Part 1: Introduction & Literature Review

1.1 Introduction

Rates of obesity and overweight continue to rise in most of the developed world including Australia, the United States and the United Kingdom. In Australia the most recent figures show that overweight affects 48% of men and 30% of women, with obesity affecting a further 19% of men and 22% of women (1). This has enormous implications for the health of Australians, increasing the risk of a number of diseases associated with or caused by obesity including cardiovascular diseases, type 2 diabetes and certain cancers. The direct health care costs from obesity alone are estimated to be in the region of $830 million (1).

The underlying reasons for this global pandemic are complex and not fully understood. Research has concentrated on three main areas: genetics, diet and physical activity. Several ‘obesity’ genes have been identified and genetic susceptibility undoubtedly plays a part. However, given the dramatic rise in overweight and obesity in the last 20 years, during a time of relative gene pool stability, other factors must be to blame. These most likely include reduced physical activity at work and during leisure time; an abundance of easily available, relatively cheap, energy-dense and overly palatable food; and social and economic influences on what and how we eat. While these broad explanations are generally accepted to be true, there is much controversy in the best means of both preventing and treating overweight and obesity, particularly concerning diet. There are currently a myriad of
dietary approaches to losing weight, as witnessed by the sheer number of diet books and regimens. These include low-fat, high or low-carbohydrate, high-protein, high-monounsaturated fat, high-fibre and low-glycaemic-index diets, or any combination of these. The conventional approach, recommended by almost all health authorities around the world, has been to reduce the total amount of fat in the diet and replace with carbohydrates.

1.2 Low Fat Diets

Reducing fat intake has been the primary focus of dietary prevention and treatment of overweight and obesity for more than 20 years. The basis of this advice is simple – namely that because fat has more than double the energy per gram than carbohydrate or protein, a reduction in fat intake will result in a reduced energy intake. Secondly, dietary fat is efficiently stored as body fat and may also lower energy expenditure, via a lower thermogenic effect compared with that of carbohydrate or protein. Finally, high-fat foods are relatively less satiating than isoenergetic portions of high-carbohydrate or high-protein foods (2, 3) and fat added to a meal does not seem to affect subsequent appetite measures or food intake (4).

Epidemiological evidence has largely supported the low fat approach. Among populations that differ in eating habits, those with a higher consumption of total fat usually have a higher prevalence of obesity (5, 6) and the percentage of total energy from fat was positively related (and percentage energy from carbohydrate was inversely related) to adiposity, even after controlling for potential confounders, in US children (7). A high fat diet combined with inactivity may be particularly obesigenic
(8). However, others have argued that the epidemiological evidence is poor and since health authorities have actively promoted low fat eating, fat intakes have declined, while rates of overweight and obesity continue to climb (9). Whether this is due to a failure of lower fat diets to prevent weight gain, or that since obesity is multifactorial, any benefit of consuming less fat has been overshadowed by other negative changes, such as a decrease in physical activity, is impossible to say.

Four meta-analyses of controlled intervention trials comparing low-fat diets with normal-fat diets under *ad libitum* conditions, consistently show that reduction of dietary fat without restriction of total energy intake, produces weight loss of around 3-4kg (6, 10-12). While this is clinically significant and known to improve health outcomes, this degree of weight loss is unlikely to satisfy an overweight dieter or have a major impact on national obesity rates. Some have argued that this is an underestimate of what can be achieved with a low-fat diet, simply due to poor adherence, particularly in a free-living situation where subjects are frequently exposed to high-fat foods (13). One long-term trial provides some evidence to support this view. Swinburn *et al.* (14) stratified subjects according to compliance and found that the range of weight loss ranged from 1kg in the poorly compliant group to about 6kg in the most compliant group over one year, with a mean loss of 3.3kg. Nevertheless, in all groups weight regain occurred as soon as the intervention phase was complete, such that after five years there was no difference to the control group. Such relapse is common, regardless of the means of weight loss, and demonstrates the need for ongoing support and reinforcement. However it could also be indicative of the fact that a low-fat diet may be difficult or unpalatable to follow in the long-term. Others have suggested that giving low-fat advice is not enough and
for effective weight loss some form of energy-restriction must also be in place. Schlundt et al. (15) reported significantly better weight and body fat loss in men and women after following a low-fat, energy restricted diet compared to those instructed to follow a low-fat ad libitum diet. However, again these differences were no longer significant at a 12-month follow-up.

There have been other dietary changes that have coincided with the apparent decline in fat intake that may also affect rates of overweight and obesity. In the 20 years or so since low fat eating has been recommended, the types of foods commonly consumed has changed dramatically. The food industry responded to the demand for lower fat foods, and a plethora of packaged foods labelled “low-fat” or “fat-free” is now widely available. Diets based on these foods are not the same as low-fat traditional diets of populations shown to have a low level of obesity. In particular many of these modern foods, while being low in fat, are not low in energy. The fat is replaced with sugars, syrups and manufactured fat-replacers which increase the energy density considerably. It may simply be therefore that the advice to lower fat intake, without concomitant advice as to which carbohydrate-rich foods to consume instead, has led to a reduction in fat with very little reduction in energy intake. Data from the US shows that carbohydrate intake declined by about 25% from 1909 to 1963, largely due to decreased consumption of wholegrain products. At the same time dietary fibre intakes decreased by around 40%. Since 1963 carbohydrate intake has steadily increased, and by the late 1990s was back to early 20th century levels. However fibre intakes have remained low (16). This is indicative of the change in most Western countries and reflects a higher consumption of refined carbohydrates,
with low intakes of wholegrains and their products. This has also meant that the glycaemic impact of today’s diet is far greater than in the past.

1.3 The Role of Carbohydrates

Carbohydrates have generally been promoted in place of fat. In theory this is a logical approach to lowering the energy density of the diet; carbohydrate provides 17kJ/g compared to 37kJ/g for fat. In addition, it is known that dietary carbohydrate promotes its own oxidation (17-19) and that under normal conditions de novo lipogenesis from carbohydrates does not occur to any great extent in humans (20-22). Carbohydrates should therefore contribute little to body fat stores. Certainly epidemiological research does not support the idea that carbohydrates contribute to excess body fat. For example, in a study of almost 3000 Europeans a higher carbohydrate intake was a significant independent predictor for lower BMI and waist circumference in both men and women (23).

However, more recent studies have shown that a habitual high-carbohydrate diet induces the enzymes involved in the de novo lipogenesis pathway. One such study demonstrated that massive carbohydrate overfeeding (2.5 x energy expenditure) in healthy males resulted in a net fat synthesis of 170g/day of which 98% took place in adipose tissue (24). Another showed that following a high-carbohydrate/low-fat meal, overweight hyperinsulinaemic men had a lower fat oxidation and a higher fractional hepatic fat synthesis than did lean men (25). Taken together such studies indicate that chronically overeating carbohydrates beyond energy requirements,
alters metabolism in such a way as to allow for greater fat storage via carbohydrate conversion pathways. Furthermore this may be more pronounced in those who are overweight. Whether this is as a result of being overweight, or an individual factor that predisposes to overweight is not currently known.

Nevertheless such massive overfeeding with carbohydrate is unlikely to occur in diets based on traditional high-carbohydrate foods. In fact there is much evidence that diets high in carbohydrate may prevent weight gain. Stubbs et al. (26) assessed the ad libitum food intake of healthy men living for seven days in a large respiration chamber offered one of three diets ranging from low fat (20%E)/high carbohydrate (67%E) to high fat (60%E)/low carbohydrate (27%E). Only when following the highest carbohydrate diet did the men achieve a negative energy balance, while on both moderate and high fat diets the men were in positive energy balance. The same diets were then tested on free-living men over two weeks to investigate any possible influence of increased physical activity. While the energy expenditure was significantly greater in the free-living situation, the energy intake on each diet was almost identical to before, with the greatest energy intake being on the high fat/low carbohydrate diet. Furthermore the men gained weight only on this diet, while they lost the most weight on the low fat/high carbohydrate diet (27). This effect is thought to be due to the bulkiness of a high-carbohydrate diet, making it difficult to overeat. However the same cannot be said of a high-carbohydrate diet based on modern processed foods with a higher energy density. The high consumption of such foods may be reducing the efficiency and impact of low fat/high carbohydrate dietary recommendations on rates of overweight and obesity.
Much debate has centred on the type of carbohydrate consumed. Conventional advice has been to reduce the intake of simple sugars while increasing the intake of complex carbohydrates, yet this advice was based more on ‘common-sense’ than sound research evidence and has become a controversial issue. A major randomised controlled multi-centre trial in Europe, the CARMEN study (28), investigated the long-term effects of simple versus complex carbohydrates as part of a low-fat diet. Moderately obese adults (n=398) were assigned for six months to a seasonal control group with no intervention, a diet control group following a diet typical of the average national intake, a low-fat high simple carbohydrate group, or a low-fat high complex carbohydrate group. Both seasonal and diet control groups gained weight over the intervention period, while both low-fat groups lost weight and body fat. The losses were modest but significant, 0.9kg and 1.8kg in the simple and complex carbohydrate groups respectively, with the difference between the two not significant. Neither were there differences in blood lipid change between any of the diet intervention groups, with no adverse effects noted. In other words this study indicated that eating sugar rather than starch had no deleterious effects in terms of weight loss or on blood lipids. A Scottish study similarly found no deleterious effect of sugar on weight control and in fact found that those with the highest sugar intake had the lowest level of obesity, while high fat intakes were positively associated with obesity (29). Vasilaras et al. (30) investigated the effects of a six months ad libitum intake of a diet rich in simple or complex carbohydrates on energy expenditure (EE) and substrate oxidation. They found no effect of diet on EE, but substrate oxidation adjusted to closely reflect the diet composition, with no differences between the simple or complex carbohydrate groups. A study from the same research group (31) reported a decreased energy intake and body weight in both post-obese and normal-
weight women, after 14 days of a high-starch diet compared to either a high-sucrose or high-fat diet. They later compared the effects of these diets on diurnal metabolic profiles in normal-weight never-obese and post-obese women (32). Again there were differences between the sugar and starch diets. Compared to the starch-rich diet, the sucrose-rich diet significantly reduced the areas under the curve (AUCs) for glucose and free fatty acids, but showed a greater triacylglycerol AUC.

These seemingly conflicting findings may be due to the classification of carbohydrates as simple or complex. Simple sugars include both foods naturally high in simple sugars, such as fruits and milk, as well as commercially produced products with added refined sugars, including confectionary, sugar-sweetened yoghurts, and soft drinks and so on. While complex carbohydrates include all breads, rice, pasta, grains and starchy vegetables. It was assumed the latter would be more filling, be digested more slowly and therefore produce smaller rises in blood glucose than simple carbohydrates. This is not always the case and in terms of the physiological effect of carbohydrate-containing foods, the classification of simple or complex tells us little. We now know that many foods rich in complex carbohydrates in fact produce a far quicker and larger effect on blood glucose levels than many foods rich in simple sugars. This physiological discovery led to the development of the Glycaemic Index (GI).
1.4 The Glycaemic Index & Glycaemic Load

The GI is defined as the blood glucose response of a 50g carbohydrate portion of food, expressed as a percentage for the same amount of carbohydrate from a reference food (glucose now recommended for international standardisation, although white bread has been used in many studies) taken by the same subject (33). It was first proposed in 1981 as an alternative means of classifying carbohydrate-containing foods (34). While initially met with scepticism, particularly regarding its practical application, there is now growing evidence for a role for using the GI in preventing and treating diabetes, reducing the risk of cardiovascular disease and certain cancers, and in weight control.

In 1997 the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organisation (WHO) convened an expert consultation on carbohydrates in human nutrition, involving scientists from thirteen countries. The subsequent FAO/WHO recommendations passed to member countries stated that the GI should be employed as a useful indicator of the impact of foods on blood glucose, but that it should be used to compare foods of similar macronutrient composition within food groups and for clinical applications including diabetes and impaired glucose tolerance (35).

The absolute blood glucose response is affected by both the quality (the glycaemic index) and quantity of carbohydrate. The glycaemic load (GL), defined as the GI/100 x grams of carbohydrate, has therefore been proposed as a measure of the overall blood glucose- and insulin-raising potential of the diet. By definition the GL can be
reduced in two ways – either by lowering the GI or by reducing the total carbohydrate in the diet. Both methods will reduce postprandial glucose and insulin responses, but may well have different metabolic effects both acutely and in the long-term.

1.4.1 GI & weight control

Two features of low-GI foods are potentially beneficial for weight control: their satiating qualities and their ability to promote fat oxidation at the expense of carbohydrate oxidation. Both of these characteristics stem from the slower rates of digestion and absorption, and correspondingly lower glycaemic and insulinaemic postprandial responses of low-GI compared to high-GI foods.

Even when appearance and nutrient content are matched, low-GI foods typically induce greater satiety than their high-GI counterparts, and are followed by less energy intake at subsequent meals (36). For example, Geliebter et al. (37) found lunch intake was markedly reduced and hunger ratings significantly lower after a low-GI compared to high-GI cereal breakfast. Jimenez-Cruz et al. (38) showed a low- compared to high-GI lunch resulted in a higher satiety perception in people with type 2 diabetes. Agus et al. (39) reported a greater energy intake from snacks on days 7 and 8, following 6 days of an energy-restricted high-GI compared to low-GI diet. Warren et al. (40) found that low-GI foods eaten at breakfast had a significant impact on food intake at lunch in normal and overweight children. Previous research from our own research unit showed that progressive refining of carbohydrate-rich foods, such as wheat grains, resulted in a step-by-step increase in the food’s GI rating and a
reciprocal decrease in satiety (41). Similarly, mixed meals with low-GI values were found to induce greater secretion of cholecystokinin, a gut peptide associated with satiation, and more fullness over a 180-minute period (42). In these studies a 50% increase in meal GI translated to a 50% decrease in satiety. And in a review of the glycaemic index, Ludwig (36), reports on 16 studies with all but one demonstrating increased satiety, delayed return of hunger and/or decreased ad libitum food intake following the consumption of low compared to high GI foods. This seems to be maintained at least over the short-term; overweight men were found to spontaneously reduce their intake by 25% when fed an ad libitum combined low-GI and high-protein, low-fat diet (43).

It has been suggested that the GI influences subsequent food intake by altering the availability of fuel sources in the postprandial period. Following a high-GI meal, relatively high plasma insulin, along with low plasma glucagon, stimulates the uptake of glucose into muscle and liver cells, while simultaneously suppressing lipolysis. Consequently circulating levels of the two major metabolic fuels, glucose and fatty acids, decline rapidly. This in turn leads to a rise in counter-regulatory hormones which stimulate hunger and promote further eating (44).

Differences in GI dictate differences in fuel partitioning and oxidation. In a recent UK study, Stevenson et al. (45) measured substrate metabolism in a 3-hour rest period following breakfast and lunch of either low- or high-GI. They confirmed that glucose and insulin responses following both meals were significantly lower, and the total amount of fat oxidised during the 3-hour rest period was significantly higher in the low-GI compared to the high-GI trial. The same research unit previously reported
significantly higher fat oxidation during exercise, following a low-GI compared to high-GI breakfast (46) and in a more recent study, a low-GI breakfast contributed less carbohydrate to glycogen stores than a high-GI, but there was better preservation of glycogen during subsequent exercise, most likely as a result of higher fat oxidation (47). This has important implications for athletes aiming for maximal performance, but also for the recreational exerciser aiming for weight control.

