1 Introduction

Latest reports regarding breast cancer in Australia show that intensive screening campaigns, more effective treatment and an aging population are all contributing to the increasing number of Australian women who are living with the experience of having had breast cancer (Australian Institute of Health and Welfare & National Breast Cancer Centre 2006; Tracey et al. 2006). While breast cancer treatments are proving more successful, they are not without considerable side-effects that may impair a woman's transition from being a 'cancer patient' to a 'cancer survivor'. After the initial physical side-effects of cancer treatment such as wound healing after surgery, alopecia and nausea after chemotherapy and localised skin reaction after radiation therapy subside about one in four women reportedly continue to experience debilitating fatigue for months and even years afterwards (Servaes et al. 2007). The question of why persistent fatigue appears to affect a certain subpopulation of women and not others remains the focus of intense research, which began with the conceptualisation of what cancer-related fatigue was thought to be (Aistars 1987; Cameron 1973; Glaus, Crow & Hammond 1996; Piper, Lindsey & Dodd 1987; Winningham et al. 1991).

Understanding the mechanisms which underpin fatigue development are very important, particularly as they directly relate to interventions that could potentially alleviate fatigue. Previous research suggests that persistent fatigue could be related to demographic characteristics such as age or menopausal status; disease and treatment variables such as a more aggressive cancer and more intense cancer treatment; psychological variables like depression; other quality of life factors including sleep quality and appetite; and biological factors like haemoglobin level and immune factors. Until now, however, studies with breast cancer patients have seldom investigated how these factors may be inter-related or

how they change over time relative to pre-treatment variables. This has resulted in equivocal findings regarding factors associated with fatigue.

One promising line of research has as its focus the circadian rhythms of the stress hormone cortisol. Recent studies have shown that fatigued breast cancer survivors have a disrupted cortisol rhythm and an inadequate cortisol response to an experimental stressor (Bower, Ganz & Aziz 2005b; Bower et al. 2005a), presumably because of prolonged inflammatory stimuli causing a disturbance in the Hypothalamic-Pituitary-Adrenal axis. What remains to be determined is how cortisol rhythm changes in the months and years after treatment recovery and importantly, the pattern of cortisol production relative to pre-treatment.

A novel line of enquiry that has not been investigated is the association between thyroid hormones and fatigue in women managed for breast cancer. Thyroid hormones are integral in regulating the metabolic rate and mobilising energy reserves in the body (Marieb & Hoehn 2007) and fatigue is one of the most common complaints in people who are diagnosed with subclinical and overt thyroid dysfunction (Gulseren et al. 2006; Watt et al. 2007). It follows that women with breast cancer who experience persistent fatigue may have an insufficiency in thyroid hormones; a condition for which relatively simple treatment is readily available. To date, studies of fatigue in breast cancer patients have not determined thyroid function and rarely have participants with a known diagnosis of thyroid dysfunction been excluded from research.

The overall aim of this research was to investigate how prevalent persistent fatigue is after radiation therapy for breast cancer and whether it is associated with cortisol rhythm and thyroid hormones.

2 Literature Review

The latest report by the Cancer Institute New South Wales (NSW) showed that breast cancer mortality rates in NSW have fallen by 18%, which has been attributed to more effective treatment and population screening (Tracey et al. 2006). While this is extremely encouraging, the incidence rates for localised breast cancer are increasing (Australian Institute of Health and Welfare & National Breast Cancer Centre 2006), meaning that many more women in Australia are living with the experience of having had breast cancer than in previous decades. This review discusses first, the quality of life of women following breast cancer treatment, second, fatigue as the most distressing symptom and one that impacts most negatively on quality of life and lastly, research evidence for potential endocrine factors (cortisol rhythm and thyroid function) that may be related to fatigue in this population.

2.1 Breast cancer survivorship and health-related quality of life

2.1.1 Breast cancer epidemiology, aetiology and treatment

It is not known what causes breast cancer, but several risk factors are likely to be related to increased incidence of the disease. Increasing age, family history, hormonal factors (i.e. age at menarche and menopause, exogenous oestrogen exposure) (Kendall, Folkerd & Dowsett 2007), obstetric history, being overweight (Connolly et al. 2002), previous benign breast disease or malignancy and previous radiation exposure (FitzGerald et al. 2006) are all thought to be involved (Washington & Leaver 2004).

In early breast cancer, the recommended treatment consists of surgery (breast conserving surgery or mastectomy) followed by adjuvant radiation therapy which significantly reduces the incidence of local recurrence and presents a small benefit in overall survival when compared to surgery alone (Fisher et al. 2002; Vinh-Hung & Verschraegen 2004). Adjuvant chemotherapy and hormonal treatment (for oestrogen receptor positive disease) also translate to significant reductions in recurrence rates and improved long-term survival (Early Breast Cancer Trialists' Collaborative Group 2005).

Advances in modern breast cancer therapies mean that women have more options regarding the management of their disease. But while treatments continue to be more effective in terms of cancer control and overall survival, a lot is yet to be learned about the short and long-term effects of treatment and how these impact on women's physical and mental well-being, as well as their ability to function in their families, workplaces and social networks.

2.1.2 Quality of life

2.1.2.1 Definition

In 2000, at the Symposium on the Clinical Significance of Quality-of-Life Measures in Cancer Patients, Quality of Life (QoL) was defined as a multidimensional construct which describes "psychological and social functioning as well as physical functioning and incorporates positive aspects of well-being as well as negative aspects of disease and infirmity" (Sloan et al. 2002). Distinctions are sometimes made between QoL and Health-Related QoL—which includes all aspects related to general health, physical symptoms and toxicity, functioning domains (physical, emotional, cognitive, role, social and sexual) and existential issues (Fayers & Machin 2007)—however, for simplicity, QoL and Health-Related QoL will be considered as synonymous throughout this thesis.

Self-reported QoL assessment is often used in clinical trials to determine the impact a new treatment may have on patients, which once translated to the clinical setting can influence clinical decision-making between different treatment options (Goodwin et al. 2003). Its measurement can also inform clinicians and other staff involved in patient care on patients' symptom control and psychosocial well-being (Perry, Kowalski & Chang 2007).

2.1.2.2 Quality of life following breast cancer treatment

QoL in cancer patients has attracted intense research interest in the past few decades and has been studied perhaps more in the breast cancer population than any other cancer group. Despite all this interest, difficulties in interpretation of QoL arise when investigators report conflicting results and use different research designs. Overall, many QoL studies are cross-sectional and if they are longitudinal, they lack an appropriate baseline or an adequate follow-up period. The number of QoL instruments in use makes choosing an appropriate tool confusing and interpreting QoL scores generated by different instruments nearly

impossible. Perry, Kowalski & Chang (2007) noted that there were over 21 different QoL instruments designed specifically for the breast cancer population. Sample populations in these studies often represent one patient stereotype (i.e. Caucasian, middle aged, married, well-educated woman) with limited information on QoL after cancer treatment of minority populations such as those who are younger or older or belong to a different racial group. With this in mind, the next few paragraphs review the breast cancer QoL literature as it pertains to the following four aspects: QoL experienced across different treatment groups, time elapsed since diagnosis (or treatment end), QoL in breast cancer patients compared to healthy controls or a reference population and finally, studies that have evaluated the effect of age on post-treatment QoL.

It is generally accepted that patients who receive more aggressive treatment—owing to the more advanced stage of their disease—will experience greater detriments in QoL. Ganz et al. (2004) compared QoL at the end of treatment of 558 women (mean age 56.9 years, range 26.9 to 87, stage I to II, any lymph node status) treated by either mastectomy only, mastectomy with adjuvant chemotherapy, lumpectomy only and lumpectomy with adjuvant chemotherapy. Most patients (69%) had also received radiation therapy, but further classification and testing of the group effect of radiation therapy was not made. The QoL instrument used was the SF–36 among a range of other questionnaires evaluating depression and other symptoms. The investigators concluded that at the end of treatment, emotional functioning was similar between treatment groups ($p = .58$), but that physical functioning was significantly worse among women who had a mastectomy versus lumpectomy (with or without chemotherapy, $p < .001$) (Ganz et al. 2004).

The impact of surgery type on QoL in early breast cancer was also investigated by Curran et al. (1998) although these investigators specifically reported only on cosmesis and body

image and did not report on other aspects of QoL (Curran et al. 1998). Comparisons were made between a breast-conserving therapy cohort (lumpectomy, axillary clearance and breast radiation therapy, n = 141) or a modified radical mastectomy cohort (no further adjuvant treatment, n = 127). Overall, Curran et al. (1998) found that the breast-conserving therapy cohort experienced significantly better body image outcomes compared to the mastectomy cohort (p = .001).

In terms of QoL after chemotherapy, two studies reported on the recovery period shortly after treatment. Browall et al. (2008) examined QoL in breast cancer patients (n = 75) at different times during chemotherapy for breast cancer and at four months after treatment. The QoL instrument used was the EORTC QLQ–C30, which includes a measure of global QoL and several functioning and symptom subscales. The main findings were that QoL at four months after chemotherapy was significantly worse in the global QoL, physical and role functioning domains (p < .05) compared to baseline (post-op) measurements (Browall et al. 2008). Similar outcomes related to QoL impairments in chemotherapy patients were reported by Galalae et al. (2005). These authors compared three treatment groups: chemotherapy followed by radiation (n = 41), radiation and hormonal treatment (n = 45) and radiation only (n = 23) at a timepoint set at six weeks post-treatment. The questionnaire was the same as that used by Browall et al. (2008). Significant differences were reported between the group of women who received chemotherapy with significantly lower scores found for global QoL and—with the exception of physical functioning—on all other functioning subscales (i.e. role, emotional, cognitive and social) (Galalae et al. 2005).

The effects of radiation therapy on QoL were also investigated using a randomised trial comparing surgery with adjuvant radiation therapy versus surgery only in women with

early breast cancer. In this study, Whelan et al. (2000) found that in the acute post-treatment period (up to two months), physical functioning was significantly worse in the radiation therapy cohort compared to the surgery only cohort (p = .0001) (Whelan et al. 2000), but that there were no differences in the other QoL domains.

QoL is assumed to improve with increasing time after breast cancer treatment. Research studies investigating the effect of elapsed time on QoL conclude that QoL either remains stable over time (Kerr et al. 2003; Wallace et al. 1993), improves several weeks after treatment completion (Deshields et al. 2005) or returns to baseline pre-treatment levels (Back et al. 2005; Dow & Lafferty 2000; Lee et al. 2008). With the exception of Kerr et al. (2003)—who included the largest sample of breast cancer survivors (n = 457) and measured QoL annually for four years—the other repeated-measures studies mentioned above followed participants only up to a maximum of seven months after treatment. Of note are studies by Back et al. (2005) and Lee et al. (2008) whose research was conducted in NSW, Australia and whose study populations (early stage breast cancer patients, n = 175 and n = 61, respectively) can be considered to be similar to the present research.

When QoL of breast cancer survivors had been compared to matched control groups or a reference population, the findings have been relatively consistent. While it seems that the overall (global) QoL in breast cancer survivors is generally high and similar to that of their healthy peers, in-depth analyses of different QoL domains reveal that breast cancer survivors do report significant detriments in some aspects of their QoL (Table 2.1).

Table 2.1 Studies comparing QoL in breast cancer survivors and controls

First author	Year	Subjects	Timepoint	QoL domain affected in breast cancer patients
Robb	2007	• Breast cancer survivors *n* = 127 (mean age 78 years) • Healthy controls *n* = 87 (mean age 77 years)	Mean 5.1 years post-treatment (range 1 to 15 years)	Psychosocial Physical
Helgeson	2005	• Breast cancer survivors *n* = 304 (mean age 51 years) • Healthy controls *n* = 187 (mean age 53 years)	Mean 5.5 years post-diagnosis (range 4.5 to 6.8 years)	Physical functioning
Arndt	2004	• Breast cancer survivors *n* = 314 (mean age 57.9 years, range 30 to 80 years) • Compared to population reference values	1 year post-diagnosis	Emotional functioning
Ganz	2002	• Breast cancer survivors *n* = 763 (mean age 55 years) • Compared to population reference values	Mean 6.3 years post-diagnosis (range 5 to 10 years)	Physical functioning Role functioning Pain General health
Dorval	1998	• Breast cancer survivors *n* = 124 • Healthy controls *n* = 262 • Age range for both groups 30 to 89 years, means not reported	8 years post-diagnosis	Physical health

Based on the studies summarised in Table 2.1, breast cancer survivors appear to have significantly different (worse) emotional functioning in the first year after diagnosis (Arndt et al. 2004). In the years that follow, emotional functioning is no longer significantly different between cancer and non-cancer populations (Dorval et al. 1998; Ganz et al. 2002;

Helgeson & Tomich 2005; Robb et al. 2007). Physical functioning is affected into the longer term, with survivors reporting significantly different (decreased) physical functioning compared to healthy controls five to eight years down the track (Dorval et al. 1998; Ganz et al. 2002; Helgeson, Snyder & Seltman 2004; Robb et al. 2007), but whether these differences are clinically significant is unclear. Only Ganz et al. (2002) evaluated the clinical significance of its statistically different QoL scores among a large sample (n = 763) of breast cancer survivors. They concluded that the differences found were not clinically significant and that their findings reflected changes that would be expected due to healthy aging.

Cancer diagnosis and treatment side-effects are likely to affect the QoL of women of different ages differently. Most QoL research has been conducted with middle-aged post-menopausal women, with only a very small number of studies reporting on the QoL of younger (< 45) and older (> 65) breast cancer patients. One recently published repeated-measures study investigated the relationship between age and various aspects of QoL at four months after cancer treatment (chemotherapy and/or radiation therapy) in middle-aged to older women (55 to 77 years). The QoL instrument was the widely used EORTC QLQ–C30 and the researchers found that with the exception of dyspnoea and sexual functioning, age had little impact on all other functioning and symptom domains of QoL (Browall et al. 2008). Somewhat conflicting findings were reported by Cimprich, Ronis & Martinez-Ramoz (2002) in their cross-sectional study which compared QoL between younger (27 to 44 years), middle-aged (45 to 65 years) and older women (66 to 79 years). Cimprich, Ronis & Martinez-Ramoz (2002) found that younger women experienced significantly worse social functioning compared with older women and that women who were older expressed significantly worse physical well-being than women who were middle-aged. In another study (Robb et al. 2007) aspects of QoL were compared between older cancer survivors

(mean age 78 years) and older women controls without cancer (mean age 77 years). While breast cancer survivors reported a similar prevalence of anxiety and depression to the older controls, cancer survivors reported significantly worse psychosocial well-being scores, lower life satisfaction, more depressed mood and a greater number of days affected by fatigue (Robb et al. 2007).

The disparity between the above studies could be due to the different study designs employed (repeated-measures versus cross-sectional, standardised time-point versus non-standardised), but another possibility could be the differences in patterns of adjustment to diagnosis and treatment among individuals. A four year follow-up study found that different levels of available personal and social supports affected QoL of middle-aged women (Helgeson, Snyder & Seltman 2004) and it is possible that this is also the case in younger and older breast cancer survivors. Finally, it appears that factors impacting on QoL of older women with breast cancer are related more to the satisfaction with processes of care and doctor-patient communication than the severity of treatment side-effects (Mandelblatt, Figueiredo & Cullen 2003).

In summary, cross-sectional and longitudinal studies of breast cancer survivors show that QoL is stable or only slightly decreased immediately after treatment, with worse detriments being experienced by women who receive more aggressive therapy. Over the months and years that follow, studies show that QoL of breast cancer survivors returns to pre-treatment levels, but paradoxically, when compared to age-matched non-cancer controls, studies show that cancer survivors report significantly worse QoL in some domains (e.g. physical functioning). Additionally, processes influencing QoL are likely to be different among women of varying ages.

Some of these conflicting findings may be related to the theory of 'response shift' which proposes that over time, people recalibrate how they perceive their symptoms and relate their current experience to what they have gone through in the past (Schwartz & Sprangers 1999). Another explanation could be that most studies use parametric statistics on data that is very rarely normally distributed and is highly prone to ceiling and floor effects (Fayers & Machin 2007). Inappropriate use of statistical methods may incorrectly reveal or conceal significant differences or associations. Another possibility may be that most studies report non-significant findings in the whole sample, while hiding the proportion of individuals who experience worst QoL. So while most breast cancer survivors report a high level of functioning and QoL, almost one in four are found to experience persistent fatigue, a symptom that many describe as the most distressing and one that impacts most on their QoL (Arndt et al. 2006). The factors mentioned above highlight the need for more research into QoL after breast cancer treatment.

2.2 Cancer-related fatigue

Fatigue as a phenomenon in health and disease has been the focus of intense research for many decades, but the underlying mechanisms that precipitate fatigue development are not well understood. Variations in fatigue definitions have arisen, at least in part, due to the number of health disciplines (e.g. physiology, pathology and psychology) attempting to conceptualise fatigue in the context of their particular field. Consequently, each discipline has a slightly different definition for fatigue; a different understanding of the proposed mechanisms leading to its development and factors perpetuating its experience. This section aims to first, provide a broad overview of theoretical frameworks and definitions that have shaped what is currently understood by the term 'fatigue', and second, to describe fatigue prevalence in women with breast cancer and factors associated with cancer-related fatigue, and lastly, limitations of previous research. While a large body of evidence deals with interventions recommended for managing cancer-related fatigue, these are beyond the scope of this chapter and will not be discussed. There are, however, two recently published meta-analyses that provide in-depth overviews of non-pharmacological (Kangas, Bovbjerg & Montgomery 2008) and pharmacological strategies (Minton et al. 2008) for dealing with cancer-related fatigue.

2.2.1 Fatigue - theoretical frameworks and definition

From a historical perspective, interest in and research into fatigue was the key focus in the context of productivity in industry. By the late 1940's fatigue was characterised in the physiological context of oxygen deprivation in muscle (Bartley & Chute 1947) and these findings led to fatigue being considered as a marker of the body's inability to adapt to stress. After this, two different lines of research have been particularly important in furthering our understanding of fatigue in cancer.

First, the General Adaptation Syndrome (GAS) developed by Selye described stress as the body's response to a demand, which if prolonged, resulted in an alarm reaction, then resistance and finally exhaustion (Selye 1952). Second, Granjean (1970) conceptualised fatigue as a non-specific state related to energy depletion and a normal sensation preceding sleep which enabled the body to recover its energy reserves. Other theoretical frameworks of cancer-related fatigue have since been published (Aistars 1987; Cameron 1973; Glaus, Crow & Hammond 1996; Olson 2007; Piper, Lindsey & Dodd 1987; Winningham et al. 1991) and are summarised in detail by Olson (2007). The current research draws on Olson's Fatigue Adaptation Model. This framework describes tiredness, fatigue and exhaustion as inter-related and on a continuum called 'adaptation' (Figure 2.1). The behavioural patterns associated with each phase of the continuum (i.e. tiredness, fatigue and exhaustion) are thought to be related to the alarm reaction, resistance and exhaustion phases of the GAS (Olson 2007).

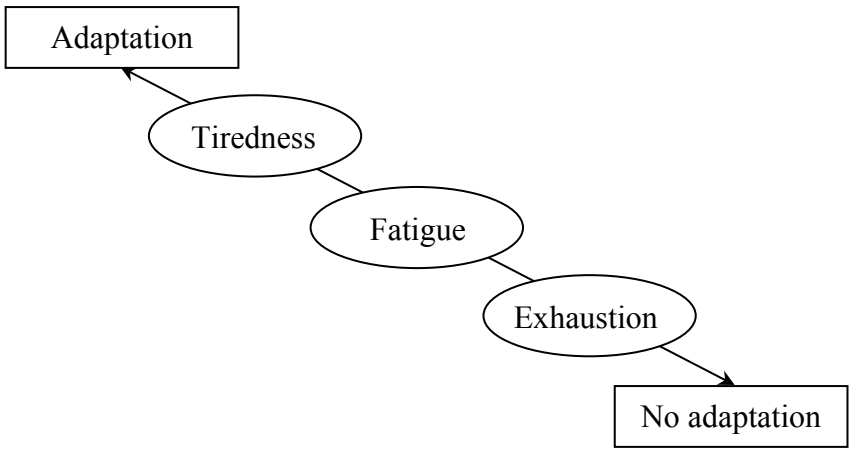


Figure 2.1 Fatigue Adaptation Model (Olson 2007)

Various authors have tried to define what cancer-related fatigue is and a proposal has been made to include cancer-related fatigue criteria in the International Classification of Diseases (ICD) database (Cella et al. 2001). The most widely accepted definition appears

to be one given by the National Comprehensive Cancer Network: *"Cancer-related fatigue is a distressing persistent, subjective sense of physical, emotional and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning."* (National Comprehensive Cancer Network 2009). This definition of fatigue encompasses the multidimensional nature of fatigue and highlights the complexity involved when trying to differentiate between 'normal' fatigue and cancer-related fatigue.

2.2.2 The experience of cancer-related fatigue

Using a qualitative methodology such as grounded theory, researchers have been able to determine what the lived experience of cancer-related fatigue is like for patients. When cancer patients' descriptions of fatigue were compared to fatigue experienced by people without cancer, it was found that fatigue was described similarly by both groups in terms of its physical, affective and cognitive dimensions (Glaus, Crow & Hammond 1996). The main difference, however, was in how the groups perceived fatigue. The healthy group described it as temporary, pleasant and alleviated by rest, whereas cancer patients experienced fatigue as distressing, with an increased need for rest. These differences between 'healthy fatigue' and 'distressing fatigue' were similar to those given by Gielissen et al. (2007). In this study, healthy participants and cancer patients were asked to indicate which list of adjectives pertaining to fatigue best described their experience. Cancer patients most often described their fatigue as anxiety provoking, whereas healthy subjects without cancer described fatigue as temporary and pleasant.

In a recent NSW study of breast cancer survivors and people suffering from chronic fatigue syndrome, both patient groups described their fatigue experience as having physical, cognitive and mood-related factors, but in contrast to chronic fatigue syndrome patients,

breast cancer patients did not indicate flu-like symptoms and musculo-skeletal pain (Bennett et al. 2007).

In summary, cancer-related fatigue in this thesis is defined as a multidimensional construct and one that is distinct from everyday tiredness and chronic fatigue syndrome in terms of its severity, the interference it causes to usual functioning and the absence of flu-like symptoms and musculo-skeletal pain.

2.2.3 Prevalence of cancer-related fatigue

For the purposes of this review, prevalence rates of fatigue are defined as the proportion of women affected by fatigue at any given time; and fatigue prevalence in women before a breast cancer diagnosis is made, is considered to be similar to fatigue prevalence in the general female population. This section of the review outlines fatigue prevalence in women in the general population, followed by fatigue prevalence, time-course and severity related to different breast cancer treatments.

2.2.3.1 Fatigue in the general population

There is a paucity of literature on the prevalence of fatigue among the general population and in particular in Australian women. However, there are two reports that describe fatigue prevalence in NSW general practice attendees and also three international studies that report fatigue prevalence among females only. The first NSW study investigated the presence of fatigue and depression among older adults (n = 124, mean age 73 years, 50% female) who attended general practice clinics in Southern Sydney (Wijeratne, Hickie & Brodaty 2007). The overall fatigue prevalence was 27.4%, but when taken alone—without co-morbid psychological symptoms—fatigue was prevalent in 10.5% of subjects surveyed (Wijeratne, Hickie & Brodaty 2007). The second and much larger study was published

eleven years earlier (Hickie et al. 1996), but showed similar results with overall fatigue prevalence of 25% and fatigue alone occurring in 7.5% of study participants (general practice attendees, *n* = 1593, mean age 37.8 years, 75% female).

While these Australian samples included both males and females, the fatigue prevalence rates specifically in women are similar to those reported by other researchers. In a large Dutch study investigating fatigue among the working population, 23% of women (*n* = 3255, mean age 38 years) scored above the validated fatigue cut-off score on the 'Checklist Individual Strength' (Bultmann et al. 2002). In 14% of these women, fatigue was associated with psychological distress, whereas 9% of women reported fatigue symptoms only. Similar findings were reported by two earlier studies by Chen (1986) and David et al. (1990), both looking at fatigue prevalence among women. Fatigue was prevalent in 11.3% (Chen 1986) and 10.6% (David et al. 1990) of participants. Taken together, these studies suggest that fatigue is prevalent in approximately 7–11% of the general population.

2.2.3.2 Before surgery

Fatigue prevalence among women prior to breast cancer diagnosis has rarely been examined; however, a recent study (De Vries, Van der Steeg & Roukema 2009) had found that 32.5% of women were fatigued even before a breast cancer diagnosis was made (*n* = 117, mean age 58.3 years). In this study, participants were recruited at an outpatient clinic after referral to the clinic due to a palpable breast lump or a mammographic abnormality. De Vries, Van der Steeg & Roukema (2009) also noted that this prevalence rate (32.5%) was similar in women who later appeared to have benign breast problems only. As there are no other studies that give fatigue prevalence before women are actually

diagnosed with breast cancer, this is the closest estimate to fatigue prevalence rates in women before breast cancer surgery.

2.2.3.3 Before adjuvant treatment

There is some evidence that even prior to adjuvant treatment, breast cancer patients experience higher levels of fatigue in comparison to adults without cancer (Ancoli-Israel et al. 2006). A small number of studies in the cancer-related fatigue literature give an indication of fatigue prevalence before the start of any adjuvant treatment (i.e. chemotherapy, radiation therapy or hormonal treatment). Six such studies were found, one of which was conducted in NSW and is thus a useful comparison to the current research. These studies suggest that between 10–28% of breast cancer patients feel fatigued before the start of adjuvant treatment (Andrykowski et al. 2005; Back et al. 2005; Berger et al. 2007; de Jong et al. 2004; Jacobsen et al. 1999; Stone et al. 2000).

Andrykowski et al. (2005) studied women with early stage breast cancer (n = 288, mean age 54.5 years), before the start of chemotherapy and found that 10.4% met the strict criteria for cancer-related fatigue (i.e. two-week period of significant fatigue and lack of energy in the past month (Cella et al. 1998)). The number of days following surgery was not reported by the authors. However, Back et al. (2005) had similar findings (10% fatigued) in a sample with comparable characteristics (n = 175, median age 56 years, breast cancer stage < II) observed 41 days (median) after surgery. Larger proportions of fatigued women were reported by both de Jong et al. (2004) and Berger et al. (2007), 25% and 28% respectively, but both of these studies included women with up to stage IIIA breast cancer. de Jong et al. (2004) in particular, reported that 6% of their sample population (n = 157, mean age 47 years, mean 35 days post-surgery) were either currently undergoing or had completed radiation therapy. In a study of cancer patients with mixed diagnoses (n = 227,

breast cancer patients n = 34), severe fatigue was prevalent in 15% of the breast cancer group (Stone et al. 2000). Finally, Jacobsen et al. (1999) found that 4% of breast cancer patients experienced severe fatigue before the start of adjuvant chemotherapy (n = 54, mean age 51 years, stage I – III) (Jacobsen et al. 1999). The variation in prevalence rates in these six studies is probably due to the different methods used to assess fatigue and differences in characteristics of the women participating. Therefore fatigue prevalence before adjuvant treatment is at least 10% and at least 4% for severe fatigue.

2.2.3.4 Prevalence due to chemotherapy

Cytotoxic treatment is known to cause unpleasant side-effects in patients including nausea, vomiting, alopecia and fatigue. Fatigue due to chemotherapy is found to increase in the week after infusion and is reported to increase in severity with the number of cycles that are administered (de Jong et al. 2004; Irvine et al. 1994; Jacobsen et al. 1999; Miller, Maguire & Kearney 2007), but by 30 days after treatment patients experience only mild levels of fatigue (Berger, Lockhart & Agrawal 2009). Jacobsen et al. (1999) reported that the prevalence of severe fatigue in their sample of 54 breast cancer patients (mean age 51 years, stage I – III) increased from 4% before the first chemotherapy cycle to 28% before the fourth cycle. de Jong et al. (2004) reported slightly higher prevalence rates compared to Jacobsen et al. (1999) at the third and fifth cycle, where 43% and 44% of participants, respectively, indicated ‘quite a bit’ to ‘very much’ fatigue. This discrepancy is probably due to the different methods used to determine fatigue prevalence; Jacobsen et al. (1999) measured fatigue severity whereas de Jong et al. (2004) asked the extent to which the participant had been bothered by fatigue. Overall, it is apparent that fatigue is a significant side-effect of chemotherapy and one that seems to worsen with each additional cycle of treatment.

2.2.3.5 Prevalence due to radiation therapy

It is well documented in longitudinal studies of cancer patients that fatigue increases over the course of radiation therapy treatment (Back et al. 2005; Geinitz et al. 2001; Greenberg et al. 1992; Haylock & Hart 1979; Irvine et al. 1998; Kobashi-Schoot et al. 1985; Lavdaniti et al. 2006; Molassiotis & Chan 2004; Smets et al. 1998; Wratten et al. 2004). Fatigue is mostly reported to increase in a linear fashion as patients progress through treatment (Lavdaniti et al. 2006; Smets et al. 1998); however, in some studies fatigue level is reported to increase in the first few weeks then plateau after week four (Geinitz et al. 2001; Greenberg et al. 1992; Wratten et al. 2004). Table 2.2 summarises the time-course of fatigue development during RT.

2.2.3.6 Prevalence after cancer treatment

While fatigue after breast cancer treatment has probably been documented the most in the cancer-related fatigue literature, many studies are limited by methodological shortcomings such as a cross-sectional design, non-standardised timepoint of measurement and heterogeneous samples that include participants with various cancer diagnoses and different disease stages. It is possible that fatigue prevalence is overestimated in these studies, particularly if samples are made up of participants both on treatment and several months after treatment, or if they received treatment for early stage disease versus palliative treatment. To overcome this limitation and therefore ascertain the most accurate fatigue prevalence after cancer treatment, this section reviews only studies that used a repeated-measures design, fatigue measurements taken at the same timepoint in the post-treatment recovery period and a homogeneous breast cancer population that excluded participants with metastatic disease.

Table 2.2 Fatigue development during Radiation Therapy

First author	Year	Subjects	Timepoints	Fatigue development
Lavdaniti	2006	• Breast cancer patients (Greece) • Stage I – II • *n* = 106 (mean age 55 years) • 58% received chemotherapy	• Day 1 or 2 of RT • Week 3 • Last week of RT	• Fatigue increased until end of RT • Linear trend
Molassiotis	2004	• Mixed diagnoses and stages (Hong Kong) • *n* = 27 (mean age 41 years) • Breast cancer patients 26% • 37% received chemotherapy	• Daily for first 14 days of RT • Measurements taken daily at 10AM, 4PM and 10PM	• Day 7 fatigue prevalence – 56% • Day 14 fatigue prevalence – 81% • Fatigue higher in afternoon and evening
Wratten	2004	• Breast cancer patients (Australia) • Non-metastatic • *n* = 52 (mean age 55.7 years) • 29% received chemotherapy	• Weekly for duration of RT	• 43% severe fatigue during RT • Fatigue increased between weeks 1 – 3 • Plateau after week 4
Geinitz	2001	• Breast cancer patients (Germany) • Stage 0 – IIB • *n* = 41 (median age 54 years) • 7% received chemotherapy	• Before RT • Weekly during RT	• Fatigue increased until week 4 • Plateau after week 4
Irvine	1998	• Breast cancer patients (Canada) • Stage I – II • *n* = 76 (mean age 60 years) • % receiving chemotherapy not reported	• Week 1 • Week 2 • Last week of RT	• Fatigue increased until week 2 • Plateau until end of RT
Smets	1998	• Mixed diagnoses and stages (Netherlands) • *n* = 250 (mean age 64 years) • Breast cancer patients 19% • No chemotherapy administered	• Before RT • 2x per week during all weeks of RT • 2 weeks post-RT	• Fatigue increased until end of RT • Linear trend

Table 2.2 *(continued)*

First author	Year	Subjects	Timepoints	Fatigue development
Greenberg	1992	• Breast cancer patients (USA) • Stage I – II • *n* = 15 (mean age 46 years) • No chemotherapy administered	• Daily for entire RT course	• Fatigue decreased between week 1 and 2, increased week 3 • Fatigue higher in afternoon and evening • Plateau week 4 until end RT

A large body of evidence suggests that fatigue returns to pre-treatment levels following adjuvant breast cancer treatment, or that there are no significant differences in fatigue compared to pre-treatment (Back et al. 2005; Geinitz et al. 2001; Geinitz et al. 2004; Greenberg et al. 1992; Irvine et al. 1998; Jacobsen et al. 2007; Michielsen et al. 2007). Even so, studies by Back et al. (2005), Jacobsen et al. (2007) and others (Bower et al. 2006; de Jong et al. 2004; De Vries, Van der Steeg & Roukema 2009; Goldstein et al. 2006) reported that a certain percentage of participants continued to experience increased fatigue that persisted into the longer term.

Using a self-report diary, 17.7% of women participating in the study by Back et al. (2005) indicated that six weeks after radiation therapy they experienced lethargy 'quite a lot' (11.4%) to 'very much' (6.3%). de Jong et al. (2004) used the 'Rotterdam Symptom Checklist' and found that 31% of women were 'quite a bit' or 'very much' fatigued at 12 weeks following adjuvant treatment. Jacobsen et al. (2007) repeated their fatigue assessments at two, four and six months following treatment and reported that 18%, 16% and 15% of participants, respectively, scored above the cut-off for fatigue. Most recently, De Vries, Van der Steeg & Roukema (2009) assessed fatigue in women after breast cancer surgery (n = 117, mean age 58.3 years) and found that 33.3% were fatigued at six months post-surgery and 25.6% at 12 months. While not noted by the authors, these higher prevalence rates could be somewhat overestimated because some participants were undergoing adjuvant treatment (i.e. chemotherapy followed by radiation therapy) at these measurement timepoints. After treatment, one Australian longitudinal study with four years of follow-up found that 18% of disease-free breast cancer survivors (n = 176, mean age 55 years, stage I – II) experienced clinically high levels of fatigue at 10 months after treatment completion (Goldstein et al. 2006). The strength of this research was that the authors also measured depression symptoms—which are known to be related to fatigue—and hence this

estimate reflects only those women suffering from fatigue without a mood disorder interaction. Lastly, in perhaps the largest and longest follow-up study to date, Bower et al. (2006) reported fatigue to be prevalent among 35% of breast cancer survivors up to five years post-treatment and in 34% between five and 10 years (n = 763, mean age 59 years). Together these studies demonstrate that fatigue remains a problem in a subpopulation of approximately 15–35% of breast cancer survivors and highlight that additional research is warranted to study persistent fatigue and factors related to it.

2.2.4 Factors related to cancer-related fatigue

Understanding the factors predicting cancer-related fatigue after curative breast cancer treatment is an important step potentially leading to interventions that alleviate fatigue. Longitudinal studies published to date have attempted to determine, using multivariate regression, the variables predicting fatigue such as demographic and psychosocial factors, clinical and medical factors, as well as disease and treatment variables. In the first year following breast cancer diagnosis and treatment, fatigue was shown to be predicted by pre-treatment fatigue (Michielsen et al. 2007), body mass index (Donovan et al. 2007), menopausal symptoms (Fan et al. 2005), receiving chemotherapy (Andrykowski et al. 2005), lower morning cortisol level (Von Ah, Kang & Carpenter 2008) and the following psychological variables: catastrophising (Andrykowski et al. 2005; Donovan et al. 2007), psychological distress and mood disturbance (Goldstein et al. 2006; Von Ah, Kang & Carpenter 2008) and neuroticism (Michielsen et al. 2007). In the one to 10 years that follow treatment, fatigue was predicted by baseline fatigue (Bower et al. 2006; Geinitz et al. 2004), pain (Bower et al. 2006; Meeske et al. 2007), type of treatment received (Bower et al. 2006), high baseline anxiety and depression (Bower et al. 2006; Geinitz et al. 2004; Servaes et al. 2007), sense of loss of control over fatigue (Servaes et al. 2007) and being bothered by cognitive problems, weight gain and appearance (Meeske et al. 2007). The

strength of these studies was their longitudinal repeated-measures design, use of a standardised timepoint and homogeneous sample populations (disease-free breast cancer survivors). Many other studies also report on the factors that are associated with fatigue post-treatment, although these are mainly of a cross-sectional design and based on a bivariate correlational analysis.

2.2.4.1 Demographic variables

Among the demographic variables, age and fatigue correlations have resulted in equivocal findings. Two studies of disease-free breast cancer survivors found a correlation between higher fatigue and younger age ($r = -.31$ (Young & White 2006) and $r = -.37$ (Winters-Stone et al. 2008)) and a third study found a non-significant trend for higher fatigue among younger participants ($r = -.30$ $p = .066$ (Geinitz et al. 2004)). And while Andrykowski et al. (2005) also concluded that younger women were more likely to experience persistent fatigue, no relationships were found between these variables by other authors (Gelinas & Fillion 2004; Mast 1998; Okuyama et al. 2000; Sugawara et al. 2005; Von Ah, Kang & Carpenter 2008). Marital status, household size and employment status have not been found to be related to fatigue (Gelinas & Fillion 2004; Okuyama et al. 2000; Sugawara et al. 2005; Young & White 2006) and with one exception (Mast 1998) neither has the level of education (Gelinas & Fillion 2004; Sugawara et al. 2005; Von Ah, Kang & Carpenter 2008; Young & White 2006). Menopausal status was not found to correlate with fatigue (Goldstein et al. 2006), although physical menopausal symptoms have been shown to be significantly related to fatigue (Fan et al. 2005; Gelinas & Fillion 2004).

