumour) was completely removed. The terms Unclear Margins or Positive Margins refer to the cases where the surgeon’s cut was through the diseased tissue (in this case the pancreatic tumour) and some of the diseased tissue was left in the patient, which may increase the likelihood of the disease recurring.
Figure 32. Curve for the effect of margins (Clear/Unclear) on survival.

Figure 32 shows that the difference in survival between the surgical group with clear and surgical group with unclear margins did not reach significance at the 0.05 level (Log Rank (Mantel-Cox) p=0.14).

10.2.2 Effects of Nitrogen Index

NI is considered to be one of the important body composition parameters affecting the well-being as well as survival of patients. It is defined as TBN expressed as a percentage of age, sex, and height-matched normal. In addition, the actual TBN content, demonstrated by changes in the NI value, has been considered to be an indicator of malnutrition and hence survival in malnourished patients. Different NI “cut-off” values have been previously demonstrated [857, 856, 855, 691, 690] to predict therapy-induced complications. These are generally around 0.85. To investigate the survival effects of NI in this group of surgical patients, NI cut-off values of 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00, and 1.05 were analysed. It should be
Chapter 10: WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

noted that at our centre, an NI of between 0.95 (or 95%) and 1.05 (or 105%) is considered to be “normal” and, generally patients with an NI less than 0.90 (or 90%) are considered to be “malnourished”. Therefore, the NI determines how well nourished is the patient.

None of the NI cut-off values that were tested were found to be statistically significant. In addition, the number of patients with an NI below the normal range was relatively low. The results for the NI values are tabulated in Table 11. Figure 33 and Figure 34 show the Kaplan-Meier analysis curves for NI with a cut-off of 0.85 and 1.00, respectively.

Table 11. Kaplan-Meier analysis results for different NI cut-off values.

<table>
<thead>
<tr>
<th>NI Cut-off Values</th>
<th>Log Rank p-Value</th>
<th>No. of Patients With</th>
<th>No. of Patients With</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NI&lt; Cut-off</td>
<td>NI&gt; Cut-off</td>
</tr>
<tr>
<td>0.90</td>
<td>0.44</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>0.95</td>
<td>0.66</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>1.00</td>
<td>0.35</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>1.05</td>
<td>0.36</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

An NI of around 0.85 is the cut-off where, in previous studies at our centre, it was demonstrated that the patients would be a greater risk of therapy-induced complications. Figure 33 demonstrates the effect of NI cut-off range of 0.85.
Chapter 10: WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

Figure 33. Survival curve for the effect of NI cut-off values of 0.85 on survival.

Figure 34. Survival curve for the effect of NI cut-off values of 1.00 on survival.

An NI of 1.00 is considered to be an ideal level of TBN. A patient with an NI of 1.00 has a 100% of TBN of an age, sex, and height-matched normal. From the total of 27 patients in the
group, 14 had an NI of less than 1.00. The result of the Kaplan-Meier analysis of setting the NI cut-off values to 1.00 is demonstrated in Figure 34. The NI did not influence survival at neither of these cut-off values.

10.2.3 Effect of Gender

The effect of gender on the survival in this group of surgical patients was also investigated. Figure 35 demonstrate the result of the Kaplan-Meier analysis (Log Rank (Mantel-Cox) p=0.73).

Figure 35. Survival curve for the effect of gender on survival.
10.3 Cox’s Regression Analysis: Methodology And Results

Cox’s Regression Analysis was carried out to investigate the effects of different body composition and parameters on the survival of the Whipple’s procedure patients. These analyses were carried out using all body composition parameters generated for this group of patients.

Initially a univariate Cox’s Regression was carried out to identify potential parameters affecting survival. To do this, the statistical analysis package’s (SPSS®) parameters were set-up with the “Time” to be the Time to End Point, the “Status” to be the Survival Status (=0) (Dead=0, Alive=1), and for the “Covariates”, each time one of the body composition parameters (shown below) were selected and analysed. The results of this regression analysis are shown in Table 12 below.
In the univariate Cox’s Regression analysis, all the parameters measured in the study on the Whipple’s procedure surgical patients were entered into SPSS® individually and Cox’s Regression (“Enter” mode) was performed. The combined results of these univariate regressions are shown in Table 12 above.

Once the univariate Cox’s Regression Analysis was completed, the parameters that had significance level of ≤0.25 were selected for the multivariate analysis part. As shown in Table 12, the body composition parameters which have a significance level of ≤0.25 are: the BMI (p=0.109), Weight (p=0.126), NI (p=0.138), Margin Status (p=0.146), FM (p=0.189), FM by TBK (p=0.195), LBM (p=0.228), and LBM by Segal (p=0.247). In addition, parameters such as Age, Sex, and TBW content, which by clinical experience are known to have the ability to affect survival (i.e. the *a priori* parameters), were also “forced” into the multivariate analysis. A “Backward Stepwise Likelihood Ratio”
Chapter 10: WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

Multivariate Cox’s Regression was then performed on the above ten parameters. Table 13 below shows the relevant results of the regression.

It should be noted that since NI is dependent and highly correlated (p<0.0001) with TBN, it was excluded from further Cox’s Regression analysis.

Table 13. Backward Stepwise Likelihood Ratio Multivariate Cox’s Regression of the parameters with Univariate Cox’s Regression \( p \leq 0.25 \).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Value</th>
<th>HR</th>
<th>95.0% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>95.0% CI for HR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.001</td>
<td>1.129</td>
<td>1.049 1.215</td>
</tr>
<tr>
<td>Margin Status</td>
<td>0.003</td>
<td>6.467</td>
<td>1.882 22.223</td>
</tr>
<tr>
<td>Age</td>
<td>0.081</td>
<td>0.962</td>
<td>0.921 1.005</td>
</tr>
</tbody>
</table>

These three parameters were then taken as the “base parameters” and Multivariate Cox’s Regression were then performed on the remaining (Sex, BMI, Weight, TBW (Kushner), Fat Mass by TBK, LBM, LBM by Van Loan, and LBM by Segal) parameters. This was done by keeping the “base parameters” constant and adding one of the eight parameters at a time, performing the regression, and then replacing the parameter with another one of the eight parameters. This, in effect, enabled exploration of the influence of these parameters using a multivariate analysis while adjusting for Margin Status, Fat Mass, and Age. The result of this multivariate analysis is shown in Table 14.
Chapter 10: WHIPPLE’S PROCEDURE SURVIVAL ANALYSIS RESULTS

Table 14. Multivariate Cox’s Regression adjusted for Fat Mass, Margin Status, and Age.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Value</th>
<th>HR</th>
<th>95.0% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM (By Segal)</td>
<td>0.279</td>
<td>1.034</td>
<td>0.973</td>
</tr>
<tr>
<td>LBM (By Van Loan)</td>
<td>0.301</td>
<td>1.037</td>
<td>0.968</td>
</tr>
<tr>
<td>LBM</td>
<td>0.321</td>
<td>1.026</td>
<td>0.975</td>
</tr>
<tr>
<td>Weight</td>
<td>0.391</td>
<td>1.025</td>
<td>0.968</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.499</td>
<td>1.031</td>
<td>0.943</td>
</tr>
<tr>
<td>Sex</td>
<td>0.775</td>
<td>0.884</td>
<td>0.379</td>
</tr>
<tr>
<td>BMI</td>
<td>0.835</td>
<td>0.969</td>
<td>0.722</td>
</tr>
<tr>
<td>Fat Mass (By TBK)</td>
<td>0.935</td>
<td>0.997</td>
<td>0.920</td>
</tr>
</tbody>
</table>

A box plot of Margin Status against Fat Mass was also made to visually demonstrate the relationship between these two parameters which were found in the above analyses to be influential in the survival of patients undergoing Whipple’s Procedure. This plot is shown in Figure 36 below. Fat Mass and Margin Status were also shown to be correlated (Pearson Correlation: 0.561; p=0.002).

This would probably point to the fact that patients in the Unclear Margin group are already depleted and low in body fat due to the severity of their disease as well as the degree of tumour progression and advancement. It is most likely that the advanced tumour, larger size and the higher degree of disease progression and tumour infiltration are the main factors in preventing the surgeon obtaining a “clear” margin in this group of patients with pancreatic cancer. Similarly, in the Clear Margin group because the disease is not too progressed and, as a result, has not affected the body composition and depleted the fat stores as much as the Unclear Margin group, the body fat content is higher. In addition, as the tumour is less advanced and there are less expansion and infiltration, the surgeon is able to excise the entire tumour and achieve a “clear” margin.
Similarly a box plot of Margin Status against Age was made to visually demonstrate the relationship between these two parameters which were also found in the above analyses to be influential in the survival of patients undergoing Whipple’s Procedure. This plot is shown in Figure 37 below.

**Figure 37. Box plot of Age against Margin Status.**
This box plot visually demonstrates that the Clear Margin group are slightly older than the Unclear Margin group. Following from the Fat Mass box-plot results above, one of the possible explanations of the observed differences in age between the two groups could be the well known higher body fat content in older individuals. Other explanation of the difference could be that tumour progression is slower in the older individuals, thus resulting in a less advanced and progressed tumour which can be more easily and completely excised by the surgeon.
10.4 Summary And Conclusions

Results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that from the three parameters tested, the Margin Status was the best parameter that could (p=0.14) determine survival in the Whipple’s Procedure surgical patients.

The results of the univariate Cox’s Regression analysis performed on all individual parameters showed that there are no parameters that have been shown to significantly influence survival in this group of patients. The closest to being statistically significant was the BMI (p=0.109). Once the parameters with significance levels of ≤0.25 were selected and multivariate Cox’s Regression performed, the Margin Status, Fat Mass, and Age were found to be statistically significant. Although the a priori parameters were then “forced” into the analysis and adjusted for Margin Status, Fat Mass, and Age none reached statistical significance.

The box plot of Fat Mass against Margin Status visually demonstrated that the patients with clear margins generally tend to have higher fat mass than the ones with unclear margins. This finding is consistent with the fact that the larger or the more advanced the tumour is, the greater would be the degree of cachexia. In addition, during cachexia there is a significant amount lipolysis (see section on Cancer Cachexia (Chapter 2) which will result in a reduced body fat.

In conclusion, it is not possible to be certain that there is an influence of Margin Status or Fat Mass (or any of the other measured body composition parameters) on survival after a Whipple’s Procedure in this population of surgical patients. In addition, when a Whipple’s Procedure patient is presented pre-operatively with a poor nutritional status, it does not necessarily mean that the patient will have a poor outcome. Therefore, it is the biological
behaviour of the tumour rather than the body composition of the patient that determines the outcome.
Chapter 11: MESOTHELIOMA PATIENT RESULTS

11. MESOTHELIOMA PATIENT RESULTS

The following chapter details the body composition results of patients who were recruited and participated in the clinical trial investigating the changes and effects of the use of thalidomide in patients with malignant mesothelioma. For this reason, the patients were separated into two groups or arms.

The Arm A patients were the patients who received chemotherapy with cisplatin and gemcitabine plus thalidomide, whereas the patients in Arm B received thalidomide alone with no other additional chemotherapy.

11.1 Patient Demographics

A total of 31 patients were recruited for this clinical trial. From this population, 11 patients had base-line measurements only and did not progress/continue with the clinical trial due to ill health. The 11 patients comprised of four patients from the Arm A group and seven from the Arm B group. The 31 base-line measurement results were used elsewhere for survival analysis calculations.

As a result, the total number of patients in the study who had repeated measurements was reduced to a total of 20, all of which completed a minimum of two chemotherapy or body composition measurement cycles. This resulted in an equal number of ten patients in each arm.

The Arm A group included eight males and two females with a mean age of 62.9 years (median: 60.8 years; range: 53.4 to 76.6 years). The Arm B group included nine males and one female with a mean age of 67.8 years (median: 68.1; range: 57.8 to 77.8 years). The fact that there were significantly more males than females with malignant mesothelioma in this
clinical trial is consistent with the reports in the literature which have indicated that in all countries and regions the rates of males with malignant mesothelioma is higher than that of the females, with the industrialised countries showing higher rates than the non-industrial countries [462, 454].

There were a total of 32 body composition measurements in Arm A and a total of 31 body composition measurements in Arm B of the clinical trial, giving a grand total of 63 body composition measurement points in the study.

### 11.2 Completion Rates

Compared with the “Whipple’s Procedure Surgical Long-Term Follow-Up” and the “Effects of EPA on Weight Loss and Cachexia” studies, the completion rates for this study was relatively poor. This is mainly due to the devastating nature of the disease which impedes patients attending hospital even for routine clinical care as well as reducing survival.

In the Arm A group, a total of seven patients (70%) completed at least three measurement cycles with only five (50%) able to successfully complete four measurement cycles. Interestingly although not required, there was one patient in this group who managed to complete seven body composition measurement cycles. However, for statistical purposes and as per original study protocol, the results of the first four measurement cycles were used in all statistical analyses.

In the Arm B group, a total of six patients (60%) completed at least three measurement cycles with only five (50%) able to successfully complete four measurement cycles. Similar to the Arm A group, there were four patients (40%) who managed to complete at least five, four patients (40%) who managed to complete at least six, three patients (30%) who managed to complete at least seven, and two patients (20%) who managed to complete at least eight
measurement cycles. In addition, there was one patient who managed and continued to complete as much as 11 body composition measurement cycles.

As such, there were a total of 20 patients enrolled in the study and commenced this clinical trial.
11.3 Body Composition Results

All relevant parameters resulting from or during the TBN and TBW measurements carried out at the CIVBC were investigated to determine the changes as well as differences in the Arm A and Arm B groups of malignant mesothelioma patients.

11.3.1 Differences In Body Composition Arm A And Arm B

The initial step in analysing the differences between the Arm A and Arm B groups was to see the differences between the average values in each group. As with the results of the Whipple’s Procedure study in the graphs demonstrating these values, for ease of viewing purposes, the body composition parameters with similar range of values were placed and displayed on the same graph.

The statistical significance of the differences between Arm A and Arm B groups at each measurement time-point is investigated and results shown in the statistical analysis sections below.

The trend for changes in the Weight and %BFat shows that the average values for the Arm A group are higher at base-line, second and the fourth chemotherapy cycle, but are lower at the third cycle. The BMI values are almost constant at all four measurement time points. None of the differences seen in Weight, %BFat, and BMI between the two study arms or between chemotherapy cycles were statistically significant. These changes are demonstrated in Figure 38.
In this group of patients with malignant mesothelioma, the TBN values also follow the same trend as that of the Weight, %BFat, and BMI where the TBN values are higher at base-line, second and the fourth chemotherapy cycle, but are lower at the third cycle. Although the differences seen in TBN between the two study arms or chemotherapy cycles were not statistically significant, the 1/slope values (Arm A: Mean: 0.001; Range: -0.009 to 0.04. Arm B: Mean: -0.027; Range: -0.133 to 0.007) were found to be almost statistically significant (p=0.052). The changes in TBN are demonstrated graphically in Figure 39.
The NI, which is TBN expressed as the percentage of age, sex, and height-matched normal, showed the same trend for both study arms. Similar to TBN, the differences seen in NI between the two study arms or between chemotherapy cycles were not statistically significant. The only exception was the difference between Arm A’s NI and Arm B’s NI at the third measurement point which was found to be statistically significant (p=0.036). The changes are demonstrated graphically in Figure 40.

The LBM values were measured by two separate methods of BIA and skinfold thickness techniques. These are demonstrated graphically in Figure 41. The LBM measurements which were carried out using the skinfold technique showed almost no difference between the two study arms and, therefore, any differences between the two study arms or between chemotherapy cycles were not statistically significant.
Figure 40. Graph of mean (±SE) TBN against time (measurement time-point) for Arm A and Arm B patient groups.

Mean (±SE) Nitrogen Index For Arm A And Arm B Groups

Figure 41. Graph of mean (±SE) LBM against time (measurement time-point) for Arm A and Arm B patient groups.

With the LBM values measured using the BIA technique, the LBM (by Lukaski), LBM (by Segal), and LBM (by Van Loan) showed a general increase in LBM for the Arm A group of
patients where as the Arm B showed an initial increase between base-line and second cycle followed by a general decline in LBM thereafter. None of these changes, neither between the two study arms nor between the measurement cycles was found to be statistically significant.

The measurements of FM derived from the above LBM measurement techniques showed the same trend between study arms as well as between measurement cycles, which was expected. In addition, as one would expect, there were no statistical differences between study arms as well as between measurement cycles. The BIA-derived FM values were found to be the same between the two study arms. However, the skinfold-derived FM values were found to be slightly higher in the Arm A group. These trends are demonstrated graphically in Figure 42 and Figure 43.

Figure 42. Graph of mean (±SE) BIA-derived FM against time (measurement time-point) for Arm A and Arm B patient groups.

Mean (±SE) Fat Mass Measurements Using BIA Technique For Arm A And Arm B Groups
The TBW measurements were found to show that there is a general increase in TBW content from the base-line to the third measurement cycle followed by a decrease at the fourth cycle. The Arm A group were found to have a higher TBW content than the Arm B group. The differences seen in TBW content between the two study arms or between chemotherapy cycles were not statistically significant. These trends are demonstrated graphically in Figure 44.
One other method of determining and statistically analysing differences between the two groups was to look at the AUCs and slopes. In another words, to statistically look at the differences in body composition parameters of the groups as a whole, regardless of the time-points where measurements were carried out. Here, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the AUC for each patient curve is calculated. These AUC values for each group is then compared statistically (Two Sample T-Test (assuming unequal variances)). Similarly, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the slope for each patient curve is measured. This slope values for each group is then compared statistically (Two Sample T-Test (assuming unequal variances)). The results of the AUC and slope for all tested parameters are summarised and tabulated in Table 15.
Table 15. Summary of statistical results for comparison between Arm A and Arm B groups using AUC and slopes. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.753</td>
<td>0.420</td>
</tr>
<tr>
<td>BMI</td>
<td>0.887</td>
<td>0.800</td>
</tr>
<tr>
<td>Body Density</td>
<td>0.832</td>
<td>0.671</td>
</tr>
<tr>
<td>Calf Circ</td>
<td>0.903</td>
<td>0.679</td>
</tr>
<tr>
<td>Chest Circ</td>
<td>0.750</td>
<td>0.921</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.268</td>
<td>0.546</td>
</tr>
<tr>
<td>FM (Lukaski)</td>
<td>0.604</td>
<td>0.961</td>
</tr>
<tr>
<td>FM (Segal)</td>
<td>0.808</td>
<td>0.796</td>
</tr>
<tr>
<td>FM (Van Loan) &lt;0.0001</td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td>LBM</td>
<td>0.834</td>
<td>0.353</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.930</td>
<td>0.912</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.798</td>
<td>0.991</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.752</td>
<td>0.996</td>
</tr>
<tr>
<td>MUA Circ</td>
<td>0.790</td>
<td>0.157</td>
</tr>
<tr>
<td>NI</td>
<td>0.772</td>
<td>0.492</td>
</tr>
<tr>
<td>TBN</td>
<td>0.711</td>
<td>0.669</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.948</td>
<td>0.874</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.924</td>
<td>0.845</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.977</td>
<td>0.874</td>
</tr>
<tr>
<td>Thigh Circ</td>
<td>0.794</td>
<td>0.849</td>
</tr>
<tr>
<td>Waist Circ</td>
<td>0.981</td>
<td>0.429</td>
</tr>
<tr>
<td>Weight</td>
<td>0.963</td>
<td>0.892</td>
</tr>
</tbody>
</table>

As it can be seen from Table 15, the only parameter that showed a statistically significant difference in AUC and slope between Arm A and Arm was the BIA-derived FM (Figure 45). The highly significant AUC (p<0.0001) and slope (p=0.048) of FM (Van Loan) suggests that when the two groups are compared as a whole, it is only the FM that is statistically different between the two groups. None of the other parameters tested under these conditions and using these methods were found to be statistically different.
11.3.2 Body Composition Changes Between Time-Points

For all parameters measured, T-Test was performed to statistically determine changes within each of the two Arm A and Arm B groups. This was used to see whether statistically there are any significant differences between body composition measurement time-points in each group. However, as approximately half of the patients of each of the study arms did not fully complete the required four body composition measurement cycles, censoring the “incomplete” cycles and performing an ANOVA test on the remaining half would not, in our opinion, have produced meaningful results that could also be used for further analysis and commenting. As a result, T-tests were instead performed between individual cycles within each of the two study arms. The results for these analyses are summarised and tabulated in Table 16.
Chapter 11: MESOTHELIOMA PATIENT RESULTS

As can be seen in Table 16, almost none of the body composition parameters tested reached significance. The only exception was the NI. This showed that there was a significant difference between the NI in cycle 1 and cycle 3 only in the Arm A group of patients (p=0.035). This can suggest that there is a late-onset but significant decrease in NI of patients with malignant mesothelioma who are receiving standard gemcitabine plus cisplatin as well as thalidomide. Although the decline in NI starts from the first cycle and continues on, these results suggest that the greatest decline occurs at the third cycle.
### Table 16. Comparison of body composition changes between different chemotherapy cycles in Arm A and Arm B. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>BC Parameter</th>
<th>Chemotherapy Cycle (p-Values) Arm A</th>
<th>Chemotherapy Cycle (p-Values) Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycles 1&amp;2</td>
<td>Cycles 2&amp;3</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>0.941</td>
<td>0.785</td>
</tr>
<tr>
<td>BMI</td>
<td>0.830</td>
<td>0.572</td>
</tr>
<tr>
<td>Body Density</td>
<td>0.486</td>
<td>0.502</td>
</tr>
<tr>
<td>Calf Circ.</td>
<td>0.893</td>
<td>0.630</td>
</tr>
<tr>
<td>Chest Circ.</td>
<td>0.843</td>
<td>0.483</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.905</td>
<td>0.627</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.573</td>
<td>0.553</td>
</tr>
<tr>
<td>(Lukaski)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.592</td>
<td>0.636</td>
</tr>
<tr>
<td>(Segal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.635</td>
<td>0.682</td>
</tr>
<tr>
<td>(Van Loan)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM</td>
<td>0.724</td>
<td>0.970</td>
</tr>
<tr>
<td>LBM</td>
<td>0.730</td>
<td>0.684</td>
</tr>
<tr>
<td>LBM</td>
<td>0.954</td>
<td>0.997</td>
</tr>
<tr>
<td>LBM</td>
<td>0.984</td>
<td>0.946</td>
</tr>
<tr>
<td>(Van Loan)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-Thigh</td>
<td>0.828</td>
<td>0.689</td>
</tr>
<tr>
<td>Circ.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-Upper-Arm Circ.</td>
<td>0.839</td>
<td>0.957</td>
</tr>
<tr>
<td>NI</td>
<td>0.593</td>
<td>0.116</td>
</tr>
<tr>
<td>TBN</td>
<td>0.600</td>
<td>0.373</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.547</td>
<td>0.589</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.747</td>
<td>0.708</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.547</td>
<td>0.589</td>
</tr>
<tr>
<td>Waist Circ.</td>
<td>0.949</td>
<td>0.609</td>
</tr>
<tr>
<td>Weight</td>
<td>0.786</td>
<td>0.773</td>
</tr>
</tbody>
</table>
11.3.3 Changes Between Groups At Specific Time-Points

In order to investigate whether each of the two study arms progressed at the same or different rates, one has to determine whether there are any differences between these groups at different measurement time-points.

To show this, body composition parameters measured at each time-point from the Arm A group was statistically (T-Test) compared with the body composition parameters measured at the same time-point in the Arm B group. The results of this comparison are tabulated in Table 17.

As it can be seen in Table 17, none of the body composition measurement parameters at any of the chemotherapy cycles showed significant difference between the two study arms. This indicates that the two groups of patients with malignant mesothelioma undergoing chemotherapy have statistically similar body composition. The results can also suggest that the presence or absence of gemcitabine and cisplatin, in the presence of thalidomide, in this group of patients with malignant mesothelioma does not significantly affect their body composition.
Table 17. Comparison of different body composition parameters between the Arm A and Arm B at the corresponding chemotherapy cycle. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Body Composition Parameters</th>
<th>Chemotherapy Cycle (p-Values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
</tr>
<tr>
<td>%Body Fat</td>
<td>0.813</td>
</tr>
<tr>
<td>BMI</td>
<td>0.923</td>
</tr>
<tr>
<td>Body Density</td>
<td>0.307</td>
</tr>
<tr>
<td>Calf Circ.</td>
<td>0.429</td>
</tr>
<tr>
<td>Chest Circ.</td>
<td>0.455</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.713</td>
</tr>
<tr>
<td>Fat Mass (Lukaski)</td>
<td>0.361</td>
</tr>
<tr>
<td>Fat Mass (Segal)</td>
<td>0.541</td>
</tr>
<tr>
<td>Fat Mass (Van Loan)</td>
<td>0.723</td>
</tr>
<tr>
<td>LBM</td>
<td>0.919</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.504</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.953</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.873</td>
</tr>
<tr>
<td>Mid-Upper-Arm Circ.</td>
<td>0.854</td>
</tr>
<tr>
<td>NI</td>
<td>0.663</td>
</tr>
<tr>
<td>TBN</td>
<td>0.751</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.351</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.464</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.351</td>
</tr>
<tr>
<td>Thigh Circ.</td>
<td>0.720</td>
</tr>
<tr>
<td>Waist Circ.</td>
<td>0.517</td>
</tr>
<tr>
<td>Weight</td>
<td>0.758</td>
</tr>
</tbody>
</table>

11.3.4 Changes Between Groups At Specific Treatment Cycle

Compared To Base-Line

In order to assess the changes in body composition parameters, for each group the pre-chemotherapy measurements were taken as the base-line values. Measurements at the next three time-points were then each compared to this base-line. Two-sample, two-tailed T-Test
was then used to compare the same time-point measurements between the Arm A and Arm B patient groups. For example, the chemotherapy Cycle 2 measurements in the Arm A group were statistically compared with the chemotherapy Cycle 2 measurements in the Arm B group. This was then repeated for the chemotherapy Cycle 3 and Cycle 4 measurements. The results for these measurements are summarised and tabulated in Table 18.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.603</td>
<td>0.832</td>
<td>0.168</td>
</tr>
<tr>
<td>BMI</td>
<td>0.469</td>
<td>0.958</td>
<td>0.267</td>
</tr>
<tr>
<td>Body Density</td>
<td>0.450</td>
<td>0.264</td>
<td>0.090</td>
</tr>
<tr>
<td>Calf Circ</td>
<td>0.729</td>
<td>0.446</td>
<td>0.306</td>
</tr>
<tr>
<td>Chest Circ</td>
<td>0.670</td>
<td>0.192</td>
<td>0.357</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.899</td>
<td>0.872</td>
<td>0.058</td>
</tr>
<tr>
<td>FM (Lukaski)</td>
<td>0.806</td>
<td>0.729</td>
<td>0.520</td>
</tr>
<tr>
<td>FM (Segal)</td>
<td>0.222</td>
<td>0.667</td>
<td>0.977</td>
</tr>
<tr>
<td>FM (Van Loan)</td>
<td>0.252</td>
<td>0.756</td>
<td>0.743</td>
</tr>
<tr>
<td>LBM</td>
<td>0.154</td>
<td>0.773</td>
<td>0.170</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.313</td>
<td>0.844</td>
<td><strong>0.031</strong></td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.658</td>
<td>0.852</td>
<td>0.117</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.638</td>
<td>0.872</td>
<td>0.126</td>
</tr>
<tr>
<td>MUA Circ</td>
<td>0.539</td>
<td>0.552</td>
<td>0.206</td>
</tr>
<tr>
<td>NI</td>
<td>1.000</td>
<td>0.410</td>
<td>0.443</td>
</tr>
<tr>
<td>TBN</td>
<td>0.999</td>
<td>0.298</td>
<td>0.423</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.419</td>
<td>0.637</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.358</td>
<td>0.763</td>
<td><strong>0.032</strong></td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.419</td>
<td>0.637</td>
<td>0.170</td>
</tr>
<tr>
<td>Thigh Circ</td>
<td>0.622</td>
<td>0.501</td>
<td>0.253</td>
</tr>
<tr>
<td>Waist Circ</td>
<td>0.467</td>
<td>0.813</td>
<td>0.331</td>
</tr>
<tr>
<td>Weight</td>
<td>0.404</td>
<td>0.942</td>
<td>0.267</td>
</tr>
</tbody>
</table>

The patterns of change in the body composition parameters with time were also investigated. In order to visually establish how and when these changes occur, the measurements at each of the chemotherapy cycle time-point were subtracted from the base-line values. These were then plotted against time and T-Test was used to compare the Arm A and Arm B patient groups.
One of the most obvious measurement parameters that one can expect to change as a result of malignant lung disease is the weight. Figure 46 shows the changes in weight with respect to the pre-chemotherapy (base-line) weight with time.

Comparing the means of the Weight change showed that there were no statistically significant differences between the two treatment arms at any of the measurement time-points.

Figure 46. Changes in Weight compared to base-line with time in patients with mesothelioma.

Graph Of Mean (±SE) Weight Against Measurement Time-Point For Arm A And Arm B Groups

In order to demonstrate changes in body composition it was, therefore, necessary to investigate the components of the Weight. The %BFat, which is derived from skin fold thickness measurements and calculated using the Durnin & Womersley’s [756, 772, 771] equations were investigated. Using this as well as the Weight, the FM was calculated in the two treatment arms. The changes in %BFat are demonstrated in Figure 47. As with the Weight, comparing the means of the %BFat change also showed that there were no statistically significant differences between the two treatment arms at any of the measurement time-points.
However, comparing the means of the FM change showed that there was an almost statistically significant difference ($p=0.058$) between the two treatment arms at the Cycle 4 measurement time-point compared to the base-line. These changes are demonstrated in Figure 48.

Figure 47. Changes in %BFat compared to base-line with time in patients with mesothelioma.

Graph Of Mean (±SE) % Body Fat Against Measurement Time-Point For Arm A And Arm B Groups
Although the changes may not be statistically significant, looking at both eth %BFat as well as the FM, one can immediately see that the patients in Arm B demonstrate a greater loss of their fat compartment than the Arm A patients. Since the Arm A patients are the patients who received chemotherapy with cisplatin and gemcitabine plus thalidomide, and the patients in Arm B received thalidomide alone, one could hypothesise that there could be a synergistic, anti cachectic effect between standard chemotherapy and thalidomide.

It is interesting to note that LBM measured using the BIA techniques and using the Lukaski’s equation \cite{797, 798}, compared to base-line, showed a statistically significant difference (p=0.031) between the two study arms at the chemotherapy Cycle 4 measurement time-point. The changes in LBM as measured using the Lukaski’s equation are shown in Figure 49.
In our previous studies in patients with breast cancer undergoing cancer chemotherapy [856, 855, 858] we had demonstrated that the body protein can change during the course of the cancer and chemotherapy. However, in this group of patients with malignant mesothelioma undergoing chemotherapy, although there were changes in TBN as compared to the base-line, they did not reach statistical significance. The changes in TBN are demonstrated in Figure 50.
As can be seen in Figure 50, there is a general decline in TBN compartment compared to base-line. The patients in both groups start with a similar loss in TBN. However, it is the patients in Arm B who have the greater overall loss of the TBN compartment. This may, again be due to a synergistic and/or anti-cachectic effect of the combination of standard chemotherapy and thalidomide.

The final component of Weight is the TBW compartment. At our centre the BIA techniques are routinely used to assess the TBW content of patients as well as research subjects. The changes in TBW content of the two arms are shown in Figure 51. Comparing the means of the TBW changes between the two arms showed that, compared to base-line, there is a statistically significant difference (Kushner: p=0.032; Fredrix: p=0.022) between Arm A and Arm B at the Cycle 4 measurement time-point.
As demonstrated in Figure 51, the patients in both arms gain TBW during the four cycles of chemotherapy. This indicates that regardless of whether or not gemcitabine / cisplatin is used in the chemotherapy, there will be some degree of water retention. However, it is the patients in Arm A who, compared to their base-line values increase their gain in TBW whereas the patients in Arm B decrease their gain in TBW during the four chemotherapy cycles. This could, therefore, mean that the use of gemcitabine / cisplatin in this group of mesothelioma patients as a part of their chemotherapy increases water retention.
11.4 Conclusions

The results of this study suggest that there is no statistically significant difference in Weight between the Arm A and the Arm B patients.

When Weight is separated into its components, there were no statistically significant differences between the two groups at the base-line. Both study arms demonstrate a steady decline in TBN and FM, although there is an apparent increase in the Arm A group at the chemotherapy Cycle 4 measurement time-point which may be due to small numbers at this time-point. The TBW content for the patients in Arm A increases steadily up to and including the chemotherapy Cycle 4 measurement time-point. The Arm B group, however, show an initial increase up to and including the chemotherapy Cycle 2 measurement time-point followed by a steady decrease. The pattern of TBW change showed a steady increase in TBW for the Arm A and a general decrease for the Arm B patient groups.

When body composition changes are compared to their base-line (pre-chemotherapy) values, a decrease in Weight is found in the Arm B patient group with the largest drop at the chemotherapy Cycle 4 measurement time-point. Compared to the base-line the Arm A patient group also shows a decrease in Weight, but there is an increase at the chemotherapy Cycle 4 measurement time-point.

Again when Weight is separated into its components, the FM shows an identical pattern to the Weight. The TBN, however, shows a general decrease for both study arms. Arm A shows little change in the decrease up to the chemotherapy Cycle 4 measurement time-point with a much larger decrease at this time-point. The Arm B patient group also shows a decline in TBN content throughout the measurement period. But, the degree of TBN loss is higher at the
chemotherapy Cycle 3 measurement time-point. The pattern of TBW change showed a
general increase in TBW for the Arm A and a steady decrease for the Arm B patient groups.
Considering the fact that Arm A group are the patients who have received standard
chemotherapy plus thalidomide and Arm B are the ones who received thalidomide alone, the
main conclusion that can be drawn is that the gemcitabine / cisplatin chemotherapy produces
water retention in this group of patients with malignant mesothelioma. In addition, the longer
the chemotherapy is continued, the more the patients are likely to be depleted in their body
weight, fat and protein. In other words, with the exception of TBW there are no statistically
significant differences between the body compositions of the two study groups. Provided that
the overall anti-cancer potential of gemcitabine / cisplatin plus thalidomide is comparable
with thalidomide alone, then by looking purely from the body composition angle one may be
able to suggest the use of thalidomide alone in the treatment of malignant mesothelioma in
this group of patients.
In summary, the results indicate that the group of patients receiving standard chemotherapy
plus thalidomide show similar weight changes to the group receiving thalidomide alone. In
addition, body composition measurements indicate that the standard chemotherapy plus
thalidomide group have a greater TBN loss and a greater TBW gain than the thalidomide-
alone group. This loss of TBN and gain in TBW looks to be “concealed” in the weight.
12. MESOTHELIOMA SURVIVAL ANALYSIS RESULTS

All survival analyses carried out on the use of thalidomide on patients with malignant mesothelioma data were performed using SPSS® Version 14 (SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois, USA). As a reminder, the Thalidomide in Mesothelioma Clinical Trial’s patients with malignant mesothelioma was separated into two arms. The Arm A group received chemotherapy plus thalidomide whereas the Arm B patients received only thalidomide as their chemotherapy.

12.1 Background

The methodology and use of the Kaplan-Meier test and the Cox’s Regression analysis including the Hazards Ratio have been detailed in section 10.1. The Kaplan-Meier test using the Log Rank test for significance was also used in the mesothelioma clinical trial for the comparison of survival data between the two arms of the trial.

For the purposes of survival analysis, the “time to end point” was considered to be either a confirmed death or the last time patient visited the clinic or the Department of Medical of Medical Oncology post-date of first diagnosis. The body composition data used for the survival analysis was the patient’s first (i.e. the pre-chemotherapy) measurements.
12.2 Kaplan-Meier Analysis: Methodology And Results

The initial step was to perform a Kaplan-Meier test on the body composition parameters measured in the study. This provided a graphical demonstration of the effects of various parameters on the survival of the patients with malignant mesothelioma undergoing chemotherapy. The following are the survival curves of these parameters measured in the patient population. In all cases the patient groups compared were the patients Arm A and patients in Arm B.

As the Kaplan-Meier analysis can only be performed on categorical variables and data, the effect of treatment arm (i.e. Arm A or Arm B), NI cut-off values, neuropathy, previous radiotherapy, previous chemotherapy, and Sex (male or female) was investigated. The NI cut-off values used were: 0.85, 0.95, 1.00, and 1.05.

12.2.1 Effects of Treatment

The initial Kaplan-Meier survival analysis was carried out to investigate the effect of treatment procedure on survival. Since both groups received thalidomide, this would, in effect, be investigating the effect of chemotherapy on mesothelioma.
Chapter 12: MESOTHELIOMA SURVIVAL ANALYSIS RESULTS

Figure 52. Curve for the effect of treatment (Arm A/Arm B) on survival.

![Survival Functions](image)

Figure 32 shows that the difference in survival between the two groups of patients with malignant mesothelioma who received chemotherapy plus thalidomide (Arm A) and thalidomide alone (Arm B), did not reach significance at the 0.05 level (Log Rank (Mantel-Cox) \( p = 0.83 \)).

12.2.2 Effects of Nitrogen Index

As mentioned previously, NI is considered to be one of the important body composition parameters that affect the well-being as well as the survival of patients with wasting diseases, such as cancer. It is calculated by expressing the TBN as a percentage of age, sex, and height-matched normal. In addition, it has been demonstrated that the actual TBN content, demonstrated by changes in the NI value, is an indicator of malnutrition and hence survival in malnourished patients. It has also been demonstrated that different NI “cut-off” values have been previously demonstrated [857, 856, 855, 691, 690] to predict therapy-induced complications.
These are generally around 0.85. The importance of the NI and TBN has also been discussed in detail in the appropriate sections.

In the malignant mesothelioma group of patients, the effects of NI on survival were investigated by utilising the NI cut-off values of 0.85, 0.95, 1.00, and 1.05. These NI cut-off values were chosen as, in our centre, and NI value between 0.95 and 1.05 (i.e.. 95% to105%) is considered to be within the “normal” range and any NI value less than 0.85 (i.e. 85%) is considered to be nutritionally “at risk”. In addition, in this population of patients with malignant mesothelioma, with the exception of two patients, all base-line NI values were above 0.85. Therefore, the use of NI cut-off values of less than 0.85 was not necessary.

Statistical analysis showed that none of the NI cut-off values that were tested were statistically significant. In addition, the number of patients with an NI below the normal range was relatively low. The results for the NI values are tabulated in Table 11 below.

An NI of around 0.85 is the cut-off where, in previous studies at our centre, it was demonstrated that the patients would be a greater risk of therapy-induced complications. The fact that in this group of patients with malignant mesothelioma undergoing chemotherapy, there were only two patients with an NI of less than 0.85 might point to the anti-cachectic properties of the thalidomide which is thought to prevent weight loss in wasting diseases. Figure 33 demonstrates the effect of NI cut-off range of 0.85.
Figure 53. Survival curve for the effect of NI cut-off values of 0.85 on survival in mesothelioma patients.

Figure 54. Survival curve for the effect of NI cut-off values of 1.00 on survival in mesothelioma patients.

An NI of 1.00 is considered to be an ideal level of TBN. A patient with an NI of 1.00 has a 100% of TBN of an age, sex, and height-matched normal. From the total of 31 patients in the
group, only 10 had an NI of less than 1.00. The result of the Kaplan-Meier analysis of setting the NI cut-off values to 1.00 is demonstrated in Figure 34. The NI did not influence survival at neither of these cut-off values. The results also indicate that there were three patients within the normal range, i.e. between NI 0.95 and 1.05, and a total of 24 “well-nourished” patients, i.e. with an NI of greater than 0.95.

Table 19. Kaplan-Meier analysis results for different NI cut-off values in patents with mesothelioma.

<table>
<thead>
<tr>
<th>NI Cut-off Values</th>
<th>Log Rank p-Value</th>
<th>No. of Patients With NI&lt; Cut-off</th>
<th>No. of Patients With NI&gt; Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85</td>
<td>0.94</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>0.95</td>
<td>0.68</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>1.00</td>
<td>0.75</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>1.05</td>
<td>0.75</td>
<td>10</td>
<td>21</td>
</tr>
</tbody>
</table>

12.2.3 Effect of Gender

Malignant mesothelioma is generally considered to be a predominantly male disease \cite{462, 454}. However, as there were a total of five females in this group of patients with malignant mesothelioma and two of these five females are the only patients currently still alive, the effect of gender on the survival was also investigated. Figure 35 demonstrate the result of the Kaplan-Meier analysis (Log Rank (Mantel-Cox) p=0.57).
As the results suggest, gender does not have a statistically significant effect on the survival in patients with malignant mesothelioma.

### 12.2.4 Effect of Previous Chemotherapy

As having a previous chemotherapy may affect the body composition, QL, and performance of patients, the effect of having chemotherapy prior to entering the Thalidomide in Mesothelioma trial was investigated. In this group of patients, there were a total of six patients with previous chemotherapy. The Kaplan-Meier survival plot is shown in Figure 56 below. The statistical analysis results indicate that having prior chemotherapy does not affect the overall survival in this group of patients with malignant mesothelioma (Log Rank (Mantel-Cox) p=0.41).
Figure 56. Survival curve for the effect of previous chemotherapy on survival of patients with malignant mesothelioma.