Physiologically these results can be explained; high-GI meals produce larger glycaemic and insulinaemic responses, which in turn produce a greater increase in carbohydrate oxidation via rapid activation of key rate-limiting enzymes. Meanwhile intermediates of glucose oxidation, such as malonyl-CoA, strongly inhibit fatty acid transport into mitochondria, resulting in decreased fatty acid oxidation (48). Longer exposure to chronic hyperglycaemia and hyperinsulinaemia results in decreased expression of the rate-limiting enzymes and alters the potential for fat oxidation. A reduced capacity to oxidise fatty acids has been found in obese insulin-resistant humans (49) and obesity-prone rats (50), and several prospective studies have linked lower rates of fat oxidation with greater weight gain (51, 52). It has further been suggested that the counter-regulatory hormone response following a high-GI meal may also have a proteolytic effect and increase the loss of lean body mass over time. This in turn may affect resting energy expenditure (REE). Agus et al. (39) reported less of a fall in REE during weight loss, with a low- compared to a high-GI diet; and recently Pereira et al. (53) showed a blunted fall in REE after 10% weight loss was achieved following a low-GL as opposed to low-fat diet, despite similar changes in body composition.
The effects of GI on metabolism and body composition can more easily be quantified in animal models, where the GI of the diet can be tightly controlled with no change in other dietary factors. After 18 weeks rats fed a high-GI diet, compared to those fed a low-GI diet, had approximately 40% more body fat and almost 10% less lean mass, despite similar body weight; and mice on a high-GI diet had almost twice the body fat of those on a low-GI diet after only 9 weeks (54). Earlier studies found a high-GI diet stimulated fatty acid synthase and lipogenesis in normal and, to a lesser extent, diabetic rats (55); while a low-GI diet decreased glucose incorporation into lipids and adipocyte diameter in both normal and diabetic rats (56).

Epidemiologic studies provide some evidence that the glycaemic effect of the diet might influence body fat control in humans. A recent observational study of 572 healthy American adults, body mass index (BMI) was found to be positively associated with GI, but not with carbohydrate intake (grams or % energy), or GL (57). In the EURODIAB Complications Study of nearly 3000 adults with type 1 diabetes, a lower GI of the diet predicted lower waist-to-hip ratio and waist circumference in men, independent of carbohydrate, fat and fibre (23).

The evidence from long-term clinical studies is building with several studies published in the last few years. Spieth et al. (58) compared the effects of a low-GI diet with a energy-restricted, low-fat diet in the management of paediatric obesity. Their results showed significantly more patients in the low-GI group achieved a decrease in BMI of at least 3kg/m² in just over four months. Similarly Ebbeling et al. (59) showed that an ad libitum, reduced-GL diet was more effective over 12 months at reducing both BMI and body fat in obese adolescents than a conventional energy-
restricted, low-fat diet. A recent study in Austrian obese adults advised to follow a high-carbohydrate, low-GI diet for 24 weeks, reported excellent weight loss with significant reduction in body fat and only small losses in lean mass, and very good adherence to the diet (60). While the diet was not directly compared to any other, the mean weight loss reported (8.9kg) after 24 weeks was exactly that of the high-protein group in Skov et al’s study (61) with similar fat mass reduction. The latter reported a mean loss of only 5.1kg in a high-carbohydrate group. Finally, Slabber et al. (62) showed that a diet designed to elicit a low-insulin response (including low-GI foods) produced significantly better weight loss than a conventional energy-restricted diet over 12 weeks in obese hyperinsulinaemic women. While not designed as a strictly low-GI diet, such results suggest that lowering postprandial insulin is important to promote better fat oxidation.

Short-term studies have shown an effect of GI on weight or body composition. A five-week low-GI, compared to a high-GI diet, resulted in a significant decrease of ~700g in body fat and a tendency to increase lean mass without any change in body weight in healthy men (63). In a cross-over study comparing the effects of four weight-maintenance diets (high-fat, low-GI, high-sucrose and high-GI) over 24 days, there was significant incidental weight loss on the low-GI, but no other diet (64).

However not all studies have shown improved weight loss with a low-GI diet. Ebbeling et al. (65) compared a low-GI diet with a conventional energy- and fat-restricted diet over 12 months (six months intensive intervention, six months follow-up) in obese young adults. Body weight in both groups had decreased significantly at six months (-8.4% and 7.8% respectively) and weight loss was reasonably well
maintained at 12 months with no significant differences between the groups at either
time point. However there were differences in other cardiovascular risk factors
favouring the low-GI diet. Similarly while there were benefits of a low-GI, compared
to high-GI diet in reducing risk factors for ischaemic heart disease, there was no
significant improvement in weight loss or body composition found in a ten-week
study of healthy, overweight women (66). In a comparison of three reduced-energy
diets varying in GI and GL (high-GI/high-GL, low-GI/low-GL and high-fat/low-
GL/high-GI), weight loss was found to be independent of diet composition (67). In
the latter study all food was provided and required to be consumed for the first 12
weeks, and thereafter subjects were advised to maintain their assigned diet while
preparing their own meals. During the first 12-weeks there was no effect of diet
composition on weight loss, although any effect of GI on satiety and subsequent food
intake would be lost under these conditions. In addition, body composition was
estimated using a four-site skin-fold measurement, which may not be sufficiently
accurate to detect changes in obese subjects. Furthermore, the calculated GIs and
GLs of the subjects’ diets during the free-living phase were similar across all groups,
despite specific dietary advice to the contrary. For these reasons, differences in body
weight and composition would be unlikely to occur.

1.4.2 GI & cardiovascular disease

In the Nurses’ Health Study (68) of approximately 75,000 women in the US, GL was
directly associated with risk of coronary heart disease (CHD) after adjustment for
known risk factors. The relative risk in the highest compared to lowest quintile of GL
was almost double. Furthermore classifying carbohydrates by their GI, as opposed to
the conventional simple-complex classification, was shown to be a better predictor of CHD risk. These associations were even stronger for women with a BMI ≥23.

GI and GL may increase the risk of CHD in a number of ways. While hyperglycaemia has long been known to increase the risk of cardiovascular disease in people with diabetes, the effect of blood glucose levels in healthy people without diabetes went unrecognised until recently. A meta-analysis of prospective studies found that post-challenge blood glucose level had a linear relationship with cardiovascular disease risk in the non-diabetic range, and the risk was higher in women (69). Moreover in both men and women, impaired post-challenge glucose levels were found to be more predictive of total and cardiovascular mortality than impaired fasting glycaemia (70).

Dietary GI and GL may also influence the plasma lipid profile. An inverse relationship was found between GI or GL and HDL-cholesterol in a US study of almost 14,000 adults, regardless of sex or BMI (71); in women in the Nurses’ Health Study (72); and in Europeans with type 1 diabetes (73). GI was the only dietary variable significantly related to HDL-cholesterol in a study of middle-aged British adults (74). While high carbohydrate diets have been associated with higher triacylglycerides (TAGs), the evidence suggests that not all carbohydrates act in the same way. The findings from the Nurses’ Health Study showed a strong positive association between GL and TAGs, with both GI and carbohydrate contributing independently to the association which was strengthened in women with high BMI (75). In a 12 month dietary intervention trial in obese young adults, a low-GI diet was also shown to be more effective at lowering TAGs than a conventional treatment.
restricted in both energy and fat (65) and after achievement of 10% weight loss (53). A short term (six days) study showed that a low-GI-low-fat-high protein diet, compared to phase 1 of the American Heart Association (AHA) diet, reduced TAGs by 35%, increased LDL peak diameter, and resulted in lower insulin in the fasting state, over the day and in response to a glucose challenge (43).

Not all studies have found such relationships. The Zutphen Elderly Study of elderly men in the Netherlands found no relationship between GI and risk of CHD, nor with blood levels of cholesterol, HDL-cholesterol, TAGs, insulin or glucose (76). And in an Italian case-control study of non-diabetic subjects with a first episode on non-fatal acute myocardial infarction, GL was not associated with increased risk, while GI was only associated with increased risk in those aged ≥ 60 years and in those who were overweight (77).

GI may affect CVD risk via effects on insulin sensitivity. Low-GI diets have been shown to improve insulin sensitivity in subjects with CHD (78, 79), diabetes (80) and obesity (62); while a high-GI diet has been shown to increase postprandial insulin resistance (64). However, in lean young men the rate of glucose infusion during a euglycaemic-hyperinsulinaemic clamp was significantly higher with a high-GI compared to low-GI diet only at the highest insulin infusion rate, while there was little or no difference at lower rates. Furthermore the study found that these differences diminished over 30 days suggesting that there may be some adaptation to a low-GI diet (81).
A high-GI diet may increase the risk of cardiovascular disease by exacerbating the pro-inflammatory process. High sensitivity C-reactive protein (hs-CRP) is a sensitive marker for systemic inflammation and has been shown in several large scale prospective studies to be related to an increased risk of ischaemic heart disease. In the Women’s Health Study dietary GL was found to be significantly and positively associated with plasma hs-CRP, independent of conventional risk factors. The association was stronger in overweight women with a BMI ≥25 (82). While in an intervention study hs-CRP levels improved more after 10% weight loss on a low-GL (and low-GI) diet, compared to low-fat diet (53).

1.4.3 GI & diabetes

It has long been postulated that a high, chronic intake of rapidly absorbed (i.e. high-GI) carbohydrates may increase the risk of developing type 2 diabetes. This could occur through metabolic changes which increase insulin resistance, and/or though increased insulin demand, ultimately leading to beta-cell decompensation (83). Several lines of evidence have lent considerable support to this hypothesis, including large-scale prospective studies.

GI was positively associated, while carbohydrate intake was inversely associated, with the incidence of diabetes in the Melbourne Collaborative Cohort Study of 36,787 men and women (84). Interestingly magnesium intake was also inversely related to the incidence of diabetes and a major source of magnesium is wholegrains, including wholegrain bread. Foods rich in magnesium are often both high fibre and low-GI. These results are in accordance with findings from the Nurses’ Health Study
II which reported a strong association between increasing GI (but not GL) and risk of diabetes. This association was more evident in those with a low cereal fibre intake (85). Similarly an ecologic study showed that increasing intakes of refined carbohydrates, alongside falling intakes of fibre, have paralleled the epidemic of type 2 diabetes in the US (16). The Framingham Offspring Study found dietary GI (and again not GL) was positively associated with insulin resistance, as measured by HOMA-IR, and the prevalence of metabolic syndrome, while an inverse relationship was found for whole grain intake (86). Finally, in men GI and/or GL were associated with several predictors of type 2 diabetes (including fasting and 2-hour glucose, fasting insulin, HbA1C, and visceral abdominal fat) in the Health, Aging and Body Composition Study (87).

Two studies, however, do not support the hypothesis that a high-GI diet leads to diabetes. In the Iowa Women’s Health Study (88) of 35,988 older women, diabetes risk was unrelated to GI or GL, nor to total carbohydrate intake, refined grains, fruit and vegetables, or soluble fibre. There were, however, strong inverse associations between incidence of diabetes and intakes of total grains, whole grains, dietary fibre, cereal fibre, and dietary magnesium. Similarly the Atherosclerosis Risk in Communities Study (89) of 12,251 American adults, found no statistically significant associations of GI or GL with incident diabetes. The data did support a protective role for cereal fibre in the development of diabetes in whites, but not in African-Americans.

There is mounting evidence to support the use of low-GI diets in the management of diabetes. In the short-term a low-GI diet was shown to significantly improve 24-hour
blood glucose profiles in adults with type 2 diabetes (90) and in a six-week crossover study, a low-GI compared to high-GI diet was shown to improve HbA1c in obese subjects with type 2 diabetes (91). Over the longer term the EURODIAB IDDM Complications Study showed that a lower GI was associated with lower HbA1c concentrations, independent of fibre intake (73), and a 2003 meta-analysis of randomized controlled trials showed that low-GI diets reduced glycated proteins 7.4% more than high-GI diets (92). Although this may seem a modest improvement, the benefit is similar to that offered by pharmacological agents designed to lower postprandial glycaemia, and may have advantages in the long-term. Finally, a low-GI diet was shown to increase the fall in LDL-cholesterol, and show a two-fold greater (although not statistically significant) improvement in glycated haemoglobin, compared to a high-GI diet during weight loss in subjects with type 2 diabetes (93).

Low-GI diets have further shown to be useful in the management of impaired glucose tolerance (IGT). Wolever & Mehling (94) found that reducing dietary GL either through consumption of low-GI foods, or through lowering total carbohydrate intake, over four months reduced postprandial glucose concentrations to a similar extent. These two diets did however have different effects on postprandial insulin, TAGs and free fatty acids. This is consistent with other studies showing that reduced-GI and GL diets may both reduce postprandial glycaemia, but have varying metabolic effects (95). The same group previously showed that a high-carbohydrate-low-GI diet improved beta-cell function in subjects with IGT significantly more than either a low-carbohydrate-high-mono unsaturated-fat diet, or a high-carbohydrate-high-GI diet (96).
1.4.4 Dietary Fibre

A diet high in fibre appears to play an important role in maintaining a healthy weight and avoiding age-related weight gain. In a prospective study over 10 years, Ludwig et al. (97) found that fat intake did not affect weight gain in young adults, but weight gain was less in those with a higher fibre intake. Fibre may also play a role in the development of insulin resistance. The Inter99 Study (98) in Denmark reported an inverse relationship between dietary fibre and insulin resistance, but no such association was found with GI or GL. Furthermore a high carbohydrate diet, including simple sugars, was not found to be associated with the development of insulin resistance. In the CARDIA study of young adults, low fibre consumption (GI was not assessed) predicted higher 10-year weight gain, waist-to-hip ratio, and 2-hour post-glucose insulin levels (a measure of insulin resistance) to a greater extent than did total or saturated fat (97).

Although fibre and GI are not precisely related, viscous dietary fibres and foods in which the natural cell wall architecture remains intact (e.g. legumes) are associated with lower GI scores (99). Given that the GI has not yet been correctly measured for the majority of foods, particularly outside of Australia, this may be one reason for the discrepancy in studies. At least part of the effect of high fibre diets may be due to their ability to slow carbohydrate absorption and lower postprandial glycaemia. The methods used in most of the large scale epidemiological studies have not been designed to assess the GI, but more accurately estimate fibre consumption.
1.5 High Protein Diets

High protein diets have been advocated for weight loss in the popular press since the 1960s, but have recently soared in popularity with the media and public, in part due to the apparent failure of low-fat diets to curb overweight and obesity trends. They were largely ignored by the scientific community until the publication of a number of trials over the last few years, showing greater weight and/or fat loss compared to conventional approaches. Skov’s study (61) of overweight Danish men and women was one of the first, and reported substantially better weight loss after 6 months following an ad libitum low-fat diet high in protein, compared to a conventional low-fat, high-carbohydrate diet (8.9kg vs 5.1kg respectively). A subsequent follow-up paper reported that at 12 months there was a far greater dropout of those on the high carbohydrate diet, and while the differences in weight loss were no longer significant, more of the high protein group lost >10kg with 10% greater reduction in intra-abdominal adipose tissue (100). Several short to medium-term studies have similarly reported better weight or fat loss with a higher protein diet (101-103) and one recently published study suggests that the combination of increased protein along with exercise may be additive in improving body composition during weight loss (104).

There have now been four major published trials in the US showing better weight loss with low-carbohydrate/high protein diets compared to conventional low-fat diets over the longer-term. Brehm et al. (105) found that obese women lost two times more weight and body fat over six months on a low-carb diet than on a restricted energy, reduced-fat plan. Two other studies published in the same year also reported
better weight loss over six months with a low-carb diet; in obese men and women (106) and in severely obese women with a high incidence of diabetes and metabolic syndrome (107). Interestingly both of these studies were followed up at 12 months and both reported no significant difference between the groups by this time point (106, 108). This was due for the most part to the low-carb group re-gaining some of the lost weight over the second 6-month period. Finally, Yancy et al. (109) reported better weight and fat loss, better muscle retention, a greater decrease in triacylglycerides and increase in HDL-cholesterol in overweight, hyperlipidaemic volunteers following a high protein, low-carbohydrate diet (with nutrition supplements) compared to those on a low-fat, reduced-calorie diet followed for 24 weeks.