2.2.4.2 Disease and treatment variables

Very few studies have found fatigue significantly related to disease and treatment factors. No correlations were found among fatigue and the following variables: nodal status and

tumour size (Goldstein et al. 2006), disease stage (Von Ah, Kang & Carpenter 2008), type of treatment received (Goldstein et al. 2006; Okuyama et al. 2000; Von Ah, Kang & Carpenter 2008; Young & White 2006) and time since treatment end (Mast 1998; Okuyama et al. 2000; Servaes, Verhagen & Bleijenberg 2002; Young & White 2006). In two studies, the number of treatment modalities received was significantly, but weakly, related to fatigue (r = .243 (Young & White 2006) and r = .27 (Winters-Stone et al. 2008); however, in another study this relationship was not found to be significant (Gelinas & Fillion 2004). Other variables significantly associated with fatigue were illness uncertainty (Mast 1998), fear of relapse (Young & White 2006) and the perceived impact of having a cancer diagnosis (Gelinas & Fillion 2004).

2.2.4.3 Psychological variables

Depression and anxiety are consistently shown to be significantly related to post-treatment fatigue (Kim et al. 2008; Kissane et al. 2004; Okuyama et al. 2000; Sugawara et al. 2005; Young & White 2006) and women who experience cancer-related fatigue are more likely to have a history of depression (Andrykowski et al. 2005). It is difficult to ascertain the causal relationship between fatigue and depression after breast cancer treatment, as each factor may contribute to or precede the other and also fatigue and depression can occur in conjunction or separately. Higher emotional distress, worse emotional well-being and impaired emotional health also correlate with fatigue (Bower et al. 2000; Gelinas & Fillion 2004; Meeske et al. 2007), underpinning the notion that fatigue is a multifaceted construct. Neuroticism (Sugawara et al. 2005; Young & White 2006) and extraversion (Sugawara et al. 2005) are other personality traits that are also significantly associated with fatigue. A study by Gelinas & Fillion (2004) found that while there was no correlation between an active coping style and fatigue, passive coping and fatigue were moderately but significantly correlated (r = .36, p < .001).

2.2.4.4 Quality of life factors

Fatigue and sleep are known to be closely related and studies investigating correlations between fatigue and sleep after breast cancer treatment conclude that fatigue is related to sleep quality and sleep disturbances (Carpenter et al. 2004; Okuyama et al. 2000; Servaes et al. 2007); however, that the overall sleep duration is not significantly related to fatigue (Carpenter et al. 2004; Geinitz et al. 2004). Another factor that has consistently been shown to be significantly associated with post-treatment fatigue is impaired physical functioning (Bower et al. 2000; Meeske et al. 2007; Servaes et al. 2007) and a reduction in physical activity (Bower et al. 2002; de Jong et al. 2004; Winters-Stone et al. 2008), but current activity level (Young & White 2006) and performance status (Sugawara et al. 2005) were not found to be related to fatigue. Other aspects of quality of life such as declines in role functioning (Bower et al. 2000; Meeske et al. 2007; Servaes et al. 2007) and social functioning (Meeske et al. 2007; Servaes et al. 2007) are also significantly related to higher fatigue. In terms of cognition, impaired cognitive function has been shown to be related to fatigue (Servaes et al. 2007), but cognitive function per se has not (Fan et al. 2005). Among other factors, pain (Gelinas & Fillion 2004; Meeske et al. 2007), sexual functioning (Broeckel et al. 2002), appetite and dyspnoea (Okuyama et al. 2000) have also been identified as significant correlates of fatigue, whereas having a co-morbidity has not (Geinitz et al. 2004).

2.2.4.5 Biomarkers

To date, various biomarkers have been studied in association with cancer-related fatigue, mainly due to their known effects in fatigued cancer free individuals. Anaemia and haemoglobin levels are significantly related to fatigue in patients with various cancer diagnoses (Stone et al. 2000), particularly during active chemotherapy (Holzner et al. 2002; Jacobsen et al. 2004; Shafqat et al. 2005). However, in studies of breast cancer

survivors who had completed their cancer treatment a correlation between haemoglobin levels and fatigue was not found (Geinitz et al. 2001; Sugawara et al. 2005). Other biological markers such as red and white blood cell counts have not been found to correlate with fatigue (Geinitz et al. 2001; Sugawara et al. 2005).

Conflicting findings have been published regarding fatigue and abnormal levels of immune function biomarkers (e.g. cytokines, tumour necrosis factor-alpha, neopterin). In one study, fatigued cancer survivors had elevated levels of interleukin-6 and tumour necrosis factor-alpha (Collado-Hidalgo et al. 2006), but other studies have not found a significant relationship between these biomarkers and fatigue (Geinitz et al. 2001; Von Ah, Kang & Carpenter 2008). Interleukin-1β has also been investigated in relation to fatigue and while in some studies a correlation with fatigue was not found (Bower et al. 2002; Geinitz et al. 2001; Gelinas & Fillion 2004), one study did report a significant correlation between interleukin-1β and fatigue (Von Ah, Kang & Carpenter 2008). A recently published quantitative review summarised 18 published studies that investigated the associations between fatigue and inflammatory markers and concluded that levels of interleukin-6 and neopterin are positively correlated with fatigue, while interleukin-1β and tumour necrosis factor-alpha were not (Schubert et al. 2007). According to this review, some difficulties in determining the fatigue-cytokine relationships may be due to, first, the nature of cytokines, because under different in-vivo conditions the same cytokine may induce pro-inflammatory and anti-inflammatory effects; second, sampling and storage issues related to blood specimens; and lastly, the inconsistent use of reliable and valid multidimensional fatigue instruments.

2.2.5 Limitations of previous research

Studying cancer-related fatigue presents unique challenges to researchers due to the subjective nature of the fatigue experience and the fact that everyone feels tired at some point. There are currently a limited number of longitudinal fatigue studies with an appropriately set base-line, which makes the interpretation of findings across studies very challenging. Many studies published to date are cross-sectional and sometimes with heterogeneous samples that include participants with different cancer diagnoses, mixed early stage and metastatic disease and patients who are undergoing active treatment and those who had completed treatment years previously. This heterogeneity makes drawing meaningful conclusions about fatigue difficult, because what may apply to patients with early stage cancer may not apply to patients with advanced disease and similarly the on-treatment fatigue experience may be completely different compared to survivorship. While many studies do report appropriate use of statistical tools, responses to subjective fatigue or quality of life questionnaires rarely follow a Gaussian (normal) distribution, hence requiring the use of non-parametric statistics or data transformation prior to analysis (Fayers & Machin 2007). Frequently, studies use parametric statistics which are likely to result in erroneous findings. Finally, there is evidence for a sub-population of breast cancer patients who experience fatigue into the longer term; however, when differences in fatigue level are measured over the entire sample, this small sub-group is likely to be 'hidden in the crowd'.

2.3 Endocrine functioning and fatigue

Endocrine regulation occurs via a feedback mechanism between the brain, blood and the peripheral endocrine organs (e.g. adrenal glands, thyroid gland, reproductive organs) (Goodman 2009). The region of the brain responsible for endocrine regulation is the hypothalamus, which releases hormones that stimulate the pituitary gland. The pituitary—in response to hypothalamic stimulation—releases hormones which target specific endocrine organs. Two hypothalamic-pituitary axes are of particular relevevance to fatigue development; the Hypothalamic-Pituitary-Adrenal axis (HPA axis) which regulates stress and the fight/flight response and the Hypothalamic-Pituitary-Thyroidal axis (HPT axis) involved in metabolism and temperature regulation. The HPA axis is thought to be involved in prolonged stress, which can result in fatigue as previously discussed in Section 2.2.1 (i.e. General Adaptation Syndrome and the Fatigue Adaptation Model). Fatigue is recognised as the main presenting symptom of thyroid dysfunction (Gulseren et al. 2006; Watt et al. 2007) suggesting that the HPT axis may be involved in fatigue development. In this section, the HPA and HPT axes are discussed first in the context of normal physiological function, followed by specific literature related to cancer and breast cancer patients, links to fatigue, and lastly limitations of previous research. It is recognised that while the HPA and HPT axes are discussed as separate entities, they do exist as complex inter-related systems in the body.

2.3.1 Hypothalamic-Pituitary-Adrenal axis

The HPA axis has long been recognised as the body system that is activated by mental and physical stressors. When activated, the pituitary releases Corticotropin-Releasing Hormone (CRH) which stimulates the adrenal gland to produce the hormone cortisol (Martini 1998). Historically, HPA axis activation was thought to result in an increased output of cortisol, which is responsible for actions in the central nervous system, metabolic system and the

immune response. More recently however, it has been shown that HPA axis dysfunction is also hallmarked by hypocortisolism, particularly in chronic stress conditions (e.g. post-traumatic stress disorder) (Miller, Chen & Zhou 2007). Chronic stress and chronic HPA axis activation are also thought to lead to increased vulnerability for a range of adverse medical outcomes (Miller, Chen & Zhou 2007).

2.3.1.1 Anatomy and physiology of adrenal glands

There are two adrenal glands in body, each positioned above the kidney. Each gland consists of an outer layer called the adrenal cortex which is made up of glandular tissue and an inner adrenal medulla which forms part of the sympathetic nervous system. The whole structure is encapsulated in a fibrous membrane (Marieb & Hoehn 2007). The cells in the adrenal cortex are arranged in three layers: the zona glomerulosa, zona fasciculata and zona reticularis as shown in Figure 2.2. Each zone is responsible for synthesising a range of different hormones (Marieb & Hoehn 2007).

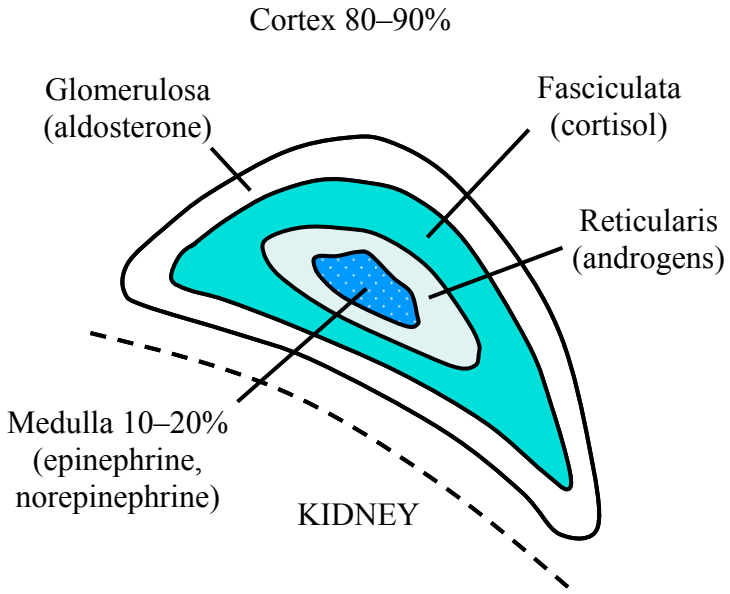


Figure 2.2 The adrenal gland, adapted from Nussey & Whitehead (2001)

The adrenal medulla produces two catecholamines, epinephrine and norepinephrine. The adrenal cortex produces three types of corticosteroids: the mineralocorticoids (mainly aldosterone), gonadocorticoids (mainly androgens or sex hormones, which are converted to testosterone or oestrogen after release) and glucocorticoids (mainly cortisol, but also in small amounts cortisone and corticosterone) (Marieb & Hoehn 2007). Glucocorticoid production is regulated by a negative feedback loop and is under the control of the anterior pituitary gland via Adrenocorticotropic Hormone (ACTH) (Marieb & Hoehn 2007). As the name suggests, glucocorticoids are important for maintaining constant blood glucose levels and in a threatening situation are responsible for mobilising glucose reserves to successfully overcome the crisis (Marieb & Hoehn 2007).

2.3.1.2 Cortisol production in cancer-free individuals

In healthy adults, cortisol release follows a 24–hour circadian pattern with highest circulating levels shortly after awakening, a gradual decrease throughout the day and lowest levels at approximately midnight. Cortisol is found in all body fluids; blood, urine, saliva, tears and cerebrospinal fluid. In the body, cortisol circulates either bound to a protein molecule (inactive) or as unbound cortisol (free, bioactive) (Martini 1998).

Salivary cortisol represents the free unbound fraction of cortisol and is an accurate measure of free circulating cortisol levels in the blood (Dorn et al. 2007; Gozansky et al. 2005). Measuring cortisol in saliva is stress-free and inexpensive and is a preferred method of sample collection in research studies compared to venepuncture, which can cause mild distress and hence temporarily elevate cortisol levels (Kirschbaum & Hellhammer 1994).

2.3.1.3 Cortisol production in breast cancer

Research evidence suggests that disturbances in circadian rhythms in patients with cancer increase with advancing disease stage (Mormont & Levi 1997; Sephton & Spiegel 2003). It is often difficult to distinguish whether the disturbances are directly related to the cancer and disease progression or are a result of treatment side-effects, patient-related factors or a combination of the above. In one study which compared salivary cortisol rhythm in breast cancer patients (early stage n = 23, metastatic disease n = 8) to healthy female controls, breast cancer patients were found to have significantly higher levels of cortisol than healthy controls (van der Pompe, Antoni & Heijnen 1996). In addition, significantly higher levels were also evident in women with metastatic breast cancer compared to those with early stage disease (van der Pompe, Antoni & Heijnen 1996). Measuring 24–hour cortisol for detection of abnormalities in its diurnal rhythm, particularly in women with advanced breast cancer, can possibly be of prognostic value as altered cortisol rhythm was found to be related to earlier mortality in this patient population (Sephton et al. 2000). Because no studies have investigated survival and diurnal cortisol in early breast cancer, it is currently not known whether alterations in cortisol rhythm can be used to predict survival in these women.

2.3.1.4 Cortisol and fatigue in breast cancer

Studies of relationships between cortisol and fatigue in breast cancer have been mixed in terms of methods and results (Table 2.3). When diurnal cortisol rhythm was assessed in breast cancer patients and compared to healthy women without a history of cancer, no significant differences were found in cortisol production (Carlson et al. 2007; Vedhara et al. 2006). However, in studies where fatigued breast cancer patients were compared to non-fatigued breast cancer patients, the fatigued group exhibited significantly flatter cortisol slopes (diurnal rhythm), significantly lower cortisol levels at awakening and significantly

higher evening levels (Bower et al. 2002; Bower et al. 2005a). The question of cortisol alteration in fatigued states remains, because a recent study of fatigued and non-fatigued breast cancer patients (Alexander et al. 2009) found conflicting results to Bower et al. (2002; 2005a), but there were important methodological differences between these studies.

Alexander et al. (2009) measured 24–hour urinary free cortisol which gives an overall cortisol output over the course of one day, whereas Bower et al. (2002; 2005a) measured cortisol in plasma and saliva at particular instants in time. The method of assigning fatigue 'caseness' differed between these studies also and it appears that Alexander et al. (2009) applied more rigorous fatigue criteria (Cella et al. 2001) and to a much larger sample population.

The evidence is also inconclusive with regards to correlations between cortisol and fatigue. Two studies reported a significant correlation between fatigue and cortisol (Bower et al. 2005a; Von Ah, Kang & Carpenter 2008); however, in a third study significant correlations were not found (Carlson et al. 2007). Collectively, these findings and those of above studies suggest that abnormal cortisol rhythm may be responsible for cancer-related fatigue after treatment in some breast cancer patients, but as it does not explain the fatigue experience in everyone, it is probably just one variable among several that relate to persistent fatigue. Another reason may be that some women are better able to adapt and cope with psychological and physical stress from a cancer diagnosis and cancer treatment than others. The ability to identify, pre-treatment, which women are at highest risk for persistent fatigue could, with appropriate interventions, improve their quality of life after treatment.

Table 2.3 Studies of cortisol and fatigue relationships in breast cancer patients

First author	Year	Subjects	Measures	Main findings
Alexander	2009	• Breast cancer survivors (UK) • Stage I – IIb • *n* = 200 (mean age 58 years) • Mean 10 months post-treatment	• 24-hour urinary free cortisol (nmoL/24hr) • Fatigue 'caseness' based on diagnostic criteria (Cella et al. 2001)	• No significant differences ($p = .305$) in urinary free cortisol between fatigued versus non-fatigued participants
Von Ah	2008	• Breast cancer patients (USA) • Stage I – IIIa • *n* = 44 (mean age 53 years) • Mean 18 days post-surgery	• Morning plasma cortisol (%), blood drawn between 9–11AM • Fatigue tool: Piper Fatigue Scale–revised	• Morning cortisol was significantly negatively correlated with fatigue level ($r = -.36$, $p < .05$) i.e. higher fatigue was related to lower morning cortisol
Carlson	2007	• Breast cancer survivors (Canada) • Stage I – III • *n* = 33 (mean age 51 years) • Mean 1.36 years post-diagnosis • Healthy control group *n* = 33 (mean age 53 years)	• Diurnal salivary cortisol (nmol/L), 4 samples collected over 1 day; at awakening, 12 noon, 5PM and 10PM • Fatigue tool: Profile of Mood States–Fatigue	• No significant differences on any cortisol indices between breast cancer survivors and controls • No significant correlations between fatigue and cortisol
Vedhara	2006	• Breast cancer patients (Netherlands) • Disease stages not reported • *n* = 85 (mean age 52.5 years) • 3 months post-diagnosis (some patients were on active treatment) • Healthy control group *n* = 59 (mean age 53 years)	• Diurnal salivary cortisol (nmol/L), 8 samples collected over 2 days; at awakening, 30 minutes after awakening, 11–1PM and 8–10PM • Fatigue not measured	• No significant differences in cortisol indices between breast cancer patients and controls

Table 2.3 *(continued)*

First author	Year	Subjects	Measures	Main findings
Bower	2005a	• Breast cancer survivors (USA) • Stage 0 – II • *n* = 29 (mean age for fatigued group 58.2 years, non-fatigued 61.8 years) • Approximately 6 years post-diagnosis	• Salivary cortisol (log ng/dL), 8 samples collected over 2 days; at awakening, 12 noon, 5PM and 10PM • Fatigue tool: SF–36 Health Survey–energy/fatigue subscale	• Fatigued group had significantly flatter slopes and significantly higher 10PM cortisol levels than non-fatigued group • Fatigue was significantly associated with flatter cortisol slope
Bower	2005b	• Breast cancer survivors (USA) • Stage 0 – II • *n* = 27 (mean age for fatigued group 55.6 years, non-fatigued 61.1 years) • Mean 8.4 years post-diagnosis • Participants underwent a 30 minute experimental stressor (Trier Social Stress Test) in the afternoon	• Salivary cortisol (μg/dL), 6 samples at 45 minute intervals; pre-test (1×), during test (2×), after test (3×) • Fatigue tool: SF–36 Health Survey–energy/fatigue subscale	• Non-fatigued group exhibited significantly higher cortisol rise following the stress test compared to fatigued group
Bower	2002	• Breast cancer survivors (USA) • Stage 0 – II • *n* = 40 (mean age for fatigued group 57.1 years, non-fatigued 58.4 years) • Approximately 5 years post-diagnosis	• Morning plasma cortisol (μg/dL), blood collection between 8–10AM • Fatigue tool: SF–36 Health Survey–energy/fatigue subscale	• Fatigued group had significantly lower morning cortisol levels compared to non-fatigued group

2.3.2 Hypothalamic-Pituitary-Thyroidal axis

The HPT axis is an important system in the body, because its main functions are to regulate the rate of metabolism and control body temperature. This happens through a negative feedback loop via thyroid hormones, which are used by virtually every cell in the body (Marieb & Hoehn 2007).

2.3.2.1 Anatomy and physiology of the thyroid gland

The thyroid gland is a butterfly-shaped organ in the anterior part of the neck. It consists of two lobes that are joined by a thin membrane called the isthmus as shown in Figure 2.3. The thyroid gland is made up of follicles—groups of cuboidal or squamous epithelial cells—inside which are stores of thyroglobulin molecules with attached iodine atoms. Thyroglobulin molecules are the basis for thyroid hormones (Marieb & Hoehn 2007).

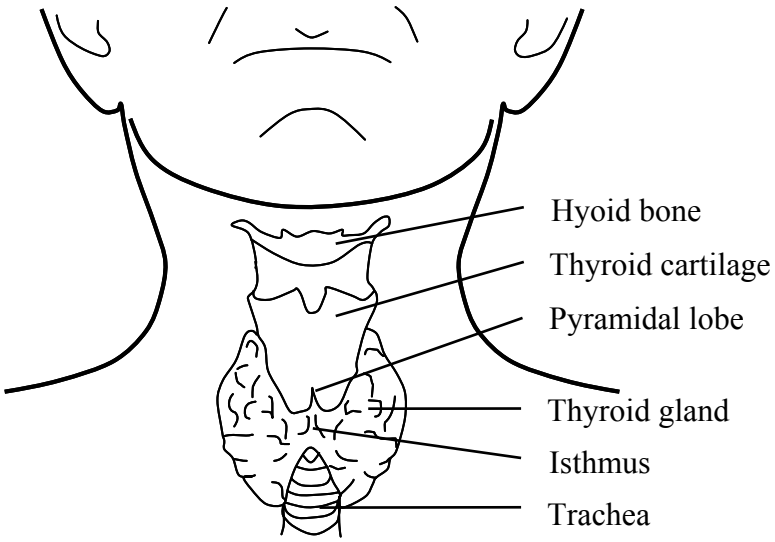


Figure 2.3 Thyroid gland (Nussey & Whitehead 2001)

The pituitary gland, in response to hypothalamic stimulation, releases Thyroid-Stimulating Hormone (TSH) which stimulates the follicular cells in the thyroid gland to release stored

thyroxine (T4) and triiodothyronine (T3) from thyroglobulin. With the aid of specific enzymes (i.e. de-iodinases) inactive T4 is converted to bioactive T3. When levels of thyroid hormones increase, the pituitary inhibits TSH release levels via a negative feed-back loop (Goodman 2009).

Most thyroid hormones circulate in the blood stream either bound to proteins called thyroid-binding globulins, while a much smaller fraction are unbound and thus able to cross the capillary endothelium to perform their biological function (Goodman 2009). Bound and free fractions in blood remain in equilibrium (Goodman 2009). Thyroid hormones are released in a circadian pattern, with highest levels shortly after the onset of sleep and lowest levels in the afternoon.

On a cellular level, after thyroid hormones cross the cell membrane, they regulate the metabolic rate by activating genes coding for glycolysis and Adenosine Triphosphate (ATP) production, as well as binding to receptors on the surface of mitochondria, thereby increasing the rate of mitochondrial ATP production (Martini 1998). Cells in the body are able to convert ATP (the high-energy compound) to Adenosine Diphosphate (ADP) and a phosphate, thus releasing energy for cell functions (Martini 1998). Thus, low thyroid hormones result in lower energy reserves, which explain why fatigue is often a symptom of hypothyroidism.

2.3.2.2 Thyroid hormones in cancer-free individuals

Population reference ranges for thyroid hormones tend to vary among diagnostic laboratories, but in general, the upper levels of the TSH reference range have been gradually decreasing due to more sensitive assays and the acknowledgement that normal reference ranges in the past were based on populations who may have had mild

undiagnosed thyroid disease (Dickey, Wartofsky & Feld 2005; Wartofsky & Dickey 2005). It is also becoming increasingly recognised that each individual may have their own set-point of normal thyroid hormone levels, because research evidence suggests that intra-individual variation is much narrower than variability within the whole population (Andersen et al. 2002).

It is estimated that approximately 10% of otherwise healthy women in the general population have an undiagnosed thyroid condition (Canaris et al. 2000). Signs of thyroid dysfunction can be obvious, such as goitre or eye problems, but often they can be vague and can overlap with other symptoms common in disease but also in health (e.g. fatigue, constipation, weight gain/loss) (Martini 1998). Subclinical thyroid dysfunction (i.e. abnormal TSH but normal T4 and T3) and overt thyroid dysfunction are often associated with detriments in certain quality of life domains (Gulseren et al. 2006; Samuels et al. 2007; Watt et al. 2007), although a recent Australian study did not find differences in quality of life between women with subclinical thyroid disease and healthy women (Bell et al. 2007). In general, when patients were asked about aspects of quality of life that were most affected by their thyroid condition, fatigue and emotional susceptibility were ranked highest overall (Watt et al. 2007). Similarly, Gulseren et al. (2006) reported that fatigue was an early complaint for 28% of patients with subclinical hypothyroidism and 36% of patients with overt thyroid dysfunction.

2.3.2.3 Thyroid hormones in breast cancer

Thyroid disorders are reported to be more prevalent among women with breast cancer than in women without cancer (Giani et al. 1996; Turken et al. 2003) and for decades now, there has been an ongoing debate regarding an association between thyroid dysfunction and breast cancer risk. One important review paper (Goldman 1990) describes the historical

basis for this apparent association, together with the inconsistent findings in clinical, epidemiological and biochemical studies conducted. The main conclusion drawn by Goldman (1990) is that due to these inconsistencies a causal relationship between thyroid disorders and breast cancer is unlikely, although the two diseases may share some etiological factors. More recently, Kuijpens et al. (2005) investigated whether decreased thyroid hormones and the presence of thyroid peroxidase antibodies were related to an increased breast cancer risk. In this study, a cohort of 2738 women without breast cancer was followed-up from 1994 until 2003. Over the course of the study, thyroid function and thyroid autoimmunity were investigated in relation to newly diagnosed breast cancer cases. Low free T4, but not thyroid autoimmunity, was found to be an independent risk factor for breast cancer development (Kuijpens et al. 2005). In summary, while the prevalence of thyroid disorders seems to be higher in women with breast cancer, further studies are needed to elucidate whether there exists a causal relationship between the two conditions.

Several studies have investigated the effect of adjuvant treatment on post-therapy thyroid function. Kumar et al. (2004) measured thyroid function at the start and end of chemotherapy in women with stage I – IIIb breast cancer (n = 178, mean age 49 years) and found that total levels of T4 significantly increased compared to pre-chemotherapy levels, although there were no significant differences in free T4 and TSH. The drug Tamoxifen is known to influence several hormones and results in increased thyroid-binding globulin (Anker et al. 1998; Kostoglou-Athanassiou et al. 1998; Mamby, Love & Lee 1995)—the protein that binds T4—however, conflicting results have been published regarding changes in TSH, total T4, free T4 and free T3 levels (Anker et al. 1998; Kostoglou-Athanassiou et al. 1998; Mamby, Love & Lee 1995; Zidan & Rubenstein 1999).

Permanent damage to the thyroid gland may occur if it is within the radiation therapy treatment field (Hancock, McDougall & Constine 1995; Nishiyama et al. 1996). While thyroid dysfunction following irradiation is most commonly reported in patients with a head and neck malignancy or Hodgkin's Disease (Jereczek-Fossa et al. 2004), thyroid gland hypo-function has also been reported in women who received nodal irradiation for breast cancer where the thyroid gland was in the direct Supraclavicular Fossa (SCF) treatment field (Bruning et al. 1985; Cutuli et al. 2000; Joensuu & Viikari 1986; Reinertsen et al. 2009; Ryu et al. 2003). Studies by Bruning et al. (1985) and Joensuu & Viikari (1986) found that 10–25% of women developed hypothyroidism some years after breast radiation therapy where part of the thyroid was in the direct radiation field. Similarly, Cutuli and colleagues (2000) published a report of five women who presented with clinical symptoms and biochemical evidence of severe hypothyroidism only weeks after completing breast RT and in whom approximately 65% of the homolateral lobe of the thyroid was assumed to have been in the direct treatment field (Cutuli et al. 2000). Ryu et al. (2003) conducted a longitudinal investigation into thyroid gland size (measured as the greatest diameter of the thyroid in the axial plane as seen on ultrasound) in 77 women who received breast radiation therapy. The authors estimated that the thyroid gland received approximately 105% of the dose prescribed to the supraclavicular field and found that significant reductions in thyroid gland size occurred for 41.5% of subjects at one year, 70.3% of subjects at two years and 71.4% of subjects at three years post-radiation therapy. Most recently, Reinertsen et al. (2009) evaluated thyroid function in women between two and six years after adjuvant chemotherapy and radiation therapy for breast cancer and concluded, that compared to an age-matched control group of healthy women, the prevalence of hypothyroidism was significantly higher in the breast cancer group (Reinertsen et al. 2009). Interestingly, one large epidemiological study (n = 38 255) did not find an increase in the risk of hypothyroidism in women who received nodal irradiation

(versus no nodal irradiation), but found that women who received any radiation therapy were more likely to develop hypothyroidism than healthy matched controls (Smith et al. 2008).

The degree of thyroid dysfunction following irradiation is likely to be influenced by the dose and the volume of thyroid gland that receives direct or scattered radiation and it may also depend on the ability of the healthy thyroid to repair any radiation damage. The thyroid gland can tolerate radiation doses of around 30 Gy, with a $TD_{5/5} - TD_{50/5} = 30 - 40$ Gy (Rubin 1989). This means there is a 5% – 50% chance of developing a serious thyroid complication in five years at doses between 30 – 40 Gy. One study reported that radiation doses of around 26 Gy may prove to have a detrimental effect (Hancock, McDougall & Constine 1995). While the typical radiation dose to a small part of the thyroid gland during breast cancer treatment is thought to be very low, the evidence mentioned above suggests that the dose and its effects in the long-term are not trivial and warrant further investigation.

The thyroid gland is not currently listed as an organ at risk in the clinical practice guidelines for breast radiation therapy (National Health and Medical Research Council 2001; The Royal Australian and New Zealand College of Radiologists 2002), nor is it routine practice to quantify radiation dose to the thyroid during treatment planning. There appear to be no direct investigations in the published literature into how much dose the thyroid receives in breast radiation therapy, although indirectly, thyroid dose has been reported in three studies comparing different breast treatment techniques (Table 2.4).

Table 2.4 Studies showing radiation dose to the thyroid during breast cancer RT

First author	Year	Subjects and methods	Dose prescription	Main findings
Ludwig	2008	• Phantom study using different sized breast-cup attachments • Dose to thyroid measured using TLDs • Comparison of wedged tangents and forward-planned segmented open fields	• Total dose 50 Gy in 25#s, 2.0 Gy/# • Thyroid gland not in direct field	• Wedged tangents: Mean thyroid dose/# = 0.028 Gy Total thyroid dose = 0.7 Gy • Segmented open fields: Mean thyroid dose/# = 0.012 Gy Total thyroid dose = 0.3 Gy
Kim	2007	• Breast cancer patients (*n* = 24) • Measured planned dose to thyroid gland • Comparison of whole breast irradiation (tangents only) and partial breast irradiation (4 non co-planar fields)	• Whole breast irradiation: Total dose 50.4 Gy in 25#s, 1.8 Gy/# • Partial breast irradiation: Total dose 38.5 Gy in 10#s, 3.85 Gy/# • Thyroid gland not in direct field	• Whole breast irradiation: Mean max. dose 25#s = 1.95 Gy Highest max. dose = 17.9 Gy • Partial breast irradiation: Mean max. dose 10#s = 0.5 Gy Highest max. dose = 5.3 Gy
Dogan	2007	• Breast cancer patients (n = 10) • Measured planned dose to thyroid gland • Comparison of 3D conformal (wide tangents, AP and PA) and IMRT (2-, 4-, 6-, 9-field step-and-shoot)	• Total dose 50 Gy in 25#s, 2.0 Gy/# • Part of thyroid gland included in direct fields for both techniques	• 3D conformal Mean thyroid D_{50} = 0.9 Gy • IMRT techniques D_{50} ranged from 11.9–32.7 Gy

Key: 3D – 3 Dimensional; # – fraction , AP – Anterio-Posterior, Gy – Gray, IMRT – Intensity Modulated Radiation Therapy, PA – Posterio-Anterior, TLDs – Thermoluminescent Devices

2.3.2.4 Thyroid hormones and fatigue in breast cancer

Thyroid dysfunction and fatigue are known to be related in the cancer-free population. The reports mentioned above examined thyroid function before and after breast cancer treatment or compared to healthy controls, but no studies to date have evaluated the relationship between thyroid function and fatigue specifically in breast cancer patients.

2.3.3 Hormone inter-relationships

An aspect regarding cancer-related fatigue that has not been examined is the affect that hormmones exert on each other. For instance, higher glucocorticoid levels are thought to suppress TSH (Samuels 2000) which could lead to decreased thyroid hormone levels and possibly higher fatigue. The pattern of cortisol and thyroid hormone regulation is depicted in Figure 2.4, including the pathway of TSH suppression by glucocorticoids. The way in which cortisol and thyroid hormones interact and may result in cancer-related fatigue will not be specifically investigated in this study, but is an important issue that warrants future research.

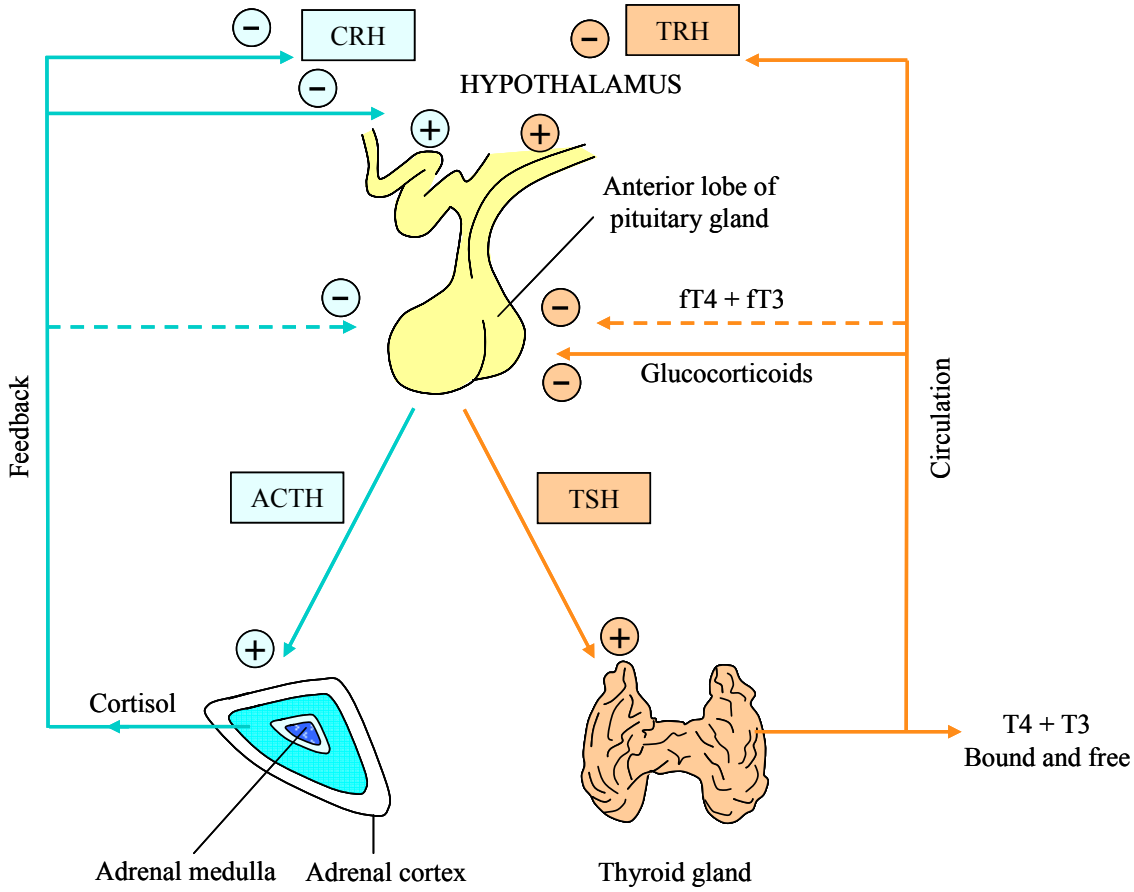


Figure 2.4 Feedback control of cortisol and thyroid hormones, modified from Nussey & Whitehead (2001)

2.4 Summary

Quality of life after breast cancer treatment is often reported to be generally good and similar to that of women without breast cancer; however, this is in contrast to the fact that many researchers find up to 30% of breast cancer patients reporting considerable fatigue. While many factors have been examined in the literature as potential contributors to higher fatigue, many of these studies are limited by methodological weaknesses such as cross-sectional designs, lack of standardised timepoints of measurement and inadequate exclusion criteria. This has been particularly true in studies looking at prevalence rates of fatigue and cortisol rhythm. At present, no studies have investigated the relationships between thyroid function and fatigue in breast cancer patients. Understanding fatigue development after breast cancer treatment is therefore important, because with improved detection and treatment outcomes after breast cancer, many more women can be expected to live with the various consequences of having had treatment. A better understanding of the factors related to persistent fatigue post-RT could help in identifying inviduals who are at risk of fatigue even before they start treatment, so that patients receive appropriate advice on how best to deal with fatigue should it occur.