12.2.5 Effect of Previous Radiotherapy

As chemotherapy, radiotherapy may also affect the body composition, QL, and performance of patients. In this group of patients, there were a total of seven patients with previous chemotherapy. The Kaplan-Meier survival plot is shown in Figure 57 below. The statistical analysis results indicate that having prior radiotherapy does not affect the overall survival in this group of patients with malignant mesothelioma (Log Rank (Mantel-Cox) p=0.89).
Figure 57. Survival curve for the effect of previous chemotherapy on survival of patients with malignant mesothelioma.
12.3 Cox’s Regression Analysis: Methodology And Results

Cox’s Regression Analysis was carried out to investigate the effects of different body composition and parameters on the survival of the Whipple’s procedure patients. These analyses were carried out using all body composition parameters generated for this group of patients.

Initially a univariate Cox’s Regression was carried out to identify potential parameters affecting survival. To do this, the statistical analysis package’s (SPSS®) parameters were set-up with the “Time” to be the Time to End Point, the “Status” to be the Survival Status (=0) (Dead=0, Alive=1), and for the “Covariates”, each time one of the body composition parameters (shown below) were selected and analysed.

In the univariate Cox’s Regression analysis, all the parameters measured in the study on the thalidomide in patients with malignant mesothelioma were entered into SPSS® individually and Cox’s Regression (“Enter” mode) was performed on each parameter. The parameters included the measurement body composition as well as haematology, ECOG performance status, and additional parameters which were thought to be relevant to the overall outcome of the trial. These additional parameters included CRP, IL-6, VEGF, the presence of neuropathy, and whether the patient has had previous chemotherapy or radiotherapy. The combined results of these univariate regressions are shown in Table 20.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Value</th>
<th>HR</th>
<th>95.0% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.050</td>
<td>1.020</td>
<td>1.000</td>
</tr>
<tr>
<td>LDH</td>
<td>0.073</td>
<td>1.008</td>
<td>0.999</td>
</tr>
<tr>
<td>Age</td>
<td>0.126</td>
<td>0.959</td>
<td>0.908</td>
</tr>
<tr>
<td>WBC</td>
<td>0.151</td>
<td>1.102</td>
<td>0.965</td>
</tr>
<tr>
<td>Neutrophil Count</td>
<td>0.215</td>
<td>1.088</td>
<td>0.952</td>
</tr>
<tr>
<td>Previous</td>
<td>0.418</td>
<td>1.477</td>
<td>0.575</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>0.456</td>
<td>0.999</td>
<td>0.996</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.507</td>
<td>1.000</td>
<td>0.999</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.576</td>
<td>0.708</td>
<td>0.211</td>
</tr>
<tr>
<td>LBM</td>
<td>0.582</td>
<td>1.017</td>
<td>0.958</td>
</tr>
<tr>
<td>Chest Circumference</td>
<td>0.587</td>
<td>1.016</td>
<td>0.960</td>
</tr>
<tr>
<td>Thigh Circumference</td>
<td>0.590</td>
<td>0.983</td>
<td>0.924</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.616</td>
<td>1.014</td>
<td>0.959</td>
</tr>
<tr>
<td>BMI</td>
<td>0.621</td>
<td>0.975</td>
<td>0.881</td>
</tr>
<tr>
<td>Mid-Upper-Arm</td>
<td>0.638</td>
<td>0.980</td>
<td>0.899</td>
</tr>
<tr>
<td>%Body Fat</td>
<td>0.648</td>
<td>0.988</td>
<td>0.937</td>
</tr>
<tr>
<td>Fat Mass (Van Loan)</td>
<td>0.653</td>
<td>0.986</td>
<td>0.928</td>
</tr>
<tr>
<td>NI&lt;95</td>
<td>0.680</td>
<td>0.823</td>
<td>0.326</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.692</td>
<td>0.990</td>
<td>0.941</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>0.697</td>
<td>0.992</td>
<td>0.951</td>
</tr>
<tr>
<td>NI</td>
<td>0.720</td>
<td>0.731</td>
<td>0.132</td>
</tr>
<tr>
<td>Calf Circumference</td>
<td>0.729</td>
<td>0.979</td>
<td>0.871</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.735</td>
<td>1.009</td>
<td>0.959</td>
</tr>
<tr>
<td>Fat Mass (Segal)</td>
<td>0.739</td>
<td>1.011</td>
<td>0.947</td>
</tr>
<tr>
<td>NI&lt;100</td>
<td>0.756</td>
<td>0.881</td>
<td>0.396</td>
</tr>
<tr>
<td>NI&lt;105</td>
<td>0.756</td>
<td>0.881</td>
<td>0.396</td>
</tr>
<tr>
<td>ECOG Performance Status</td>
<td>0.794</td>
<td>0.898</td>
<td>0.402</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.802</td>
<td>1.009</td>
<td>0.942</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.807</td>
<td>1.006</td>
<td>0.962</td>
</tr>
<tr>
<td>Study Arm</td>
<td>0.830</td>
<td>1.089</td>
<td>0.500</td>
</tr>
<tr>
<td>CRP</td>
<td>0.835</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.863</td>
<td>1.006</td>
<td>0.937</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.863</td>
<td>1.005</td>
<td>0.946</td>
</tr>
<tr>
<td>Fat Mass (Lukaski)</td>
<td>0.870</td>
<td>0.997</td>
<td>0.956</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>0.881</td>
<td>1.118</td>
<td>0.259</td>
</tr>
<tr>
<td>TBN</td>
<td>0.888</td>
<td>1.000</td>
<td>0.999</td>
</tr>
<tr>
<td>Previous</td>
<td>0.893</td>
<td>1.065</td>
<td>0.426</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>0.940</td>
<td>1.057</td>
<td>0.247</td>
</tr>
<tr>
<td>NI&lt;85</td>
<td>0.948</td>
<td>1.000</td>
<td>0.986</td>
</tr>
<tr>
<td>IL6</td>
<td>0.958</td>
<td>1.001</td>
<td>0.969</td>
</tr>
</tbody>
</table>
Once the univariate Cox’s Regression Analysis was completed, the parameters that had significance level of ≤0.25 were selected for the multivariate analysis part. As shown in Table 20, the parameters which have a significance level of ≤0.25 are: the haemoglobin levels (p=0.050), LDH levels (p=0.073), Age (p=0.126), WBC count (p=0.151), and neutrophil count (p=0.215). It is interesting to note that none of these five parameters are “body composition” parameters. In addition to the above five parameters, other important parameters such as Fat Mass, NI, Sex, TBW (by Kushner) content, VEGF levels, and study arm, which by clinical experience are known to have the ability to affect survival (i.e. the \textit{a priori} parameters), were also “forced” into the multivariate analysis. A “Backward Stepwise Likelihood Ratio” Multivariate Cox’s Regression was then performed on the above 11 parameters. Table 21 below shows the relevant results of the regression analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Value</th>
<th>HR</th>
<th>95.0% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>0.001</td>
<td>1.046</td>
<td>1.020 1.074</td>
</tr>
<tr>
<td>Age</td>
<td>0.007</td>
<td>0.919</td>
<td>0.864 0.978</td>
</tr>
<tr>
<td>NI</td>
<td>0.008</td>
<td>0.040</td>
<td>0.004 0.437</td>
</tr>
</tbody>
</table>

These three parameters were then taken as the “base parameters” and Multivariate Cox’s Regression were then performed on the remaining eight (LDH levels, WBC count, neutrophil count, Fat Mass, Sex, TBW by Kushner, VEGF, and study arm) parameters. This was done by keeping the “base parameters” constant and adding one of the eight parameters at a time, performing the regression, and then replacing the parameter with another one of the eight parameters. This, in effect, enabled exploration of the influence of these parameters using a multivariate analysis while adjusting for haemoglobin levels, Age, and NI. The result of this multivariate analysis is shown in Table 22.
The results demonstrated that none of the tested parameters has statistically significant influence on the survival of this group of patients with malignant mesothelioma undergoing chemotherapy.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Value</th>
<th>HR</th>
<th>95.0% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Study Arm</td>
<td>0.373</td>
<td>1.520</td>
<td>0.606 3.814</td>
</tr>
<tr>
<td>WBC Count</td>
<td>0.616</td>
<td>1.048</td>
<td>0.871 1.262</td>
</tr>
<tr>
<td>LDH</td>
<td>0.705</td>
<td>0.998</td>
<td>0.989 1.008</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.759</td>
<td>1.013</td>
<td>0.930 1.104</td>
</tr>
<tr>
<td>Neutrophil Count</td>
<td>0.766</td>
<td>1.028</td>
<td>0.858 1.231</td>
</tr>
<tr>
<td>Sex</td>
<td>0.866</td>
<td>1.117</td>
<td>0.307 4.069</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.957</td>
<td>1.000</td>
<td>0.999 1.001</td>
</tr>
<tr>
<td>TBW(by Kushner)</td>
<td>0.994</td>
<td>1.000</td>
<td>0.917 1.091</td>
</tr>
</tbody>
</table>
12.4 Summary And Conclusions

Results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that none of the categorical parameters tested reached statistical significance. From these six categorical parameters tested, having previous chemotherapy \( (p=0.41) \) was the closest parameter that could determine survival in this group of patients with malignant mesothelioma receiving chemotherapy. This was closely followed by the effect of gender \( (p=0.57) \).

The results of the univariate Cox’s Regression analysis performed on all individual parameters showed that haemoglobin levels \( (p=0.050) \) and LDH levels \( (p=0.073) \) could significantly influence survival in this group of patients. Once the parameters with significance levels of \( \leq 0.25 \) were selected and multivariate Cox’s Regression performed, the haemoglobin levels \( (p=0.001) \), Age \( (p=0.007) \), and NI \( (p=0.008) \) were found to be statistically significant. Although the \textit{a priori} parameters were then “forced” into the analysis and adjusted for haemoglobin levels, Age, and NI, none reached statistical significance.

The overall results, thus, suggest that haemoglobin levels, Age, and NI are the parameters that can influence the survival of patients with malignant mesothelioma undergoing chemotherapy. The fact that the Study Arm parameter did not reach statistical significance could indicate that survival in these patients is not affected by the presence or absence of chemotherapy with gemcitabine and cisplatin. Whether the presence or absence of thalidomide influences survival in these patients requires a separate design and clinical trial to address this issue.
Chapter 13: EPA CLINICAL TRIAL PATIENT RESULTS

13. EPA CLINICAL TRIAL PATIENT RESULTS

As mentioned elsewhere, the EPA clinical trial was designed and carried out as a double-blind, placebo-controlled, randomised trial in a pancreatic cancer patient population undergoing standard cytotoxic cancer chemotherapy. The randomisation process which allocated the patients into EPA or placebo arms took the disease extent also into consideration. As a result, once the randomisation codes were revealed, the data were analysed in two separate ways: as EPA versus placebo as well as metastatic versus locally advanced disease.

13.1 Patient Demographics

A total of 39 patients were initially recruited for this clinical trial. From these patients, two refused to commence the trial due to the inability to swallow the required seven EPA / placebo capsules which were essential for the trial. Therefore, the total number of patients in the trial was actually 37.

From the 37 patients, there were a total of 20 males (54.1%) and 17 females (45.9%). The mean age for the whole patient population was 68.2 years (Median: 68.1 years; Range: 53.2 to 83.4 years). The mean age for the males was 67.9 years (Median: 67.2 years; Range: 56.7 to 83.4 years). The mean age for females was 68.6 years (Median: 70.2 years; Range: 53.2 to 80.5 years).
Chapter 13: EPA CLINICAL TRIAL PATIENT RESULTS

There were 19 patients (51.4%) who received EPA and 18 (48.6%) who received the placebo. Amongst the males, there were equal number of 10 patients (50%) who received EPA and 10 patients (50%) who received the placebo. In the female population, there were nine patients (52.9%) who received EPA and eight (47.1%) who received the placebo.

The extent of disease progression at the time of diagnosis often affects the outcome of therapy as well as the patient’s QL. In our group of 37 patients with pancreatic cancer, there were a total of 20 patients (54.1%) with locally advanced disease and 17 (45.9%) with metastatic disease. In the male population, there were 12 patients (60%) with locally advanced disease and eight (40%) with metastatic disease. In the female population, there were eight (47.1%) with locally advanced disease and nine (52.9%) with metastatic disease.

There were also an almost equal distribution of patients who received EPA and placebo in the locally advanced and the metastatic groups. The number of patients in the locally advanced group who received EPA was nine (45%) and was 10 (58.8%) in the metastatic group. The number of patients who received the placebo was 11 (55%) in the locally advanced group and seven (41.2%) in the metastatic group.
13.2 Completion Rates

Considering the devastating nature of the disease, the overall completion rates were relatively good. There were a total of three patients deaths while still on the trial. Two deaths were due to disease progression and infiltration and one was due to stroke. None of the deaths were due to the EPA administration or the body composition measurements.

There were also a number of “drop-outs” which were due to a number of different reasons. The major reasons for the non-death related drop-outs were the progressive ill health and the inability to cope with cancer therapy as well as attending the four-weekly body composition measurement sessions. There were five patients who refused to continue giving no specific reason other than that they cannot cope with cancer therapy and various different tests as well as attending the body composition measurement sessions. An additional four patients requested to be taken out of the study as they felt that they are too ill to continue with the trial. One patient was also taken out of the study due to an accidental fall and hip fracture at home.

The final number of patients that completed the required five body composition measurements was 24. The number of patient “drop-outs” and the stage of the trial that the event occurred has been summarised and is demonstrated in Figure 58 as a flowchart.
Figure 58. Flowchart demonstrating the number of patient “drop-outs” and the stage of the event.

- **39 Patients Recruited** (up to 01/09):
  - 2 patients dropped out due to inability to take the required EPA/Placebo capsules.

- **37 Completed at least baseline (pre-chemotherapy) measurements**:
  - 8 patients dropped out due to refusal (5) and death (1).

- **32 Patients completed at least two measurements**:
  - 4 patients dropped out due to ill health (2), accidental hip fracture (1) and death (1).

- **28 Patients completed at least three measurements**:
  - 2 patients dropped out due to ill health (2) and death (1).

- **25 Patients completed at least four measurements**:

- **24 Patients completed all the required five measurements**:
  - 1 patient dropped out due to refusal to continue until health.
13.3 Body Composition Results: Metastatic Versus Locally Advanced Disease

All relevant parameters resulting from or during the TBN, TBK and TBW measurements carried out at the CIVBC were investigated to determine the changes in body composition. Initially, the pancreatic cancer patient population was separated into patients who had been diagnosed with a locally advanced disease and those who were diagnosed with metastatic disease.

13.3.1 Differences In Body Composition Between Metastatic And Locally Advanced

The initial step in analysing the difference between the metastatic and locally advanced groups was to see the differences between the average IVBC values in each group. Please note that in the graphs demonstrating these values, for ease of viewing purposes, the body composition parameters with similar range of values were placed and displayed on the same graph.

The differences between the two groups were also investigated statistically. The results for the statistical differences, if any, has been detailed and tabulated in the sections below.

The trend for changes in Weight, %BFat, and BMI are demonstrated in Figure 59. As it can be seen, the locally advanced group consistently has a higher weight than the metastatic group. However, both the %BFat and BMI are higher in the metastatic group only in the first two measurement time-points.
Figure 59. Mean (±SE) changes in Weight (Kg), %BFat (%), and BMI (Kg/\(\text{Ht}^2\)) with time in patients with metastatic and locally advanced disease.

Statistical analyses did not show a statistically significant difference between the two groups for Weight, %BFat, or BMI at any measurement time-point. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

The TBN is one of the four important compartments in a four compartmental body composition model. It can be affected as a result of different disease conditions. As with the Weight, the TBN was shown to also be consistently higher in the locally advanced group. Although the general and overall trend was a decrease in the TBN content, but both group’s TBN peaked at the third measurement time-point, i.e. at the Cycle 3. These changes are shown in Figure 60.
There were no statistically significant differences in TBN between the two groups at any of the measurement time-point. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

At our centre, the NI and TBK/Ht are used as a measure of the patients’ TBN and TBK, respectively, compared to the “normal” range for their gender. The NI is the TBN expressed as a percentage of age, sex, and height-matched “normal” and the “normal” range at our centre is generally considered to be from 0.95 to 1.05, i.e. 95% to 105% of normal. The TBK/Ht’s “normal” range is considered to be $0.86 \pm 0.09 \text{ g/cm}$ (or 0.72 to 1.00 g/cm) for males and $0.59 \pm 0.05 \text{ g/cm}$ (or 0.52 to 0.66 g/cm) for females \cite{813, 818, 812, 814}.

In our pancreatic cancer population, the metastatic group was shown to have a higher NI up to and including the third measurement time-point, but was lower than the locally advanced
group in the fourth and fifth measurement time-points. Neither the metastatic nor the locally advanced group show a definite trend in NI gain or loss. The TBK/Ht was, however, shown to be very similar in both groups but slightly higher in the locally advanced group. These differences are shown in Figure 61.

**Figure 61.** Graph of mean (±SE) NI and TBK/Ht against time for the patients with metastatic and locally advanced disease.

The differences between the two groups in NI and TBK/Ht were not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

Although the “traditional” method of measuring LBM in the clinical setting is by using the skinfold technique, other methods have been developed and are now been utilised. One of these alternative methods of measuring the LBM is using the BIA technique. The details of
this method are given in the section on BIA (Chapters 7.3.2 and 8.1.2). The three most common equations used at our centre to calculate the LBM using the BIA technique are the Lukaski [797, 798], Segal [799, 754], and Van Loan [800, 780] equations. The changes in the LBM of patients with metastatic and locally advance disease are shown in Figure 62.

The LBM (By Lukaski) showed a general overall decrease in LBM for both groups with the locally advanced group having a higher LBM. However, both groups showed an increase in LBM at the second and third measurement time-points before decreasing.

The differences between the two groups in the BIA-derived LBM were not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.
Chapter 13: EPA CLINICAL TRIAL PATIENT RESULTS

The traditional, and perhaps the most common, method of measurement of LBM is to measure skinfold thicknesses and calculate the %BFat using the Durnin and Womersley’s equations \[756, 772, 771\] and then calculate the LBM from weight and %BFat. Details of skinfold thickness measurement technique and measurements of %BFat are given in the anthropometry section (Chapters 7.3 and 8.1). This method is also non-invasive and does not require any specialised or expensive equipment. The LBM can also be calculated from TBK. At our centre, the TBK-derived LBM is reported as a part of the TBK measurement results. Details of TBK measurement using the Whole Body Counter technique are given in the TBK section (Chapters 7.4 & 8.4 and Appendix E 1.3.4.4).

Figure 63. Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for the patients with metastatic and locally advanced disease.

Mean (±SE) LBM Measurements Using Skinfolds And TBK Techniques
For Metastatic And Locally Advanced Groups

![Graph of mean (±SE) LBM measurements using skinfolds and TBK techniques against time for the patients with metastatic and locally advanced disease.](image-url)
Chapter 13: EPA CLINICAL TRIAL PATIENT RESULTS

The LBM was shown to be higher in the locally advanced than the metastatic group. The locally advanced group, in addition, showed a general upward trend in LBM, with the LBM reaching its maximum at the third measurement time-point. The metastatic group, however, showed an increase in LBM up to the second measurement time-point followed by a decrease. The TBK-derived LBM showed an almost identical pattern to the skinfold-derived LBM for both groups. These changes are shown in Figure 63.

The differences between the two groups in the skinfold-derived LBM as well as the TBK-derived LBM were also not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

The fat compartment is another one of the four compartments of the body in a four compartmental model body composition. As with the other patient groups investigated here, the FM was measured by subtracting the LBM from the total body weight. In addition, FM can also be obtained from TBK measurements. At our centre the TBK-derived FM is obtained as a part of the TBK measurements. The Figure 64 demonstrates the differences between the metastatic and locally advanced disease groups at different measurement time-points.

The FM in the locally advanced group showed an overall decrease throughout the five measurement time-points with the exception of the third measurement time-point where there was an increase in FM that was higher than at any of the other time-points. With the metastatic group, there was a consistent decline in FM with the exception of the fifth measurement time-point where there was a slight increase in FM.

The TBK-derived FM did not show a consistent trend throughout the five measurement time-points. The FM for the locally advanced group reached its maximum at the second measurement time-point but was decreased at the third. This was then followed by an upward
trend in the fourth and fifth measurement time-points. The metastatic group had a slightly lower FM in general. There was, however, a consistent downward trend up to the fourth measurement time-point followed by an increase in the fifth measurement time-point.

**Figure 64.** Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for the patients with metastatic and locally advanced disease.

The differences between the two groups in the skinfold-derived FM as well as the TBK-derived FM were not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

The TBK measurements did not show any specific pattern. However, both the locally advanced and the metastatic groups demonstrated increases and decreases in TBK at the
corresponding measurement time points. Both the locally advanced and metastatic groups had their maximum TBK at the third measurement cycle. However, the only difference between the two groups was the lowest TBK which occurred at the fifth measurement time-point for the metastatic and the second measurement time-point for the locally advanced groups. These changes and differences are shown in Figure 65.

![Figure 65. Graph of mean (±SE) TBK measurements against time for the patients with metastatic and locally advanced disease.](image)

The differences between the two groups in TBK were not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

The TBW is one of the main and another important body compartment in a four compartmental model body composition. It can be affected as a result of different therapies as...
well as disease conditions. At our centre, the TBW is measured using the BIA technique and
the equations of Fredrix [753], Pullicino [755], or Kushner & Schoeller [746, 748, 745, 747] are used to
calculate the final TBW from the resistance and reactance values obtained from the BIA
instrument. These were validated at our centre in gastrointestinal cancer patients [758, 851, 846] as
well as renal dialysis patients [686].

The changes in TBW as measured using the equations of Fredrix [753], Pullicino [755], or
Kushner & Schoeller [746, 748, 745, 747] in the locally advanced and metastatic disease patients are
shown in Figure 66.

**Figure 66.** Graph of mean (±SE) TBW measurements against time for metastatic and locally advanced
disease patient groups using the Fredrix, Pullicino, and Kushner equations.

Using the Fredrix [753] equation, an increase in TBW was seen in both the metastatic as well as
the locally advanced groups between the first and second measurement time-points. For the
locally advanced group, this increase continues to the fourth measurement time-point but
declines at the fifth measurement time-point. With the metastatic group, the decline in TBW content occurs after the third measurement time-point and continues to the fifth measurement time-point.

Using the Pullicino [755] equation, a consistent upward trend is seen in the locally advanced group. With the metastatic group, the upward trend continues only up to and including the fourth measurement time-point. At the fifth measurement time-point, there is a decline in the TBW content of the metastatic group.

Using the Kushner & Schoeller [746, 748, 745, 747] equation, the locally advanced group shows an increase in TBW content up to the third measurement time-point. However, the TBW levels decline at the fourth measurement time-point, although it slightly picks-up at the fifth measurement time-point. With the metastatic group, there is a downward trend in TBW although there is a slight increase at the third measurement time-point.

The differences between the two groups in TBW, regardless of which equation was used, were found to be not statistically significant at any of the measurement time-points. Statistical comparison of changes between different cycles are summarised and tabulated in Table 24 and Table 25.

In order to determine and statistically analyse differences between the two groups one can also plot and analyse the AUCs and slopes. Here, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the AUC for each patient curve is calculated. These AUC values for each group is then compared statistically (Two Sample T-Test (assuming unequal variances)). Similarly, the measurement results for individual patients (y-axis) are plotted against their respective time-points (x-axis) and the slope for each patient curve is measured. This slope values for each group is then compared
Chapter 13: EPA CLINICAL TRIAL PATIENT RESULTS

statistically (Two Sample T-Test (assuming unequal variances)). The results of the AUC and slope for all tested parameters are summarised and tabulated in Table 23.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Met</td>
<td>SD Met</td>
</tr>
<tr>
<td>%BFat</td>
<td>101.8</td>
<td>41.7</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>102.8</td>
<td>44.9</td>
</tr>
<tr>
<td>BMI</td>
<td>80.4</td>
<td>25.9</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>67.4</td>
<td>28.2</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>68.8</td>
<td>31.4</td>
</tr>
<tr>
<td>LBM</td>
<td>153.7</td>
<td>46.2</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>154.5</td>
<td>52.1</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>151.6</td>
<td>48.0</td>
</tr>
<tr>
<td>LBM (TBK)</td>
<td>143.4</td>
<td>51.7</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>140.9</td>
<td>43.0</td>
</tr>
<tr>
<td>NI</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>TBK</td>
<td>323.9</td>
<td>116.9</td>
</tr>
<tr>
<td>TBK/HT</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>TBN</td>
<td>6089.2</td>
<td>2011.6</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>114.6</td>
<td>37.8</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>114.3</td>
<td>37.1</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>105.4</td>
<td>36.8</td>
</tr>
<tr>
<td>Weight</td>
<td>240.1</td>
<td>87.6</td>
</tr>
</tbody>
</table>

As can be seen in Table 23, none of the AUC comparisons between the two groups were statistically significant. However, the slope values for seven of the tested parameters were found to be statistically significant. Here all three BIA-derived LBM were found to be statistically significant indicating that the trends in LBM change mentioned above may be significantly different between patients with metastatic and locally advanced diseases.
Similarly, the trends in TBW changes show significant differences between the two groups of patients suggesting the increase in TBW is statistically significant. Although the NI did not show any specific trend, the slope values for the two groups demonstrate a high statistical difference.

13.3.2 Body Composition Changes Between Time-Points

For all parameters measured, T-Test was performed to statistically determine changes within each of the two metastatic and locally advanced groups. This was used to see whether statistically there are any significant differences between body composition measurement time-points in each group. The changes in body composition parameters in each group between measurement time-points have been described in section 13.3.1 above.

The results for these analyses on the metastatic group are summarised and tabulated in Table 24 and for the locally advanced group in Table 25.

As seen in Table 24, in the metastatic group almost all the statistically significant changes were between the base-line (C1) and the other four measurement time-points. None of the tested LBM parameters, except for the TBK-derived LBM, showed any statistically significant changes between any of the measurement time-points. This was also true for the TBW, TBN, NI, and TBK-derived FM. Weight showed statistically significant decreases in the second (p=0.021) and third (p=0.024) measurement time-point only when it was compared to the base-line values. The %BFat showed statistically significant decreases for all measurement time-points compared with the base-line values. Similarly, the FM showed statistically significant decreases between the base-line values and the second (p=0.009), third (p=0.011), and fourth (p=0.009) measurement time-point. The BMI, which is derived from height and weight, showed statistically significant decline between the base-line values and
the third (p=0.013) and fourth (p=0.031) measurement time-points. The TBK (p=0.008), TBK/Ht (p=0.009), and LBM (By TBK) (p=0.008) showed statistically significant increase between the base-line values and the third measurement time-points.

It is interesting to note that the majority of the statistically significant changes are changes that occur in the third measurement time-point compared to the base-line values. Also the fact that the Weight, %BFat, and FM decrease as compared to the base-line values and an increase in the TBK, TBK/Ht, and LBM (By TBK) can suggest that there is a loss in the fat compartment and an increase in the FFM of the patients with metastatic pancreatic cancer.
Table 24. Comparison of body composition changes between different chemotherapy cycles in metastatic patients. Results (p-Values) sorted by parameter.

| Parameter       | C1 & C2 | C1 & C3 | C1 & C4 | C1 & C5 | C2 & C3 | C2 & C4 | C2 & C5 | C3 & C4 | C3 & C5 | C4 & C5 |
|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| %BFat           | 0.018   | 0.044   | 0.004   | 0.033   | 0.905   | 0.040   | 0.224   | 0.071   | 0.302   | 0.852   |
| BMI             | 0.649   | 0.993   | 0.416   | 0.554   | 0.488   | 0.615   | 0.730   | 0.925   | 0.856   | 0.873   |
| Fat Mass (TBK)  | 0.057   | 0.013   | 0.031   | 0.185   | 0.072   | 0.237   | 0.492   | 0.389   | 0.938   | 0.330   |
| Fat Mass (TBK)  | 0.960   | 0.356   | 0.300   | 0.449   | 0.454   | 0.756   | 0.882   | 0.970   | 0.952   | 0.945   |
| LBM             | 0.089   | 0.071   | 0.317   | 0.391   | 0.183   | 0.954   | 0.968   | 0.513   | 0.855   | 0.709   |
| LBM (Lukaski)   | 0.709   | 0.724   | 0.481   | 0.943   | 0.941   | 0.682   | 0.889   | 0.370   | 0.804   | 0.989   |
| LBM (Segal)     | 0.127   | 0.092   | 0.287   | 0.637   | 0.663   | 0.911   | 0.858   | 0.522   | 0.949   | 0.977   |
| LBM (TBK)       | 0.139   | 0.008   | 0.209   | 0.531   | 0.767   | 0.769   | 0.928   | 0.542   | 0.980   | 0.671   |
| LBM (Van Loan)  | 0.102   | 0.084   | 0.263   | 0.538   | 0.655   | 0.904   | 0.909   | 0.504   | 0.948   | 0.912   |
| NI              | 0.696   | 0.846   | 0.931   | 0.414   | 0.751   | 0.665   | 0.421   | 1.000   | 0.095   | 0.465   |
| TBK             | 0.139   | 0.008   | 0.209   | 0.531   | 0.767   | 0.769   | 0.928   | 0.542   | 0.980   | 0.671   |
| TBK/Ht          | 0.160   | 0.009   | 0.226   | 0.575   | 0.809   | 0.813   | 0.927   | 0.561   | 0.988   | 0.712   |
| TBN             | 0.423   | 0.830   | 0.810   | 0.529   | 0.565   | 0.847   | 0.473   | 0.908   | 0.145   | 0.639   |
| TBW (Fredrix)   | 0.865   | 0.971   | 0.816   | 0.432   | 0.763   | 0.892   | 0.890   | 0.640   | 0.675   | 0.899   |
| TBW (Kushner)   | 0.652   | 0.327   | 0.540   | 0.829   | 0.625   | 0.831   | 0.959   | 0.592   | 0.786   | 0.894   |
| TBW (Pullicino) | 0.865   | 0.971   | 0.816   | 0.432   | 0.763   | 0.892   | 0.890   | 0.640   | 0.675   | 0.899   |

The main observable difference between the metastatic and locally advance group’s results are the fact that none of the parameters that showed statistically significant changes in the metastatic group showed statistically significant changes in the locally advanced group. One similarity is that almost all the statistically significant differences are when the measurement time-points are compared to the base-line values. As seen in Table 25, the LBM (By Lukaski) was the only LBM parameters that showed statistically significant change. The LBM (By Lukaski) showed a statistically significant increase (p=0.041) between the second...
measurement time-point compared to base-line, but a statistically highly significant decrease (p=0.002) when the fifth measurement time-point was compared to the base-line values. The TBW (Fredrix) and TBW (Pullicino) both showed statistically significant increase at the second, third, and fourth measurement time-point when compared to the base-line values. However, they both showed a highly significant increase at the fifth measurement time-point when compared to the base-line. A statistically significant increase (p=0.023) between the second measurement time-point and base-line and a statistically significant decrease (p=0.006) between the fifth measurement time-point and base-line was also true for TBW (By Kushner).

The above results can suggest that the patients with locally advanced disease maintain their Weight, FM, and TBN but are more likely to have a lower TBW by the end of the four month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but are more likely to have a decreased fat compartment and a higher FFM.
Table 25. Comparison of body composition changes between different chemotherapy cycles in locally advanced patients. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C1 &amp; C2</th>
<th>C1 &amp; C3</th>
<th>C1 &amp; C4</th>
<th>C1 &amp; C5</th>
<th>C2 &amp; C3</th>
<th>C2 &amp; C4</th>
<th>C2 &amp; C5</th>
<th>C3 &amp; C4</th>
<th>C3 &amp; C5</th>
<th>C4 &amp; C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.085</td>
<td>0.211</td>
<td>0.124</td>
<td>0.095</td>
<td>0.439</td>
<td>0.144</td>
<td>0.113</td>
<td>0.205</td>
<td>0.173</td>
<td>0.666</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.238</td>
<td>0.638</td>
<td>0.217</td>
<td>0.471</td>
<td>0.404</td>
<td>0.156</td>
<td>0.287</td>
<td>0.694</td>
<td>0.977</td>
<td>0.170</td>
</tr>
<tr>
<td>BMI</td>
<td>0.372</td>
<td>0.779</td>
<td>0.211</td>
<td>0.825</td>
<td>0.914</td>
<td>0.166</td>
<td>0.922</td>
<td>0.062</td>
<td>0.802</td>
<td>0.295</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.161</td>
<td>0.576</td>
<td>0.221</td>
<td>0.241</td>
<td>0.700</td>
<td>0.173</td>
<td>0.192</td>
<td>0.133</td>
<td>0.262</td>
<td>0.967</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.261</td>
<td>0.778</td>
<td>0.229</td>
<td>0.483</td>
<td>0.491</td>
<td>0.144</td>
<td>0.278</td>
<td>0.621</td>
<td>0.865</td>
<td>0.118</td>
</tr>
<tr>
<td>LBM</td>
<td>0.969</td>
<td>0.856</td>
<td>0.451</td>
<td>0.853</td>
<td>0.977</td>
<td>0.458</td>
<td>0.762</td>
<td>0.631</td>
<td>0.634</td>
<td>0.239</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.041</td>
<td>0.083</td>
<td>0.085</td>
<td>0.002</td>
<td>0.373</td>
<td>0.560</td>
<td>0.049</td>
<td>0.753</td>
<td>0.433</td>
<td>0.197</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.155</td>
<td>0.347</td>
<td>0.586</td>
<td>0.203</td>
<td>0.974</td>
<td>0.564</td>
<td>0.801</td>
<td>0.550</td>
<td>0.817</td>
<td>0.427</td>
</tr>
<tr>
<td>LBM (TBK)</td>
<td>0.204</td>
<td>0.869</td>
<td>0.664</td>
<td>0.912</td>
<td>0.504</td>
<td>0.223</td>
<td>0.575</td>
<td>0.930</td>
<td>0.933</td>
<td>0.230</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.193</td>
<td>0.396</td>
<td>0.708</td>
<td>0.299</td>
<td>0.996</td>
<td>0.501</td>
<td>0.925</td>
<td>0.486</td>
<td>0.892</td>
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</tr>
<tr>
<td>NI</td>
<td>0.260</td>
<td>0.550</td>
<td>0.307</td>
<td>0.429</td>
<td>0.068</td>
<td>0.560</td>
<td>0.441</td>
<td>0.457</td>
<td>0.725</td>
<td>0.861</td>
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<td>TBK</td>
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<td>0.664</td>
<td>0.912</td>
<td>0.504</td>
<td>0.223</td>
<td>0.575</td>
<td>0.930</td>
<td>0.933</td>
<td>0.230</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.176</td>
<td>0.847</td>
<td>0.623</td>
<td>0.879</td>
<td>0.451</td>
<td>0.202</td>
<td>0.483</td>
<td>0.924</td>
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<td>TBN</td>
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<td>0.253</td>
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<td>0.607</td>
<td>0.449</td>
<td>0.429</td>
<td>0.973</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.006</td>
<td>0.022</td>
<td>0.014</td>
<td>0.001</td>
<td>0.421</td>
<td>0.504</td>
<td>0.073</td>
<td>0.914</td>
<td>0.408</td>
<td>0.243</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.023</td>
<td>0.054</td>
<td>0.076</td>
<td>0.006</td>
<td>0.454</td>
<td>0.739</td>
<td>0.138</td>
<td>0.688</td>
<td>0.532</td>
<td>0.273</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.006</td>
<td>0.022</td>
<td>0.014</td>
<td>0.001</td>
<td>0.421</td>
<td>0.504</td>
<td>0.073</td>
<td>0.914</td>
<td>0.408</td>
<td>0.243</td>
</tr>
</tbody>
</table>
13.3.3 Changes Between Groups At Specific Time-Points

In order to investigate whether each of the two metastatic and locally advanced disease groups progress at the same or different rates, one has to determine whether there are any differences between these groups at different measurement time-points.

To show this, body composition parameters measured at each time-point from the metastatic group was statistically (T-Test) compared with the body composition parameters measured at the same time-point in the locally advanced group. The results of these comparisons are summarised and tabulated in Table 26.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.927</td>
<td>0.936</td>
<td>0.709</td>
<td>0.678</td>
<td>0.997</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.815</td>
<td>0.704</td>
<td>0.733</td>
<td>0.814</td>
<td>0.939</td>
</tr>
<tr>
<td>BMI</td>
<td>0.754</td>
<td>0.772</td>
<td>0.666</td>
<td>0.483</td>
<td>0.533</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.985</td>
<td>0.881</td>
<td>0.489</td>
<td>0.340</td>
<td>0.441</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.938</td>
<td>0.734</td>
<td>0.702</td>
<td>0.548</td>
<td>0.663</td>
</tr>
<tr>
<td>LBM</td>
<td>0.706</td>
<td>0.689</td>
<td>0.507</td>
<td>0.319</td>
<td>0.158</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.670</td>
<td>0.563</td>
<td>0.473</td>
<td>0.259</td>
<td>0.140</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.771</td>
<td>0.650</td>
<td>0.493</td>
<td>0.290</td>
<td>0.190</td>
</tr>
<tr>
<td>LBM (TBK)</td>
<td>0.883</td>
<td>0.867</td>
<td>0.654</td>
<td>0.452</td>
<td>0.259</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.783</td>
<td>0.668</td>
<td>0.470</td>
<td>0.283</td>
<td>0.175</td>
</tr>
<tr>
<td>NI</td>
<td>0.960</td>
<td>0.465</td>
<td>0.905</td>
<td>0.418</td>
<td>0.778</td>
</tr>
<tr>
<td>TBK</td>
<td>0.883</td>
<td>0.867</td>
<td>0.654</td>
<td>0.452</td>
<td>0.259</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.940</td>
<td>0.970</td>
<td>0.709</td>
<td>0.468</td>
<td>0.327</td>
</tr>
<tr>
<td>TBN</td>
<td>0.674</td>
<td>0.975</td>
<td>0.732</td>
<td>0.257</td>
<td>0.307</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.614</td>
<td>0.481</td>
<td>0.414</td>
<td>0.272</td>
<td>0.162</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.580</td>
<td>0.487</td>
<td>0.419</td>
<td>0.245</td>
<td>0.145</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.614</td>
<td>0.481</td>
<td>0.414</td>
<td>0.272</td>
<td>0.162</td>
</tr>
<tr>
<td>Weight</td>
<td>0.793</td>
<td>0.731</td>
<td>0.442</td>
<td>0.264</td>
<td>0.200</td>
</tr>
</tbody>
</table>

The results of the comparison between the two groups (see Table 26) indicate that the two groups are statistically not different at any of their corresponding measurement time-points.

This could, however, also suggest that the two groups may not progress at the same rate.
13.3.4 Changes Between Groups At Specific Time-Points Compared To Base-Line

In order to assess the changes in body composition parameters as compared to their pre-trial status, for each group the pre-chemotherapy measurements were taken as the base-line values. Measurements at the next four time-points were then each compared to this base-line. Paired T-Test was then used to compare the same time-point measurements between the metastatic and locally advanced groups. The results are summarised in Table 27.

The patterns of change in the body composition parameters with time were also investigated. In order to visually establish out how and when these changes occur, the measurements at each of the chemotherapy cycle time-point were subtracted from the base-line values. These were then plotted against time and T-Test was used to compare the metastatic and locally advanced patient groups.

The most common of all measurements that one can expect to change as a result of cancer and cancer chemotherapy is the weight. The Figure 67 shows the changes in weight with respect to the pre-chemotherapy base-line weight with time (Therapy Cycle).

As can be seen in Figure 67, both groups lose weight throughout the four month of the clinical trial. In addition, the amount of weight lost at each measurement time-point compared to the base-line for the locally advanced group is considerably less than that of the metastatic group. The differences in weight loss compared to base-line between the two groups is statistically significant (p=0.039) at the second measurement time-point. The other measurement time-points showed no statistically significant differences.
Figure 67. Changes in Weight with time in pancreatic cancer patients with metastatic and locally advanced disease.

![Mean (±SE) Weight Changes Compared To Base-Line In Pancreatic Cancer Patients With Metastatic & Locally Advanced Disease](image)

The FM is from skin fold thickness measurements and calculated using the Durnin & Womersley’s [756, 772, 771] equations. Both the metastatic and the locally advanced groups...
showed loss of FM during the clinical trial period. The metastatic group showed a greater loss as compared to the base-line values. The difference between the two groups was shown to be statistically highly significant at the second measurement time-point (p=0.016).

As can be seen in Figure 69, the locally advanced group was found to gain LBM where as the metastatic group was shown to be losing LBM. When compared to the base-line values the differences in LBM (By Lukaski) between the two groups were shown to be statistically significant at the fifth measurement time-point.