However results have not been consistent. In a review of high protein diets, only seven out of fifteen studies reported significantly greater weight loss with a higher protein diet (110). There is some suggestion that a high protein diet may increase body fat loss, even where there is no difference in weight. In the same review ten studies which looked at body composition were identified, with most reporting improvements in body composition with the high protein diet, but only three of these reached statistical significance. Three Australian studies from the CSIRO failed to find that increasing the ratio of protein to carbohydrate improved weight or fat loss in patients with type 2 diabetes (111), obese, hyperinsulinaemic adults (112), or obese women (113). The latter two studies did report beneficial metabolic changes in the high protein groups. Alford et al. (114) found no difference in body composition change in overweight women following hypo-caloric diets with 25%, 45% or 75% energy from carbohydrate. Landers et al. (115) reported no significant differences in
weight or body composition change in obese adults following the Atkins, Zone or conventional diets (<10%, 40% and 50% energy from carbohydrate respectively). Dansinger et al. (116) reported that weight loss was associated with self-reported dietary adherence and not with diet type in subjects randomly assigned to one of four popular diets varying in macronutrient content (Atkins, Zone, Weight Watchers and Ornish). In a systematic review of low carbohydrate diets, including 107 articles and involving more than 3000 participants, weight loss was not associated with reduced carbohydrate content. Weight loss was associated with diet duration and restriction of energy intake, and interestingly as the intervention period increased, there were few diets with a low carbohydrate content (117). Perhaps this suggests that low carbohydrate diets are difficult to adhere to in the long term. Finally vegetarians, with a diet inevitably higher in carbohydrate with modest amounts of protein, tend to have a lower BMI compared to non-vegetarians (118).

There is some evidence to suggest that high protein, reduced-carbohydrate diets may be more efficacious in subjects with features of the metabolic syndrome. Hwalla Baba et al. (102) reported better weight loss (although greater water loss) in obese hyperinsulinaemic subjects following a high protein, compared to high carbohydrate hypo-energetic diet. Although fasting insulin decreased in both groups, only in the high protein group did it reach the normal range. The same group of investigators also showed better weight loss with a high protein diet in hyper- compared to normo-insulinaemic obese men (119). In the CSIRO study (113) there were no significant differences in weight loss or body composition change in the subject group as a whole, but a sub-analysis showed that the obese women with high baseline
triacylglycerides lost significantly more body fat on the high-protein versus high-carbohydrate diet.

Several mechanisms may explain why high protein diets may be better than high carbohydrate diets in improving body composition and weight loss. A number of studies, but not all (111), have shown a smaller fall in REE during weight loss with a higher protein diet (102). This may be due to the alleged ability of a high protein diet to spare lean muscle tissue during weight loss (112, 120-122). Protein may also increase total energy expenditure via increased thermogenesis. Mikkelsen et al. (123) showed that 24-hour energy expenditure, measured in a respiratory chamber, was 3% higher in overweight men with the substitution of carbohydrate with 17-18% of energy as protein. Furthermore, animal protein (pork) produced a higher energy expenditure than plant protein (soy). Johnston et al. (124) showed a two-fold increase in postprandial thermogenesis on a high-protein versus high-carbohydrate diet. In a review of high protein diets, fifteen studies showed a higher thermogenic effect with a high protein intake (110). Protein is also considered to be the most satiating macronutrient. In the same review, out of fourteen studies to examine the satiating effect of protein compared to one other macronutrient, eleven reported a higher subjective satiety rating following a protein preload. Longer term at least one study has reported better satiety ratings in subjects following high protein diets for weight loss (125). Finally high-protein diets may improve fat oxidation and reduce fat storage by reducing insulin demand. Layman et al. (126) showed that a high-protein, compared to a high-carbohydrate, diet stabilises blood glucose responses across the course of the day and reduces the postprandial insulin response.
Part 1

Introduction & Literature Review

A different line of evidence for increased protein diets comes from the diet of past and present hunter-gatherer societies, which has been proposed as a good model for the optimum diet for humans. Ethnographic and anthropologic studies have showed that animal foods dominate their diets, providing some 19%-35% of the total dietary energy as protein, whereas carbohydrate in the diet, estimated to contribute 22%-40% of the dietary energy, came from wild plant foods, including large quantities of fruits, vegetables, and legumes (127). Others have argued that the intake of plant foods may have been greater than suggested because plant foods leave less trace in the archaeological record than animal foods (128). Nevertheless, whatever the exact contributions, both the animal and plant foods consumed were rich in vital nutrients but had a low energy density. As such they may have contributed to the low incidence of cardiovascular disease and other so-called “diseases of civilisation” in these societies. Interestingly these foods would also have had predominately low GI scores, and the diet overall would have had a low GL compared to modern Western diets (129).

While many of the popular high protein diets have used this argument to support their dietary approach, there are some key differences between the two. The diets of hunter-gatherers had lower levels of saturated fatty acids (SFA) and higher intakes of n-3 polyunsaturated fatty acids (PUFA) (130). Primarily this is due to differences in the fat profile of wild versus domesticated meat - modern domesticated meats tend to have more saturated fat and less n-3 PUFA – but cuts of meat and meat products that are popular today, and promoted in many high protein diet plans, are particularly high in total and saturated fat e.g. sausages, burgers, salami and bacon. In addition hunter-gatherer diets had far greater intakes of antioxidants, fibre and other
micronutrients including vitamin C, than modern diets due to their large intake of wild plant foods. This is contrary to the high protein modern diet plans which tend to severely limit plant food intake in order to reduce dietary carbohydrate. Finally, there are important differences in energy and nutrient requirements between hunter-gatherer and modern man. The former is estimated to have been active for more than eight hours a day compared to the sedentary lifestyle typical of today (128).

Nevertheless, despite these differences there is sound evidence that humans are genetically designed to eat animal foods. We have a relatively high requirement for the minerals iron and zinc, with the richest and most readily absorbed sources being animal foods; we have a small but significant requirement for vitamin B₁₂ found only in animal foods; we have a limited ability to synthesize the amino acid taurine found only in animal foods; while we can synthesize vitamin A from beta-carotene, we have a finite ability to do so, and can only obtain pre-formed vitamin A from animal foods; and finally we have an inefficient ability to chain elongate and desaturate 18 carbon fatty acids to their long chain counterparts (such as the long chain n-3 fats known to be important for health, brain development and function) found only in animal foods (130).

In seeming contradiction however, health recommendations have in general advised the public to consume less meat and there is a general perception that red meat is less healthy than other protein sources. This advice has been borne from epidemiological evidence associating meat consumption with cancers of the breast, prostate and the bowel (131). The strongest line of evidence is for red and processed meat consumption and the risk of colon, particularly colorectal, cancer. This was initially
shown in the Nurses’ Health Study (132) and has subsequently been supported by several studies as reviewed in two meta-analyses (133, 134) and in a recent report from the European Prospective Investigation into Cancer and Nutrition (135). The latter also reported an inverse association with fish intake. However these associations are complicated by the Western diet and lifestyle pattern e.g. those who eat more meat also tend to eat fewer vegetables, are less active, have higher BMIs and consume less fibre, all factors that have been associated with risk of colon cancer (136). Meat is also a major source of saturated fat in Western diets and as such has been targeted as a food to reduce in ‘heart health’ campaigns. However lean cuts of red meat are not major suppliers of saturated fats and diets high in lean meat have been shown to be effective in improving lipid profiles of hyperlipidaemic men (137).

It seems clear that man has evolved to eat meat, but it is the type of meat, along with other aspects of our diet (e.g. low fruit and vegetable consumption), that may have increased our risk of several chronic diseases including CVD and certain cancers. The challenge of modern nutrition science is to find way to marry the protective aspects of the diet of our evolutionary past, with today’s food supply, modern man’s nutritional requirements and the practical considerations of obtaining food in today’s industrialised world.

**1.5.1 Safety of high protein diets**

The Recommended Dietary Intakes (RDIs) for protein are designed to meet the physiological requirements for protein in healthy people. In Australia this is set at 0.75g protein per kilogram body weight for adults. This is easily met with modern
diets and data from the National Nutrition Survey shows that the mean daily intake for both men and women exceeds the RDI (112g and 76g respectively). With the popularity of high protein diets for weight loss, the concept of an optimal protein intake that may be higher than the RDI has been discussed, but concern has centred over what the upper limit on protein intake should be. The concerns regarding the safety of high protein diets are primarily on three major areas: heart, kidney and bone health.

1.5.1.1 Protein & heart health

General public health messages have advised a reduction in animal foods, particularly red meat, largely due to the fact that many of such foods are a rich source of saturated fat. The relationship between saturated fat and blood cholesterol profiles, and subsequently heart health, is well documented (138). For these reasons concerns have been raised that high protein diets, with an increased intake of animal foods, may increase the risk of CVD. Nonetheless, the vast majority of the high-protein trials mentioned above have reported an improvement in blood lipid profiles, at least during weight loss. In particular, high-protein diets seem to result in a better reduction in triacylglycerides (101, 106, 107, 109, 113, 139), while a major criticism of high carbohydrate, low-fat diets has been that they tend to raise triacylglycerides (140).

There is less known about the effect of a high protein diet in the long term or during weight maintenance. At least one study has demonstrated that high protein diets, at least in the form popularised by diet books, may be detrimental to those with existing
Part 1

Introduction & Literature Review

heart disease. Fleming (141) examined the effects of either conventional dietary advice or a popular high protein diet on patients with coronary artery disease (CAD). Those in the conventional group showed a reduction in each of the independent variables indicative of an improvement in the extent and severity of their CAD, while those in the high protein diet group demonstrated a worsening of risk factors and progression of CAD. However not all high protein diets are the same, and there is evidence that a higher protein diet, without concomitant increase in saturated fat and excessively low carbohydrate (as tends to be the case with the popular diet books), may improve cardiovascular health. A recently published randomized control trial, the OmniHeart study, showed that partial replacement of dietary carbohydrate with either protein or monounsaturated fat (with weight kept constant) resulted in lower blood pressure, improved lipid profiles and reduced cardiovascular risk in a group of adults with pre- or stage 1 hypertension (142). Similarly in a review of protein and cardiovascular health, Hu (143) reported that in general higher protein intakes have been associated with lower blood pressure, but ecological studies have revealed inconsistent results. Some suggest a positive association of coronary heart disease (CHD) and animal protein, but a negative association with plant protein consumption, including recent data from the Iowa Women’s Health Study (144). Yet data from the Nurses’ Health Study showed a lower relative risk of CHD in the highest quintile of protein intake compared the lowest (145).

Part of the inconsistency may come from confounding factors in studies not specifically designed to examine protein intake independently. For example countries with a higher protein intake also tend to have a higher total and saturated fat intake, combined with low fibre consumption. There are also major differences in the type
of animal foods eaten and in the contribution of other foods to total saturated fat intake. Meat and other animal products were not the major source of saturated fat in an Australian study - hidden fats in fast food, snack foods and other processed foods were the major source of saturated fat (146). The type of meat is also clearly important. Lean red meat, trimmed of visible fat, is not high in saturated fat and in a recent review (147) of 54 studies, lean red meat did not increase cardiovascular risk factors (plasma cholesterol levels or thrombotic risk factors).

1.5.1.2 Protein & bone health

Excessive dietary protein may contribute to demineralisation of bone and hence there is concern that high protein diets may increase the risk of osteoporosis. Dietary protein interacts with calcium metabolism and it is well documented that urinary calcium tends to increase with protein intake (148-151). The proposed mechanism is that the greater intake of sulphur-containing amino acids, increase renal acid load and results in a decreased re-absorption of calcium, to act as a buffer, from the renal tubules (152). However, whether this calcium is coming from resorption of bone or from increased calcium absorption is unclear. The epidemiological evidence linking high protein intakes to osteoporosis is not conclusive. Abelow et al. (153) examined cross-cultural variations in animal protein consumption and hip fracture incidence. Using data from 34 published studies in 16 countries, they reported a positive association in women between animal protein intake and fracture rates. Similarly, Sellmeyer et al. (154) found an increased rate of bone loss and risk of fracture in elderly women with a high ratio of animal to vegetable protein. Yet others have pointed out that contradictory to these results, women with the highest animal to
vegetable protein ratio also had the highest bone mineral densities (155). Similarly high protein intakes have been linked to improvements in bone mineral density where calcium intake is also high (156, 157).

The acidosis effect of a high protein intake appears to be counter-acted in part by dietary phosphorus, which increases along with animal protein and meat consumption. Phosphorus increases the renal re-absorption of calcium, thereby reducing calcium excretion in urine, regardless of calcium intake (158). A simultaneous high intake of fruits and vegetables may also be important. Barzel and Massey (159) propose that a typical Western diet containing a relatively high amount of animal protein with a low consumption of fruits and vegetables has a detrimental effect on calcium balance due to the increased renal acid load. They hypothesise that the ability to deal with the increased acid load of a high meat diet by providing a buffer from bone resorption, was a survival advantage in hunter-gatherer societies. However, overall these societies had a large intake of plant foods which would act as alkali-buffers, reducing urine acidity and subsequent calcium loss (129).

Finally, the total amount of protein is clearly important. Orwoll et al. (160) reported that only very high protein intakes induced hypercalcuria, and this effect was blunted if the protein is part of a mixed diet and is not found at more moderate increases in dietary protein.
1.5.1.3 Protein & renal health

It is well documented that high protein diets increase glomerular filtration rate (GFR), intraglomerular pressure and kidney size (158, 161, 162). There is also firm evidence that this is harmful in those with pre-existing kidney disorders, resulting in a progression of their disease. In such individuals, decreasing the protein content of the diet has been shown to slow progression of the disease and kidney function is maintained for longer, presumably by lowering the work load on each remaining nephron (162). What is not known is whether these effects are damaging to those with healthy kidneys. Some argue that the increase in GFR is merely a physiological indictor of protein intake and is not detrimental (163), whereas others raise the possibility that over a lifetime a high GFR may be related to the decline in renal function associated with age, by literally “wearing out” the kidneys (158). The available evidence does not support this argument. In a recent review paper, Eisenstein et al. (164) concluded that there was little evidence for adverse effects of high protein diets on renal function in individuals without established renal disease. However there is some evidence that a high protein intake can significantly increase the risk of kidney stones (165-167). Overall there seems little adverse risk of high protein diets in healthy individuals but they may be contraindicated for those susceptible to, or with existing kidney disease, including people with diabetes and a family history of kidney stones.
1.6 Study Aims & Hypotheses

Taking all of the above evidence together, reducing total and particularly saturated fat intakes are warranted, but the question to be answered is whether the efficacy of reduced-fat diets can be improved by reducing the GL of the diet. While initially low-GI and high-protein diets may seem diametrically opposed, they are in fact both reducing the dietary GL and trying to reduce post-prandial glycaemia and insulinaemia.

The principal aims of the present study therefore were to assess whether:

1. reducing the GL of reduced-fat diets can improve weight loss and body composition change;
2. there are differences between the two approaches to lower GL (by consuming low-GI foods, or by replacing some carbohydrate with protein) in weight loss, body composition change and cardiovascular disease risk factors;
3. there are further advantages to combining both approaches (i.e. low-GI and increased protein) to produce the lowest GL; and
4. to carry out a profile day assessment in order to confirm that our test diets did indeed result in the predicted lowering of glycaemic and insulinaemic responses.

Lean red meat was specifically chosen to provide the additional protein component of the two higher protein diets for a number of reasons. Principally to assess whether
an increase in red meat intake could improve the nutritional quality of diets designed for weight loss, particularly in increasing levels of iron and zinc which are often low in such diets, while simultaneously assessing the effect on cardiovascular risk factors such as blood lipids. This is important given that a current top selling diet book in Australia advocates high lean red meat consumption.