3 Methodology

3.1 Study design, aims and hypotheses

This study was observational, longitudinal with repeated measures and was exploratory in nature. Participants attended identical fatigue assessment sessions on two occasions, approximately six months apart. There were four main aims:

1. To measure the level and prevalence of fatigue six months after breast cancer RT
2. To determine whether persistent fatigue was related to cortisol rhythm and thyroid function indices
3. To measure radiation dose to the thyroid gland on breast RT treatment plans and compare the mean thyroid dose, thyroid function and fatigue between 'tangents only' RT treatment and 'tangents + SCF' RT treatment
4. To investigate whether there was a relationship between mean thyroid dose, fatigue and thyroid function six months after RT treatment

The above aims were the basis for the following six hypotheses:

H_1: The level of persistent fatigue at six months after breast cancer RT will be significantly different to the level of fatigue experienced pre-treatment.

H_0: The level of persistent fatigue at six months after breast cancer RT will not be significantly different to the level of fatigue experienced pre-treatment.

H_2: Fatigue level measured at six months after breast cancer RT will be positively correlated with levels of cortisol measured at awakening, 30 minutes after awakening and in the evening.

H_0: Fatigue level measured at six months after breast cancer RT will not correlate with levels of cortisol measured at awakening, 30 minutes after awakening and in the evening.

H_3: Fatigue level measured at six months after breast cancer RT will be negatively correlated with thyroid hormones (fT4 and fT3) and positively correlated with TSH.

H_0: Fatigue level measured at six months after breast cancer RT will neither correlate with thyroid hormones (fT4 and fT3) nor TSH.

H_4: Radiation dose to the thyroid gland will be significantly different (lower) in participants treated with a standard 'tangents only' RT treatment compared to participants treated with 'tangents + SCF' RT treatment.

H_0: Radiation dose to the thyroid gland will not be significantly different in participants treated with a standard 'tangents only' RT treatment compared to participants treated with 'tangents + SCF' RT treatment.

H_5: Thyroid function and fatigue in participants treated with 'tangents + SCF' RT treatment will be significantly different compared to participants treated with 'tangents only' RT treatment.

H_0: Thyroid function and fatigue in participants treated with 'tangents + SCF' RT treatment will not be significantly different compared to participants treated with 'tangents only' RT treatment.

H_6: Radiation dose to the thyroid gland will be positively correlated with fatigue and TSH and negatively correlated with thyroid hormones at six months after breast cancer RT.

H_0: Radiation dose to the thyroid gland will neither correlate with fatigue nor thyroid function indices at six months after breast cancer RT.

Each aim was linked to one or more hypotheses as shown in Table 3.1.

Table 3.1 Links between aims and hypotheses

Study aim	Hypothesis	Cohort studied
1	H_1	1
2	H_2 and H_3	1 and 2
3	H_4 and H_5	1 and 2
4	H_6	1 and 2

3.2 Setting

The setting for this study was the Department of Radiation Oncology, Northern Sydney Cancer Centre, Royal North Shore Hospital (RNSH), which is a public teaching hospital, located approximately 8 km north of Sydney City CBD, NSW, Australia. This department was chosen as the study site because of its participation in evidence-based practice in breast cancer treatment, state-of-the-art radiation therapy equipment and the willingness of medical and allied health staff to collaborate in this study.

Salivary cortisol specimens were stored at the Brain and Mind Research Institute (BMRI) imaging laboratory (The University of Sydney, 100 Mallett Street, Camperdown, NSW, Australia) and cortisol analysis took place at the School of Exercise and Sports Science biochemistry laboratory (Faculty of Health Sciences, The University of Sydney, Cumberland Campus, Lidcombe, NSW, Australia). Blood tests were performed at the RNSH and were sent for analysis to PaLMS, a commercial laboratory at the hospital.

3.3 Participants

3.3.1 Study population

Participants were women diagnosed with non-metastatic breast cancer. Two cohorts of participants were studied as shown in Figure 3.1. Cohort 1 comprised women who were about to receive RT treatment for breast cancer. The first fatigue assessment, T_0, occurred prior to RT and was deemed a baseline measure. These participants were then followed-up at T_1, which was six months after their last RT treatment (± fortnight). Cohort 2 comprised women who had completed RT six months earlier. These participants were assessed initially at T_1, six months after their last treatment fraction and again at T_2, 12 months post-RT (± fortnight). The rationale for including a second cohort of participants was to increase the potential sample size of participants, particularly at the six month post-RT timepoint. The length of the researcher's candidature was a limiting factor in the possible duration of data acquisition and a second cohort was deemed an acceptable compromise in this instance.

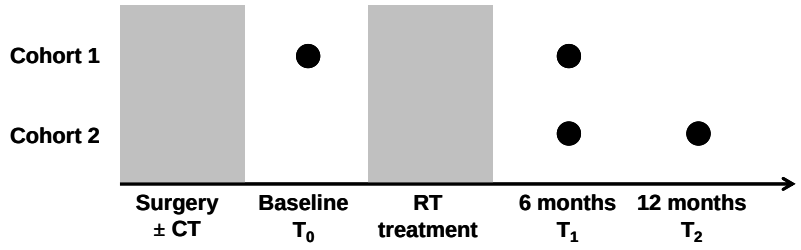


Figure 3.1 Assessment timepoints. CT – Chemotherapy; RT – Radiation Therapy

3.3.2 Eligibility

Participants were eligible if they had a primary breast cancer diagnosis, were referred for adjuvant RT (Cohort 1) or had completed RT six months earlier (Cohort 2), were able to read, write and speak English or agreed to communicate via an interpreter and provided written informed consent. Participants were ineligible if they had proven metastatic

disease, had a prior history of RT treatment, had a known diagnosis (in the past 12 months) of thyroid dysfunction, serious mental illness (e.g. bipolar disorder, schizophrenia), chronic fatigue syndrome or another condition known to be related to fatigue (e.g. fibromyalgia), had used medication to treat any of the aforementioned conditions, or had a history of substance abuse in the past 12 months.

3.3.3 Sample size estimates

The estimated sample sizes were calculated based on the typical number of newly presenting breast cancer cases seen in one year at the Department of Radiation Oncology, Northern Sydney Cancer Centre, RNSH. This was estimated to be approximately 250 new cases. Taking into account the fact that around 30% of people decline participation in research studies and clinical trials (Shimm & Spece 1992) and that the recruitment period was planned for approximately six months, the sample sizes were estimated to be 85 participants in each cohort. Power calculations were not performed as this was an observational study.

3.3.4 Radiation therapy treatment protocol

All participants completed radiation therapy at the Northern Sydney Cancer Centre according to standard protocols. Treatment planning consisted of a computed tomography planning scan using a GE Lightspeed scanner. Participants were in a supine position, lying on an immobilisation board with arms raised and resting in adjustable arm and wrist supports. Slices were acquired at 0.25 cm thickness. Computer planning was performed using the VARiAN Eclipse treatment planning system using 3-Dimensional Conformal Radiation Therapy (3D CRT). Medial and lateral borders were marked on the computed tomography data set by the Radiation Oncologist, with treatment to regional lymphatics (if required) marked on Digitally Reconstructed Radiographs (DRR).

Treatment consisted of both medial and lateral tangents with or without an electron boost field (‘tangents only’), medial and lateral tangents with treatment of the supraclavicular fossa with or without a posterior axilla field (‘tangents + SCF’) or Accelerated Partial Breast Irradiation (APBI) with the use of multiple non-coplanar beams. Conventional dose prescriptions were 50 Gy in 25 treatment fractions to the tangents (2 Gy per fraction, ± 10 Gy in 5 fractions for electron boost), 50 Gy in 25 fractions to the supraclavicular field (2 Gy per fraction), hypofractionated RT dose prescription with 42.4 Gy given in 16 fractions (2.65 Gy per fraction) and for the APBI technique delivering 38.5 Gy in 10 fractions given twice daily (3.85 Gy per fraction).

Treatment was delivered using a 6 MV VARiAN linear accelerator fitted with multi-leaf collimators and an electronic portal imaging device for treatment verification. All participants were reviewed by their Radiation Oncologist once a week throughout their treatment course as per standard treatment protocol at this department.

3.4 Ethics approval and funding

Ethics approval to conduct this study was sought and granted on the 29 November 2007 from the Human Research Ethics committee at the Northern Sydney Central Coast Health (NSCCH) service. An amendment was added to the original application requesting approval to assess the endocrine status as well as quality of life and impact of fatigue on all participants. This was approved on the 8 May 2008. The complete ethics application and amendment were also ratified by the Human Research Ethics Committee at The University of Sydney. Research funding for this study was obtained from the Cancer Institute NSW under the Scholar Award Scheme. Ethics approval documents, consent forms and funding approval are shown in Appendix A.

3.5 Data acquisition

3.5.1 Recruitment

Recruitment of participants commenced on the 16 January 2008 and finished on the 4 August 2008 (201 calendar days; six months and three weeks). Potential participants were given verbal and written information about the study at either a new patient clinic appointment or RT planning appointment (Cohort 1) or at the follow-up consultation with their Radiation Oncologist (Cohort 2). In each instance, women were asked to consider participating and were contacted a short while later to confirm whether they would like to take part in the study.

At this point, if the woman chose not to give consent she was thanked for her time. Medical and demographic information of women declining participation was collected from the patients' medical record to determine whether there were any differences between women who consented or declined participation. If verbal consent was obtained, a convenient time for conducting the first fatigue session was agreed upon and written informed consent was completed at the start of the first fatigue assessment session before any data collection.

Recruitment progress was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation 1983-2003) and an electronic schedule of appointments was also kept up-to-date using this software. The recruitment spreadsheet was used to summarise participant accrual on a monthly basis.

3.5.2 Fatigue assessment session

Fatigue assessments were conducted in a quiet room located at the Radiation Oncology Department. Once written informed consent was obtained, the session commenced with a

demographics questionnaire. Fatigue is a subjective phenomenon and is experienced differently by individuals. For this reason four fatigue instruments were used in this study to measure subjective fatigue, each with a particular and distinct focus on the experience. The demographics questionnaire and all fatigue instruments are shown in Appendix B.

Prior to answering the questionnaires, participants were instructed to read each question carefully and to complete it honestly. They were assured that there were no right or wrong answers. Participants were told to take as much time as they needed to complete each questionnaire and to ask questions if they were unsure or did not understand a particular item. Some participants chose to complete the questionnaires at home due to work or family commitments.

3.5.2.1 Demographics questionnaire

A data sheet was developed to record each participant's demographic information. Questions were asked by the researcher and participants were invited to respond. The questionnaire consisted of demographic information, anthropometrics, risk behaviours, medical history and cancer and treatment history. Specific information about participant's cancer and cancer treatment history was completed separately using medical records.

Anthropometric measurements consisted of participants' weight (kg) and height (m) to enable calculation of the Body Mass Index (BMI), as well as waist and hip circumferences (cm) which were used to calculate the Waist to Hip Ratio (WHR). The BMI was used to measure the level of obesity and the WHR to measure body fat distribution. Both of these measures were surrogate markers of participants' nutritional or physical activity status over the study period. Measurements were taken according to the World Health Organisation

(WHO) guidelines and were conducted at the Radiation Oncology Department (World Health Organisation 1987).

Participants' weight was measured to the nearest 0.1 kg with digital scales placed on a stable floor surface (linoleum, not carpet). WHO recommend that weight is measured with subjects wearing no clothes or only light underwear. Participants in this study were not asked to disrobe, but only to remove heavy clothing i.e. shoes, coat. Height was measured using a wall-mounted tape measure and a small flat metal ruler. Participants were asked to remove their shoes, stand against the wall with their feet together, back straight and looking straight ahead. The measure was taken with the participants reaching their maximum height, without taking their feet off the ground and holding their head in the Frankfurt plane (World Health Organisation 1987). Height was recorded to the nearest 0.5 cm. Calculation of the BMI was carried out using the standard formula weight/height^2 (kg/m^2) and classified according to the WHO categories of underweight < 18.5 kg/m^2, normal weight 18.5 to 24.9 kg/m^2, grade 1 overweight 25 to 29.9 kg/m^2, grade 2 overweight 30 to 39.9 kg/m^2 and obese > 40 kg/m^2.

WHR measurements were taken with subjects standing upright, with feet approximately 25–30cm apart and weight equally distributed on both feet (World Health Organisation 1987). The waist circumference (cm) was taken at the level of the umbilicus and the hip circumference (cm) at the maximal width of the hips (Houmard et al. 1991). For waist circumference measurements, participants were asked to breathe in and then gently breathe out, with the measurement taken after full expiration as this reduces abdomen muscle contraction (World Health Organisation 1987). Waist and hip measurements on the first 36 participants were performed in duplicate to assess intra-observer reliability in measurement

taking. The WHR was calculated by dividing the waist circumference by the hip circumference. Being a ratio, this measurement does not have units.

3.5.2.2 Multidimensional Fatigue Symptom Inventory–Short Form

The Multidimensional Fatigue Symptom Inventory–Short Form (MFSI–SF) is a 30 item questionnaire developed for use in cancer patients by David Stein and colleagues (Stein et al. 2004; Stein et al. 1998). It consists of five empirically derived fatigue subscales (physical fatigue, emotional fatigue, mental fatigue, general fatigue and vigor), each with six items. Participants are asked to consider each statement and indicate whether they experienced it over the past seven days to the following degree: 'not at all' = 0, 'a little' = 1, 'moderately' = 2, 'quite a bit' = 3 or 'extremely' = 4. The score for each subscale is summed and thus can range between 0 and 24. A higher score indicates worse level of fatigue, with the exception of the vigor subscale, where a higher score indicates higher level of vigor. The total fatigue score can also be calculated by summing the physical, mental, emotional and general subscales and subtracting the score for the vigor subscale.

The MFSI–SF questionnaire has been shown to be valid in people with cancer and it has good test-retest reliability as well as internal consistency reliability; in a previous study, Cronbach's alpha coefficients for all subscales ranged between .87 and .96 (Stein et al. 2004). The questionnaire has been used by breast cancer patients in the past, allowing for meaningful comparisons to be made with previous studies. The time interval (past seven days) is relatively short, which is favourable as it reduces the potential for recall bias. The questionnaire was chosen for use in this study because it allows one to determine which fatigue dimension participants are experiencing most predominantly, but does not assume that fatigue is actually present.

3.5.2.3 Somatic and Psychological HEalth REport–12

The Somatic and Psychological HEalth REport–12 (SPHERE–12) contains 12 questions and was developed in Australia by Professor I. Hickie (Hickie et al. 2001) as a tool to screen for depression by General Practitioners. The SPHERE–12 is made up of two subscales (somatic symptoms, SOMA–6 and psychological symptoms, PSYCH–6). Participants are asked to respond to each statement and how it related to them over the past few weeks by using one of the following three responses: 'never or some of the time', 'a good part of the time' or 'most of the time', which attract a score of '0', '1' and '2' respectively. Higher scores are interpreted as worse symptoms. The total score for each subscale is summed and compared to a predefined cut-off on each subscale. This enables respondents to be classified into one of four categories: Soma case (score of > 3 on the SOMA–6), Psych case (score of > 2 on the PSYCH–6), Both (above cut-off on both subscales) or Neither (below cut-off on both subscales).

The SPHERE–12 has been shown to be reliable and valid and has been previously used in the NSW breast cancer population to differentiate between fatigue and depression, which are known to be related (Bennett et al. 2004). The SPHERE–12 was included in the study to enable significant fatigue to be measured as separate from psychological symptoms or mood disturbance.

3.5.2.4 EORTC Quality of Life Questionnaire–C30

The EORTC QLQ–C30 questionnaire was developed by the European Organisation for Research and Treatment of Cancer for specific use in cancer patients (Aaronson et al. 1993). The instrument is made up of 30 items which fit into one of three domains; functioning (15 questions), symptoms (13 questions) and global quality of life (two questions). The functioning domain is further subdivided into five subscales (role

functioning, physical functioning, social functioning, emotional functioning and mental functioning) and the symptom domain into nine subscales (fatigue, nausea and vomiting, appetite, diarrhoea, constipation, dyspnoea, sleep quality, pain and financial problems). The response categories for the functioning and symptom domains are 'not at all', 'a little', 'quite a bit' and 'very much' with scores of '1' to '4', respectively. Global quality of life uses two anchors 'very poor' and 'excellent' and is scored on a scale of '1' to '7'. Calculation of overall scores is done using a scoring manual provided with the questionnaire document (Fayers et al. 2001). In short, the total score is summed for each subscale and converted to a standardised score of between '0' and '100'. A higher score on the functioning subscale indicates better functioning, a higher score on the symptom subscales indicates worse symptoms and higher scores on the global quality of life subscale are interpreted as better quality of life. An overall total score for this instrument is not calculated.

The EORTC QLQ–C30 has been in wide use since 1993 and hence there is a lot of normative data and breast cancer patient data available for comparison with the current sample. Its psychometric properties are very good, particularly in terms of its reliability and validity. The advantage of this instrument is that it measures a variety of factors affecting quality of life in one simple, easy and relatively short questionnaire. It was chosen for use in this study because it measures participants' global quality of life, which is not included in the MFSI–SF or SPHERE–12. The questionnaire was included in the study only after an ethics amendment, therefore not all participants completed this questionnaire at their first fatigue assessment.

3.5.2.5 Fatigue Impact Scale

The Fatigue Impact Scale (FIS) was developed by Fisk et al. (1994) to measure the impact of fatigue on the social, cognitive and physical functioning of people with multiple sclerosis. It is a 40 item questionnaire which is made up of three subscales: social (20 items), cognitive (10 items) and physical (10 items). Respondents are asked to consider the past few weeks of their life and to indicate for each item how much of a problem their fatigue level has been. The response categories are 'no problem', 'small problem', 'moderate problem', 'big problem' and 'extreme problem' and these are scored from '0' through to '4'. Scores for each subscale are summed and a total fatigue impact score can be calculated by summing all items.

It is likely that this is the first study using the FIS in a breast cancer population to measure fatigue impact. A Medline citation search conducted in May 2008 found no studies that had used the FIS in breast cancer patients. Table 3.2 summarises the types of samples in which the FIS instrument has been validated.

Table 3.2 Citations using the Fatigue Impact Scale

Sample	**Number of citations per year**									
	Total	'08	'07	'06	'05	'04	'03	'02	'01	'00
Multiple Sclerosis	51		8	9	15	3	6	7	1	2
Hepatitis C	9		2	1	3		1	2		
1º Biliary Cirrhosis	7	1		3	2					1
CFS	4	2	1	1						
Post-polio Syndrome	4		2	2						
COPD	3		1	1		1				
IBS	2	1	1							
Post-stroke Fatigue	2		1		1					
Fibromyalgia	1		1							
Myasthenia Gravis	1			1						
Parkinson's disease	1		1							
Sclerosing Cholangitis	1					1				
Traumatic brain injury	1			1						

Key: CFS – Chronic Fatigue Syndrome; COPD – Chronic Obstructive Pulmonary Disease; IBS – Irritable Bowel Syndrome

Nevertheless, inclusion of the FIS was deemed appropriate because it has been used in people with chronic fatigue syndrome (Amsterdam, Shults & Rutherford 2008; Blazquez et al. 2008; Newton et al. 2007; Weatherley-Jones et al. 2004), which is similar to the experience of cancer-related fatigue. The FIS psychometrics, including internal consistency and reliability, were assessed by making comparisons between the FIS social, cognitive and physical functioning subscales and the corresponding EORTC QLQ–C30 functioning subscales (social, mental and physical functioning). Although conducting a more in-depth analysis of the FIS psychometrics would have been desirable (e.g. asking healthy age-matched female volunteers to complete the questionnaire), it was not possible due to time limitations.

3.5.2.6 Additional fatigue question

An additional question, not included in any of the fatigue questionnaires, was asked to ascertain how fatigued participants felt in the past seven days compared to before their breast cancer diagnosis. Participants were asked the following question:

"How do your fatigue levels in the past seven days compare with those before your cancer diagnosis?"

Responses were 'worse', 'no different' or 'better' and participants were asked to indicate the appropriate answer by circling one of the categories. This additional question was asked at both time points and was considered necessary, because it contained a common set-point anchored at a time before the participant's cancer diagnosis. This timepoint was deemed as the participant's usual level of fatigue.

3.5.3 Saliva collection protocol and cortisol analysis

3.5.3.1 Specimen collection

Measurement of cortisol in saliva has several advantages over the measurement of cortisol in other body fluids. Compared to cortisol measurement from a blood serum sample, saliva collection is non-invasive, posing minimum discomfort to the participant. It is inexpensive, which allows for repeated sampling and can be performed by the participant at home without their attendance at the research site. 24–hour salivary cortisol levels have been repeatedly shown to closely correspond with cortisol levels in serum (Dorn et al. 2007; Gozansky et al. 2005; Kirschbaum & Hellhammer 1994).

As outlined in the previous chapter, cortisol release in the body follows a 24–hour circadian rhythm. The highest concentrations are in the morning, with a peak in hormone levels occurring at approximately 30 minutes after awakening and thereafter a slow reduction in circulating cortisol over the course of the day and into the night. For this reason, participants were asked to collect three saliva samples per day for a total of three days to account for the random occurrence of an unusually stressful day or instance during sampling. Participants were asked to collect the first saliva sample at awakening, the second sample exactly 30 minutes after awakening and the last sample in the evening before bedtime. Specific collection times were not prescribed, mainly because morning cortisol levels peak at different times for different people (Bailey & Heitkemper 2001) and also because this might introduce problems with protocol adherence (e.g. forgetting to take a sample at a given time, then sampling later/earlier). Participants were not asked to collect additional specimens during the day to minimise subject burden.

Saliva was collected by participants with single use collection tubes called Salivettes® (Sarstedt, Aktiengesellschaft & Co., 16 Park Way, Technology Park, SA 5095, Australia).

The Salivette® consists of a neutral flavoured cotton swab inside a small plastic tube with a pierced base, which are suspended within a larger plastic tube that enables easy centrifugation (Figure 3.2). To collect a saliva specimen, the cotton swab is removed, placed in the mouth and allowed to absorb saliva for a few minutes. Once fully saturated, the swab is removed and placed inside the small plastic tube and the lid closed. Approximately 1 mL of saliva is collected using this method.

Figure 3.2 Salivette saliva collection tube

Care was taken to ensure that participants understood the collection protocol and they were encouraged to ask any questions for clarification before commencing. Each participant received a sampling package which consisted of a detailed instruction sheet, including a chart to record the time each sample was collected (Appendix C) and 10 Salivettes (labelled 1–9 for sampling and one unlabelled spare Salivette) inside a zip-lock bag. If required, a postage-paid padded envelope (size 2) was provided for return of samples, in which case the participant was advised to perform sampling on a Friday, Saturday and Sunday, to ensure samples were posted early in the week to avoid delays in postage over the weekend. If not posting, participants returned their samples directly to the researcher at the Radiation Oncology department the day after completing sampling.

3.5.3.2 Storage

Throughout the three day sampling period at home, participants were asked to store their saliva samples in the refrigerator. Samples that were returned to the researcher in person were temporarily stored in an insulated padded cooler bag (Sistema Plastics Ltd, Australia, style 4570, 185 mm x 115 mm x 240 mm) with a reusable gel ice pack to keep the samples cool until they were transported to the laboratory at the BMRI. Posted samples were immediately taken to the laboratory inside the cooler bag. It has been shown that saliva specimens do not degrade if kept at room temperature for seven days (Aardal & Holm 1995) and up to 14 days (Gröschl et al. 2001) or when transferred by the postal service over five days (Clements & Parker 1998). All posted samples were returned to the researcher between two to five days. Once at the laboratory, samples were centrifuged at 3000 revolutions per minute for four minutes using a Heraeus Sepatech Megafuge 1.0 centrifuge (Foss Electric Pty Ltd.). After centrifugation, specimens were aliquoted into labelled microcentrifuge tubes and frozen at −80°C (−86°C Ultralow Freezer, NUAIRE Inc.) until analysis.

3.5.3.3 Cortisol analysis

Saliva specimens were analysed between November 2008 and April 2009 at the School of Exercise and Sports Science using Diagnostic Systems Laboratories Inc. (Texas, USA) DSL-10-67100i ACTIVE® Cortisol Enzyme Immunoassay (EIA) kits, according to the methods contained in the package insert (Appendix D). All analyses were performed with the assistance and under supervision of the laboratory manager and biochemist Dr. Patricia Ruell. In brief, participants' samples from both timepoints were analysed using the same kit. Samples were thawed at room temperature, then thoroughly mixed prior to use with a vortex mixer (Bio-Rad, BR-2000 Vortexer). Standards, controls, and saliva specimens were pipetted in duplicate (Gilson pipetman and Eppendoft multipipette plus) in amounts

outlined in the package insert. Plate shakers used were the IKA MS3 digital and Roto Mix Type 50800 Thermolyre. The washing step was performed manually and repeated six times. Microplates were read using a Bio-Rad Benchmark Plus microplate spectrophotometer and data processing and reduction was done using Microplate Manager Software V5.2.1 (Bio-Rad Laboratories 2002, Life Science Group, California). The overall intra-assay coefficients of variation were below 5.6% and inter-assay coefficients of variation were below 4%. Analyses were repeated if any samples were above 15% coefficient of variation.

3.5.3.4 Population reference values

Several publications have shown a reference range or mean values for salivary cortisol based on sampling time (Aardal & Holm 1995, p. 930; Hansen et al. 2003, p. 307; Patel et al. 2004, p. 1425; Wust et al. 2000, p. 82). Two of these studies used recommendations by the International Federation of Clinical Chemistry (IFCC) and International Union of Pure and Applied Chemistry (IUPAC) to estimate population reference ranges (Hansen et al. 2003; Patel et al. 2004), giving overall the most reliable reference values. Table 3.3 lists the reference ranges published in the literature.

In all four of these studies, age was not found to be related to salivary cortisol levels. Wust et al. (2000) found significantly higher awakening cortisol in women than in men; however, the size of this effect was small. Hansen et al. (2003) also found significantly higher cortisol levels in women, but only at the 60 minutes after awakening sample. Thus, in this study, awakening salivary cortisol was compared to the reference range by Patel et al. (2004) due to the female sample in that study, the 30 minute samples were compared to the pooled reference range derived by Hansen et al. (2003) and the evening levels were considered normal if they were below 6 nmol/L as given by Aardal & Holm (1995).

3.5.4 Blood tests

Consenting participants underwent blood collection either at the PaLMS specimen collection centre (Clinic 13) at the Royal North Shore Hospital, or in some cases (e.g. participant had difficulty walking) the blood collection was performed at the Radiation Oncology Department by one of the nurses. For convenience, no restriction was placed on the time of day the blood test was performed.

3.5.4.1 Specimen collection

In total 10 mL of blood was collected by venepuncture by an experienced and accredited nurse following standard hospital procedures. For a complete differential blood count and haemoglobin analysis blood was collected into an EDTA blood collection tube for both testing and storage at PaLMS haematology. Endocrine samples (TSH, fT4 and fT3) were collected into serum gel tubes, one for analysis and one for storage at PaLMS endocrinology. In all cases, blood specimens were delivered to the laboratory for testing immediately after collection.

Table 3.3 Population reference values for salivary cortisol

First author	Year	Subjects	Methods	Salivary cortisol reference values (nmol/L)		
				Awakening	*20 or 30 minutes after awakening*	*Evening*
Patel	2004	• Healthy adults, *n* = 248 • Males *n* = 128, median age 41 years • Females *n* = 120, median age 44 years	• 1 sample taken at awakening only for 2 consecutive days • RIA	Males 10.9 – 40.3 Females 9.3 – 40.3	nd	nd
Hansen	2003	• Healthy adults, *n* = 120 • Males *n* = 37, mean age 45 years • Females *n* = 83, mean age 44 years	• 4 samples taken on a single day; awakening, 20 and 60 minutes after awakening, 6PM in evening • RIA	Pooled M/F data 3.5 – 35.7	Pooled M/F data 7.5 – 39.9	Pooled M/F data 6PM 1.1 – 10.5
Wust	2000	• Healthy adults, *n* = 509 mean age 37 years • Males *n* = 319 • Females *n* = 190	• 4 samples taken for 2 consecutive days; awakening, 30, 45 and 60 minutes after awakening • Time-resolved fluorescence immunoassay	Pooled M/F data Mean 15.1	Pooled M/F data Mean 22.9	nd
Aardal	1995	• Healthy adults, *n* = 197 • Males *n* = 123 (age range 20–70 years) • Females *n* = 74 (age range 20–70 years)	• 2 samples taken on 1 day only; 8AM and 10PM • RIA	Pooled M/F data 8AM 3.5 – 27.0	nd	Pooled M/F data 10PM < 6.0

Key: RIA – radioimmunoassay; M/F – Male/Female; nd – no data.

3.5.4.2 Full blood count and thyroid function analysis

Tests were performed according to standard commercial laboratory protocols, such as when blood and thyroid function tests are ordered for diagnosis. Routine quality controls were conducted in compliance with the required standards for commercial laboratory procedures. Test results were posted directly from the laboratory to the researcher within a week of the test being performed.

3.5.4.3 Laboratory reference values

PaLMS laboratories used reference values as shown in Table 3.4.

Table 3.4 PaLMS laboratory reference values

Parameter	Units	Reference Interval
Red cells		
Haemoglobin	g/L	115 – 160
Red cell count	$\times 10^{12}$/L	3.80 – 5.20
White cells		
White cell count	$\times 10^{9}$/L	4.0 – 11.0
TSH	mIU/L	0.350 – 3.60
Free T4	pmol/L	10.5 – 25.2
Free T3	pmol/L	2.5 – 7.3

Key: T4 – thyroxine; T3 – triiodothyronine; TSH – Thyroid-Stimulating Hormone

If any differential blood count parameters were found to be outside the reference interval for any participant at any timepoint, this information was communicated to the Radiation Oncologist within 24 hours for further management. If any thyroid function indices were found to be outside of the reference interval at any timepoint, the Radiation Oncologist was notified and the participant's eligibility was reassessed based on whether thyroid medication was required. Participants remained eligible if no thyroid medication was used during the course of the study.

3.5.5 Radiation dose to the thyroid gland

Dose to the thyroid gland was estimated retrospectively from participants' RT computed tomography scans using the Eclipse Treatment Planning System (Varian Medical Systems, Inc.). The data set of each participant was first checked by the researcher to ensure the superior aspect of the thyroid gland was visible. Next, a senior Radiation Oncologist specialising in breast radiation therapy and a Radiation Oncology Registrar, both trained in thyroid gland volume delineation, manually outlined the thyroid on participants' treatment plans. To ensure face validity, a third Radiation Oncologist, highly experienced in head and neck anatomy, verified a small random sample ($n = 5$) of thyroid gland volumes. Only participants who underwent a baseline and/or six month post-RT thyroid function blood test were included in this analysis.

Once the delineation step had been completed, Dose-Volume Histograms (DVHs) were created for each participant using Eclipse software and printed out in hard-copy. The following data was collected from the DVHs: thyroid gland volume (cm^3), mean, minimum and maximum thyroid gland dose (Gy) and dose-volume parameters (V_5, V_{10}, V_{15}, V_{20}, V_{25}, V_{30}, V_{35}, V_{40}, V_{45} and V_{50}, where V_x represents the volume of the thyroid gland receiving x Gy). The treatment plan was visually inspected to determine whether any part of the thyroid gland was in the direct RT field (Y/N). Thyroid function data included T_0 and T_1 thyroid function tests (TSH, fT4 and fT3). Additional calculations were carried out to determine absolute changes in hormone levels from T_0 to T_1 and percentage changes in hormone levels from T_0 to T_1. This was done using Equations 3.1 and 3.2, where 'T_x' represented the first measurement and 'T_y' the second measurement.

Equation 3.1 $\quad$ Absolute change $= T_y - T_x$

Equation 3.2 Relative percentage change = $[(T_y - T_x)/T_x] \times 100$

Additional data used in this analysis were age (years), fatigue at six months post-RT (MFSI–SF general fatigue subscale) and the prescribed treatment dose (Gy).

3.6 Missing data

Behavioural data collected using self-report questionnaires commonly contains some percentage of missing data (Fayers & Machin 2007). Missing data can be random, where items are missed by accident, or participants can deliberately decide not to respond to particular items (Fayers & Machin 2007). Any missing data can be a source of bias, hence it is important to minimise it if at all possible. One of the strategies used in this study was to ask that participants completed all questionnaires during the fatigue assessment session. Once filled-out, all questionnaire pages were checked for completeness with participants being asked to complete any missing items. Most of the missing data in this study was in the form of missing single items rather than entire forms and seemed to occur more when participants took questionnaires home to complete. Overall, the missing data in this study comprised 0.28% of all questionnaire items and was considered to have occurred completely at random.

Responses to any missing items were imputed based on methods described in the EORTC QLQ–C30 scoring manual (Fayers et al. 2001). Using this method, the score for a missing item was assumed to have the same value as the average of the remaining completed items in the subscale. Due to a later ethics amendment, entire questionnaires were missing at the first timepoint for some participants. In this case, subsequent questionnaires were used for cross-sectional analyses only, because a base-line was unavailable for repeated-measures analysis.

In some cases biological data (thyroid function tests, salivary cortisol) were missing for some participants due to either participant declining the procedure (e.g. saliva collection; had ‘bad’ veins and venepuncture was not possible). In addition, as a result of a later ethics

amendment, some participants were missing biological data at the first timepoint, in which case their follow-up biological data was used in cross-sectional analyses only.

3.7 Data analysis

All data analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows, Graduate Student Version 15.0 (SPSS Inc. 1989-2006). Charts and graphs were created with GraphPad Prism, Version 4.0 for Windows (GraphPad Software Inc. 1992-2005) or Microsoft Excel (Microsoft Corporation 1983-2003) and Microsoft PowerPoint (Microsoft Corporation 1983-2003). The distribution of all variables was visually inspected and tested for normality using the Shapiro-Wilk statistic. Parametric statistics were used if the distribution was found to be approximately normal (Shapiro-Wilk statistic > .05) and non-parametric statistics were used for non-normal distributions (Shapiro-Wilk statistic < .05) (Allen & Bennett 2008). As this was an exploratory study, statistical significance was set at $p < .05$ and no additional statistical tests were used to correct for multiple tests.

3.7.1 Recruitment

Recruitment summaries were produced on a monthly basis using Microsoft Excel (Microsoft Corporation 1983-2003) to track the progress of participant accrual, as well as any eligible patients who declined participation in the study. Information regarding either the reason for exclusion or participation refusal were recorded. Differences between demographic and disease variables were compared between consenting and declining participants (Cohort 1) to ensure that the study sample was unbiased with respect to these variables. Continuous data were analysed using independent samples *t* tests and categorical variables using the Chi-Square (x^2) Test of Contingencies (Allen & Bennett 2008).

3.7.2 Reliability studies

3.7.2.1 *Questionnaire psychometrics*

All four questionnaires were tested for internal consistency reliability using Cronbach's alpha. Questionnaire data for all participants and timepoints were pooled and subscales (only those consisting of more than two items) were analysed separately. A Cronbach's alpha > .70 was considered acceptable (Allen & Bennett 2008).

3.7.2.2 Intra-observer reliability of waist and hip circumference measurements

Intra-observer reliability in measuring the waist and hip circumferences was carried out on the first 36 measurements taken. Absolute and percentage changes in measurements were calculated using Equations 3.1 and 3.2 and summarised using descriptive statistics. Reliability was measured using Pearson's correlation coefficient r between the first and second measurement. An $r > .50$ was considered a strong correlation. The coefficient of determination r^2 was used to assess the degree to which the first measurement predicted the second measurement.

3.7.2.3 Coefficients of variation in salivary cortisol assays

Data was collected on coefficients of variation at each cortisol assay to compare intra-assay and inter-assay variation. Any cortisol values found to exceed the 15% coefficient of variation were not used, but were repeated at the next assay.

3.7.3 Aim 1 – fatigue level and prevalence

Questionnaire data were first analysed descriptively. Each timepoint was examined in isolation, with data at the common timepoint for both cohorts (T_1) pooled. Fatigue prevalence was determined using the SPHERE–12 classification system with predetermined cut-off points. Changes in prevalence were estimated using the Chi-Square

(x^2) Test for Goodness of Fit, which compared the observed frequencies in SPHERE–12 membership and the expected frequencies in membership (i.e. T_1 versus T_0 and T_2 versus T_1). If a significant result was found, Cohen's *w* was used as an index of effect size (Allen & Bennett 2008). Repeated-measures analyses were performed to test for differences in fatigue over time using the Wilcoxon Signed Ranks test and follow-up calculations of effect size based on Cohen's *r* (Allen & Bennett 2008). Relationships between fatigue (MFSI–SF) at different timepoints, fatigue and quality of life (EORTC QLQ–C30), and fatigue (MFSI–SF) and continuous variables (age, BMI, WHR and tumour size) were calculated using Spearman's correlation coefficient Rho. Correlations coefficients of .1 were considered weak, .3 medium and .5 and above were considered strong (Allen & Bennett 2008). The coefficient of determination r^2 was used to calculate the degree to which one variable predicted the other. Finally, the Mann-Whitney *U* test was used to compare differences in fatigue level (MFSI–SF) between different demographic categories.

3.7.4 Aim 2 – cortisol rhythm and thyroid function indices

Data from the three day salivary cortisol sampling procedure were averaged for each participant. Cortisol data was then interpreted in four ways. First, salivary cortisol was expressed in absolute levels of awakening, 30 minutes post-awakening and evening cortisol (nmol/L). These values were $\log_{10}$-transformed using Equation 3.3 due to the skewed distribution of data, with the transformed data used for statistical analysis (Zar 1996).