The TBW is one of the other body composition parameters that can often be disrupted as a result of disease or therapy. The patterns of TBW change with respect to the base-line values were also investigated. These are demonstrated in Figure 70.
The results indicate that the locally advanced group of patients gain TBW whereas the metastatic group lose TBW as compared with the base-line values. These changes were not found to be statistically significant.
Table 27. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the metastatic and locally advanced groups. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.070</td>
<td>0.235</td>
<td>0.235</td>
<td>0.614</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.451</td>
<td>0.908</td>
<td>0.908</td>
<td>0.677</td>
</tr>
<tr>
<td>BMI</td>
<td>0.153</td>
<td>0.222</td>
<td>0.222</td>
<td>0.316</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.016</td>
<td>0.202</td>
<td>0.202</td>
<td>0.496</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.276</td>
<td>0.904</td>
<td>0.904</td>
<td>0.761</td>
</tr>
<tr>
<td>LBM</td>
<td>0.166</td>
<td>0.530</td>
<td>0.530</td>
<td>0.399</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.292</td>
<td>0.109</td>
<td>0.109</td>
<td>0.041</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.247</td>
<td>0.177</td>
<td>0.177</td>
<td>0.250</td>
</tr>
<tr>
<td>LBM (TBK)</td>
<td>0.406</td>
<td>0.929</td>
<td>0.929</td>
<td>0.396</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.255</td>
<td>0.187</td>
<td>0.187</td>
<td>0.268</td>
</tr>
<tr>
<td>NI</td>
<td>0.788</td>
<td>0.640</td>
<td>0.640</td>
<td>0.239</td>
</tr>
<tr>
<td>TBK</td>
<td>0.406</td>
<td>0.929</td>
<td>0.929</td>
<td>0.396</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.431</td>
<td>0.888</td>
<td>0.888</td>
<td>0.390</td>
</tr>
<tr>
<td>TBN</td>
<td>0.950</td>
<td>0.677</td>
<td>0.677</td>
<td>0.225</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.296</td>
<td>0.132</td>
<td>0.132</td>
<td>0.070</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.290</td>
<td>0.127</td>
<td>0.127</td>
<td>0.069</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.277</td>
<td>0.132</td>
<td>0.132</td>
<td>0.070</td>
</tr>
<tr>
<td>Weight</td>
<td>0.039</td>
<td>0.279</td>
<td>0.279</td>
<td>0.411</td>
</tr>
</tbody>
</table>

The results of the base-line-adjusted comparisons are summarised and tabulated in Table 27. The fact that the only body composition parameters that showed statistically significant differences between the two groups were the Weight, FM, and LBM (Lukaski) and no other tested parameter was significantly different, suggests that the metastatic group can lose Weight more than the locally advanced group and that the FM is the major component of the Weight that is being lost. A similar trend/pattern in Weight loss (Figure 67) and FM loss (Figure 68) may be such an indicator.
13.3.5 Summary And Conclusions: Metastatic Versus Locally Advanced

The 37 pancreatic cancer patients undergoing cancer chemotherapy were separated into the metastatic and locally advanced groups to enable a body composition analysis and comparison of disease extent ignoring whether the patient was receiving EPA or placebo as a part of the clinical trial.

Comparing the measurement time-points between the two groups showed that, statistically, there were no group differences at any measurement time-points for the body composition measurements (unpaired T-Test).

However, the base-line-corrected body composition parameters showed changes and differences between the two groups for the Weight, FM, and LBM (Lukaski) at one measurement time-point only (unpaired T-Test) suggesting that the metastatic group lost more FM and Weight than the locally advanced group.

Results of the analyses of body composition changes between measurement time-points for the metastatic and locally advanced groups separately, suggested that the patients with locally advanced disease maintain their Weight, FM, and TBN but are more likely to have a lower TBW by the end of the four month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but are more likely to have a decreased fat compartment and a higher FFM.
13.4 Body Composition Results: EPA Versus Placebo

13.4.1 Differences In Body Composition Between EPA And Placebo Administration

The initial step in analysing the difference between the EPA and placebo groups was to see the differences between the average IVBC values in each group. Please note that in the graphs demonstrating these values, for ease of viewing purposes, the body composition parameters with similar range of values were placed and displayed on the same graph.

The differences between the two groups were also investigated statistically. The results for the statistical differences, if any, has been detailed and tabulated in the sections below.

The trend for changes in Weight, %BFat, and BMI (Figure 71) suggests that the EPA group initially start with an apparently higher Weight and %BFat, and a lower BMI than the placebo group, but these are not significantly different.

In addition, there are no significant differences in Weight, %BFat, and BMI between the two groups throughout the trial period (see Table 31).
Figure 71. Mean (±SE) Weight (Kg), %BFat (%), and BMI (Kg/Ht²) with time in patients receiving EPA or Placebo.

Similarly, the TBN in the EPA group seems apparently higher than the placebo with the differences reversing after the fourth measurement time-point. However, statistically both groups are not significantly different at any time-point during the course of the trial (see Table 31). These changes are demonstrated in Figure 72.
The changes in NI and TBK/Ht are demonstrated in Figure 73. The NI showed the same pattern of change as the TBN and NI where the apparent difference becomes less with time to the point that by the fourth measurement time-point, the placebo group has an apparently higher NI value. The TBK/Ht, however, was apparently lower for the EPA group than the placebo group.

Statistically, there were no differences in NI and TBK/Ht between the two groups at the start or any measurement time-point during the trial. (see Table 31).

It is interesting to point out that the TBN, NI, and TBK/Ht all measure the “metabolic” compartments of the body and all showed an apparent but non-significant drop at the fourth measurement time-point.
This drop at the fourth measurement time-point was also true for the three BIA-derived LBM (Figure 74) as well as the skinfold-derived LBM (Figure 75), but not TBK-derived LBM (Figure 75) measurements. As with the previous parameters, there were apparent changes throughout the trial, but none were statistically significant demonstrating that not only both groups start with similar and non-significantly different body composition, but this difference remains non-significant at all measurement time-points (see Table 31). The changes are demonstrated in Figure 74.
Figure 74. Graph of mean (±SE) LBM measurements using BIA technique against time for the patients receiving EPA or Placebo.

Mean (±SE) LBM Measurements Using BIA Technique For Pancreatic Cancer Patients Receiving Placebo Or EPA
In this clinical trial the FM was estimated in two different ways of either using the TBK or skinfold technique. Both methods showed the FM content of the EPA group to be consistently but non-significantly higher than the placebo group throughout the trial period. Both groups, in fact start the trial with non-significantly different FM content (see Table 31). There was also no specific pattern to the increase or decrease in FM of either group. These are shown in Figure 76.
Figure 76. Graph of mean (±SE) FM measurements using skinfolds and TBK techniques against time for the patients receiving EPA or Placebo.

Base-line values of TBK are statistically similar (Table 31). The TBK is maintained in the EPA group with a higher upward trend but there is a trend indicating lower TBK content for the placebo group. However, the groups were not statistically and significantly different.
Figure 77. Graph of mean (±SE) TBK measurements against time for the patients receiving EPA or Placebo.

Mean (±SE) TBK Measurements For Pancreatic Cancer Patients Receiving Placebo Or EPA

The changes in TBW as measured using the equations of Fredrix [753], Pullicino [755], or Kushner & Schoeller [746, 748, 745, 747] in the EPA and placebo groups are demonstrated in Figure 78.

All three BIA-derived TBW measurements showed identical patterns of differences between the two groups. The trend in the differences between the EPA and placebo groups was identical to that seen with the TBN (Figure 72) and was not statistically significant at any measurement time-point (see Table 31, Figure 78).
Figure 78. Graph of mean (±SE) TBW measurements against time for the EPA and Placebo disease patient groups using the Fredrix, Pullicino, and Kushner equations.

As an alternate method of determining changes and differences between the EPA and placebo groups, the AUC and slope values were plotted and statistically analysed (T-Test). The results have been summarised and tabulated in Table 28. The results of the analyses demonstrate that there are no statistically significant differences in the AUC and slope values of the EPA and placebo group. This would, therefore, suggest that there might not be any differences in the changes of body composition measurements in these two groups.
Table 28. Summary of statistical results for comparison between EPA and Placebo groups using AUC and slopes. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.957</td>
<td>0.183</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.632</td>
<td>0.921</td>
</tr>
<tr>
<td>BMI</td>
<td>0.506</td>
<td>0.485</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.915</td>
<td>0.913</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.433</td>
<td>0.944</td>
</tr>
<tr>
<td>LBM</td>
<td>0.404</td>
<td>0.363</td>
</tr>
<tr>
<td>LBM (By TBK)</td>
<td>0.349</td>
<td>0.744</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.378</td>
<td>0.849</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.513</td>
<td>0.392</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.513</td>
<td>0.413</td>
</tr>
<tr>
<td>NI</td>
<td>0.566</td>
<td>0.270</td>
</tr>
<tr>
<td>TBK</td>
<td>0.349</td>
<td>0.744</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.314</td>
<td>0.717</td>
</tr>
<tr>
<td>TBN</td>
<td>0.494</td>
<td>0.434</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.340</td>
<td>0.868</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.336</td>
<td>0.743</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.356</td>
<td>0.868</td>
</tr>
<tr>
<td>Weight</td>
<td>0.610</td>
<td>0.582</td>
</tr>
</tbody>
</table>

The above comparisons demonstrate that the two groups were well randomised and the differences between them at the same measurement time-point remained essentially non-significant.

### 13.4.2 Body Composition Changes Between Time-Points

For all parameters measured, T-Test was performed to statistically determine changes within each of the two EPA and placebo groups. This was used to see whether there are any significant differences between body composition measurement time-points in each group. The changes in body composition parameters in each group between measurement time-points have been described in section 13.4.1 above.

The results for these analyses on the EPA group are summarised and tabulated in Table 30 and for the placebo group in Table 29.
As tabulated in Table 29 for the placebo group, there were statistically significant changes between different measurement time-points of TBN, NI, %BFat, FM, and TBW. However, Weight and TBK did not show any statistically significant differences between any of the measurement time-points.

Both the TBN (p=0.005) and NI (p=0.002) showed statistically highly significant increases between the second and fourth measurement time-points. In addition the NI showed statistically significant increase between the third and fifth measurement time-points (p=0.034). The %BFat showed statistically significant decreases between a number of different measurement time-points. There was a highly significant change between the baseline and fifth measurement time-point (p=0.0098) as well as significant decreases between the baseline and the fourth (p=0.031), second and fifth (p=0.024), third and fifth (p=0.040), and fourth and fifth (p=0.042) measurement time-points. The statistically significant decreases in FM was only between the base-line and second (p=0.047) and the base-line and fifth (p=0.033) measurement time-points. Both the TBW (By Fredrix) and TBW (By Pullicino) showed statistically significant increases between the base-line and second measurement time-point (p=0.019 and p= 0.019, respectively).
The EPA group showed statistically significant changes in more parameters than the placebo group. As demonstrated in Table 30 for the EPA group these include Weight, TBN, %BFat, FM, LBM (By Lukaski), and all three TBW measurements. The statistically significant decreases in Weight and TBN were both between the third and fourth measurement time-points (p=0.012 and p=0.048, respectively). The %BFat showed a statistically significant increase only between the base-line and second measurement time-points (p=0.022). Although

| Parameter          | C1 & C2 | C1 & C3 | C1 & C4 | C1 & C5 | C2 & C3 | C2 & C4 | C2 & C5 | C3 & C4 | C3 & C5 | C4 & C5 |
|--------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| %BFat              | 0.078   | 0.068   | 0.031   | 0.010   | 0.238   | 0.114   | 0.024   | 0.182   | 0.040   | 0.042   |
| %BFat (TBK)        | 0.215   | 0.957   | 0.493   | 0.738   | 0.476   | 0.091   | 0.680   | 0.537   | 0.672   | 0.168   |
| BMI                | 0.150   | 0.123   | 0.089   | 0.314   | 0.312   | 0.269   | 0.779   | 0.376   | 0.705   | 0.421   |
| Fat Mass           | 0.047   | 0.082   | 0.077   | 0.033   | 0.295   | 0.185   | 0.068   | 0.257   | 0.107   | 0.215   |
| Fat Mass (TBK)     | 0.404   | 0.974   | 0.446   | 0.863   | 0.677   | 0.115   | 0.745   | 0.515   | 0.808   | 0.125   |
| LBM                | 0.230   | 0.261   | 0.273   | 0.546   | 0.741   | 0.956   | 0.617   | 0.586   | 0.379   | 0.453   |
| LBM (By TBK)       | 0.128   | 0.390   | 0.778   | 0.089   | 0.758   | 0.156   | 0.826   | 0.493   | 0.502   | 0.144   |
| LBM (Lukaski)      | 0.112   | 0.434   | 0.533   | 0.305   | 0.847   | 0.899   | 0.900   | 0.748   | 0.987   | 0.726   |
| LBM (Segal)        | 0.921   | 0.797   | 0.788   | 0.782   | 0.776   | 0.787   | 0.842   | 0.992   | 0.994   | 0.998   |
| LBM (Van Loan)     | 0.947   | 0.729   | 0.686   | 0.628   | 0.759   | 0.731   | 0.748   | 0.953   | 0.923   | 0.914   |
| NI                 | 0.632   | 0.553   | 0.752   | 0.795   | 0.342   | **0.002** | **0.034** | 0.248   | 0.232   | 0.968   |
| TBK                | 0.128   | 0.390   | 0.778   | 0.089   | 0.758   | 0.156   | 0.826   | 0.493   | 0.502   | 0.144   |
| TBK/HT             | 0.115   | 0.402   | 0.806   | 0.106   | 0.707   | 0.130   | 0.905   | 0.490   | 0.519   | 0.151   |
| TBN                | 0.546   | 0.445   | 0.947   | 0.692   | 0.359   | **0.005** | 0.157   | 0.266   | 0.646   | 0.442   |
| TBW (Fredrix)      | **0.019** | 0.184   | 0.105   | 0.057   | 0.921   | 0.803   | 0.724   | 0.872   | 0.816   | 0.812   |
| TBW (Kushner)      | 0.108   | 0.412   | 0.371   | 0.256   | 0.914   | 0.925   | 0.886   | 0.997   | 0.954   | 0.917   |
| TBW (Pullicino)    | **0.019** | 0.184   | 0.105   | 0.057   | 0.921   | 0.803   | 0.724   | 0.872   | 0.816   | 0.812   |
| Weight             | 0.094   | 0.149   | 0.122   | 0.105   | 0.476   | 0.349   | 0.369   | 0.488   | 0.542   | 0.792   |
the FM showed a statistically significant increase between the base-line and the second measurement time-points (p=0.039), there was a statistically significant decrease between the third and fourth measurement time-points (p=0.039). Between the same measurement time-points, the BMI also showed a statistically significant decrease (p=0.041). There was a statistically significant increase in LBM (By Lukaski) between the base-line and fifth measurement time-points (p=0.011). The TBW measurements by Fredrix (p=0.014) and Pullicino (p=0.014) both showed statistically significant increases in TBW between the base-line and the fifth measurement time-points. All TBW measurements displayed a statistically significant decrease between the second and third measurement time-points.
Table 30. Comparison of body composition changes between different chemotherapy cycles in patients receiving EPA. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C1 &amp; C2</th>
<th>C1 &amp; C3</th>
<th>C1 &amp; C4</th>
<th>C1 &amp; C5</th>
<th>C2 &amp; C3</th>
<th>C2 &amp; C4</th>
<th>C2 &amp; C5</th>
<th>C3 &amp; C4</th>
<th>C3 &amp; C5</th>
<th>C4 &amp; C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.022</td>
<td>0.147</td>
<td>0.053</td>
<td>0.414</td>
<td>0.888</td>
<td>0.117</td>
<td>0.842</td>
<td>0.174</td>
<td>0.969</td>
<td>0.395</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.650</td>
<td>0.243</td>
<td>0.103</td>
<td>0.073</td>
<td>0.337</td>
<td>0.812</td>
<td>0.317</td>
<td>0.780</td>
<td>0.454</td>
<td>0.793</td>
</tr>
<tr>
<td>BMI</td>
<td>0.153</td>
<td>0.211</td>
<td>0.076</td>
<td>0.787</td>
<td>0.310</td>
<td>0.095</td>
<td>0.642</td>
<td>0.041</td>
<td>0.910</td>
<td>0.244</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.039</td>
<td>0.189</td>
<td>0.065</td>
<td>0.689</td>
<td>0.948</td>
<td>0.115</td>
<td>0.949</td>
<td>0.039</td>
<td>0.855</td>
<td>0.298</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.668</td>
<td>0.117</td>
<td>0.096</td>
<td>0.116</td>
<td>0.196</td>
<td>0.725</td>
<td>0.352</td>
<td>0.816</td>
<td>0.540</td>
<td>0.978</td>
</tr>
<tr>
<td>LBM</td>
<td>0.677</td>
<td>0.525</td>
<td>0.501</td>
<td>0.852</td>
<td>0.402</td>
<td>0.431</td>
<td>0.656</td>
<td>0.524</td>
<td>0.755</td>
<td>0.768</td>
</tr>
<tr>
<td>LBM (By TBK)</td>
<td>0.238</td>
<td>0.905</td>
<td>0.578</td>
<td>0.208</td>
<td>0.350</td>
<td>0.922</td>
<td>0.367</td>
<td>0.410</td>
<td>0.629</td>
<td>0.881</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.839</td>
<td>0.184</td>
<td>0.773</td>
<td>0.011</td>
<td>0.379</td>
<td>0.710</td>
<td>0.012</td>
<td>0.419</td>
<td>0.276</td>
<td>0.268</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.807</td>
<td>0.892</td>
<td>0.716</td>
<td>0.090</td>
<td>0.976</td>
<td>0.656</td>
<td>0.370</td>
<td>0.225</td>
<td>0.704</td>
<td>0.385</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.733</td>
<td>0.812</td>
<td>0.656</td>
<td>0.115</td>
<td>0.960</td>
<td>0.625</td>
<td>0.412</td>
<td>0.195</td>
<td>0.763</td>
<td>0.396</td>
</tr>
<tr>
<td>NI</td>
<td>0.311</td>
<td>0.827</td>
<td>0.223</td>
<td>0.713</td>
<td>0.353</td>
<td>0.302</td>
<td>0.849</td>
<td>0.074</td>
<td>0.697</td>
<td>0.508</td>
</tr>
<tr>
<td>TBK</td>
<td>0.238</td>
<td>0.905</td>
<td>0.578</td>
<td>0.208</td>
<td>0.350</td>
<td>0.922</td>
<td>0.367</td>
<td>0.410</td>
<td>0.629</td>
<td>0.881</td>
</tr>
<tr>
<td>TBK/HT</td>
<td>0.245</td>
<td>0.926</td>
<td>0.524</td>
<td>0.189</td>
<td>0.362</td>
<td>0.969</td>
<td>0.368</td>
<td>0.443</td>
<td>0.607</td>
<td>0.814</td>
</tr>
<tr>
<td>TBN</td>
<td>0.207</td>
<td>0.965</td>
<td>0.184</td>
<td>0.725</td>
<td>0.248</td>
<td>0.299</td>
<td>0.903</td>
<td>0.048</td>
<td>0.521</td>
<td>0.490</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.397</td>
<td>0.134</td>
<td>0.583</td>
<td>0.014</td>
<td>0.571</td>
<td>0.723</td>
<td>0.031</td>
<td>0.535</td>
<td>0.267</td>
<td>0.331</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.689</td>
<td>0.450</td>
<td>0.864</td>
<td>0.021</td>
<td>0.698</td>
<td>0.920</td>
<td>0.043</td>
<td>0.406</td>
<td>0.337</td>
<td>0.309</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.397</td>
<td>0.134</td>
<td>0.583</td>
<td>0.014</td>
<td>0.571</td>
<td>0.723</td>
<td>0.031</td>
<td>0.535</td>
<td>0.267</td>
<td>0.331</td>
</tr>
<tr>
<td>Weight</td>
<td>0.172</td>
<td>0.272</td>
<td>0.122</td>
<td>0.874</td>
<td>0.515</td>
<td>0.190</td>
<td>0.729</td>
<td>0.012</td>
<td>0.735</td>
<td>0.298</td>
</tr>
</tbody>
</table>
13.4.3 Changes Between Groups At Specific Time-Points

Trends previously described, influence the groups’ mean values. As a result comparisons of the mean values were made at each measurement time-point (Table 31).

To determine this, body composition parameters measured at each time-point from the EPA group was statistically (T-Test) compared with the body composition parameters measured at the same time-point in the placebo group. The results of these comparisons are summarised and tabulated in Table 31.

Table 31. Statistical comparison of body composition parameters of the EPA and placebo groups at the corresponding time-points. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.855</td>
<td>0.348</td>
<td>0.418</td>
<td>0.322</td>
<td>0.098</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.632</td>
<td>0.575</td>
<td>0.338</td>
<td>0.105</td>
<td>0.597</td>
</tr>
<tr>
<td>BMI</td>
<td>0.785</td>
<td>0.467</td>
<td>0.763</td>
<td>0.883</td>
<td>0.999</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.512</td>
<td>0.219</td>
<td>0.393</td>
<td>0.562</td>
<td>0.317</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.320</td>
<td>0.165</td>
<td>0.218</td>
<td>0.182</td>
<td>0.578</td>
</tr>
<tr>
<td>LBM</td>
<td>0.577</td>
<td>0.576</td>
<td>0.855</td>
<td>0.576</td>
<td>0.412</td>
</tr>
<tr>
<td>LBM (By TBK)</td>
<td>0.995</td>
<td>0.909</td>
<td>0.903</td>
<td>0.171</td>
<td>0.615</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.631</td>
<td>0.747</td>
<td>0.930</td>
<td>0.543</td>
<td>0.661</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.491</td>
<td>0.458</td>
<td>0.720</td>
<td>0.804</td>
<td>0.824</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.470</td>
<td>0.454</td>
<td>0.728</td>
<td>0.837</td>
<td>0.839</td>
</tr>
<tr>
<td>NI</td>
<td>0.408</td>
<td>0.358</td>
<td>0.525</td>
<td>0.328</td>
<td>0.761</td>
</tr>
<tr>
<td>TBK</td>
<td>0.995</td>
<td>0.909</td>
<td>0.903</td>
<td>0.171</td>
<td>0.615</td>
</tr>
<tr>
<td>TBK/Ht</td>
<td>0.967</td>
<td>0.949</td>
<td>0.783</td>
<td>0.124</td>
<td>0.600</td>
</tr>
<tr>
<td>TBN</td>
<td>0.468</td>
<td>0.565</td>
<td>0.659</td>
<td>0.272</td>
<td>0.492</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.616</td>
<td>0.776</td>
<td>0.958</td>
<td>0.463</td>
<td>0.639</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.674</td>
<td>0.785</td>
<td>0.999</td>
<td>0.446</td>
<td>0.546</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.616</td>
<td>0.776</td>
<td>0.958</td>
<td>0.463</td>
<td>0.639</td>
</tr>
<tr>
<td>Weight</td>
<td>0.492</td>
<td>0.341</td>
<td>0.598</td>
<td>0.950</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The results of the comparison between the two groups (see Table 31) indicate that the two groups are statistically not different at their corresponding measurement time-points. This could, however, also suggest that the two groups may not progress at the same rate.
13.4.4 Changes Between Groups At Specific Time-Points Compared To Base-Line

In order to assess the changes in body composition parameters as compared to their pre-trial status, for each of the two groups the pre-chemotherapy measurements were taken as the base-line values. Measurements at the next four time-points were then each compared to this base-line. Two-sample, two-tailed T-Test was then used to compare the same time-point measurements between the EPA and placebo groups. The results are summarised in Table 32. The patterns of change in the body composition parameters with time were also investigated. In order to visually establish out how and when these changes occur, the measurements at each of the chemotherapy cycle time-point were subtracted from the base-line values. These were then plotted against time and T-Test was used to compare the EPA and placebo groups.

The patients’ weight is one of the parameters that probably is most likely to change as a result of cancer and/or cancer therapy. As demonstrated in Figure 79, both groups lose weight throughout the four month of the clinical trial. In addition, the amount of weight lost at each measurement time-point compared to the base-line for the patients receiving placebo is apparently more than that of the patients receiving EPA. In other word, the placebo seems to increase the rate of weight loss. However, the differences between the two groups were not found to be statistically significant at any of the tested measurement time-points.
The change in FM compared to the base-line followed the same trend as the Weight (Figure 79). The placebo group was shown (see Figure 80) to lose FM more rapidly than the EPA group. As with the Weight, the loss for EPA was least at the last measurement time-point.
compared to base-line. Again, the differences between the two groups were not found to be statistically significant at any of the tested measurement time-points.

Similarly, there was no apparent change in TBN at all measurement time-points for both groups (see Figure 81).

Figure 81. Changes in TBN with time in pancreatic cancer patients receiving EPA or Placebo.

Mean (±SE) TBN Changes Compared To Base-Line In Pancreatic Cancer Patients Receiving Placebo Or EPA

The third of the four compartments in a four compartmental body composition analysis is the TBW. The TBW levels of both groups tested here showed an apparent increase, although not significantly, during the course of the clinical trial (Figure 82).
Figure 82. Changes in TBW with time in pancreatic cancer patients receiving EPA or Placebo.

Mean (±SE) TBW Changes Compared To Base-Line In Pancreatic Cancer Patients Receiving Placebo Or EPA

Compared to base-line, the only parameters whose differences between the two groups were statistically significant were the TBK, TBK/Ht, and TBK-derived LBM (LBM (TBK)) (see Table 32). For all three parameters the change was only statistically significant at the last measurement time-point. In all three parameters, the placebo group showed a statistically significant decrease where as the EPA showed a statistically significant increase (see Figure 83).
Figure 83. Changes in TBK and LBM (by TBK) with time in pancreatic cancer patients receiving EPA or Placebo.

Mean (±SE) TBK And LBM (TBK) Changes Compared To Base-Line In Pancreatic Cancer Patients Receiving Placebo Or EPA

Figure 84. Changes in TBK/Ht with time in pancreatic cancer patients receiving EPA or Placebo.

Mean (±SE) TBK/Ht Changes Compared To Base-Line In Pancreatic Cancer Patients Receiving Placebo Or EPA
Table 32. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the EPA and Placebo groups. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BFat</td>
<td>0.884</td>
<td>0.627</td>
<td>0.666</td>
<td>0.066</td>
</tr>
<tr>
<td>%BFat (TBK)</td>
<td>0.871</td>
<td>0.551</td>
<td>0.926</td>
<td>0.343</td>
</tr>
<tr>
<td>BMI</td>
<td>0.809</td>
<td>0.642</td>
<td>0.643</td>
<td>0.562</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>0.771</td>
<td>0.645</td>
<td>0.783</td>
<td>0.103</td>
</tr>
<tr>
<td>Fat Mass (TBK)</td>
<td>0.968</td>
<td>0.468</td>
<td>0.807</td>
<td>0.285</td>
</tr>
<tr>
<td>LBM</td>
<td>0.506</td>
<td>0.731</td>
<td>0.777</td>
<td>0.715</td>
</tr>
<tr>
<td>LBM (By TBK)</td>
<td>0.310</td>
<td>0.529</td>
<td>0.946</td>
<td>0.042</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.417</td>
<td>0.340</td>
<td>0.895</td>
<td>0.087</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.367</td>
<td>0.332</td>
<td>0.911</td>
<td>0.226</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.363</td>
<td>0.331</td>
<td>0.938</td>
<td>0.212</td>
</tr>
<tr>
<td>NI</td>
<td>0.780</td>
<td>0.802</td>
<td>0.238</td>
<td>0.706</td>
</tr>
<tr>
<td>TBK</td>
<td>0.310</td>
<td>0.529</td>
<td>0.946</td>
<td>0.042</td>
</tr>
<tr>
<td>TBK/HT</td>
<td>0.297</td>
<td>0.556</td>
<td>0.987</td>
<td>0.044</td>
</tr>
<tr>
<td>TBN</td>
<td>0.770</td>
<td>0.598</td>
<td>0.278</td>
<td>0.883</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.416</td>
<td>0.372</td>
<td>0.634</td>
<td>0.214</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.390</td>
<td>0.368</td>
<td>0.697</td>
<td>0.160</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.434</td>
<td>0.382</td>
<td>0.634</td>
<td>0.214</td>
</tr>
<tr>
<td>Weight</td>
<td>0.777</td>
<td>0.663</td>
<td>0.757</td>
<td>0.224</td>
</tr>
</tbody>
</table>

13.4.5 Summary And Conclusions: EPA Versus Placebo

The 37 pancreatic cancer patients undergoing cancer chemotherapy were separated into patients who received EPA and those who received the placebo to enable a body composition analysis and comparison of treatment ignoring the patients’ disease extent.

All statistical comparisons between the two groups at all of the measurement time-points pointed to the fact that the two groups are not statistically different in their body composition, indicating that EPA does not influence changes in body composition in this group of pancreatic cancer patients.
Comparisons of the body composition parameters with the base-line values showed that the patients receiving the placebo show an apparent higher loss of Weight and FM during the entire measurement periods than the EPA group. The apparent gain in TBW was, with the exception of the fourth time-point, greater in the EPA group. The TBK, TBK/Ht, LBM (By TBK) showed statistically significant differences at the last measurement time-point with the EPA showing increases in all three parameters.

Both the EPA and placebo groups showed an interesting pattern in the “metabolic” compartments of the body where there was a statistically non-significant drop at the fourth measurement-time-point.

The overall conclusions that can be drawn from the above results is that firstly, the two groups were well randomised with both starting the trial with similar and statistically non-significantly different body composition parameters. Secondly, the two groups were also statistically not different at their corresponding measurement time-points. And thirdly, the patients receiving placebo compared to those receiving EPA lose more Weight, and FM but less TBW throughout the trial. The TBK/Ht (p=0.044), TBK (p=0.042), and LBM (By TBK) (p=0.042), however, showed statistically significant differences where in all three parameters the EPA showed an increase compared to the base-line. As such, the use of EPA in this group of pancreatic cancer patients undergoing cancer chemotherapy with gemcitabine results in a non-significant reduction in weight loss, FM loss, and TBW gain with a statistically significant increase in FFM.
Chapter 14: EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

14. EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

As mentioned elsewhere, the EPA clinical trial was designed and carried out as a double-blind, placebo-controlled, randomised trial in a pancreatic cancer patient population undergoing standard cytotoxic cancer chemotherapy. The QL data of this trial will, therefore, be analysed in a similar fashion to the body composition data. Hence, the QL data are analysed in two separate ways: as EPA versus placebo as well as metastatic versus locally advanced disease.

14.1 Patient Demographics

The patient demographics as well as the drop-out and completion rates are identical to the ones described in the EPA Clinical Trial Patient Results section (Chapter 13).

14.2 Scoring Quality Of Life Questionnaires

The QL questionnaire that was used for the EPA was the EORTC’s QLQ-C30 and its pancreatic module QLQ-PAN26. These questionnaires consisted of a total of 56 questions addressing a variety of general cancer-related as well as specific pancreatic cancer-related questions. Once the questionnaires were completed, the replies were “scored” as a percentage value based on the strict guidelines obtained directly from EORTC, Southampton, UK. This resulted in a total of 22 categories which were then analysed.

405
In addition, the replies from the Pain Card, ECOG Performance Status, and Karnofsky Performance Status were also added to the 22 QL scores and analysed. This resulted in a total of 28 categories of replies. The results of these are statistically analysed and detailed in the relevant sections below.
14.3 Quality Of Life Results: Metastatic Versus Locally Advanced Disease

All relevant parameters resulting from questionnaires completed by the pancreatic cancer patients as a part of the clinical trial were investigated to determine the changes in the QL. Initially, the pancreatic cancer patient population was separated into patients who had been diagnosed with a locally advanced disease and those who were diagnosed with metastatic disease. The EORTC QL questionnaires were completed by the patients at home whereas the Pain Card was completed at the time of body composition measurements at the CIVBC by the patient. The ECOG Performance Status and Karnofsky Performance Status were completed by the investigator on the day of body composition measurements.

14.3.1 Differences In Quality Of Life Between Metastatic And Locally Advanced

The initial step in analysing the difference between the metastatic and locally advanced groups was to see the differences between the average QL scores in each group. The differences between the two groups were also investigated statistically. The results for the statistical differences, if any, has been detailed and tabulated in the relevant sections below.

The initial step in analysing the data was to see whether there are any statistically significant differences between the two groups when the corresponding measurement time-points are compared. Here, T-Test was used to compare the two groups and the results (p-values) have been summarised and tabulated in Table 33.
Table 33. Statistical comparison of QL parameters of metastatic and locally advanced groups at the corresponding time-points. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.651</td>
<td>0.086</td>
<td>0.398</td>
<td>0.784</td>
<td>0.918</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.317</td>
<td>0.924</td>
<td>0.376</td>
<td>0.584</td>
<td>0.509</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.712</td>
<td>0.555</td>
<td>0.523</td>
<td>0.887</td>
<td>0.937</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.229</td>
<td>0.721</td>
<td>0.890</td>
<td>0.583</td>
<td>0.531</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.456</td>
<td>0.650</td>
<td>0.872</td>
<td>0.741</td>
<td>0.216</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.556</td>
<td>0.254</td>
<td>0.511</td>
<td>0.894</td>
<td>0.349</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.672</td>
<td>0.397</td>
<td>0.609</td>
<td>0.927</td>
<td>0.826</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.045</td>
<td>0.794</td>
<td>0.528</td>
<td>0.095</td>
<td>0.157</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.051</td>
<td>0.832</td>
<td>0.806</td>
<td>0.722</td>
<td>0.408</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.445</td>
<td>0.856</td>
<td>0.631</td>
<td>0.807</td>
<td>0.847</td>
</tr>
<tr>
<td>Financial</td>
<td>0.939</td>
<td>0.948</td>
<td>0.169</td>
<td>0.228</td>
<td>0.197</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.557</td>
<td>0.610</td>
<td>0.516</td>
<td>0.940</td>
<td>0.846</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.742</td>
<td>0.361</td>
<td>0.470</td>
<td>0.702</td>
<td>0.970</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.814</td>
<td>0.972</td>
<td>0.357</td>
<td>0.080</td>
<td>0.508</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.143</td>
<td>0.625</td>
<td>0.629</td>
<td>0.878</td>
<td>0.840</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.460</td>
<td>0.120</td>
<td>0.888</td>
<td><strong>0.028</strong></td>
<td>0.791</td>
</tr>
<tr>
<td>Pain</td>
<td>0.124</td>
<td>0.915</td>
<td>0.469</td>
<td>0.323</td>
<td>0.941</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.077</td>
<td>0.523</td>
<td>0.206</td>
<td>0.152</td>
<td>0.585</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.776</td>
<td>0.382</td>
<td>0.845</td>
<td>0.814</td>
<td>0.765</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.673</td>
<td>0.956</td>
<td>0.833</td>
<td>0.991</td>
<td>0.394</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.641</td>
<td>0.395</td>
<td><strong>0.022</strong></td>
<td>0.156</td>
<td>0.136</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.252</td>
<td>0.783</td>
<td>0.476</td>
<td>0.795</td>
<td>0.778</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.657</td>
<td>0.240</td>
<td>0.362</td>
<td>0.634</td>
<td>0.433</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.867</td>
<td>0.757</td>
<td>0.467</td>
<td>0.399</td>
<td>0.275</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.341</td>
<td>0.398</td>
<td>0.192</td>
<td>0.056</td>
<td>0.613</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.496</td>
<td>0.541</td>
<td>0.733</td>
<td>0.442</td>
<td>0.133</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.160</td>
<td>0.245</td>
<td>0.201</td>
<td>0.426</td>
<td>0.272</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.100</td>
<td>0.099</td>
<td><strong>0.021</strong></td>
<td><strong>0.017</strong></td>
<td>0.383</td>
</tr>
</tbody>
</table>

As seen from Table 33 there are only a few QL categories that are significantly different between the metastatic and locally advanced disease patients. The feeling of Dyspnoea was shown to be statistically different (p=0.045) between the two groups at the base-line.
Looking at the Figure 85 one can see that, with the exception of the second measurement time-point, the metastatic group of patients felt that they had more breathing problems than the locally advanced group. This is probably expected as the metastatic patients are more likely to have disease infiltration as well as multiple disease sites.

The other QL category that was found to be statistically different between the two groups was the Nausea & Vomiting ($p=0.028$). The statistically significant difference was at the fourth measurement time point. In general, the metastatic group was seen to be scoring higher or at best similar to the locally advanced group for Nausea & Vomiting. Since one of the symptoms of pancreatic cancer and, indeed, the progress of the disease is nausea and vomiting, the considerably higher scores given by the metastatic group is probably expected. The trends in change in Nausea & Vomiting scores are demonstrated in Figure 86.
One of the effects of advancing pancreatic cancer is the loss of interest in the previously-interested activities. The EORTC QL questionnaire has incorporated this “symptom” of the disease progression as the “Sexuality” score. The metastatic group scored considerably lower than the locally advanced group with the differences being statistically significant at the third measurement time-point. The trends in the progress of these scores are demonstrated in Figure 87.
Chapter 14: EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

Figure 87. Graph of mean (±SE) Sexuality scores against time (measurement time-point) in patients with metastatic and locally advanced disease.

Mean (±SE) In Sexuality Scores In Pancreatic Cancer Patients With Metastatic & Locally Advanced Disease

There statistically significant differences between the two groups in their Karnofsky Performance Status scores at the third (p=0.021) and fourth (p=0.017) measurement time-point. The locally advanced group had higher scores throughout the trial period indicating that this group is “performing better than the metastatic group. These are demonstrated in Figure 88.
The data from the QL questionnaires, Pain Card, ECOG Performance Status, and Karnofsky Performance Status were also analysed by statistically comparing their AUCs and slopes (Two Sample T-Test (assuming unequal variances)). The results of the comparisons for all tested parameters are summarised and tabulated in Table 34.
Table 34. Summary of statistical results for comparison between metastatic and locally advanced groups using AUC and slopes. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>AUC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.397</td>
<td>0.624</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.738</td>
<td>0.916</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.478</td>
<td>0.517</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.374</td>
<td>0.189</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.994</td>
<td>0.404</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.463</td>
<td>0.324</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.301</td>
<td>0.339</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.345</td>
<td>0.185</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.305</td>
<td>0.033</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.619</td>
<td>0.550</td>
</tr>
<tr>
<td>Financial</td>
<td>0.263</td>
<td>0.428</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.437</td>
<td>0.303</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.237</td>
<td>0.917</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.652</td>
<td>0.277</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.929</td>
<td>0.427</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.334</td>
<td>0.781</td>
</tr>
<tr>
<td>Pain</td>
<td>0.905</td>
<td>0.619</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.599</td>
<td>0.715</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.511</td>
<td>0.914</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.370</td>
<td>0.183</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.076</td>
<td>0.361</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.557</td>
<td>0.086</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.738</td>
<td>0.820</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.704</td>
<td>0.060</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.537</td>
<td>0.536</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.463</td>
<td>0.531</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.298</td>
<td>0.377</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.450</td>
<td>0.442</td>
</tr>
</tbody>
</table>

As can be seen in Table 34, none of the AUC comparisons between the two groups were statistically significant. However, the slope values for one of the tested parameters were found to be statistically significant. Here, the Emotional Functioning was found to be statistically significant (p=0.033) indicating that the upward trend in the Emotional Functioning score of the metastatic group is statistically different to the locally advanced group.
The comparison between the two groups between their corresponding measurement time-points shows that the metastatic group are performing worse than the locally advanced group in term of their Dyspnoea, Nausea & Vomiting, and Sexuality. In addition, the Karnofsky score, which gives an “overall” performance score, also showed that the metastatic group are not performing as good as the locally advanced group.

### 14.3.2 Quality Of Life Changes Between Time-Points

For all parameters measured, T-Test was performed to statistically determine changes within each of the two metastatic and locally advanced groups. This was used to see whether statistically there are any significant differences between QL measurement time-points in each group. The changes in QL parameters in each group between the statistically significant measurement time-points have been described in section 13.3.1 above.

The results for these analyses on the metastatic group are summarised and tabulated in Table 35 and for the locally advanced group in Table 36.

As seen in Table 35, in the metastatic group there is a statistically significant increase (p=0.049) between the second and fifth measurement time-point indicating an improvement in the patients’ self satisfaction. There were statistically significant increases in the constipation scores between the third and fifth (p=0.049), and fourth and fifth (p=0.049) measurement time-point indicating a probable worsening and/or progression of disease. Another indicator of worsening disease is the increase in episodes of nausea and vomiting. There was a significant (p=0.033) increase in Nausea & Vomiting between the base-line and the fourth measurement time-point. This increase in Nausea & Vomiting was the largest during the clinical trial. The statistically significant drop in the Sexuality score (p=0.015) between the base-line and the third measurement time-point is also an indicator of the worsening health.
Chapter 14: EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

An increase in Pain Card Q1 score is an indicative of increase in pain. A statistically significant (p=0.024) was found between the base-line and fifth measurement time-point indicating that by the end of the trial, the patients with metastatic disease were experiencing an increase in pain as compared to base-line. The general trend in Pain Card Q1 score for this group has been an increase during the period of the trial. The Pain Card Q4 score measures the patients’ mood. For the metastatic group there has been a general decline in mood throughout the period of the trial with statistically significant decreases between the second and fifth (p=0.026), and third and fifth (p=0.004) measurement time-points.