Specifically the hypotheses tested were:

1. that replacing high-GI foods in a conventional reduced-fat diet with their low-GI counterparts would increase weight and body fat loss in a group of overweight adults;
2. the partial replacement of dietary carbohydrate with protein in a reduced-fat diet would result in a similar improvement in weight and fat loss as the low-GI approach since both reduce dietary GL to the same degree;
3. the both reduced GL diets would result in similar improvements in cardiovascular disease risk factors;
4. that a diet with the lowest GL, combining both low-GI foods and high protein, would produce the greatest improvements in weight, body composition and cardiovascular risk factors; and
5. that in a 10-hour profile day, glycaemic and insulinaemic responses would be reduced in a step-wise fashion with decreasing GL.
Part 2: Methods

2.1 Subjects

Volunteers were recruited locally using noticeboards and newspaper advertisements. Young adults, 18 to 40 y, body mass index (BMI) ≥25 kg/m², body weight <150kg (weight limit of DEXA scanning machine), with weight fluctuation of <5kg in the previous two months and willing to eat red meat and maintain current physical activity, were recruited. Exclusion criteria were chronic illness, regular medication other than birth control, eating disorders, special diets, pregnancy, food allergy and insufficient English. In total 148 individuals were screened between July 2002 and July 2004, of whom 129 (98 women, 31 men) met the inclusion criteria. Of those excluded, two were taking medications with weight-related side effects, five were older than 40 y, three were non-red meat eaters, five had weight >150kg and four were accepted but withdrew before randomisation (Figure 1).

Subjects were stratified by weight and sex and then randomly assigned to one of the four diets, which resulted in four well-matched groups with no significant differences in baseline characteristics (Table 1).
Figure 1: Flow of participants through the trial.
Table 1: Baseline characteristics of the study participants in each diet group\textsuperscript{1}.

<table>
<thead>
<tr>
<th></th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>31.8 ± 1.7</td>
<td>30.5 ± 1.4</td>
<td>30.2 ± 1.5</td>
<td>34.6 ± 1.5</td>
<td>0.15</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>25 (78)</td>
<td>23 (72)</td>
<td>24 (75)</td>
<td>26 (79)</td>
<td>0.91</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.0 ± 1.9</td>
<td>87.1 ± 2.7</td>
<td>87.7 ± 2.9</td>
<td>88.4 ± 3.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>0.66</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>30.9 ± 0.6</td>
<td>30.6 ± 0.8</td>
<td>31.3 ± 0.8</td>
<td>32.1 ± 0.9</td>
<td>0.59</td>
</tr>
<tr>
<td>Dropouts</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>0.24</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.79 ± 0.19</td>
<td>4.71 ± 0.19</td>
<td>5.15 ± 0.18</td>
<td>4.83 ± 0.14</td>
<td>0.30</td>
</tr>
<tr>
<td>HDL-chol (mmol/L)</td>
<td>1.29 ± 0.07</td>
<td>1.17 ± 0.05</td>
<td>1.16 ± 0.05</td>
<td>1.36 ± 0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol: HDL ratio</td>
<td>3.94 ± 0.25</td>
<td>4.16 ± 0.24</td>
<td>4.75 ± 0.32</td>
<td>3.83 ± 0.26</td>
<td>0.08</td>
</tr>
<tr>
<td>LDL-chol (mmol/L)</td>
<td>2.87 ± 0.16</td>
<td>2.90 ± 0.14</td>
<td>3.33 ± 0.15</td>
<td>2.89 ± 0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.37 ± 0.15</td>
<td>1.39 ± 0.13</td>
<td>1.41 ± 0.13</td>
<td>1.25 ± 0.12</td>
<td>0.84</td>
</tr>
<tr>
<td>Free fatty acids (μmol/L)</td>
<td>510 ± 33</td>
<td>436 ± 32</td>
<td>545 ± 42</td>
<td>520 ± 53</td>
<td>0.26</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.04 ± 0.11</td>
<td>4.95 ± 0.07</td>
<td>4.92 ± 0.14</td>
<td>5.04 ± 0.09</td>
<td>0.78</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>79 ± 7</td>
<td>83 ± 10</td>
<td>101 ± 12</td>
<td>81 ± 8</td>
<td>0.32</td>
</tr>
<tr>
<td>HOMA1-IR\textsuperscript{2}</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>0.59</td>
</tr>
<tr>
<td>HOMA2-IR\textsuperscript{2}</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>0.46</td>
</tr>
<tr>
<td>HOMA2-IS\textsuperscript{3}</td>
<td>81 ± 8</td>
<td>85 ± 6</td>
<td>70 ± 6</td>
<td>82 ± 6</td>
<td>0.41</td>
</tr>
<tr>
<td>HOMA2-%B\textsuperscript{3}</td>
<td>123 ± 6</td>
<td>122 ± 8</td>
<td>164 ± 24\textsuperscript{4}</td>
<td>125 ± 10</td>
<td>0.10</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>26 ± 2</td>
<td>22 ± 2</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
<td>0.46</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.6 ± 0.8</td>
<td>4.3 ± 0.7</td>
<td>3.1 ± 0.6</td>
<td>4.3 ± 0.9</td>
<td>0.57</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Mean ± SEM. \textsuperscript{2}Homeostasis model assessment of insulin resistance 1 (original model) and 2 (computer model) (168) using fasting glucose (mmol/L) and insulin concentration (mU/mL) . \textsuperscript{3}HOMA2 insulin sensitivity (%) and β-cell function % (168). \textsuperscript{4}One subject was an outlier. Without this subject, baseline HOMA2-%B in the HP/HGI group was 142 ± 10.
2.2 Intervention Diets

All four diets were designed as reduced-energy, low fat (30% E), moderate fibre (30 g/day), eating plans with differences in the quantity and quality of available carbohydrate. DIET 1 was a high carbohydrate (55% E) and average protein (15% E) diet based on high GI foods (i.e. a ‘conventional’ low fat diet), including fibre-rich breakfast cereals and breads. DIET 2 had the same macronutrient proportions but was based on previously verified low GI foods (169). DIET 3 was a higher protein (25% E), carbohydrate-reduced (45% E) diet based on lean red meat (to supply the additional protein) and high GI foods. DIET 4 had the same macronutrient proportions as DIET 3 but specified low GI food choices (Figure 2). Target glycaemic load was highest in DIET 1 and lowest in DIET 4 (Table 2).

![DIETS 1 & 2](image1.png)  
![DIETS 3 & 4](image2.png)

Figure 2: Target macronutrient contributions in the high carbohydrate groups (DIETS 1 & 2) and the high protein groups (DIETS 3 & 4). CHO = carbohydrate.
Table 2: Target macronutrient energy distribution (% total energy), glycaemic index (GI) and glycaemic load (GL)\(^1,2\).

<table>
<thead>
<tr>
<th></th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO (%)</td>
<td>55</td>
<td>55</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>15</td>
<td>15</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>GI (^1)</td>
<td>HIGH (67)</td>
<td>LOW (40)</td>
<td>MOD-HIGH (57)</td>
<td>LOW (34)</td>
</tr>
<tr>
<td>GL(^1) (g)</td>
<td>HIGH (127)</td>
<td>LOW (75)</td>
<td>LOW (87)</td>
<td>LOWEST (54)</td>
</tr>
</tbody>
</table>

\(^1\) values calculated from dietary instruction sheets

2.3 Dietary Instructions

Subjects were instructed by a dietitian and given dietary instruction sheets specifying the number of serves they should eat from each food group (Appendix 1), with details as to the size of one serve. The number of serves in each food group was calculated to achieve the desired macronutrient distribution and was designed to help them lose weight (providing \(~6000\) kJ for women and \(8000\) kJ for men). Dairy intake was held constant to minimise confounding from this source. This meant that the GI of DIET 3 could not be as high as DIET 1 since all dairy foods have a low-GI. This subsequently reduced the overall GI of DIET 3, despite the inclusion of other high GI foods, since dairy foods provided a greater proportion of their carbohydrate than DIET 1. All groups were also provided with additional lists of suitable meals.
and snacks, including appropriate choices when eating out, and a sample 7-day plan. Within this framework, they were told ‘eat to appetite’ and stop eating when full.

The diets were not therefore strictly *ad libitum* since subjects were advised as to the appropriate number of serves they should aim to eat from each food group (necessary to achieve the desired macronutrient balance). But in order to allow satiety and appetite factors to function, subjects were not required to eat all the specified serves or portions of foods provided, and if hungry they were permitted to increase the number of serves proportionally from each food group.

Alcohol was not forbidden but all subjects were asked to limit alcohol consumption to no more than two units per day. Given that the majority of subjects were university students we did not feel that banning alcohol for three months was feasible, nor acceptable to most subjects.

To maximise compliance, all key carbohydrate and protein foods and some pre-prepared meals were provided. A colour-coded ‘shop’ system (different colour for each diet) with bar code reader (illustrated in Picture 1) was utilised and subjects collected their foods on a weekly basis. All key foods of low or high GI were provided including bread, breakfast cereal (illustrated in Picture 3), crackers, snack bars, oats, legumes, pasta, basmati, Doongara, jasmine or calrose rice. Red meat (lean beef and ‘Trim’ lamb) was supplied vacuum packed to all four diet groups in specified portions (illustrated in Picture 2) with the high protein groups receiving four weekly serves of 150g for women, 220g for men, and the high carbohydrate groups two weekly serves of 75g for women and 100g for men. To assist with
compliance meat was supplied both as raw fillets and as marinaded, ready-cut strips for stir-frying. A home economist was employed to prepare specified recipes (low or high GI and carbohydrate) suitable for each diet group (illustrated in Picture 2). These were supplied frozen at each weekly meeting.

On the same day they met with a dietitian who encouraged compliance, answered queries and carried out the measurements required. Study personnel were not blinded to dietary assignment but cognisant of the need for impartiality and equivalent treatment for all groups.

![Picture 1: barcode system used in the ‘shop’ to ensure subjects received low or high GI foods, according to their diet prescription](image-url)
Picture 2: a selection of frozen meals specially prepared in-house, and fresh lean red meat provided in specific portions for each diet group.

Picture 3: a selection of the low and high GI cereals and biscuits provided by the investigators.
2.4 Exercise & Physical Activity

The aim of the intervention was to investigate the effect of diet alone without any influence of exercise or physical activity. During screening subjects were asked what their current physical activity and exercise levels were, and their weekly activities (including walking to/from university, attendance at gym or group fitness classes, swimming and sports) were recorded. An activity level low, moderate or high was assigned. It was then stressed to the participants that they must stick to this activity level for the course of the intervention. Since the vast majority were sedentary and classified in the low level, this was not a major problem and was viewed with relief by most subjects!
2.5 Outcome Measures

2.5.1 Weight

Weight was measured weekly, with subjects in bare feet and light clothing, on an electronic scale (Tanita Corporation, Japan, illustrated in Picture 4).

Picture 4: a subject being weighed at a weekly meeting.
2.5.2 Body composition

Body composition (fat mass and lean mass) were determined at baseline and on completion of the 12-wk intervention, using dual-energy x-ray absorptiometry (Lunar Prodigy, GE Health Care, UK). This was performed at the metabolic unit, Royal Prince Alfred Hospital, Sydney, with subjects wearing only underwear and a hospital gown. Quality control and calibration checks are performed daily by hospital staff.

2.5.3 Waist circumference

Waist circumference was measured at baseline, week 6 and week 12 using standard protocol; on bare mid-riff, standing posture, mid-way between the lower rib and the iliac crest, following exhalation.

2.5.4 Biochemical analyses

Venous blood samples were drawn from an ante-cubital vein after an overnight fast at baseline, week 6 and week 12. Two standard 8.5ml SST II (serum separator) tubes and one 6ml EDTA (K2) tube were collected on each occasion and held on ice (for no more than two hours) until centrifugation. SST tubes were spun at 1500G for ten minutes and EDTA tubes at 3000G for five minutes. Aliquots were stored at -20°C prior to analysis within four weeks of sampling.
Total cholesterol (TC), high density lipoprotein (HDL)-cholesterol and triacylglycerides (TAG) were measured enzymatically by a Roche PPE modular machine (Roche, USA). Plasma non-esterified free fatty acids (FFA) were determined using a NEFAC test kit (Wako Pure Chemical Industries, Osaka, Japan) adapted to use on Roche Cobas Fara instrument (F Hoffmann-La Roche Ltd, Basel, Switzerland). The glucose-hexokinase method was used to measure glucose on the Roche PPE modular machine (Roche, USA). High sensitivity C-reactive protein (hs-CRP) was measured using near infra-red immunonephelometry on an Innage automated analyzer (Beckman-Coulter, Gladesville, NSW, Australia), and insulin determined using the Microparticles Enzyme Immuno Assay on an autoanalyser AXSYM using Abbott Diagnostic kit (Japan, Minato-ku, Tokyo).

LDL-cholesterol was calculated by difference using the Friedewald calculation: LDL = TC – TG/2.18 – HDL.

Homeostatic model assessment (HOMA) was used to assess β-cell function and insulin resistance (IR) from fasting glucose and insulin concentration. HOMA1-IR (the original model (fasting glucose x fasting insulin/22.5) as well as HOMA2-IR, HOMA2-insulin sensitivity (%S) and HOMA2-β-cell function (the non-linear computer model) were calculated as described by Wallace et al. (168).

2.5.5 Dietary compliance & measurement of food intake

Adherence to the allocated diet was maximised by the provision of key foods and weekly checks with the dietitian. In addition subjects were asked to keep a three-day
food diary, including two weekdays and one weekend day, at baseline and during weeks 4 and 8, to assess dietary compliance and estimate energy and macronutrient intake. A dietitian entered the data into a customised database which incorporated the Australian food composition tables (FoodWorks™ Professional 2005, Xyris Software, Australia). GI and GL values of the food diaries were calculated using a specially designed Excel spreadsheet using carbohydrate data for each food from the FoodWorks™ analysis and manually entering published GI values (169) using the glucose = 100 scale. Additional GI data were obtained from an online database (www.glycemicindex.com). A GI value of 68, equal to that for sucrose, was used for foods with an unknown GI.

2.5.6 End of study questionnaire

On completion of the 12-week intervention, all subjects filled out an end of study questionnaire (Appendix 3). This comprised five questions which used a linear 0-120mm scale, with five categories as markers, to assess:

1. the difficulty in following the prescribed diet,
2. like/dislike of the prescribed diet,
3. how filling the meals were,
4. how hungry they were between meals, and
5. how difficult they would find following their prescribed diet permanently.

A final question asked them to list any benefits or side effects they experienced while following their prescribed diet.
2.6 *Ethical Approval*

The study was approved by the Human Research Ethics Committee of the University of Sydney (approved 2001, reference number 01/05/04, project number 2223) and subjects gave written, informed consent.

2.7 *Statistical Treatment*

Power calculations indicated that 120 subjects (30 in each arm) provided 90% power to detect a 2.0 kg difference in body weight change among groups using significance = 5%. The primary end points were mean absolute change from baseline in body weight and fat mass at week 12. Chi-square analysis was used to compare the proportion in each group who achieved 5% or more weight loss. Univariate and repeated-measures ANOVA were used to assess the changes in weight, body composition and blood parameters. Changes were assessed with and without adjustment for baseline differences. Missing data were replaced with the last known value for the primary intention-to-treat analysis and excluded in the secondary analysis. SPSS version 12.0 (SPSS Inc, Chicago, Ill) was used for all statistical analyses and statistician Associate Professor Peter Petocz at Macquarie University, Sydney, supervised and confirmed all results.
2.8 Profile Days

2.8.1 Subjects

To confirm that the diets produced differences in day-long glycaemia and insulinaemia, mixed meals representative of each diet were fed over a 10 h period to eleven weight stable volunteers (mean age 26.5 ± 4.4 y, BMI: 30.0 ± 4.3 kg/m²) who had completed the weight loss intervention. To reduce variation all subjects recruited were of Caucasian ethnic background. Previous exclusion and inclusion criteria applied. In particular subjects had to have been weight stable for a minimum of three months after completing the weight loss phase before taking part. The Human Research Ethics Committee of the University of Sydney gave separate approval for this section of the study and all subjects gave written informed consent.