Equation 3.3 Log_{10} transformation $x = \log(x+1)$

Second, cortisol data was represented as the Awakening Cortisol Response (ACR) also known as the Cortisol Awakening Response (CAR), both as an absolute change in cortisol

(nmol/L) from awakening to 30 minutes post-awakening and as relative percentage change, using Equations 3.1 and 3.2. Averaged over a number of days, the ACR with respect to time at awakening is considered a reliable marker of adreno-cortical activity (Pruessner et al. 1997). Third, the slope of cortisol decline from awakening to evening was calculated. For this calculation the 30 minute post-awakening sample was ignored. To calculate cortisol slope, linear regression was used to regress cortisol level on the time of sampling, with the awakening sample being assigned the value of $t = 0$. The resulting slope was then interpreted as the degree of diurnal variation in cortisol (Sephton et al. 2000; Vedhara et al. 2006). Finally, cortisol data was used to determine the Area Under Curve (AUC) parameters. Specifically, two variations of the AUC were calculated as described by Pruessner et al. (2003). The AUC_g was defined as the 'area under curve with respect to ground' which was used as a measure of the overall free cortisol output over the course of the day and the AUC_i was defined as the 'area under curve with respect to increase' which gives a measure of change over time (Pruessner et al. 2003). Figure 3.3 illustrates these two parameters.

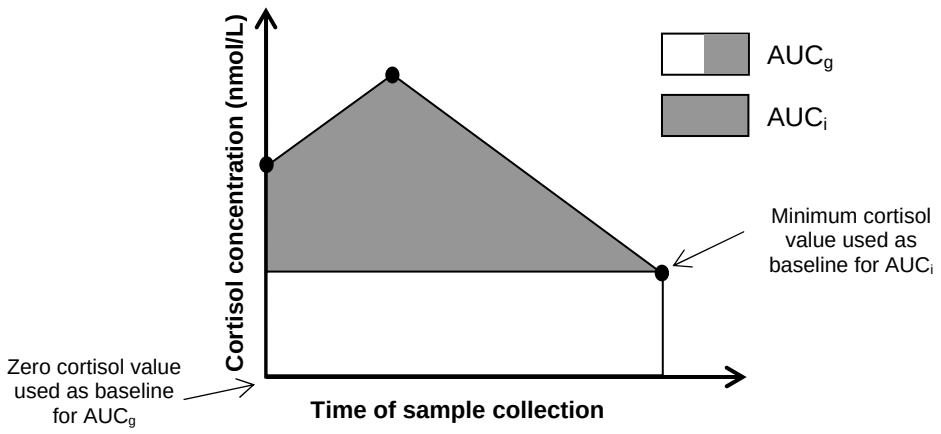


Figure 3.3 Difference between AUC_g and AUC_i (adapted from Vedhara et al. 2006)

Each cortisol index was first analysed descriptively. Changes over time were compared using the paired samples *t* test. Correlations between fatigue and cortisol indices were assessed using Spearman's correlation coefficient Rho. Significant differences in cortisol indices between fatigued versus non-fatigued participants ('Soma' versus 'Neither' cases on the SPHERE–12) were measured using the Mann-Whitney *U* test.

Thyroid hormones were first analysed descriptively. Similar to the cortisol indices, changes over time were compared using paired samples *t* tests. Correlations between fatigue and thyroid function were assessed with Spearman's correlation coefficient Rho. Significant differences in thyroid function between fatigued versus non-fatigued participants ('Soma' versus 'Neither' cases on the SPHERE–12) were measured using the Mann-Whitney *U* test.

3.7.5 Aims 3 and 4 – Radiation dose to the thyroid gland

Descriptive statistics were used to summarise age, thyroid gland size, thyroid function, fatigue at T_1 and radiation dose to the thyroid. As well as evaluating these variables for the whole group that had thyroid function data available at T_0 and/or T_1 (n = 29), participants were also grouped based on RT treatment technique (i.e. 'tangents only' and 'tangents + SCF'). Longitudinal changes in thyroid hormones were assessed using paired samples *t* tests for both the entire group and the 'tangents only' group and with the Wilcoxon Signed Ranks test for the 'tangents + SCF' group due to the small sample (n = 5). Differences in age, thyroid gland volume, T_1 fatigue and radiation dose to the thyroid between the two treatment groups were compared using the Mann-Whitey *U* test. Effect sizes were calculated for any significant differences. Correlations between the mean thyroid gland dose, T_1 thyroid function and T_1 fatigue were investigated using the Spearman's

correlation coefficient Rho. Correlations coefficients of .1 were considered small, .3 medium and .5 large (Allen & Bennett 2008).

4 Results

4.1 Introduction

Recruitment of participants into the study was performed over six months. In total, 39 women consented to the study before commencing radiation therapy treatment (Cohort 1) and 15 women were recruited at six months after treatment (Cohort 2). Some loss to follow-up occurred over the duration of the study. This chapter begins with a summary of the recruitment phase including reasons for declining participation, the demographic characteristics of consenting participants and differences between those women who did and did not give consent. The next section details the reliability studies undertaken to ensure repeatable data collection, specifically an analysis of questionnaire psychometrics, waist and hip circumference measurements and the reliability of salivary cortisol assays. The remainder of the chapter is then structured according to the individual aims of the research as set out in the methods chapter, including a description of fatigue, relationships between fatigue, salivary cortisol and thyroid function as well as a study of radiation dose to the thyroid gland.

4.2 Recruitment

4.2.1 Overall summary

The recruitment period lasted from 16 January 2008 until 4 August 2008. In that time, Cohort 1 comprised a final sample of 48 participants and Cohort 2 a sample of 15 participants. At follow-up, nine additional participants from Cohort 1 declined participation and one was excluded because of being diagnosed with a new primary cancer. In Cohort 2, two participants had been diagnosed with a recurrence at follow-up and one participant was unable to be contacted. Reasons for declining participation and being ineligible for participation are shown in Table 4.1. Detailed recruitment summaries for both cohorts are shown in Figure 4.1 and Figure 4.2. Participants who had a haemoglobin level below the normal reference range at any timepoint were not included in fatigue analyses at that timepoint, but they were not excluded entirely from the study.

Table 4.1 Reasons for non-participation and exclusion

Reason for:	***n* (%)**
Declining participation	
Too busy with work or family	13 (21)
Not interested in research study	13 (21)
Did not return phone call after two attempts	12 (20)
Personal reasons (not disclosed)	10 (16)
Difficulty attending sessions, resides in rural NSW	8 (13)
Unwell	5 (8)
Total	61 (100)
Ineligibility / exclusion	
Thyroid dysfunction	13 (33)
Recurrent disease / metastasis	7 (18)
Mental illness	6 (15)
Language barrier	5 (13)
Previous radiation therapy	4 (10)
Adrenal hyperplasia	1 (3)
Chronic fatigue syndrome	1 (3)
Stroke	1 (3)
Systemic Lupus Erythromatosis	1 (3)
Total	39 (100)

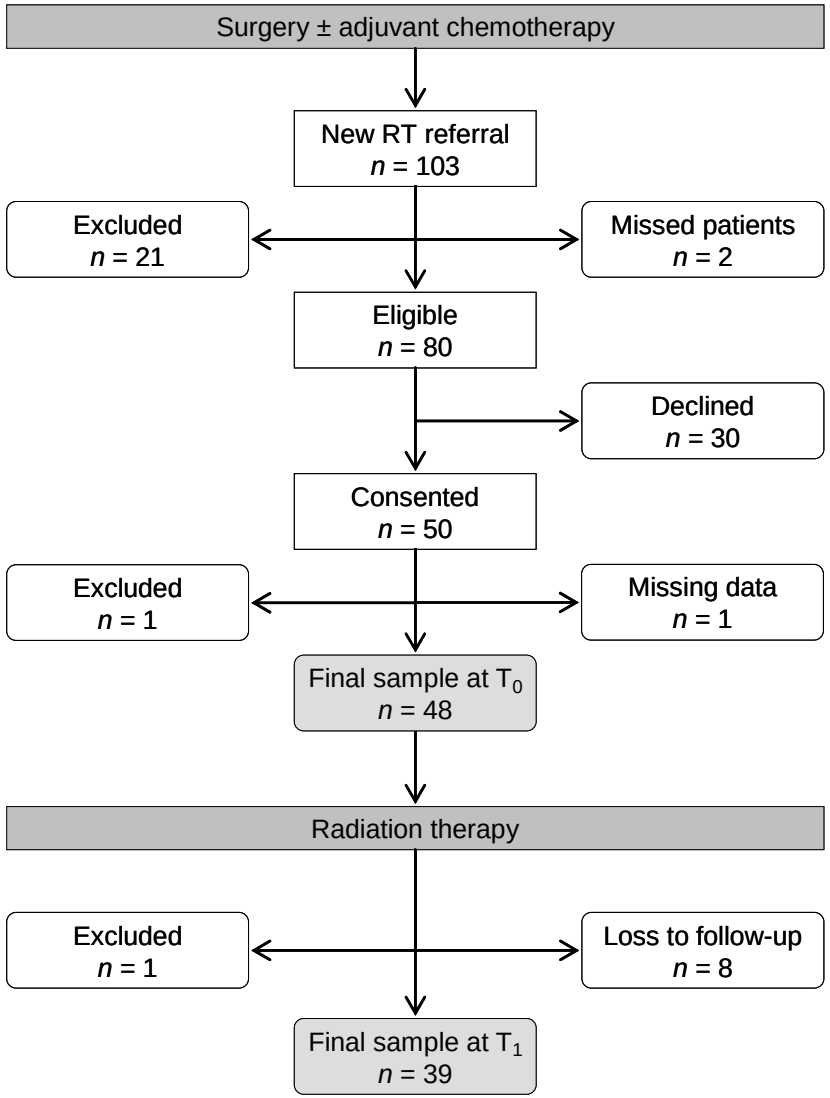


Figure 4.1 Cohort 1 recruitment summary

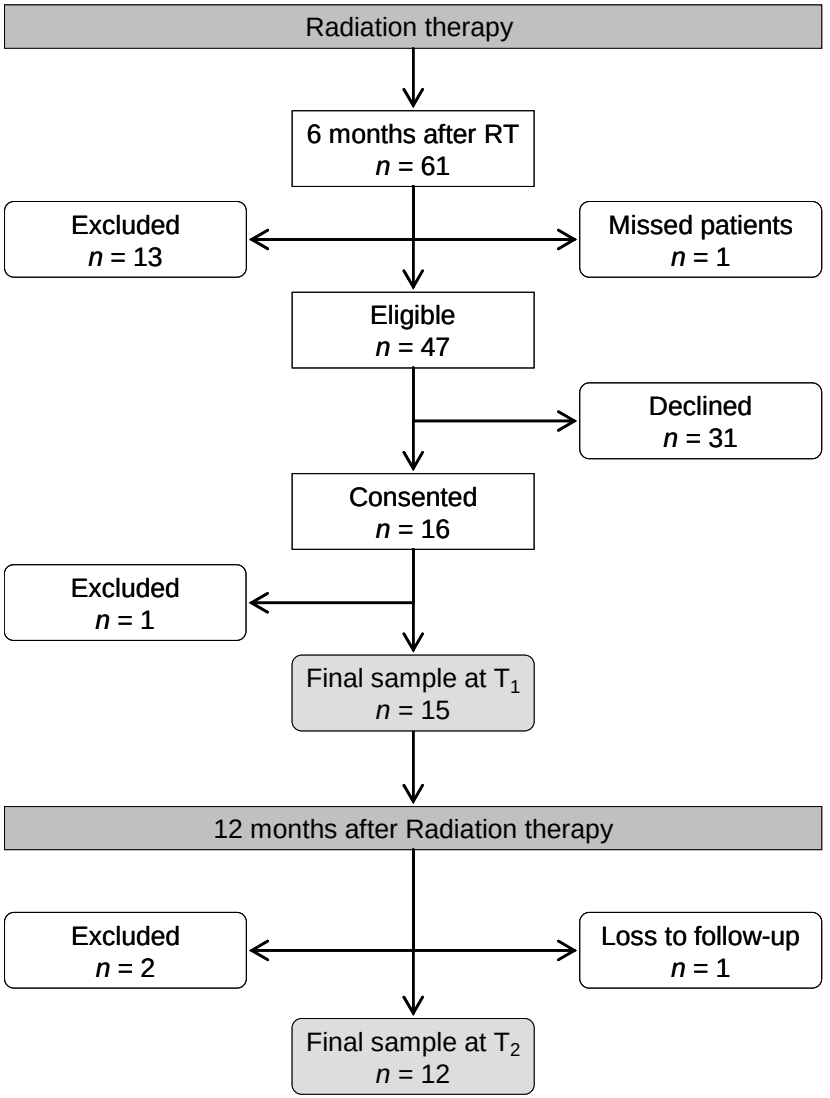


Figure 4.2 Cohort 2 recruitment summary

4.2.2 Participant characteristics

Demographic characteristics of both cohorts are presented in Table 4.2. The baseline T_0 assessment was conducted between 14 – 70 days post-surgery for participants who did not have chemotherapy (mean 36 days, *SD* = 15.5) and was between 1 – 60 days after the last chemotherapy cycle for participants who had received chemotherapy (mean 29 days, *SD* = 16.4). The T_1 assessments (six months post-RT) were between 24.3 – 35.7 weeks after the last RT treatment fraction for participants in Cohort 1 (mean 27 weeks, *SD* = 2.0) and between 24.4 – 31.7 weeks after the last RT treatment fraction for participants in Cohort 2 (mean 27 weeks, *SD* = 1.8). The T_2 assessments (12 months post-RT) were conducted between 50.4 and 57.3 weeks after the last RT treatment fraction (mean 54 weeks, *SD* = 2.1).

Table 4.2 Demographics of Cohort 1 and Cohort 2

Variables	**Cohort 1**		**Cohort 2**	
Mean age in years (*SD*)	58.0 (11.8)		62.9 (9.9)	
Mean tumour size in mm (*SD*)	25.5 (28.9)		14.3 (9.2)	
	T_0	**T_1**	**T_1**	**T_2**
Mean Body Mass Index (*SD*)	27.7 (5.81)	28.1 (5.87)	25.9 (4.03)	24.9 (3.34)
Mean Waist:Hip Ratio (*SD*)	0.88 (0.08)	0.86 (0.07)	0.88 (0.06)	0.88 (0.06)
	***n* (%)**		***n* (%)**	
Ethnicity				
Australian	30 (62.5)		12 (80.0)	
European	10 (20.8)		2 (13.4)	
Other	8 (16.7)		1 (6.6)	
Marital status				
Married / de-facto	34 (70.8)		10 (66.7)	
Divorced / separated / widowed	14 (29.2)		5 (33.3)	
Educational level				
Secondary education	28 (58.3)		10 (66.7)	
Tertiary education	20 (41.7)		5 (33.3)	
Employment status				
Employed (full-time / part-time)	29 (60.4)		8 (53.3)	
Retired / unemployed / on-leave	19 (39.6)		7 (46.7)	
Menopausal status				
Pre- or peri-menopausal	18 (37.5)		1 (6.7)	
Post-menopausal	30 (62.5)		14 (93.3)	
Number of co-morbidities				
Nil	27 (56.3)		3 (20.0)	
One or more	21 (43.7)		12 (80.0)	
Current smoker				
Yes	5 (10.4)		1 (6.7)	
No	43 (89.6)		14 (93.3)	
Alcohol intake per week				
Nil	17 (35.4)		2 (13.3)	
< 5 standard drinks	17 (35.4)		4 (26.7)	
5 – 10 standard drinks	12 (25.0)		5 (33.3)	
10 – 15 standard drinks	2 (4.2)		3 (20.0)	
+15 standard drinks	0 (0.0)		1 (6.7)	
Previous oestrogen exposure				
Nil	10 (20.8)		4 (26.7)	
OCP only	23 (47.9)		6 (40.0)	
HRT only	2 (4.2)		0 (0.0)	
Both OCP and HRT	13 (27.1)		5 (33.3)	
T-Staging				
T_{is}	5 (10.4)		3 (20.0)	
T_1	25 (52.1)		8 (53.3)	
T_2	13 (27.1)		3 (20.0)	
T_3	4 (8.3)		1 (6.7)	
T_4	1 (2.1)		0 (0.0)	

Table 4.2 (*continued*)

Variables	Cohort 1 *n* (%)	Cohort 2 *n* (%)
Histopathological diagnosis		
DCIS	6 (12.5)	3 (20.0)
Invasive ductal carcinoma	31 (64.6)	9 (60.0)
Invasive lobular carcinoma	3 (6.3)	1 (6.7)
Invasive tubular carcinoma	4 (8.3)	2 (13.3)
Inflammatory	1 (2.1)	0 (0.0)
Mixed type	2 (4.2)	0 (0.0)
Other	1 (2.1)	0 (0.0)
Left of right sided tumour		
Left	21 (43.8)	8 (53.3)
Right	26 (54.2)	7 (46.7)
Bi-lateral	1 (2.1)	
ER status[a]		
Positive	37 (77.1)	12 (80.0)
Negative	11 (22.9)	2 (13.3)
HER-2 status[b, c]		
Positive	4 (8.3)	1 (6.7)
Negative	37 (77.1)	10 (66.7)
Surgery type		
Lumpectomy	39 (81.3)	12 (80.0)
Mastectomy	9 (18.7)	3 (20.0)
Chemotherapy		
Yes	17 (35.4)	5 (33.3)
No	31 (64.6)	10 (66.7)
Radiation therapy treatment		
Tangents only	35 (72.9)	13 (86.7)
Tangents + SCF	13 (27.1)	2 (13.3)
Hormone treatment[d]		
Nil	11 (22.9)	4 (26.7)
Tamoxifen	16 (33.3)	2 (13.3)
Arimidex	10 (20.8)	6 (40.0)
Herceptin	2 (4.2)	1 (6.7)
Femara	0 (0.0)	1 (6.7)

Key: [a]Cohort 2, missing data *n* = 1, [b]Cohort 1, missing data *n* = 7, [c]Cohort 2, missing data *n* = 4, [d]Cohort 2, missing data *n* = 1; DCIS – Ductal Carcinoma In Situ; HRT – Hormone Replacement Therapy; OCP – Oral Contracaptive Pill; SCF – Supraclavicular Fossa

4.2.3 Differences between consenting and declining patients

Differences between demographic and disease variables were compared between consenting and declining patients to ensure that the study sample was unbiased and representative of the NSW breast cancer population. Variables tested were age, menopausal status, employment, current smoker status, number of co-morbid conditions,

histopathological diagnosis, tumour size, ER status, HER-2 status, surgery type and undergoing chemotherapy treatment.

An independent samples *t* test was used to compare the mean age of consenting participants (n = 48, mean 58.0 years, SD = 11.84) and declining patients (n = 30, mean 59.8 years, SD = 14.65). The *t* test was not statistically significant, $t(76) = .596$, $p = .553$, two-tailed. The mean tumour size between the two groups was also compared using the independent samples *t* test. Mean tumour size of participants (n = 48) was 25.5 mm (SD = 28.9) and of declining patients (n = 27, 3 missing data) was 26.9 mm (SD = 20.27) which was not significantly different ($t(73) = .233$, $p = .816$, two-tailed).

A Chi-Square (x^2) Test of Contingencies was used to compare selected demographic and disease variables of consenting participants and declining patients, where a statistically significant Chi-Square would indicate that the groups differed. No significant differences were found for any of the variables tested as shown in Table 4.3, indicating that the sample population was representative of the NSW breast cancer patient population during the study period.

Table 4.3 Results of the Chi-Square (x^2) test between consenting and declining patients

Variable	x^2 **(df, *n*)**	***p*-value**
Menopausal status (Pre / Post)	0.31 (1, 77)	.58
Employment status (Y / N)	0.05 (1, 72)	.83
Current smoker (Y / N)	2.20 (1, 69)	.14
Number of co-morbidities (Nil / 1+)	0.83 (1, 73)	.36
Histopathological diagnosis (Invasive ductal ca / Other)	1.14 (1, 79)	.29
ER status (Positive / Negative)	2.49 (1, 72)	.11
HER-2 status (Positive / Negative)	0.99 (1, 64)	.32
Surgery type (Lumpectomy / Mastectomy)	0.91 (1, 78)	.34
Chemotherapy (Y / N)	1.68 (1, 78)	.19

Key: Y – Yes; N – No; df – degrees of freedom

4.3 Reliability studies

4.3.1 Questionnaire psychometric analyses

The internal consistency of questionnaire subscales was very high and within the acceptable level of Cronbach's alpha > 0.7 for all, except two subscales as shown in Table 4.4.

Table 4.4 Internal consistency reliability of questionnaires

Questionnaire and subscale	Cronbach's alpha
MFSI–SF	
Mental fatigue	.90
Physical fatigue	.78
Emotional fatigue	.93
General fatigue	.94
Vigor	.89
SPHERE–12	
PSYCH subscale	.83
SOMA subscale	.81
EORTC QLQ–C30	
Physical functioning	.77
Role functioning	.83
Cognitive functioning	.62
Emotional functioning	.87
Social functioning	.81
Pain	.85
Fatigue	.82
Nausea / Vomiting	.11
Global Quality of Life	.86
FIS	
Impact on cognitive functioning	.96
Impact on social functioning	.95
Impact on physical functioning	.95

The two exceptions were the nausea/vomiting subscale and cognitive functioning subscale of the EORTC QLQ–C30. These subscales consisted of only two items each and seemed to ask slightly different (and somewhat unrelated) questions, i.e. "Q14. Have you felt nauseated?" and "Q15. Have you vomited?"; "Q20. Have you had difficulty in

concentrating on things, like reading a newspaper or watching television?" and "Q25. Have you had difficulty remembering things?". This may explain the low Cronbach's alpha values for these subscales.

4.3.2 Intra-observer reliability of waist and hip circumference measurements

Intra-observer reliability in measurement taking was investigated on the first 36 participants to determine whether the measurement method was repeatable. The mean absolute change in waist measurements (second minus first measurement) was −0.07 cm (*SD* 1.66) and the mean percentage change was 0.11% (*SD* 1.81). There was a strong positive and significant correlation between the two waist measurements as indicated by $r = .99$, $p < .001$. The coefficient of determination was found to be $r^2 = .99$, which showed that the first measurement strongly predicted the second. The mean absolute change in hip measurements was −0.08 cm (*SD* 1.16) and the mean percentage change was 0.09% (*SD* 1.03). Similar to the waist measurements, there was a strong positive and significant correlation between the two hip measurements where $r = .99$, $p < .001$ and $r^2 = .99$. The negative absolute change for both waist and hips indicated that the second measurement was on average slightly lower than the first, but this was less than one millimetre. The high correlation coefficients confirmed that the measurement method for both waist and hip circumferences was reliable.

4.3.3 Coefficients of variation in salivary cortisol assays

In total 22 salivary cortisol assays were performed between 5 November 2008 and 22 April 2009. Any values exceeding a coefficient of variation of 15% were repeated at the next assay. The overall intra-assay coefficients of variation ranged between 2.14% to 5.58%. The inter-assay coefficient of variation was 3.76%.

4.4 Aim 1 – Fatigue level and prevalence

4.4.1 Descriptive summary of fatigue dimensions and fatigue prevalence

Data from Cohorts 1 and 2 at the common timepoint (T_1) were pooled, so sample sizes in this cross-sectional analysis were not equal at each timepoint. Each questionnaire was initially analysed descriptively. In general, multidimensional fatigue and functioning improved with increasing time after treatment or remained at pre-treatment levels as shown in Figure 4.3 – Figure 4.7; each box-and-whisker plot shows the median, inter-quartile range and the absolute range.

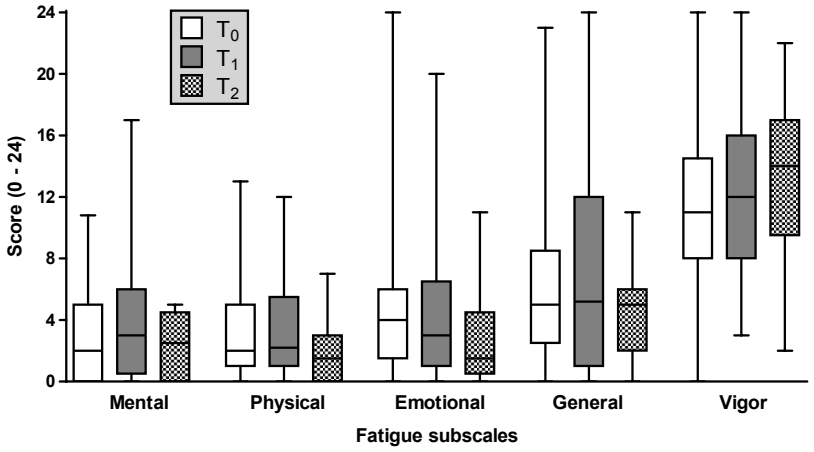


Figure 4.3 Fatigue dimensions at each timepoint: MFSI–SF

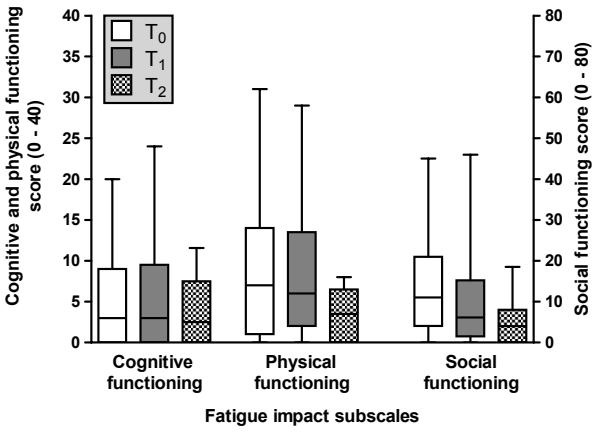


Figure 4.4 Fatigue impact on function: FIS

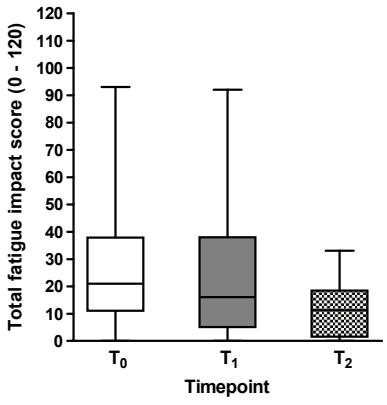


Figure 4.5 Total fatigue impact: FIS

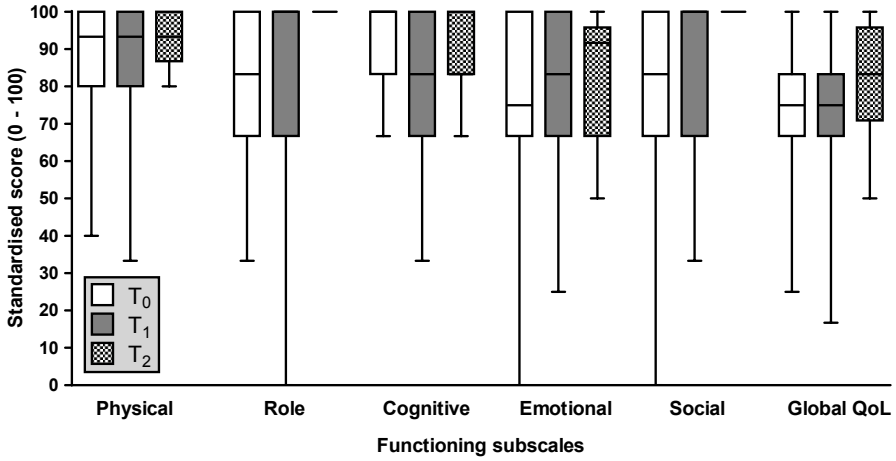


Figure 4.6 Functioning and quality of life at each timepoint: EORTC QLQ–C30

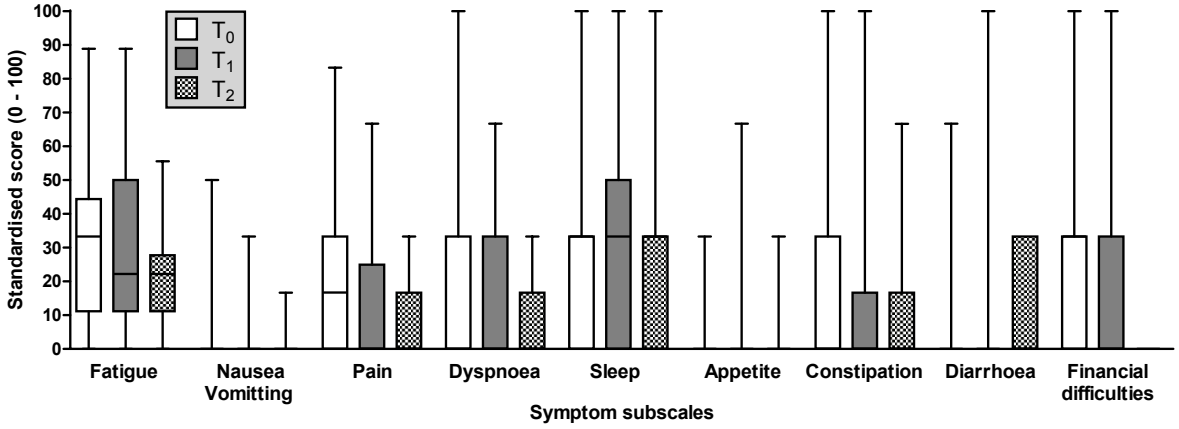


Figure 4.7 Symptoms experienced at each timepoint: EORTC QLQ–C30

Using the classification system of the SPHERE–12 questionnaire the prevalence of fatigue (without associated mood disorder) increased over time (T_0 = 12.5%, T_1 = 29.1%, T_2 = 33.3%). A Chi-Square (x^2) Test for Goodness of Fit was used to compare the observed frequencies of SPHERE–12 group membership at T_1 to the expected group membership frequencies (T_0). Similarly, observed group membership frequencies at T_2 were compared to the expected frequencies (T_1). Table 4.5 lists the percentages of participants at each timepoint. For the purpose of the Chi-Square analysis, categories

'Psych' and 'Both' were merged to satisfy the assumption of less than 20% of expected frequencies being below five.

Table 4.5 SPHERE–12 category membership in Cohort 1 and 2 at each timepoint

Category	**T_0** ***n* (%)**	**T_1** ***n* (%)**	**T_2** ***n* (%)**
Neither	25 (52.1)	29 (52.7)	5 (41.7)
Soma	6 (12.5)	16 (29.1)	4 (33.3)
Psych	7 (14.6)	2 (3.6)	2 (16.7)
Both	10 (20.8)	8 (14.6)	1 (8.3)

For T_0 to T_1 comparisons, the Chi-Square test was statistically significant, $x^2 = 30.35$, $p < .001$ indicating that the frequency of group membership was significantly different between these two timepoints. As an index of effect size, Cohen's *w* was .55 which can be considered large. The Chi-Square test was not statistically significant for T_1 and T_2 comparisons, $x^2 = 5.24$, $p = .073$. As a general observation, a large proportion of participants at each timepoint (18–35%) scored above the 'Psych' subscale cut-off score, indicating a higher risk of experiencing depression among this group.

At each timepoint, participants were asked about their current level of fatigue in relation to fatigue they experienced pre-diagnosis. This data is summarised descriptively in Table 4.6.

Table 4.6 Current fatigue compared to fatigue pre-diagnosis

Current fatigue	**T_0** ***n* (%)**	**T_1** ***n* (%)**	**T_2** ***n* (%)**
No different / same as at pre-diagnosis	21 (44.7)	21 (42.0)	6 (54.5)
Better than pre-diagnosis	3 (6.4)	6 (12.0)	3 (27.3)
Worse than pre-diagnosis	21 (48.9)	23 (46.0)	2 (18.2)

4.4.2 Fatigue level and prevalence over time – longitudinal analyses (Hypothesis 1)

Differences in fatigue, functioning and quality of life between pre-treatment versus six months and six months versus 12 months post-RT were determined using the Wilcoxon Signed Ranks test. Only participants who completed questionnaires at both timepoints were included. Those participants with a low haemoglobin level were excluded from this analysis because low haemoglobin at one or both timepoints could confound the differences observed after treatment. Means, standard deviations and *p*-values for the MFSI–SF, FIS and EORTC QLQ–C30 questionnaires for each pair of timepoints are shown in Table 4.7.

Overall, these significant differences showed improvements in fatigue and functioning from pre-treatment to six months post-RT levels. Specifically, emotional functioning (MFSI–SF) had significantly improved by six months after treatment, $T = 124.0$, $z = -2.239$, n – ties = 30, $p = .025$ with 21 participants reporting lower emotional fatigue at T_1, whilst only nine reported higher fatigue. Follow-up analyses showed that this effect could be considered medium ($r = .41$). The level of social functioning (EORTC QLQ–C30) had significantly improved by six months after treatment, $T = 30.0$, $z = -2.216$, n – ties = 17, $p = .027$ with 13 participants reporting improved social functioning compared to four reporting worse social functioning. This effect can be considered large, $r = .54$. In addition, this finding was supported by the significantly reduced impact of fatigue on social functioning (FIS), $T = 41.5$, $z = -2.372$, n – ties = 20, $p = .018$ with 12 participants reporting less fatigue impact on social functioning at T_1 compared to five who indicated higher impact, also a large effect ($r = .53$). Participants had also experienced significant improvements in role functioning (EORTC QLQ–C30) at T_1 compared with T_0, $T = 33.5$, $z = -2.05$, n – ties = 17, $p = .04$. Follow-up effect size calculations showed that this was a large effect, $r = .50$.

Table 4.7 Differences in fatigue, functioning and quality of life over time

Questionnaire and subscales	**T_0** mean (*SD*)	**T_1** mean (*SD*)	*p*-value	**T_1** mean (*SD*)	**T_2** mean (*SD*)	*p*-value
MFSI–SF		***n* = 36**			***n* = 11**	
Mental fatigue	3.1 (3.06)	4.2 (4.27)	.193	3.7 (3.42)	2.4 (2.07)	.326
Physical fatigue	3.5 (3.60)	3.9 (3.76)	.263	2.5 (2.32)	2.1 (2.35)	.797
Emotional fatigue	5.0 (5.32)	4.2 (4.84)	.025*	5.9 (6.93)	3.0 (3.49)	.166
General fatigue	6.2 (5.62)	7.1 (6.59)	.198	6.2 (6.18)	4.6 (3.23)	.592
Vigor	11.7 (5.44)	13.1 (5.51)	.054	10.8 (4.85)	12.9 (5.71)	.213
FIS		***n* = 24**			***n* = 10**	
Impact on cognitive functioning	5.2 (6.24)	5.0 (5.69)	.285	6.7 (6.78)	4.2 (4.53)	.051
Impact on physical functioning	9.0 (8.28)	8.9 (8.39)	1.00	7.5 (6.27)	3.7 (3.20)	.035*
Impact on social functioning	14.6 (12.37)	10.2 (10.74)	.018*	8.5 (7.51)	5.5 (5.80)	.092
Total fatigue impact	28.8 (24.00)	24.2 (23.39)	.127	22.7 (18.22)	13.4 (11.04)	.059
EORTC QLQ–C30		***n* = 31**			***n* = 11**	
Physical functioning	86.1 (17.08)	87.1 (15.74)	.968	88.3 (10.29)	92.8 (6.63)	.302
Role functioning	77.0 (23.58)	84.8 (22.60)	.040*	90.3 (22.99)	100.0 (0.00)	.180
Cognitive functioning	86.8 (13.46)	78.9 (19.81)	.057	81.9 (13.20)	88.9 (10.86)	.230
Emotional functioning	77.7 (21.20)	79.9 (20.01)	.151	81.3 (13.82)	83.4 (16.66)	.309
Social functioning	73.0 (27.22)	87.3 (17.90)	.027*	84.7 (24.06)	100.0 (0.00)	.102
Global Quality of Life	71.1 (17.31)	73.3 (21.00)	.690	78.5 (11.48)	81.9 (16.61)	.405
Fatigue	32.0 (24.74)	30.1 (25.16)	.930	26.8 (13.78)	20.4 (15.59)	.477
Nausea/Vomiting	2.9 (9.60)	2.5 (6.00)	.180	4.2 (7.55)	2.8 (6.49)	.269
Pain	18.1 (22.61)	16.2 (20.30)	.149	13.9 (17.16)	8.3 (13.29)	.126
Dyspnoea	13.7 (26.10)	13.7 (20.39)	.477	27.8 (19.24)	8.3 (15.07)	.087
Sleep	33.3 (29.60)	37.7 (35.14)	.761	22.2 (21.71)	27.8 (31.25)	.590
Appetite	7.8 (14.34)	2.9 (9.59)	.180	13.9 (22.28)	2.8 (9.62)	.141
Constipation	11.8 (21.52)	13.7 (21.89)	.527	8.3 (28.77)	11.1 (21.71)	.655
Diarrhoea	5.9 (15.28)	4.9 (18.59)	.655	0.0 (0.00)	11.1 (16.41)	.046*
Financial difficulties	32.3 (31.24)	19.6 (28.56)	.053	16.7 (22.47)	0.0 (0.00)	.034*

Key: *Denotes significant difference, $p < .05$; NB: Sample sizes differ because not all participants completed all questionnaires due to an ethics amendment.