There no observable or statistical change in the ECOG Performance Status scores. However, there was a statistically significant decrease in the Karnofsky Performance Status scores between the base-line and the second measurement time-point (p=0.027). Declining Karnofsky scores may be indicative of worsening and/or progressing disease.
## Table 35. Comparison of QL changes between different chemotherapy cycles in metastatic patients. Results (p-Values) sorted by parameter.

| QL Categories       | C1 & C2 | C1 & C3 | C1 & C4 | C1 & C5 | C2 & C3 | C2 & C4 | C2 & C5 | C3 & C4 | C3 & C5 | C4 & C5 |
|---------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Altered Bowel Habit| 1.000   | 0.591   | 0.563   | 0.626   | 1.000   | 0.528   | 0.815   | 0.487   | 0.351   | 0.756   |
| Appetite           | 0.714   | 0.758   | 0.685   | 0.451   | 0.432   | 0.080   | 0.104   | 0.351   | 0.197   | 0.351   |
| Body Image         | 0.410   | 0.309   | 0.285   | 0.316   | 0.779   | 0.170   | 0.049   | 0.197   | 0.104   | 0.732   |
| Cognitive Functioning| 0.831 | 0.726   | 0.111   | 0.351   | 0.277   | 0.351   | 0.104   | 0.080   | 0.451   | 0.095   |
| Constipation       | 0.588   | 0.104   | 0.351   | 0.104   | 0.192   | 0.080   | 0.563   | 1.000   | 0.049   | 0.049   |
| Diarrhoea          | 0.192   | 0.081   | 1.000   | 0.598   | 1.000   | 0.351   | 1.000   | 0.351   | 1.000   | 0.351   |
| Digestive System   | 0.264   | 0.555   | 0.329   | 0.262   | 0.360   | 0.140   | 0.087   | 0.476   | 0.329   | 0.451   |
| Dyspnoea           | 0.167   | 0.279   | 0.598   | 0.451   | 1.000   | 0.170   | 0.197   | 0.170   | 0.197   | 0.598   |
| Emotional Functioning| 0.258 | 0.134   | 0.476   | 0.749   | 0.296   | 0.285   | 0.763   | 0.094   | 0.227   | 0.893   |
| Fatigue            | 0.871   | 0.298   | 0.563   | 0.549   | 0.258   | 1.000   | 0.862   | 0.316   | 0.405   | 0.802   |
| Financial          | 0.640   | 0.213   | 0.351   | 0.563   | 0.176   | 0.351   | 0.351   | 0.598   | 0.351   | 1.000   |
| Global Health Status| 0.337 | 0.187   | 0.805   | 0.930   | 0.779   | 0.080   | 0.351   | 0.170   | 0.443   | 0.528   |
| Healthcare         | 0.068   | 0.671   | 1.000   | 1.000   | 0.145   | 0.351   | 0.351   | 0.265   | 0.265   | 1.000   |
| Hepatic            | 0.690   | 0.279   | 0.442   | 0.516   | 0.779   | 0.487   | 0.476   | 0.402   | 0.451   | 0.836   |
| Insomnia           | 0.553   | 0.343   | 0.197   | 0.732   | 0.724   | 0.285   | 0.763   | 0.598   | 0.685   | 0.451   |
| Nausea & Vomiting  | 0.371   | 0.555   | 0.033   | 0.451   | 0.756   | 0.402   | 0.197   | 0.275   | 0.351   | 0.080   |
| Pain               | 0.271   | 0.121   | 0.649   | 0.516   | 0.192   | 0.277   | 0.571   | 0.351   | 0.815   | 0.775   |
| Pancreatic Pain    | 0.248   | 0.104   | 1.000   | 1.000   | 0.620   | 0.483   | 0.598   | 0.344   | 0.465   | 1.000   |
| Physical Functioning| 0.706 | 0.775   | 0.402   | 0.286   | 0.360   | 0.111   | 0.225   | 1.000   | 0.483   | 0.503   |
| Role               | 0.432   | 1.000   | 0.598   | 0.217   | 0.756   | 0.197   | 0.265   | 1.000   | 0.549   | 0.476   |
| Social Functioning | 0.277   | 0.193   | 1.000   | 1.000   | 0.437   | 0.180   | 0.180   | 0.451   | 0.626   | 1.000   |
| Sexuality          | 0.871   | 0.015   | 0.170   | 0.080   | 0.068   | 0.763   | 0.685   | 0.080   | 0.170   | 0.351   |
| Pain Card Q1       | 0.318   | 0.240   | 0.199   | 0.024   | 0.807   | 0.848   | 0.207   | 0.886   | 0.381   | 0.301   |
| Pain Card Q2       | 1.000   | 0.701   | 0.780   | 0.060   | 0.615   | 0.443   | 0.120   | 0.726   | 0.066   | 0.084   |
| Pain Card Q3       | 0.409   | 0.710   | 0.124   | 0.156   | 0.476   | 0.597   | 0.873   | 0.190   | 0.176   | 0.606   |
| Pain Card Q4       | 0.830   | 0.169   | 0.830   | 0.162   | 0.208   | 0.705   | 0.026   | 0.064   | 0.004   | 0.094   |
| ECOG               | 0.337   | 0.674   | 0.168   | 0.195   | 0.586   | 0.678   | 1.000   | 0.081   | 0.681   | 0.447   |
| Karnofsky          | 0.027   | 0.089   | 0.081   | 0.139   | 0.754   | 0.591   | 1.000   | 0.343   | 1.000   | 0.622   |

The locally advanced group showed statistically significant changes in a wider variety of QL categories. These are summarised in Table 36.
Table 36. Comparison of QL changes between different chemotherapy cycles in locally advanced patients. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>C1 &amp; C2</th>
<th>C1 &amp; C3</th>
<th>C1 &amp; C4</th>
<th>C1 &amp; C5</th>
<th>C2 &amp; C3</th>
<th>C2 &amp; C4</th>
<th>C2 &amp; C5</th>
<th>C3 &amp; C4</th>
<th>C3 &amp; C5</th>
<th>C4 &amp; C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.058</td>
<td>0.308</td>
<td>0.302</td>
<td>0.171</td>
<td>0.313</td>
<td>0.510</td>
<td>0.711</td>
<td>0.860</td>
<td>0.391</td>
<td>0.609</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.608</td>
<td>0.068</td>
<td>0.068</td>
<td><strong>0.047</strong></td>
<td>0.189</td>
<td>0.271</td>
<td>0.302</td>
<td>1.000</td>
<td>0.793</td>
<td>0.720</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.136</td>
<td>0.709</td>
<td>0.709</td>
<td>0.885</td>
<td>0.089</td>
<td>0.131</td>
<td>0.306</td>
<td>1.000</td>
<td>0.856</td>
<td>0.720</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.369</td>
<td>0.546</td>
<td>0.265</td>
<td><strong>0.040</strong></td>
<td>1.000</td>
<td>0.458</td>
<td>0.263</td>
<td>0.271</td>
<td>0.136</td>
<td>0.720</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.422</td>
<td>0.290</td>
<td>0.253</td>
<td>0.315</td>
<td>0.719</td>
<td>0.582</td>
<td>0.752</td>
<td>0.582</td>
<td>1.000</td>
<td>0.583</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.835</td>
<td>0.818</td>
<td>0.458</td>
<td>1.000</td>
<td>1.000</td>
<td>0.334</td>
<td>1.000</td>
<td>0.433</td>
<td>0.720</td>
<td>0.082</td>
</tr>
<tr>
<td>Digestive System</td>
<td>1.000</td>
<td>0.499</td>
<td>0.334</td>
<td>0.873</td>
<td>0.827</td>
<td>0.719</td>
<td>0.883</td>
<td>0.458</td>
<td>0.533</td>
<td>0.336</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.096</td>
<td>1.000</td>
<td>1.000</td>
<td>0.435</td>
<td>0.271</td>
<td>0.271</td>
<td>0.752</td>
<td>1.000</td>
<td>0.720</td>
<td>0.165</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.916</td>
<td>0.361</td>
<td>0.068</td>
<td>0.283</td>
<td>0.173</td>
<td><strong>0.021</strong></td>
<td>0.092</td>
<td>0.442</td>
<td>0.389</td>
<td>0.630</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.571</td>
<td>0.344</td>
<td>0.373</td>
<td>0.924</td>
<td>0.872</td>
<td>0.751</td>
<td>0.635</td>
<td>0.872</td>
<td>0.598</td>
<td>0.306</td>
</tr>
<tr>
<td>Financial Status</td>
<td><strong>0.041</strong></td>
<td>0.104</td>
<td>0.189</td>
<td>0.104</td>
<td>0.433</td>
<td>0.719</td>
<td>0.435</td>
<td>0.334</td>
<td>1.000</td>
<td>0.336</td>
</tr>
<tr>
<td>Global Health</td>
<td>0.466</td>
<td>0.383</td>
<td>0.815</td>
<td>0.449</td>
<td>0.324</td>
<td>0.084</td>
<td>0.596</td>
<td>0.164</td>
<td>1.000</td>
<td>0.477</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.431</td>
<td>0.292</td>
<td>0.709</td>
<td>0.443</td>
<td>0.237</td>
<td>0.818</td>
<td>0.416</td>
<td>0.164</td>
<td>0.096</td>
<td>0.234</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.894</td>
<td>0.271</td>
<td>0.095</td>
<td>0.873</td>
<td>0.212</td>
<td>0.070</td>
<td>1.000</td>
<td>0.334</td>
<td>0.174</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>Insomnia</td>
<td>1.000</td>
<td>0.217</td>
<td>0.499</td>
<td>1.000</td>
<td>0.486</td>
<td>0.751</td>
<td>0.583</td>
<td>0.499</td>
<td>0.302</td>
<td>0.500</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.579</td>
<td>0.189</td>
<td>0.433</td>
<td>0.487</td>
<td>0.054</td>
<td>0.751</td>
<td>0.355</td>
<td>0.055</td>
<td>1.000</td>
<td>0.292</td>
</tr>
<tr>
<td>Pain</td>
<td>0.269</td>
<td>0.182</td>
<td>0.189</td>
<td>0.890</td>
<td>0.486</td>
<td>0.610</td>
<td>0.292</td>
<td>0.751</td>
<td><strong>0.045</strong></td>
<td>0.104</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.076</td>
<td><strong>0.035</strong></td>
<td>0.071</td>
<td>0.404</td>
<td>0.344</td>
<td>0.404</td>
<td>0.365</td>
<td>1.000</td>
<td><strong>0.032</strong></td>
<td>0.114</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.579</td>
<td>0.900</td>
<td>0.547</td>
<td>0.807</td>
<td>0.860</td>
<td>0.384</td>
<td>0.618</td>
<td>0.484</td>
<td>0.443</td>
<td>0.265</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.533</td>
<td>0.465</td>
<td>0.860</td>
<td>0.373</td>
<td>0.413</td>
<td>0.104</td>
<td>0.699</td>
<td>0.413</td>
<td>0.828</td>
<td>0.302</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.529</td>
<td>0.303</td>
<td><strong>0.038</strong></td>
<td>0.174</td>
<td>0.849</td>
<td>0.279</td>
<td>0.593</td>
<td>0.237</td>
<td>0.664</td>
<td>0.234</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.543</td>
<td>0.903</td>
<td>0.705</td>
<td>0.900</td>
<td>0.865</td>
<td>0.595</td>
<td>0.699</td>
<td>0.452</td>
<td>0.710</td>
<td>0.612</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.062</td>
<td>0.182</td>
<td>0.138</td>
<td>0.267</td>
<td>0.818</td>
<td>0.826</td>
<td><strong>0.003</strong></td>
<td>0.952</td>
<td><strong>0.014</strong></td>
<td><strong>0.011</strong></td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.331</td>
<td>0.119</td>
<td><strong>0.039</strong></td>
<td>0.918</td>
<td>0.083</td>
<td><strong>0.025</strong></td>
<td>0.803</td>
<td>0.253</td>
<td>0.160</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.516</td>
<td>0.691</td>
<td>0.499</td>
<td>0.132</td>
<td>0.230</td>
<td>0.163</td>
<td>0.265</td>
<td>0.530</td>
<td><strong>0.049</strong></td>
<td><strong>0.043</strong></td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.790</td>
<td>0.887</td>
<td>0.172</td>
<td>0.143</td>
<td>0.391</td>
<td>0.193</td>
<td>0.061</td>
<td>0.083</td>
<td><strong>0.039</strong></td>
<td>0.386</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.331</td>
<td>0.669</td>
<td>0.217</td>
<td>0.670</td>
<td>1.000</td>
<td>0.384</td>
<td>1.000</td>
<td>0.271</td>
<td>1.000</td>
<td>0.189</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.494</td>
<td>0.432</td>
<td>0.334</td>
<td>0.384</td>
<td>0.188</td>
<td>0.238</td>
<td>0.582</td>
<td>0.546</td>
<td>0.096</td>
<td>0.068</td>
</tr>
</tbody>
</table>

The locally advanced group showed a decline in appetite with a statistically significant decrease (p=0.047) between the base-line and fifth measurement time-point. In this group of patients, declines in Cognitive Functioning and Emotional Functioning was detected with a statistically significant decrease in the Cognitive Functioning between the base-line and fifth (p=0.040) and Emotional Functioning between the second and fourth measurement time-
Chapter 14: EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

points (p=0.021). This group also experienced more financial difficulties which significantly increased by the second measurement time-point (p=0.041) and stayed at a high level throughout the trial period. Although Social Functioning scores were stable, there was a statistically significant increase between the base-line and fourth measurement time-point.

The physical symptoms experienced by this group of patients were in general pain and Pancreatic Pain both of which showed steady decline through the trial period. The decline in pain was statistically significant between the third and fifth (p=0.045), and in Pancreatic Pain between the base-line and third (p=0.035), and third and fifth (p=0.035) measurement time-points.

In contrast the Pain Card Q1 showed an overall increase in pain with the increase between the second and fifth (p=0.003), third and fifth (p=0.014), and fourth and fifth (p=0.011) measurement time-points being statistically significant. Pain Card Q2 which prompts the patient to choose a word indicative of their pain, showed a general decline with a sharp increase at the fifth measurement time-point. The decline between the base-line and fourth (p=0.039), and second and fourth (p=0.025) as well as the increase between fourth and fifth (p=0.016) measurement time-points were shown to be statistically significant. Similarly, the Pain Card Q3 scores showed a steady increase with time followed by a sharp decrease by the end of the trial. This indicates that the patients increasingly get pain relief up to the last measurement time-point where there is a sharp decrease in pain relief. The decreases in pain relief between the third and fifth (p=0.049), and fourth and fifth (p=0.043) measurement time-points were found to be statistically significant. The mood scores of Pain Card Q4 were found to be decreasing with time. The decline between the third and fifth (p=0.039) measurement time-point was found to be statistically significant.
Overall, the QL results for the metastatic group have indicated that there is an increase in the patients’ pain with a decline in mood and general performance. The gastrointestinal symptoms have also been shown to have increased. These, collectively may point to a worsening and/or progressing disease which is consistent with a metastatic aetiology. The locally advanced group were found to have more emotional difficulties than the metastatic group. However, as measured by the EORTC questionnaire, the patients progressively experienced less pain. In contrast, the Pain Card, which is mostly a linear analogue scale, showed an increase in pain and a decline in mood.

14.3.3 Changes Between Groups At Specific Time-Points

Compared To Base-Line

In order to assess the changes in QL parameters as compared to their pre-trial status, for each group the pre-chemotherapy measurements were taken as the base-line values. Measurements at the next four time-points were then each compared to this base-line. Two-sample, two-tailed T-Test was then used to compare the same time-point measurements between the metastatic and locally advanced groups. The results are summarised in Table 37.

The patterns of change in the QL parameters with time were also investigated. In order to visually establish out how and when these changes occur, the measurements at each of the chemotherapy measurement time-point were subtracted from the base-line values. These were then plotted against time and T-Test was used to compare the metastatic and locally advanced patient groups.
### Table 37. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the metastatic and locally advanced groups. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.130</td>
<td>0.309</td>
<td>0.874</td>
<td>0.659</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.947</td>
<td>0.237</td>
<td>0.380</td>
<td>0.386</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.938</td>
<td>0.317</td>
<td>0.618</td>
<td>0.511</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.330</td>
<td>0.279</td>
<td>0.421</td>
<td>0.069</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.808</td>
<td>0.935</td>
<td>0.452</td>
<td>0.059</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.359</td>
<td>0.523</td>
<td>0.542</td>
<td>0.864</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.611</td>
<td>0.910</td>
<td>0.169</td>
<td>0.221</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.035</td>
<td>0.334</td>
<td>0.624</td>
<td>0.622</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.238</td>
<td>0.070</td>
<td>0.517</td>
<td>0.487</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.377</td>
<td>0.901</td>
<td>0.291</td>
<td>0.381</td>
</tr>
<tr>
<td>Financial</td>
<td>0.969</td>
<td>0.237</td>
<td>0.146</td>
<td>0.339</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.981</td>
<td>0.753</td>
<td>0.740</td>
<td>0.592</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.984</td>
<td>0.766</td>
<td>0.741</td>
<td>0.239</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.968</td>
<td>0.623</td>
<td>0.108</td>
<td>0.519</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.827</td>
<td>0.986</td>
<td>0.470</td>
<td>0.894</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.213</td>
<td>0.957</td>
<td>0.021</td>
<td>0.788</td>
</tr>
<tr>
<td>Pain</td>
<td>0.623</td>
<td>0.964</td>
<td>0.615</td>
<td>0.857</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.947</td>
<td>0.511</td>
<td>0.181</td>
<td>0.485</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.247</td>
<td>0.384</td>
<td>0.313</td>
<td>0.962</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.719</td>
<td>0.964</td>
<td>0.596</td>
<td>0.191</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.561</td>
<td>0.042</td>
<td>0.012</td>
<td>0.035</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.138</td>
<td>0.152</td>
<td>0.784</td>
<td>0.937</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.066</td>
<td>0.077</td>
<td>0.033</td>
<td>0.351</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.907</td>
<td>0.391</td>
<td>0.044</td>
<td>0.090</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.786</td>
<td>0.217</td>
<td>0.498</td>
<td>0.679</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.726</td>
<td>0.166</td>
<td>0.530</td>
<td>0.984</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.986</td>
<td>0.693</td>
<td>0.693</td>
<td>0.332</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.143</td>
<td>0.046</td>
<td>0.046</td>
<td>0.369</td>
</tr>
</tbody>
</table>

The results of the base-line-adjusted comparisons are summarised and tabulated in Table 27. Compared to the base-line, the two groups were only significantly different in their Dyspnoea, Nausea & Vomiting, and Sexuality EORTC scores.

As demonstrated in Figure 89, Dyspnoea did not show any specific trend or pattern for either of the two groups. However, the two groups were significantly different in their score at the second measurement time-point (p=0.035).
Figure 89. Changes in Dyspnoea scores with time in pancreatic cancer patients with metastatic and locally advanced disease.

Mean Changes (±SE) In Dyspnoea Compared To Base-Line In Pancreatic Cancer Patients With Metastatic & Locally Advanced Disease

Figure 90. Changes in Nausea & Vomiting scores with time in pancreatic cancer patients with metastatic and locally advanced disease.

Mean Changes (±SE) In Nausea & Vomiting Compared To Base-Line In Pancreatic Cancer Patients With Metastatic & Locally Advanced Disease

Similarly, although, there was no specific trend or pattern with the Nausea & Vomiting scores either, the metastatic group seemed to always show an increase during the trial (see Figure
90). As such the difference between the two groups was statistically significant (p=0.021) at the fourth measurement time-point.

The trend or pattern for the EORTC’s Sexuality scores was not clear. However, what was obvious was the fact that the locally advanced group was consistently demonstrating an increase in scores with time while the metastatic group was consistently demonstrating a decrease with time as compared to the base-line values (see Figure 91). The differences between the two groups were statistically significant at the third (p=0.042), fourth (p=0.012), and fifth (p=0.035) measurement time-points.

Figure 91. Changes in Sexuality scores with time in pancreatic cancer patients with metastatic and locally advanced disease.

The results from the Pain Card Q1 (see Figure 92) show that the metastatic group progressively experience more pain whereas the pain is reduced (except at fifth measurement time-point) in the locally advanced group, as compared to the base-line values. These changes were statistically significant at the fourth measurement time-point (p=0.033). With the Pain Card Q2 results the trend is not clear and seems that the patients’ perception of the words
describing their pain widely varies with time as well as being contradictory to the Pain Card Q1 results.

Figure 92. Changes in Pain Card Q1 scores with time in pancreatic cancer patients with metastatic and locally advanced disease.

The changes in the Karnofsky Performance Status demonstrated a reduction in performance compared to the base-line for the metastatic group. The locally advanced group, however, demonstrated a decrease in the second and fifth and an increase in the third and fourth measurement time-points. The differences between the two groups were statistically significant at the third (p=0.046) and fourth (p=0.046) measurement time-points where the locally advanced group showed an increase in the scores.
Figure 93. Changes in Karnofsky Performance Status scores with time in pancreatic cancer patients with metastatic and locally advanced disease.

The comparison of base-line-corrected measurement time-points of the two groups showed that there is no obvious trend or pattern in Dyspnoea and Nausea & Vomiting scores for either group, although the metastatic group demonstrated consistent, non-significant increase in Nausea & Vomiting scores. The locally advanced group showed a general increase and the metastatic group showed a general decrease in EORTC’s Sexuality scores. Pain scores from the Pain Card Q1 showed a general increase for the metastatic group and a general decrease for the locally advanced group. The Karnofsky Performance Status scores showed a decrease in performance for the metastatic group.
14.3.4 Summary And Conclusions: Metastatic Versus Locally Advanced

The 37 pancreatic cancer patients undergoing cancer chemotherapy were separated into the metastatic and locally advanced groups to enable a QL analysis and comparison of disease extent ignoring whether the patient was receiving EPA or placebo as a part of the clinical trial.

The comparison between the two groups between their corresponding measurement time-points shows that the metastatic group are performing worse than the locally advanced group especially in term of their Dyspnoea, Nausea & Vomiting, and Sexuality. In addition, the Karnofsky score, which gives an “overall” performance score, also showed that the metastatic group are not performing as well as the locally advanced group. Furthermore, for the metastatic group there is an increase in the patients’ pain with a decline in mood and general performance as well as increase in gastrointestinal symptoms.

Base-line-corrected comparisons demonstrated that the metastatic group have consistent, non-significant increase in Nausea & Vomiting scores. The locally advanced group showed a general increase and the metastatic group showed a general decrease in EORTC’s Sexuality scores. Pain scores from the Pain Card Q1 showed a general increase for the metastatic group and a general decrease for the locally advanced group.

These results seen in the metastatic group, collectively may point to a worsening and/or progressing disease which is consistent with a classic metastatic disease aetiology.
14.4 Quality Of Life Results: EPA Versus Placebo

The changes and differences in the QL of patients were also investigated based on whether they received EPA or placebo as a result of the randomisation process. The only difference between this section and 14.3 is the fact that any changes, differences, or similarities in the patients’ QL have been investigated on the basis of their treatment rather than the extent of disease.

14.4.1 Differences In Quality Of Life Between EPA And Placebo Administration

The same analytical processes as described in section 13.3.1 were carried out to determine the differences between the EPA and placebo groups. The results for the statistical differences, if any, has been detailed and tabulated in the relevant sections below.

The initial step in analysing the data was to see whether there are any statistically significant differences between the two groups when the corresponding measurement time-points are compared. Here, T-Test was used to compare the two groups and the results (p-values) have been summarised and tabulated in Table 38.

As seen in Table 38 there are only a few categories at a few measurement time-point that is different between the EPA and placebo groups. The EPA group show more satisfaction with the healthcare that they are receiving than the placebo group with the second (p=0.019) and fifth (p=0.005) measurement time-point showing statistically significant differences. The degree of satisfaction varies with different measurement time-points (see Figure 94).
Table 38. Statistical comparison of QL parameters of EPA and Placebo groups at the corresponding time-points. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.132</td>
<td>0.280</td>
<td>0.349</td>
<td>0.068</td>
<td>0.380</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.877</td>
<td>0.825</td>
<td>0.839</td>
<td>0.230</td>
<td>1.000</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.429</td>
<td>0.658</td>
<td>0.599</td>
<td>0.768</td>
<td>0.914</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.658</td>
<td>0.819</td>
<td>0.129</td>
<td>0.782</td>
<td>0.408</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.069</td>
<td>0.311</td>
<td>0.504</td>
<td>0.235</td>
<td>0.959</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.332</td>
<td>0.264</td>
<td>0.308</td>
<td>0.775</td>
<td>0.575</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.675</td>
<td>0.766</td>
<td>0.473</td>
<td>0.173</td>
<td>0.603</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.296</td>
<td>0.484</td>
<td>0.512</td>
<td>1.000</td>
<td>0.254</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.449</td>
<td>0.276</td>
<td>0.572</td>
<td>0.747</td>
<td>0.732</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.334</td>
<td>0.105</td>
<td>0.529</td>
<td>0.549</td>
<td>0.816</td>
</tr>
<tr>
<td>Financial</td>
<td>0.230</td>
<td>0.990</td>
<td>0.497</td>
<td>0.232</td>
<td>0.403</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.770</td>
<td>0.783</td>
<td>0.178</td>
<td>0.848</td>
<td>0.286</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.060</td>
<td>0.019</td>
<td>0.225</td>
<td>0.233</td>
<td>0.005</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.022</td>
<td>0.227</td>
<td>0.033</td>
<td>0.177</td>
<td>0.398</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.330</td>
<td>0.202</td>
<td>0.808</td>
<td>0.807</td>
<td>0.190</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.308</td>
<td>0.636</td>
<td>0.359</td>
<td>0.281</td>
<td>0.160</td>
</tr>
<tr>
<td>Pain</td>
<td>0.402</td>
<td>0.940</td>
<td>0.537</td>
<td>0.575</td>
<td>0.472</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.298</td>
<td>0.656</td>
<td>0.665</td>
<td>0.952</td>
<td>0.729</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.737</td>
<td>0.272</td>
<td>0.231</td>
<td>0.579</td>
<td>0.230</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.811</td>
<td>0.216</td>
<td>1.000</td>
<td>0.339</td>
<td>0.422</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.021</td>
<td>0.882</td>
<td>0.683</td>
<td>0.431</td>
<td>0.556</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.366</td>
<td>0.216</td>
<td>1.000</td>
<td>1.000</td>
<td>0.818</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.512</td>
<td>0.189</td>
<td>0.284</td>
<td>0.202</td>
<td>0.873</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.996</td>
<td>0.571</td>
<td>0.021</td>
<td>0.053</td>
<td>0.549</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.706</td>
<td>0.057</td>
<td>0.273</td>
<td>0.288</td>
<td>0.073</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.773</td>
<td>0.809</td>
<td>0.190</td>
<td>0.094</td>
<td>0.949</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.132</td>
<td>0.280</td>
<td>0.349</td>
<td>0.068</td>
<td>0.380</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.877</td>
<td>0.825</td>
<td>0.839</td>
<td>0.230</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Figure 94. Graph of mean (±SE) Healthcare Satisfaction scores against time (measurement time-point) in patients receiving EPA or Placebo.

Mean (±SE) Healthcare Satisfaction Scores In Pancreatic Cancer Patients Receiving EPA Or Placebo

Figure 95. Graph of mean (±SE) Hepatic Symptoms scores against time (measurement time-point) in patients receiving EPA or Placebo.

Mean (±SE) Hepatic Symptoms Scores In Pancreatic Cancer Patients Receiving EPA Or Placebo
Both groups show similar trends in Hepatic Symptoms scores with a gradual decrease in the scores followed by a sharp increase at the fifth measurement time-point (see Figure 95). The EPA group, however, had lower Hepatic Symptoms scores. The differences between the groups were statistically significant at the base-line (p=0.022) and third (p=0.033) measurement time-point.

The EORTC’s Sexuality scores did not show a specific trend or pattern (see Figure 96). However, the placebo group consistently scored higher than the EPA group with the base-line differences being statistically significant (p=0.021). This can indicate a lower incidence or a lesser degree of disease related symptoms experienced by the placebo group.

The Pain Card Q2 scores which requires the patients to select words closest to their degree of pain was found to be generally lower in the placebo group than the EPA group. This difference was statistically significant at the third (p=0.021) measurement time-point.
As with the metastatic versus locally advanced comparisons, the differences in the AUC and slopes values for the EPA and placebo groups were calculated and statistically analysed. The results are summarised and tabulated in Table 39.

The mean Cognitive Functions of the placebo group was shown to gradually increase with time during the trial period. This was in contrast to the EPA group where there was a gradual decline. In addition, the mean scores were higher for the placebo than the EPA group. Comparing the slopes statistically showed that there is a highly significant difference between the two groups (p<0.0001).

The Pain Card Q3 scores which address pain relief were slightly higher for the EPA group suggesting a better pain relief in this group. The difference in slopes were also found to be statistically highly significant (p<0.0001) (Table 39). The trends against time to demonstrate slopes and AUCs for Altered Bowel Habit, Cognitive Functioning, Hepatic Symptoms, and Pain Card Q3 scores are shown in Figure 97.

The overall results of the above comparisons suggest that the EPA group are more satisfied with the amount/degree of healthcare that they are receiving, have lower hepatic symptoms, a lower perception of pain, and get more relief from pain than the placebo group. However, the EPA group have lower EORTC Sexuality scores and declining cognitive functions.
Table 39. Summary of statistical results for comparison between EPA and Placebo groups using AUC and slopes. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>AUC</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>&lt;0.0001</td>
<td>0.336</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.595</td>
<td>0.337</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.914</td>
<td>0.341</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.235</td>
<td>0.532</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.962</td>
<td>0.351</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.299</td>
<td>0.182</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.924</td>
<td>0.343</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.339</td>
<td>0.326</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.492</td>
<td>0.798</td>
</tr>
<tr>
<td>Financial</td>
<td>0.357</td>
<td>0.131</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.195</td>
<td>0.761</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.145</td>
<td>0.337</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.052</td>
<td>0.984</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.515</td>
<td>0.320</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.192</td>
<td>0.054</td>
</tr>
<tr>
<td>Pain</td>
<td>0.664</td>
<td>0.336</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.843</td>
<td>0.995</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.128</td>
<td>0.334</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.180</td>
<td>0.470</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.175</td>
<td>0.547</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.122</td>
<td>0.173</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.356</td>
<td>0.549</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.677</td>
<td>0.682</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.604</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.232</td>
<td>0.094</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.487</td>
<td>0.754</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.205</td>
<td>0.078</td>
</tr>
</tbody>
</table>
14.4.2 Quality Of Life Changes Between Time-Points

The same analytical processes as described in section 14.3.2 were performed to statistically determine whether there are any significant differences between QL measurement time-points in each group. The changes in QL parameters in each group between the statistically significant measurement time-points have been described in section 13.4.1 above.

The results of the analyses carried out on the EPA group are summarised and tabulated in Table 41 and in Table 40 for the placebo group.
Results in Table 40 shows that the statistically significant differences between different time-points within the placebo group are mainly involve the emotional/behavioural categories. These include the Cognitive, Emotional, Role, and Social Functioning as well as satisfaction with healthcare. The non-emotional/behavioural category showing significant difference was the Altered Bowel Habit.

The Role, Cognitive, and Social Functioning categories all showed a general increase in scores during the trial. The significant differences as indicated in Table 40 were all statistically significant increases in these scores. Although Role Functioning showed generalised increase in scores during the trial, the drop in scores between the second and third measurement time-points was statistically significant (p=0.025). The Healthcare Satisfaction scores also showed a similar pattern where there was a decrease in scores during the trial but a sharp increase between the second and third measurement time-points was statistically significant (p=0.044) ending the trial with a score equal to the base-line. The Altered Bowel habit showed an almost “sinusoidal” pattern which may be related to the chemotherapy and other associated medications which may explain the statistically significant sharp increase between the base-line and the second measurement time-point (p=0.038).

All four Pain Card scores had at least two statistically significant changes. The changes seen can be best explained as the Q1 and Q2 which both address the perception of pain follow each other and the Q3 and Q4 which address pain relief and mood follow each other (see Figure 98).
Table 40. Comparison of QL changes between different chemotherapy cycles in patients receiving the Placebo. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>C1 &amp; C2</th>
<th>C1 &amp; C3</th>
<th>C1 &amp; C4</th>
<th>C1 &amp; C5</th>
<th>C2 &amp; C3</th>
<th>C2 &amp; C4</th>
<th>C2 &amp; C5</th>
<th>C3 &amp; C4</th>
<th>C3 &amp; C5</th>
<th>C4 &amp; C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.038</td>
<td>0.222</td>
<td>0.529</td>
<td>0.059</td>
<td>0.095</td>
<td>0.065</td>
<td>0.365</td>
<td>0.220</td>
<td>0.339</td>
<td>0.111</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.844</td>
<td>0.636</td>
<td>0.795</td>
<td>0.266</td>
<td>0.673</td>
<td>0.674</td>
<td>0.266</td>
<td>0.586</td>
<td>0.220</td>
<td>0.191</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.095</td>
<td>0.436</td>
<td>0.638</td>
<td>0.772</td>
<td>0.137</td>
<td>0.339</td>
<td>0.136</td>
<td>1.000</td>
<td>0.438</td>
<td>0.438</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.365</td>
<td>0.687</td>
<td>0.012</td>
<td>0.054</td>
<td>0.337</td>
<td>0.166</td>
<td>0.504</td>
<td>0.054</td>
<td>0.339</td>
<td>0.438</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.512</td>
<td>0.139</td>
<td>0.266</td>
<td>0.586</td>
<td>0.337</td>
<td>0.339</td>
<td>1.000</td>
<td>0.166</td>
<td>0.166</td>
<td>0.166</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1.000</td>
<td>0.209</td>
<td>0.463</td>
<td>0.586</td>
<td>0.082</td>
<td>0.166</td>
<td>0.339</td>
<td>0.339</td>
<td>0.166</td>
<td>0.339</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.830</td>
<td>0.893</td>
<td>0.633</td>
<td>0.408</td>
<td>0.053</td>
<td>0.096</td>
<td>1.000</td>
<td>0.723</td>
<td>0.674</td>
<td></td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.096</td>
<td>0.337</td>
<td>0.082</td>
<td>0.275</td>
<td>0.584</td>
<td>0.674</td>
<td>0.754</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.058</td>
<td>0.347</td>
<td>0.223</td>
<td>0.732</td>
<td>0.436</td>
<td>0.014</td>
<td>0.103</td>
<td>0.241</td>
<td>0.408</td>
<td>0.408</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.865</td>
<td>0.632</td>
<td>0.457</td>
<td>0.204</td>
<td>0.570</td>
<td>0.615</td>
<td>0.191</td>
<td>0.838</td>
<td>0.429</td>
<td>0.152</td>
</tr>
<tr>
<td>Financial Status</td>
<td>0.583</td>
<td>1.000</td>
<td>0.339</td>
<td>0.339</td>
<td>1.000</td>
<td>0.674</td>
<td>0.674</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.378</td>
<td>0.819</td>
<td>0.470</td>
<td>0.840</td>
<td>0.266</td>
<td>0.059</td>
<td>0.267</td>
<td>0.365</td>
<td>0.815</td>
<td>0.570</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.151</td>
<td>0.213</td>
<td>0.809</td>
<td>0.410</td>
<td>0.044</td>
<td>0.515</td>
<td>0.795</td>
<td>0.033</td>
<td>0.020</td>
<td>0.318</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.542</td>
<td>0.337</td>
<td>0.376</td>
<td>0.862</td>
<td>0.844</td>
<td>0.551</td>
<td>1.000</td>
<td>0.638</td>
<td>0.586</td>
<td>0.457</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.612</td>
<td>0.673</td>
<td>0.339</td>
<td>0.674</td>
<td>0.613</td>
<td>1.000</td>
<td>0.777</td>
<td>0.723</td>
<td>1.000</td>
<td>0.723</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.671</td>
<td>0.387</td>
<td>0.339</td>
<td>0.210</td>
<td>0.584</td>
<td>0.820</td>
<td>0.723</td>
<td>0.820</td>
<td>1.000</td>
<td>0.862</td>
</tr>
<tr>
<td>Pain</td>
<td>0.710</td>
<td>0.515</td>
<td>0.633</td>
<td>1.000</td>
<td>0.794</td>
<td>0.862</td>
<td>0.732</td>
<td>0.820</td>
<td>0.417</td>
<td>0.623</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.363</td>
<td>0.312</td>
<td>0.547</td>
<td>0.923</td>
<td>1.000</td>
<td>0.898</td>
<td>0.351</td>
<td>0.920</td>
<td>0.261</td>
<td>0.255</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.500</td>
<td>0.307</td>
<td>0.324</td>
<td>0.153</td>
<td>0.416</td>
<td>0.777</td>
<td>0.317</td>
<td>0.586</td>
<td>0.660</td>
<td>0.309</td>
</tr>
<tr>
<td>Role</td>
<td>0.699</td>
<td>0.527</td>
<td>0.830</td>
<td>0.586</td>
<td>0.025</td>
<td>0.429</td>
<td>0.368</td>
<td>0.339</td>
<td>0.571</td>
<td>0.777</td>
</tr>
<tr>
<td>Functioning</td>
<td>0.165</td>
<td>0.025</td>
<td>0.862</td>
<td>0.295</td>
<td>0.691</td>
<td>0.301</td>
<td>0.608</td>
<td>0.067</td>
<td>0.207</td>
<td>0.111</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.759</td>
<td>0.669</td>
<td>0.447</td>
<td>0.410</td>
<td>0.549</td>
<td>0.689</td>
<td>0.555</td>
<td>0.339</td>
<td>0.600</td>
<td>0.795</td>
</tr>
<tr>
<td>Sexuality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.488</td>
<td>0.814</td>
<td>0.710</td>
<td>0.079</td>
<td>0.806</td>
<td>0.918</td>
<td>0.013</td>
<td>0.830</td>
<td>0.065</td>
<td>0.024</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.475</td>
<td>0.080</td>
<td>0.053</td>
<td>0.245</td>
<td>0.019</td>
<td>0.005</td>
<td>0.198</td>
<td>0.776</td>
<td>0.011</td>
<td>0.006</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.073</td>
<td>0.982</td>
<td>0.879</td>
<td>0.033</td>
<td>0.010</td>
<td>0.004</td>
<td>0.547</td>
<td>0.748</td>
<td>0.019</td>
<td>0.031</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.703</td>
<td>0.367</td>
<td>0.912</td>
<td>0.143</td>
<td>0.049</td>
<td>0.513</td>
<td>0.087</td>
<td>0.251</td>
<td>0.009</td>
<td>0.012</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.334</td>
<td>0.673</td>
<td>0.219</td>
<td>0.721</td>
<td>0.673</td>
<td>0.584</td>
<td>0.721</td>
<td>0.190</td>
<td>1.000</td>
<td>0.273</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.271</td>
<td>0.502</td>
<td>0.837</td>
<td>0.436</td>
<td>0.436</td>
<td>0.461</td>
<td>0.502</td>
<td>0.753</td>
<td>1.000</td>
<td>0.776</td>
</tr>
</tbody>
</table>
Unlike the placebo group, the EPA showed significant changes in a wider variety of QL categories with the majority of changes falling between the base-line and third measurement time-point (see Table 41). The decrease in Loss of Appetite was gradual during the trial and only began to increase towards the end of trial. Statistically significant decreases were seen between the base-line and third (p=0.026), base-line and fourth (p=0.002), and second and fourth (p=0.012) measurement time-points indicating an improvement in the appetite of these patients. Although Dyspnoea showed a generalised decrease in this group, there was a statistically significant increase between the third and fifth measurement time-point (p=0.037). There was also a general decrease in Fatigue scores through the trial with the decrease being statistically significant between the base-line and third measurement time-point (p=0.016). This could also be indicative of an improvement in performance and QL. Both Pain and Pancreatic Pain showed general decreases through the trial with the decrease being statistically significant between the base-line and third measurement time-point (p=0.032 and p=0.002, respectively). There were also improvements in Role Functioning and EORTC’s Sexuality scores with the improvement being statistically significant between the
base-line and third measurement time-point (p=0.032 and p=0.015, respectively). This was, however, followed by a decline in Role Functioning after the third measurement time-point.