2.8.2 Design

A crossover design was used where the four menus were given in random order on separate days 3-7 days apart. The standard glycaemic index testing protocol was followed: on the day prior to testing, subjects were asked to abstain from alcohol and to eat a low-fat, carbohydrate-based evening meal that did not include legumes. The latter is in order to reduce the known ‘second meal effect’ where blood glucose responses are improved following breakfast when a low-GI meal is consumed the evening before (170, 171). Subjects reported at around 0800 hours following a 10-hour overnight fast where only water was permitted. Four meals, representative of a typical eating pattern and of the sample menu plans supplied during the weight loss
phase, were provided: breakfast at 0800 hours (0 min), morning snack at 1100 hours (180 min), lunch at 1300 hours (300 min) and dinner at 1700 hours (540 min). A second snack was not included (as per the weight loss menus) since monitoring finished at 1800 hours and it was assumed the second snack would be eaten in the evening. Subjects were instructed to consume meals within 20 minutes along with 250ml water. No additional foods or drinks were permitted. Subjects remained within the research unit for the entire profile period and took part in light activities only (reading, studying, watching TV).

2.8.3 Profile Day Menus

The menus (Table 3) were carefully chosen, using foods with a known GI, and prepared to match total energy, fat and fibre across all four diets, and carbohydrate and protein to be similar in each diet pair i.e. the two high carbohydrate diets (DIETS 1 and 2) and the two high protein diets (DIETS 3 and 4). In order to match the fibre intake of DIET 2 it was necessary to add unprocessed wheat bran to the other three diets. This is an insoluble fibre previously shown to have no effect on glycaemic index or glucose absorption, and only minor effects on gastric emptying (172, 173). Energy was also matched across all four diets at each meal; breakfast provided 21%, morning snack 14%, lunch 28% and dinner 38% of the total day’s energy. Energy, macronutrient and fibre content of each menu were calculated using FoodWorks™ Professional Edition software (Xyris Software, Highgate Hill, Qld, Australia, Version 3.02, 2003). Glycaemic indices and load were calculated using published GI values (169), using Australian data where possible, and available carbohydrate per serve. Where an Australian value was not available, the mean of published GI values
from other countries that employed standard GI methodology was used. Energy, macronutrient profile, fibre, GI and GL of each menu are shown in Table 4.

2.8.4 Blood collection & analysis on the profile days

Finger-prick capillary blood samples were collected at 30-60 min intervals throughout the course of the day, starting prior to eating breakfast and finishing one hour after eating dinner (Figure 3). Responses were quantified as the incremental areas under the curve (AUC, mean ± SE).

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Breakfast</th>
<th>Snack</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>0  1  2  3  4  5  6  7  8  9  10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of sampling (min)</td>
<td>0  30  60  120  180  240  300  330  360  420  480  540  570  600</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: Timing of meals and finger-prick blood sampling for each profile day.

The samples were collected from warmed hands using an automatic lancet device (Safe-T-Pro®, Boehringer Mannheim, Australia). Participants warmed their hands for approximately two minutes in hot water to improve blood circulation to the fingers prior to sampling. At each time point, an aliquot of approximately 800μL of capillary blood was collected into 1.5mL microcentrifuge tubes coated with heparin (10 U heparin sodium salt; Sigma Chemical, St Louis, MO). The tubes were
immediately centrifuged at 13 400 g for 45 sec and the plasma drawn off and stored at –20°C prior to analysis. All samples were analysed within three weeks of collection.

Plasma samples were thawed at room temperature prior to glucose and insulin analysis. Plasma glucose was measured in duplicate using a glucose hexokinase enzymatic assay on a spectrophotometric analyser (Roche/Hitachi 912, Boehringer Mannheim, Germany). The mean intra-assay and inter-assay coefficients of variation (CV) were both below 5%. Plasma insulin concentrations were assayed using the Coat-A-Count Insulin Radioimmunoassay Kit protocol (Diagnostic Products, Los Angeles, CA). The mean intra-assay CV for the insulin assay was 5% and the mean inter-assay CV was 3%.

2.8.5 Data & statistical analysis of profile days

The crossover design with eleven subjects gave the study >93% power to discriminate two standard deviations between arms. Incremental area under the curve (AUC) for glucose and insulin were calculated geometrically to quantify the cumulative responses across the 10-hour monitoring period (33, 174). Any area below the fasting level was ignored. Results are expressed as the mean response to each diet.

Statistical analysis was performed in SPSS for Windows statistical package (Version 11.0.0, 2003). Results were analysed using a general linear model analysis of variance (ANOVA) with diet as a fixed factor and subject as a random factor. Post
Part 2

Methods

hoc comparisons between the mean glucose, insulin and satiety area responses for each diet were carried out with a Dunnett’s adjustment for comparing the effects of each treatment diet with DIET 1 (significance, P < 0.017). To determine the effect of GI, amount of carbohydrate and their [GI x carbohydrate] interaction on glucose, insulin and satiety responses for the diets, a second general linear model was fitted. Linear regression was used to measure correlation between variables.
## Table 3: Profile day menus for each of the four intervention diets.

<table>
<thead>
<tr>
<th></th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td>45g Sultana Bran&lt;sup&gt;1&lt;/sup&gt; cereal</td>
<td>45g Guardian&lt;sup&gt;6&lt;/sup&gt; cereal</td>
<td>80g Egg Omelette</td>
<td>80g Egg Omelette</td>
</tr>
<tr>
<td></td>
<td>120ml Milk&lt;sup&gt;2&lt;/sup&gt; (1.4% fat)</td>
<td>120ml Milk&lt;sup&gt;2&lt;/sup&gt; (1.4% fat)</td>
<td>40g Wholemeal bread&lt;sup&gt;4&lt;/sup&gt;</td>
<td>38g Oatbran &amp; honey bread&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>80g Rockmelon</td>
<td>50g Strawberry</td>
<td>80g Spinach</td>
<td>80g Spinach</td>
</tr>
<tr>
<td></td>
<td>80g Pineapple</td>
<td>100g Grapefruit</td>
<td>20g Red Capsicum</td>
<td>20g Red Capsicum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80g Tomato, grilled</td>
<td>80g Tomato, grilled</td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td>40g White bagel&lt;sup&gt;1&lt;/sup&gt;, toasted</td>
<td>40g Fruit Bread&lt;sup&gt;7&lt;/sup&gt;, toasted</td>
<td>120g Low-fat fruit yoghurt&lt;sup&gt;11&lt;/sup&gt;</td>
<td>120g Low-fat fruit yoghurt&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5g Butter</td>
<td>4g Butter</td>
<td>5g Wheat bran†</td>
<td>5g Wheat bran†</td>
</tr>
<tr>
<td></td>
<td>10g Strawberry Jam</td>
<td>15g Fruit Spread</td>
<td>100g Banana</td>
<td>180g Green Apple</td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td>446g Potato &amp; Leek soup†</td>
<td>50g Oatbran &amp; honey bread&lt;sup&gt;9&lt;/sup&gt;</td>
<td>90g Wholemeal bread&lt;sup&gt;4&lt;/sup&gt;</td>
<td>84g Oatbran &amp; honey bread&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>10g Wheat bran†</td>
<td>290g Minestrone soup&lt;sup&gt;7&lt;/sup&gt;</td>
<td>60g Lean Ham</td>
<td>60g Lean Ham</td>
</tr>
<tr>
<td></td>
<td>28g Wholemeal bread&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10g Butter</td>
<td>25g Lettuce</td>
<td>25g Lettuce</td>
</tr>
<tr>
<td></td>
<td>5g Butter</td>
<td>140g Green Apple</td>
<td>45g Tomato</td>
<td>45g Tomato</td>
</tr>
<tr>
<td></td>
<td>50g Banana</td>
<td></td>
<td>20g Cheddar Cheese</td>
<td>20g Cheddar Cheese</td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td>120g Potato gnocchi&lt;sup&gt;5&lt;/sup&gt;</td>
<td>160g Spaghetti&lt;sup&gt;10&lt;/sup&gt;</td>
<td>120g Brown rice&lt;sup&gt;12&lt;/sup&gt;</td>
<td>160g Wholemeal Spaghetti&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120g Bolognais sauce</td>
<td>120g Bolognais sauce</td>
<td>200g Bolognais sauce</td>
<td>200g Bolognais sauce</td>
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<tr>
<td></td>
<td>10g Wheat bran†</td>
<td>58g Lettuce</td>
<td>15g Wheat bran†</td>
<td>5g Wheat bran†</td>
</tr>
<tr>
<td></td>
<td>58g Lettuce</td>
<td>68g Tomato</td>
<td>58g Lettuce</td>
<td>58g Lettuce</td>
</tr>
<tr>
<td></td>
<td>68g Tomato</td>
<td>25g Cucumber</td>
<td>68g Tomato</td>
<td>68g Tomato</td>
</tr>
<tr>
<td></td>
<td>25 g Cucumber</td>
<td>18g Olive Oil</td>
<td>25g Cucumber</td>
<td>25g Cucumber</td>
</tr>
<tr>
<td></td>
<td>18g Olive oil</td>
<td>10g Balsamic Vinegar</td>
<td>10g Olive Oil</td>
<td>10g Olive Oil</td>
</tr>
<tr>
<td></td>
<td>10g Balsamic Vinegar</td>
<td></td>
<td>6g Balsamic Vinegar</td>
<td>6g Balsamic Vinegar</td>
</tr>
</tbody>
</table>

<sup>1</sup> Sultana Bran™ Kellogg’s, Australia.  
<sup>2</sup> Lite White™, Dairy Farmers, Australia.  
<sup>3</sup> White Bagel: Bagel House Bakery, Australia.  
<sup>4</sup> Sunblest™ Wholemeal Bread, Tip Top Bakeries, Australia.  
<sup>5</sup> Potato Gnocchi, Golden Pasta, Australia.  
<sup>6</sup> Guardian™ Kellogg’s, Australia.  
<sup>7</sup> Bürger™ Fruit and Mixed Grain Bread, Tip Top Bakeries, Australia.  
<sup>8</sup> Bürger™ Oat Bran and Honey Bread, Tip Top Bakeries, Australia.  
<sup>9</sup> Campbell’s Country Ladle™ Traditional Minestrone Soup, Australia.  
<sup>10</sup> Spaghetti, San Remo, Australia.  
<sup>11</sup> Ski D’lite™ low fat Wild Strawberry yoghurt, Dairy Farmers, Australia.  
<sup>12</sup> SunRice™ Brown Calrose medium grain rice, Australia.  
<sup>13</sup> Wholemeal Spaghetti, San Remo, Australia.  
† Unprocessed Wheat bran, Nature’s Own, Australia. Wheat bran has no effect on glycemic responses, added to equalise the fibre contents between the four diets.
Table 4: Energy, macronutrient, fibre, glycaemic index and glycaemic load of the four profile day menus.

<table>
<thead>
<tr>
<th>MEAL</th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
</tr>
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<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1010</td>
<td>1000</td>
<td>1000</td>
<td>990</td>
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<td>Carbohydrate (g)</td>
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<td>19</td>
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<td>Protein (g)</td>
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<td>17</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>GI</td>
<td>59</td>
<td>34</td>
<td>71</td>
<td>31</td>
</tr>
<tr>
<td>GL (g)</td>
<td>29</td>
<td>15</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td><strong>Morning Snack</strong></td>
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<td></td>
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<tr>
<td>Energy (kJ)</td>
<td>740</td>
<td>740</td>
<td>780</td>
<td>750</td>
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<td>Carbohydrate (g)</td>
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<tr>
<td>GI</td>
<td>62</td>
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<td>43</td>
<td>36</td>
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<tr>
<td>GL (g)</td>
<td>20</td>
<td>13</td>
<td>15</td>
<td>12</td>
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<tr>
<td><strong>Lunch</strong></td>
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<tr>
<td>Energy (kJ)</td>
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<td>31</td>
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<td>GL (g)</td>
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<td>24</td>
<td>11</td>
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<tr>
<td><strong>Dinner</strong></td>
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<tr>
<td>Energy (kJ)</td>
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<td>2010</td>
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<td>GI</td>
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<td>GL (g)</td>
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<td>175</td>
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<td>141</td>
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<tr>
<td>Protein (g)</td>
<td>49</td>
<td>47</td>
<td>81</td>
<td>81</td>
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<tr>
<td>Fat (g)</td>
<td>42</td>
<td>42</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>CHO/PRO/FAT ratio</td>
<td>56 / 15 / 29</td>
<td>53 / 15 / 29</td>
<td>42 / 26 / 30</td>
<td>42 / 26 / 29</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>34</td>
<td>37</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>GI</td>
<td>65</td>
<td>40</td>
<td>68</td>
<td>34</td>
</tr>
<tr>
<td>GL (g)</td>
<td>116</td>
<td>65</td>
<td>84</td>
<td>43</td>
</tr>
</tbody>
</table>

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Section 3: Results

3.1 Completion Rate

Of the 129 enrolled subjects, 13 dropped out (all female): one fell pregnant, one failed to complete the final analysis, two moved interstate and nine cited disappointment with rate of weight loss. The distribution of dropouts is shown in Figure 4.

Figure 4: Completers (shown in solid colour) and dropouts (shown with diagonal stripe) in each dietary intervention group. Percentage of each intervention group to complete the full 12 week intervention shown in brackets. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
3.2 Weight & Body Composition Change

3.2.1 Weight change

In the primary intention-to-treat analysis (Table 5a), all four diets resulted in significant weight reduction over 12 weeks (p<0.001), but the differences among diet groups were not significant (-3.7 ± 0.5 kg, -4.8 ± 0.5 kg, -5.3 ± 0.5 kg, -4.4 ± 0.5kg, DIETS 1, 2, 3 and 4 respectively, p=0.17, Figure 5). Neither were there significant differences among the groups in mean percentage body weight change (-4.2 ± 0.6 %, -5.5 ± 0.5 %, -6.2 ± 0.4 % and -4.8 ± 0.7 % for DIETS 1, 2, 3 and 4 respectively, p=0.09). There were, however, significant differences in the proportion of individuals who lost >5% of initial body weight: 31% of subjects on DIET 1, 56% on DIET 2, 66% on DIET 3 and 33% on DIET 4 group (p=0.011, Figure 6).

The pattern of findings was essentially unchanged in a secondary analysis of data to exclude subjects who did not complete the full 12-week intervention (Table 5b). The proportion of subjects with >5% weight loss remained significant (37%, 60%, 68% and 32% for DIETS 1, 2, 3 and 4 respectively, n = 116, p = 0.015).
### Table 5: Changes in weight and body composition\(^1\).