Further significant improvements over time were observed from six months to 12 months post-RT, with a significantly reduced impact of fatigue on physical functioning (FIS) (p = .035) and significantly decreased financial difficulties (EORTC QLQ–C30) (p = .034). The effect size of both differences can be considered large; r = .74 and r = .54, respectively. Participants also reported a significantly different level of diarrhoea symptoms at the 12 month post-RT timepoint, with seven reporting the same degree of the symptom, four reporting increased symptoms and none reporting improvements, but follow-up calculations showed that this effect was small (r = .20). It should be noted that these T_1 to T_2 repeated-measures comparisons included only a very small sample size (n = 11) and so should be interpreted with caution.

Fatigue prevalence based on SPHERE–12 classifications was compared at each timepoint and cohort. The main observation was that over time, 58% of participants in Cohort 1 did not change group membership and that clinically high fatigue developed as a new symptom in two (5.3%) women in this cohort as shown in Table 4.8. There did not appear to be any distinguishing demographic characteristics (e.g. chemotherapy) in these two individuals that may have accounted for their increased fatigue symptoms. In contrast, all three participants who were 'Soma' cases at both T_0 and T_1 had received chemotherapy.

Table 4.8 SPHERE–12 classifications at T_0 and T_1; n (%)

		T_0				
		Neither	Soma	Psych	Both	
T_1	Neither	16 (42.1)	-	3 (7.9)	3 (7.9)	
	Soma	2 (5.3)	3 (7.9)	2 (5.3)	3 (7.9)	
	Psych	1 (2.6)	-	1 (2.6)	-	
	Both	1 (2.6)	1 (2.6)	-	2 (5.3)	No change
						22 (58%)

Key: Shading indicates new fatigue symptoms at T_1

Table 4.9 shows SPHERE–12 group membership for Cohort 2. Neither of the two individuals who were in the 'Soma' category both at T_1 and T_2 had received chemotherapy.

Table 4.9 SPHERE–12 classifications at T_1 and T_2; *n* (%)

		T_1				
		Neither	Soma	Psych	Both	
T_2	Neither	5 (45.5)	-	-		
	Soma	-	2 (18.2)	-	1 (9.1)	
	Psych	1 (9.1)	-	-	1 (9.1)	
	Both	-	-	-	1 (9.1)	No change
						8 (73%)

4.4.3 Demographic variables, quality of life and fatigue

The next set of analyses investigated correlations between multidimensional fatigue (MFSI–SF) at different timepoints and differences in fatigue between demographic categories at each timepoint. Fatigue and quality of life comparisons were performed using data from the EORTC QLQ–C30 only. Women who had a low haemoglobin level or scored above the cut-off for depression on the SPHERE–12 were excluded in order to minimise bias, because both factors are known to be related to fatigue.

Spearman's Rho correlations were used to first, test correlations between T_1 general fatigue versus fatigue dimensions at T_0 and T_2 general fatigue versus fatigue dimensions at T_1, second, test correlations between fatigue dimensions and continuous variables (i.e. age, BMI, WHR and tumour size) and third, test correlations between EORTC QLQ–C30 fatigue and its relationship to quality of life.

Significant relationships were seen between T_1 general fatigue and baseline general fatigue and baseline vigor. Using the coefficient of determination (r^2), 29% and 22% of the variance in T_1 general fatigue scores was predicted by baseline general fatigue and baseline

vigor scores, respectively. At T_2, strong positive correlations were evident between T_2 general fatigue and T_1 mental fatigue and T_1 general fatigue. Sixty-six percent and 74% of the variance in T_2 general fatigue scores was explained by T_1 mental fatigue and T_1 general fatigue, respectively. A summary of correlation coefficients and *p*-values is given in Table 4.10

Table 4.10 Significant correlates of multidimensional fatigue (MFSI–SF)

Subscale	Spearman's Rho (r_s)	*p*-value
T_0 Physical fatigue, $n = 28$		
Body Mass Index	.40	.035*
T_1 General fatigue, $n = 20$		
T_0 General fatigue	.54	.014*
T_0 Vigor	−.47	.035*
T_1 Physical fatigue, $n = 42$		
Tumour size	.35	.025*
T_2 General fatigue, $n = 7$		
T_1 General fatigue	.86	.013*
T_1 Mental fatigue	.82	.026*
T_2 Emotional fatigue, $n = 7$	−.79	.033*
Tumour size		

Key: *Denotes significant difference, $p < .05$

Relationships were tested between each MFSI–SF fatigue subscale and the continuous variables age, BMI, WHR and tumour size at each timepoint. Age and WHR were not related to any fatigue dimension at any timepoint. Significant relationships were seen between T_0 physical fatigue and BMI, T_1 physical fatigue and tumour size and T_2 emotional fatigue and tumour size as shown in Table 4.10. This suggests that increased BMI was related to higher physical fatigue at baseline and that larger tumour size (surrogate for disease stage) was related to higher physical fatigue six months post-RT. At

12 months post-RT larger tumour size was related to lower emotional fatigue; however, this result was based on a very small sample (n = 7) and requires further investigation.

The EORTC QLQ–C30 questionnaire was used to investigate relationships between fatigue and quality of life at each timepoint, as well as predictors of six and 12 months post-RT fatigue. Results of the correlations are shown in Table 4.11 for each timepoint.

Table 4.11 Significant correlates of fatigue (EORTC QLQ–C30) at each timepoint

Subscale	**Spearman's Rho (r_s)**	***p*-value**
T_0, n = 27		
Physical functioning	−.43	.026*
Role functioning	−.53	.005***
Cognitive functioning	−.41	.034*
Social functioning	−.45	.018*
Global quality of life	−.60	.001***
Pain	.52	.006***
Sleep	.47	.014*
Appetite	.43	.024*
T_1, n = 42		
Physical functioning	−.53	< .001***
Role functioning	−.58	< .001***
Cognitive functioning	−.57	< .001***
Social functioning	−.46	.002***
Global quality of life	−.61	< .001***
Pain	.41	.006***
Dyspnoea	.36	.019*
Appetite	.37	.016*
T_2, n = 8		
Global quality of life	−.75	.032*

Key: *Denotes significant difference, $p < .05$; ***denotes significant difference, $p < .001$

Overall, higher fatigue correlated with worse functioning (except emotional functioning) and worse quality of life, as well as with increasing symptoms of pain, poorer sleep, worse

appetite and increased shortness of breath at some timepoints. Fatigue was unrelated to emotional function, nausea/vomiting, constipation, diarrhoea and financial difficulties.

Post-RT fatigue at T_1 and T_2 was significantly predicted by several quality of life parameters. These are shown in Table 4.12. Baseline role functioning and baseline pain symptoms were the strongest predictors of fatigue six months post-RT, explaining 60% and 51% of the variance in fatigue scores respectively. The third strongest predictor of fatigue at six months post-RT was baseline fatigue, accounting for 49% of the variance seen in the T_1 fatigue scores. Shortness of breath (dyspnoea) at T_1 was the only predictor of T_2 fatigue in a small sample of seven participants. Similar to the results shown above, this is likely to be a trait of the small sample ($n = 7$) that completed both T_1 and T_2 assessments and not a real effect among the general breast cancer population.

Table 4.12 Quality of life predictors of T_1 and T_2 fatigue

Quality of life subscales	r^2
Predictors of T_1 fatigue	
T_0 Role functioning	.60
T_0 Pain	.51
T_0 Fatigue	.49
T_0 Cognitive functioning	.47
T_0 Dyspnoea	.34
T_0 Global quality of life	.34
T_0 Physical functioning	.34
T_0 Social functioning	.23
Predictor of T_2 fatigue	
T_1 Dyspnoea	.57

Finally, differences in MFSI–SF subscale scores were compared between different categorical variables using the Mann-Whitney *U* test. Significantly different results are shown in Table 4.13. Data at the common timepoint (T_1) were pooled to boost the sample

size. No significant differences were found in any fatigue subscales for the following variables: marital status (married/de-facto versus divorced/separated), education level (up to secondary versus tertiary and above), employment status (employed versus retired/unemployed/on-leave), menopausal status (pre-/peri-menopausal versus post-menopausal), previous oestrogen exposure (nil versus any), T-staging (T_{is} – T_1 versus > T_2), ER-status (positive versus negative), surgery type (lumpectomy versus mastectomy), RT type ('tangents only' versus 'tangents + SCF') and hormone therapy (yes versus no).

At baseline, participants who smoked reported significantly higher emotional fatigue and, counter-intuitively, those with no co-morbidities experienced more general fatigue. At T_1, women with a left-sided tumour reported worse emotional fatigue and less vigor than women with a right-sided tumour. Participants with a positive HER-2 status and those who received chemotherapy experienced worse physical fatigue. At T_2, participants diagnosed with an infiltrating ductal carcinoma reported significantly higher vigor than those with another diagnosis. In contrast to T_1 results, women who had received chemotherapy reported a significantly lower level of physical fatigue at T_2 and significantly lower emotional fatigue compared to those who did not have chemotherapy. The effect sizes for these differences were small to medium, with the exception of T_2 results with large effect sizes; however, this was most likely due to the small T_2 sample size.

Table 4.13 Significant differences in MFSI–SF fatigue between demographic categories

Subscale	Demographic variable	*Mean Rank*	*U*	*z*	*p*-value	Effect size
T_0 Emotional	Current smoker					
	Yes, *n* = 3	25.83	3.50	−2.568	.004**	.49
	No, *n* = 25	13.14				
T_0 General	Number of co-morbidities					
	Nil, *n* = 15	17.67	50.0	−2.210	.029*	.42
	1+, *n* = 13	10.85				
T_1 Emotional	Left or right sided tumour[a]					
	Left, *n* = 19	26.84	136.0	−2.274	.023*	.35
	Right, *n* = 24	18.17				
T_1 Vigor	Left or right sided tumour[a]					
	Left, *n* = 19	17.24	137.5	−2.218	.027*	.34
	Right, *n* = 24	25.77				
T_1 Physical	HER-2 status					
	Positive, *n* = 4	28.50	16.0	−2.379	.015*	.41
	Negative, *n* = 30	16.03				
T_1 Physical	Chemotherapy					
	Yes, *n* = 15	27.07	134.0	−1.958	.05*	.30
	No, *n* = 28	19.29				
T_2 Vigor	Histopathological diagnosis					
	Infiltrating Ductal, *n* = 4	6.38	.50	−2.178	.029*	.77
	Other, *n* = 4	2.63				

Table 4.13 *(continued)*

Subscale	Demographic variable	*Mean Rank*	*U*	*z*	*p*-value	Effect size
T_2 Physical	Chemotherapy					
	Yes, *n* = 3	2.17	.50	−2.125	.036*	.75
	No, *n* = 5	5.90				
T_2 Emotional	Chemotherapy					
	Yes, *n* = 3	2.17	.50	−2.112	.036*	.75
	No, *n* = 5	5.90				

Key: [a]Participant with bi-lateral cancer excluded; *denotes significant difference, $p < .05$; **denotes significant difference, $p < .01$

4.4.4 Aim 1 conclusions

Across the two cohorts, average levels of fatigue before, six and 12 months after treatment were generally low and the level of reported functioning and quality of life was quite high. That being said, additional significant improvements in emotional fatigue, social functioning and impact of fatigue on social functioning were seen six months post-RT compared to pre-treatment levels. Higher fatigue was associated with worse functioning and poorer quality of life and an increased presence of some symptoms. Fatigue prevalence (not related to depression) increased from 12.5% at baseline to 29.1% at six months and to 33.3% at 12 months post-RT. Hypothesis 1 was supported, with a significantly different fatigue prevalence at T_1 compared to baseline. No significant differences were seen in prevalence at T_2. After closer analysis only two participants (5.3%) developed severe fatigue by the T_1 follow-up assessment. The other participants in the 'Soma' category at T_1 reported having fatigue, depression or both sets of symptoms (i.e. 'Soma', 'Psych' or 'Both') at the baseline assessment.

4.5 Aim 2 – Cortisol rhythm and thyroid function indices

4.5.1 Salivary cortisol rhythm overview

Salivary cortisol collection was performed only by participants consenting to the procedure. Some participants completed the first assessment but were lost to follow-up at the subsequent assessment. At baseline, three participants were excluded from Cohort 1 based on non-compliance to the saliva collection procedure, two provided inadequate saliva volumes for accurate analysis and one participant performed collection at incorrect times. At T_1, one participant from Cohort 1 was excluded from analysis because her cortisol values exceeded the upper limit of the assay and thus the results were likely to be invalid. No participants were excluded from Cohort 2.

The following two sub-sections summarise, first, the salivary cortisol parameters at each timepoint (cortisol rhythm, awakening cortisol response, cortisol slope and area under curve) and second, correlations between fatigue and salivary cortisol together with differences in cortisol between fatigued versus non-fatigued participants.

4.5.2 Descriptive summary of salivary cortisol indices

The means and standard deviations of all four cortisol indices are listed in Table 4.14.

Absolute cortisol concentration at awakening, 30 minutes post-awakening and evening were within the reference ranges quoted in the literature. The mean increase in cortisol from awakening to 30 minutes post-awakening (ACR) was 18.2% at T_0, 19.2% at T_1 and 31.1% at T_2, but the variability between participants was very high as shown by the high standard deviations. The slopes of cortisol decrease over the course of the day were comparable between all three timepoints as were the AUC parameters.

Table 4.14 Descriptive summary of salivary cortisol indices

Cortisol Index	**T_0, *n* = 28**	**T_1, *n* = 41**	**T_2, *n* = 10**
	HH:MM	HH:MM	HH:MM
Average cortisol sampling time			
Awakening sample	7:00	06:57	06:30
30 minutes post-awakening sample	7:32	07:28	07:01
Evening sample	22:07	22:15	22:03
	Mean (*SD*)	Mean (*SD*)	Mean (*SD*)
Cortisol rhythm			
Awakening cortisol (nmol/L)	30.0 (0.51)	28.9 (0.47)	28.8 (0.37)
30 minutes post-awakening (nmol/L)	34.0 (0.46)	33.0 (0.46)	35.1 (0.76)
Evening cortisol (nmol/L)	5.4 (1.05)	5.1 (0.69)	5.4 (0.44)
Awakening Cortisol Response			
Absolute change (nmol/L)	3.7 (8.82)	4.3 (8.93)	9.5 (11.89)
Relative change (%)	18.2 (35.54)	19.2 (35.52)	31.1 (47.92)
Cortisol slope	−1.65 (0.87)	−1.66 (0.84)	−1.58 (0.54)
Area Under Curve			
Relative to ground (AUC_g)	56.8 (25.15)	54.38 (26.78)	57.4 (19.85)
Relative to increase (AUG_i)	41.4 (18.69)	42.2 (19.56)	45.8 (19.17)

Key: HH:MM – Hours:Minutes of sampling collection; AUC_g – Area Under Curve with respect to ground; AUC_i – Area Under Curve with respect to increase

Changes over time in cortisol indices were compared using paired samples *t* tests and found to be statistically non-significant as shown in Table 4.15. Cortisol rhythms for both cohorts (mean and 95% CI) are presented in Figure 4.8 and Figure 4.9.

Table 4.15 Salivary cortisol indices; changes over time for each cohort

Cortisol index	**Mean difference**	**95% CI**	***t***	***p*-value**
Cohort 1: T_0 versus T_1, *n* = 21				
Cortisol rhythm				
Awakening (nmol/L)	−0.02	−0.16 to 0.14	−0.272	.789
30 mins post-awakening (nmol/L	−0.06	−0.19 to 0.08	−0.973	.342
Evening (nmol/L)	−0.04	−0.25 to 0.24	−0.318	.754

Table 4.15 *(continued)*

Cortisol index	Mean difference	95% CI	*t*	*p*-value
Cohort 1: T_0 versus T_1, *n* = 21				
Awakening Cortisol Response				
Absolute change (nmol/L)	−2.0	−7.88 to 3.87	−0.711	.485
Relative change (%)	−5.6	−27.27 to 16.10	−0.537	.597
Cortisol slope	0.086	−0.20 to 0.37	0.629	.537
Area Under Curve				
Relative to ground (AUC_g)	−3.97	−11.9 to 3.98	−1.042	.310
Relative to increase (AUG_i)	−4.28	−12.37 to 3.81	−1.103	.283
Cohort 2: T_1 versus T_2, *n* = 10				
Cortisol rhythm				
Awakening (nmol/L)	−0.05	−0.20 to 0.12	−0.754	.470
30 mins post-awakening (nmol/L	−0.06	−0.35 to 0.37	−0.372	.719
Evening (nmol/L)	−0.17	−0.34 to 0.04	−1.830	.100
Awakening Cortisol Response				
Absolute change (nmol/L)	−3.3	−12.42 to 5.78	−0.825	.431
Relative change (%)	−4.2	−35.25 to 26.89	−0.304	.768
Cortisol slope	−0.007	−0.37 to 0.36	−0.044	.966
Area Under Curve				
Relative to ground (AUC_g)	−6.14	−20.28 to 8.00	−0.983	.351
Relative to increase (AUG_i)	−3.8	−8.46 to 10.86	−0.586	.572

Key: AUC_g – Area Under Curve with respect to ground; AUC_i – Area Under Curve with respect to increase

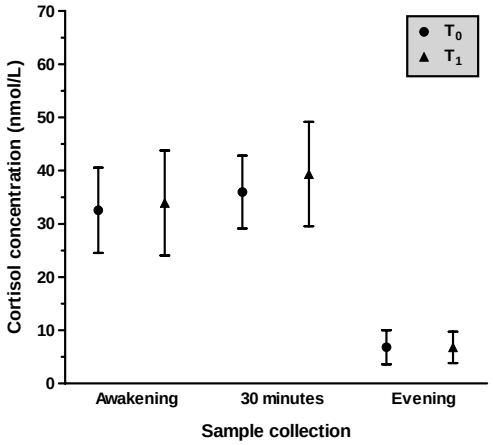


Figure 4.8 Salivary cortisol rhythm; Cohort 1 *n* = 21

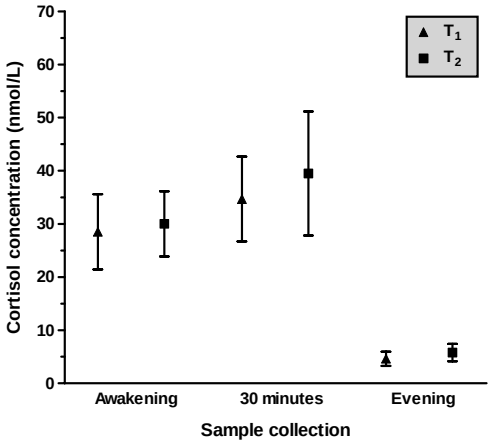


Figure 4.9 Salivary cortisol rhythm; Cohort 2 *n* = 10

4.5.3 Salivary cortisol and fatigue (Hypothesis 2)

Cortisol data for each SPHERE–12 category were first examined descriptively. Descriptive statistics for these analyses are presented in Table 4.16.

Next, Mann-Whitney *U* tests were used to determine whether there were any significant differences in cortisol indices between fatigued and non-fatigued participants ('Soma' versus 'Neither' groups) at each timepoint. Participants with a low haemoglobin level or those who scored above the cut-off for depression on the SPHERE–12 were excluded in the statistical analyses to minimise bias, because both factors are known to be related to fatigue. Table 4.17 lists the results of these analyses.

Table 4.16 Descriptive statistics of salivary cortisol indices for SPHERE–12 categories

Cortisol Index	Neither Mean (*SD*)	Soma Mean (*SD*)	Psych Mean (*SD*)	Both Mean (*SD*)
T_0	***n* = 15**	***n* = 6**	***n* = 3**	***n* = 4**
Cortisol rhythm				
Awakening cortisol (nmol/L)	27.2 (0.40)	38.8 (0.24)	29.9 (0.31)	27.8 (1.27)
30 minutes post-awakening (nmol/L)	32.1 (0.43)	40.7 (0.32)	35.3 (0.24)	32.1 (0.94)
Evening cortisol (nmol/L)	3.9 (0.44)	9.2 (1.65)	4.9 (1.78)	8.1 (1.48)
Awakening Cortisol Response				
Absolute change (nmol/L)	5.1 (8.77)	1.7 (12.08)	4.3 (2.12)	1.0 (8.26)
Relative change (%)	23.9 (43.78)	6.2 (26.73)	15.0 (8.98)	16.9 (26.67)
Cortisol slope	−1.65 (0.66)	−1.65 (1.08)	−1.58 (0.43)	−1.71 (1.64)
Area Under Curve				
Relative to ground (AUC_g)	50.4 (16.30)	69.1 (19.45)	54.8 (14.38)	63.9 (55.70)
Relative to increase (AUG_i)	41.9 (15.33)	41.6 (24.21)	39.9 (11.09)	40.6 (31.37)
T_1	***n* = 22**	***n* = 11**	***n* = 1**	***n* = 7**
Cortisol rhythm				
Awakening cortisol (nmol/L)	29.9 (0.48)	29.9 (0.33)	-	21.9 (0.43)
30 minutes post-awakening (nmol/L)	33.7 (0.52)	36.2 (0.25)	-	26.5 (0.51)
Evening cortisol (nmol/L)	5.5 (0.79)	4.5 (0.63)	-	5.2 (0.61)
Awakening Cortisol Response				
Absolute change (nmol/L)	3.8 (7.70)	6.1 (9.23)	-	5.3 (10.83)
Relative change (%)	13.6 (25.27)	26.2 (37.15)	-	32.0 (56.20)
Cortisol slope	−1.71 (0.87)	−1.73 (0.72)	-	−1.14 (0.67)

Table 4.16 *(continued)*

Cortisol Index	Neither Mean (*SD*)	Soma Mean (*SD*)	Psych Mean (*SD*)	Both Mean (*SD*)
T_1	***n* = 22**	***n* = 11**	***n* = 1**	***n* = 7**
Area Under Curve				
Relative to ground (AUC_g)	56.8 (34.45)	55.1 (11.21)	-	42.7 (12.91)
Relative to increase (AUG_i)	43.4 (23.53)	45.0 (11.70)	-	31.0 (12.07)
T_2	***n* = 4**	***n* = 4**	***n* = 1**	***n* = 1**
Cortisol rhythm				
Awakening cortisol (nmol/L)	33.7 (0.22)	24.1 (0.52)	-	-
30 minutes post-awakening (nmol/L)	39.7 (0.46)	30.6 (1.38)	-	-
Evening cortisol (nmol/L)	5.0 (0.45)	5.8 (0.36)	-	-
Awakening Cortisol Response				
Absolute change (nmol/L)	7.6 (11.00)	12.5 (15.24)	-	-
Relative change (%)	20.5 (29.65)	43.0 (70.13)	-	-
Cortisol slope	−1.89 (0.42)	−1.33 (0.67)	-	-
Area Under Curve				
Relative to ground (AUC_g)	61.9 (17.88)	54.3 (28.55)	-	-
Relative to increase (AUG_i)	51.4 (18.28)	42.1 (25.66)	-	-

Key: AUC_g – Area Under Curve with respect to ground; AUC_i – Area Under Curve with respect to increase
NB: Means and standard deviations were not computed for sample sizes *n* =1

Table 4.17 Differences in salivary cortisol indices between fatigued and non-fatigued participants

Cortisol index	Group	*Mean Rank*	*U*	*z*	*p*-value
T_0 Awakening cortisol level	Fatigued, *n* = 4 Non-fatigued, *n* = 14	13.25 8.43	13.0	−1.593	.127
30 minutes post- awakening cortisol level	Fatigued, *n* = 4 Non-fatigued, *n* = 14	12.50 8.64	16.0	−1.274	.233
Evening cortisol level	Fatigued, *n* = 4 Non-fatigued, *n* = 14	11.00 9.07	22.0	−0.638	.574
Awakening Cortisol Response (% change)	Fatigued, *n* = 4 Non-fatigued, *n* = 14	8.75 9.71	25.0	−0.319	.798
Cortisol slope	Fatigued, *n* = 4 Non-fatigued, *n* = 14	9.00 9.64	26.0	−0.212	.878
AUC_g	Fatigued, *n* = 4 Non-fatigued, *n* = 14	13.25 8.43	13.0	−1.593	.127
AUC_i	Fatigued, *n* = 4 Non-fatigued, *n* = 14	10.5 9.21	24.0	−0.425	.721
T_1 Awakening cortisol level	Fatigued, *n* = 9 Non-fatigued, *n* = 22	16.89 15.64	91.0	−0.348	.749
30 minutes post- awakening cortisol level	Fatigued, *n* = 9 Non-fatigued, *n* = 22	18.06 15.16	80.5	−0.805	.428
Evening cortisol level	Fatigued, *n* = 9 Non-fatigued, *n* = 22	12.83 17.30	70.5	−1.241	.219
Awakening Cortisol Response (% change)	Fatigued, *n* = 9 Non-fatigued, *n* = 22	16.44 15.82	95.0	−0.174	.881

Table 4.17 *(continued)*

Cortisol index	Group	*Mean Rank*	*U*	*z*	*p*-value
Cortisol slope	Fatigued, *n* = 9 Non-fatigued, *n* = 22	14.89 16.45	89.0	−0.435	.685
AUC_g	Fatigued, *n* = 9 Non-fatigued, *n* = 22	17.22 15.50	88.0	−0.479	.654
AUC_i	Fatigued, *n* = 9 Non-fatigued, *n* = 22	18.90 14.82	73.0	−1.132	.273
T_2 Awakening cortisol level	Fatigued, *n* = 3 Non-fatigued, *n* = 4	2.67 5.00	2.0	−1.414	.229
30 minutes post- awakening cortisol level	Fatigued, *n* = 3 Non-fatigued, *n* = 4	4.00 4.00	6.0	0.000	1.000
Evening cortisol level	Fatigued, *n* = 3 Non-fatigued, *n* = 4	3.50 4.38	4.5	−0.535	.629
Awakening Cortisol Response (% change)	Fatigued, *n* = 3 Non-fatigued, *n* = 4	4.67 3.50	4.0	−0.707	.629
Cortisol slope	Fatigued, *n* = 3 Non-fatigued, *n* = 4	5.00 3.25	3.0	−1.061	.400
AUC_g	Fatigued, *n* = 3 Non-fatigued, *n* = 4	3.67 4.25	5.0	−0.354	.857
AUC_i	Fatigued, *n* = 3 Non-fatigued, *n* = 4	3.67 4.25	5.0	−0.354	.857

Key: AUC_g – Area Under Curve with respect to ground; AUC_i – Area Under Curve with respect to increase

Correlations were evaluated between salivary cortisol indices (cortisol rhythm, ACR, slope, AUC_g and AUC_i, change in cortisol levels between timepoint) and fatigue dimensions measured by the MFSI–SF. Participants with a low haemoglobin level or those who scored above the cut-off for depression on the SPHERE–12 were excluded in the statistical analyses to minimise bias, because both factors are known to be related to fatigue.

At T_0, significant Spearman's Rho coefficients indicated that general fatigue was positively correlated with awakening cortisol level, $r_s = .48$, $p = .042$, $n = 18$ and that physical fatigue was related to cortisol at 30 minutes post-awakening, $r_s = .50$, $p = .036$, $n = 18$ and AUC_i, $r_s = .50$, $p = .034$, $n = 18$. This suggests that before the start of radiation therapy, greater general and physical fatigue were related to higher morning cortisol and thus increased stress, because cortisol was used as a surrogate biomarker for prolonged stress. The significant positive relationship between AUC_i values and physical fatigue shows that participants with higher physical fatigue experienced larger decreases in cortisol over the course of the day. These results are presented in Figure 4.10 to Figure 4.12. All other parameters were statistically non-significant.

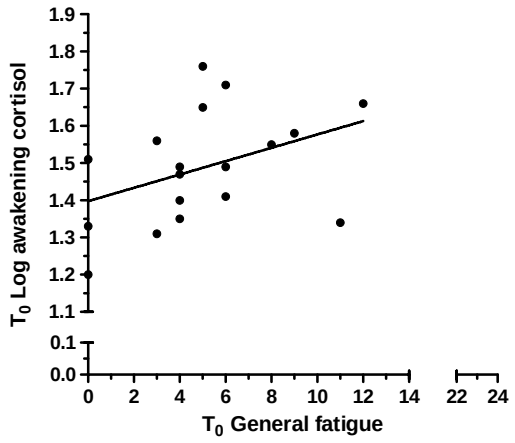


Figure 4.10 Correlation between T_0 general fatigue and T_0 awakening time

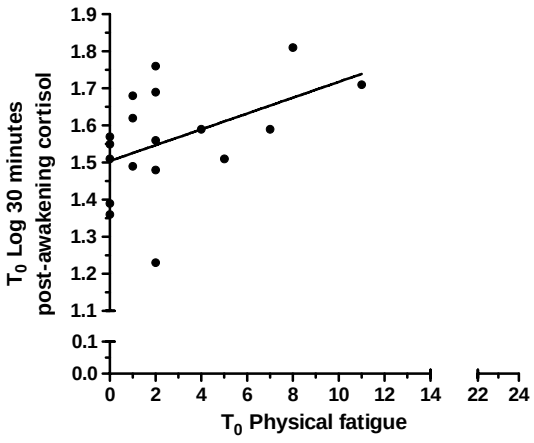


Figure 4.11 Correlation between T_0 physical fatigue and T_0 30 minutes cortisol

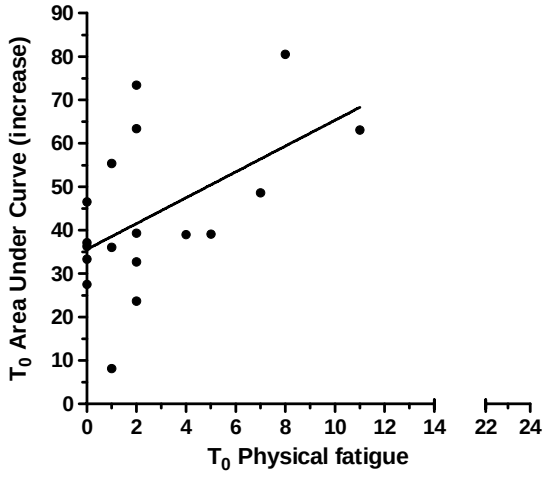


Figure 4.12 Correlation between T_0 physical fatigue and T_0 AUC_i

Several cortisol indices correlated with fatigue parameters at T_1 and these relationships are shown in Figure 4.13 to Figure 4.16. Upon closer inspection, however, an outlier was evident requiring further analysis. In each instance, the outlier was found to be beyond mean ± 2SD and therefore the correlations were repeated with the outlier excluded.

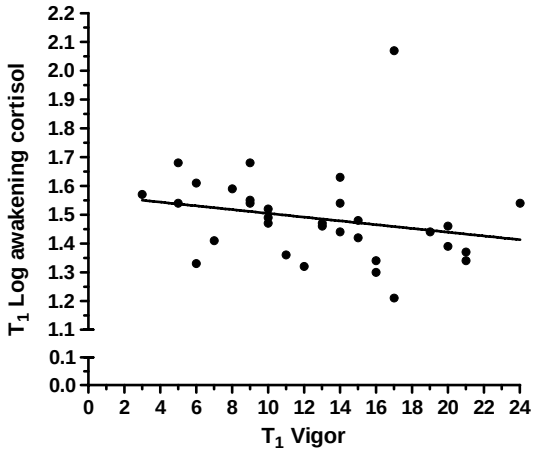


Figure 4.13 Correlation between T_1 vigor and T_1 awakening cortisol

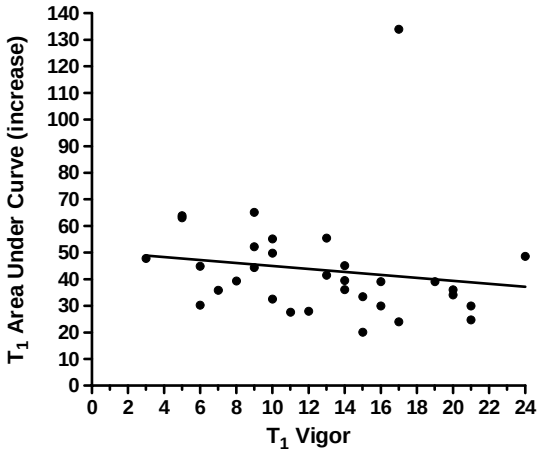


Figure 4.14 Correlation between T_1 vigor and T_1 AUC_i

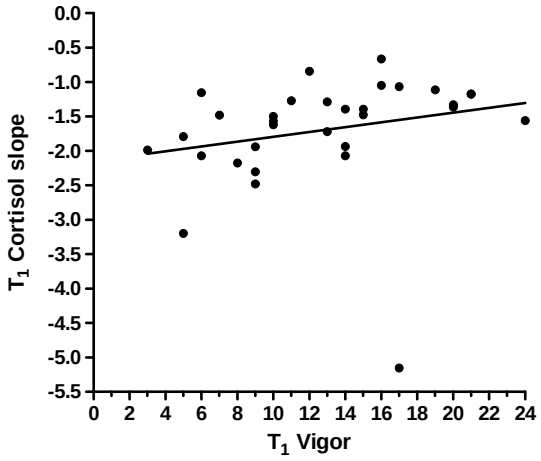


Figure 4.15 Correlation between T_1 vigor and T_1 cortisol slope

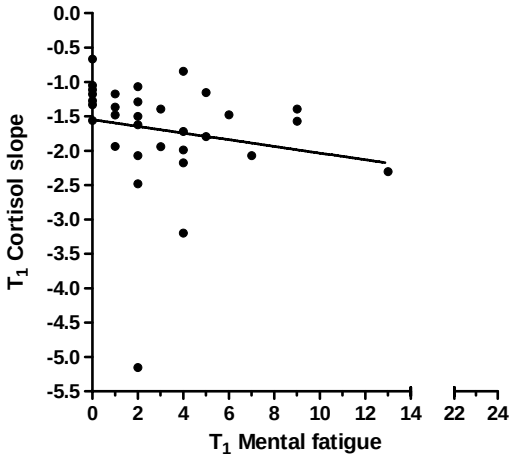


Figure 4.16 Correlation between T_1 mental fatigue and T_1 cortisol slope

After the outlier was removed, there was a significant negative correlation found between vigor and awakening cortisol, $r_s = -.51$, $p = .004$, $n = 31$ and vigor and AUC_i, $r_s = -.48$, $p = .006$, $n = 31$. Vigor was also positively correlated with cortisol slope, $r_s = .60$, $p = .004$, $n = 31$. A significant negative relationship was seen between mental fatigue and cortisol slope, $r_s = -.47$, $p = .007$, $n = 31$. All other parameters were statistically non-significant. Figure 4.17 to Figure 4.20 show correlations when the outlier was removed.

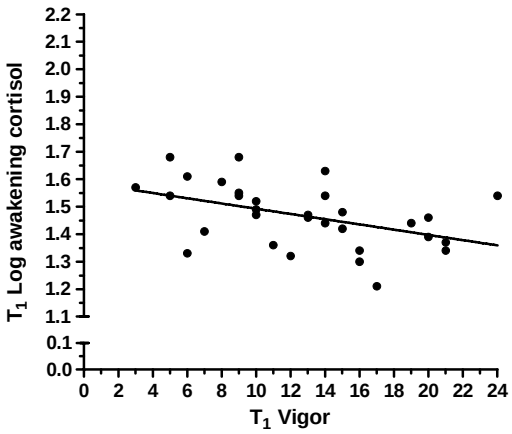


Figure 4.17 Correlation between T_1 vigor and T_1 awakening cortisol – minus outlier

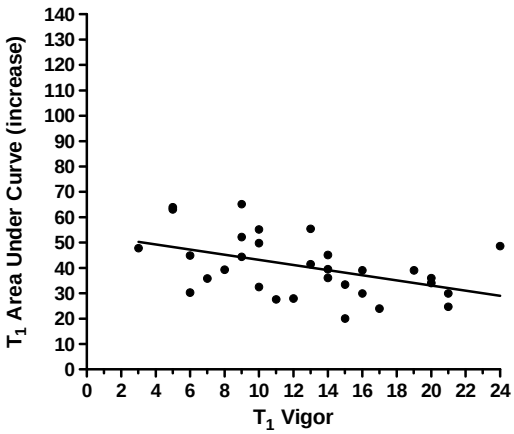


Figure 4.18 Correlation between T_1 vigor and T_1 AUC_i – minus outlier

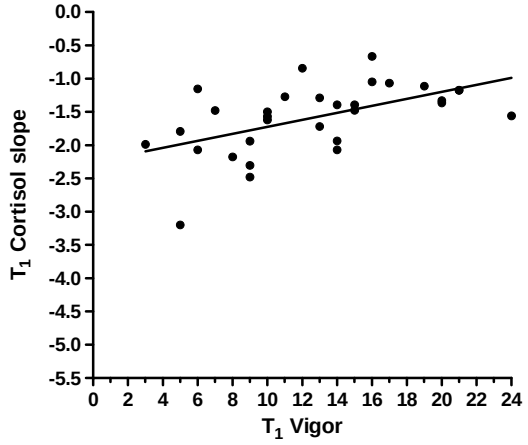


Figure 4.19 Correlation between T_1 vigor and T_1 cortisol slope – minus outlier

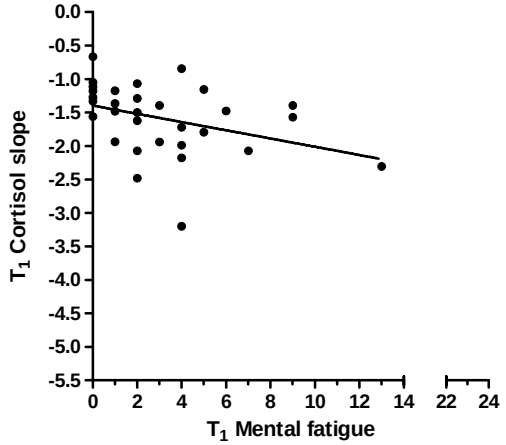


Figure 4.20 Correlation between T_1 mental fatigue and T_1 cortisol slope – minus outlier

These findings indicate that more vigor six months post-RT was associated with lower awakening cortisol level and a 'flatter' overall cortisol slope and hence smaller decreases in cortisol by the end of the day. The relationship between mental fatigue and cortisol slope suggests that participants who experienced higher mental fatigue had 'steeper' cortisol slopes (i.e. sharper decreases in cortisol from awakening to evening).