Figure 99. Mean Pain Card scores against time (Therapy Cycle) for patients receiving EPA.
Table 41. Comparison of QL changes between different chemotherapy cycles in patients receiving the EPA. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>C1 &amp; C2</th>
<th>C1 &amp; C3</th>
<th>C1 &amp; C4</th>
<th>C1 &amp; C5</th>
<th>C2 &amp; C3</th>
<th>C2 &amp; C4</th>
<th>C2 &amp; C5</th>
<th>C3 &amp; C4</th>
<th>C3 &amp; C5</th>
<th>C4 &amp; C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.869</td>
<td>1.000</td>
<td>0.320</td>
<td>0.703</td>
<td>0.730</td>
<td>0.266</td>
<td>0.522</td>
<td>0.447</td>
<td>0.468</td>
<td>0.405</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.487</td>
<td>0.026</td>
<td>0.002</td>
<td>0.052</td>
<td>0.794</td>
<td>0.012</td>
<td>0.168</td>
<td>0.275</td>
<td>1.000</td>
<td>0.591</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.807</td>
<td>0.638</td>
<td>0.089</td>
<td>0.468</td>
<td>0.489</td>
<td>0.600</td>
<td>0.394</td>
<td>0.809</td>
<td>0.758</td>
<td>0.780</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.729</td>
<td>0.638</td>
<td>0.441</td>
<td>0.223</td>
<td>1.000</td>
<td>0.586</td>
<td>0.555</td>
<td>0.723</td>
<td>1.000</td>
<td>0.555</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.435</td>
<td>0.389</td>
<td>0.465</td>
<td>0.823</td>
<td>0.502</td>
<td>0.210</td>
<td>0.798</td>
<td>0.339</td>
<td>0.279</td>
<td>0.168</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.336</td>
<td>0.674</td>
<td>1.000</td>
<td>0.591</td>
<td>0.273</td>
<td>1.000</td>
<td>0.343</td>
<td>0.275</td>
<td>0.726</td>
<td>0.591</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.365</td>
<td>0.152</td>
<td>0.437</td>
<td>0.647</td>
<td>1.000</td>
<td>0.347</td>
<td>0.853</td>
<td>0.517</td>
<td>0.140</td>
<td>0.138</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.165</td>
<td>0.054</td>
<td>0.167</td>
<td>0.678</td>
<td>0.337</td>
<td>0.674</td>
<td>0.193</td>
<td>0.586</td>
<td>0.037</td>
<td>0.081</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.355</td>
<td>0.438</td>
<td>0.136</td>
<td>0.300</td>
<td>0.856</td>
<td>1.000</td>
<td>0.557</td>
<td>1.000</td>
<td>0.210</td>
<td>0.893</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.583</td>
<td>0.016</td>
<td>0.120</td>
<td>0.204</td>
<td>0.193</td>
<td>0.466</td>
<td>0.583</td>
<td>1.000</td>
<td>0.616</td>
<td>1.000</td>
</tr>
<tr>
<td>Financial Status</td>
<td>0.239</td>
<td>0.777</td>
<td>0.676</td>
<td>1.000</td>
<td>0.427</td>
<td>0.275</td>
<td>1.000</td>
<td>0.389</td>
<td>0.343</td>
<td>0.591</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.397</td>
<td>0.059</td>
<td>0.457</td>
<td>0.343</td>
<td>0.798</td>
<td>0.077</td>
<td>0.726</td>
<td>0.075</td>
<td>0.485</td>
<td>0.399</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.254</td>
<td>0.754</td>
<td>0.796</td>
<td>1.000</td>
<td>0.711</td>
<td>0.305</td>
<td>0.726</td>
<td>0.430</td>
<td>0.726</td>
<td>0.343</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.583</td>
<td>0.220</td>
<td>0.796</td>
<td>0.309</td>
<td>0.219</td>
<td>0.376</td>
<td>0.394</td>
<td>0.674</td>
<td>0.052</td>
<td>0.168</td>
</tr>
<tr>
<td>Insomnia</td>
<td>1.000</td>
<td>0.111</td>
<td>0.341</td>
<td>1.000</td>
<td>0.053</td>
<td>0.082</td>
<td>1.000</td>
<td>0.674</td>
<td>0.096</td>
<td>0.279</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>1.000</td>
<td>0.339</td>
<td>0.441</td>
<td>1.000</td>
<td>0.273</td>
<td>1.000</td>
<td>1.000</td>
<td>0.438</td>
<td>0.343</td>
<td>0.343</td>
</tr>
<tr>
<td>Pain</td>
<td>0.116</td>
<td>0.032</td>
<td>0.120</td>
<td>0.464</td>
<td>0.436</td>
<td>0.612</td>
<td>0.309</td>
<td>0.820</td>
<td>0.390</td>
<td>0.616</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.058</td>
<td>0.002</td>
<td>0.058</td>
<td>0.367</td>
<td>0.841</td>
<td>0.520</td>
<td>0.601</td>
<td>1.000</td>
<td>0.156</td>
<td>0.581</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.848</td>
<td>0.291</td>
<td>0.221</td>
<td>0.932</td>
<td>0.746</td>
<td>0.497</td>
<td>0.423</td>
<td>0.345</td>
<td>0.318</td>
<td>0.434</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.355</td>
<td>0.032</td>
<td>1.000</td>
<td>0.743</td>
<td>0.489</td>
<td>0.795</td>
<td>0.509</td>
<td>0.474</td>
<td>0.213</td>
<td>0.662</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.064</td>
<td>0.147</td>
<td>0.086</td>
<td>0.191</td>
<td>0.632</td>
<td>0.295</td>
<td>1.000</td>
<td>0.305</td>
<td>0.798</td>
<td>0.619</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.486</td>
<td>0.015</td>
<td>0.167</td>
<td>0.226</td>
<td>0.136</td>
<td>0.447</td>
<td>1.000</td>
<td>0.740</td>
<td>0.394</td>
<td>0.758</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.711</td>
<td>0.835</td>
<td>0.952</td>
<td>0.169</td>
<td>0.857</td>
<td>0.981</td>
<td>0.051</td>
<td>0.701</td>
<td>0.080</td>
<td>0.145</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.743</td>
<td>1.000</td>
<td>0.489</td>
<td>0.617</td>
<td>0.546</td>
<td>0.612</td>
<td>0.580</td>
<td>0.417</td>
<td>0.749</td>
<td>0.216</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.079</td>
<td>0.640</td>
<td>0.916</td>
<td>0.564</td>
<td>0.280</td>
<td>0.572</td>
<td>0.602</td>
<td>0.588</td>
<td>0.618</td>
<td>0.718</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.467</td>
<td>0.522</td>
<td>0.101</td>
<td>0.134</td>
<td>0.824</td>
<td>0.013</td>
<td>0.018</td>
<td>0.016</td>
<td>0.026</td>
<td>0.670</td>
</tr>
<tr>
<td>ECOG</td>
<td>0.332</td>
<td>0.670</td>
<td>0.166</td>
<td>0.082</td>
<td>1.000</td>
<td>0.339</td>
<td>0.588</td>
<td>0.191</td>
<td>0.585</td>
<td>0.341</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.096</td>
<td>0.458</td>
<td>0.723</td>
<td>0.140</td>
<td>1.000</td>
<td>0.674</td>
<td>0.341</td>
<td>0.674</td>
<td>0.138</td>
<td>0.371</td>
</tr>
</tbody>
</table>

The Pain Card Q1 and Q2 scores showed gradual increases with Q3 remaining stable (see Figure 99). The Pain Card Q4 showed sharp decreases after the third measurement time-point which were statistically significant (see Table 41 and Figure 99).
The placebo group showed improvements in the functional scales, with the exception of Healthcare satisfaction which showed no change compared to the base-line values. Significant changes in the physical symptoms were demonstrated by increases in Altered Bowel Habit. As measured by the Pain Card, the perception of pain was increased and the relief from pain was decreased in the placebo group.

In the EPA group, the results showed an overall improvement in QL. Here, Loss of appetite, Dyspnoea, and Fatigue was reduced and Role Functioning and Sexuality improved. Both Pain and Pancreatic Pain were decreased. However, as measured by Pain Card, pain was increased while the mood was decreased.

### 14.4.3 Changes Between Groups At Specific Time-Points

**Compared To Base-Line**

In order to assess the changes in QL parameters as compared to their pre-trial status, for each group the pre-chemotherapy measurements were taken as the base-line values. The same procedure for data and statistical analysis as described in section 14.3.3 was followed. The results were summarised and tabulated in Table 42.

The base-line-corrected results show that only two QL categories show statistically significant differences between the two groups. At the second measurement time-point, the Altered Bowel Habit demonstrated a statistically significant difference (p=0.037) where the placebo group show an increase and the EPA group show a decrease in scores. This could, therefore, suggest that the EPA may have some beneficial effect. The Dyspnoea also showed a statistically significant difference (p=0.018) where, again, the placebo group show an increase and the EPA group show a decrease in scores. These changes are also demonstrated in Figure 100 and Figure 101.
Table 42. Summary of the comparison of body composition parameters at different time-points with the base-line (pre-chemotherapy) values between the EPA and Placebo groups. Results (p-Values) sorted by parameter.

<table>
<thead>
<tr>
<th>QL Categories</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered Bowel Habit</td>
<td>0.037</td>
<td>0.174</td>
<td>0.805</td>
<td>0.911</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.810</td>
<td>0.307</td>
<td>0.408</td>
<td>0.416</td>
</tr>
<tr>
<td>Body Image</td>
<td>0.119</td>
<td>0.530</td>
<td>0.706</td>
<td>0.484</td>
</tr>
<tr>
<td>Cognitive Functioning</td>
<td>0.922</td>
<td>0.250</td>
<td>0.482</td>
<td>0.119</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.385</td>
<td>0.115</td>
<td>0.534</td>
<td>0.524</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.463</td>
<td>0.509</td>
<td>0.337</td>
<td>0.342</td>
</tr>
<tr>
<td>Digestive System</td>
<td>0.669</td>
<td>0.104</td>
<td>0.456</td>
<td>0.700</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>0.136</td>
<td>0.018</td>
<td>0.151</td>
<td>0.384</td>
</tr>
<tr>
<td>Emotional Functioning</td>
<td>0.538</td>
<td>0.223</td>
<td>0.515</td>
<td>0.071</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.850</td>
<td>0.128</td>
<td>0.442</td>
<td>0.541</td>
</tr>
<tr>
<td>Financial</td>
<td>0.201</td>
<td>0.900</td>
<td>0.955</td>
<td>0.669</td>
</tr>
<tr>
<td>Global Health Status</td>
<td>0.959</td>
<td>0.103</td>
<td>0.884</td>
<td>0.606</td>
</tr>
<tr>
<td>Healthcare</td>
<td>0.816</td>
<td>0.151</td>
<td>0.529</td>
<td>0.633</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0.527</td>
<td>0.530</td>
<td>0.880</td>
<td>0.150</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0.960</td>
<td>0.066</td>
<td>0.097</td>
<td>0.631</td>
</tr>
<tr>
<td>Nausea &amp; Vomiting</td>
<td>0.969</td>
<td>0.193</td>
<td>0.290</td>
<td>0.297</td>
</tr>
<tr>
<td>Pain</td>
<td>0.285</td>
<td>0.363</td>
<td>0.538</td>
<td>0.861</td>
</tr>
<tr>
<td>Pancreatic Pain</td>
<td>0.322</td>
<td>0.389</td>
<td>0.369</td>
<td>0.666</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>0.927</td>
<td>0.550</td>
<td>0.858</td>
<td>0.367</td>
</tr>
<tr>
<td>Role Functioning</td>
<td>0.586</td>
<td>0.840</td>
<td>0.856</td>
<td>0.464</td>
</tr>
<tr>
<td>Sexuality</td>
<td>0.129</td>
<td>0.319</td>
<td>0.772</td>
<td>0.931</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>0.967</td>
<td>0.982</td>
<td>0.840</td>
<td>0.402</td>
</tr>
<tr>
<td>Pain Card Q1</td>
<td>0.890</td>
<td>0.444</td>
<td>0.371</td>
<td>0.476</td>
</tr>
<tr>
<td>Pain Card Q2</td>
<td>0.730</td>
<td>0.138</td>
<td>0.660</td>
<td>0.171</td>
</tr>
<tr>
<td>Pain Card Q3</td>
<td>0.088</td>
<td>0.391</td>
<td>0.125</td>
<td>0.512</td>
</tr>
<tr>
<td>Pain Card Q4</td>
<td>0.955</td>
<td>0.186</td>
<td>0.097</td>
<td>0.286</td>
</tr>
<tr>
<td>ECOG</td>
<td>1.000</td>
<td>0.876</td>
<td>0.862</td>
<td>0.972</td>
</tr>
<tr>
<td>Karnofsky</td>
<td>0.687</td>
<td>0.847</td>
<td>0.520</td>
<td>0.217</td>
</tr>
</tbody>
</table>
Figure 100. Changes in Altered Bowel Habit scores with time in pancreatic cancer patients receiving EPA or Placebo.

Figure 101. Changes in Dyspnoea scores with time in pancreatic cancer patients receiving EPA or Placebo.
Results of the base-line-corrected comparison between the two groups, therefore, demonstrate that the patients receiving EPA have lower Dyspnoea and Altered Bowel Habit scores pointing to a possible beneficial effect of EPA.

### 14.4.4 Summary And Conclusions: EPA Versus Placebo

The 37 pancreatic cancer patients undergoing cancer chemotherapy were separated into the EPA and placebo groups to enable a QL analysis and comparison of treatment effects in these patients.

Comparing individual measurement time-points between the groups suggested that the EPA group are more satisfied with the amount/degree of healthcare that they are receiving, have lower hepatic symptoms, a lower perception of pain, and get more relief from pain than the placebo group. However, the EPA group showed a lower EORTC Sexuality scores and declining cognitive functions.

Comparing individual measurement time-points within each group separately demonstrated both positive and negative QL effects in the placebo group. The placebo group showed improvements in the functional scales, with the exception of Healthcare satisfaction which showed no change compared to the base-line values. Significant undesirable changes in the physical symptoms were demonstrated by increases in Altered Bowel Habit. As measured by the Pain Card, the perception of pain was increased and the relief from pain was decreased in the placebo group. The EPA group, however, showed positive results. In the EPA group, the results showed an overall improvement in QL. Here, Loss of Appetite, Dyspnoea, and Fatigue was reduced and Role Functioning and Sexuality improved. Both Pain and Pancreatic Pain were decreased. However, as measured by Pain Card, pain was increased while the mood was decreased.
Chapter 14: EPA CLINICAL TRIAL PATIENT QUALITY OF LIFE RESULTS

The base-line-adjusted comparisons of individual measurement time-points between the two groups demonstrated that the patients receiving EPA have lower Dyspnoea and Altered Bowel Habit scores pointing to a possible beneficial effect of EPA.

The overall results obtained from the comparison of the QL effects of EPA and placebo shows a beneficial effect of EPA administration on QL in this group of pancreatic cancer patients undergoing cancer chemotherapy.
15. EPA CLINICAL TRIAL SURVIVAL ANALYSIS RESULTS

All survival analyses carried out on the EPA clinical trial ("Fish Oil Trial") patient data were performed using SPSS® Version 14 (SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois, USA).

15.1 Background

The methodology and use of the Kaplan-Meier \cite{852} test and the Cox’s Regression \cite{853} analysis including the Hazards Ratio \cite{853} have been detailed in section 10.1.

In our study, the Kaplan-Meier survival analysis test using the Log Rank test for significance was used for the comparison of survival data between the EPA and placebo groups.

In our study, for the purposes of survival analysis, the “time to end point” was considered to be either a confirmed death or the last time patient visited clinic up to 01/08/2008. The body composition data used for the survival analysis was the patient’s first (i.e. the pre-chemotherapy) or Cycle 1 measurements.

15.2 Kaplan-Meier Analysis: Methodology And Results

The initial step was to perform a Kaplan-Meier test on the body composition parameters measured in the study. This provided a graphical demonstration of the effects of various parameters on the survival of the pancreatic cancer patients. The following are the survival curves of these parameters measured in the patient population. In all cases the patient groups
compared were the patients who received EPA or placebo as a part of their double-blind, randomised, placebo-controlled clinical trial.

As the Kaplan-Meier analysis can only be performed on categorical variables and data, the effect of treatment (i.e. EPA or placebo), NI cut-off values, Sex (male or female), and extent of disease (metastatic or locally advanced) were investigated. The NI cut-off values used were: 0.85, 0.90, 0.95, 1.00, and 1.05 as well as the fact that whether they were with the NI’s “normal range” or not.

15.2.1 Effects of Treatment

The initial Kaplan-Meier survival analysis was carried out to investigate the effect of treatment on survival.

The actual treatments that the patients received were either capsules filled with EPA or capsules filled with vegetable oil (Soya oil) administered orally. These capsules were administered in addition to their standard gemcitabine cytotoxic chemotherapy.
Figure 32 shows that the difference in survival between the EPA and placebo groups did not reach significance at the 0.05 level (Log Rank (Mantel-Cox) p=0.200).

### 15.2.2 Effects of Nitrogen Index

NI is considered to be one of the important body composition parameters affecting the well-being as well as survival of patients. It is defined as TBN expressed as a percentage of age, sex, and height-matched normal. In addition, the actual TBN content, demonstrated by changes in the NI value, has been considered to be an indicator of malnutrition and hence survival in malnourished patients. Different NI “cut-off” values have been previously demonstrated to predict therapy-induced complications. These are generally around 0.85. For this reason, the following NI cut-off values were investigated: 0.85, 0.90, 0.95, 1.00, and 1.05. Also the fact that the patient’s NI was within the normal range or not
were analysed using this test. At our centre, an NI of between 0.95 (or 95%) and 1.05 (or 105%) is considered to be “normal” and, generally patients with an NI less than 0.90 (or 90%) are considered to be “malnourished”.

None of the NI cut-off values that were tested were found to be statistically significant. In addition, the number of patients with an NI below the normal range was relatively low. The results for the NI values are tabulated in Table 11. Figure 33 and Figure 34 show the Kaplan-Meier analysis curves for NI with a cut-off of 0.90 and 1.00, respectively.

<table>
<thead>
<tr>
<th>NI Cut-off Values</th>
<th>Log Rank p-Value</th>
<th>No. of Patients With NI&lt; Cut-off</th>
<th>No. of Patients With NI&gt; Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85</td>
<td>0.359</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>0.90</td>
<td>0.073</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>0.95</td>
<td>0.376</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>1.00</td>
<td>0.719</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>1.05</td>
<td>0.36</td>
<td>22</td>
<td>15</td>
</tr>
</tbody>
</table>

An NI of around 0.85 is the cut-off where, in previous studies at our centre, it was demonstrated that the patients would be a greater risk of therapy-induced complications. However, statistically this was not found to be significant.
Figure 103. Survival curve for the effect of NI cut-off values of 0.90 on survival.

Figure 104. Survival curve for the effect of NI cut-off values of 1.00 on survival.
An NI of 1.00 is considered to be an ideal level of TBN. A patient with an NI of 1.00 has a 100% of TBN of an age, sex, and height-matched normal. From the total of 37 patients in the group, 15 (40.5%) had an NI of less than 1.00. The result of the Kaplan-Meier analysis of setting the NI cut-off values to 1.00 is demonstrated in Figure 34. The NI did not influence survival at neither of these cut-off values.

The influence of whether the patient’s NI is within the normal range affects their survival was also investigated. Results demonstrated that there were a total of 31 patients outside the normal range (above or below) and six within the normal range. The influence, however, was not statistically significant (Log Rank (Mantel-Cox) p=0.318).

15.2.3 Effect of Gender

As some therapeutic agents have been known to affect males and females differently, the effect of gender on the survival in this group of pancreatic cancer patients was also investigated. Figure 35 demonstrate the result of the Kaplan-Meier analysis (Log Rank (Mantel-Cox) p=0.285).
15.2.4 Effect of Disease Extent

One of the important factors that affect the survival of pancreatic cancer patients is how advanced the disease has progressed. The survival rates of pancreatic cancer patients in relation to the extent of their diseases have been detailed in the section on pancreatic cancer (Chapter 3.2).

Our results indicate that the extent of the disease (metastatic or locally advanced) is a statistically significant Log Rank (Mantel-Cox) p=0.017) factor and does influence the survival in this group of patients. The Figure 106 shows the Kaplan-Meier plot for the extent of the disease.
Figure 106. Survival curve for the effect of the extent of disease on survival.
15.3 Cox’s Regression Analysis: Methodology And Results

Cox’s Regression Analysis was carried out to investigate the effects of different body composition and parameters on the survival of the pancreatic cancer patients receiving either EPA or placebo. These analyses were carried out using all body composition parameters generated for this group of patients.

Initially a univariate Cox’s Regression was carried out to identify potential parameters affecting survival. To do this, the statistical analysis package’s (SPSS®) parameters were set-up with the “Time” to be the Time to End Point, the “Status” to be the Survival Status (=0) (Dead=0, Alive=1), and for the “Covariates”, each time one of the body composition parameters (shown below) were selected and analysed. The results of this regression analysis are shown in Table 44 below.
### Table 44. Cox’s Regression univariate analysis (sorted by p-values).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Values</th>
<th>HR</th>
<th>95% CI For HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Extent</td>
<td>0.022</td>
<td>3.148</td>
<td>1.181 - 8.385</td>
</tr>
<tr>
<td>Age</td>
<td>0.030</td>
<td>1.079</td>
<td>1.007 - 1.156</td>
</tr>
<tr>
<td>NI&lt;90</td>
<td>0.081</td>
<td>0.438</td>
<td>0.173 - 1.107</td>
</tr>
<tr>
<td>%BFat</td>
<td>0.119</td>
<td>0.939</td>
<td>0.868 - 1.016</td>
</tr>
<tr>
<td>Study Arm</td>
<td>0.207</td>
<td>0.546</td>
<td>0.214 - 1.397</td>
</tr>
<tr>
<td>Sex</td>
<td>0.290</td>
<td>0.606</td>
<td>0.239 - 1.533</td>
</tr>
<tr>
<td>LBM</td>
<td>0.323</td>
<td>1.021</td>
<td>0.979 - 1.065</td>
</tr>
<tr>
<td>NI Normal Range</td>
<td>0.329</td>
<td>2.096</td>
<td>0.475 - 9.256</td>
</tr>
<tr>
<td>TBW (Fredrix)</td>
<td>0.356</td>
<td>1.034</td>
<td>0.963 - 1.109</td>
</tr>
<tr>
<td>TBW (Pullicino)</td>
<td>0.356</td>
<td>1.029</td>
<td>0.969 - 1.093</td>
</tr>
<tr>
<td>NI&lt;85</td>
<td>0.364</td>
<td>0.645</td>
<td>0.251 - 1.661</td>
</tr>
<tr>
<td>NI&lt;95</td>
<td>0.380</td>
<td>0.658</td>
<td>0.259 - 1.674</td>
</tr>
<tr>
<td>TBW (Kushner)</td>
<td>0.384</td>
<td>1.027</td>
<td>0.967 - 1.090</td>
</tr>
<tr>
<td>LBM (Lukaski)</td>
<td>0.404</td>
<td>1.017</td>
<td>0.977 - 1.060</td>
</tr>
<tr>
<td>TBK/Height</td>
<td>0.476</td>
<td>2.876</td>
<td>0.157 - 52.703</td>
</tr>
<tr>
<td>%BFat (By TBK)</td>
<td>0.483</td>
<td>0.986</td>
<td>0.946 - 1.027</td>
</tr>
<tr>
<td>CRP</td>
<td>0.494</td>
<td>1.004</td>
<td>0.992 - 1.017</td>
</tr>
<tr>
<td>TBK</td>
<td>0.524</td>
<td>1.005</td>
<td>0.990 - 1.020</td>
</tr>
<tr>
<td>LBM (By TBK)</td>
<td>0.524</td>
<td>1.011</td>
<td>0.978 - 1.045</td>
</tr>
<tr>
<td>Weight</td>
<td>0.554</td>
<td>1.009</td>
<td>0.979 - 1.040</td>
</tr>
<tr>
<td>LBM (Segal)</td>
<td>0.605</td>
<td>1.009</td>
<td>0.975 - 1.045</td>
</tr>
<tr>
<td>LBM (Van Loan)</td>
<td>0.617</td>
<td>1.011</td>
<td>0.968 - 1.056</td>
</tr>
<tr>
<td>NI</td>
<td>0.637</td>
<td>0.699</td>
<td>0.158 - 3.087</td>
</tr>
<tr>
<td>NI&lt;100</td>
<td>0.720</td>
<td>0.841</td>
<td>0.325 - 2.172</td>
</tr>
<tr>
<td>NI&lt;105</td>
<td>0.720</td>
<td>1.190</td>
<td>0.460 - 3.074</td>
</tr>
<tr>
<td>FM</td>
<td>0.748</td>
<td>0.987</td>
<td>0.914 - 1.067</td>
</tr>
<tr>
<td>BMI</td>
<td>0.753</td>
<td>1.018</td>
<td>0.912 - 1.135</td>
</tr>
<tr>
<td>CEA</td>
<td>0.782</td>
<td>1.000</td>
<td>0.999 - 1.001</td>
</tr>
<tr>
<td>CA19.9</td>
<td>0.841</td>
<td>1.000</td>
<td>1.000 - 1.000</td>
</tr>
<tr>
<td>FM (By TBK)</td>
<td>0.925</td>
<td>1.003</td>
<td>0.945 - 1.065</td>
</tr>
<tr>
<td>TBN</td>
<td>0.934</td>
<td>1.000</td>
<td>0.999 - 1.001</td>
</tr>
</tbody>
</table>

In the univariate Cox’s Regression analysis, all body composition parameters measured in the study as well as the CEA, CRP, and CA19.9 were entered into SPSS® individually and Cox’s Regression ("Enter" mode) was performed. The combined results of these univariate regressions are shown in Table 44 above.
Once the univariate Cox’s Regression Analysis was completed, the parameters that had significance level of ≤0.25 were selected for the multivariate analysis part. As shown in Table 12, the body composition parameters which have a significance level of ≤0.25 are: the Disease Extent (p=0.0.022), Age (p=0.030), NI<90 (p=0.081), %BFat (p=0.119), and Study Arm (p=0.207). In addition, parameters such as TBW content, Weight, Study Arm, NI<90, Sex, CEA, CRP, and CA19.9 which by clinical experience are known to have the ability to affect survival (i.e. the *a priori* parameters), were also “forced” into the multivariate analysis.

A “Backward Stepwise Likelihood Ratio” Multivariate Cox’s Regression was then performed on the above ten parameters. Table 13 below shows the relevant results of the regression.

It should be noted that since TBN is dependent and correlated with NI, it was excluded from further Cox’s Regression analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Values</th>
<th>HR</th>
<th>95% CI For HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Disease Extent</td>
<td>0.002</td>
<td>6.361</td>
<td>1.938</td>
</tr>
<tr>
<td>%BFat</td>
<td>0.010</td>
<td>0.886</td>
<td>0.808</td>
</tr>
<tr>
<td>Age</td>
<td>0.089</td>
<td>1.070</td>
<td>0.990</td>
</tr>
</tbody>
</table>

These three parameters were then taken as the “base parameters” and Multivariate Cox’s Regression were then performed on the remaining (TBW (By Kushner), Weight, Study Arm, NI<90, Sex, CEA, CRP, and Ca 19.9) parameters. This was done by keeping the “base parameters” constant and adding one of the eight parameters at a time, performing the regression, and then replacing the parameter with another one of the eight parameters. This, in effect, enabled exploration of the influence of these parameters using a multivariate analysis while adjusting for Margin Status, Fat Mass, and Age. The result of this multivariate analysis is shown in Table 14.
Table 46. Multivariate Cox’s Regression adjusted for Disease Extent, %BFat, and Age.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p-Values</th>
<th>HR</th>
<th>95% CI For HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>Weight</td>
<td>0.253</td>
<td>1.022</td>
<td>0.985</td>
</tr>
<tr>
<td>Sex</td>
<td>0.253</td>
<td>2.256</td>
<td>0.560</td>
</tr>
<tr>
<td>Study Arm</td>
<td>0.361</td>
<td>0.602</td>
<td>0.202</td>
</tr>
<tr>
<td>TBW</td>
<td>0.428</td>
<td>1.031</td>
<td>0.956</td>
</tr>
<tr>
<td>CEA</td>
<td>0.577</td>
<td>1.000</td>
<td>0.999</td>
</tr>
<tr>
<td>CRP</td>
<td>0.620</td>
<td>1.004</td>
<td>0.988</td>
</tr>
<tr>
<td>NI&lt;90</td>
<td>0.677</td>
<td>0.757</td>
<td>0.204</td>
</tr>
<tr>
<td>Ca 19.9</td>
<td>0.967</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
15.4 Summary And Conclusions

Results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that from the three parameters tested, the Disease Extent was the best parameter that could (p=0.017) determine survival in the pancreatic cancer patients.

The results of the univariate Cox’s Regression analysis performed on all individual parameters showed that the Disease Extent (p=0.022) and Age (p=0.030) were the parameters that could significantly influence survival in this group of patients. Once the parameters with significance levels of ≤0.25 were selected and multivariate Cox’s Regression performed, the Disease Extent, Age, and %BFat were found to be statistically significant. Although the a priori parameters were then “forced” into the analysis and adjusted for Disease Extent, Age, and %BFat, none reached statistical significance.

Results of the survival analysis also demonstrate that the use of EPA in this group of pancreatic cancer patients did not provide any benefit. In fact, as shown in Figure 102 the group of patients receiving the EPA had a “worse” survival than the group receiving the placebo.

In conclusion, the use of EPA in patients with pancreatic cancer undergoing gemcitabine chemotherapy is not recommended. The extent of disease progression and age of the patient at the onset of chemotherapy are the factors that influence the survival. In terms of body composition, the percentage of body fat, obtained by the skinfold technique, influences these patients’ survival. Therefore, it is more the biological behaviour of the tumour rather than the body composition of the patient that determines the outcome.
16. DISCUSSION AND CONCLUSIONS

16.1 Discussion

This project investigated the changes in body composition of three patient groups receiving different therapies for their cancers. This project also investigated the effect(s) of the treatments used on the survival and investigated the body composition parameters involved which can influence survival in these patients. Two of the three patient groups studied had pancreatic cancer and one group had malignant mesothelioma. The particular methods used and/or developed in these studies and their respective results have been reported in detail in their respective Methods section. In addition, the results of the analyses for each patient group has been summarised at the end of the relevant chapters.

The four compartmental model was used to investigate the changes in body composition during the measurement time-points in all patients. Three out of the four compartments were measured while the fourth, the mineral, was assumed not to be changing during the duration of each trial. Levels of the protein compartment was measured using the IVNCA technique, the fat compartment using the skinfolds, TBK, and BIA techniques, and the water compartment using BIA techniques. As we were investigating the potential benefits of an anti-cachectic agent, EPA, its influence on the patients’ QL and pain scores were also investigated using the well established EORTC questionnaires as well as Pain Card.

The primary aim of this project was to assess the outcome of the therapies and to investigate their effect(s) and influence(s) on cachexia, which is often present in these patients and affect survival as well as response to therapy. The characteristics, hallmarks, aetiology, symptoms,
Chapter 16: DISCUSSION AND CONCLUSIONS

prognosis, and possible treatment procedures have already been detailed elsewhere in this project.

As such, in setting out to investigate the effects of the therapies we expected the classical characteristics and hallmarks of cachexia, albeit to different degrees, to be present at the start of the trials or to surface at different time-points during the course of the measurements and therapies. The findings for each patient group is, therefore discussed.

The first group of patients that was tested were pancreatic cancer patients who were undergoing Whipple’s Procedure to resect their pancreatic tumour. The treatment given to these patients was, therefore, surgery. The extent by which their tumour was resected has been reported\cite{340} to influence the survival of these patients and has also been discussed in detail in the section on “Pancreas And Pancreatic Cancer” (Chapter 3). Recent study of Moon et al\cite{345} reported that the adequacy of resection (R0) with a rate of 73% was the most significant factor for predicting long-term survival. Other studies investigating the effects of resection in tumours of the head of pancreas reported that negative resection margin or a complete resection status were the important predictors of outcome\cite{351, 348, 349, 347, 350, 352, 346}.

For example, the study of Benassai et al\cite{348} reported a five year survival rate of almost 53% in their most favourable subset of patients. Similarly, a large population study reported that following a multivariate analysis of the results of their peri-ampullary adenocarcinoma patients who had Whipple’s Procedure, the most powerful independent predictors favouring long-term survival included a pathologic diagnosis of duodenal adenocarcinoma, tumour diameter less than three centimetres, negative resection margins, absence of lymph node metastases, well-differentiated histology, and no re-operation\cite{350}.

In general, the reason why some patients’ surgery results in Clear Margins whereas others result in Unclear Margins is multifactorial. The most common reasons include the size,
location, grade, and the degree of advancement at the time of surgery. As such, a Clear Margin was expected to result in the maintenance of body composition and increase survival. Therefore, the initial measurements were carried out pre-operatively, and then followed-up at two, five, 14 and 26 weeks post-operatively. In addition, the patients were followed-up for up to 72 months to assess survival.

Surprisingly, there is very little information or reports in the literature with regards to changes in body composition following a Whipple’s Procedure and differences between resection margins. In fact, this has been one of the aims of this project. One of the few that is available is a report by Gupta et al [859] who, as a part of a report on the nutritional effects of upper gastrointestinal cancer, describe their “in press” results of the short-term (seven days) body composition changes post-Whipple’s Procedure. Their report suggests that there is a small, but statistically significant, decrease in TBF (by DeXA), small and statistically non-significant increase in weight, and decrease in serum proteins. These authors [859] associated the loss of weight to the depletion of body fat. Also, the study of McLeod et al [860] concluded that, compared to controls, their Whipple’s Procedure patient population showed post-operative increases in weight with no significant differences in LBM. In term of the long-term changes, to our knowledge there is only one report from the 1990s [861] that comes close to investigating these changes, but still do not consider margin differences. The study of Curran et al [861] reported that in their patient group all patients had below normal body fat and post-operatively, the body fat levels decreased with no change in protein or weight.

In our project when the Clear Margin and Unclear Margin groups were compared by statistically analysing the differences between different time-points within each group, the results showed that although the Unclear Margin group had lower body composition values than the Clear Margin group, both groups seemed to begin to gradually “equalise” around the 14 weeks post-operative time-point. This suggested that regardless of whether a curative or a
palliative surgery is performed on patients with pancreatic cancer, at least by around 14 weeks, their body composition statuses could become relatively similar. These changes were predominantly in weight, the body fat, and water compartments. The trend in body composition changes also showed that, although both groups may start with non-significantly different body composition, they tended to grow closer around the 14 week time-point indicating that the Clear Margin group may lose more than Unclear Margin group. The implications of these findings, therefore, were that once the most appropriate surgical procedure was performed, an adjuvant therapy regimen (such as chemotherapy) at around 14 weeks may have the most impact on the patient’s overall treatment outcome.

However, the fact that the Unclear Margin group consistently had lower body composition parameters indicated that this group of patients, although technically not malnourished, start major surgery with lower “stores”. In addition, the fact that none of the body composition parameters’ slope values were significantly different between the two groups indicated that the rate of change of the measured body composition parameters were similar for both the Clear as well as the Unclear resection margin groups.

The loss of weight and body fat measured in these pancreatic cancer patients was consistent with the classical hallmark of cancer cachexia where there is a loss of weight, LBM, and FM and the finding of Gupta et al [859]. In fact, there is a direct link between the loss of weight and an increase in the rate of morbidity and mortality in diseases demonstrating symptoms of cachexia. Studies of Windsor et al [14] have shown that with patients in these states, death usually occurs when the total body weight loss reaches 30% and that this appears to be as a result of respiratory failure through the erosion of the diaphragm causing hypostatic pneumonia.

The survival in the Whipple’s Procedure patients was also investigated. Results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that from the three categorical parameters tested, the Margin Status was the best parameter that could
determine survival in the Whipple’s Procedure surgical patients. The multivariate Cox’s Regression demonstrated the Margin Status, Fat Mass, and Age to be statistically significant in influencing survival.

As a follow-up or sequel to the Whipple’s Procedure resection margin trial, a second study was carried out on another cohort of patients with pancreatic cancer. This group of pancreatic cancer patients were due to receive the standard cytotoxic chemotherapy with gemcitabine. These patients were, therefore, randomised into receiving an anti-cachectic agent, EPA, or placebo. The anti-cachectic properties of EPA have been detailed in the section on “EPA And Omega 3 Fatty Acid” (Chapter 5). As an additional investigation, the extent of the disease, i.e. metastatic or locally advanced, was also considered. It was our hypothesis that the EPA group would demonstrate an improved body composition and survival, and the patients with metastatic disease would show a “worse” body composition status, QL, and survival. These were based on the fact that EPA is an anti-cachectic agent and, as such, would reduce or reverse cachexia and/or similar catabolic conditions. Similarly, we hypothesised that the patients with a more advanced disease would, as one would expect, be performing “worse” than those with less advanced disease.

In this group of pancreatic cancer patients, when the effects of disease extent was investigated there were no group differences at any measurement time-points for the body composition parameters. However, compared to the base-line values there were changes and differences between the two groups for the Weight, FM, and LBM (By Lukaski) suggesting that the metastatic group lost more FM and Weight than the locally advanced group. In addition, body composition changes between measurement time-points for the metastatic and locally advanced groups suggested that the patients with locally advanced disease maintain their Weight, FM, and TBN but were more likely to have a lower TBW by the end of the four
month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but were more likely to have a decreased fat compartment and a higher FFM. These findings were consistent with the findings that patients with more advanced disease are likely to have body composition-related symptoms of cachexia. In fact, one of the characteristics of cancer cachexia is the loss of body fat and an altered lipid metabolism with the main two cytokines implicated being the TNF-α and IL-1 \[37, 39, 141\]. Here, there is a large and significant loss of white adipose tissue. This is primarily due to a decrease in the lipoprotein lipase activity and an increase in the activity of the hormone-sensitive lipase \[37, 39\]. In addition, there is an inhibition of glucose and de novo lipogenesis in the tissues which has been shown to be due to the cytokines TNF-α and IL-1 \[37, 39\]. The loss of fat in patients with cancer cachexia is due mainly to the fatty acids and glycerol from the host’s adipose tissue being mobilised rather than a decrease in its production or synthesis \[143\]. This is most likely due to the release of LMF by the tumour to supply lipids to the tumour \[111\]. In addition, in patients with cancer cachexia there is an increased rate of oxidation of fatty acids. This, together with the reduced food and calorie intake, results in the depletion of fat stores which is often seen in patients with cancer cachexia.

When the same cohort of pancreatic cancer patients were investigated on the basis of whether they received EPA or placebo, a number interesting conclusions were reached. Firstly, due to the fact that the patients were well randomised, the two groups commenced the double-blind, placebo-controlled, randomised trial with similar and statistically non-significantly different body composition parameters. Secondly, the two groups were also found to be statistically not different at their corresponding measurement time-points. And thirdly, the patients receiving placebo compared to those receiving EPA lost more Weight, and FM but less TBW throughout the trial. The TBK/Ht (p=0.044), TBK (p=0.042), and LBM (By TBK) (p=0.042), however, showed statistically significant differences where in all three parameters the EPA
showed an increase compared to the base-line (pre-chemotherapy). As such, it was concluded that the use of EPA in this group of pancreatic cancer patients undergoing cancer chemotherapy with gemcitabine results in a non-significant reduction in weight loss, FM loss, and TBW gain with a statistically significant increase in FFM.

Therefore, in this part of the project we demonstrated that pancreatic cancer patients receiving the standard gemcitabine chemotherapy show cachexia-related symptoms. In addition, we demonstrated that the use of the anti-cachexia agent, EPA, can alleviate these symptoms.

Although the use of EPA showed positive effects in this group of pancreatic cancer patients, its effect on survival was contradictory. Results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that from the three parameters tested, the Disease Extent was the best parameter that could (p=0.017) determine survival in the pancreatic cancer patients. In addition univariate Cox’s Regression analysis performed on all individual parameters showed that the Disease Extent (p=0.022) and Age (p=0.030) were the parameters that could significantly influence survival in this group of patients. In fact, the results of the Kaplan-Meier plot demonstrated that the group of patients receiving the EPA had a “worse” survival than the group receiving the placebo resulting in the conclusion that the use of EPA in patients with pancreatic cancer undergoing gemcitabine chemotherapy may not be recommended.

As the aim of therapy in cancer patients is to improve the well-being of patients, the QL of these patients was also investigated. When the disease extent was investigated, the differences between the two group’s corresponding measurement time-points showed that the metastatic group are performing “worse” than the locally advanced group especially in term of their Dyspnoea, Nausea & Vomiting, and Sexuality. In addition, the Karnofsky score, which gives an “overall” performance score, showed that the metastatic group are not performing as well as the locally advanced group. Furthermore, for the metastatic group there was an increase in
Chapter 16: DISCUSSION AND CONCLUSIONS

the patients’ pain with a decline in mood and general performance as well as increase in gastrointestinal symptoms. Base-line-corrected comparisons demonstrated that the metastatic group have consistent, non-significant increase in Nausea & Vomiting scores. The locally advanced group showed a general increase and the metastatic group showed a general decrease in EORTC’s Sexuality scores. Pain scores from the Pain Card Q1 showed a general increase for the metastatic group and a general decrease for the locally advanced group. These findings were consistent with the expected metastatic or a progressing disease status.

When the effects of EPA on QL was investigated, EPA group showed more satisfaction with the amount/degree of healthcare that they were receiving, had lower hepatic symptoms, a lower perception of pain, and got more relief from pain than the placebo group. Base-line-corrected results showed that placebo group improved in their functional scales, but increased their Altered Bowel Habit scores with an increase in the perception of pain and decrease in relief from pain. The EPA group, however, showed a decrease in the Loss of Appetite, Dyspnoea, Pain, Pancreatic Pain, and Fatigue, and improvements in Role Functioning and Sexuality. As detailed in the sections on “Cachexia” (Chapter 2) and “Pancreas And Pancreatic Cancer” (Chapter 3), almost all the symptoms that got worse with placebo but improved with EPA administration are cachexia-induced and pancreatic cancer-induced symptoms. As a result, it was concluded that EPA does improve the QL of this group of pancreatic cancer patients.

Since EPA showed some promise, another anti-cachectic agent was also investigated. For this purpose, thalidomide, which has anti-cachectic as well as anti-neoplastic properties, was tested in patients with malignant mesothelioma. The properties of thalidomide are detailed in the section on “Thalidomide In Cancer Therapy” (Chapter 6). The mesothelioma patients were randomised into two groups. One group received thalidomide plus the standard
gemcitabine / cisplatin combination chemotherapy (Arm A) and one group received thalidomide alone (Arm B). Both groups showed no statistically significant differences between their weights throughout the four cycles of the trial. When the weight was separated into its components, both groups showed a steady decline in the TBN and FM components. The TBW component showed an increase for the Arm A and a steady decrease for the Arm B patient groups.

The base-line-corrected body composition measurements also showed a decrease in weight for both arms. However, towards the last measurement time-point, the Arm A showed an increase whereas the Arm B showed a decrease in weight. The base-line-corrected components of weight, the TBN and FM, also showed a decline. However, the pattern of TBW change, again, showed an increase in TBW for the Arm A and a decrease for the Arm B patient groups.

The overall results of the body composition analysis suggested that the group of patients receiving standard chemotherapy plus thalidomide show similar weight changes to the group receiving thalidomide alone. In addition, body composition measurements indicated that the standard chemotherapy plus thalidomide group have a greater TBN loss and a greater TBW gain than the thalidomide-alone group. This loss of TBN and gain in TBW looked to be “concealed” in the weight.