(a) **Primary intention-to-treat analysis of all subjects (n = 129).**

<table>
<thead>
<tr>
<th></th>
<th>DIET 1 (n=32)</th>
<th>DIET 2 (n=32)</th>
<th>DIET 3 (n=32)</th>
<th>DIET 4 (n=33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight (kg)</td>
<td>-3.7 ± 0.5</td>
<td>-4.8 ± 0.5</td>
<td>-5.3 ± 0.5</td>
<td>-4.4 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>% weight change</td>
<td>-4.2 ± 0.6</td>
<td>-5.5 ± 0.5</td>
<td>-6.2 ± 0.4</td>
<td>-4.8 ± 0.7</td>
<td>0.09</td>
</tr>
<tr>
<td>% subjects with &gt;5% loss</td>
<td>31</td>
<td>56</td>
<td>66</td>
<td>33</td>
<td>0.011</td>
</tr>
<tr>
<td>Change in waist (cm)</td>
<td>-4.3 ± 0.7</td>
<td>-5.6 ± 0.7</td>
<td>-6.3 ± 0.6</td>
<td>-5.0 ± 0.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Change in fat mass (kg)</td>
<td>-2.8 ± 0.5</td>
<td>-4.5 ± 0.5</td>
<td>-4.3 ± 0.5</td>
<td>-3.7 ± 0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Change in lean mass (kg)</td>
<td>-0.5 ± 0.2</td>
<td>-0.3 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>-0.4 ± 0.2</td>
<td>0.75</td>
</tr>
</tbody>
</table>

(b) **Secondary analysis of subjects who completed the study (n = 116).**

<table>
<thead>
<tr>
<th></th>
<th>DIET 1 (n=27)</th>
<th>DIET 2 (n=30)</th>
<th>DIET 3 (n=31)</th>
<th>DIET 4 (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight (kg)</td>
<td>-4.2 ± 0.6</td>
<td>-5.0 ± 0.4</td>
<td>-5.4 ± 0.4</td>
<td>-4.7 ± 0.4</td>
<td>0.51</td>
</tr>
<tr>
<td>% weight change</td>
<td>-4.8 ± 0.7</td>
<td>-5.7 ± 0.5</td>
<td>-6.3 ± 0.5</td>
<td>-5.1 ± 0.8</td>
<td>0.32</td>
</tr>
<tr>
<td>% subjects with &gt;5% loss</td>
<td>37</td>
<td>60</td>
<td>68</td>
<td>32</td>
<td>0.015</td>
</tr>
<tr>
<td>Change in waist (cm)</td>
<td>-5.0 ± 0.8</td>
<td>-5.9 ± 0.8</td>
<td>-6.3 ± 0.8</td>
<td>-5.8 ± 0.8</td>
<td>0.67</td>
</tr>
<tr>
<td>Change in fat mass (kg)</td>
<td>-3.4 ± 0.5</td>
<td>-4.8 ± 0.5</td>
<td>-4.4 ± 0.5</td>
<td>-4.4 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Change in lean mass (kg)</td>
<td>-0.5 ± 0.3</td>
<td>-0.2 ± 0.3</td>
<td>-0.7 ± 0.3</td>
<td>-0.5 ± 0.2</td>
<td>0.63</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM, all data other than % weight change were adjusted for baseline values.
**Part 3**

**Results**

![Graph showing weight loss over weeks for different diets](image)

**Figure 5:** Changes in weight for ALL SUBJECTS over the 12 week intervention period. Circles represent the high carbohydrate diets; squares the high protein diets; dashed line the low-GI diets; and solid line the high-GI diets. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

![Bar chart showing percentage of group loss](image)

**Figure 6:** Percentage of each diet group who lost more than 5% and more than 10% of initial body weight. There were significant differences between groups in those who lost >5% body weight (p=0.011) but not for >10%. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
There were differences between men and women, although there were insufficient men in each group in order to carry out a meaningful sub-analysis. However the differences were in response to DIETS 1 and 4, with men tending to lose more weight than women on these diets. Figure 7 shows the mean weight loss for men and women in each group and Figure 8 the mean weight changes for men over the 12-week intervention. From these it can be seen that men performed similarly on DIETS 1, 2 and 3 but lost more weight on DIET 4, although this difference did not reach statistical significance due to the small number and large variation within the men in each group.

**Figure 7:** Differences in weight loss between men (n=31, labelled M) and women (n=98, labelled F) in each dietary group. Values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
A sub-analysis of the women (Table 6a) showed significant differences among the four groups in weight loss (-3.1 ± 0.5kg, -4.8 ± 0.5kg, -5.4 ± 0.5kg, -3.5 ± 0.5kg, DIETS 1, 2, 3 and 4 respectively, p=0.006) and percentage weight change (-3.7 ± 0.6%, -5.7 ± 0.6%, -6.5 ± 0.5%, -4.1 ± 0.7%, DIETS 1, 2, 3 and 4 respectively, p=0.005). These differences remained statistically significant in a secondary analysis of women who completed the 12-week intervention (n=85, Table 6b).

Figure 9 shows the weight changes for women over the 12-week intervention. In contrast to the men, women lost more weight on DIETS 2 and 3, but did not lose more weight on the combined intervention DIET 4 (high protein and low-GI).
Table 6: Changes in weight and body composition in WOMEN\textsuperscript{1}

(a) Primary intention-to-treat analysis all women (n = 98).

<table>
<thead>
<tr>
<th></th>
<th>DIET 1 (n=25)</th>
<th>DIET 2 (n=23)</th>
<th>DIET 3 (n=24)</th>
<th>DIET 4 (n=26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight (kg)</td>
<td>-3.1 ± 0.5</td>
<td>-4.8 ± 0.5</td>
<td>-5.4 ± 0.5</td>
<td>-3.5 ± 0.5</td>
<td>0.006</td>
</tr>
<tr>
<td>% weight change</td>
<td>-3.7 ± 0.6</td>
<td>-5.7 ± 0.6</td>
<td>-6.5 ± 0.5</td>
<td>-4.1 ± 0.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Change in waist (cm)</td>
<td>-3.2 ± 0.7</td>
<td>-5.8 ± 0.8</td>
<td>-6.2 ± 0.8</td>
<td>-3.9± 0.7</td>
<td>0.017</td>
</tr>
<tr>
<td>Change in fat mass (kg)</td>
<td>-2.5 ± 0.5</td>
<td>-4.5 ± 0.5</td>
<td>-4.6 ± 0.5</td>
<td>-2.9 ± 0.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Change in lean mass (kg)</td>
<td>-0.2 ± 0.2</td>
<td>-0.3 ± 0.4</td>
<td>-0.2 ± 0.3</td>
<td>-0.1 ± 0.2</td>
<td>0.95</td>
</tr>
</tbody>
</table>

(b) Secondary analysis of women who completed the study (n = 85).

<table>
<thead>
<tr>
<th></th>
<th>DIET 1 (n=20)</th>
<th>DIET 2 (n=21)</th>
<th>DIET 3 (n=23)</th>
<th>DIET 4 (n=21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight (kg)</td>
<td>-3.7 ± 0.6</td>
<td>-5.1 ± 0.5</td>
<td>-5.5 ± 0.5</td>
<td>-3.7 ± 0.5</td>
<td>0.038</td>
</tr>
<tr>
<td>% weight change</td>
<td>-4.4 ± 0.7</td>
<td>-6.1 ± 0.6</td>
<td>-6.6 ± 0.5</td>
<td>-4.2 ± 0.9</td>
<td>0.025</td>
</tr>
<tr>
<td>Change in waist (cm)</td>
<td>-4.1 ± 0.8</td>
<td>-6.1 ± 0.8</td>
<td>-6.3 ± 0.8</td>
<td>-4.5 ± 0.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Change in fat mass (kg)</td>
<td>-3.1 ± 0.5</td>
<td>-4.9 ± 0.5</td>
<td>-4.8 ± 0.5</td>
<td>-3.6 ± 0.5</td>
<td>0.044</td>
</tr>
<tr>
<td>Change in lean mass (kg)</td>
<td>-0.2 ± 0.3</td>
<td>-0.3 ± 0.4</td>
<td>-0.3 ± 0.3</td>
<td>-0.2 ± 0.3</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\textsuperscript{1}All data other than % weight change are adjusted for baseline values.
Figure 9: Changes in weight for WOMEN (n=98) over the 12 week intervention period. Circles represent the high carbohydrate diets; squares the high protein diets; dashed line the low-GI diets; and solid line the high-GI diets. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

3.2.2 Body composition change

Reductions in fat mass were significant within each group (p <0.001), but not among the four diets (p=0.08, Table 5a, Figure 10). There was a trend for DIET 1 to lose less fat than the other three groups (-2.8 ± 0.5 kg, -4.5 ± 0.5 kg, -4.3 ± 0.5 kg, -3.7 ± 0.5 kg, for DIETS 1, 2, 3 and 4 respectively, p=0.08). Declines in lean body mass reached significance in the two high GI groups (p=0.03 for DIET 1, p=0.017 for DIET 3), but overall there were no significant differences among the four diets (p=0.75, Table 5a, Figure 10). In all groups the loss of lean mass was a small proportion of overall weight loss accounting for less than 15% of total weight loss (14%, 6%, 11% and 9% for DIETS 1, 2, 3 and 4 respectively). These findings were
essentially unchanged in the secondary analysis of subjects who completed the study (Table 5b).

![Diagram showing changes in fat (solid colour) and lean mass (diagonal stripe) in each diet group (n=129). The differences among groups were not significant for fat mass (p=0.08) or lean mass (p=0.75). Values expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.]

There were gender-influenced fat mass changes with a significant interaction with diet (p = 0.008). In a sub-analysis of women (n = 98), there were highly significant differences in fat mass change (-2.5 ± 0.5kg, -4.5 ± 0.6kg, -4.6 ± 0.4kg, -2.8 ± 0.7kg for DIETS 1, 2, 3 and 4 respectively, p = 0.007, Table 6a, Figure 11) with DIETS 2 and 3 achieving the biggest fat mass losses.

In both sexes, fat mass change displayed a significant interaction of GI with carbohydrate content (p = 0.024), more strongly so in women (p = 0.001). In other words GI became more important with increasing carbohydrate intake.
Figure 11: Changes in fat mass (solid colour) and lean mass (diagonal stripe) in WOMEN (n=98). Differences in fat mass were highly significant among groups (p=0.007) with no significant differences in lean mass change (p=0.95). Values expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

In the sub-analysis of women there were no differences among diet groups in loss of lean mass and all groups lost very small amounts (-0.2 ± 0.2kg, -0.3 ± 0.4kg, -0.2 ± 0.3kg, -0.1 ± 0.2kg for DIETS 1, 2, 3 and 4 respectively, n=98, p=0.95, Table 6b, Figure 11).

3.2.3 Waist circumference change

Figure 12 shows the changes in waist circumference for each group. All groups had a significant reduction (p<0.001), but the differences among diet groups were not significant (-4.3 ± 0.7cm, -5.6 ± 0.7cm, -6.3 ± 0.6cm, -5.0 ± 0.7cm, DIETS 1, 2, 3 and 4 respectively, p=0.22). Nevertheless the pattern of loss was similar to that for other measures with DIETS 2 and 3 achieving the biggest change.
Figure 12: Changes in waist circumference from week 0 to 12 (n=129). Values expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

In the sub-analysis of women the changes in waist circumference were statistically significant with more pronounced change in DIETS 2 and 3 (-3.2 ± 0.7cm, -5.8 ± 0.8cm, -6.2 ± 0.8cm, -3.9 ± 0.7cm, DIETS 1, 2, 3 and 4 respectively, p=0.017, Table 6a, Figure 13).

Figure 13: Changes in waist circumference in WOMEN (n=98) from week 0 to 12. Values expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
3.3 Secondary Sensitivity Analysis

The pattern of findings were similar when analysis was confined to those with high fasting insulin (> 110 pmol/L, n = 37) or high fasting TAG level (> 1.5 mmol/L, n = 38). These cut-off levels were chosen to match previous studies where a difference was shown (102, 113, 119). DIET 1 performed least well in both sub-groups. Among those with hyperinsulinaemia at baseline the differences among groups were not statistically different, although there was a trend for better fat loss on DIET 2 (Figure 14). Among those with high baseline fasting TAG, differences were significant; the diet with the highest glycaemic load (DIET 1) achieved the least fat loss and the diet with the lowest glycaemic load (DIET 4) the greatest fat loss (-2.0 ± 0.8kg, -4.9 ± 0.8kg, -4.4 ± 0.9kg, -5.6 ± 1.0kg for DIETS 1, 2, 3 and 4 respectively, p = 0.028, Figure 15).

Figure 14: Changes in fat mass in a sub-analysis of subjects with baseline insulin >110 pmol/L (n=37). Differences among the groups were not significant (p=0.236). DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
Figure 15: Changes in fat mass in a sub-analysis of subjects with baseline TAG >1.5 mmol/L (n=38). Differences among the groups were significant (p=0.028). DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
3.4 Blood Parameter Changes

Table 8 shows the blood parameter and cardiovascular risk factor changes for the four diet groups. All four diets improved the cardiovascular risk factors HDL-cholesterol, total: HDL cholesterol ratio, TAG, hs-CRP and glucose homeostasis with no differential effect of diet composition. There were however divergent effects on total- and LDL-cholesterol and significant differences in changes in leptin. There were no statistically significant interactions of gender on any of the blood parameters measured and results were essentially unchanged in a sub-analysis of women (Table 9).

3.4.1 Blood lipids

There were significant differences among groups in total cholesterol change (+0.05 ± 0.10mmol/L, -0.18 ± 0.10mmol/L, +0.24 ± 0.10, -0.05 ± 0.10mmol/L, DIETS 1, 2, 3 and 4 respectively, p=0.04) and LDL-cholesterol change (+0.04 ± 0.10mmol/L, -0.17 ± 0.10mmol/L, +0.26 ± 0.10, -0.04 ± 0.09mmol/L, DIETS 1, 2, 3 and 4 respectively, p=0.019, Figure 16). Both parameters worsened on DIET 3 (+5% and +8% respectively) in contrast to an improvement on DIET 2 (-4% and -6% respectively, p = 0.033 and 0.013 for pair-wise comparisons). The increase in LDL-cholesterol on DIET 3 was exaggerated in women (+10%) and significantly different to the fall on DIET 2 (-9%, p = 0.001, Table 9). In terms of the percentage of each group with high LDL-cholesterol (defined as ≥2.6mmol/L), DIET 2 were the only group to show an improvement from baseline (Figure 17). Overall, there was a significant effect of GI,
but not protein content, on change in total cholesterol (p = 0.019) and LDL cholesterol (p = 0.009).

HDL-cholesterol rose significantly within all groups (p=0.007), but there were no differences among the four diets (+0.08 ± 0.04 mmol/L, +0.03 ± 0.04 mmol/L, +0.05 ± 0.04 mmol/L, +0.07 ± 0.04, DIETS 1, 2, 3 and 4 respectively, p=0.82, Figure 16). Similarly, changes in the ratio of total cholesterol: HDL-cholesterol were not significantly different among groups (-0.23 ± 0.11, -0.21 ± 0.11, +0.02 ± 0.11, -0.37 ± 0.11, DIETS 1, 2, 3 and 4 respectively, p=0.11), but DIET 4 were the only group to show a significant fall in the ratio within the study period (p=0.0008 in the intention-to-treat analysis and 0.0004 for completers).

Triacylglycerides (TAG) fell significantly in all dietary groups (p=0.001), with no differential effect of diet (-0.14 ± 0.07mmol/L, -0.05 ± 0.07 mmol/L, -0.18 ± 0.07mmol/L, -0.19 ± 0.07 mmol/L, DIETS 1, 2, 3 and 4 respectively, p=0.39, Figure 16). The most significant falls in TAG, however, occurred in the two high protein groups (p=0.009 for DIET 3 and p=0.008 for DIET 4).
Figure 16: Changes in blood lipid fractions from week 0 to week 12 (n=129). TC = Total cholesterol. All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

Figure 17: Percentage in each diet group with high LDL-cholesterol (≥2.6mmol/L) at baseline and on completion of the 12-week intervention. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
There were no differences among groups in change in fasting free fatty acids (FFA), although there was an interesting trend for little change in DIET 2 while all other groups fell (-63 ± 35µmol/L, +3 ± 36µmol/L, -44 ± 35µmol/L, -57 ± 34 µmol/L, DIETS 1, 2, 3 and 4 respectively, p=0.56, Figure 18).

Figure 18: Change in fasting FFA from week 0 to week 12 (n=129). All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
3.4.2 Measures of insulin sensitivity & glucose metabolism

Fasting insulin levels fell in all groups with no significant differences among groups (-8.1 ± 6.9 pmol/L, -13.3 ± 6.9 pmol/L, -17.1 ± 7.0pmol/L, -10.4 ± 6.8pmol/L, DIETS 1, 2, 3 and 4 respectively, Table 8, Figure 19). The changes in fasting insulin were significantly correlated with changes in fat mass (r = 0.19, p = 0.03, Figure 20).