At T_2, the ACR (% change) was related to mental fatigue, $r_s = .80$, $p = .031$, $n = 7$ and vigor, $r_s = -.78$, $p = .041$, $n = 7$. These associations show that participants with higher mental fatigue exhibited greater percentage change in cortisol shortly after awakening, whereas those with higher vigor had a relatively smaller percentage change in cortisol levels 30 minutes after awakening. These results are shown in Figure 4.21 and Figure 4.22. All other parameters were statistically non-significant.

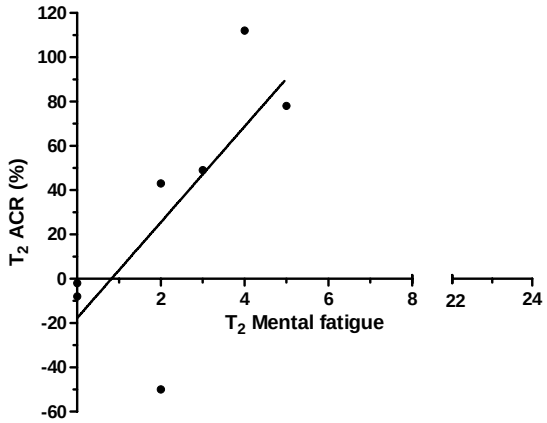


Figure 4.21 Correlation between T_2 mental fatigue and T_2 ACR

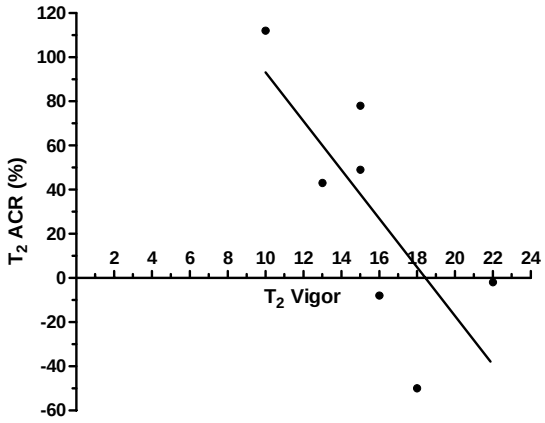


Figure 4.22 Correlation between T_2 vigor and T_2 ACR

Lastly, an analysis was undertaken of the relationships between changes in cortisol levels over time (relative percentage change) and MFSI–SF fatigue dimensions. The mean percentage changes in cortisol from baseline to T_1 and from T_1 to T_2 are shown in Figure 4.23 and Figure 4.24. Scatterplots show the mean (horizontal line) and descriptives in the tables for each sampling timepoint.

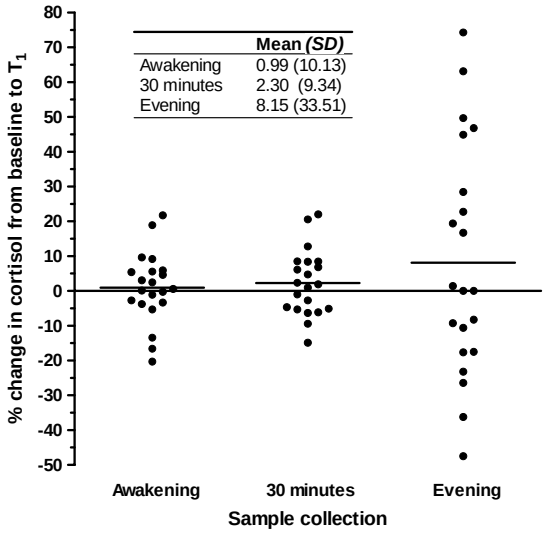


Figure 4.23 Percentage change in cortisol levels from baseline to T_1

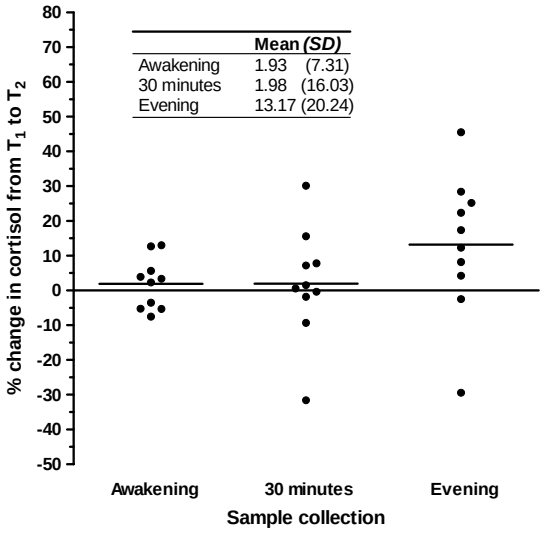


Figure 4.24 Percentage change in cortisol levels from T_1 to T_2

Spearman's Rho correlation coefficients were determined between relative percentage change from baseline in awakening, 30 minutes after awakening and evening cortisol levels (log_{10}-transformed) and T_1 MFSI–SF subscales. Significant negative correlations were seen between a change in evening cortisol from baseline and T_1 physical fatigue, $r_s = -.52$, $p = .026$, $n = 18$ and T_1 general fatigue $r_s = -.55$, $p = .017$, $n = 18$, as shown in Figure 4.25 and Figure 4.26. These correlations suggest that increased fatigue at six months post-RT was related to decreases in evening cortisol from baseline. No other significant correlations were observed as shown in Table 4.18.

Table 4.18 Spearman's Rho coefficients for T_1 MFSI–SF subscales and salivary cortisol; Cohort 1 $n = 18$

% Change from baseline to T_1	Mental fatigue	Physical fatigue	Emotional fatigue	General fatigue	Vigor
Awakening cortisol	.12	−.35	−.19	−.15	.02
30 mins post-awakening	−.11	−.13	.07	−.11	.03
Evening cortisol	−.26	−.52*	−.47	−.55*	.41

Key: *denotes significant difference, $p < .05$

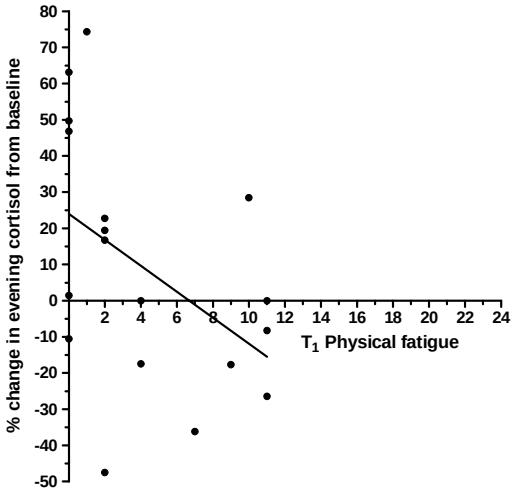


Figure 4.25 Correlation between % change in evening cortisol from baseline and T_1 physical fatigue

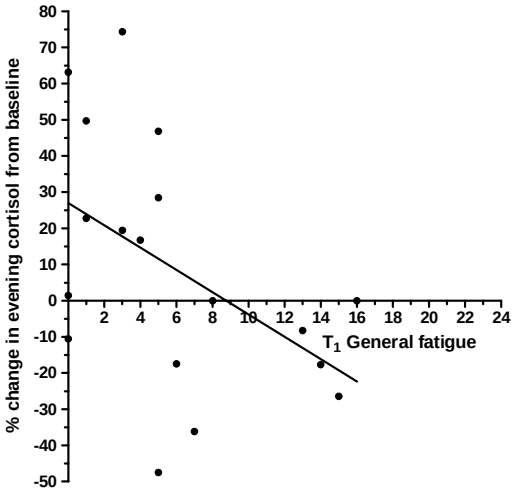


Figure 4.26 Correlation between % change in evening cortisol from baseline and T_1 general fatigue

A second analysis was conducted to examine the relationships between relative percentage change from T_1 to T_2 in awakening, 30 minutes after awakening and evening cortisol levels (log_{10}-transformed) and T_2 MFSI–SF subscales. No significant correlations were observed as shown in Table 4.19.

Table 4.19 Spearman's Rho coefficients for T_2 MFSI–SF subscales and salivary cortisol; Cohort 2 $n = 7$

% Change from T_1 to T_2	Mental fatigue	Physical fatigue	Emotional fatigue	General fatigue	Vigor
Awakening cortisol	.04	.22	−.07	.26	−.20
30 minutes post-awakening	.36	.31	.02	.46	−.27
Evening cortisol	−.15	−.04	.20	−.40	.49

4.5.4 Thyroid function indices overview

Thyroid function tests were performed using a blood test, with the blood samples sent to a commercial laboratory for testing of TSH, free T4 and free T3. Only participants consenting to the venepuncture procedure underwent the test. One participant in Cohort 2 had missing data for the free T3 hormone at T_1, but there were no other missing data or exclusions.

4.5.5 Descriptive summary of thyroid function indices

At T_0, two participants had a TSH level below the reference range and one had TSH above the reference range. In addition, another two participants had a high free T3 level. At T_1, one participant had a TSH level below the reference range (same participant as at T_0) and two different participants were found to have high free T3. All participants at T_2 had thyroid function indices within the reference range. Each of these test results was communicated to the treating Radiation Oncologist but none of these incidental findings required further treatment other than monitoring. The mean thyroid function indices at

each timepoint were found to be within the laboratory reference ranges. Table 4.20 lists the descriptive statistics of the thyroid function tests at each timepoint.

Table 4.20 Descriptive summary of thyroid function indices

Thyroid function Index	**T_0, *n* = 31**	**T_1, *n* = 43**	**T_2, *n* = 10**
	Mean (*SD*)	Mean (*SD*)	Mean (*SD*)
TSH (mIU/L)	1.319 (0.857)	1.347 (0.698)	1.546 (0.905)
Free T4 (pmol/L)	16.1 (2.18)	16.2 (2.24)	15.4 (2.04)
Free T3 (pmol/L)	5.7 (1.10)	5.6 (1.02)[a]	5.0 (1.40)

Key: [a]Missing data *n* = 1

Differences over time were tested using paired samples *t* tests. With the exception of free T4 which was found to be significantly different (lower) at T_2 compared to T_1, no other significant differences were found as shown in Table 4.21. The mean and 95% confidence intervals for each pair of comparisons are shown from Figure 4.27 to Figure 4.30.

Table 4.21 Thyroid function indices; changes over time for each cohort

Thyroid function Index	**Mean difference**	**95% CI**	***t***	***p*-value**
Cohort 1: T_0 versus T_1, *n* = 25				
TSH (mIU/L)	−0.193	−0.422 to 0.035	−1.744	.094
Free T4 (pmol/L)	0.44	−0.26 to −1.14	1.288	.210
Free T3 (pmol/L)	0.18	−0.39 to 0.76	0.656	.518
Cohort 2: T_1 versus T_2, *n* = 10				
TSH (mIU/L)	−0.181	−0.683 to 0.322	−0.813	.437
Free T4 (pmol/L)	1.48	0.46 to 2.50	3.283	.009**
Free T3 (pmol/L)	0.59	−0.25 to 1.43	1.588	.147

Key: **denotes significant difference, $p < .01$

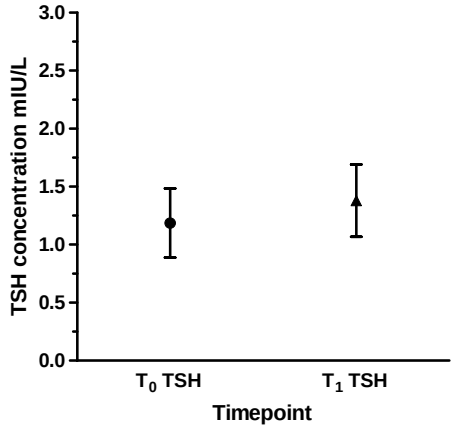


Figure 4.27 TSH; Cohort 1 *n* = 25

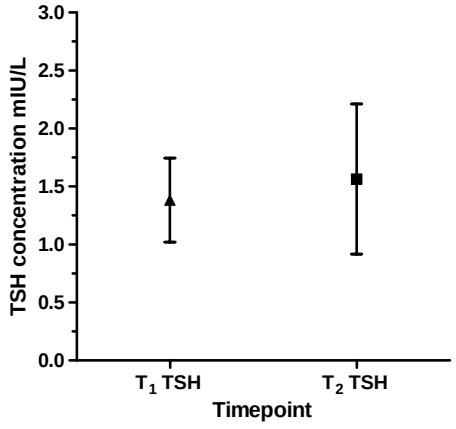


Figure 4.28 TSH; Cohort 2 *n* = 10

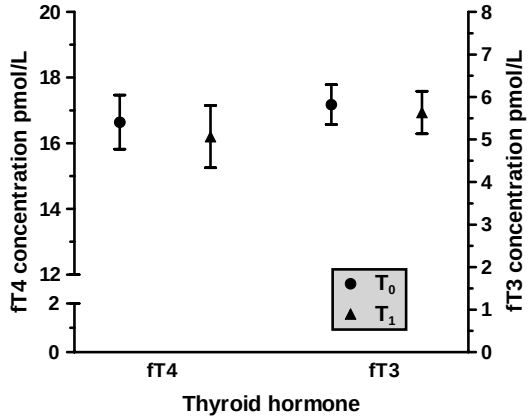


Figure 4.29 Free T4 and free T3; Cohort 1 *n* = 25

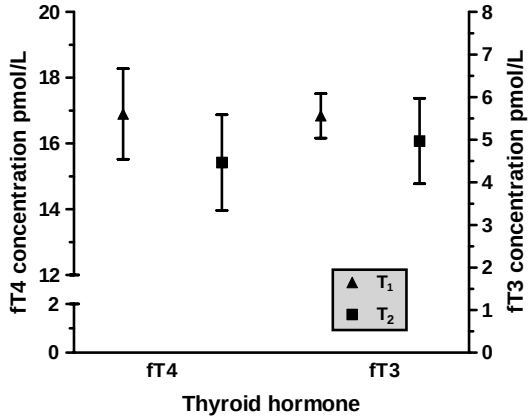


Figure 4.30 Free T4 and free T3; Cohort 2 *n* = 10

4.5.6 Thyroid function and fatigue (Hypothesis 3)

Thyroid function data for each SPHERE–12 category were first examined descriptively. Descriptive statistics for these analyses are presented in Table 4.22.

Table 4.22 Descriptive statistics of thyroid function indices for SPHERE–12 categories

Thyroid Function Index	**Neither** Mean (*SD*)	**Soma** Mean (*SD*)	**Psych** Mean (*SD*)	**Both** Mean (*SD*)
T_0	***n* = 16**	***n* = 6**	***n* = 3**	***n* = 6**
TSH (mIU/L)	1.493 (1.007)	1.075 (0.961)	0.967 (0.177)	1.274 (0.421)
Free T4 (pmol/L)	16.5 (2.42)	16.8 (1.15)	15.2 (1.77)	14.8 (2.21)
Free T3 (pmol/L)	5.7 (1.02)	6.1 (1.17)	5.3 (0.64)	5.3 (1.46)
T_1	***n* = 21**	***n* = 12**	***n* = 1**	***n* = 8**
TSH (mIU/L)	1.347 (0.696)	1.172 (0.623)	-	1.519 (0.814)
Free T4 (pmol/L)	16.5 (2.36)	16.1 (1.97)	-	15.3 (2.20)
Free T3 (pmol/L)	5.8 (0.935)	5.4 (1.01)	-	5.5 (1.17)
T_2	***n* = 3**	***n* = 4**	***n* = 2**	***n* = 1**
TSH (mIU/L)	1.626 (0.749)	1.948 (1.137)	1.011 (0.875)	-
Free T4 (pmol/L)	16.4 (2.80)	13.7 (0.68)	16.7 (0.565)	-
Free T3 (pmol/L)	6.2 (0.95)	4.0 (1.25)	4.5 (0.848)	-

NB: Means and standard deviations were not computed for sample sizes $n = 1$

Next, Mann-Whitney *U* tests were used to determine whether there were any significant differences in thyroid function between fatigued and non-fatigued participants ('Soma' versus 'Neither' groups) at each timepoint (Table 4.23). Similar to the cortisol analyses, participants with a low haemoglobin level or those who scored above the cut-off for depression on the SPHERE–12 were excluded to minimise bias, because both factors are known to be related to fatigue. No statistically significant differences were found between the two groups.

Spearman's Rho correlation coefficients were calculated between thyroid function indices and multidimensional fatigue as measured by the MFSI–SF. No significant correlations were found at any timepoint between thyroid function and any fatigue subscales.

Finally, an analysis of the relationships between changes in thyroid function over time (relative percentage change) and MFSI–SF fatigue dimensions was undertaken. The mean percentage changes in thyroid function from baseline to T_1 and from T_1 to T_2 are shown in Figure 4.31 and Figure 4.32. Scatterplots show the mean (horizontal line) and descriptives in the tables for each sampling timepoint.

Table 4.23 Differences in thyroid function between fatigued and non-fatigued participants

Thyroid function index	Group	*Mean Rank*	*U*	*z*	*p*-value
T_0 TSH (mIU/L)	Fatigued, *n* = 4 Non-fatigued, *n* = 15	8.00 10.53	22.0	−0.800	.469
Free T4 (pmol/L)	Fatigued, *n* = 4 Non-fatigued, *n* = 15	11.38 9.63	24.5	−0.551	.596
Free T3 (pmol/L)	Fatigued, *n* = 4 Non-fatigued, *n* = 15	13.25 9.13	17.0	−1.302	.221
T_1 TSH (mIU/L)	Fatigued, *n* = 12 Non-fatigued, *n* = 21	15.75 17.71	111.0	−0.561	.593
Free T4 (pmol/L)	Fatigued, *n* = 12 Non-fatigued, *n* = 21	14.83 18.24	100.0	−0.974	.345
Free T3 (pmol/L)	Fatigued, *n* = 12 Non-fatigued, *n* = 21	14.64 17.48	95.0	−0.815	.434
T_2 TSH (mIU/L)	Fatigued, *n* = 3 Non-fatigued, *n* = 3	3.33 3.67	4.0	−0.218	1.000
Free T4 (pmol/L)	Fatigued, *n* = 3 Non-fatigued, *n* = 3	2.50 4.50	1.5	−1.328	.200
Free T3 (pmol/L)	Fatigued, *n* = 3 Non-fatigued, *n* = 3	2.33 4.67	1.0	−1.528	.200

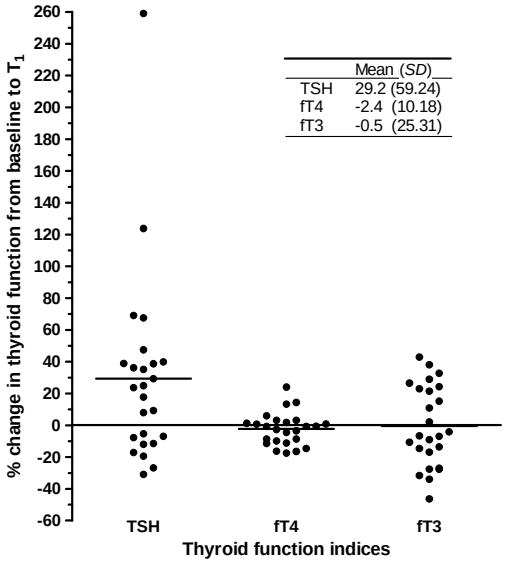


Figure 4.31 Percentage change in thyroid function from baseline to T_1

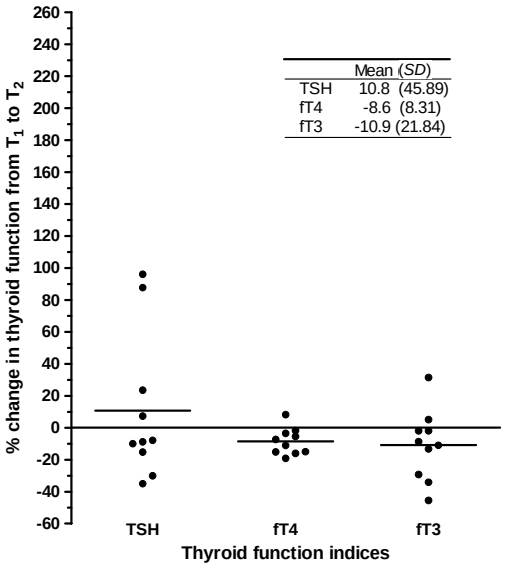


Figure 4.32 Percentage change in thyroid function from T_1 to T_2

Spearman's Rho correlations showed that there were no significant relationships between percentage change in thyroid function indices from baseline to T_1 and any of the MFSI–SF subscales (Table 4.24).

Table 4.24 Spearman's Rho coefficients for T_1 MFSI–SF subscales and thyroid function; Cohort 1 $n = 21$

% Change from baseline to T_1	Mental fatigue	Physical fatigue	Emotional fatigue	General fatigue	Vigor
TSH	.03	.14	.28	.07	−.10
Free T4	.04	.26	.08	.09	.12
Free T3	−.09	.09	−.18	−.04	−.05

A significant relationship, however, was evident between the percentage change in free T4 from T_1 to T_2 and T_2 physical fatigue, $r_s = -.84$, $p = .036$, $n = 6$, as well as between percentage change in free T4 from T_1 to T_2 and T_2 emotional fatigue, $r_s = -.83$, $p = .042$, $n = 6$ shown in Figure 4.33 and Figure 4.34. This relationship indicated that higher levels of physical and emotional fatigue at T_2 were associated with greater decreases in free T4

levels from six to 12 months post-RT. This finding should be interpreted with caution however, because of the small number of paired data sets available for this correlation. The remaining correlations were not statistically significant as given in Table 4.25.

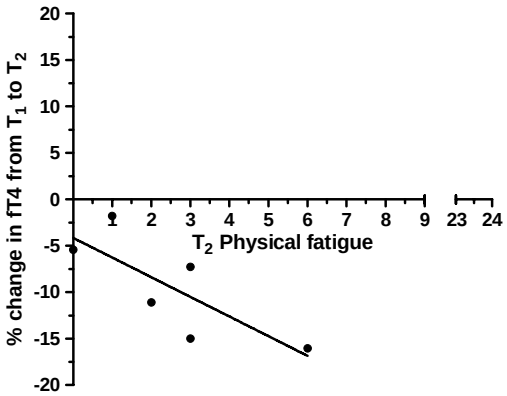


Figure 4.33 Correlation between % change in free T4 from T_1 to T_2 and T_2 physical fatigue

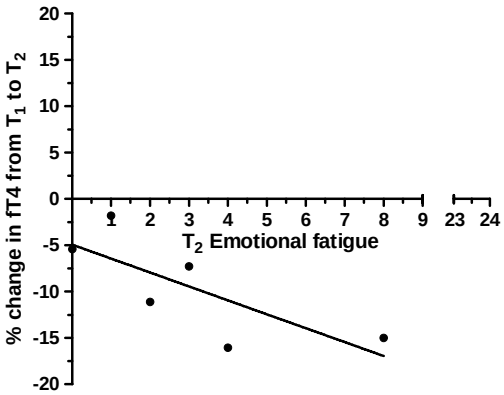


Figure 4.34 Correlation between % change in free T4 from T_1 to T_2 and T_2 emotional fatigue

Table 4.25 Spearman's Rho coefficients for T_2 MFSI–SF subscales and thyroid function; Cohort 2 $n = 6$

% Change from T_1 to T_2	Mental fatigue	Physical fatigue	Emotional fatigue	General fatigue	Vigor
TSH	.17	−.61	−.37	−.53	−.03
Free T4	.12	−.84*	−.83*	−.41	.38
Free T3	−.15	−.20	−.49	.18	−.06

Key: *denotes significant difference, $p < .05$

4.5.7 Aim 2 conclusions

In summary, salivary cortisol indices did not change over time or after radiation therapy for either cohort. There were no significant differences in cortisol indices between fatigued and non-fatigued participants. Overall, higher fatigue was associated with higher morning salivary cortisol, steeper cortisol slopes and heightened awakening cortisol response in the morning, partially supporting Hypothesis 2. Increased vigor was associated with reduced morning cortisol levels, flatter cortisol slopes and lower awakening cortisol responses. Additional correlational analyses revealed that the percentage change in evening cortisol levels from baseline to six months post-RT was associated with physical and general fatigue at six months post-RT.

Regarding thyroid function, only free T4 was significantly lower at T_2 compared to T_1; however all other indices were not significantly different over time or after radiation therapy. There were no significant differences in thyroid function between fatigued and non-fatigued participants. Hypothesis 3 was partially supported as the levels of physical and emotional fatigue at 12 months post-RT significantly correlated with changes in free T4 levels from six to 12 months post-RT, such that higher fatigue was related to decreased levels of free T4 at 12 months post-RT.

4.6 Aim 3 – Radiation dose to the thyroid gland

4.6.1 Overview of available data

In total, 32 participants had thyroid function test data at baseline (T_0) and/or six months post-RT (T_1). Of these, three participants were excluded due to a computer problem with producing Dose-Volume Histograms (DVHs), therefore 29 participants were included with 23 having thyroid function data at both T_0 and T_1 timepoints. Age (years) and thyroid gland volumes (cm^3) were available for all 29 participants. In this sub-group, 20 participants had received RT using 'tangents only' technique and nine were treated using the 'tangents + SCF' technique.

4.6.2 Descriptive summary of age, thyroid size, thyroid function and radiation dose

This sub-sample of participants (n = 29) had a mean age of 57.5 years (SD = 12.7) and a mean thyroid gland volume of 11.1 cm^3 (SD = 4.9). The minimum radiation dose to the thyroid gland was 0.3 Gy (SD = 0.16), the mean maximum dose was 13.6 Gy (SD = 20.9) and the average mean dose was 2.8 Gy (SD = 5.23).

Changes over time in TSH, free T4 and free T3 were analysed within the group as a whole (paired samples t tests), within the 'tangents only' group (paired samples t tests) and the 'tangents + SCF' group (Wilcoxon Signed Rank tests). The only significant difference over time was observed in TSH level among the 'tangents + SCF' treatment group (higher TSH at T_1 compared to T_0, large effect size r = .90). The thyroid hormones free T4 and free T3 were slightly lower post-RT (T_1), although this difference was not significant and all thyroid function indices were within the normal reference range. These results are shown in Table 4.26.

Table 4.26 Differences in thyroid function from T_0 to T_1 between different breast RT techniques

Group and thyroid function index	**T_0** Mean (*SD*)	**T_1** Mean (*SD*)	**Mean difference**	**95% CI of difference**	***t***	***p*-value**
Whole group, *n* = 23						
TSH (mIU/L)	1.18 (0.74)	1.38 (0.79)	−0.20	−0.45 to 0.05	−1.689	.105
Free T4 (pmol/L)	16.9 (1.89)	16.3 (2.35)	0.55	−0.19 to 1.29	1.546	.136
Free T3 (pmol/L)	5.9 (1.15)	5.6 (1.23)	0.27	−0.34 to 0.89	0.923	.366
Tangents only, *n* = 18						
TSH (mIU/L)	1.25 (0.75)	1.27 (0.66)	−0.02	−0.20 to 0.16	−0.259	.799
Free T4 (pmol/L)	16.9 (2.13)	16.4 (2.6)	0.49	−0.43 to 1.42	1.130	.274
Free T3 (pmol/L)	5.8 (1.2)	5.7 (1.22)	0.09	−0.58 to 0.77	0.296	.771
Tangents + SCF, *n* = 5					***z***	
TSH (mIU/L)	0.94 (0.74)	1.79 (1.14)			−2.023	.043*
Free T4 (pmol/L)	16.7 (0.65)	16.0 (1.1)	-	-	−0.813	.416
Free T3 (pmol/L)	6.1 (0.99)	5.2 (1.33)			−1.214	.225

Key: *Denotes significant difference, $p < .05$

4.6.3 Changes in radiation dose, thyroid function and fatigue between treatment groups (Hypotheses 4 and 5)

The next set of analyses compared age, thyroid gland volume, T_1 general fatigue (MFSI–SF), thyroid function and mean radiation dose to the thyroid between the two RT treatment groups using the Mann-Whitney *U* test. The mean, minimum and maximum radiation dose were significantly higher in the 'tangents + SCF' treatment group compared to the 'tangents only' group, with effect sizes between $r = .66 – .79$ which can be considered large. The other variables were not significantly different as shown in Table 4.27.

Table 4.27 Differences in age, thyroid gland size, thyroid function and radiation dose between different breast RT techniques

Variable	Tangents only	Tangents + SCF	*p*-value
	Mean (*SD*)	Mean (*SD*)	
Age (years)	59.3 (11.3)	53.4 (15.3)	.122
Thyroid gland volume (cm^3)	10.98 (4.95)	11.5 (5.06)	.982
T_1 general fatigue	7.0 (7.4)	9.3 (4.8)	.335
T_0 thyroid function			
TSH (mIU/L)	1.25 (0.75)	0.94 (0.74)	.724
Free T4 (pmol/L)	16.9 (2.13)	16.7 (0.65)	.238
Free T3 (pmol/L)	5.8 (1.2)	6.1 (0.99)	.683
T_1 thyroid function			
TSH (mIU/L)	1.27 (0.66)	1.79 (1.14)	.773
Free T4 (pmol/L)	16.4 (2.6)	16.0 (1.1)	.655
Free T3 (pmol/L)	5.7 (1.22)	5.2 (1.33)	.896
Mean dose to thyroid (Gy)	0.4 (0.14)	8.2 (6.9)	< .001***
Minimum dose to thyroid (Gy)	0.2 (0.09)	0.5 (0.18)	< .001***
Maximum dose to thyroid (Gy)	0.5 (0.19)	42.8 (12.1)	< .001***

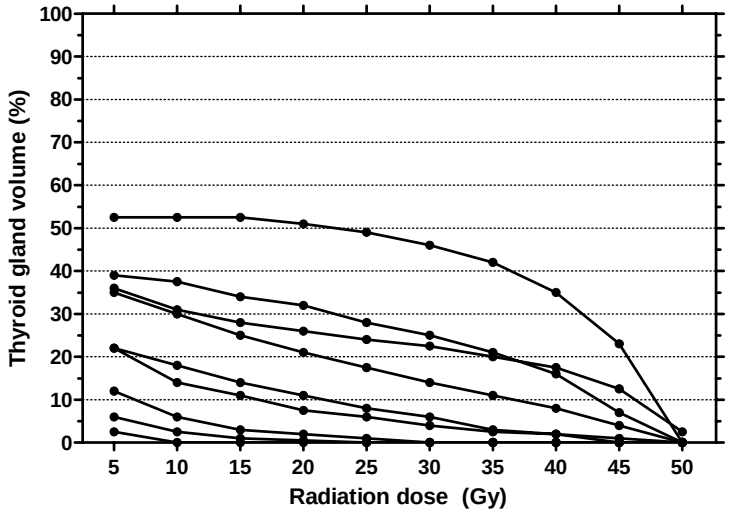
Key: ***Denotes significant difference, $p < .001$

4.6.4 Radiation dose received by the thyroid gland in supraclavicular fossa RT

Radiation doses to the entire thyroid gland in the 'tangents only' group were very low (equivalent to scattered radiation), such that the maximum dose to the thyroid for any of

the participants in this group was below 1.0 Gy and for nine out of 20 (45%) the maximum dose was below 0.5 Gy. More in-depth DVH analyses were therefore not conducted for this group.

Radiation doses to the thyroid gland within the 'tangents + SCF' group were variable. Figure 4.35 shows the combined DVH for all nine participants within the 'tangents + SCF' RT treatment group together with the mean and standard deviations of each DVH parameter. Large standard deviations for each DVH parameter indicated that the degree of variability in the dose and volume of thyroid gland potentially irradiated was very high.



Parameter	Mean dose (Gy)	*SD*
V_5	25.2	16.7
V_{10}	21.3	17.7
V_{15}	18.7	17.7
V_{20}	16.8	17.3
V_{25}	14.8	16.5
V_{30}	13.1	15.7
V_{35}	11.1	14.3
V_{40}	8.9	11.9
V_{45}	5.3	7.9
V_{50}	0.3	0.8

Figure 4.35 DVH of participants in the 'tangents + SCF' RT treatment; $n = 9$

The Beam's Eye View (BEV) images of all nine participants were visually inspected and it was observed that eight out of nine participants (89%) had at least some part of the thyroid gland within the direct SCF radiation field. Figure 4.36 shows a BEV of the participant whose thyroid gland was not within the SCF field (hence lowest dose) and Figure 4.37 shows a BEV of the participant with the highest thyroid gland dose. In these images the purple region corresponds to the thyroid and the yellow box represents the SCF radiation treatment field.

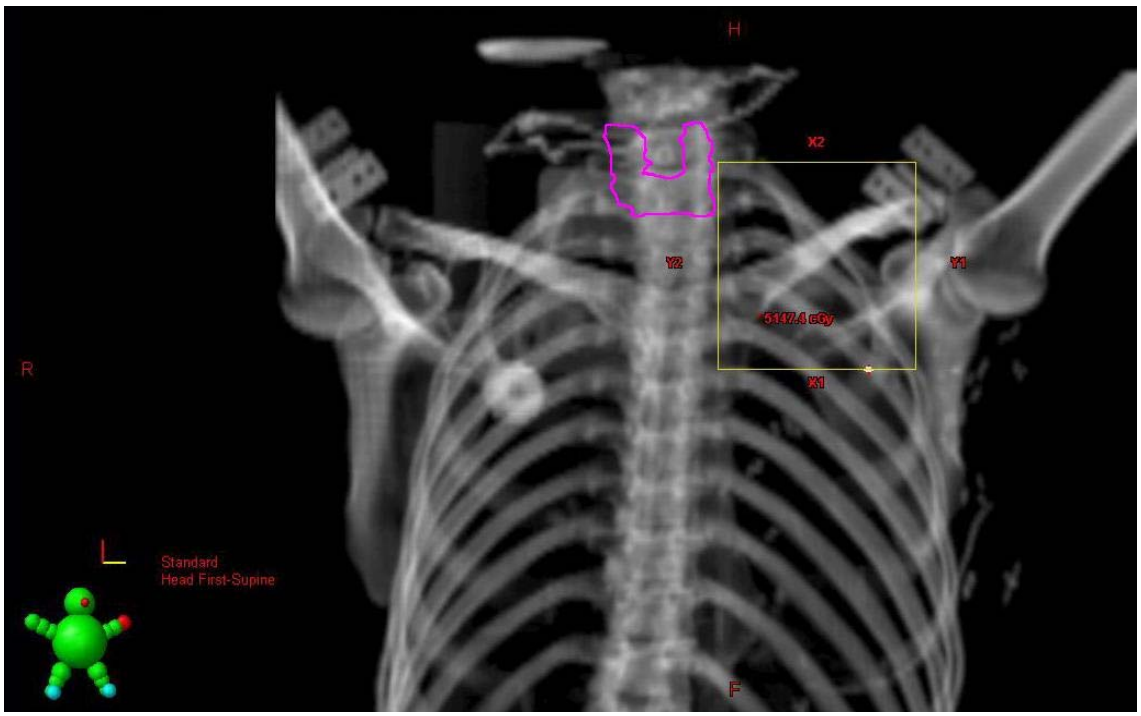


Figure 4.36 BEV of participant with lowest radiation dose to the thyroid gland

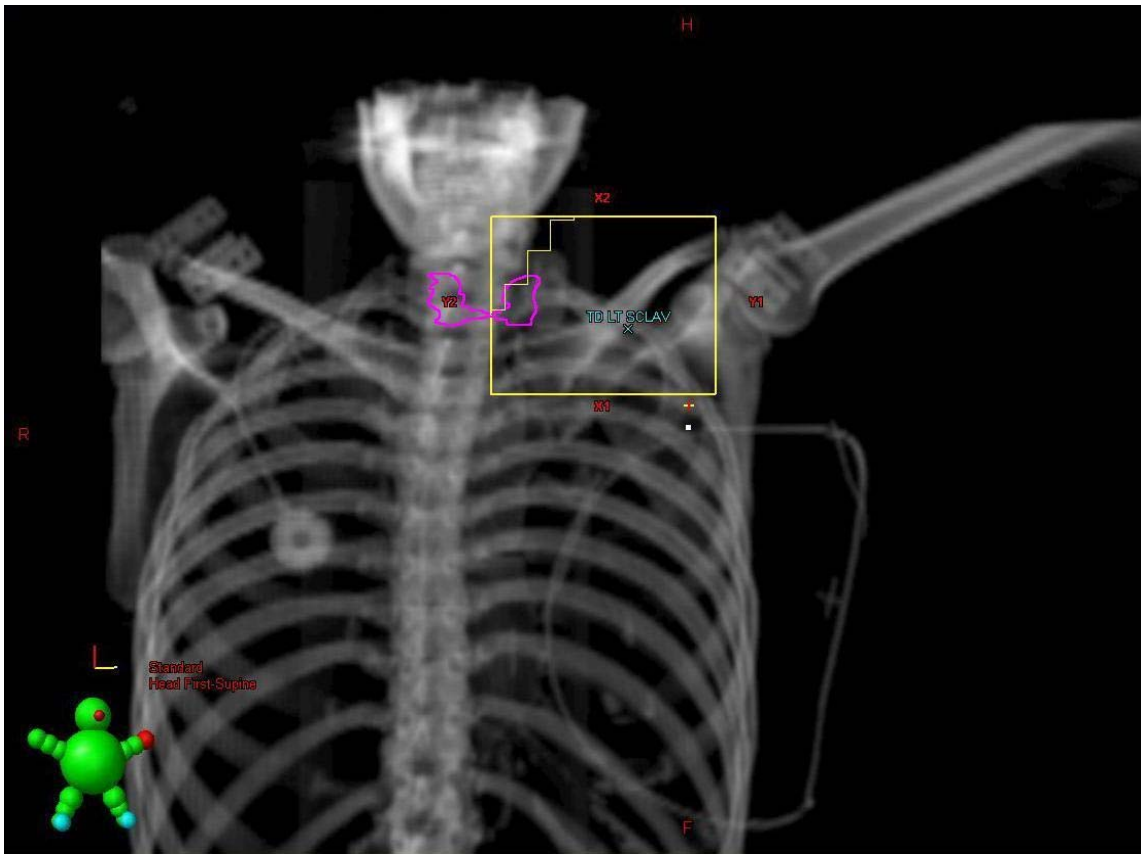


Figure 4.37 BEV of participant with highest radiation dose to the thyroid gland

4.6.5 Aim 3 conclusions

Hypothesis 4 was supported, in that radiation doses to the thyroid gland as seen on RT treatment plans were significantly different (lower) in the 'tangents only' group compared to participants treated with a 'tangents + SCF' RT technique.