Provided that the overall anti-cancer potential of gemcitabine / cisplatin plus thalidomide is comparable with thalidomide alone, then by looking purely from the body composition angle one may be able to suggest the use of thalidomide alone in the treatment of malignant mesothelioma in this group of patients. In addition, the changes in body composition parameters seen here, such as the weight loss, loss of FM and protein are consistent with the reported characteristics and hallmarks of cachexia.
The survival of this group of patients with malignant mesothelioma was also investigated. The results of the Kaplan-Meier survival analysis test using the Log Rank test for significance showed that none of the categorical parameters tested reached statistical significance. The results of the multivariate Cox’s Regression showed the haemoglobin levels (p=0.001), Age (p=0.007), and NI (p=0.008) to be statistically significant. The fact that the Study Arm parameter did not reach statistical significance could indicate that survival in these patients is not affected by the presence or absence of chemotherapy with gemcitabine and cisplatin.

This loss of body protein seen in both our malignant mesothelioma and pancreatic cancer patients undergoing chemotherapy is consistent with the reports in the literature. It has been reported that probably the most destructive and detrimental aspect of cancer cachexia is the increased loss of body protein the degree of which is associated with poor survival [18]. It is, in fact, another cardinal feature of cancer cachexia which cannot be reversed by an increase in food and calorie intake or through parenteral nutritional intervention [107]. In cancer cachexia the net protein loss is caused by a combination of increased protein degradation and a decrease in protein synthesis [147, 148].

The clinical aspects and consequences of cachexia can simply be summarised as morbidity, debilitating conditions, and mortality. The conditions such as loss of muscle mass, impaired muscle function, fatigue, reduced activity and functional capacity by themselves are enough to severely and significantly affect the patients’ QL. As hypothesised, all three groups of patients with malignancies showed symptoms of cachexia which were detected using our body composition analysis techniques. The interventional methods and/or therapies used on these patients were similarly evaluated and all three procedures were found to be promising and, thus, warrants further investigations in larger cohorts of patients. Our body composition analysis techniques also uncovered the determinants of survival in these patients. These will
enable future therapies to be better designed, administered, and individualised for these patients.

### 16.2 Summary And Conclusions

#### Whipple’s Procedure Group

The results of the margin comparisons in the patients undergoing Whipple’s Procedure demonstrated that that post-operatively, with the exception of FM, there were no significant changes in the measured body composition parameters at the two and five weeks time-points. However, the main time-points where changes did occur were around the five and 14 weeks post-operative time-point. Compared to the base-line, there were highly significant changes in Weight, BMI, and FM followed by significant changes in %BFat, TBK/Ht and LBMs at the 14 week time-point. At the 26 weeks post-operative time point, the only significant changes were in the FM, %BFat and BMI parameters. These suggested that the only body compartment that was affected post-operatively may be the fat compartment. This would, therefore, suggest that in pancreatic cancer patients undergoing Whipple’s Procedure the major long term change in their body composition is in the fat compartment.

There was also a deviation between the two groups in their TBN, LBM and TBW content observable in a long-term setting and fat content in the relatively shorter-term. To further investigate this, a larger patient population and a 12 or 18 month follow-up is required, which may not be possible due to the nature of this disease.

Comparing the differences between the two groups, showed that although the Unclear Margin group had lower body composition values, both groups seem to begin to gradually “equalise” around the 14 weeks post-operative time-point. This may suggest that regardless of whether a curative or a palliative surgery is performed on patients with pancreatic cancer, at least by
around 14 weeks, their body composition statuses can become relatively similar. These changes were predominantly in Weight and the body fat and water compartments. The trend in body composition changes showed that, although both groups may start with non-significantly different body composition, they tended to grow closer around the 14 week point indicating that the Clear Margin group may lose more than Unclear Margin group. The implications of these findings, therefore, are that once the most appropriate surgical procedure is performed, an adjuvant therapy regimen (such as chemotherapy) at around 14 weeks may have the most impact on the patient’s overall treatment outcome.

The survival analysis results for the Whipple’s Procedure patients demonstrated that Margin Status, Fat Mass, and Age were statistically significant and can influence survival.

**Malignant Mesothelioma Group**

The main conclusion that was drawn was that the gemcitabine / cisplatin chemotherapy produced water retention in this group of patients with malignant mesothelioma. In addition, the longer the chemotherapy is continued, the more the patients were likely to be depleted in their body weight, fat and protein. The final outcome demonstrated that both study arms show similar weight changes. In addition, body composition measurements indicated that the gemcitabine / cisplatin chemotherapy plus thalidomide group had a greater TBN loss and a greater TBW gain than the thalidomide-alone group. This loss of TBN and gain in TBW looked to be “concealed” in the weight.

Provided that the overall anti-cancer potential of gemcitabine / cisplatin plus thalidomide is comparable with that of thalidomide-alone, then by looking purely from the body composition angle one may be able to suggest the use of thalidomide alone in the treatment of malignant mesothelioma in this group of patients.
The results of the survival analysis carried out on the mesothelioma patient group suggested that haemoglobin levels, Age, and NI are the parameters that can influence the survival of patients with malignant mesothelioma undergoing chemotherapy. The fact that the Study Arm parameter did not reach statistical significance could indicate that survival in these patients is not affected by the presence or absence of chemotherapy with gemcitabine and cisplatin. Whether the specific presence or absence of thalidomide influences survival in these patients requires a separate design and clinical trial to address this issue.

**Pancreatic Cancer Chemotherapy Group**

When the pancreatic cancer patients were separated according to the extent of their disease, i.e. into metastatic and locally advanced groups, results suggested that there were no statistically significant differences between the two groups. Results of the analyses of body composition changes between measurement time-points for the metastatic and locally advanced groups separately, suggested that the patients with locally advanced disease maintain their Weight, FM, and TBN but are more likely to have a lower TBW by the end of the four month of chemotherapy. However, the patients with metastatic pancreatic cancer maintain their TBW but are more likely to have a decreased fat compartment and a higher FFM.

When the pancreatic cancer patients were separated according to whether they received EPA or placebo, the results demonstrated that firstly, due to the fact that the patients were well randomised, the two groups commenced the trial with similar and statistically non-significantly different body composition parameters. Secondly, the two groups were also found to be statistically not different at their corresponding measurement time-points. And
thirdly, the patients receiving placebo compared to those receiving EPA lost more Weight, and FM but less TBW throughout the trial. The TBK/Ht (p=0.044), TBK (p=0.042), and LBM (By TBK) (p=0.042), however, showed statistically significant differences where in all three parameters the EPA showed an increase compared to the base-line (pre-chemotherapy). As such, it was concluded that the use of EPA in this group of pancreatic cancer patients undergoing cancer chemotherapy with gemcitabine results in a non-significant reduction in weight loss, FM loss, and TBW gain with a statistically significant increase in FFM.

Results of the survival analysis demonstrated that the use of EPA in this group of pancreatic cancer patients did not provide any benefit. In fact, as it was shown in the Kaplan-Meier plot, the group of patients receiving the EPA had a “worse” survival than the group receiving the placebo. Therefore, the use of EPA in patients with pancreatic cancer undergoing gemcitabine chemotherapy should be made with caution. The extent of disease progression and age of the patient at the onset of chemotherapy were the factors that influenced the survival. In terms of body composition, the percentage of body fat, obtained by the skinfold technique, influenced these patients’ survival.

The comparison of QL scores between the EPA and placebo groups demonstrated that the placebo group showed improvements in the functional scales. Significant undesirable changes in the physical symptoms were demonstrated by increases in Altered Bowel Habit. As measured by the Pain Card, the perception of pain was increased and the relief from pain was decreased in the placebo group. In the EPA group, the results showed an overall improvement in QL. Here, Loss of appetite, Dyspnoea, and Fatigue were reduced and Role Functioning and Sexuality improved. Both Pain and Pancreatic Pain were decreased. However, as measured by Pain Card, pain was increased while the mood was decreased. The base-line-corrected
comparisons of individual measurement time-points between the two groups demonstrated that the patients receiving EPA had lower Dyspnoea and Altered Bowel Habit scores pointing to a possible beneficial effect of EPA.

The overall results obtained from the comparison of the QL effects of EPA and placebo shows a beneficial effect of EPA administration on QL in this group of pancreatic cancer patients undergoing cancer chemotherapy.

However, when the pancreatic cancer patients are separated into metastatic and locally advanced groups, the QL results seen in the metastatic group, collectively may point to a worsening and/or progressing disease which is consistent with classic metastatic disease aetiology.

As seen from the results of the three patient groups, body composition techniques were used here as a tool to monitor changes in various body composition parameters to assess the outcomes, including survival, of the use of different therapies and interventional procedures in these three groups of cancer patients. For these purposes, they were demonstrated to be an effective and invaluable tool.

**16.3 Future Directions**

As seen from these and our previous trials, body composition analysis is a useful tool in the prediction and management of therapy-induced toxicities. Although body composition analysis facilities, such as IVNCA and Whole Body Counting, may not yet be widely available, other surrogate body composition analysis techniques may be used to indirectly measure those parameters. As such, we highly recommend the ongoing clinical assessment of patients who are or are suspected of malnutrition.
Chapter 16: DISCUSSION AND CONCLUSIONS

The post-Whipple’s procedure clinical trial demonstrated the changes in body composition and survival of these pancreatic cancer patients. We anticipate similar long term changes in patients with malignancies undergoing other types of major surgery where malnutrition and/or cachexia are present and affect survival. As such, further research into these procedures using a larger cohort is required. In fact, as mentioned earlier there are virtually no reports in the literature looking at long term changes in body composition and/or survival of patients undergoing major surgery.

Both the mesothelioma and EPA clinical trials investigated the use of anti-cachectic agents in patients who were receiving standard cancer chemotherapy. In these settings, body composition analysis was demonstrated to have the ability to detect these changes. However, for this thesis due to the shortage of funding and hence the small patient groups, the strength of the statistical analyses were not sufficient to demonstrate certain aspects of survival. Armed with the current information, these trials, especially the EPA trial, needs to be repeated with a larger cohort of patients. There is also some evidence in the literature that DHA might have effects similar to EPA. This also needs to be investigated. In addition, this clinical trial should also be carried out on other groups of cancer patients who are at risk of malnutrition and/or cachexia to assess the degree of malnutrition and/or cachexia as well as survival.

The mesothelioma trial was originally designed to evaluate the use of thalidomide as an agent with both chemotherapeutic as well as anti-cachectic properties in patients with malignant mesothelioma. As indicated in section 12.4 whether the presence or absence of thalidomide influences survival in these patients requires a separate design and clinical trial to address this issue.

Although additional investigations were considered for these clinical trials, they were not implemented due to the lack of funding. As some aspects of cachexia also involves altered food intake, with the aid of a professional dietician, future research should include the change
in food intake during the course of treatment and correlated with changes in body composition, QL, and survival.
REFERENCES


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References


REFERENCES


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APPENDIX A: Quality Of Life Questionnaire

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: [ ]
Your birthdate (Day, Month, Year): [ ]
Today's date (Day, Month, Year): 31 [ ]

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at All</th>
<th>A Little</th>
<th>Quite a Bit</th>
<th>Very Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. Do you have any trouble taking a long walk?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. Do you have any trouble taking a short walk outside of the house?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. Do you need to stay in bed or a chair during the day?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. Do you need help with eating, dressing, washing yourself or using the toilet?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

During the past week:

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at All</th>
<th>A Little</th>
<th>Quite a Bit</th>
<th>Very Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Were you limited in doing either your work or other daily activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. Were you limited in pursuing your hobbies or other leisure time activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. Were you short of breath?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. Have you had pain?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. Did you need to rest?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11. Have you had trouble sleeping?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12. Have you felt weak?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13. Have you lacked appetite?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14. Have you felt nauseated?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15. Have you vomited?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
**APPENDIX A: Quality Of Life Questionnaire**

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at All</th>
<th>A Little</th>
<th>Quite a Bit</th>
<th>Very Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>16. Have you been constipated?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Have you had diarrhea?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Were you tired?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Did pain interfere with your daily activities?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. Did you feel tense?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. Did you worry?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. Did you feel irritable?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Did you feel depressed?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. Have you had difficulty remembering things?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. Has your physical condition or medical treatment interfered with your family life?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. Has your physical condition or medical treatment interfered with your social activities?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. Has your physical condition or medical treatment caused you financial difficulties?</td>
<td>1 2 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**For the following questions please circle the number between 1 and 7 that best applies to you**

29. How would you rate your overall health during the past week?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor</td>
<td>Excellent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

30. How would you rate your overall quality of life during the past week?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor</td>
<td>Excellent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX A: Quality Of Life Questionnaire**

**EORTC QLQ - PAN26**

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

<table>
<thead>
<tr>
<th>During the past week:</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>31. Have you had abdominal discomfort?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>32. Did you have a bloated feeling in your abdomen?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>33. Have you had back pain?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>34. Did you have pain during the night?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>35. Did you find it uncomfortable in certain positions (e.g. lying down)?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>36. Were you restricted in the types of food you can eat as a result of your disease or treatment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>37. Were you restricted in the amounts of food you could eat as a result of your disease or treatment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>38. Did food and drink taste different from usual?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>39. Have you had indigestion?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>40. Were you bothered by gas (flatulence)?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>41. Have you worried about your weight being too low?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>42. Did you feel weak in your arms and legs?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>43. Did you have a dry mouth?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>44. Have you had itching?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>45. To what extent was your skin yellow?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>46. Did you have frequent bowel movements?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>47. Did you feel the urge to move your bowels quickly?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>48. Have you felt physically less attractive as a result of your disease and treatment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Please go to the next page*
## APPENDIX A: Quality Of Life Questionnaire

**During the past week:**

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>49. Have you been dissatisfied with your body?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>50. To what extent have you been troubled with side-effects from your treatment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>51. Were you worried about your health in the future?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>52. Were you limited in planning activities, for example meeting friends, in advance?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>53. Have you received adequate support from your health care professionals?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>54. Has the information given about your physical condition and treatment been adequate?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>55. Have you felt less interest in sex?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>56. Have you felt less sexual enjoyment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
APPENDIX B: Pain Card

2

Mild

Moderate

Just Noticeable

No Pain

Severe

Strong

Excruciating

Weak

RELIEF

SCALE

NO relief of pain

COMPLETE relief of pain
APPENDIX B: Pain Card
APPENDIX C: Publications And Presentations

The following is the list of publications made during the course of this project:

**Publications**

**Peer-Reviewed Journal Articles:**


APPENDIX C: Publications And Presentations


**Peer-Reviewed Abstracts & Short Papers**


The following is the list of presentations made during the course of this project:

**Conference Presentations:**


5. Malignant mesothelioma (MM) and cachexia: Description of body composition changes in 27 patients receiving chemotherapy and/or thalidomide treatment. *N Pavlakis, A Aslani, R Harvie, RC Smith, HR Wheeler*. 8th International Conference of the International Mesothelioma Interest Group, 19th to 22nd October 2006, Chicago, Illinois, USA.


APPENDIX C: Publications And Presentations


527

17. The use of fish oil in pancreatic cancer patients with cachexia. A Aslani. Nuclear Medicine Continuing Education Sessions, Department of Nuclear Medicine, RNS Hospital, Sydney. 30th October 2002.


23. Longitudinal comparison of nutritional state in patients with ESRF treated with HD or PD. BA Cooper, A Aslani, LS Ibels, CA Pollock. ASN/ISN World Congress of Nephrology. 2001, San Francisco, USA.


30. Residual renal function at dialysis entry significantly determines nutritional state and survival on dialysis. BA Cooper, A Aslani, M Ryan, BJ Allen, LS Ibels, CA Pollock. 36th Annual Scientific Meeting of the Australian and New Zealand Society of Nephrology, Melbourne, 15th-17th March 2000.


APPENDIX D: Total Body Water Validation Articles

Authors’ Contributions

In the article titled “Comparison of methods of body water determination in gastrointestinal cancer patients undergoing surgery” all patients were referred and supplied by Prof Ross C Smith. Prof Smith also provided intellectual support and study design. Dr Bruce A Cooper assisted in data analyses as well as assisting in putting the processed patient blood plasma samples through FTIR analysis at the University of Sydney. Dr André Sevette assisted in patient recruitment and blood sample collections. Dr Ross D Hansen assisted in BIA data interpretation. All body composition and anthropometric measurements as well as all remaining study procedures, laboratory preparations and analyses were performed by Mr Alireza Aslani. This included the writing and submission of the article.

In the article titled “Changes in body composition during upper gastrointestinal cancer surgery” all patients were referred and supplied by Prof Ross C Smith. Prof Smith also provided intellectual support and study design. Dr Bruce A Cooper assisted in data analyses as well as assisting in putting the processed patient blood plasma samples through FTIR analysis at the University of Sydney. Dr André Sevette assisted in patient recruitment and blood sample collections. All body composition and anthropometric measurements as well as all remaining study procedures, laboratory preparations and analyses were performed by Mr Alireza Aslani. This included the entire writing and submission of the article.
Comparison of methods of body water determination in gastrointestinal cancer patients undergoing surgery

Alireza Aslani¹, Bruce A Cooper², Andre Sevete³, Ross D Hansen⁴ and Ross C Smith³

¹Department of Nuclear Medicine, ²Department of Renal Medicine, ³Department of Surgery, ⁴Gastrointestinal Investigation Unit, Royal North Shore Hospital, Pacific Highway, St Leonards, NSW 2065, Australia.

Excess fluid retention can result in major morbidity in surgical patients. Understanding the degree of fluid retention is critical to developing better strategies for fluid management. The aim of this study was, therefore, to identify the most efficient method of total body water (TBW) estimation in gastrointestinal cancer patients undergoing surgery. The TBW of 39 gastrointestinal cancer patients (19 females) was measured using the reference standard deuterium oxide dilution technique (TBW₁₈_O₂) and compared to estimates derived via several equations: bioelectrical impedance analysis (Kushner, Pollicino, and Fredrix equations; Watson's equations; 73.2% of fat-free mass; and 58% body weight. All measurements were carried out concurrently. Analysis of variance and post-hoc tests showed that the 58% body weight method overestimated TBW₁₈_O₂ by 0.7 ± 10.1 kg (p = 0.002). The Fredrix, then the Kushner equations mean overestimation of TBW₁₈_O₂ by 0.5 ± 7.4 kg and 1.2 ± 7.5 kg respectively; both (P < 0.5) produced the narrowest limits of agreement and the least bias. These results indicate that the Fredrix equation may be an accurate, less time-consuming alternative to TBW₁₈_O₂ in determining TBW for groups of gastrointestinal cancer surgery patients. However, due to relatively wide limits of agreement, such measurements are probably of limited value for individual patient assessment.

Key words: total body water, cancer, surgery, bioelectrical impedance analysis, body composition

Introduction

Water disturbances are a recognised consequence of severe illness in surgical patients [1,2]. Water retention can conceal losses of body protein, and therefore weight is a poor reflection of malnutrition. Available nomograms such as the Watson’s equations [3] and 58% body weight do not account for this malnourished state that frequently occurs in all surgical patients. Clinical assessment mainly relies on physical signs of oedema and gross symptoms from fluid retention, and on dehydration in patients suffering fluid loss. Excess fluid is not always apparent until pulmonary oedema occurs, which can be fatal [4].

Protein loss in critically-ill patients is triggered and maintained by cell shrinkage secondary to cellular dehydration [1]. Post-operative pulmonary oedema is also a well-documented occurrence, [2] and incorrect fluid management in the critically ill, such as in gastrointestinal cancer patients undergoing major surgery, can lead to fluid overload, which in turn leads to complications including cardiac failure.

Although the causes of post-operative pulmonary oedema are many, one of the most common causes has been reported to be fluid overload with high hydrostatic pressures [2]. Large volumes of fluid are often administered post-operatively to compensate for the fluid lost during the operation. However, information regarding the correct amount of fluid required post-operatively, and the maximum amount of fluid that can be safely administered post-operatively is currently scarce [3]. Decisions on the amount of fluid replacement are largely based on experience, intuition, or approximation. In addition, the presence or absence of different disease conditions, such as cardiovascular or renal dysfunction, plus other factors such as age and body weight, contribute in determining the correct amount of fluids required to be administered post-operatively. Again, the exact extent of the contribution of these factors is still not clear [3]. There is, therefore, a considerable need for fluid replacements to be tailored to the individual patient’s physiological requirements and post-operative conditions, i.e. to be individualized.

It has been shown by Plank and Hill [4] that patients with sepsis retained approximately twice as much fluid as patients with major trauma, but the latter took longer to return to normal hydration levels. Recent studies of Cheng et al [5] have also demonstrated that in critically-ill elderly patients the prolonged expansion of body water contributes to a poorer outcome from critical illness.

Regulated bedside monitoring of patients' fluid levels

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is therefore an essential part of clinical management if complications are to be avoided. Hence, a patient’s fluid levels should be assessed pre-operatively and monitored post-operatively such that fluid replacement changes can be made on a dynamic basis. A rapid, painless and non-invasive fluid monitoring technique would, therefore, be invaluable.

Bioelectrical impedance analysis (BIA) has been developed to allow rapid bedside measurements of total body water (TBW), as few clinically-accessible methods are available to evaluate fluid losses during surgery [5]. The reports on the use and acceptance of BIA as a clinical tool to measure TBW and/or its components have, however, been controversial. While some researchers in the early to mid 1990s have found BIA to be a useful and viable tool [6,7] others have concluded that it is neither useful nor sufficiently accurate for clinical use [8].

The aim of this study was to compare different rapid bedside methods of TBW estimation, including the BIA technique, with the reference standard deuterium oxide dilution technique (TBW<sub>TDO</sub>) in patients who had presented with upper gastrointestinal malignancies and were undergoing major surgery.

**Methods**

Protocols were approved by the Human Research Ethics Committee of the Royal North Shore Hospital. All subjects gave written, informed consent to participate in the studies.

**Patient selection**

The patients for the study were recruited consecutively from the Department of Surgery when scheduled for major upper gastrointestinal surgery. Measurements were carried out prior to surgery.

**Anthropometric measurements**

Height and weight were measured using standard stadiometer and electronic scales (Wedderburn Scales, Sydney), respectively. Skinfold thickness measurements were carried out on the patient’s non-dominant side using skinfold callipers (Holtain Ltd, Crymych, UK) at three standardized sites: triceps, biceps, and subscapular, with the patient in a standing position.

Percentage body fat was estimated from the skinfold thickness measurements at the three sites. The equations of Durnin and Womersley [9] were used to calculate the percentage body fat, using an in-house computer program that calculates percentage body fat and body density. Fat-free mass (FFM) was calculated from weight and percentage body fat.

Inter-observer errors were avoided for the anthropometric and BIA measurements as these were all performed by a single researcher.

**Total body water measurements**

_deuterium oxide dilution (TBW<sub>TDO</sub>). Although different isotopes are currently available, deuterium oxide (D<sub>2</sub>O) was chosen because of its lower cost and non-radioactivity. D<sub>2</sub>O (1 µmol·kg<sup>-1</sup>·FMM) was given orally, at night, before sleeping. This dose was chosen as it has a safe therapeutic threshold that also results in post-dose plasma concentrations detectable by the analysis technique. Blood samples were collected, in triplicates, in the morning in heparinised tubes containing 143 USP units of lithium heparin (Vacutainer PST, Becton Dickinson & Co., USA). Fluid input and output were measured during the equilibration period to permit corrections to the final TBW estimation. The blood samples were centrifuged at 1520 g at 4°C for 20 min (Beckman Model TJ-6 centrifuge with a TH-4 rotor at 2700 rpm). The ultrafiltration technique of Adani et al [10] was used to separate the D<sub>2</sub>O/H<sub>2</sub>O mixture from the plasma. The resulting D<sub>2</sub>O/H<sub>2</sub>O mixture was then analysed using Fourier transform infra-red (FTIR) analysis [11] to determine the final D<sub>2</sub>O concentration in the patient’s plasma. A correction factor of 1.04 was also applied to correct for non-aqueous hydrogen exchange. The accuracy of the isotope dilution techniques has been reported to be 1% to 4% [5]. The precision, expressed as a coefficient of variation, was 1%.

**Bioelectrical impedance analysis (BIA).** For the BIA method, measurements were carried out on the patient in the supine position using two leads on the non-dominant hand and two leads on the ipsilateral foot (tetrapolar arrangement). Leads were attached to the skin using Red Dot Ag/AgCl Resting Electrodes (3M Health Care, St Paul, MN, USA). The patient’s skin was cleaned with an alcohol swab and then dried prior to electrode attachment. Patients were rested in the supine position for at least five minutes, and the measurements were performed with their arms parallel but separate to the trunk and their legs apart far enough so that their thighs were not touching. The body’s resistance was measured in triplicate using a sweep frequency bioelectrical impedance meter (SEAC SFR25, UniQuest Limited, Queensland, Australia) at 50 kHz. The precision of the BIA measurement, expressed as a coefficient of variation, is <0.2%, as reported previously [13]. Resistance was used in three different equations of Kushner [6] (TBW<sub>K</sub>), Pullicino [12] (TBW<sub>P</sub>), and Fredrix [13] (TBW<sub>F</sub>), as given below, to calculate the TBW.

**Other total body water estimation techniques.** TBW was additionally estimated from 58% body weight (58%BW), Worsnop equations [14] (TBW<sub>W</sub>), and FFM (eq 73.2%FFM) [15] (TBW<sub>FFM</sub>). The latter three techniques did not require BIA measurements. It should also be noted that each of the three BIA equations utilise corrected dilution spaces whereas the TBW<sub>F</sub> equations do not.

**Total body water estimation equations**

The TBW<sub>W</sub> equation [14] (Equation 3) was developed and derived from healthy subjects, whereas the TBW<sub>F</sub> equation [14] (Equation 4) was developed in patients
with cancer. As indicated in the original article, for the \( \text{TBW}_{\text{F}} \) equations [5] (Equations 5 and 6) ... most of the individuals were healthy volunteers, some were patients hospitalised for minor disorders with no clinical evidence of oedema ... The \( \text{TBW}_{\text{F}} \) equations [6] (Equations 1 and 2) included a wider range of subjects, which included patients with obesity, diabetes, inflammatory bowel syndrome, and patients on intravenous nutrition.

In the following equations, \( H \) is the height in centimetres, \( W \) is the weight in kilograms, \( A \) is the age in years, \( R \) is the resistance in ohms, and \( \text{TBW} \) is in liters.

Equation 1. Kushner [6] equation for \( \text{TBW} \) estimation for males:

\[
\text{TBW} = 8.399 + 0.399(A^{1/2}) + 0.145(W)
\]

Equation 2. Kushner [6] equation for \( \text{TBW} \) estimation for females:

\[
\text{TBW} = 8.515 + 0.382(A^{1/2}) + 0.127(W)
\]

Equation 3. Pullicino [12] equation for \( \text{TBW} \) estimation:

\[
\text{TBW} = 0.958(A^{1/2}) + 1.825
\]

Equation 4. Fredrix [14] equation for \( \text{TBW} \) estimation:

\[
\text{TBW} = 8.9 + 0.5(A^{1/2})
\]

Equation 5. Watson [5] equation for \( \text{TBW} \) estimation for males:

\[
\text{TBW} = 2.447 - 0.09516A + 0.1074H + 0.3362W
\]


\[
\text{TBW} = 0.1059H + 0.2466W - 2.097
\]

Statistical analyses

Data collected during the study were stored in a computer database (Microsoft Access 2000 for Windows) and analysed using SPSS for Windows Version 11 (Chicago, USA).

Single factor analysis of variance (ANOVA) was used to test the hypothesis that means from the different techniques of \( \text{TBW} \) determination were equal. Post-hoc Bonferroni tests were then used to compare the means of individual methods with the reference standard technique (\( \text{TBW}_{\text{F}} \)). The difference in \( \text{TBW} \) values derived by the different methods was also plotted against the mean of the methods by the model described by Bland and Altman [16]. Unless otherwise stated, results are presented as mean values ± SD.

Results

Thirty-nine patients (19 females, 20 males) were recruited for this study. The mean and median age was 65 and 68 years, respectively, with a range of 40.7 to 81.6 years. Surgical procedures were distal pancreatectomy in eight patients, oesophagectomy in ten, palliative laparotomy in one, partial gastrectomy in two, total gastrectomy in two, and a Whipple's procedure in 16. The mean anthropometric measurements for the patients at the time of the study are provided in Table 1.

Mean total body water values

The overall comparisons of the \( \text{TBW} \) estimation techniques are demonstrated in Figure 1 and Table 2. ANOVA revealed a significant difference in the means of the various \( \text{TBW} \) measurements (\( P<0.001 \)). Post-hoc tests showed that the \( \text{TBW} \) method significantly overestimated \( \text{TBW}_{\text{F}} \) (by \( 6.7 \pm 10.1 \) kg; \( P <0.002 \)). The mean of this method was also significantly greater (\( P<0.05 \)) than each other method except \( \text{TBW}_{\text{F}} \). The means of \( \text{TBW}_{\text{F}} \), \( \text{TBW}_{\text{exp}} \), \( \text{TBW}_{\text{exp}} \), and \( \text{TBW}_{\text{exp}} \) agreed to within 2.2 kg with the mean \( \text{TBW}_{\text{F}} \) value (Table 2). The corresponding 95% confidence intervals for the differences between these methods and \( \text{TBW}_{\text{F}} \) are also given in Table 2.

Table 1. Patients' (n=39) body composition characteristics at the time of the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.3 ± 12.4</td>
<td>66.3</td>
<td>40.7 to 81.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.6 ± 13.8</td>
<td>68.8</td>
<td>45.0 to 100.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.6 ± 9.3</td>
<td>166.8</td>
<td>151.4 to 181.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 4.3</td>
<td>25.4</td>
<td>15.9 to 36.0</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>30.9 ± 6.6</td>
<td>31.8</td>
<td>20.5 to 45.8</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>48.6 ± 9.9</td>
<td>48.5</td>
<td>34.0 to 68.7</td>
</tr>
<tr>
<td>( \text{TBW}_{\text{F}} ) (kg)</td>
<td>34.3 ± 9.5</td>
<td>34.4</td>
<td>18.2 to 54.4</td>
</tr>
<tr>
<td>TBW m² (g)</td>
<td>1759 ± 192</td>
<td>1759</td>
<td>1129 to 2014</td>
</tr>
<tr>
<td>NI</td>
<td>1.01 ± 0.12</td>
<td>0.99</td>
<td>0.73 to 1.48</td>
</tr>
</tbody>
</table>

\( \text{TBW} \) = total body water; \( \text{NI} \) = nitrogen index (\( \text{TBW} \) expressed as a % of normal).

Figure 1. Mean values (± SE) of the different methods of \( \text{TBW} \) estimation. **\( P<0.05 \) vs all other methods except Watson equations.
APPENDIX D: Total Body Water Validation Articles

<table>
<thead>
<tr>
<th>Measurement technique</th>
<th>BIAS (kg)</th>
<th>95% CI</th>
<th>P</th>
<th>Limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>58% body weight</td>
<td>-6.7 ± 10.1</td>
<td>-11.9 to -1.5</td>
<td>0.002</td>
<td>-26.9 to 11.5</td>
</tr>
<tr>
<td>73.2% of FFM</td>
<td>-1.3 ± 8.6</td>
<td>-6.5 to 3.9</td>
<td>1.0</td>
<td>-18.5 to 15.9</td>
</tr>
<tr>
<td>Fredrix</td>
<td>-0.5 ± 7.4</td>
<td>-5.7 to 4.7</td>
<td>1.0</td>
<td>-15.3 to 14.3</td>
</tr>
<tr>
<td>Kushner</td>
<td>-1.2 ± 7.5</td>
<td>-6.3 to 4.0</td>
<td>1.0</td>
<td>-16.2 to 13.9</td>
</tr>
<tr>
<td>Pullicino</td>
<td>2.5 ± 7.5</td>
<td>-3.0 to 7.1</td>
<td>1.0</td>
<td>-12.9 to 17.2</td>
</tr>
<tr>
<td>Watson</td>
<td>-1.9 ± 8.1</td>
<td>-7.1 to 3.3</td>
<td>1.0</td>
<td>-18.1 to 14.3</td>
</tr>
</tbody>
</table>

Bias = mean ± SD difference between TBW_{D2O} and the technique.
CI = confidence interval for the mean difference between TBW_{D2O} and the technique.
P = significance level for the difference between TBW_{D2O} and the technique (ANOVA with post-hoc Bonferroni tests).
Limits of agreement = those obtained from Bland and Altman plots, defined as the mean difference ± 2 SD.

Bland and Altman tests
Figure 2 and Table 2 summarize results of the Bland and Altman [16] analyses. These tests showed relatively large limits of agreement between each method of body water assessment and the reference standard TBW_{D2O} technique (Table 2). The TBW_{W} technique (Fig. 2) yielded values that agreed best with TBW_{D2O} reflecting the least bias and one of the narrowest limits of agreement. TBW_{P} and TBW_{D2O} also produced relatively narrow limits of agreement, and TBW_{w} produced the narrowest limits of agreement amongst the non-BIA equations.

Discussion
In surgical patients, fluid status can be disrupted, influencing drug pharmacokinetics. This may result in the requirement for a change in drug dosing and therapy requirements. Knowledge of fluid status and body composition, if available, influences fluid management and nutritional support and intervention. This influence becomes particularly important when water-soluble drugs, which are distributed in the water compartment, are used. Therefore, knowledge of the patient’s TBW content and/or its constituents is invaluable in establishing a goal for appropriate preventative intervention [1,2,4].

Isotope dilution techniques are considered to be the ‘reference standards’ in TBW determination. The isotope dilution techniques currently available utilize either tritium oxide (H₂O), oxygen-18 (H¹⁸O), or D₂O. Following their administration, these isotopes are detected in various body fluids, such as plasma, urine, and saliva, using appropriate analytical techniques [17]. In this study, the D₂O isotope dilution technique was used, as it is relatively inexpensive and is not radioactive. Fourier transform infra-red (FTIR) analysis [1] was undertaken to determine D₂O levels in patients’ plasma.

The technique of TBW estimation by D₂O dilution (or isotope dilution in general) and FTIR analysis technique may not be suitable for use when patient or subject numbers are large, such as in routine clinical practice [11,18,19]. This is due to the long preparation phase required for FTIR analysis, the reliance on full cooperation from patients for D₂O administration as well as fluid balance recordings, and the need for access to complex and expensive equipment that is not available in most laboratories and hospitals. There is, therefore, a need for bedside measurements of TBW for its assessment in individual patients and for studying therapeutic outcome in groups of patients.

Estimation of TBW from FFM is amongst the non-invasive bedside techniques currently available. Here body fat is calculated from skinfold thickness using Durnin and Womersley’s [9] equations and then FFM is, in turn, calculated from the body fat. The error for the measurement of body fat is around 10%. However, as fat can be 25% to 45% of body mass, it implies an error of 3% to 5% of body weight. In addition, this measure does not take individual variation in the hydration of fat-free mass into account.

BIA is an electrical method of assessing human body composition by quantifying TBW, fluid volumes, body cell mass (BCM), and fat-free mass through measuring tissue conductivity. This measurement has the advantage of assessing different degrees of hydration of lean tissue. BIA works on the basic principle that under stable conditions the conductivity of a body segment is directly proportional to the amount of electrolyte-rich fluid present. It assumes that the
Body water measurement in cancer surgery patients

Similarly, the other methods of TBW estimation included (Watson formula, and 73.2% FFM) did not differ significantly from TBW_{est}. The Bland and Altman analyses showed that although all methods had relatively wide limits of agreement, the method with the narrowest limits of agreement as well as the least bias was TBW_{est}. TBW_{est} and TBW_{est} produced similar limits of agreement. From the non-BIA equations, TBW_{est} produced the narrowest limits of agreement. Also, in practice, it would appear from reviewing each of the Bland and Altman plots that the inaccuracies are greater for patients with larger TBW values (Fig. 2).

Given the need to assess TBW and other body composition parameters in surgical patients, especially patients receiving TPN, chemotherapy, and other nutritional interventions and supports, accurate and reliable methods are necessary. In clinical practice, there is a need for quick and reliable methods that preferably are also user-friendly. Amongst the different methods tested here, TBW_{est} and TBW_{est} were found to have the best limits of agreement and low bases. The BIA-based equations appeared to have clear advantage over the other methods of assessing TBW in this group of surgical patients. However, not all equations are suitable and reliable for all patient populations. Therefore, as noted by others [22], it is also our recommendation that if the BIA or non-BIA techniques are to be used, specific equations should be derived and validated for the given patient or study population.

We have shown that the TBW_{est} and TBW_{est} produced similar mean TBW results for these surgical patients. However, the variance of measurements makes for wide limits of agreement. This would, in turn, imply that the BIA measurements are of limited value for individual patient measurements, but are of value in assessing groups of patients. This is in agreement with recent, comparable studies in patients with gastrointestinal disorders [23,24].

In summary, we have demonstrated that in cancer surgery patients, TBW_{est} may be used as an alternative method to TBW_{est} to assess and monitor patients’ TBW, therefore reducing fluid-related complications. BIA-based techniques showed advantages over non-BIA equations in these patients. However, due to wide limits of agreement with the reference TBW_{est}, BIA techniques are recommended for group, rather than individual, assessment of fluid status.

References

APPENDIX D: Total Body Water Validation Articles


APPENDIX D: Total Body Water Validation Articles

Changes in body composition during upper gastrointestinal cancer surgery

A. Aslani, B.A. Cooper, A. Sevette and R.C. Smith

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Changes in weight and body composition are expected after most major abdominal surgery. Excess fluid retention especially may result in major morbidity in the post-operative patients. Loss of weight, itself, has been generally addressed by administration of appropriate intervention procedures. However, the components of the weight change has not yet determined if an efficient and effective intervention procedure is to be successfully administered. Total body protein, body fat and body water of 15 patients undergoing major upper gastrointestinal (GI) surgery were measured pre-operatively and two weeks post-operatively. Total body protein (TBP) was measured using the in-vivo neutron capture technique (INCAT) technique and total body water (TBW) using the isotope dilution technique. The nitrogen index (NI) was calculated from TBP and total body fat (TBF) was calculated from TBF and total body fat (TBF) was calculated from TBF. Fat-free mass (FFM) and percentage body fat (%BF) as well as fat-free mass (FFM) and percentage body fat (%BF) as well as from anthropometric measurements. Although there was a general increase in weight post-operatively, the majority of BC parameters (TBP, NI, FM, %BF, FFM and BMI) decreased, with the exception of TBW, which increased from 33.6 l to 38.1 l. The findings suggest that, in this group of surgical patients, the increase in weight observed post-operatively is due to an increase in the TBW. The findings also demonstrate the need to validate bedside techniques to measure the crucial TBW, which may be essential to the overall recovery and survival of this group of patients.

Key words: surgery, body composition, gastrointestinal cancer, nutrition, total body nitrogen.

Introduction

Water disturbances are a recognized consequence of severe illness in surgical patients [1, 2]. This includes the patient’s loss in body protein and, therefore, is the reason why weight is a poor reflection of malnutrition. Clinical assessment mainly relies on physical signs of oedema and congestive heart failure for fluid retention, and on dehydration for patients suffering fluid loss. Excess fluid is not always apparent until pulmonary oedema occurs which can sometimes be fatal [3]. The present study, therefore, sets out to investigate the components of weight change during major upper gastrointestinal surgery.

Methods

Protocols were approved by the Human Research Ethics Committee of the Royal North Shore Hospital. All subjects gave written, informed consent to participate in the study.

The patients for the study were recruited consecutively from the Department of Surgery when scheduled for major upper gastrointestinal surgery. Measurements were carried out prior to surgery and one week post-operatively (day 14).

Height and weight were measured using standard stadiometer and electronic scales respectively. Anthropometric measurements were carried out on the patient’s non-dominant side by trained medical staff. Skinfold thickness measurements were carried out using skinfold calipers (Holtain Ltd, Crymych, UK) at three sites: triceps, biceps, and subscapular area, with the patient in a standing position. Percentage body fat (%BF) was estimated from the skinfold thickness measurements at the three sites of biceps, triceps and subscapular area. The equations of Durnin and Womersley [4] were used to estimate %BF, using a computer program that enabled easy estimation of %BF as well as body density. The fat-free mass (FFM) was calculated from weight and %BF.

Total body water (TBW) measurements were carried out using the isotope dilution technique. The isotope dilution technique is considered to be the ‘reference standard’ for TBW determination. Deuterium oxide (D₂O) (1 g/kg FFM) was given orally at night, before sleeping. This dose was chosen as it has a safe therapeutic threshold that also resulted in post-dose plasma concentrations detectable by the analysis technique. Blood samples were collected in the morning in heparinized tubes containing 1-13 U/rf units of lithium heparin (Vacutainer® PST, Becton Dickinson & Company, USA). Blood samples were analysed for total body water content.

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538
APPENDIX D: Total Body Water Validation Articles

Co., USA). The blood samples were centrifuged at 1520 g at 4 °C for 20 min (Beckman® Model TJ-6 centrifuge: with a TH-4 rotor at 2700 rpm). The ultrafiltration technique of Aslani et al. [5] was used to separate the D₂O/H₂O mixture from the plasma, which was then analysed using the analytical method of Blagojevic et al. [6] using Fourier transform infra-red (FTIR) analysis to determine the final D₂O concentration in the patient's plasma. A correction factor [17] of 1.04 was applied to correct for the non-aqueous hydrogen exchange. The accuracy of the isotope dilution technique has been reported to be 1% to 4% [8]. The coefficient of variation was 1%.

Total body nitrogen (TBN) was measured by in-vivo neutron capture analysis (IVNCA) [9–11] using fast neutrons emitted from a neutron source. The overestimation observed by the patient is approximately one tenth of the annual background radiation.