Insulin sensitivity improved to varying degrees on all four diets as measured by the computer model HOMA2-IS, with no significant difference among groups (+1.7 ± 7.3%, +9.3 ± 7.4%, +25.7 ± 7.4%, +16.4 ± 7.2%, DIETS 1, 2, 3 and 4 respectively, p=0.13, Table 8). In the total dataset, there was a marginally significant (p<0.049) effect of carbohydrate content on HOMA2-IS. In women only, HOMA2-IS showed significant differences among the groups (p<0.025), with those following DIET 3 showing higher sensitivity than those following DIET 1 in pairwise comparisons (p<0.029). Change in fat mass correlated with changes in HOMA2-IS (r = 0.20, p = 0.025). There were no significant differences among groups using the original HOMA model (HOMA1-IR), or the newer computer models to assess insulin resistance (HOMA2-IR) and beta-cell function (HOMA2-β, Table 8).
Figure 19: Changes in fasting insulin from week 0 to week 12 (n=129). All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.

Figure 20: Correlation between change in fasting insulin and change in fat mass (r=0.19, p=0.03).
Fasting glucose changed little overall and there were no significant differences among the groups (-0.04 ± 0.10mmol/L, -0.06 ± 0.10mmol/L, -0.05 ± 0.10mol/L, +0.02 ± 0.10mmol/L; DIETS 1, 2, 3 and 4 respectively, p=0.94, Table 8, Figure 21).

![Figure 21: Changes in fasting glucose from week 0 to week 12 (n=129). All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.](image)

3.4.3 Leptin

Diet had significant effects on changes in leptin (p=0.006), falling more on DIETS 2 and 3 (-1.9 ± 1.5ng/ml, -7.5 ± 1.5ng/ml, -5.4 ± 1.5ng/ml, -1.0 ± 1.4ng/ml; DIETS 1, 2, 3 and 4 respectively, Table 8, Figure 22), with a significant interaction between GI and CHO content (p = 0.003). On an individual basis, there was a significant correlation between change in fat mass and change in leptin (r=0.31, p<0.001, Figure 23). In a secondary analysis of completers only, this correlation remained significant.
(r = 0.27, p =0.003), with no additional effect of GI or CHO content. There were no significant correlations between leptin and fasting insulin at any time point (baseline, 6 weeks or 12 weeks), or in change in either blood parameter (r=0.15, p=0.09, Figure 24).

Figure 22: Change in leptin from week 0 to week 12 (n=129). All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
Figure 23: Correlation between change in fasting leptin and change in fat mass (r=0.31, p<0.001).

Figure 24: Correlation between change in leptin and change in fasting insulin, not significant (r=0.15, p=0.09).
3.4.4 High sensitivity C-reactive protein (hs-CRP)

Hs-CRP fell significantly in all groups except DIET 4, but there were no significant differences among groups (-0.8 ± 0.4mg/L, -1.1 ± 0.4mg/L, -0.8 ± 0.4mg/L, -0.01 ± 0.4mg/L, DIETS 1, 2, 3 and 4 respectively, p=0.18, Table 8, Figure 25). There was a marginally significant correlation between change in hs-CRP and change in fat mass (r=0.18, p=0.05, Figure 26).

Figure 25: Change in hs-CRP from week 0 to week 12 (n=129). All values are expressed as mean ± SEM. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
Figure 26: Correlation between change in hs-CRP and change in fat mass (r=0.18, p=0.05).
Table 8: Primary intention-to-treat analysis of changes in cardiovascular risk factors from baseline to end of week 12. Expressed as mean ± SEM changes adjusted for baseline differences.

<table>
<thead>
<tr>
<th>Absolute change</th>
<th>DIET 1 (n=32)</th>
<th>DIET 2 (n=32)</th>
<th>DIET 3 (n=32)</th>
<th>DIET 4 (n=33)</th>
<th>P value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.05 ± 0.10</td>
<td>-0.18 ± 0.10</td>
<td>+0.24 ± 0.10$^2$</td>
<td>-0.05 ± 0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.04 ± 0.10</td>
<td>-0.17± 0.10$^3$</td>
<td>+0.26 ± 0.10$^3$</td>
<td>-0.04 ± 0.09</td>
<td>0.019</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>+0.08 ± 0.04</td>
<td>+0.03 ± 0.04</td>
<td>+0.05 ± 0.04</td>
<td>+0.07 ± 0.04</td>
<td>0.82</td>
</tr>
<tr>
<td>Cholesterol: HDL ratio</td>
<td>-0.23 ± 0.11</td>
<td>-0.21 ± 0.11</td>
<td>+0.02 ± 0.11</td>
<td>-0.37 ± 0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Triacylglycerides (mmol/L)</td>
<td>-0.14 ± 0.07</td>
<td>-0.05 ± 0.07</td>
<td>-0.18 ± 0.07</td>
<td>-0.19 ± 0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Free fatty acids (μmol/L)</td>
<td>-63 ± 35</td>
<td>+3 ± 36</td>
<td>-44 ± 35</td>
<td>-57 ± 34</td>
<td>0.56</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.04 ± 0.10</td>
<td>-0.06 ± 0.10</td>
<td>-0.05 ± 0.10</td>
<td>+0.02 ± 0.10</td>
<td>0.94</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-8.1 ± 6.9</td>
<td>-13.3 ± 6.9</td>
<td>-17.1 ± 7.0</td>
<td>-10.4 ± 6.8</td>
<td>0.82</td>
</tr>
<tr>
<td>HOMA1-IR$^5$</td>
<td>-0.3 ± 0.2</td>
<td>-0.5 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>-0.3 ± 0.2</td>
<td>0.72</td>
</tr>
<tr>
<td>HOMA2-IR$^5$</td>
<td>-0.1 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>0.77</td>
</tr>
<tr>
<td>HOMA2-1S$^6$</td>
<td>+1.7 ± 7.3</td>
<td>+9.3 ± 7.4</td>
<td>+25.7 ± 7.4</td>
<td>+16.4 ± 7.2</td>
<td>0.13</td>
</tr>
<tr>
<td>HOMA2-β$^6$</td>
<td>-11.3 ± 17.8</td>
<td>-15.0 ± 17.8</td>
<td>-16.5 ± 18.1</td>
<td>+11.8 ± 17.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>-1.9 ± 1.5</td>
<td>-7.5 ± 1.5$^4$</td>
<td>-5.4 ± 1.5</td>
<td>-1.0 ± 1.4</td>
<td>0.006</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>-0.8 ± 0.4</td>
<td>-1.1 ± 0.4</td>
<td>-0.8 ± 0.4</td>
<td>-0.01 ± 0.4</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$^1$Univariate ANOVA of the absolute change between 0 time and week 12. Repeated measures ANOVA using 0, 6 and 12-week data gave similar findings (data not shown).

$^2$p = 0.033 DIET 2 v DIET 3.

$^3$p = 0.013 DIET 2 v DIET 3.

$^4$p = 0.011 DIET 2 v DIET 4.

$^5$Homeostasis model assessment of insulin resistance 1 (original model) and 2 (computer model) using fasting glucose (mmol/L) and insulin concentration (mU/mL) .

$^6$HOMA2 insulin sensitivity (%) and β-cell function %.
Table 9: Primary intention-to-treat analysis of changes in cardiovascular risk factors in WOMEN from baseline to end of week 12 (n=98). Expressed as mean ± SEM changes adjusted for baseline differences.

<table>
<thead>
<tr>
<th>Absolute change</th>
<th>DIET 1 (n=25)</th>
<th>DIET 2 (n=23)</th>
<th>DIET 3 (n=24)</th>
<th>DIET 4 (n=26)</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.05 ± 0.12</td>
<td>-0.19 ± 0.13</td>
<td>+0.33 ± 0.13(^2)</td>
<td>-0.06 ± 0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.04 ± 0.11</td>
<td>-0.19± 0.12(^3)</td>
<td>+0.34 ± 0.12(^3)</td>
<td>-0.02 ± 0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>+0.06 ± 0.04</td>
<td>+0.04 ± 0.05</td>
<td>+0.05 ± 0.05</td>
<td>+0.05 ± 0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>Cholesterol: HDL ratio</td>
<td>-0.23 ± 0.11</td>
<td>-0.21 ± 0.11</td>
<td>+0.02 ± 0.11</td>
<td>-0.37 ± 0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Triacylglycerides (mmol/L)</td>
<td>-0.09 ± 0.07</td>
<td>-0.02 ± 0.07</td>
<td>-0.18 ± 0.07</td>
<td>-0.08 ± 0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Free fatty acids (µmol/L)</td>
<td>-64 ± 42</td>
<td>+22± 45</td>
<td>-14 ± 42</td>
<td>-36 ± 41</td>
<td>0.56</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.04 ± 0.14</td>
<td>-0.01 ± 0.14</td>
<td>-0.04 ± 0.14</td>
<td>+0.05 ± 0.13</td>
<td>0.94</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-7.4 ± 5.9</td>
<td>-14.8 ± 6.1</td>
<td>-29.2 ± 6.0</td>
<td>-14.9 ± 5.7</td>
<td>0.08</td>
</tr>
<tr>
<td>HOMA1-IR(^5)</td>
<td>-0.3 ± 0.2</td>
<td>-0.5 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>-0.3 ± 0.2</td>
<td>0.72</td>
</tr>
<tr>
<td>HOMA2-IR(^5)</td>
<td>-0.1 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>0.77</td>
</tr>
<tr>
<td>HOMA2-1S(^6)</td>
<td>+1.7 ± 7.3</td>
<td>+9.3 ± 7.4</td>
<td>+25.7 ± 7.4</td>
<td>+16.4 ± 7.2</td>
<td>0.13</td>
</tr>
<tr>
<td>HOMA2-β(^5)</td>
<td>-11.3 ± 17.8</td>
<td>-15.0 ± 17.8</td>
<td>-16.5 ± 18.1</td>
<td>+11.8 ± 17.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>-2.9 ± 1.9</td>
<td>-8.9 ± 1.9(^4)</td>
<td>-6.8 ± 1.9</td>
<td>-0.13 ± 1.8</td>
<td>0.005</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>-0.8 ± 0.5</td>
<td>-1.2 ± 0.5</td>
<td>-0.8 ± 0.5</td>
<td>-0.02 ± 0.5</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\(^1\)Univariate ANOVA of the absolute change between 0 time and week 12. Repeated measures ANOVA using 0, 6 and 12-week data gave similar findings (data not shown).
\(^2\)\(^p\) = 0.031 DIET 2 v DIET 3.
\(^3\)\(^p\) = 0.013 DIET 2 v DIET 3.
\(^4\)\(^p\) = 0.006 DIET 2 v DIET 4.
\(^5\)Homeostasis model assessment of insulin resistance 1 (original model) and 2 (computer model)(168) using fasting glucose (mmol/L) and insulin concentration (mU/mL) .
\(^6\)HOMA2 insulin sensitivity (%) and β-cell function % .
3.5 Secondary Sensitivity Analysis of Blood Parameters

A secondary analysis of the blood results to exclude subjects who did not complete the full 12-week intervention was carried out (Table 10). The pattern of findings was mostly similar, with and without adjustment for baseline differences. When unadjusted for baseline differences, fasting FFA rose significantly (30%) in DIET 2 compared to a decline in the other three groups (-73 ± 38 µmol/L, +61 ± 47 µmol/L, -78 ± 40 µmol/L, -110 ± 54 µmol/L for DIETS 1, 2, 3 and 4 respectively, p=0.04). These differences were no longer significant when adjusted for baseline differences (Table 10). Changes in total cholesterol, LDL-cholesterol and leptin remained significant with a similar pattern to the primary analysis.
Table 10: Secondary analysis of changes in cardiovascular risk factors for COMPLETERS (excluding dropouts) from baseline to end of week 12. Expressed as mean ± SEM changes adjusted for baseline differences.

<table>
<thead>
<tr>
<th>Absolute change</th>
<th>DIET 1 (n=27)</th>
<th>DIET 2 (n=30)</th>
<th>DIET 3 (n=31)</th>
<th>DIET 4 (n=28)</th>
<th>P value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>+0.01 ± 0.12</td>
<td>-0.22 ± 0.11²</td>
<td>+0.24 ± 0.11</td>
<td>-0.07 ± 0.11³</td>
<td>0.036</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>+0.02 ± 0.10</td>
<td>-0.22 ± 0.10⁴</td>
<td>+0.25 ± 0.10</td>
<td>-0.06 ± 0.10⁵</td>
<td>0.015</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.08 ± 0.04</td>
<td>0.03 ± 0.04</td>
<td>0.06 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>Cholesterol: HDL ratio</td>
<td>-0.24 ± 12</td>
<td>-0.24 ± 0.12</td>
<td>-0.04 ± 0.12</td>
<td>-0.42 ± 0.12</td>
<td>0.17</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>-0.18 ± 0.07</td>
<td>-0.04 ± 0.07</td>
<td>-0.19 ± 0.07</td>
<td>-0.21 ± 0.07</td>
<td>0.27</td>
</tr>
<tr>
<td>Free fatty acids (µmol/L)</td>
<td>-68 ± 35</td>
<td>12 ± 34</td>
<td>-46 ± 33</td>
<td>-97 ± 35</td>
<td>0.15</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.04 ± 0.10</td>
<td>-0.08 ± 0.09</td>
<td>-0.07 ± 0.09</td>
<td>0.00 ± 0.10</td>
<td>0.94</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-10.9 ± 7.4</td>
<td>-12.8 ± 7.0</td>
<td>-15.7 ± 6.9</td>
<td>-13.8 ± 7.2</td>
<td>0.97</td>
</tr>
<tr>
<td>HOMA1-IR⁵</td>
<td>-0.36 ± 0.24</td>
<td>-0.45 ± 0.23</td>
<td>+0.53 ± 0.22</td>
<td>-0.41 ± 0.23</td>
<td>0.96</td>
</tr>
<tr>
<td>HOMA2-IR⁵</td>
<td>-0.19 ± 0.13</td>
<td>-0.23 ± 0.12</td>
<td>+0.29 ± 0.12</td>
<td>-0.24 ± 0.13</td>
<td>0.95</td>
</tr>
<tr>
<td>HOMA2-IS⁶</td>
<td>1.8 ± 8.0</td>
<td>9.3 ± 7.6</td>
<td>26.0 ± 7.5</td>
<td>15.8 ± 7.8</td>
<td>0.16</td>
</tr>
<tr>
<td>HOMA2-β⁷</td>
<td>-13.1 ± 20.0</td>
<td>-13.6 ± 18.9</td>
<td>-15.3 ± 19.0</td>
<td>-13.1 ± 19.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>-2.5 ± 21.6</td>
<td>-7.8 ± 1.6</td>
<td>-5.4 ± 1.5</td>
<td>-1.2 ± 1.6</td>
<td>0.018</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>-0.8 ± 0.4</td>
<td>-1.2 ± 0.4</td>
<td>-0.9 ± 0.4</td>
<td>0.0 ± 0.4</td>
<td>0.22</td>
</tr>
</tbody>
</table>

¹ Univariate ANOVA of the absolute change between 0 time and 12 week. Repeated measures ANOVA using 0, 6 and 12-week data gave similar findings (data not shown).
² p = 0.004 for DIET 2 vs DIET 3.
³ p = 0.06 for DIET 4 vs DIET 3.
⁴ p = 0.002 for DIET 2 vs DIET 3.
⁵ P = 0.039 for DIET 4 vs DIET 3.
⁶ Homeostasis model assessment of insulin resistance 1 (original model) and 2 (computer model) (168) using fasting glucose (mmol/L) and insulin concentration (mU/mL).
⁷ HOMA2 insulin sensitivity (%) and β-cell function % (168).
3.6 Dietary Adherence

3.6.1 Macronutrients & energy intake

The analysis of the food diaries showed that all 4 groups achieved their intended carbohydrate and protein distributions, exceeding their respective targets. Both high carbohydrate groups consumed >55% energy from carbohydrate and less than 20% energy from protein, whereas both high protein groups consumed <45% energy from carbohydrate and >25% energy from protein (Table 11, Figure 27). The differences among groups were highly significant (p<0.001).