No significant differences were found in thyroid function or six months post-RT fatigue between the two treatment techniques, thus Hypothesis 5 was not supported. There was however a significant difference observed in TSH levels of participants treated with 'tangents + SCF' with TSH being significantly higher at six months post-RT compared to pre-treatment. The thyroid hormones had slightly decreased, and while this did not reach statistical significance, taken together with the significantly higher TSH these results may indicate very early changes in thyroid function.

An important observation was made regarding the radiation dose to the thyroid gland when participants were treated with a supraclavicular fossa radiation field. The variability in the dose to the thyroid was very high, suggesting that some participants may have received thyroid doses at close to or above the tolerance level of the organ. It should be noted that the radiation doses quoted as part of this study represent only an estimate, not the actual radiation doses received as part of treatment. The actual doses were not measured, but the differences between the estimated and actual values were assumed to be very small.

4.7 Aim 4 – Relationships between radiation dose, thyroid function and fatigue

4.7.1 Correlations summary (Hypothesis 6)

Spearman's Rho correlation coefficients were used to investigate correlations between the mean thyroid gland dose, post-RT thyroid function and post-RT fatigue. In the whole group overall, a strong positive correlation was found between the mean thyroid gland dose and percentage change in TSH, r_s = .55, p = .012, n = 20, indicating that higher thyroid gland dose was related to increases in TSH level from baseline to six months post-RT. This finding is presented in Figure 4.38. No other significant correlations were found between mean thyroid gland dose and TSH, free T4, free T3 levels or post-RT fatigue.

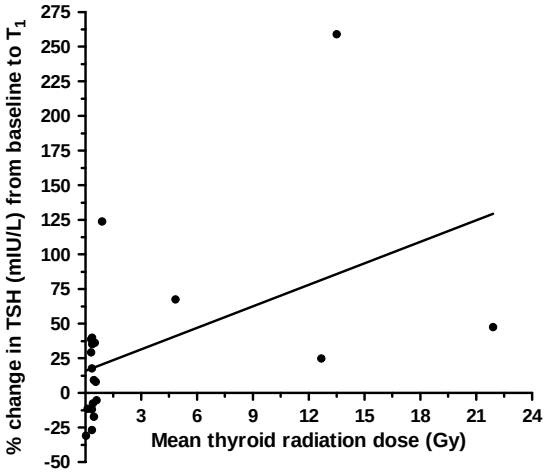


Figure 4.38 Correlation between percentage change in TSH and mean thyroid gland dose

Correlations were also tested between the above variables in the 'tangents only' group and 'tangents + SCF' group separately, because the mean dose to the thyroid between the two groups was found to be significantly different as presented in the previous section. No correlations were evident for the 'tangents only' group. In the 'tangents + SCF' group, a significant correlation was apparent between mean thyroid gland dose and T_1 free T3 levels, r_s = −.83, p = .042, n = 6 and mean thyroid gland dose and percentage change in

free T3, $r_s = -.90$, $p = .037$, $n = 5$, suggesting that higher thyroid gland dose was related to decreases in free T3 from baseline to six months post-RT. These results are shown in Figure 4.39 and Figure 4.40. It should be acknowledged that these findings were based on a very small sample and therefore need to be interpreted with caution.

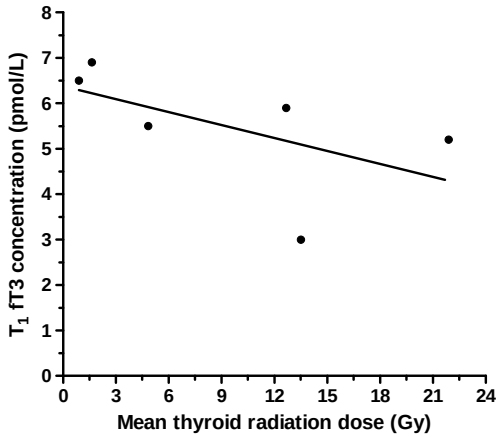


Figure 4.39 Correlation between T_1 free T3 and mean thyroid gland dose

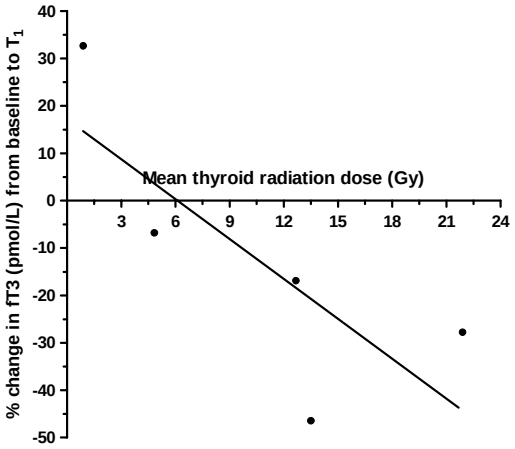


Figure 4.40 Correlation between percentage change in free T3 and mean thyroid dose

4.7.2 Aim 4 conclusions

No correlations were evident between fatigue at six months post-RT and any of the thyroid hormones, hence Hypothesis 6 was not supported. A relationship was found between change in TSH between T_0 and T_1 and mean radiation dose to the thyroid gland, as well as associations between T_1 free T3 levels and percentage change versus mean thyroid radiation dose. These relationships suggest that thyroid function may be affected in breast cancer patients receiving RT to the supraclavicular fossa. These findings were based on a very small sample size with a short follow-up period (six months) and require further investigation.

5 Discussion

5.1 Introduction

This prospective study evaluated fatigue, quality of life, diurnal cortisol rhythm and thyroid function in women prior to receiving radiation therapy for breast cancer and six months after treatment completion. In addition, a second cohort of women was recruited and assessed at six months and 12 months after radiation therapy. Study participants completed a demographics questionnaire, three self-report fatigue questionnaires (MFSI–SF, SPHERE–12, FIS), the EORTC QLQ–C30 questionnaire, a blood test measuring thyroid function (TSH, free T4 and free T3) and three day saliva collection to assess cortisol rhythm. Radiation doses to the thyroid gland were estimated from radiation therapy treatment planning scans. The main findings discussed in this chapter are:

1. Recruitment process and participant attrition
2. Prevalence of post-RT fatigue in this study compared to previous research with breast cancer patients and survivors
3. Cortisol rhythm, thyroid function and how these changed over time and how they were related to post-RT fatigue
4. Findings related to radiation dose to the thyroid in breast RT

These sections are then followed by a discussion of the study's limitations and finally, additional questions that would benefit from future research.

5.2 Recruitment and attrition

5.2.1 Recruitment process

The most common reasons for declining participation were time commitments with work or family and a lack of interest in the research. The number of women in Cohort 1 who were eligible (n = 80), but refused to participate was 30 (37.5%) and was within the range of 14 – 44% of people refusing participation in research (Shimm & Spece 1992). A higher rate of women declined participation in Cohort 2 (66%), which was most likely due to the extended time between being approached and given information about the study (shortly after treatment completion) and being contacted several months later about attending a fatigue assessment session at six months post-RT.

Having many people decline participation in a research study is of concern due to bias. To verify whether the sample population was unbiased in this study, selected demographic characteristics and disease variables were compared between study refusers and consenting participants. No significant differences were found between these two groups ($p > .05$), providing evidence that the study population was similar to the population of women who declined in terms of age, menopausal status, tumour size, diagnosis and treatment type.

5.2.2 Attrition

The number of women consenting to participation and remaining in the study over the six month follow-up period was 53 out of 63 (84%), which can be considered a very good result. For comparison, retention rates at the approximate six month follow-up after treatment described in other longitudinal studies looking at fatigue in breast cancer patients were 70% (Jacobsen et al. 2007) and 72.9% (Michielsen et al. 2007).

While the rate of participant drop-out at the second fatigue assessment session was fairly low (nine out of 63, 14%) and participants were free to withdraw from the study at any time without providing a justification, possible reasons for discontinuing with the study do warrant some discussion. In six cases, women who did not complete the second session were unable to be contacted or did not respond to two telephone call attempts from the researcher. Before making telephone contact, a letter was sent to each participant reminding them that their second assessment was due in the near future and that they would be contacted by the researcher about arranging a suitable time for this appointment. One participant posted a 'Withdrawal from participation' form to the researcher. So in these seven instances the reason for attrition was uncertain. The remaining two participants gave verbal confirmation of their intention to withdraw; one participant lived outside of the Sydney metropolitan area and could not arrange travel to attend the research session at the hospital, the other was suffering from influenza at the time of the assessment and could not reschedule the assessment due to planned travel overseas.

There may be many possible reasons why some women chose to discontinue their participation. Being diagnosed with early stage breast cancer and completing treatment was likely to be a major milestone in the journey towards recovery for these women, meaning that some women were too busy to attend the hospital or were wanting to 'move on' and not revisit the cancer experience. It is possible that some participants were discouraged by the invasive nature of some of the procedures (i.e. three day saliva sampling or discomfort associated with a blood test). Upon closer investigation, three of the nine women who withdrew had not undertaken these procedures at baseline, because this was introduced only after a later ethics amendment. The other six participants did take part in saliva collection and a blood test at their first assessment and for these women completing those procedures may have been a disincentive in continuing with the study.

Another possibility may be that some participants were simply 'too tired' to complete the study. If correct this point is particularly important, because it may mean that the women most vulnerable to experiencing post-RT fatigue were the same ones who failed to be captured in the final analysis of post-RT fatigue and its relationships. This research confirmed previous studies' findings that baseline fatigue was a strong predictor of post-RT fatigue (Bower et al. 2006; Geinitz et al. 2004; Servaes et al. 2007; Stone et al. 2001), which here accounted for 29% of the variance in post-RT fatigue scores. Taking this into consideration, only two of the women who withdrew their participation had a baseline MFSI–SF general fatigue score above the T_0 group mean of 6.2 and so possibly withdrew due to high fatigue at T_1, but it remains unclear whether being too fatigued was the main cause for failing to complete the second assessment.

In conclusion, the women who took part in this study did not differ in terms of age, menopausal status, disease status, surgery and chemotherapy, from those who declined to participate, indicating that the chance of selection bias was minimal. The attrition rate was low with the majority of consenting participants completing both fatigue sessions. While somewhat unlikely, it cannot be ruled out that some women were too fatigued to attend the second assessment, meaning that the levels of fatigue reported in this study may be slightly underestimated.

5.3 Fatigue level and prevalence

5.3.1 Introduction

This study investigated the experience of fatigue among women with breast cancer before the start of RT, at six months following the last RT treatment fraction and also at 12 months after RT. Fatigue was defined as a multidimensional construct and fatigue levels were measured using a reliable and valid questionnaire (MFSI–SF). In addition, this study compared the prevalence of fatigue at each of the aforementioned timepoints. Participants were classified as 'fatigued' using the SPHERE–12 questionnaire which was developed in New South Wales to be used by General Practitioners in screening for mental illness (Hickie et al. 2001). This reliable and valid instrument had the advantage of measuring both mood disorder and fatigue symptoms, allowing for a distinction to be made between fatigue experienced alongside mood disorders such as depression, as these two symptoms often co-occur. Based on the SPHERE–12 classification system and cut-off scores, participants were categorised at each timepoint as either at risk of increased psychological symptoms (depression), somatic symptoms (fatigue), both symptoms or neither symptom.

Fatigue prevalence in this study was defined in terms of the percentage of participants scoring above the 'fatigue' cut-off point, but without any associated depression symptoms. The response categories were 'never or some of the time', 'a good part of the time' and 'most of the time' and within a timeframe of the past few weeks. This was useful because feeling tired, fatigued, unhappy or under strain is experienced by everyone sometimes, but this does not necessarily mean being at risk of clinically high fatigue or mental illness. Participants accumulated points only when their responses indicated they were experiencing these symptoms more than sometimes, which overall meant that only participants who were above the cut-off score were experiencing a high severity of

symptoms. To this end, this classification system was deemed conservative in assigning participants a fatigue 'caseness'.

Fatigue is also known to be related to Quality of Life and hence two questionnaires were used to assess quality of life including levels of functioning (EORTC QLQ–C30) and the impact fatigue may have on functioning domains (FIS). The addition of these two questionnaires provided extra information on the effect fatigue was having on participants' lives and enabled the identification of specific domains that were most affected at each timepoint.

The next few sub-sections discuss results pertaining to fatigue levels, prevalence rates and relationships between fatigue, quality of life and other factors at each measured timepoint. The study's limitations are outlined, before a concluding statement is given on this part of the study.

5.3.2 Fatigue before adjuvant RT

Mean fatigue levels prior to the start of RT were generally low and corresponded to the MFSI–SF mean scores reported by Ancoli-Israel et al. (2006). In that study, women with breast cancer (n = 76, mean age 51.2 years) were asked to complete the MFSI–SF after surgery but before the start of any adjuvant treatment; a timepoint which was common for 65% of participants in the present study. In contrast, a study by Lim et al. (2005) evaluated fatigue in healthy subjects (n = 70, mean age 36.0 years, 51.4% female) using the same questionnaire and the fatigue subscale means were lower (with the exception of the mental fatigue mean score in the present study) and vigor was higher in healthy participants compared to breast cancer patients. It should be noted that the sample population of healthy subjects was younger and almost half of the participants were male (Lim et al.

2005). For comparison, Table 5.1 lists the MFSI–SF subscale means found in the present study and the means reported by Ancoli-Israel et al. (2006) and Lim et al. (2005).

Table 5.1 MFSI–SF subscale means in participants, breast cancer patients and healthy adults

Study	**Current study**	**Ancoli-Israel et al. 2006**	**Lim et al. 2005**
Subjects	Breast cancer patients	Breast cancer patients	Healthy adults
	***n* = 36**	***n* = 76**	***n* = 70**
MFSI–SF subscale	Mean (*SD*)	Mean (*SD* not reported)	Mean (*SD*)
Mental fatigue	3.1 (3.06)	3.7	3.54 (3.49)
Physical fatigue	3.5 (3.60)	2.8	1.94 (2.24)
Emotional fatigue	5.0 (5.32)	5.3	3.84 (3.84)
General fatigue	6.2 (5.62)	5.9	5.77 (5.44)
Vigor	11.7 (5.44)	11.1	14.4 (4.14)

Using the SPHERE–12 classification method, 12.5% of participants were classified as highly fatigued before the start of RT. This prevalence rate was found to be slightly higher than fatigue prevalence in the Australian general adult population (10.5%) reported by Wijeratne, Hickie & Brodaty (2007) who also used the SPHERE–12 instrument to define fatigue cases. The current results also confirm the findings of Back et al. (2005) who found that fatigue was prevalent among 10% of breast cancer patients in NSW before the start of RT (n = 175, median 41 days post-surgery). The main difference between the study by Back et al. (2005) and the present research—apart from using a different method to categorise fatigued from non-fatigued participants—was that Back et al. (2005) excluded women who had adjuvant chemotherapy after surgery, whereas in the present research 35% of participants had completed chemotherapy, with a mean of 29 days (range 1 – 60 days) before the baseline fatigue assessment. It is unclear, however, whether the increased fatigue prevalence in the present study was a reflection of post-chemotherapy recovery processes, because no significant differences in the level of multidimensional fatigue

(MFSI–SF) were found between women who had received chemotherapy versus those who did not ($p > .05$).

Another factor that needs to be considered is recovery from surgical intervention, which in this study was a mean of 36 days (range 14 – 70) before the first fatigue assessment. A control group of healthy women was not included in this study and so it is impossible to know how surgery may have impacted on the fatigue prevalence rates, but it would be reasonable to assume that it had some effect, particularly for those participants who were tested in the early post-operative period. Having said that, the prevalence rates before RT reported by women with breast cancer were very similar to fatigue prevalence in the Australian population and it may be that in general, a small proportion of people suffer from higher than usual fatigue whether burdened by a recent cancer diagnosis or not.

In this study, a higher BMI was significantly related to the experience of worse physical fatigue before the start of adjuvant RT ($r_s = .40$, $p = .035$), an association which has been reported previously and, interestingly, also in a sample of NSW breast cancer patients (Wratten et al. 2004). Another study compared BMI and fatigue during radiation therapy and found that BMI correlated with fatigue during radiation therapy only, but not before (Geinitz et al. 2001). Used alone, the BMI does not provide sufficient information on the location of adipose tissue on the body and so the WHR was used in addition to the BMI, each giving complementary information about body composition. The WHR, however, was not related to fatigue at any timepoint ($p > .05$), so the relationships between body composition and fatigue require further study.

When pre-RT fatigue levels were compared between participants belonging to different demographic groups (e.g. married versus divorced/separated; employed versus

unemployed/retired) participants who were smokers had significantly higher levels of emotional fatigue compared to non-smokers (p = .004) and higher general fatigue levels were indicated by participants with no co-morbidities compared to those with at least one co-morbid condition in addition to having breast cancer (p = .029). No other significant differences were found between any other demographic variables before RT (p > .05). That people with more co-morbidities experience lower fatigue than those without any such conditions is counter-intuitive and may be a spurious finding. Data collection on the number of co-morbidities in this study was done by participant self-report only and so the completeness of the data gathered cannot be guaranteed. A more accurate way of collecting co-morbidity data would have been to use the Charlson Co-morbidity Index (Charlson et al. 1987), a widely used measure of co-morbidity, however additional time would have been necessary to administer this questionnaire and so this option was not pursued.

In terms of quality of life parameters related to fatigue before adjuvant RT, higher fatigue correlated with worse functioning in multiple domains (r_s = .41 – .53), with worse global quality of life (r_s = .60), increased symptoms of pain, poorer sleep and poorer appetite (r_s = .43 – .52). Results of the FIS instrument showed that the presence of fatigue impacted most on participants' physical functioning, then social functioning and lastly cognitive functioning. These results confirmed findings reported previously between pre-treatment fatigue versus functioning and quality of life (Ancoli-Israel et al. 2006; Lavdaniti et al. 2006; Stone et al. 2001), pain (Jacobsen et al. 1999; Stone et al. 2001) and sleep disturbances (Ancoli-Israel et al. 2006; Berger et al. 2007; Stone et al. 2001).

5.3.3 Fatigue at six months after RT

At six months post-RT, significant improvements were noted in the levels of emotional fatigue (MFSI–SF, p = .025), significant improvements in role and social function

(EORTC QLQ–C30, $p = .04$ and $p = .027$, respectively) and a significant reduction in the impact fatigue had on social functioning (FIS, $p = .018$). Other fatigue dimensions and quality of life parameters were not significantly different compared to the baseline measures ($p > .05$). Together, these results confirm previous studies (Back et al. 2005; Geinitz et al. 2001; Geinitz et al. 2004; Greenberg et al. 1992; Irvine et al. 1998; Jacobsen et al. 2007; Michielsen et al. 2007), which suggest that most women experience improvements in fatigue and quality of life several months post-RT.

A question often posed when statistically significant differences are observed over time in self-report instruments is whether the differences are also clinically significant. The scoring systems used in self-report questionnaires vary from instrument to instrument and for ease of completion, they rely on numerical scales with intervals that are equally graded and which progress in a linear fashion. A problem that arises is that the phenomenon tested may not increase linearly or in uniform gradients, such that an increase of one point on the scale at the lower extreme may mean a smaller (or greater) change, than the change when an increase of one point occurs at the higher end of the scale. This idea, as well as the idea of clinically meaningful change is important in interpreting any behavioural results. With this in mind, most measures do not have levels of clinical significance that are agreed upon, although in previous studies a difference of more than 10 points on the EORTC QLQ–C30 has been used to describe a clinically important difference (Lee et al. 2008; Michelson et al. 2000; Osoba et al. 1998). Using the difference of 10 points to represent clinical significance, improvements in the EORTC QLQ–C30 social functioning subscale can be considered clinically significant post-RT, whereas there was a less than 10-point change in role functioning and it is therefore not clinically significant (Table 3.7). In the absence of established levels of clinical significance in the MFSI–SF scores, a judgment on the clinical significance of improved emotional fatigue can be based on effect size

calculations. These showed that the effect size of the difference was 'medium' which may also be potentially clinically significant.

Cross-sectional analyses showed that the fatigue prevalence rate using the SPHERE–12 questionnaire was 29.1% at six months post-RT. This is in concordance with several previously published reports on fatigue prevalence rates (15 – 35%) after successful cancer treatment (Back et al. 2005; Bower et al. 2006; de Jong et al. 2004; De Vries, Van der Steeg & Roukema 2009; Goldstein et al. 2006; Jacobsen et al. 2007). As mentioned earlier, the advantage of our calculation was that the prevalence rate excluded participants who were experiencing mood disorder or depression symptoms concurrently with fatigue. A second advantage was the longitudinal analysis and a baseline measure for comparison.

Based on a cross-sectional analysis, differences in frequencies of participants in each of the SPHERE–12 categories were evaluated at six months and compared to the baseline frequencies. A statistically significant difference was found in group membership between these timepoints ($p < .001$), suggesting that significantly more women were experiencing high levels of fatigue and a significantly lower proportion showed depression symptoms post-RT. A longitudinal analysis showed that the SPHERE–12 status was unchanged from baseline to six months post-RT in over half of the participants (58%). A similar rate was found by Goldstein et al. (2006), whose sample also comprised NSW breast cancer patients, and where 47% of participants did not change their SPHERE–12 status from the first assessment (10 months post-treatment) to the second assessment (24 months post-treatment). Finally, a recent study reported that patients who experience tiredness before being diagnosed with breast cancer continue to report tiredness afterwards (De Vries, Van der Steeg & Roukema 2009).

A strength of the present study was the inclusion of a pre-RT baseline to assess fatigue prevalence, particularly as published reports often quote high rates of 'unexplained' fatigue post-treatment without taking into account the fatigue state pre-treatment. In addition, prevalence rates excluded participants who had a low haemoglobin level, known thyroid dysfunction, a medical condition known to be related to fatigue or depressive symptoms. In the present research, new fatigue symptoms—that is, those not present at baseline—were reported by just two women (5.3%) and of the remaining eight participants who scored above the SPHERE–12 cut-off for fatigue at six months post-RT, three had been already fatigued at baseline, two indicated depressive symptoms and three had both fatigue and depression. Goldstein et al. (2006) reported newly developed fatigue symptoms in 12.9% of participants, but this was not in relation to a pre-treatment measurement timepoint. In summary, with strict exclusion criteria and use of a pre-RT baseline, newly acquired 'unexplained' fatigue was reported by 5.3% of participants at six months post-RT in this study. The rates of 'unexplained' persistent fatigue after cancer treatment as reported in the literature may be overestimated, because strict exclusions as those used here have seldom been applied in previous studies and rarely with a pre-treatment baseline for comparison.

At six months post-RT, higher general fatigue was most strongly related to higher general fatigue at baseline (r_s = .54), confirming previous findings (Bower et al. 2006; Geinitz et al. 2004; Michielsen et al. 2007; Servaes et al. 2007; Stone et al. 2001). Increased physical fatigue post-RT was significantly related to larger tumour size in this study (r_s = .35), but correlations between disease variables and fatigue have rarely been found in previous research and in fact, one study reported that no correlation existed between fatigue and tumour size (Goldstein et al. 2006). Larger tumour size corresponds with increased cancer staging and it follows that more advanced staging is likely to be associated with worse fatigue. While there were no significant differences in the level of fatigue between women

with different disease stages (T_{is} – T_1 versus > T_2), fatigue levels six months post-RT were significantly higher in women who had received chemotherapy (p = .05) and those who were HER-2 positive (p = .015), both being indicators of a less favourable diagnosis. It should be noted, however, that only a small number of women belonged to these subgroups. One explanation for the discrepancies between this study and others that had not found any disease factors related to fatigue could be the type of fatigue instrument that was used. Cancer-related fatigue is known to be a multidimensional construct meaning that certain aspects of the fatigue experience (e.g. physical, mental or emotional) may be more strongly related to certain disease factors than others and this may not be captured with unidimensional fatigue instruments often employed in previous studies.

Higher post-RT fatigue was also related to poorer scores in several EORTC QLQ–C30 quality of life domains. Specifically, higher fatigue was significantly associated with poorer physical functioning, role functioning, cognitive functioning, social functioning and an overall worse global quality of life (r_s = −.46 – −.61). These relationships confirm findings that have been previously reported by others using the same quality of life tool (Kim et al. 2008; Servaes et al. 2007; Stone et al. 2001). Post-RT fatigue was also significantly related to more pain, dyspnoea and worse appetite (r_s = .36 – .41), once again confirming findings that have been reported previously (Gelinas & Fillion 2004; Meeske et al. 2007; Okuyama et al. 2000).

Majority of women in this study (Cohort 1 77%, Cohort 2 73%) were on anti-oestrogen hormonal treatment after radiation therapy. These drugs are not without side-effects, some of which include nausea, vomiting, pain and diarrhoea (MIMS Australia Pty Ltd 2010). Testing whether side-effects of hormonal therapies contribute to the experience of post-RT

fatigue was beyond the scope of this study, but would be an important topic of future research.

There were several factors at baseline that predicted the level of fatigue six months post-RT. As mentioned in an earlier section, the level of fatigue at baseline was a strong predictor of fatigue after treatment (r^2 = .49). Other longitudinal studies have also reported this predictive relationship (Geinitz et al. 2004; Servaes et al. 2007; Stone et al. 2001) and found similar coefficients of determination (r^2 = .49, r^2 = .51, r^2 = .45, respectively). Other baseline factors that significantly predicted post-RT fatigue were the level of functioning (r^2 = .23 – .60), global quality of life (r^2 = .34) and increased symptoms of pain (r^2 = .51) and dyspnoea (r^2 = .34).

The Fatigue Adaptation Model (Olson 2007) can be used to explain these observations. The model posits that the fatigue experience lies on a continuum from adaptation to no adaptation and can range from mild tiredness, through to fatigue and finally exhaustion. It may be that some women are more physically and psychologically vulnerable than others, particularly in the early stages of being diagnosed with cancer, and find it more difficult to cope with the burdens of that diagnosis. Those women who cope better and therefore adapt have a higher quality of life, better overall functioning and fewer symptoms before commencing radiation therapy and this ability lasts into the longer term. On the other hand, women who are initially more tired experience greater impairments in physical, social and emotional function and generally have a poorer quality of life before commencing adjuvant treatment are likely to have substantial problems with coping and adapting after treatment, which if persistent may result in exhaustion. It seems that these women would benefit from baseline screening for fatigue and psychosocial well-being, which could assist in targeting

appropriate interventions to help women who are likely to experience problems later down the track.

5.3.4 Fatigue at 12 months after RT

In the small cohort of participants who were tested at six months and 12 months after treatment, differences in multidimensional fatigue and quality of life were not statistically significant ($p > .05$). The general trends observed over time were decreases in fatigue and increases in vigor. A significant improvement was found in the impact fatigue had on participants' physical functioning by 12 months after completion of adjuvant RT ($p = .035$).

The overall rate of fatigue prevalence at 12 months post-RT was 33.3%. This rate was higher than at the six months post-RT timepoint, but was similar to the prevalence rate found in other studies with long-term follow-up of breast cancer patients after treatment (Bower et al. 2006; Goldstein et al. 2006). A closer repeated-measures analysis showed that no new cases of fatigue had developed over the study period and that in fact, 73% of participants had not changed their SPHERE–12 category membership. In addition, the proportions of participants in the 'Neither', 'Soma' and 'Psych/Both' categories were not significantly different between six and 12 months post-RT ($p > .05$).

Significant relationships were seen between general fatigue at 12 months post-RT and general fatigue ($r_s = .86$) and mental fatigue ($r_s = .82$) six months earlier. A negative relationship was evident between emotional fatigue and tumour size ($r_s = -.79$) implying that higher emotional fatigue was related to smaller tumour size. Similar to the results obtained at six months post-RT, the 12 months post-RT fatigue level was also significantly associated with worse quality of life ($r_s = -.75$). It should be noted, however, that the

validity of these results is uncertain due to the small sample size used in the calculations and for this reason additional research is required with an adequate sample to confirm whether these relationships show a real effect.

In summary, fatigue levels reported after adjuvant RT were generally quite low and improvements in emotional fatigue as well as some functioning subscales were seen after treatment. Higher fatigue was related to poorer quality of life and a greater degree of functional impairment. The longitudinal analysis of fatigue prevalence revealed that while a considerable proportion of women (29%) experience persistent fatigue at six months after treatment completion—a finding consistent with previous research—the clinically high levels of unexplained fatigue were a newly developed symptom only for a small fraction of breast cancer patients (5%).

5.4 Cortisol rhythm and thyroid function indices

5.4.1 Salivary cortisol indices

Previous studies of cortisol and breast cancer related fatigue have been cross-sectional in design and with one exception (Von Ah, Kang & Carpenter 2008), had been conducted without a standardised measurement timepoint in the participants' cancer recovery process (Alexander et al. 2009; Bower, Ganz & Aziz 2005b; Bower et al. 2002; Bower et al. 2005a; Carlson et al. 2007; Vedhara et al. 2006). This study was the first to investigate longitudinal changes in salivary cortisol rhythm in women with breast cancer and with a baseline (pre-RT) measure of cortisol rhythm for comparison. Standardised timepoints of measurement were incorporated into the design of the study, with assessments carried out pre-RT and as close as possible to six months and 12 months after the last RT treatment fraction. Repeated-measures of several cortisol indices (i.e. cortisol rhythm, awakening cortisol response, cortisol slope and area under curve analyses) did not show any statistically significant differences between baseline versus six months post-RT and six months versus 12 months post-RT timepoints. This section first discusses the cortisol findings as they relate to population reference values, followed by a discussion of the results with respect to previous studies of fatigued versus non-fatigued breast cancer survivors and an overall summary of the relationships between cortisol indices and fatigue.

While this study did not have a control group of healthy women to compare salivary cortisol indices to, some general comparisons can be made with reference to previously published work and population reference values (Table 3.3). Participants in this study had mean salivary cortisol levels within the population reference ranges of two reports (Hansen et al. 2003; Patel et al. 2004), but the awakening levels at each timepoint were higher than the 8AM morning reference range given by Aardal & Holm (1995). It should be noted that the reference ranges by Hansen et al. (2003) and Aardal & Holm (1995) were based on

pooled data from males and females, whereas Patel et al. (2004) reported a separate reference range for females only. The morning cortisol levels in the present study were also higher when compared to salivary cortisol levels of exhausted and non-exhausted working adults (non-patients) (Lindeberg et al. 2008). One other previous study showed that salivary cortisol levels were not significantly different between breast cancer survivors (mean 1.36 years post-diagnosis) and healthy controls (Carlson et al. 2007). Compared to awakening and evening cortisol levels of the control and breast cancer groups in Carlson et al.'s study (2007), the mean cortisol levels in the current research, particularly the morning samples, tended to be higher. Table 5.2 lists these observations; results are shown for the T_1 timepoint because of the largest sample size.

The awakening cortisol response, a distinct phenomenon that is characterised by an increase in cortisol levels that peak at 30 minutes after awakening, was first described by Pruessner et al. (1997) and was shown to vary widely among individuals with levels rising between 50–75% after awakening (Pruessner et al. 1997). In one review paper, 12 studies of healthy adults were summarised and the average ACR was shown to be a rise in cortisol of 9.3 nmol/L ± 3.1 nmol/L from awakening to 30 minutes after awakening (Clow et al. 2004). This rise is thought to represent three secretory episodes in the 30 minute period after awakening (Clow et al. 2004; Wust et al. 2000) and therefore absolute levels of cortisol increase may be a more reliable measure of early morning HPA reactivity than relative percentage change, which is dependent on the level of cortisol at awakening. The physiological significance of the ACR is yet to be defined, but one recently published review hypothesised that the increase in ACR is associated with the anticipation of the upcoming day (Fries, Dettenborn & Kirschbaum 2009).

Table 5.2 Salivary cortisol levels in the current study compared to previous research

First author	Year	Subjects	Measure	Salivary cortisol (nmol/L)		
				Awakening	30 minutes after awakening	Evening
Current study (T_1)	2010	Breast cancer patients *n* = 41	Mean (*SD*)	28.9 (0.47)	33.0 (0.46)	5.1 (0.69)
Lindeberg	2008	Non-patient adults *n* = 78				
		Exhausted	Median	16.8	22.0	3.9
		Non-exhausted	Median	18.5	28.5	3.7
Carlson	2007	Breast cancer patients *n* = 33	Mean (*SD*)	14.2 (6.84)	nd	1.86 (0.89)
		Healthy controls *n* = 33	Mean (*SD*)	13.9 (8.99)	nd	2.7 (3.22)
Patel	2004	Healthy females *n* = 120	Range	9.3 – 40.3	nd	nd
Hansen	2003	Healthy adults *n* = 120	Range	3.5 – 35.7	7.5 – 39.9[a]	1.1 – 10.5
Aardal	1995	Healthy adults *n* = 197	Range	3.5 – 27.0[b]	nd	< 6.0

Key: [a]Range was based on 20 minutes after awakening; [b]Range was based on an 8AM saliva sample, not awakening; nd – no data

With the exception of the T_2 ACR (mean increase = 9.5 nmol/L), the baseline and six months post-RT mean ACRs (T_0 mean ACR = 3.7 nmol/L, T_1 mean ACR = 4.3 nmol/L) were lower than those described in the above literature of an expected cortisol increase of 9.3 nmol/L ± 3.1 nmol/L in healthy individuals. Additionally, the morning cortisol rises were also lower compared to the mean ACR of breast cancer patients in a previous study (11.1 nmol/L) (Vedhara et al. 2006).

These discrepancies may be related to selection bias due to the small sample sizes and also the uncertainty of participants' compliance in carrying out the saliva sampling protocol. Participants completed all saliva sampling at home without additional monitoring or electronic tracking of when a sample was collected. The ACR phenomenon was, however, carefully explained to each participant in the fatigue assessment session with a particular emphasis placed on the importance of recording the exact time participants took each saliva sample. A previous study had found a high compliance rate of 84% when participants were unaware that the time of sampling was being monitored using electronic microchips inside the lid of the Salivette (Kudielka, Broderick & Kirschbaum 2003). In the current study, when the time of sample collection was collated from participants' record sheets, the average saliva sampling time at each timepoint was within one to two minutes of the requested 30 minutes after awakening, but as the recorded times had not been verified objectively, the effect of non-compliance cannot be ruled out as a potential confounder.

Two Area Under Curve parameters were measured; AUC_g and AUC_i which represent the overall cortisol output and the reactivity of the HPA system, respectively (Pruessner et al. 2003). There were no significant differences over time in these parameters in each cohort. The AUC analyses in this study were based on three data points calculated from the mean

of awakening, 30 minutes and evening samples over three days. The sampling protocol did not include saliva collection during the day in order to keep subject burden and costs to a minimum, but was for this reason limited and more accurate AUC values would have been obtained if additional saliva collection was requested throughout the day.

The AUC findings for each timepoint in this study were not dissimilar to the values of the salivary cortisol AUC parameters in breast cancer patients and healthy controls (Carlson et al. 2007; Vedhara et al. 2006), but the absence of day and afternoon saliva samples are a likely explanation for the overall differences. Table 5.3 shows these comparisons, with current study's results given for T_1 due to the largest sample size at that timepoint. While neither Vedhara et al. (2006) nor Carlson et al. (2007) found any significant differences in AUC parameters between breast cancer patients and controls, the AUC parameters differ considerably between these two studies and of particular interest are the differences between the healthy control groups. While both studies included four saliva collection timepoints throughout the day, these were performed at a different stage in participants' cancer journey (three months post-diagnosis and some patients were on active treatment in Vedhara et al.'s (2006) study; 1.36 years post-treatment in Carlson et al.'s (2007) study). In addition, there was a difference in sample sizes and much lower standard deviations in Vedhara et al.'s (2006) study which could explain the lower AUC parameters.