Total body protein (TBP) was then calculated by multiplying TBN by a factor of 6.25. This is on the basis of 1 gram of nitrogen in 6.25 g of protein [9]. The nitrogen index (NI) was calculated by expressing the TBN as a percentage of normal [12].

Data collected during the study were stored in a computer database (Microsoft Access® 7.0 for Windows® 95) and analysed using a computer spreadsheet (Microsoft Excel® 7.0 for Windows® 95). The paired two-sample T-test was used to determine whether the changes in body composition parameters were statistically significant.

Results

Fifteen (eight male, seven female) patients were recruited for this study. The mean and median age was 65 and 69 years, respectively, with a range from 40 to 78 years. Surgical procedures were distal pancreatectomy in two, gastrectomy in three, and Whipple's procedure in five.

The mean values of body composition and anthropometric measurements for the pre-operative and post-operative patients at the time of the study are given in Table 1 and Table 2 respectively.

Although there were changes in mean body composition and anthropometric parameters, none were found to be statistically significant, with the exception of TBW (%) [p<0.09].

Discussion

In surgical patients, the fluid status can be disrupted influencing the drug pharmacokinetics that may result in the requirement for a change in drug doses and therapy requirements. The knowledge of fluid status and body composition, if available, influences fluid management and nutritional support and intervention. This influence becomes particularly important when water-soluble drugs, i.e. the drugs that are distributed in the water compartment, are used. Therefore, knowledge of the patients' TBW content and/or its constituents is important in establishing a goal for a potential and appropriate preventative intervention.

The isotope dilution technique is considered to be the 'reference standards' in TBW determination. The isotope dilution techniques currently available for use in biological samples use either tritium oxide (T₂O), oxygen-18 (H₂¹₈O), or deuterium oxide (D₂O). Following their administration, these isotopes are then detected in various body fluids, such as plasma, urine or saliva, using appropriate analytical techniques. In this study, the D₂O isotope dilution technique was used as it is relatively cheap and the isotope is not radioactive. The analytical method of Blagojevic et al. [6] was used with the addition of the 1.04 correction factor [17], which involved the use of Fourier transform infra-red (FTIR) analysis for the determination of D₂O levels in the patient’s plasma. The technique of TBW estimation by D₂O dilution and the FTIR analysis technique, as pointed out by Blagojevic et al. [6] as well as other authors [13, 14], may not be suitable for use when patient or subject numbers are large, such as in routine clinical practice. This is mainly due to the long preparation phase required for FTIR analysis and the reliance on full cooperation from patients for D₂O administration as well as fluid balance recordings, and access to complex and expensive equipment not available in most laboratories and hospitals. Therefore, there is a need for validation of quick and efficient bedside techniques, such as those reported by Cooper et al. [15], for use in this group of patients.

The population examined in this study was

### Table 1. Pre-operative body composition and anthropometric measurements.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>SE</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>25.6</td>
<td>1.2</td>
<td>25.3</td>
<td>18.4 to 33.3</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.9</td>
<td>1.9</td>
<td>28.8</td>
<td>21.6 to 45.8</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>22.0</td>
<td>1.7</td>
<td>20.6</td>
<td>11.4 to 32.0</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>48.9</td>
<td>2.7</td>
<td>49.6</td>
<td>36.6 to 68.7</td>
</tr>
<tr>
<td>NI</td>
<td>1.02</td>
<td>0.04</td>
<td>1.04</td>
<td>0.02 to 1.34</td>
</tr>
<tr>
<td>TBP (kg)</td>
<td>11.3</td>
<td>0.8</td>
<td>10.6</td>
<td>7.5 to 17.6</td>
</tr>
<tr>
<td>TBW (l)</td>
<td>33.6</td>
<td>2.2</td>
<td>34.0</td>
<td>19.4 to 49.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.9</td>
<td>3.5</td>
<td>69.1</td>
<td>52.0 to 100.7</td>
</tr>
</tbody>
</table>

### Table 2. Post-operative body composition and anthropometric measurements.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>SE</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>25.6</td>
<td>1.0</td>
<td>24.4</td>
<td>21.7 to 33.9</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.3</td>
<td>1.9</td>
<td>26.6</td>
<td>22.3 to 44.3</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>21.6</td>
<td>1.7</td>
<td>18.4</td>
<td>12.2 to 31.7</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>49.4</td>
<td>2.3</td>
<td>50.0</td>
<td>37.8 to 62.3</td>
</tr>
<tr>
<td>NI</td>
<td>0.99</td>
<td>0.03</td>
<td>0.96</td>
<td>0.81 to 1.22</td>
</tr>
<tr>
<td>TBP (kg)</td>
<td>11.1</td>
<td>0.8</td>
<td>11.0</td>
<td>7.6 to 16.1</td>
</tr>
<tr>
<td>TBW (l)</td>
<td>38.1</td>
<td>2.7</td>
<td>35.7</td>
<td>24.3 to 53.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.0</td>
<td>2.8</td>
<td>71.9</td>
<td>51.8 to 93.8</td>
</tr>
</tbody>
</table>

539
APPENDIX D: Total Body Water Validation Articles

Changes in body composition during upper gastrointestinal cancer surgery 81

patients with upper gastrointestinal cancer undergoing major surgery. They represented a wide age range with approximately equal numbers of males and females. The patient population had a wide range of height and weight, which is reflected in the BMI, % body fat, and TBN (see Tables 1 and 2). The results show that the patients had a varying level of nutritional status, both pre-operatively as well as post-operatively, ranging from severely malnourished (pre-operative NI = 0.82; post-operative NI = 0.81) to well nourished (pre-operative NI = 1.34; post-operative NI = 1.22). The mean NI, however, indicated that the patient group seemed to have TBW close to the normal level (NI = 1.00).

Conclusion

Generally, there was an increase of body weight post-operatively as compared to the pre-operative weight. In addition, there was a statistically significant increase in TBW. This, together with the fact that all other measured parameters were decreased post-operatively as compared to their corresponding pre-operative values, one may conclude that the post-operative increase in body weight is mainly due to the increase in TBW. One may also speculate that the reason for this fluid retention could be due to a combination of the disease, treatment, or fluid management techniques.

References

APPENDIX E: Selection, Recruitment And Measurement Protocols

There were a total of three patient studies and clinical trials carried out. Each one of these studies or clinical trials had a slightly different patient group investigating different aspects of body composition, therapy, and the effects of therapy on body composition and QL. As a result, each had a different protocol and study design. This methods section was, therefore, separated into three sections with each section being dedicated to a different patient study or clinical trial.

1.1 Body Composition Measurement Protocols

The following protocols were initially set-up at our centre for the measurements of the particular component(s) of body composition.

1.1.1 Total Body Nitrogen Measurement Protocol

An almost identical protocol is followed for all patients attending the Body Protein Monitor Unit for TBN measurement. This protocol was designed to ensure the procedure runs smoothly and efficiently.

As a part of this protocol, all subjects were required to sign appropriate consent forms agreeing to participate in the relative research project and be subjected to TBN measurements by the IVNCA technique. In the case of the pancreatic cancer patients for this study, their consent forms were signed on the day of recruitment and was sufficient for five TBN
measurements. Requirements of the Royal North Shore Hospital’s policy on female patients (both in-patients and out-patients) indicated that a second member of staff must be present in the room for the full duration of the procedure. As a result, either the Department of Surgery’s secretary or one of the Department of Nuclear Medicine’s technologists were asked to remain in the room for this purpose.

The following protocol was followed for the core TBN measurements in all patients measured for all projects and clinical trials:

1) On arrival, patient’s details were checked and it was ensured that the referral form is completed and the appropriate consent form is present. In almost all cases the patients were personally approached, met, and escorted down to the Body Protein Monitor. On completion of the measurements, the patients were again personally escorted back to their meeting place.

2) The patient was then asked to strip down to their underwear and then put on a standard hospital gown, if they were not already in hospital gown. The purpose of the hospital gown was to keep the patient warm and, at the same time, standardise the amount of clothing worn by the patients while on the IVNCA instrument.

3) The height, weight, and other anthropometric measurements were made and the values noted down on the Body Protein Monitor Unit’s TBN form.

4) After all the above measurements were carried out, the patient was asked to sit while the IVNCA table was brought back and the phantom box removed.

5) A thin mattress was then placed on the bed and two hospital towels were folded to act as a pillow for the patient. A thin disposable multipurpose medical towel (Kimberly-Clark Versa® Towel 49.0 x 41.5cm) was placed over the mattress and the pillows to provide a hygienic surface for the patient to lie on.
6) The patient laid in a supine position on the movable table, and appropriate measurements required for the TBN calculations were made on the patient. These measurements were the width of the body at the shoulder, hip, and knee positions.

7) These measurements were noted down on the TBN data form for later entry into the TBN data base for TBN calculations. They also, in a way, ensured that the patient is lying exactly in the middle of the table.

8) Depending on the patient’s neck to knee distance, the motorised table was set to make the appropriate number of stops while passing over the source. This usually was either three or four stops. These stops or increments are approximately 26cm, which is the effective width of the thermal neutron beam at the top of the motorised table.

9) The MCA S100 System was then initiated from the computer and the motorised table from its respective switch. The MCA S100 System was set to count for 200 seconds. Once the table’s magnet reached the appropriate “gate” magnetic sensor on the main body of the IVNCA instrument, the MCA S100 System started the 200 seconds count.

10) Once the scan was completed, the motorised table automatically returned to its original position.

11) The above procedure was then repeated for a second supine scan. A “double supine” protocol was followed for the patients in these projects and clinical trials as a result of most patients having had surgery performed in the abdominal region, thus being unable to lie in the prone position.

12) On completion of the TBN measurements, the patient was accompanied back to the ward or meeting place.
1.1.2 Total Body Water Measurement By D₂O Dilution Protocols

Before TBW measurements are carried out, it was ensured that all patient documentation are present and all appropriate and necessary forms are filled and signed accordingly.

1.1.2.1 D₂O Dosing

The D₂O was dosed orally at 1.0 gram per kilogram of LBM. As a result, the weight and %BFat was required for dosing. These values were obtained from the TBF and anthropometric measurements carried out as a part of the TBN measurements, which could also be used for LBM calculation.

In order to prepare a D₂O dose the following protocol was carefully followed:

1) The general patient identification information on the TBW Analysis Data Sheet was completed, making sure that the weight, %BFat, and LBM were also included.

2) The required and correct number/amount of consumables, glassware, and equipment were assembled.

3) Patient's full name and hospital unit number were written on the sticky label and attached to the glass bottle.

4) The bottle and its cap were then weighed and the empty bottle weight was noted on the TBW Analysis Data Sheet.

5) The bottle cap was then removed and the balance tared.

6) Approximately 50mL of D₂O was poured in the glass beaker.

7) D₂O was then transferred to the glass bottle making sure the required weight is not exceeded.

8) Once the required amount was transferred, the D₂O weight was noted on the TBW Analysis Data Sheet.

9) The glass bottle was topped-up with drinkable water, fruit cordial, or fruit juice.
10) The cap was closed tightly and bottle was gently shaken.

11) The full bottle was then weighed and the full bottle weight was noted on the TBW Analysis Data Sheet.

12) The patient was then requested pass urine and fully empty their bladder.

13) The bottle was then given to the patient to drink straight from the bottle, ensuring that it is drunk slowly with no loss from the sides of the mouth.

14) The time and date of drinking were noted on the bottle and on the TBW Analysis Data Sheet.

15) The bottle was then re-weighed and the empty bottle weight was noted on the TBW Analysis Data Sheet.

The patient was monitored (for at least 4 hours) for urine output and it was ensured that there was also no fluid input (for example, drinking). In the case of the pre-operative and post-operative patients, the dose was given on the hospital ward and the fluid inputs and outputs were measured by the research nursing staff and noted on the patient’s “Fluid Charts”. The fluid input and output results were collected the next day.

1.1.2.2 Patient Sample Collection

Blood was used to detect the concentration of D₂O and, hence, estimate the TBW. Samples were collected at least 4 hours post D₂O administration. For legal/ethical reasons, blood samples were collected by trained medical staff. To collect blood samples:

1) It was ensured that latex examination gloves were worn.

2) Two 10mL blood samples were collected in heparinized Vacutainer PST® blood collecting tubes, two hours apart [727].

3) Patient's name, hospital unit number and the time/date when the blood samples were taken were written on the collection tube.
4) The time and date the blood samples were collected were written down on the TBW Analysis Data Sheet.

5) The blood was then centrifuged for 20 minutes at 1500g and 4°C to separate the plasma.

6) Using a glass Pasteur pipette, the plasma layer was carefully removed and place it in an appropriate plastic container and store at -20°C or lower for the preparation phase at a later date.

1.1.2.3 Patient and Standard Sample Processing

The D$_2$O/H$_2$O mixture in the plasma samples collected were required to be extracted before FTIR analysis can be performed. To extract the mixture:

1) It was ensured that latex examination gloves were worn as potentially hazardous biological materials were to be handled.

2) The frozen plasma samples were to allowed thaw overnight at room temperature.

3) The Sartorius Centrisart® I tube’s cap was removed.

4) The inner plastic tube was taken out and carefully placed on a clean, dry surface without touching the membrane end.

5) Approximately 2.5mL of plasma was placed in the outer tube, ensuring that the maximum 2.5mL limit was not exceeded.

6) The inner tube was then carefully replace and allowed to slowly slide down under gravity.

7) If more than one patient's samples were being prepared, the patient's name, date of sample and sample number were written on the tube using a permanent marker to avoid errors.
8) The tube(s) was then placed in the high speed centrifuge and centrifuged at 2500 g and 4°C for 2 hours.

9) Using tweezers, the inner tube was carefully removed without touching the membrane end.

10) Using glass Pasteur pipettes, the D₂O/H₂O mixture was removed and placed in an Eppendorf® plastic container, ensuring that the membrane does not get perforated.

11) The container was appropriately labelled using sticky labels noting the patient's name and date of the sample.

12) The outer tube was again topped-up with more plasma and, again, ensuring that the 2.5 mL limit was not exceeded.

13) The inner tube was replaced and placed back in the centrifuge.

14) The sample(s) were centrifuged at 2500 g and 4°C for a further 2 hours.

15) Using twicers, the inner tube was carefully removed without touching the membrane end.

16) Using glass Pasteur pipettes, the D₂O/H₂O mixture was removed and added to the D₂O/H₂O mixture already in the Eppendorf® plastic container, again ensuring that the membrane does not get perforated.

17) Usually, this was all the maximum D₂O/H₂O mixture that can be obtained from the plasma sample. The D₂O concentration in the mixture could then be measured using FTIR analysis.

18) The processed samples were finally stored at -20°C or lower until analysed using FTIR analysis.
1.1.2.4 Standard Concentrations Preparation

A number of different D$_2$O concentrations were prepared in human plasma to be used as "standards" during the FTIR analysis from which the patients’ plasma D$_2$O concentration was estimated. The concentrations used were: 0.0, 0.1, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 g/L. In order to make-up the standard solutions, the following were carried out:

1) It was ensured that latex examination gloves were worn as potentially hazardous biological materials were to be handled.

2) The required expired human plasma was obtained from the hospital's blood bank.

3) The frozen plasma was allowed to be thawed overnight at room temperature.

4) The plasma batch numbers were noted for future reference.

5) The contents of the plasma bags were emptied in a clean 2L glass beaker to be transferred to the volumetric flasks as necessary.

6) Starting from the highest concentration, the 7 D$_2$O concentrations (0.0, 0.1, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 g/L.) were prepared in volumetric flasks using serial dilution technique.

7) It was ensured that enough plasma was kept for the 0.0g/L concentration.

8) Once all concentrations were made, 50mL aliquots were made in 50mL plastic yellow-top containers. It should be noted that as many aliquots as possible were made as these aliquots could be used as standards for future analyses.

9) The D$_2$O concentration and the preparation date were written on the plastic containers and frozen at -20°C or lower until required.

10) On the day of FTIR analysis, 10mL of each of the D$_2$O concentrations were placed in scintillation vials to be taken to the FTIR facility.


1.1.2.5 Sample Analysis

The FTIR analysis of the processed plasma samples were carried out to measure the D$_2$O concentration in the resulting D$_2$O/H$_2$O mixture. Both the patient and the standard samples were analysed on the same day. To carry out the FTIR analysis:

1) It was ensured that latex examination gloves were worn as potentially hazardous biological materials were to be handled.

2) It was ensured that the same settings were set on the FTIR instrumentation and used for all samples. These were: A Bruker® IFS66v FTIR Analyser (MCT detector) running the Opus® version 2.01 software capable of 4 wave number resolution, 256 scans, at mid infra-red with a Calcium Fluoride liquid cell (0.1mm path length).

3) The D$_2$O standards were put through the FTIR once before the patient samples and once at the end of the day/session and the mean values were used.

4) The D$_2$O standards and patients' absorption values were entered in a Microsoft Excel® spread sheet on location and saved.

5) For the final estimation of D$_2$O concentrations in the patient’s samples, the D$_2$O standards’ absorption values were used to plot a concentration-absorption curve from which the patients' D$_2$O concentration were estimated from their respective absorption values.

The measurement of D$_2$O standards before and at the end of patient samples ensured that the quality of the results was kept constantly good. The FTIR facility at the University of Sydney's School of Chemistry had their own strict quality control protocols ensuring smooth and accurate running of the facility.
The resulting data were stored in a Microsoft Excel® data sheet in an IBM-Compatible Pentium PC. All calculations for patient’s final plasma D$_2$O concentrations were also made using Microsoft Excel® data sheet in an IBM-Compatible Pentium PC.

### 1.1.3 Total Body Water Measurements By BIA Protocols

BIA measurements were carried out to primarily measure the TBW. However, they can also be used to estimate the patient’s TBF content or LBM. Therefore, in these projects and clinical trials, the BIA technique was also utilised as an additional method of TBF estimation. Before TBW measurements by BIA were carried out, it was ensured that all the patient documentations were present and all the required forms were filled and signed accordingly.

#### 1.1.3.1 Pre-Measurement Preparations

The following steps were followed prior to taking patient’s BIA measurements:

1) The SFB23 was turned-on and checked to see whether the battery condition was adequate.

2) If the battery levels were adequate (>11.0 volts), the SFB23 was turned-off and proceeded to the next step. However, if battery levels were not adequate (<11.0 volts), the SFB23's was connected to the charger and was left overnight to charge.

3) As the BIA instrument has a limited capacity to store data, it was ensured that enough measurement spaces were available to be able to store the new measurements. If there were not enough spaces available, the SFB23 was purged, i.e. the previous BIA files downloaded to the computer.

4) The electrodes were prepared by cutting 2 Red Dot electrodes in half to make 4 electrodes.
5) The electrode leads were finally attached to the back of the SFB23, ready to carry out BIA measurements.

1.1.3.2 Electrode Attachments And Data Acquisition

The following steps were followed in order to attach the electrodes to the patient and take BIA measurements:

1) Patient's height and weight was measured. The \textit{height} was measured using a KabiVitrum™ Stadiometer, by standing the patient up right, bare feet, and with their back to the wall and the back of their heels touching the wall. The weight was measured using electronic medical scales. The one used at the Body Protein Monitor unit was the Wedderburn™ Scales, UWBW-150.

2) It was ensured that the patient was lying down in the supine position for at least 5 minutes with their legs slightly apart so the inside of their thighs did not touch.

3) The patient's full name, height, weight, sex, date of birth, project/clinical status, and the BIA file numbers were noted in the logbook.

4) The electrode attachment areas were carefully cleaned with alcohol swabs to clean and remove any cream, or greasy substance and ensure good contact.

5) On the non-dominant side, one electrode was attached to the back of the hand on the knuckles between the index finger and the first finger. The second electrode was attached to the back of the wrist parallel to the first electrode.

6) On the same side of the body (non-dominant side) one electrode was attached to the foot, between the first toe and the second toe. The second electrode was then attached to the ankle parallel to the first electrode.
APPENDIX E: Selection, Recruitment And Measurement Protocols

7) the port end of the electrode leads were then connected to the back of the SFB23 and the coloured ends to the electrodes (i.e. the patient).

8) The BIA instrument was then turned-on and measurements (in triplicates) were taken.

9) The BIA File numbers were the noted in the logbook under the patient’s details.

Once measurements were taken, the BIA instrument was connected to the IBM-Compatible PC where the BIA instrument’s own analytical software had been installed. The stored patient files were then transferred to the PC and analysed.

The resulting files contained the “raw data” which included the resistance, reactance, and impedance values. These were then copied into a Microsoft Excel® spread sheet. The equations to estimate TBF and TBW (Kushner [747], Pullicino [755] and Fredrix [753]) were incorporated where the TBF and TBW can be easily calculated from the patients’ height, weight, age, sex as well as the reactance, and impedance values. Although the TBF was automatically calculated from the raw BIA data, it was only the TBW which was utilised in our projects and clinical trials.

1.1.4 Total Body Potassium Measurement Protocol

As the TBK measurements were carried out in a small confined chamber, it was important to ensure the investigators/clinicians referring the patient as well as the patients themselves were fully aware of possible patient discomfort and/or refusal due to the feeling of claustrophobia. Before TBK measurements are carried out, it was ensured that all patient documentation were present and all the appropriate forms are filled and signed accordingly.
1.1.4.1 Pre-Measurement Preparations

Before the patient is measured, system calibrations were carried out. To carry out system calibration:

1) It was ensure that the High Voltage read 500 volts on the coarse dial and 400 volts on the fine dial, i.e. a total of 900 volts.
2) It was ensured that that the red lights on the right side of the Canberra MCA40 unit showed M/R, ADD, and PHA and the unit was running normally.
3) The counting chamber's mattress was rolled back and the calibration circle located on the bed.
4) The potassium source was placed within the calibration circle.
5) The chamber door was then closed. As there is no lock or latch on the Whole Body Counter chamber door, the door was secured shut with a wooden wedge.
6) It was ensured that the MCA40's memory dial is at correct position (position $\frac{1}{1}$) and the unit was running normally.
7) The system was then started to count. When the count time reached 1000 seconds the system automatically stopped displaying the final spectra.
8) Once the 1000 seconds counting had been completed, the two detector's regions of interest were checked and the integral values for each detector noted by moving the dial to $\frac{1}{4}$ for detector 1 and $\frac{3}{4}$ for detector 2.
9) These results were then recorded in the logbook under Potassium Calibrations.
10) If the regions of interest were correct, it was clearly indicated in the logbook that the regions of interest were ok and no changes/adjustments were required.
11) If not correct, then the regions of interest were adjusted accordingly ensuring that the total number of channels for each detector is always kept constant. Any changes/adjustments (pre-and post change) were be clearly noted in the logbook.
12) Once changes/adjustments have been made, the final integral counts were noted in the logbook.

1.1.4.2 Patient Measurements

On arrival at the Whole Body Counter, it was ensured that all the required forms are signed and in order. The patient was then asked to remove all leather, as they contain potassium. To carry out patient TBK measurements:

1) It was ensured that the High Voltage read 500 volts on the coarse dial and 400 volts on the fine dial, i.e. a total of 900 volts.

2) It was ensured that the red lights on the right side of the Canberra MCA40 unit showed M/R, ADD, and PHA and the unit was running normally.

3) Patient’s details, including their full name, project/disease status, hospital unit number, and date of birth, were written in the TBK logbook.

4) The patient was then guided to enter the chamber and lay in the supine position with their head touching the right hand side wall at the centre mark position. The centre mark was marked with a small black arrow to assist in positioning the patient on the bed.

5) The patient was centred on the bed by measuring 365mm (using the wooden meter rule) in from the back wall of the chamber. The centring was checked at the nose, sternum, and feet.

6) The patient's anterior-posterior thickness and transverse thickness was measured and noted in the TBK logbook under the patient's details.

7) Patient was assured that although the door is closed shut, it can be pushed open from the inside in case of an emergency and that the members of staff are present outside at all times.
8) The chamber door was then closed. As there is no lock or latch on the Whole Body Counter chamber door, the door was secured shut with a wooden wedge.

9) Final check was made to ensure that the MCA40's memory dial is at the correct position (position $1/1$).

10) The system counting was then started to count for 1000 seconds.

11) Once the 1000 seconds was completed, the system stopped automatically and the two detector's regions of interest were checked by moving the memory dial to $1/4$ for detector 1 and $3/4$ for detector 2. The integral values were then written in the logbook under the patient's details.

12) Once the patient had left, the chamber door was then closed and held in position using the wooden wedge.

13) The background measurements were then carried out as soon as possible after the patient measurements.

### 1.1.4.3 Background Measurements

In almost all cases the background measurements were carried out as soon as possible immediately before patients attending the Whole Body Counter or immediately after the patient had completed their TBK measurements. This was to ensure that the “background counts” are as close as possible to the time of patient measurements.

To carry out system background measurements:

1) It was ensure that the High Voltage read 500 volts on the coarse dial and 400 volts on the fine dial, i.e. a total of 900 volts.

2) It was ensured that that the red lights on the right side of the Canberra MCA40 unit showed M/R, ADD, and PHA and the unit was running normally.
APPENDIX E: Selection, Recruitment And Measurement Protocols

3) The chamber door was then closed. As there is no lock or latch on the Whole Body Counter chamber door, the door was secured shut with a wooden wedge.

4) It was ensured that the MCA40's memory dial is at correct position (position 1/1) and the unit was running normally.

5) The system was then started to count. When the count time reached 1000 seconds the system automatically stopped displaying the final spectra.

6) Once the 1000 seconds counting had been completed, the two detector's regions of interest were checked and the integral values for each detector noted by moving the dial to 1/4 for detector 1 and 3/4 for detector 2.

7) These results were then recorded in the logbook under Background.
1.2 Pre- & Post-Operative Surgical ("Water") Validation Study

The Pre-operative and Post-operative Gastrointestinal Surgery Patient study was a prospective trial to look at the changes in the body composition of patients with upper gastrointestinal cancer undergoing major surgery. This study was set out to investigate the components of weight that changes as a result of major surgery. In addition, the results of this study was also utilised to validate BIA techniques against the isotope dilution technique (i.e. the "gold standard").

The results from this study have been published in two peer-reviewed international scientific journal \cite{758,848} and is also included in the Appendix D section.

Body composition measurements were performed to investigate the changes in TBN, TBW, and %BFat in the pre-operative and post-operative setting. As a part of the TBN measurements, anthropometric measurements were also carried out on all subjects. In this study the TBW was estimated using BIA techniques as well as the D$_2$O isotope dilution technique.

All measurements were carried out at the Body Protein Monitor Unit and the Whole Body Counter facility of the Department of Nuclear Medicine, Royal North Shore Hospital. Procedures requiring medical laboratory and laboratory equipment (including sample storage) were carried out at the Surgical Laboratories of the Department of Surgery, Royal North Shore Hospital. The only equipment utilised out side the Royal North Shore Hospital was the Fourier Transform Infra Red analysis machine which was located at the University of
Sydney’s Optical Spectroscopy section of the School of Chemistry. The facility was used under the supervision of the Optical Spectroscopy Laboratory technician.

The necessary patient information were kept at the Body Protein Monitor Unit, some as hard copies, some electronically, and some in both formats. The results of the TBN measurements, including all the anthropometric measurements, were kept electronically in a computer database (Microsoft FoxPro® version 2.5 for DOS) at the Body Protein Monitor Unit. Microsoft Access® 2000 for Windows® and later Microsoft Access® 2003 for Windows® were used to store all patients’ TBW information. Microsoft Excel® 2000 for Windows® and later Microsoft Excel® 2003 for Windows® were used for data manipulation, calculation, and some statistical analysis purposes. Other statistical analyses were also carried out using the SPSS® statistical software for Windows®.

1.2.1 Ethical Approval

This study was approved by the Royal North Shore Hospital’s Medical (Human) Research Ethics Committee and the Radiation Ethics Committee. All subjects gave informed, written consent to participate in the study.

1.2.2 Recruitment And Follow-up Process

At recruitment, all patients recruited for this study were pre-operative patients with upper gastrointestinal cancers on whom major upper gastrointestinal surgery was performed by one (i.e. the same) upper gastrointestinal surgeon at the Royal North Shore Hospital.

The recruitment process involved the referral or introduction of patient by the surgeon. The patient was then met, either at the surgeons’ rooms or the surgical ward and the trial was fully explained to them. During this meeting the purpose, conduct and, the nature of the trial was fully explained. It was also made clear that participation in the trial was voluntary and they
can drop-out of the trial at any time point. In majority of cases a family member or friend was also present. If agreeable, the patient was then asked to read and sign the trial’s consent form. The pre-operative measurements were carried out one day before the scheduled surgery. This was taken to be day= -1 with the actual surgery day to be day= 0. The post-operative day was two weeks after surgery, i.e. day= 14.

On the measurement day, the following procedures were generally followed:

1) The necessary calibration and setting-up processes for the TBN, TBK and TBW measurements were performed;

2) The patient was met at the surgical ward;

3) The patient was then escorted to the Department of Nuclear Medicine’s Whole Body Counter facility for TBK assessments. The pre-operative patients were generally mobile and, at most, required to be taken using a wheelchair. Majority of the post-operative patients required significant assistance and some required to be taken using their hospital bed;

4) Once the TBK assessments were completed, the patient was escorted to the Body Protein Monitor Unit for the TBN, anthropometry, TBF, and TBW (by BIA) measurements to be carried out.

5) Once the measurements at the Body Protein Monitor Unit was carried out, the patient was escorted back to the ward;

6) Due to the patients’ ill health, a member of medical staff was always present to assist in the transfer of patients as well as in the cases of any emergency assistance;

7) The D$_2$O dose for TBW assessment was the prepared and the relevant worksheets were completed as soon as the patient was returned to the surgical ward;

8) The D$_2$O dose was then taken to the surgical ward;

9) Patient was asked to their bladder;
10) The D\textsubscript{2}O dose was given to him/her to drink while waiting, to ensure that all the liquid dose is ingested;  
11) The nursing staff on the surgical ward were also notified;  
12) The empty D\textsubscript{2}O dose bottle was taken back and re-weighed to calculate the exact volume of D\textsubscript{2}O ingested;  
13) Depending on the time of the scheduled surgery, two blood sample were either taken the next day or approximately four hours after D\textsubscript{2}O dose administration;  
14) Depending on the time of the scheduled surgery, the fluid chart was checked the next day or approximately four hours after D\textsubscript{2}O dose administration to assess the patient’s fluid input and output;  
15) The results were then documented for the final TBW by D\textsubscript{2}O calculations.

1.2.3 Inclusion Criteria

In order to be recruited and included in this clinical trial, the patients had to fulfil the following strict criteria:

1) Patients with a pathologic diagnosis of an upper gastrointestinal cancer that required surgery;  
2) Signed informed consent.  
3) Patients with an age greater than 18 years;  
4) The ability to understand the nature of the project and to provide informed consent;  
5) Adequate haematologic and biochemical functioning, including liver function tests, permitting suitability for surgery;  
6) Life expectancy of greater than four weeks;  
7) Ability to complete the scheduled body composition measures.
1.2.4 Exclusion Criteria

Following conditions were sufficient to exclude patient from the study:

1) Patients who had received and/or were already receiving cancer chemotherapy;
2) Patients with an age less than 18 years
3) Inability to understand the nature of the project and to provide informed consent;
4) Pregnant and lactating women;
5) Serious intercurrent medical illness or psychological, familial, sociological, geographical, or other concomitant conditions that did not permit adequate follow-up and compliance with the study protocol;
6) Participation in clinical trials of other experimental agents within 30 days of study entry;
7) Women of childbearing potential not using effective contraception;
8) Radiotherapy within six weeks.

1.2.5 Body Composition Measurements

Body composition measurements and anthropometry were carried out pre-operatively one day prior to surgery (base-line) and then at 2 weeks post-operatively.

Body composition was determined by three different measures: a measure of TBN (or TBP), TBW, and TBF. The TBW was measured using the BIA technique as well as the D₂O isotope dilution technique. All measurements were non-invasive and were carried out at the Department of Nuclear Medicine, Royal North Shore Hospital. The total duration of the body composition measurements were approximately one hour.
1.2.5.1 Anthropometric And Total Body Fat Measurements

Anthropometric measurements were carried out at the Body Protein Monitor Unit of the Department of Nuclear Medicine, Royal North Shore Hospital. These measurements were considered to be part of and were taken prior to TBN measurements on the patient's non-dominant side. These measurements included skinfold thicknesses, circumferences (chest, waist, mid-thigh, mid-calf, and mid-upper-arm), height, and weight.

Skinfold thickness measurements were carried out, in triplicates, using skinfold callipers at three sites of triceps, biceps, and subscapular area, with the patient in a standing position. The equations of Durnin and Womersley [756] were then used to estimate the %BFat, using a computer program that enabled easy estimation of %BFat as well as body density.

The LBM and TBF were then calculated from weight and %BFat.

1.2.5.2 Total Body Nitrogen And Total Body Protein Measurements

The TBP was measured non-invasively using IVNCA technique [691, 698, 207]. In this technique a small dose of radiation of less than 0.2 mSv of fast neutrons was delivered to the patient. The overall radiation received by the patient was approximately one tenth of the annual background radiation. Characteristic γ-rays emitted from nitrogen and hydrogen is detected by two bilateral sodium iodide detectors. The intensity of these γ-rays, after correction for attenuation and background, allowed the TBN to be determined. The TBP could then be calculated by multiplying the TBN by a factor of 6.25 [207]. This is on the basis of 1 gram of nitrogen in 6.25 grams of protein [207]. The nitrogen index (NI) was also calculated by expressing the TBN as a percentage of age, sex, and height-matched normal [857]. These measures allowed the assessment of TBP with a precision and accuracy of 3% and 4.5%, respectively [696, 698, 207]. This technique required the patient to be lying in the supine position on a movable table for a total of approximately 20 minutes while he/she was carefully centred.
on the table followed by the table moving between the two sodium iodide detectors. The total radiation exposure from this measurement was approximately 0.2mSv and was the only measurement in this project, which involved radiation.

1.2.5.3 **Total Body Water Measurements**

TBW measurements were carried out directly after TBN measurements while the patient was still on the IVNCA bed. This test was also non-invasive and utilised the BIA technique. The BIA measurements were carried out on the patient in the supine position using two leads attached on the non-dominant hand and two leads attached on the ipsilateral foot (tetrapolar arrangement). Leads are attached to the skin using Red Dot™ Ag/AgCl Resting EKG Electrodes (3M Health Care, St Paul, MN 55144-1000). Patient's skin was required to be cleaned with alcohol swab and then dried prior to electrode attachment to ensure a good connection. Measurements were performed with the patient's arms parallel but separate to the trunk, and their legs apart far enough so their thighs were not touching.

The body's resistance was then measured in triplicates using a Swept Frequency Bioelectrical Impedance meter (SEAC® SFB23, UniQuest Limited, Queensland, Australia). The 50 kHz measurements were then used with three different equations of Kushner [747], Pullicino [755] and Fredrix [753] to calculate the patient’s TBW.

In addition, the TBW was measured using the D₂O isotope dilution technique. The technique used here involved the original method developed by Blagojevic et al [727] incorporating our own plasma preparation phase [851, 846] which was used to speed-up the overall process. The D₂O dose (1 g kg⁻¹ of LBM) was given orally as a D₂O/fruit cordial mixture. The dose was always prepared after all the rest of body composition measurements were completed. It was taken to the patient on the surgical ward and the patient was closely observed while ingesting the mixture to ensure that the patient does drink the mixture and does not simply discard it.
Preoperatively, depending on the time of that the patient was scheduled for surgery, two blood samples were either taken early the next day or approximately four hours after D$_2$O dose administration. For the post-operative patients, the blood samples were taken approximately four hours after D$_2$O dose administration. The four hour period was required for the D$_2$O dose to equilibrate with the patient’s body water producing a D$_2$O/H$_2$O mixture. The blood samples were then centrifuged for 20 minutes at 1500g and 4°C to separate the plasma which contained the D$_2$O/H$_2$O mixture.

The D$_2$O/H$_2$O mixture was separated from the plasma using our own published technique [851, 846]. The resulting D$_2$O/H$_2$O mixture was then taken to the University of Sydney’s FTIR facility at the Faculty of Chemistry to measure the D$_2$O concentration.

To reduce cost and time associated with access to FTIR facility as well as high speed centrifuges, the patients’ plasma samples were stored at -70°C and batch-processed and batch-analysed.
1.3 Pancreatic Cancer Surgery Follow-up Study

This study, also known as the “Whipple’s Procedure Study”, was carried out to investigate the survival and body composition changes in pancreatic cancer patients who underwent major surgery. This was a homogeneous patient population on whom Whipple’s Procedure (pancreaticoduodenectomy) was performed by one (i.e. the same) surgeon and the changes in survival and body composition was followed-up post-operatively for a period of 26 weeks. For further analysis, the patient population were later separated into the patients who had a clear resection margin and those who had an unclear resection margin. The survival rate and body composition changes as well as QL was then determined in each group to statistically assess the differences.

Body composition measurements were performed to investigate the changes in TBN, TBW, and %BFat in the pre-operative and post-operative setting. As a part of the TBN measurements, anthropometric measurements were also carried out on all subjects. As the use of BIA technique was validated against D₂O isotope dilution technique in surgical patients and the results were published in a peer-reviewed scientific journal [758], only the BIA techniques were used to estimate the TBW.

All measurements were carried out at the Body Protein Monitor Unit and the Whole Body Counter facility of the Department of Nuclear Medicine, Royal North Shore Hospital. The necessary patient information were kept at the Body Protein Monitor Unit, some as hard copies, some electronically, and some in both formats. The results of the TBN measurements, including all the anthropometric measurements, were kept electronically in a computer database (Microsoft FoxPro® version 2.5 for DOS) at the Body Protein Monitor Unit. Microsoft Access® 2000 for Windows® and later Microsoft Access® 2003 for Windows® were used to store all patients’ TBW information. Microsoft Excel® 2000 for Windows® and later
Microsoft Excel® 2003 for Windows® were used for data manipulation, calculation, and some statistical analysis purposes. Other statistical analyses were also carried out using the SPSS® statistical software for Windows®.

1.3.1 Ethical Approval

This study was approved by the Royal North Shore Hospital’s Medical (Human) Research Ethics Committee and the Radiation Ethics Committee (Protocol Number: 9605-067M). All subjects gave informed, written consent to participate in the study.

1.3.2 Recruitment And Follow-up Process

At recruitment, all patients recruited for this study were pre-operative patients with pancreatic cancers on whom Whipple’s Procedure (pancreateicoduodenectomy) was performed by one (i.e. the same) upper gastrointestinal surgeon at the Royal North Shore Hospital.

The recruitment process involved the referral or introduction of patient by the surgeon. The patient was then met, either at the surgeon’s rooms or the surgical ward and the trial was fully explained to them. During this meeting the purpose, conduct and, the nature of the trial was fully explained. It was also made clear that participation in the trial was voluntary and they can drop-out of the trial at any time point. In majority of cases a family member or friend was also present. If agreeable, the patient was then asked to read and sign the trial’s consent form.

The pre-operative measurements were carried out one day before the scheduled day of surgery. This was taken to be day= -1 with the actual surgery day to be day= 0. The post-operative day was two weeks after surgery, i.e. day= 14, which for most patients corresponded to the day of their discharge. The follow-up measurements were carried out at 5, 14 and 26 weeks post-operatively when the patients attended the surgeon’s rooms for their scheduled check-ups. The appointment was made at a date and time which was most
convenient for the patient to attend so as to minimise any stress and inconvenience to the patient as well as ensuring patient compliance for the duration of the trial.

All body composition and QL assessments and methods were identical at each measurement point.

On the measurement day, the following procedures were generally followed:

1) The necessary calibration and setting-up processes for the TBN, TBK and TBW measurements were performed and the necessary CRFs were prepared;

2) The patient was met at a pre-designated location usually at the surgeon’s rooms. The day=-1 and day= 14 patients (i.e. the pre-operative and two weeks post-operative patients) were met on the surgical ward;

3) The patient was then escorted to the Department of Nuclear Medicine’s Whole Body Counter facility for TBK assessments. Depending on the condition of the patient, wheelchair or hospital bed were used. Most patients in their follow-up phase (i.e. five weeks or more post-operative) did not require such assistances;

4) Once the TBK assessments were completed, the patient was escorted to the Body Protein Monitor Unit for the TBN, anthropometry, TBF, and TBW measurements to be carried out.

5) The QL questionnaire, together with a return envelope (for the follow-up patients), was given to the patient and were requested to complete and post back;

6) The patient was then escorted either back to the surgical ward or to the surgeon’s rooms (for the follow-up patients).

**1.3.3 Inclusion And Exclusion Criteria**

The same inclusion and exclusion criteria as in sections 1.2.3 and 1.2.4 were used and followed.
1.3.4 Body Composition Measurements

Body composition measurements and anthropometry were carried out pre-operatively one day prior to surgery (base-line) and then at 2 weeks post-operatively. The follow-up measurements were carried out at 5, 14 and 26 weeks post-operatively when the patients attended the surgeon’s rooms for their scheduled check-ups. Body composition was determined by three different measures: a measure of TBN (or TBP), TBW, and TBF.

1.3.4.1 Anthropometric And Total Body Fat Measurements

Anthropometric and body fat measurements were carried out as outlined in section 1.2.5.1.

1.3.4.2 Total Body Nitrogen And Total Body Protein Measurements

The TBN and TBP were measured as outline in section 1.2.5.2.

1.3.4.3 Total Body Water Measurements

TBW measurements were carried out as outlined in section 1.2.5.3.