Fatigue 'caseness' as determined by the SPHERE–12 questionnaire was used to compare differences in cortisol indices between fatigued and non-fatigued participants in each cohort. The strength of this approach was the possibility of differentiating between fatigue symptoms and depressed mood, which are known to be related and could potentially confound the results. The overall findings were that none of the measures of salivary cortisol rhythm differed significantly between participants who were fatigued versus those

who were not ($p > .05$). Similar findings have been reported recently in a study of breast cancer survivors (mean 10 months post-treatment) (Alexander et al. 2009). Other studies investigating differences in salivary cortisol between fatigued and non-fatigued breast cancer survivors have shown conflicting results: specifically, that fatigued breast cancer survivors have significantly flatter cortisol slopes or a decline in cortisol over time (Bower et al. 2005a) and that fatigued survivors exhibit a significantly lower level of awakening cortisol than non-fatigued survivors (Bower et al. 2002).

Table 5.3 Cortisol AUC parameters in the current study compared to previous research

First author	**Year**	**Subjects**	**Area Under Curve, Mean (*SD*)**	
			AUC_g	AUC_i
Current study (T_1)	2010	Breast cancer patients *n* = 41	54.38 (26.78)	42.2 (19.56)
Carlson	2007	Breast cancer patients *n* = 33	83.70 (24.49)	56.43 (30.40)
		Healthy controls *n* = 33	92.21 (38.95)	51.10 (42.33)
Vedhara	2006	Breast cancer patients *n* = 85	20.58 (6.81)	12.94 (4.45)
		Healthy controls *n* = 59	21.22 (5.06)	12.71 (4.85)

It needs to be acknowledged that there are important differences in both studies by Bower et al. (2002; 2005a) mentioned above compared to the present research, which may explain the conflicting results. First, Bower et al. (2002; 2005a) measured cortisol at a single timepoint at approximately five to six years post-diagnosis giving a single snap shot of cortisol rhythm, but no information on HPA axis function prior to adjuvant treatment. Second, fatigue 'caseness' was assigned differently, with participants being classified as fatigued if they scored above the midpoint of 50 on the SF–36 energy/fatigue subscale (score range 0 – 100). This was less rigorous than the method of determining fatigue cases

in the current study. In addition, it was unclear whether participants with depressive symptoms were excluded from analysis. Third, it appeared as though participants in both studies by Bower et al. (2002; 2005a) were recruited from the same cohort of individuals taking part in a large survey of quality of life and it was unclear whether a separate sub-set of individuals participated in the 2002 study compared with the 2005 study. Finally, it is possible that the differences in morning cortisol and flatter cortisol slopes observed by Bower et al. (2002; 2005a) may be apparent only several years after cancer treatment, whereas the follow-up intervals of six and 12 months in this study may have been too short for detecting small differences between fatigued and non-fatigued survivors.

To summarise, the mean levels of salivary cortisol in the current study tended to be towards the higher end of the population reference ranges quoted in the literature. This suggests that if higher cortisol levels are used as a surrogate measure of greater subjective stress, breast cancer patients in the current study were experiencing, on average, higher levels of stress than a reference population without cancer. The ACRs were lower than those observed in healthy adults without cancer, supporting the notion that the experience of cancer can affect circadian rhythms, although it is acknowledged that this study did not include a control group or a pre-diagnosis baseline for comparison and so this claim is only speculative. Differences between fatigued and non-fatigued participants were not statistically significant, which was in agreement with a similar study conducted at 10 months post-treatment (Alexander et al. 2009), but further research with a longer follow-up period is required to elucidate the long-term differences in cortisol rhythm between breast cancer survivors indicating persistent fatigue compared to survivors without persistent fatigue.

5.4.2 Cortisol and fatigue

This study was the first to evaluate salivary cortisol indices in relation to fatigue before and after adjuvant treatment in women with breast cancer using a longitudinal repeated-measures study design in the short term period following RT. Also unique to this study was the investigation of relationships between salivary cortisol rhythm and multidimensional fatigue, without the possible confounding interaction of depressive symptoms.

At baseline, higher fatigue levels correlated with higher levels of morning cortisol (r_s = .48) and the AUC_i parameter (r_s = .50). At six months post-RT, higher levels of vigor were related to lower levels of awakening cortisol (r_s = −.51), flatter cortisol slopes (r_s = .50) and smaller AUC_i (r_s = −.48), whereas higher mental fatigue was associated with steeper cortisol slopes (r_s = −.47) or more diurnal variation. At 12 months post-RT, relationships were evident between higher awakening cortisol responses (r_s = .80), which were associated with increased mental fatigue and lower vigor. To summarise, prior to adjuvant RT, increases in cortisol were associated with higher general and physical fatigue and after RT higher cortisol was associated with higher mental fatigue and lower vigor.

The relationships found in the current study between salivary cortisol rhythm and fatigue appear to contradict previous research, but it is important to mention that the research design of this study, the inclusion of a pre-RT baseline and measures of multidimensional fatigue do make comparisons of the results with previous research difficult. Compared with two recently published studies of healthy participants and non-patient adults, increased fatigue was related to lower morning cortisol levels and a lower ACR (Adam et al. 2006) and higher fatigue was associated with flatter cortisol slopes (Lindeberg et al. 2008). Opposite relationships were observed in the current study, but it is very likely that these differences were due to the different sample populations. The participants in the

aforementioned studies were unlikely to be experiencing the degree of stress that breast cancer patients do, due to the associated physical and psychological burdens of having a cancer diagnosis. Being told that one has cancer can be a trigger for major stress and according to the Fatigue Adaptation Model, fatigue could be a likely outcome of that stressor (Olson 2007), explaining the positive correlation found between fatigue and morning cortisol at baseline. In addition, important review papers synthesising research on circadian rhythms suggest that HPA axis function can increase at the onset of a stressor, but can also decrease when the stressor persists into the long term (Miller, Chen & Zhou 2007; Sapolsky, Romero & Munck 2000). The period during which participants in this study were assessed can be considered to be the acute phase of the stressor and may explain the observed hyperactivation of the HPA axis and its relationship with increased fatigue.

Support for this notion comes from previous studies with breast cancer survivors, where fatigue and cortisol relationships were investigated at a timepoint more than one year after diagnosis. Carlson et al. (2007) investigated fatigue and salivary cortisol relationships at a mean period of 1.36 years post-diagnosis and did not find any significant relationships between these two variables. When Bower et al. (2005a) investigated cortisol rhythm and fatigue in breast cancer survivors at approximately six years post-diagnosis, significant relationships were apparent between higher fatigue and flatter cortisol slope; a relationship that has been reported in non-patient adults as mentioned earlier (Lindeberg et al. 2008). This seems to indicate that several years after diagnosis breast cancer patients exhibit similar patterns of HPA axis function as adults without cancer.

Examination of the relationships between percentage change in cortisol levels and multidimensional fatigue resulted in an unexpected finding. Decreases in evening cortisol

levels from baseline to six months post-RT were significantly related to higher levels of physical fatigue (r_s = −.52) and general fatigue (r_s = −.55) experienced six months post-RT. In other words, participants whose evening cortisol levels were lower at the six months post-RT follow-up compared to baseline had reported higher physical and general fatigue after treatment. Due to various limitations of this study, for instance the small sample size, it is unknown whether this was a real effect or a random finding. In the case of the former, the theory of higher stress being related to increased fatigue after cancer treatment is open to question. Comparisons with prior work cannot be made, as there are no previous studies that have looked at the relationship between fatigue and changes in salivary cortisol over time. Additional research needs to be undertaken to determine whether this finding can be replicated and also to provide an explanation for this observation.

An explanation for why the current results appear to be inconsistent with the cortisol and fatigue relationships found in previous research may be, that the relationships between fatigue and HPA axis function are variable and that they change over time as women progress through the period of diagnosis, intensive treatment, followed by a period of recovery and adaptation.

While this study fills a gap in knowledge regarding the relationships between cortisol and fatigue immediately post-diagnosis and in the acute post-RT period—showing that fatigue is associated with increases in morning HPA axis activation during this time—one aspect warranting further study is the causal link between stress and fatigue. It is likely that fatigue can be a contributing factor in the experience of stress in a similar way to stress being able to induce feelings of fatigue. Currently, this complex causal relationship is yet to be elucidated.

5.4.3 Thyroid function indices

Undiagnosed thyroid dysfunction is prevalent in about 10% of the female population (Canaris et al. 2000), with symptoms of fatigue occurring in up to 35% of people with thyroid dysfunction (Gulseren et al. 2006). Thyroid hormones are involved in regulating metabolism and controlling body temperature and on a cellular level, they are needed for mitochondrial ATP production and energy release (Martini 1998). For this reason, it was hypothesised that low thyroid hormone reserves were related to persistent fatigue in women following radiation therapy for breast cancer.

Throughout the study, having a previously diagnosed thyroid condition or an incidental finding of thyroid dysfunction were grounds for exclusion. The thyroid function indices tested were TSH, free T4 and free T3 at each timepoint and overall, almost all participants had hormone levels within the reference ranges specified by the commercial laboratory that conducted the tests. The treating Radiation Oncologist was informed in instances where women's thyroid function was outside of the reference range, but, other than monitoring, no further medical intervention was required and these participants were not excluded from the study.

Over the past few years, there has been some debate regarding the validity of thyroid function reference ranges, particularly for TSH, where the upper limit has been gradually decreasing with the development of more sensitive TSH assays, the recognition that past reference intervals were based on populations who may have had undiagnosed thyroid problems and the finding that individual variation in normal thyroid function is much narrower than initially thought (Andersen et al. 2002; Dickey, Wartofsky & Feld 2005; Wartofsky & Dickey 2005). New research evidence has also emerged that points to genetic influences in TSH variation rather than environmental factors (Panicker et al. 2008),

meaning that for some people their TSH can be 'abnormal' even if it is found to be within the normal population reference range. Ideally, several monthly tests conducted over the course of one year would have been required to ascertain individuals' 'normal' TSH set-point, but this would have involved additional blood testing, willingness and time on the participants' behalf as well as more resources to conduct these tests. Hence, this study relied on the population reference ranges on account of the short follow-up period and two assessment timepoints.

None of the thyroid function indices (TSH, free T4 and free T3) changed significantly from baseline to six months after RT ($p > .05$). However, over the six to 12 month follow-up period (Cohort 2, $n = 10$), mean free T4 levels significantly declined ($p = .009$). Similar findings have been reported by Nishiyama et al. (1996) who also found that free T4 levels were significantly lower six months after head and neck radiation therapy in a group of patients with various cancers ($n = 22$). In the current study, the 12 months mean free T4 level was still within the population reference range and so this difference was not deemed clinically significant. The statistical difference was also based on a very small sample size and therefore it is not known whether this may have been a spurious finding that was unique only to this sample.

Mean TSH, free T4 and free T3 levels of the women in this study were found to be similar to those in previously published studies of women with breast cancer tested at various times over active breast cancer treatment and post-treatment recovery (Alexander et al. 2009; Kuijpens et al. 2005; Kumar et al. 2004; Saraiva et al. 2005). These are shown in Table 5.4. Thyroid function was not significantly different between fatigued and non-fatigued participants in this study ($p > .05$), which was in agreement with a similar recently published study of breast cancer survivors who were a mean of 10 months post-treatment

(Alexander et al. 2009). The sample sizes and follow-up intervals in the current study were quite modest and so may not have had enough statistical power to detect significant changes in thyroid function. A study incorporating a larger sample size and longer follow-up is needed to investigate this question in more depth.

Table 5.4 Thyroid function indices in the current study compared to previous research

First author	Year	Subjects	Thyroid function indices (mean)		
			TSH (mIU/L)	Free T4 (pmol/L)	Free T3 (pmol/L)
Current study	2010	Breast cancer patients			
		T_0 (n = 31)	1.319	16.1	5.7
		T_1 (n = 43)	1.347	16.2	5.6
		T_2 (n = 10)	1.546	15.4	5.0
Alexander	2009	Breast cancer patients 10 months post-treatment			
		Fatigued (n = 60)	2.96	15.23	nd
		Non-fatigued (n = 104)	2.01	14.82	nd
Kuijpens	2005	Breast cancer patients Timepoint unspecified			
		(n = 37)	1.85	15.5	nd
		Healthy control group (n = 2 738)	1.99	15.3	nd
Saraiva	2005	Breast cancer patients Before adjuvant treatment			
		(n = 26)	1.36	18.0[a]	3.56
		Healthy control group (n = 22)	2.41	14.2[a]	2.87
Kumar	2004	Breast cancer patients Before chemotherapy			
		(n = 22)	1.60	19.9[a]	nd
		After chemotherapy (n = 22)	1.56	18.5[a]	nd

Key: [a]denotes value was converted from conventional units reported in study (ng/dL) to SI units (pmol/L) by multiplying by a factor of 12.87 (The Journal of the American Medical Association 2009); nd – no data

5.4.4 Thyroid function and fatigue

Relationships between fatigue and thyroid function are known to be significant in people who have a thyroid disorder. The possibility that persistent fatigue is also related to problems with normal thyroid functioning in breast cancer patients has begun to gain recognition only in the past couple of years. One very recent preliminary report showed promising results in alleviating cancer-related fatigue by using Thyrotropin-Releasing Hormone (TRH), although the first four participants did not have impaired thyroid function to warrant TRH use (Kamath et al. 2009). The underpinning hypothesis behind therapeutic TRH administration is that TRH, which is released from the pituitary gland, will stimulate the production of TSH which in turn assists in thyroid hormone release. One other recently published study had compared thyroid function between fatigued and non-fatigued breast cancer patients (Alexander et al. 2009), but similar to the current findings no significant differences could be observed. A substantial limitation of the current study was the small number of participants who were classified as 'fatigue cases' and a much larger research study is warranted to test whether thyroid function indices differ between fatigued and non-fatigued breast cancer survivors.

While no significant correlational relationships were found between thyroid function and fatigue in the cross-sectional analyses at any timepoint, some interesting observations were evident when changes in thyroid function over time were taken into account. In particular, strong negative correlations were seen between physical fatigue at 12 months post-RT and changes in free T4 levels from six to 12 months post-RT ($r_s = -.84$) as well as between emotional fatigue at 12 months post-RT and changes in free T4 levels from six to 12 months post-RT ($r_s = -.83$). It must be acknowledged that these correlations were based on a sample size of only six participants; however, they highlighted that larger decreases in free T4 levels over time were significantly related to higher self-reported fatigue. These

findings, together with the observed significant decreases in free T4 in the same cohort appear to—at least in part—support the hypothesis that persistent post-RT fatigue is related to decreases in specific thyroid hormones. Changes in TSH were unrelated to fatigue, which from a physiological point of view makes sense, because it is the direct availability of thyroid hormones that regulates metabolism and processes involved in releasing energy for cell functions, not specific levels of TSH. That no relationship between fatigue and change in thyroid function was seen at six months post-RT is likely to be due to the short time interval between tests.

In summing up, thyroid function of fatigued versus non-fatigued participants was not significantly different, although some thyroid disturbances were found to be significantly associated with physical and emotional fatigue dimensions. As this is the first study to test the relationships between thyroid function and cancer-related fatigue at standardised timepoints after RT, additional experimental research such as a controlled comparison is needed to substantiate these observations.

5.5 Radiation dose to the thyroid and fatigue

5.5.1 Thyroid gland tolerance doses

The thyroid gland can tolerate radiation doses of around 30 Gy, with a $TD_{5/5} - TD_{50/5} = 30 - 40$ Gy (Rubin 1989). This means there is a 5% – 50% chance of developing a serious thyroid complication in five years at doses between 30 – 40 Gy. The most common thyroid complication after external beam radiation therapy is hypothyroidism (Jereczek-Fossa et al. 2004) which is thought to result from a combination of direct damage to the follicular cells, damage to the structure of blood vessels supplying blood to the thyroid and a possible autoimmune reaction (National Council on Radiation Protection and Measurements (NCRP) 2009). In previous studies, up to 25% of women who received supraclavicular fossa treatment as part of breast cancer radiation therapy experienced thyroid hypofunction in the long term (Bruning et al. 1985; Joensuu & Viikari 1986). Compared to women without supraclavicular fossa irradiation, the prevalence of thyroid disorders was significantly higher in women who did receive this treatment (Reinertsen et al. 2009).

In this study, radiation doses to the thyroid gland—estimated from the Dose-Volume Histogram— varied significantly between radiation therapy treatment techniques ($p < .001$). Participants who were treated with tangents had only minimal thyroid doses (mean 0.4 Gy), whereas women who had treatment to the supraclavicular fossa in addition to tangents had mean thyroid doses that were 20-fold higher (mean 8.2 Gy). This study found that thyroid doses with the tangential technique were of a similar magnitude to previous studies reporting thyroid dose estimates with this treatment (Dogan et al. 2007; Kim et al. 2007; Ludwig et al. 2008) and it is the first study to document thyroid doses in supraclavicular fossa treatment in detail.

The main observation among the cohort of women who had treatment to the supraclavicular fossa was that the thyroid dose substantially varied within this sub-population; doses ranged from a minimum of 0.5 Gy in one individual to a maximum of 42.8 Gy in another. While no participants had whole organ doses above 30 Gy, in three participants the estimated dose to 20% of the thyroid gland was 35 Gy and in one woman 20% of the thyroid was receiving more than 45 Gy. At present it is not known how damaging high radiation doses to a small part of the organ are, but the risk of developing a complication is expected to be much higher than if the doses were minimal. With the exception of one participant whose thyroid gland was outside of the supraclavicular fossa RT treatment field, all other participants (8/9) would have benefitted from a reduction in the mean and maximum doses to the thyroid gland if health professionals were aware that the organ was within the treatment field.

Such extreme variability in doses to the thyroid in different patients using the same treatment technique can be due to many factors, some that relate to the treatment technique itself and which can be manipulated to reduce the dose and others that are unique to the patient and thus not amenable to change. The position of the treatment field itself, particularly the medial and superior field margins, as well as the use of shielding, can have a substantial impact on thyroid dose. In Australia, the majority of Radiation Oncologists (49%) typically place the superior border of the supraclavicular fossa field at the lateral end of clavicle and 84% place the medial border at the clavicular head/pedicles as shown in Figure 5.1 and Figure 5.2 (Morgia, Lamoury & Morgan 2009). The more the treatment field is superior and medial across midline, the greater the chance that more of the thyroid gland is going to be inadvertently irradiated. But as supraclavicular lymph nodes are known to vary in position and depth between patients (Goodman et al. 2001), it may be more appropriate to outline the supraclavicular lymphatics on RT planning scans rather

than to use a standardised protocol where field margins are based on bony anatomy (Goodman et al. 2001; Liengsawangwong et al. 2007). In this way, optimum dose coverage to the supraclavicular nodes can be better achieved and dose to healthy tissue, including the thyroid, can be reduced. The introduction of shielding in the superior/medial corner could possibly spare the thyroid gland altogether.

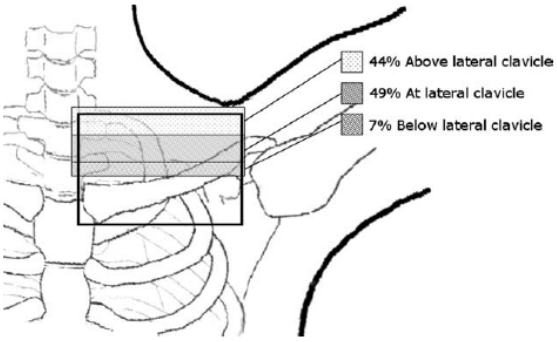


Figure 5.1 Superior border placement of the SCF field (Morgia, Lamoury & Morgan 2009)

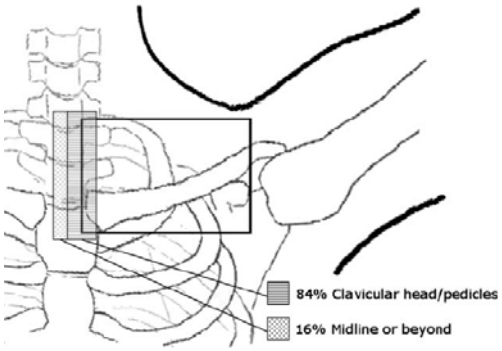


Figure 5.2 Medial border placement of the SCF field (Morgia, Lamoury & Morgan 2009)

The size of the thyroid is characteristic to each individual and to a large degree so is its anatomical position, although slight thyroid movement may be achieved by increasing arm abduction or turning the head to one side. Manually outlining the thyroid gland on RT planning scans and estimating radiation dose to this structure is not routine clinical practice in Australia, although it is a requirement in some clinical trial protocols, e.g. STARS trial. The assumption being made here is that thyroid position relative to the treatment field is rarely determined in breast RT, which explains the large degree of variability in the doses observed in this study. It is acknowledged, however, that there would be circumstances where altering the patient's arm or head position or the addition of shielding would not be desirable if it was to compromise adequate tumour control.

To conclude, this study showed that doses to the thyroid gland in supraclavicular fossa RT are not trivial and dose-volume variations between patients treated with this technique can be substantial. One recent study found that while Intensity Modulated Radiation Therapy (IMRT) improved the dose coverage of the breast and reduced doses to the heart, lungs and contralateral breast, radiation dose to the thyroid gland was much higher with IMRT (D_{50} = 11.9 Gy) compared to conventional 3D CRT (D_{50} = 0.9 Gy) (Dogan et al. 2007). It is therefore important that additional research is performed to determine what dose-volume parameters are critical and can be safely tolerated by the thyroid gland, especially when breast RT is delivered using IMRT techniques.

5.5.2 Mean thyroid dose and thyroid function

Damage to the thyroid from external beam radiation therapy is dose dependent (National Council on Radiation Protection and Measurements (NCRP) 2009). Monitoring thyroid function after head and neck radiation therapy and mantle technique treatments—where it is known that irradiating the thyroid will be unavoidable—is a routine part of patient follow-up practice, but this is not the case after breast cancer radiation therapy. The presumed reasons for this are: (1) in the majority of cases, breast cancer treatment involves the use of tangential treatment fields only, which are well inferior of the thyroid and so in general, the thyroid is not considered an organ that warrants 'at risk' status due to radiation exposure; (2) the prevalence of thyroid dysfunction increases as women age, and so the relationships between thyroid dysfunction after radiation exposure or due to normal aging processes can overlap; (3) it may be that damage to the thyroid gland is deemed less 'critical' compared to other specialised organs (e.g. spinal cord), because thyroid dysfunction due to radiation exposure can be treated in contrast to spinal cord damage which is permanent.

The significant strong positive correlation between mean thyroid gland dose and percentage change in TSH from pre-RT to post-RT (r_s = .55) confirms that changes in thyroid function are dose-dependent and can be detected as soon as six months following irradiation. Such changes have been reported previously by Nishiyama et al. (1996) in a study of 22 cancer patients who had received external radiation to the head and neck with part of the thyroid gland in the treatment field. Patients were treated for head and neck, oesophageal, lung, breast and miscellaneous cancer, and the radiation doses ranged between 40 – 70 Gy. Nishiyama et al. (1996) reported significant increases in TSH six months after irradiation, stable total T4, total T3 and free T3 hormones and significantly lower free T4 levels (Nishiyama et al. 1996).

The increase in TSH after treatment would indicate insufficient thyroid hormones and overall thyroid hypofunction, which is reported as the most common problem following radiation damage to the thyroid gland (Jereczek-Fossa et al. 2004). It needs to be mentioned, however, that the majority of participants in this study received very little radiation to the thyroid and this correlation may have been affected by the small number of participants in whom thyroid dose was estimated to be significantly higher. Additional research needs to be undertaken to further validate this observation, because changes in thyroid function following radiation therapy are rarely anticipated in breast cancer patients.

Mean thyroid dose, when compared between participants who received tangential treatment versus those who were also treated with a supraclavicular fossa field, was found to be significantly different. In the former group, there were no significant correlations between mean thyroid dose and any of the thyroid function indices ($p > .05$). In contrast, in participants treated with a supraclavicular fossa field, mean thyroid dose was significantly

related to lower free T3 levels ($r_s = -.83$) and decreases in free T3 from baseline to six months post-RT ($r_s = -.90$).

These correlations, together with the relationship between mean dose and increased TSH seem to indicate that impairments in thyroid function were beginning to emerge, but the data does not allow for further definitive conclusions to be made regarding thyroid impairments, because the period of follow-up was very short. A diagnosis of hypothyroidism (elevated TSH and decreased thyroid hormones) would probably not have been made at this stage, as thyroid function was within population reference limits for all of these participants, but this should be interpreted in the context of new research indicating that thyroid hormones can be within population limits and yet be 'abnormal' (Andersen et al. 2002). In addition, the risk of developing hypothyroidism following RT to the supraclavicular region is thought to increase at around two years after treatment (Kaffel et al. 2001; Reinertsen et al. 2009). Nevertheless, the development of hypothyroidism following supraclavicular fossa RT has been consistently reported in breast cancer patients (Bruning et al. 1985; Cutuli et al. 2000; Joensuu & Viikari 1986; Reinertsen et al. 2009; Ryu et al. 2003) and the relationships observed in this study appear to indicate early changes in thyroid function. The findings of this study, together with previous research support the practice of monitoring thyroid function as part of patient follow-up after radiation therapy to the supraclavicular fossa.

5.5.3 Radiation dose and fatigue

The hypothesis that increased thyroid dose would be related to greater fatigue post-RT was not supported by the data in this study and neither was fatigue level significantly different between participants treated with tangents compared with participants treated with supraclavicular fossa RT in addition to tangents. One possible explanation for these

observations could be that the level of reported fatigue was related to a mechanism other than thyroid function, but which was not measured in this study. Other reasons could be related to the study design, in that the small sample size and a relatively short period of follow-up prevented such relationships and differences from being detected and future research into this area should be conducted with an aim to overcome these shortcomings.

5.6 Limitations of research

There were several limitations in this research which warrant discussion. The overall sample sizes were quite small and participants were recruited from a single radiation oncology facility. The main barrier to obtaining a larger sample or conducting a multi-centre research study was the research candidature timeframe. Another reason was that the number of new patient referrals for radiation therapy in the six month recruitment period was lower than initially expected, which meant that a smaller number of women could be approached about potential research participation. With this in mind, the longitudinal design of the study was nevertheless deemed to be robust enough in overcoming this limitation, at least in part. Some participant attrition did occur over the course of the follow-up assessments and this affected the extent to which results from Cohort 2 can be generalised.

The basis for this study was the specific investigation of the effect that radiation therapy treatment had on fatigue and thus a pre-RT baseline was chosen. It is acknowledged that conducting the baseline assessment before the start of adjuvant radiation therapy cannot be considered a 'true' baseline, because all participants who were tested at this timepoint had earlier undergone surgical intervention to remove the tumour and 35% of participants had also completed several cycles of chemotherapy treatment. In order to recruit the largest sample possible, women who had undergone chemotherapy prior to radiation therapy were not excluded from this study and in some ways this sub-sample provided valuable additional information on the fatigue experience.

A different approach would have been to recruit potential participants prior to breast cancer surgery, but this alternative is not without its problems. First, there is often an extremely short amount of time between the discovery of a suspected tumour and having

surgery. Potential research participants should be given adequate time to consider their involvement in a research study, which under these circumstances may not have been possible. Second, prior to having surgery women are likely to be in shock; they may be distressed, worried, emotionally vulnerable and from an ethical viewpoint, their ability to give informed consent could be compromised. Finally, it is unknown whether an assessment at this stage would provide a valid baseline, because the uncertainty of a cancer diagnosis causes a range of feelings and emotions that may not necessarily reflect the usual 'baseline' of the individual. It could be argued that the only true baseline assessment is one that is conducted even before a tumour is suspected, but due to logistic limitations this is not an option in most studies and would require an overwhelming amount of time and resources.

The use of self-report questionnaires poses a range of limitations in behavioural studies. In this study, it was important that the completion of questionnaires was not too tiresome, especially because this study focused on measuring fatigue. For this reason the current study employed only four questionnaires to get the balance between adequate data collection and time required to complete the questionnaires right; however, it may be argued that the use of more questionnaires would have enabled a richer data-set. Another limitation was the potential for recall bias. The MFSI–SF and EORTC QLQ–C30 questionnaires asked participants to consider their answer in the context of the past week, whereas the SPHERE–12 and FIS questionnaires referred to the past few weeks. For some participants, this longer time interval may have posed some difficulties in terms of recall bias and so to an unknown extent, some of the responses may have actually related to participants' immediate or very recent fatigue or non-fatigue state.

Quite a few participants commented on the unclear wording of the FIS questionnaire. The questionnaire asked participants to consider the extent to which fatigue had impacted on various tasks or aspects of their lives, but the response categories were in the format 'no problem', 'small problem', 'moderate problem', 'big problem' and 'extreme problem'. This created some confusion, particularly for participants who in previous questionnaires indicated that they were not experiencing any fatigue. While it was known that the questionnaire had not been formally validated for use by cancer patients, it was included in the study because it had been validated in other fatigue conditions (i.e. Chronic Fatigue Syndrome) and also in order to gauge the impact fatigue had on a range of different areas in participants' functioning. It was not possible to conduct a validation study of this questionnaire in breast cancer patients due to time constraints. Taking into consideration the fact that many participants commented that the FIS was somewhat ambiguous, its results should be interpreted with caution.

While most participants completed all four questionnaires on the same day as the blood test, this was not the case for cortisol sampling which was conducted by participants at home, sometimes a few days after attending the fatigue assessment at the hospital and always over a three day period. To a very small extent this may have compromised the validity of fatigue and cortisol correlations, because they were not performed at exactly the same time. In addition, relationships between cortisol levels and fatigue could have been affected by the time interval between the two measures, particularly if a participant was having a stressful couple of days or fell ill during saliva collection, but felt different while completing questionnaires. To overcome this limitation, each participant was asked to record any such experiences in the 'comments' section of the saliva sampling record sheet and upon examination, there were no instances which may have posed a serious threat to the validity of the results. Nevertheless, the cortisol levels and fatigue questionnaires were

completed at different times and therefore provide information that relate to that given time only.

The schedule and procedure of salivary cortisol sampling had some additional limitations. First, there was no method of verifying compliance to the sampling procedure as non-compliance has been shown to influence the accuracy of measured diurnal cortisol (Kudielka, Broderick & Kirschbaum 2003). One way of increasing compliance is to use a microchip within the lid of the Salivette to create a timestamp at the time that the lid was removed. In the study mentioned above, 84% of participants were found to be compliant in their sample collection without knowing that their sampling was being monitored, but informing participants of electronic monitoring increased compliance to 97% (Kudielka, Broderick & Kirschbaum 2003). Due to the costs involved, it was not possible to use electronic tracking to determine compliance of saliva sampling in this study. It is uncertain to what degree non-compliance had influenced the findings here, but its effect cannot be disregarded and the conclusions drawn about cortisol and fatigue should be interpreted with this in mind.

Second, there were no saliva sampling times scheduled during the day and so there was no additional information regarding the overall rhythm throughout the middle of the day and afternoon. The reason for only collecting saliva early in the morning and evening was to minimise participant burden and the time required to perform the sampling. It was also important that participants were able to store collected samples in the refrigerator immediately after sampling and having to perform saliva sampling during the day may have created unwanted restrictions on participants' activities. Costs were also a consideration, because extra sampling timepoints would require additional consumables and cortisol EIA kits. Finally, it was thought that prescribing specific times of saliva

collection may in fact increase non-compliance if a participant forgot to sample at a given time. Overall, the saliva collection schedule aimed to collect as much information on diurnal cortisol rhythm as possible, but without excess subject burden and a possible disincentive to participation in the research study.

One limitation of the thyroid function tests was that there was no control for the time of day that the blood test was performed. The time of blood collection was based on convenience (i.e. when participant was attending hospital) and varied between different participants as well as timepoints for the same participant. It is well documented that TSH follows a circadian rhythm, with lowest levels during the day and largest variations in TSH occurring during the night (Andersen et al. 2003; Fisher 1996). This indicates that the time of blood collection should not have had a great impact on the validity of thyroid function data as the diurnal variation during the day, when all blood tests were performed, would have been at its lowest. Another limitation was the short amount of time between baseline and follow-up testing. Changes in thyroid function after RT may not have been apparent after only six months and may be masked by seasonal changes in thyroid function which can also occur in healthy adults (Andersen et al. 2003). Finally, due to budget restrictions, thyroid function tests did not include other measures related to thyroid function such as thyroid autoantibodies, TRH, total T4 and T3 hormone levels, reverse T3 or enzyme activity.

It should be noted that the radiation doses to the thyroid gland were estimated from the RT treatment plans, not measured during active treatment. Reports suggest that some uncertainty exists in dosimetric calculation when the region of interest is outside of the treatment field as was the case in this study. One way of measuring the actual dose to the thyroid gland would have been with the use of TLDs; however, this part of the research

was undertaken after all other data collection was completed and so by this stage all participants had finished their RT course. Nevertheless, the data that was collected as part of this sub-study highlighted the need for additional research and a critical evaluation of the treatment plan, particularly for women who are treated with radiation to the supraclavicular fossa, because the healthy thyroid gland may be inadvertently within the treatment field.

6 Conclusion

The experience of fatigue during and after radiation therapy treatment is a recognised side-effect which can affect an individual's physical, emotional and mental well-being. Radiation therapy patients are often informed that fatigue will be transient in nature; however, it can become a debilitating problem if it does not resolve after treatment completion. The aims of this study were to investigate the prevalence of persistent fatigue in a population of women receiving radiation therapy for breast cancer and whether fatigue was related to changes in the stress hormone cortisol and thyroid hormones.

The participants in this study experienced significant improvements in emotional fatigue, role functioning and social functioning six months after completing radiation therapy for breast cancer. Consistent with previous research, high fatigue levels were prevalent in 29% of women at six months and 33% of women at 12 months after treatment. A new finding was that at six months after treatment 5% of women experienced fatigue that was not present at the pre-treatment baseline, whereas the current literature suggests that unexplained fatigue is experienced by a considerably larger proportion of patients. This conservative estimate of unexplained fatigue prevalence after radiation therapy can be attributed to the strict exclusion of patients who either had a known thyroid disorder, another condition where fatigue was a presenting complaint or the differentiation between fatigue and depression interactions.

In contrast to previous studies conducted with this patient population, no significant differences were observed in salivary cortisol levels and diurnal cortisol rhythm between breast cancer survivors who were fatigued compared to those who were not. This study was unique, however, in that participants were assessed longitudinally and at standardised timepoints. This was the first study to show that higher fatigue was associated with an

increased activation of the HPA axis before adjuvant radiation therapy, suggesting that women who were more stressed were likely to experience more fatigue.

Thyroid function in women who were fatigued was not significantly different to women who were not fatigued; however, important new information regarding associations between fatigue and thyroid function after radiation therapy was found. In the small cohort studied, free T4 hormone levels decreased significantly from six to 12 months following treatment and larger decreases in free T4 were related to higher fatigue.

In addition, an investigation of the effects of radiation dose on thyroid function revealed that women who had radiation therapy to the supraclavicular fossa received significantly higher radiation doses to the thyroid gland than women who received localised treatment to the breast only; however, the variability in mean doses between participants with supraclavicular fossa treatment was considerable. Changes in thyroid function were observed in this treatment group, with significant increases in TSH from baseline to six months after treatment suggesting an insufficiency in thyroid hormones. No corresponding changes in fatigue were observed. When examining the relationships between radiation dose and thyroid function in this sub-group, higher mean radiation dose to the thyroid was significantly related to decreases in free T3 levels. Together, these findings indicate that changes in thyroid function as early as six months after treatment were beginning to emerge. A recommendation based on these findings is that radiation dose to the thyroid gland should be calculated at the time of treatment planning and, if found unacceptable, that thyroid function tests are included as part of patient follow-up investigations after treatment.

The impact of these findings is two-fold. The prevalence rate of newly developed unexplained fatigue in women following radiation therapy for breast cancer can be considered to be similar to the prevalence rate of fatigue in the general population; however, other fatigue related factors including depression and thyroid dysfunction should be investigated in women who experience high levels of fatigue before and after cancer treatment. Radiation doses to the thyroid gland can be highly variable when supraclavicular fossa treatment fields are used in addition to tangential fields and should therefore be examined at treatment planning before the start of treatment.

Future research into this area should include: a larger sample size with a comparison control group; longitudinal data acquisition at regular intervals following cancer treatment; more frequent saliva sampling throughout the day to ascertain a more accurate representation of cortisol rhythm; a long-term study of thyroid function and fatigue in women who receive supraclavicular fossa irradiation; more comprehensive thyroid function testing (e.g. inclusion of thyroid autoantibodies, TRH).

Questions remain about the relationships between post-treatment fatigue and endocrine changes in women managed for breast cancer. The processes at play when stressors such as cancer and the associated aggressive treatments are placed upon the body are very complex. The inter-relationships between various hormones, as well as the immune system, and the possible physical and psychological outcomes of these processes are important aspects that warrant future research. The problem of causation and whether hormone changes precede fatigue or whether the very experience of fatigue becomes a stressor in its own right is also worth investigation.