1.3.4.4 Total Body Potassium Measurements

TBK was measured using the supine geometric sodium iodide counting \[^{[814]}\]. These measurements were carried out at the Royal North Shore Hospital at the Department of Nuclear Medicine’s Whole Body Counter facility. This test was also non-invasive and did not involve any external radiation. In fact, it relied on the natural \(^{40}\)K radiation from the body to estimate the TBK. The TBK was then used to estimate the patients' FFM on the assumption that the potassium content of FFM is 2.26 g/kg and 2.52 g/kg in women and men, respectively \[^{[204]}\].
The precision and accuracy of this technique is 1.5% and 4.5%, respectively \[818\]. This procedure involved the patient to lie on a bed in a small steel chamber for approximately 20 minutes while his/her levels of $^{40}\text{K}$ was measured. Within the steel chamber, the background radiation was reduced to a minimum.
1.4 Thalidomide In Mesothelioma Clinical Trial

Malignant mesothelioma is a devastating disease. Most patients present with advanced or inoperable disease. The national rate of malignant mesothelioma is one of the highest in the world and NSW has the largest number of cases in Australia *. Unfortunately, the incidence of malignant mesothelioma is increasing in NSW. In 1999 the incidence rate was 3.2 cases per 100,000 person years *. Most cases are asbestosis related and the latency from exposure to diagnosis is measurable in decades, usually around 40 years. This accounts for the mean age of presentation in NSW being 66 years. Unfortunately, survival from diagnosis is short with a median in NSW of only 40 weeks and in NSW between 1985 and 1995, only 15% of 503 cases survived more than two years *. Survival has been demonstrated to be dependent on specific prognostic factors and can vary from four to 18 months depending on these factors *. Significant poor prognostic factors have been shown to be male sex, older age, weight loss, chest pain, poor performance status, low haemoglobin, leucocytosis, thrombocytosis and non-epithelial cell type [862, 504]. Most patients experience progressive symptoms and rapid deterioration in QL.

(* Dust Diseases Board Mesothelioma, Australia official statistics).

Rationale

Thalidomide has been shown to inhibit bFGF and VEGF mediated angiogenesis as well as affect the immune system by modulating cytokine release, in particular IL-6 and TNF-α [516, 515, 863, 657]. It has been shown to produce palliative benefits in HIV and in cancer-associated anorexia-cachexia and has demonstrated anti-tumour activity and palliative benefit in some patients with advanced solid tumours [864]. In animal studies it has been shown to have an analgesic effect, presumably by modulating cytokine release [865]. Its role in palliating the
tumour wasting syndrome as seen in advanced malignant mesothelioma is yet to be established.

Preclinical studies evaluating chemotherapy and anti-angiogenic drug combination therapy have in almost all instances demonstrated enhanced anti-tumour activity without additional toxicity \cite{866}. Low-dose scheduling of cisplatin has itself been demonstrated to have an anti-angiogenic affect. Anti-angiogenic agents combined with low dose “anti-angiogenic” scheduled chemotherapy has been shown to overcome conventional drug resistance in animal models, by targeting tumour associated endothelial cells\cite{868, 867, 866}. The rationale for combining thalidomide with low dose intermittent cisplatin/gemcitabine is the hope of greater activity with reduced toxicity. It is also hypothesized that this combination will have additive symptomatic/palliative benefit in patients with malignant mesothelioma.

The primary objective of this trial was, therefore, to determine the effect(s) on nutritional status of patients (as measured by TBN) with advanced malignant mesothelioma treated with thalidomide alone or in combination with cisplatin and gemcitabine and to assess the value of nutritional status as a prognostic marker and in predicting toxicity.

All measurements for this clinical trial were carried out at the Body Protein Monitor Unit and the Whole Body Counter facility of the Department of Nuclear Medicine, Royal North Shore Hospital.

The necessary patient information were kept at the Body Protein Monitor Unit, some as hard copies, some electronically, and some in both formats. The results of the TBN measurements, including all the anthropometric measurements, were kept electronically in a computer database (Microsoft FoxPro® version 2.5 for DOS) at the Body Protein Monitor Unit. Microsoft Access® 2000 for Windows® and later Microsoft Access® 2003 for Windows® were
used to store all patients’ TBW information. Microsoft Excel® 2000 for Windows® and later Microsoft Excel® 2003 for Windows® were used for data manipulation, calculation, and some statistical analysis purposes. Other statistical analyses were also carried out using the SPSS® statistical software for Windows®.

1.4.1 Ethical Approval

This study was approved by the Royal North Shore Hospital’s Medical (Human) Research Ethics Committee and the Radiation Ethics Committee. All subjects gave informed, written consent to participate in the study.

1.4.2 Recruitment And Follow-up Process

All patients were recruited by the Department of Medical Oncology, Royal North Shore Hospital’s medical, nursing, and data manager staff.

The patients recruited for this trial attended the out-patients clinic of the Department of Medical Oncology. The recruitment process involved the medical oncologist interviewing and examining the patient and the trial being fully explained to them. During this meeting the purpose, conduct and, the nature of the trial was fully explained. It was also made clear that participation in the trial was voluntary and they can drop-out of the trial at any time point. In majority of cases a family member or friend was also present. If the patient satisfied the inclusion criteria (see below), he/she was then referred to nursing and/or data manager staff who would in turn liaise with and carry out all the necessary measurements. The nursing and/or data manager staff were also responsible for escorting the patients to the BPM facility for full body composition measurements and analysis. If agreeable, the patient was then asked to read and sign the trial’s consent form.
The pre-operative measurements were carried out prior to the first chemotherapy cycle. The subsequent or follow-up measurements were carried out at the beginning of each chemotherapy cycle. The follow-up appointments were made at a date and time which was most convenient for the patient to attend so as to minimise any stress and inconvenience to the patient as well as ensuring patient compliance for the duration of the trial.

All body composition and QL assessments and methods were identical at each measurement point.

1) On the measurement day, the following procedures were generally followed:

2) The necessary calibration and setting-up processes for the TBN and TBW measurements were performed;

3) The patient was met at a pre-designated location by the oncology nurse/data manager;

4) The patient was then escorted to the Department of Nuclear Medicine’s Body Protein Monitor Unit for the TBN, anthropometry, TBF, and TBW measurements to be carried out;

5) The patient was then escorted either back to the Department of Medical Oncology’s out-patients clinic or to the oncology specialist’s rooms, as required.

1.4.3 Study Design

This study involved two parallel open-label single institution Phase II trials. The choice of trial for a given patient depended on their overall fitness and trial eligibility criteria. There was no randomization involved.

Schema of treatment protocols was offered to patients with inoperable or relapsed advanced malignant mesothelioma referred to the Department of Medical Oncology, Royal North Shore Hospital or other participating centres. Patients were considered for either arm A or arm B
depending on their suitability and eligibility for either study as indicated below. Patients considered unfit or ineligible for both studies were treated palliatively according to best local practice.

Patient with advanced malignant mesothelioma considering trial participation.

Does the patient fulfil the eligibility criteria for study A without exclusions being met?  

Arm A-Thalidomide and cisplatin/gemcitabine

Yes  

No

Does the patient fulfil the criteria for inclusion into study B, without exclusions being met?  

Standard palliative treatments at the discretion of the treating physician.

No  

Yes

Arm B Thalidomide alone.

Arms A and B were conducted as standard Phase II studies.

1.4.3.1 Patient Selection

Any patient with advanced malignant mesothelioma referred to a medical oncologist within the Department of Medical Oncology, Royal North Shore Hospital and other participating centres was considered for this study. Eligible consenting patients had their chemotherapy administered in the well-established oncology outpatients’ clinics of Royal North Shore Hospital and the Northern Cancer Institute of North Shore Private Hospital. All assessments were performed on an outpatient basis.

Written informed consent was obtained from each patient after the study was fully explained.
1.4.4 Inclusion Criteria

In order to be recruited and included in this clinical trial, the patients had to fulfil all of the following common criteria and criteria specific to arm A or arm B for inclusion into the arm A or arm B, respectively. The revised TNM staging system of the International Mesothelioma Interest Group was used to stage patient’s disease. Performance status was assessed using the ECOG criteria.

1.4.4.1 Common Inclusion Criteria

In order to be recruited and included in the clinical trial, the patients were required to satisfy the following inclusion criteria:

1) Pathologically (histological or cytological) confirmed advanced inoperable (any stage) or recurrent malignant pleural mesothelioma.

2) Adequate underlying renal function (creatinine <1.5 times the ULRR or creatinine clearance >60 ml/min) hepatic and bone marrow function.

3) Adequate hepatic function with serum bilirubin ≤1.5 times ULRR, ALT or AST ≤2.5 times the ULRR if no liver metastases or ≤5 times the ULRR in the presence of liver metastases.

4) Adequate bone marrow reserve with absolute neutrophil ≥ 2.0 x 10^9 / L or platelet count of ≥100 x 10^9 / L.

5) Ability to complete QL questionnaire and attend clinic for assessments, including TBN and body composition assessments.

6) Life expectancy greater than eight weeks.
7) Measurable disease on CT imaging.

8) Written informed consent to participate in this study.

### 1.4.4.2 Inclusion Criteria Specific For Arm A

1) Age 18 to 75 years.

2) No prior systemic cytotoxic chemotherapy.

3) ECOG performance status ≤2.

### 1.4.4.3 Inclusion Criteria Specific For Arm B

1) Age 18-80.

2) Prior cytotoxic chemotherapy or medically unfit for chemotherapy.

3) ECOG performance status ≤2.

### 1.4.5 Exclusion Criteria

Following conditions were sufficient to exclude patient from the study:

#### 1.4.5.1 Common Exclusion Criteria

If any of the patients satisfied one or more of the following criteria, they were automatically excluded from the clinical trial:

1) Immunotherapy or investigational therapy other than chemotherapy within the last six weeks.

2) Less than three weeks since prior radiotherapy.

3) Brain metastases or spinal cord compression that has not been stable for at least two
APPENDIX E: Selection, Recruitment And Measurement Protocols

months since prior surgery and/or radiotherapy.

4) Pre-existing motor or sensory neuropathy ≥CTC grade 2.

5) Absolute neutrophil count less than $2.0 \times 10^9 / \text{L}$ or platelet count less than $100 \times 10^9 / \text{L}$.

6) Serum bilirubin greater than 1.5 times the ULRR.

7) ALT or AST greater than 2.5 times the ULRR if no liver metastases or greater than 5 times the ULRR in the presence of liver metastases.

8) Serum creatinine greater than 1.5 times the ULRR or creatinine clearance ≤ 60 ml/min.

9) Planned or current pregnancy or breast feeding.

10) Any severe or uncontrolled systemic disease or psychiatric illness e.g. uncompensated or unstable cardiac failure, respiratory, renal or liver disorder or uncontrolled significant active infections.

11) Evidence of any other significant clinical disorder or laboratory finding that makes it undesirable for the patient to participate in the trial.

12) History of other malignancy within the last five years that could affect the diagnosis or assessment.

1.4.5.2 Exclusion Criteria Specific For Arm A

1) Creatinine clearance ≤ 60 ml/min.

2) Greater than trace of blood or protein on repeat urinalysis on dipstick.

3) Hypersensitivity to mannitol.

4) Patients with recent documented myocardial infarction or stroke within 12 months of study entry or severe or unstable angina pectoris or recurrent transient ischaemic
attacks.

5) Patients with recent coronary artery bypass grafting, percutaneous coronary or carotid stenting with 12 months of study entry.

6) Patients with diabetes mellitus with clinical evidence of severe peripheral vascular disease.

1.4.5.3 Patient Restrictions

Warnings about the teratogenic effects of thalidomide were given, and women who may have been pregnant or intending to become pregnant were considered to be ineligible and, therefore, were excluded from the study. Otherwise it was a requirement of recruitment into the study that sexually active women and men use contraception to prevent pregnancy.

1.4.6 Materials And Supplies

Gemcitabine HCl (Gemzar®) is a nucleoside analogue that inhibits tumour activity. It is available commercially and is supplied as a 200mg or 1000mg white, lyophilized powder in sterile single use vials.

Cisplatin is available commercially and supplied as sterile lyophilized white powder in single-dose vials containing 10 mg or 50 mg of cisplatin for intravenous administration.

Thalidomide was purchased directly from Penn Pharmaceuticals. It came as a 100 mg capsule. Quantities of thalidomide were dispensed on a month by month basis by the Royal North Shore Hospital pharmacy. Unused thalidomide capsules were asked to be returned.
1.4.7 Body Composition Measurements

Body composition measurements and anthropometry were carried out pre-chemotherapy (base-line) and then at the beginning of each chemotherapy cycle. The follow-up measurements were carried when the patients attended the out-patients clinic for their scheduled chemotherapy treatment.

Body composition was determined by three different measures: a measure of TBN (or TBP), TBW, and TBF. The TBW was measured using the BIA technique. All measurements were non-invasive and were carried out at the Department of Nuclear Medicine, Royal North Shore Hospital.

1.4.7.1 Anthropometric And Total Body Fat Measurements

Anthropometric and body fat measurements were carried out as outlined in section 1.2.5.1.

1.4.7.2 Total Body Nitrogen And Total Body Protein Measurements

The TBN and TBP were measured non-invasively as outlined in section 1.2.5.2.

1.4.7.3 Total Body Water Measurements

TBW measurements were carried out using BIA techniques directly after TBN measurements as outlined in section 1.2.5.3 while the patient was still on the IVNCA bed.

1.4.8 Study drug administration

All patients received their thalidomide commencing at a dose of 100 mg nocte. Dose escalation occurred weekly by 100 mg until 500 mg or five capsules was reached or until maximum tolerated dose. The maximum tolerated dose was defined as the dose of thalidomide at which there was no intolerable (≥ CTC grade 2) neurotoxicity or other toxicity.
Patients in arm A will also receive Gemcitabine 800mg/m² intravenously over 30 to 60 minutes on days 1, 8 and 15 and Cisplatin 25mg/m² intravenously on days 1, 8 and 15, to be repeated every 28 days for a maximum of six cycles. Chemotherapy only commenced after a one-week run in period with thalidomide alone at 100 mg nocte. Patients remained on this dose of thalidomide during the week one of chemotherapy before being considered for dose escalation at the end of the second week. Patients in arm A could continue on thalidomide after completion of chemotherapy until disease progression.

All patients received cisplatin with a program of forced diuresis, including mannitol and intravenous fluid with normal saline and magnesium supplementation, in keeping with the current protocol for cisplatin administration at the Royal North Shore Hospital.

Eligible patients with a documented vascular history were permitted to continue on their anti-platelet or anti-coagulant therapy.

All patients who received cisplatin also received a prophylactic dose of heparin (5000 units) subcutaneously at completion of their cisplatin infusion.

### 1.4.8.1 Dose Reductions And Modifications

There was no increase of either gemcitabine or cisplatin dose for any reason, eg. weight gain. However dose reduction for weight loss or toxicity was recommended and carried out as required. Dose reductions were made according to the system with the greatest toxicity.

Toxicities were graded using the National Cancer Institute CTC.

If toxicity did occur, appropriate supportive treatment was instituted to ameliorate signs and symptoms. A maximum of two dose reductions for cisplatin and gemcitabine was allowed. Patients requiring further dose reductions were withdrawn from the study.
Patients intolerant of 100mg of thalidomide were withdrawn from the study.

1.4.9 Serious Adverse Events

An adverse event was defined and considered to be any untoward medical event that occurs in conjunction with the use of the drug thalidomide or its combination with the known protocol of chemotherapy cisplatin and gemcitabine, whether or not it is considered drug related.

Serious adverse events are defined below:
1.5 EPA Clinical Trial Protocols

The EPA Trial (also known as the “Fish Oil Trial”) was a double-blind, placebo-controlled, randomized clinical trial where patients with pancreatic cancer were treated with Gemcitabine alone (Gemcitabine plus placebo) or in a combination regimen with EPA (Gemcitabine plus EPA). For the whole study a minimum number of 30 patients in total were initially anticipated (15 in each arm).

Body composition measurements were performed to investigate the changes in TBN, TBW, FFM, and %BFat during the course of cancer chemotherapy for pancreatic cancer. As a part of the TBN measurements, anthropometric measurements were also carried out on all subjects.

All measurements were carried out at the Body Protein Monitor Unit and the Whole Body Counter facility of the Department of Nuclear Medicine, Royal North Shore Hospital.

The necessary patient information were kept at the Body Protein Monitor Unit, some as hard copies, some electronically, and some in both formats. The results of the TBN measurements, including all the anthropometric measurements, were kept electronically in a computer database (Microsoft FoxPro® version 2.5 for DOS) at the Body Protein Monitor Unit. Microsoft Access® 2000 for Windows® and later Microsoft Access® 2003 for Windows® were used to store all patients’ TBK and TBW information. Microsoft Excel® 2000 for Windows® and later Microsoft Excel® 2003 for Windows® were used for data manipulation, calculation, and some statistical analysis purposes. Other statistical analyses were also carried out using the SPSS® version 14 statistical software for Windows®.
1.5.1 Ethical Approval And Clinical Trial Registration

This clinical trial was approved by the Royal North Shore Hospital’s Medical (Human) Research Ethics Committee and the Radiation Ethics Committee (HREC Protocol No: 0303-067M). Approval was also obtained from the North Shore Private Hospital.

This clinical trial was also registered with the Australian Clinical Trials Registry (Registration Number: ACTRN012607000323426).

1.5.2 Recruitment And Follow-up Process

Majority of the patients recruited for this clinical trial were post-operative patients on whom pancreatic resection was performed by either of the two upper gastrointestinal surgeons at the Royal North Shore Hospital or the North Shore Private Hospital.

The recruitment process involved the referral of patient by one of the surgeons or oncologist prior to the commencement of Gemcitabine chemotherapy. The patient was then met, either at the surgeons’ rooms, surgical ward, or at the chemotherapy clinic, and the trial was fully explained to them. During this meeting the purpose, conduct and, the nature of the trial (i.e. double-blind, placebo-controlled, and randomised) was fully explained. It was also made clear that participation in the trial was voluntary and they can drop-out of the trial at any time point. In majority of cases a family member or friend was also present. If agreeable, the patient was asked to read and sign the trial’s consent form. An information sheet detailing the trial procedure was also provided. The patient was then asked to contact the office as soon as they have been notified (by the chemotherapy clinic) of the date and time of their first chemotherapy so that an appointment for body composition measurements could be made. The appointment was made at a date and time which was most convenient for the patient to attend so as to minimise any stress and inconvenience to the patient as well as ensuring patient compliance for the duration of the trial.
On the measurement day, the following procedures were generally followed:

1) The necessary calibration and setting-up processes for the TBN, TBK and TBW measurements were performed and the necessary CRFs were prepared;

2) The patient was met at a pre-designated location;

3) The patient was escorted to the Department of Nuclear Medicine’s Whole Body Counter facility for TBK assessments;

4) If the patient was being measured for the first time, the Department of Clinical Oncology’s designated data manager was contacted by telephone and the patient’s full name, date of birth, sex, hospital medical record number, ECOG status, and disease status (locally advanced or metastatic) was provided for randomisation purposes;

5) Once the randomisation process was completed, the data manager would automatically contact the hospital’s pharmacy so that the correct capsules would be dispensed and ready to be pick-up. The dispensed capsules, depending on the randomisation, were either EPA or placebo.

6) Once the TBK assessments were completed, the patient was escorted to the Body Protein Monitor Unit for the TBN, anthropometry, TBF, and TBW measurements to be carried out.

7) At the Body Protein Monitor Unit, in addition to the above mentioned body composition measurements, the patient’s vital signs, symptoms, performance status, and pain levels were also assessed. These were recorded on the appropriate case report forms and charts and the measurement check list completed;

8) The pharmacy was contacted by telephone to ensure the capsules are being dispensed;

9) Once the measurements at the Body Protein Monitor Unit was carried out, an appointment for next cycle’s measurements was made and the date and time written for the patient;
10) The QL questionnaire together with a return envelope was given to the patient and were requested to complete and post back;

11) A completed pathology request form was given for the required blood tests to be carried out and the patient was requested to have the blood tests done as soon as possible;

12) The patient was then escorted to the pharmacy where the capsules were picked-up. If this was not the base-line measurement, last cycle’s capsule jar was also returned to the pharmacy;

13) The patient was then escorted either to the pathology laboratory (for blood tests) or back to the meeting place.

1.5.3 Randomisation Process

Randomisation took place centrally at the Royal North Shore Hospital's Department of Medical Oncology by computer at a 1:1 ratio to receive Gemcitabine alone (plus placebo) or combined with EPA (Gemcitabine plus EPA).

Eligible patients will be stratified at inclusion according to age (≥ 70 and <70 years), ECOG performance status (0, 1, 2), and locally advanced or metastatic. After having checked the patient’s eligibility criteria, patient’s randomisation was requested by telephone or fax. Computer generated randomisation logs were used for each stratum. Patients were allocated to his/her stratum and were randomly assigned to the therapeutic arm according to the next consecutive number in the stratum log.

In order to ensure that the double-blind and randomised nature of the trial is enforced and maintained, only the designated Department of Clinical Oncology data manager randomising patients and the hospital’s research pharmacist dispensing the capsules were aware of the randomisation code. Also, the white plastic one litre container containing the EPA or placebo capsules were all identical regardless of its contents. The containers were labelled only with
the patient’s full name, hospital medical record number, trial number, and appropriate instructions on administrating the capsules.

To ensure compliance, each cycle’s dispensed capsule container contained a variable number of capsules. The number of capsules was always well in excess of what was actually required by the patient for the cycle (four weeks) period, the exact number of which was only known to the designated research pharmacist. When the patient returned the used container to the pharmacy, the pharmacist then counted and hence determined the exact number of capsules taken by the patient during that particular cycle.

Based on the studies of Barber et al \cite{228, 251, 253, 252} and Wigmore et al \cite{249, 605, 250} reported in the literature on pancreatic cancer patients, we expected 50\% of patients in the placebo arm to have a minimum of 15\% weight loss. With the administration of Fish Oil, we expected only 10\% of patients (i.e. an absolute improvement of 40\%) receiving fish oil treatment arm to have 15\% weight loss. In order for the study to have a power of 80\% to yield a statistically significant result, a sample size of 25 patients in each arm was required. The criterion for significance (alpha) was set at 0.05. Therefore for this study, a sample size of 30 patients for each study arm (total of 60) was initially proposed to also allow for any drop-outs, although a minimum of 50 patients is required overall.

1.5.4 Inclusion Criteria

In order to be recruited and included in this clinical trial, the patients had to fulfil the following strict criteria:

1) Patients with a pathologic diagnosis of pancreas cancer that was locally advanced and unresectable, partially resected or metastatic;

2) Signed informed consent;
3) Patients with an age greater than 18 years;
4) The ability to understand the nature of the project and to provide informed consent;
5) Patients with ECOG performance status of ≤ 2, as determined by the referring physician;
6) Patients who were more than four weeks post-operative or post radiotherapy;
7) Adequate haematologic and biochemical functioning, including liver function tests, permitting suitability for Gemcitabine chemotherapy;
8) Life expectancy of greater than 16 weeks;
9) Ability to complete the scheduled body composition measures;
10) Ability to complete the QL questionnaire.

1.5.5 Exclusion Criteria

Following conditions were sufficient to exclude patient from the study:

1) Patients who had received and/or were already receiving chemotherapy for pancreatic cancer;
2) Patients with an age less than 18 years;
3) Inability to understand the nature of the project and to provide informed consent;
4) Progressing or untreated brain metastases;
5) Pregnant and lactating women;
6) Serious intercurrent medical illness or psychological, familial, sociological, geographical, or other concomitant conditions that did not permit adequate follow-up and compliance with the study protocol;
7) Participation in clinical trials of other experimental agents within 30 days of study entry;
8) Women of childbearing potential not using effective contraception;
9) Radiotherapy within six weeks;

10) Surgery within four weeks.

### 1.5.6 Termination Criteria

Pancreatic cancer and its chemotherapy not only rapidly reduces the patients’ QL, it also has a relatively short median survival [869, 296]. Therefore, Subjects were withdrawn if:

1) They were unwilling to continue with the study;

2) They moved and started living too far from the Royal North Shore Hospital, thus inconvenient for them to attend the hospital;

3) Intolerance of study medication due to the progress of disease.

### 1.5.7 Study Discontinuation

The following events were considered to be sufficient reason for discontinuing treatment:

1) Disease progression;

2) Development of unacceptable or recurrent grade 3 - 4 toxicity despite dose modification as directed by the protocol, or the occurrence of a single episode of grade 3 – 4 neurotoxicity;

3) Treatment delay of greater than three weeks;

4) Administration of any other experimental drug during the trial not specified within the study protocol;

5) Significant protocol violations including the appearance of any of the exclusion criteria not related to toxicities of the study drugs (i.e. development of a significant psychiatric disorder which would affect the status of the patient’s informed consent);

6) Conditions requiring therapeutic intervention not permitted by the protocol;
7) Consent withdrawn by the patient;
8) Any other situation where, in the opinion of the investigator, continued participation in the study would not be in the best interest of the patient;
9) Confirmed pregnancy in a study subject during the course of the trial.

1.5.8 Chemotherapy And EPA

All treatments and measurements for this clinical trial were carried out within the Royal North Shore Hospital and/or the North Shore Private Hospital. Gemcitabine was administered in an outpatient setting. Drug ordering followed the PBS guidelines. The patient was discharged after the infusions were completed and after the standard period of observation. In no instances in this trial, Gemcitabine was administered in an inpatient setting.

All patients received the same standard chemotherapy regimen of Gemcitabine (1000mg/m² iv D1, 8, 15, Q4 weekly). Treatment/placebo administrations were carried out during the first 16 weeks of the chemotherapy.

1.5.8.1 EPA And Placebo

The EPA and placebo (Soya oil) were dispensed by the Department of Pharmacy, Royal North Shore Hospital. The EPA or placebo capsules for a period of four weeks were given to the patient by the hospital pharmacy and instructed to be taken home and taken orally. In addition, the patients were instructed to return the empty capsule containers with any remaining capsules.

The doses of EPA used in various clinical studies involving cachexia or weight loss in pancreatic cancer patients reported in the literature ranges from 2g/day [251, 252] and 2.2g/day [251, 250] to 6g/day [249]. The effects and side effects of these dose levels were, somewhat,
similar. In our personal communications with Prof Michael J Tisdale, (a leading authority from the UK on EPA and fish oil administration in pancreatic cancer patients) with regards to the safe and effective dose of EPA to give our cancer patients, it was suggested that: "the minimum effective dose is 2g EPA per day, (not total fatty acids; DHA has no effect) and there is no toxicity up to 6g EPA per day. Patients would require this dose daily throughout the study. It would not be sufficient to administer it at the start of chemotherapy and at 6 and 12 weeks post chemotherapy. We have found that patients can take the EPA themselves at home."

In our clinical trial, EPA (2.7g/day) was given orally as soft gel capsules for 16 weeks commencing on the first day of chemotherapy.

Our primary reason for choosing to administer high-purity EPA preparations instead of EPA as a part of an oral nutritional supplement was to allow the effects measured and/or observed in the trial to be attributed to the EPA rather than to a cocktail of nutritional supplement mixture and/or various fatty acids. In addition, the currently available protein and energy-enriched nutritional supplements containing EPA and other omega-3 fatty acids (such as Prosure® and Ensure®) were found to have low concentrations of EPA (approximately 1g) per 250ml can which meant that patients were required to drink a minimum of 2.5 cans per day. Since most pancreatic cancer patients have poor appetite as well as nausea and vomiting due to the presence of the tumour and the administration of chemotherapy, the ingestion of 2.5 cans of the often rich nutritional supplements would have been almost impossible.

1.5.8.2 Treatment And Placebo Arm Regimens

In the treatment arm, Gemcitabine was administered iv (1000mg/m² iv D1, 8, 15, Q4 weekly) over 30 minutes. EPA (2.7g/day) was then taken orally by the patient as 7 capsules per day. The administration of EPA continued for the next 4 weeks of the Gemcitabine chemotherapy.
The cycle was then repeated until the four cycles were completed. This, therefore, resulted in a total of 16 weeks of Gemcitabine plus EPA therapy and a total of five body composition and QL measurements.

In the placebo arm, Gemcitabine was administered iv (1000mg/m² iv D1, 8, 15, Q4 weekly) over 30 minutes. Placebo capsules (containing Soya oil) were taken orally by the patient as 7 capsules per day. The administration of placebo continued for the next 4 weeks of the Gemcitabine chemotherapy. The cycle was then repeated until the four cycles were completed. This, therefore, resulted in a total of 16 weeks of Gemcitabine plus placebo therapy and a total of five body composition and QL measurements.

1.5.9 Body Composition And Quality Of Life

Body composition measurements, anthropometry and QL measurements were carried out prior to chemotherapy (base-line) and then at 4, 8, 12, and 16 weeks post-chemotherapy.

Body composition was determined by three different measures: a measure of TBN (or TBP), TBW, and TBF. All measurements were non-invasive and were carried out at the Department of Nuclear Medicine, Royal North Shore Hospital. The total duration of the body composition measurements was approximately 1.5 hours.

1.5.9.1 Anthropometric And Total Body Fat Measurements

Anthropometric and body fat measurements were carried out as outlined in section 1.2.5.1.

1.5.9.2 Total Body Nitrogen And Total Body Protein Measurements

The TBN and TBP were measured non-invasively as outlined in section 1.2.5.2.
1.5.9.3 **Total Body Water Measurements**

TBW measurements were carried out using BIA techniques directly after TBN measurements as outlined in section 1.2.5.3 while the patient was still on the IVNCA bed.

1.5.9.4 **Total Body Potassium Measurements**

The TBK were measured non-invasively using the Whole Body Counting technique as outlined in section 1.3.4.4.

1.5.9.5 **Quality Of Life Measurements**

The QL of these pancreatic cancer patients undergoing chemotherapy was assessed using the EORTC’s QLQ-C30 core instrument together with their pancreatic cancer module QLQ-PAN26 \[841, 840, 839\]. The QLQ-C30 core questionnaire consisted of 30 questions and the QLQ-PAN26 pancreatic module consisted of an additional 26 questions. The two questionnaires were stapled together and given to the patient to complete at home. They were instructed to answer the questions with their last four weeks’ QL and feelings in mind. A return envelope was also provided for the patients to return their completed questionnaire.

Results were then analysed according to the strict guidelines and instructions provided directly by the EORTC, Southampton, UK.

1.5.10 **Blood Tests And Vital Signs**

Blood tests were carried out at the same time points as the body composition and QL assessments, i.e. prior to the start of chemotherapy (base-line) and then at 4, 8, 12, and 16 weeks after the start of chemotherapy. The blood test carried out were the haematology (full blood counts), biochemistry, liver function tests, glucose, calcium, magnesium, phosphates, CRP, CEA, and CA 19.9.
While at the Body Protein Monitor Unit, the heart rate and blood pressure were also measured. An electronic automatic blood pressure monitor (Omron® Model T5, Omron Health Care Co. Ltd, 24 Yamanouchi, Yamanoshita-cho, Ukyo-ku, Kyoto 615-0084, Japan) was used to avoid operator bias. The vital signs were recorded on the patient’s symptoms case report form.
APPENDIX F: EPA & Placebo Data Sheets

Product Data

ROPUFA® ‘75’ n-3 EE

Product identification
Refined ethyl esters of fish oil
Eicosapentaenoic acid ethyl ester
Docosahexaenoic acid ethyl ester
Product code: 50 0249 4

Description
ROPUFA ‘75’ n-3 EE is a yellowish liquid. It contains at least 75% n-3 polyunsaturated fatty acids (PUFAs) in the form of ethyl esters, predominantly as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is treated and stabilized with rosemary extract, ascorbyl palmitate, mixed tocopherols and citric acid.

Chemical structure

Eicosapentaenoic acid (EPA) ethyl ester
\[\text{C}_{22}\text{H}_{34}\text{O}_{3}\]
\[M, 330.5\]
CAS No.: 86227-47-6
Chemical name: eicosapenta-5,8,11,14,17-enoic acid ethyl ester

Docosahexaenoic acid (DHA) ethyl ester
\[\text{C}_{22}\text{H}_{36}\text{O}_{4}\]
\[M, 356.5\]
CAS No.: 81926-94-5
Chemical name: docosahexa-4,7,10,13,16, 19-enoic acid ethyl ester

Specifications
Appearance: yellowish liquid
Acid value: max. 3 mg KOH per gram
Peroxide value: max. 10.0 mEq/kg
Anisidine value: max. 20
Oligomers: max. 2%
Conjugated dienes: max. 1.5%
Iron: max. 1 ppm
Copper: max. 0.1 ppm
Lead: max. 0.1 ppm
Arsenic: max. 0.1 ppm

EPA content (gas chromatography): min. 42%
EPA content (weight as ethyl ester): min. 380 mg/g
DHA content (gas chromatography): min. 22%
DHA content (weight as ethyl ester): min. 200 mg/g
Total content of n-3 PUFAs (gas chromatography): min. 75%
Total content of n-3 PUFAs (weight as ethyl ester): min. 720 mg/g

Stability and storage
ROPUFA ‘75’ n-3 EE is sensitive to air, heat, light and humidity. The product may be stored for 18 months from the date of manufacture in the unopened original container (which is sealed under inert gas) and at a temperature below 15 °C. The ‘best used before’ date is printed on the label. Keep container tightly closed. Once opened, use contents quickly.

Uses
For health food supplements.
Product Data

ROPUFA® ‘75’ n-3 EE

Safety
This product is safe for the intended use.
For full safety information and necessary precautions, please refer to the respective Roche Safety Data Sheet.

The information given in this publication is based on our current knowledge and experience, and may be used at your discretion and risk. It does not relieve you from carrying out your own precautions and tests. We do not assume any liability in connection with your product or its use. You must comply with all applicable laws and regulations, and observe all third party rights.

Roche Vitamins Ltd
CH-4070 Basel (Switzerland)
Tel. +41 61 68 73030
Fax +41 61 68 81592
Product Information

ROPUFA ’75’ n-3 EE

“Roche” Specifications and Tests

1. **Appearance:** yellowish liquid

2. **Acid-value:** max. 3.0 mg KOH/g
   Proceed according to the general method described in the Ph. Eur.

3. **Peroxide value:** max. 10.0 mEq/kg
   Proceed according to the general method described in the Ph. Eur. This test is the first to be done after the opening of the sample.

4. **P-Anisidine value:** max. 10

**Definition**

The p-anisidine value is defined as 100 times the absorbance measured in a 1 cm cell of a solution containing 1.00 g of the oil in 100 ml of a mixture of solvent and reagent according to the method described.

**Principle**

Reaction in an acetic solution of the aldehydic/ketonic compounds in oil with p-anisidine, followed by determination of the absorbance at 350 nm.

**Reagents**

Hexane, HPLC grade.

Glacial acetic acid, HPLC grade.

Moisture content less than 0.1 percent by Karl Fischer determination.

p-Anisidine AR, 0.025 g/100 ml glacial acetic acid.

The moisture content of the reagent should be checked before use each day and must be less than 0.1% by Karl Fischer determination.
**Procedure**

The sample should be clear and dry.

Determine the sample size according to the following table:

<table>
<thead>
<tr>
<th>Expected p-Anisidine value</th>
<th>Test sample (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>5</td>
</tr>
<tr>
<td>5-10</td>
<td>3</td>
</tr>
<tr>
<td>10-20</td>
<td>2</td>
</tr>
<tr>
<td>20-30</td>
<td>1</td>
</tr>
</tbody>
</table>

Weigh the sample to the nearest 0.1 mg into a 25 ml volumetric flask. Dissolve and dilute to volume with hexane.

Zero the spectrophotometer for transmission and absorbance. Measure the absorbance difference between the two empty cells ($A_0$). Measure the absorbance ($A_s$) of the solution at 350 nm in a cell using the reference cell filled with solvent as the blank. Fill to volume a 5 ml volumetric flask with the oil solution.

Repeat with solvent in a second 5 ml, volumetric flask. Add 1 ml (Gilson micro-pipette) of the p-anisidine reagent to each flask and mix well. After exactly 10 minutes measure the absorbance ($A_s$) of the solution in the first flask in a cell at 350 nm, using the solution from the second flask as a blank in the reference cell.

Correct $A_0$ and $A_s$ for the absorbance difference $A_0$, between the two cells.

**Expression of Results**

The p-anisidine (p-AV) value is given by the formula:

$$p-AV = \frac{25 \times (1.2 A_s - A_0)}{m}$$

Where $m$ is the mass of the test sample (in g).

**5. Iron, Copper, Lead, Arsenic:**

Iron: max. 1 ppm  
Copper: max. 0.1 ppm  
Lead: max. 0.1 ppm  
Arsenic: max. 0.1 ppm

Measured by Graphite Furnace Atomic Absorption (method available on request).
6. Oligomers: max. 2.0 %
Proceed according to the general method described in the Ph.Eur.

7. Conjugated dienes: max. 1.5 %
Proceed according to the general method described in the Ph.Eur.

8. Fatty acid composition:
Proceed according to AOCS Official Method Ce 1b-89

Total content of n-3 PUFAs: min. 720 mg/g min. 75% (gas chromatography)
EPA content: min. 380 mg/g min. 42% (gas chromatography)
DHA content: min. 200 mg/g min. 22% (gas chromatography)

Operating Conditions
CAPILLARY COLUMN
Manufacturer: J. & W. Scientific
Liquid phase: DB225
Length x I.D.: 30 m x 0.25 mm
Film thickness: 0.25 micron
Column material: Fused silica

CARRIER GAS
Helium: BOC Grade A
Inlet Pressure: 170 kPa

TEMPERATURE
Column temperature programme:
170°C
170°C to 240°C at 1.5°C/min
hold 13.33 mins

Total run time: 60 min.
Injector temperature: 270°C
Detector temperature: 270°C

SAMPLE
Injection mode: Split
Injection volume: 2 microlitres
Concentration: 1.5 - 2.0% (w/v)
ROPUFA '75' n-3 EE

"Roche" Specifications and Tests

Typical sample chromatogram
APPENDIX F: EPA & Placebo Data Sheets

SOFTGEL PRODUCT SPECIFICATION

Customer: University of Sydney (Prof. Ross Smith)  Code: MIKTOP
Product Name: Neutral Taste Ropufa Softgel Capsule  Version: 26th February, 2004

Size: 22 Minim  Shape: Oblong  Print: Not Printed
Fill Colour: Clear Pale Yellow solution  Shell Colour: Clear Natural (CLR)
Weights (mg): Fill: 1150  Shell (approx.): 489  Total: 1639

Disintegration Time: Not More Than 30 Minutes
Uniformity of Mass: Complies to Current Therapeutic Goods Order

<table>
<thead>
<tr>
<th>Ingredient(s):</th>
<th>Per Softgel</th>
<th>Standard**</th>
<th>Overage % Claim</th>
<th>QA Release % Input</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Softgel Fill:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish Oil - Natural</td>
<td>1 g</td>
<td>CARDINAL</td>
<td>-</td>
<td>92.5-107.5*</td>
</tr>
<tr>
<td>containing approx:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Fatty Acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl Esters 750mg as:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl Ester 360mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl Ester 200mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: This material is</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ropufa &quot;S&quot; N-3 EE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Excipients:**
- Sorbitan Mono-Oleate 60 mg BP
- Polysorbate 80 17 mg BP
- Orange Oil 73 mg CARDINAL

**2. Softgel Shell:**
(Standardised composition: per softgel)
- Carrageenan 61.1 mg CARDINAL
- Sodium Phosphate Dibasic Anhydrous 8.33 mg CARDINAL
- Hydroxypropyl starch 191 mg CARDINAL
- Glycerol 189 mg BP
- Water - Purified 39.1 mg BP

* Identified then quantified by input
** Unless specified all Standards comply to the most recent Monograph.

Hung Truong
R&D Manager
APPENDIX F: EPA & Placebo Data Sheets

VEGCAP PRODUCT SPECIFICATION

Customer: Placebo Veggicap

Product Name: Placebo for Maxepa ® 1000 mg - Veggicap

Code: PBVMXC

Version: 22nd December 2003 (1)

Size: 20 Minim

Shape: Oblong

Print: Unprinted

Fill Colour: Clear pale yellow solution

Shell Colour: Clear Natural (CLR)

Weights (mg): Fill: 1000

Shell (approx.): 379

Total: 1379

Disintegration Time: Not More Than 30 Minutes

Uniformity of Mass: Complies to Current Therapeutic Goods Order

<table>
<thead>
<tr>
<th>Ingredient(s):</th>
<th>Per Vegicap</th>
<th>Standard**</th>
<th>Overage % Claim</th>
<th>QA Release % Input</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excipients(s):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya Oil</td>
<td>1000 mg</td>
<td>BP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Vegicap Shell
(Standardised Composition: per vegicap)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (mg)</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrageenan</td>
<td>54.6</td>
<td>CARDINAL</td>
</tr>
<tr>
<td>Hydroxypropyl Starch</td>
<td>169</td>
<td>CARDINAL</td>
</tr>
<tr>
<td>Sodium Phosphate – Dibasic Anhydrous</td>
<td>4.96</td>
<td>CARDINAL</td>
</tr>
<tr>
<td>Glycerol</td>
<td>131</td>
<td>BP</td>
</tr>
<tr>
<td>Water – Purified</td>
<td>18.9</td>
<td>BP</td>
</tr>
</tbody>
</table>

** Unless specified all Standards comply with the most recent Monograph.

Jon Athanasopoulos

Regulatory Affairs Manager