Table 11: Target and actual macronutrient energy distribution (% total energy), glycaemic index (GI) and glycaemic load (GL)\(^1,2\).

<table>
<thead>
<tr>
<th></th>
<th>DIET 1</th>
<th></th>
<th>DIET 2</th>
<th></th>
<th>DIET 3</th>
<th></th>
<th>DIET 4</th>
<th></th>
<th>P value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Actual</td>
<td>Target</td>
<td>Actual</td>
<td>Target</td>
<td>Actual</td>
<td>Target</td>
<td>Actual</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CHO (% E)</strong></td>
<td>55</td>
<td>60±1</td>
<td>55</td>
<td>56±1</td>
<td>45</td>
<td>42±1</td>
<td>45</td>
<td>40±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Protein (%</strong></td>
<td>15</td>
<td>18±1</td>
<td>15</td>
<td>19±0</td>
<td>25</td>
<td>28±1</td>
<td>25</td>
<td>26±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Fat (% E)</strong></td>
<td>30</td>
<td>19±1</td>
<td>30</td>
<td>22±1</td>
<td>30</td>
<td>27±1</td>
<td>30</td>
<td>29±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Alcohol (%)</strong></td>
<td>0</td>
<td>2±1</td>
<td>0</td>
<td>3±1</td>
<td>0</td>
<td>2±1</td>
<td>0</td>
<td>3±1</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>GI</strong></td>
<td>67</td>
<td>70±1</td>
<td>40</td>
<td>45±1</td>
<td>57</td>
<td>59±1</td>
<td>34</td>
<td>44±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>GL (g)</strong></td>
<td>127</td>
<td>129±8</td>
<td>75</td>
<td>89±5</td>
<td>87</td>
<td>75±3</td>
<td>54</td>
<td>59±4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± s.e.m. \(^2\) Target values were the calculated values from sample menus. Actual values were calculated from food diaries completed during weeks 4 and 8 of the intervention. \(^3\) P value for comparison of actual values among the 4 diets.

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Table 12: Nutrient intake determined from food diaries completed at baseline and during the intervention\(^1,2\).

<table>
<thead>
<tr>
<th></th>
<th>HGI</th>
<th>LGI</th>
<th>HP/HGI</th>
<th>HP/LGI</th>
<th>P value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kJ)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>9630 ± 470</td>
<td>9030 ± 460</td>
<td>9220 ± 450</td>
<td>8890 ± 470</td>
<td>0.41</td>
</tr>
<tr>
<td>intervention</td>
<td>6010 ± 240</td>
<td>6150 ± 190</td>
<td>5950 ± 170</td>
<td>5970 ± 190</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>CHO (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>262 ± 14</td>
<td>239 ± 13</td>
<td>248 ± 14</td>
<td>251 ± 19</td>
<td>0.78</td>
</tr>
<tr>
<td>intervention</td>
<td>209 ± 9</td>
<td>200 ± 7</td>
<td>146 ± 6</td>
<td>143 ± 7</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>99 ± 5</td>
<td>93 ± 5</td>
<td>89 ± 5</td>
<td>93 ± 4</td>
<td>0.54</td>
</tr>
<tr>
<td>intervention</td>
<td>63 ± 3</td>
<td>69 ± 2</td>
<td>95 ± 2</td>
<td>93 ± 3</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Total fat (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>93 ± 6</td>
<td>82 ± 6</td>
<td>88 ± 5</td>
<td>73 ± 5</td>
<td>0.06</td>
</tr>
<tr>
<td>intervention</td>
<td>32 ± 2</td>
<td>36 ± 2</td>
<td>44 ± 2</td>
<td>48 ± 2</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Saturated fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>36 ± 3</td>
<td>33 ± 3</td>
<td>32 ± 3</td>
<td>27 ± 3</td>
<td>0.16</td>
</tr>
<tr>
<td>intervention</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Monounsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>33 ± 2</td>
<td>29 ± 2</td>
<td>31 ± 2</td>
<td>26 ± 2</td>
<td>0.10</td>
</tr>
<tr>
<td>intervention</td>
<td>9 ± 1</td>
<td>11 ± 1</td>
<td>16 ± 2</td>
<td>17 ± 1</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 2</td>
<td>10 ± 1</td>
<td>0.03</td>
</tr>
<tr>
<td>intervention</td>
<td>4 ± 0</td>
<td>8 ± 0</td>
<td>6 ± 1</td>
<td>7 ± 0</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Fibre (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>23 ± 1</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>21 ± 1</td>
<td>0.14</td>
</tr>
<tr>
<td>intervention</td>
<td>23 ± 1</td>
<td>30 ± 1</td>
<td>21 ± 1</td>
<td>24 ± 1</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>984 ± 69</td>
<td>844 ± 79</td>
<td>824 ± 61</td>
<td>932 ± 62</td>
<td>0.30</td>
</tr>
<tr>
<td>intervention</td>
<td>648 ± 47</td>
<td>637 ± 42</td>
<td>697 ± 44</td>
<td>719 ± 42</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Iron (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>14 ± 1</td>
<td>0.21</td>
</tr>
<tr>
<td>intervention</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Zinc (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>13 ± 2</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
<td>0.19</td>
</tr>
<tr>
<td>intervention</td>
<td>8 ± 0</td>
<td>7 ± 0</td>
<td>11 ± 0</td>
<td>12 ± 1</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SE intake/day.  \(^2\) Intakes were based on the analysis of 3-day food diaries completed at baseline and during week 4 and week 8 (average is shown as ‘intervention’).  \(^3\) P value for differences among the 4 diets, one-way analysis of variance with Bonferroni adjustment.
Figure 27: Target and actual macronutrient distribution (% total energy) in each of the four diets.

There were however differences in fat intake during the intervention. All groups reduced fat intake but the high protein groups ate more fat, achieving closer to the target of 30% energy, than the high carbohydrate groups who consumed closer to 20% energy from fat. In absolute terms this difference was between 8 and 16g per day (p < 0.001, Table 12). Accordingly, the high protein groups ate more of each type of fat although the ratio of saturated to unsaturated fatty acids remained constant (0.7, 0.5, 0.5, 0.5, DIETS 1, 2, 3 and 4 respectively). Cholesterol intake was relatively low (less than the recommended daily intake of 300mg) in all groups but as expected there were significant differences among the groups (p<0.001), with higher intake in the two high protein groups (DIET 3 293 ± 18mg, DIET 4 239 ± 18mg) than the two high carbohydrate groups (DIET 1 125 ± 10mg, DIET 2 119 ± 14 mg).

Figure 28 illustrates the changes from baseline in grams per day of each macronutrient. All groups reduced their carbohydrate intake, but to a greater extent
in the high protein groups (-20%, -16%, -41%, -43%, DIETS 1, 2, 3 and 4 respectively); the high carbohydrate groups (DIETS 1 and 2) reduced their protein intake, while the high protein groups maintained their usual protein intake; all groups reduced their fat intake but to a greater extent in the high carbohydrate groups. There was no difference in apparent energy intake during the intervention (p = 0.41, Table 12, Figure 29).

In summary therefore, the reduction in energy in the high carbohydrate groups (DIETS 1 and 2) came primarily from a dramatic reduction in fat with smaller reductions in carbohydrate and protein, whereas the reduction in energy in the high protein groups (DIETS 3 and 4) came from similar reductions in both carbohydrate and fat, with protein intake remaining constant.
Figure 28: Changes in grams of carbohydrate, protein and fat per day from baseline in each diet group as calculated from food diaries. Values expressed as means ± SEM.

Figure 29: Change in energy intake from baseline in each diet group calculated from food diaries. Values expressed as means ± SEM.
3.6.2 Glycaemic index and glycaemic load

All groups appeared to achieve their target GI and GL, and the difference among groups was highly significant (p<0.001, Table 11). In pairwise comparisons, there was a 2-fold difference in glycaemic load between the two extreme diets (129 ± 8 vs 59 ± 4 g/day, DIET 1 vs DIET 4 respectively, p < 0.001), but the intermediate diets were not different from each other (89 ± 5 vs 75 ± 3 g/day, DIET 2 vs DIET 3 respectively, p = 0.25). The glycaemic index was significantly different (p<0.001) between all group pairs except DIETS 2 and 4. In other words, as intended, DIETS 2 and 4 had the same GI but varied in GL, whereas DIETS 2 and 3 had the same GL but varied in GI (Figure 30).
Figure 30: Box plots of calculated GI and GL of the four diets based on food diaries completed during weeks 4 and 8 of the intervention. DIET 1 = high CHO, high GI; DIET 2 = high CHO, low GI; DIET 3 = high protein, high GI; DIET 4 = high protein, low GI.
3.6.3 Dietary fibre

Mean dietary fibre intake was similar in DIETS 1, 3 and 4, but significantly higher in DIET 2 being the only group to achieve the target intake (30 g/day, p < 0.001, Table 12). Compared to baseline this was a significant improvement (an extra 10g per day), whereas in all other groups, intervention fibre intakes were similar to baseline diet (Figure 31).

![Graph showing dietary fibre intake across different diets.](image)

**Figure 31:** Mean daily fibre intakes at baseline and during the intervention in each diet group. Values are calculated from food diaries and expressed as mean ± SEM.
3.6.4 Minerals: calcium, iron and zinc

Mean calcium intake was lower during the intervention than at baseline in all four groups, with no significant differences among groups in intake during the intervention (p=0.50, Table 12, Figure 30). There were however, significant differences among groups in the intake of iron and zinc (p<0.01 and p<0.001 respectively), being higher in both high protein groups (Table 12, Figure 32).

Figure 32: Mean daily intakes of the minerals calcium, iron and zinc during the intervention for each of the four diets. Values are calculated from food diaries and are expressed as mean ± SEM.
3.7 Prevalence of Metabolic Syndrome Risk Factors

The International Diabetes Federation (IDF) definition of the metabolic syndrome is the presence of three or more of the following risk factors:

- high waist circumference of ≥ 94cm for men; ≥ 80cm for women
- raised triacylglycerols >1.7 mmol/l
- low HDL-cholesterol <0.9mmol/l for men; <1.1 mmol/l for women
- raised fasting plasma glucose >5.6 mmol/l

Using this definition the percentage in each group with two or more of the above risk factors for metabolic syndrome reduced in all groups with no significant differences between groups (Figure 33).

![Graph showing percentage of each diet group with two or more metabolic syndrome risk factors at baseline and on completion of the 12-week intervention. Circles represent the high carbohydrate diets; squares the high protein diets; solid line the high-GI diets; and dashed line the low-GI diets.](image)

Figure 33: The percentage of each diet group with two or more metabolic syndrome risk factors at baseline and on completion of the 12-week intervention. Circles represent the high carbohydrate diets; squares the high protein diets; solid line the high-GI diets; and dashed line the low-GI diets.
The most prevalent risk factor at baseline was central obesity, followed by low HDL-cholesterol, high TAG and the least prevalent was high fasting plasma glucose (FPG). Within each group the percentage with each individual risk factor improved, except the percentage with high TAG in DIET 3 which remained unchanged (Figure 34).

Figure 34: Percentage in each diet group with metabolic syndrome risk factors (IDF definition) central obesity, high TAG, low HDL-cholesterol and high fasting plasma glucose (FPG) at baseline and on completion of the 12-week intervention. (NB identical figures for percentage with high FPG on DIETS 2 & 3)
3.8 Subjective responses to assigned diet

There were no statistically different responses among the groups to any of the questions in the end of study questionnaire (Table 13). In response to whether the diet was difficult to follow, the mean of each diet group was around the “slightly difficult” marker. All groups responded similarly to the question regarding the liking of foods and meals, selecting around the “like slightly” category most often. The diets appeared to be equally filling, with subjects in all groups selecting between the “moderately filling” and “very filling” categories most often. There was a tendency for subjects in DIET 2 (the high carbohydrate/low-GI group) to report less hunger between meals, while those in DIET 1 (the high carbohydrate/high-GI group) tended to report the greatest hunger between meals, however this difference was not statistically significant (p=0.53).

The most notable difference, although again not statistically different, was in response to the question regarding the perceived difficulty in following this type of diet permanently. Subjects in DIET 2 and DIET 3 tended to report they would find it “slightly difficult”, whereas those in DIET 1 and DIET 4 rated a greater degree of perceived difficulty, responding closer to the “moderately difficult” category.
Table 13: Participant responses to end of study questionnaire\(^1\)

<table>
<thead>
<tr>
<th>Question</th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
<th>(p) value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you find the diet difficult to follow?</td>
<td>33 ± 5</td>
<td>30 ± 4</td>
<td>30 ± 4</td>
<td>37 ± 4</td>
<td>0.70</td>
</tr>
<tr>
<td>(not at all difficult – extremely difficult)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Considering the diet as a whole, how much did you like the foods/meals?</td>
<td>103 ± 3</td>
<td>107 ± 2</td>
<td>99 ± 4</td>
<td>98 ± 6</td>
<td>0.33</td>
</tr>
<tr>
<td>(dislike extremely – like extremely)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were the meals sufficiently filling?</td>
<td>86 ± 3</td>
<td>86 ± 3</td>
<td>84 ± 5</td>
<td>78 ± 4</td>
<td>0.40</td>
</tr>
<tr>
<td>(not at all filling – extremely filling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rating the diet as a whole, how hungry were you between meals?</td>
<td>41 ± 5</td>
<td>32 ± 5</td>
<td>36 ± 4</td>
<td>37 ± 4</td>
<td>0.53</td>
</tr>
<tr>
<td>(not at all hungry – extremely hungry)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How difficult would you find it to follow this type of diet permanently?</td>
<td>48 ± 7</td>
<td>35 ± 5</td>
<td>36 ± 5</td>
<td>50 ± 7</td>
<td>0.17</td>
</tr>
<tr>
<td>(not at all difficult – extremely difficult)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values expressed as mean ± SEM. Calculated from measured response on a 0-120mm scale with five marker categories. \(^2\) One way ANOVA for each of the numerical questions.
Question 5 asked subjects to list any benefits or side effects from their assigned diet (Table 14). On the whole the responses were positive with subjects in all groups reporting some benefits, such as less bloating, more energy (particularly in DIET 2), better skin or an increased awareness of their diet.

Of the side effects listed, the most notable difference among the groups was that five subjects in each of the high protein groups (DIETS 3 and 4) reported constipation being a problem, whereas only one subject (from DIET 2) in the high carbohydrate groups (DIETS 1 and 2) noted this effect. In fact two subjects in each of the latter groups noted better bowel movements as a benefit of their assigned diet, whereas no-one in either higher protein group reported such a benefit. Increased flatulence was noted only in the low-GI groups with four subjects in DIET 2 and one in DIET 4 reporting this side-effect. Interestingly, while increased energy was noted by several subjects in all groups, only DIET 1 had any reports of tiredness as a side-effect, albeit by only three subjects. One subject in DIET 3 reported bad breath as a side effect, and one in DIET 4 reported migraines as a problem. The subject attributed this to the increased red meat consumption but of course no cause effect relationship can be established.
### Table 14: Reported benefits and side effects of each diet group

<table>
<thead>
<tr>
<th>Benefits</th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better bowel movements</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less bloating</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>More energy</td>
<td>4</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Better skin</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased awareness of diet</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Better concentration</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Side effects</th>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipation</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tired</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cravings for other foods</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Bad breath</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

1 Number in each group reporting benefit/side effect.
3.9 Profile Days

3.9.1 Day-long glucose and insulin excursions

Postprandial glucose (Figure 35) and insulin (Figure 37) concentrations fluctuated across the course of the day as predicted by the calculated GI and glycaemic load of the meals. The incremental glucose AUC over 10 hours was highest for DIET 1 (315 ± 36 mmol/L.min) and lowest for DIET 4 (196 ± 30 mmol/L.min with significant differences among the groups (p=0.023, Figure 36). In pairwise comparisons only these two extremes were significantly different from each other (p=0.004).

The insulin profiles followed the same pattern with the insulin AUC being highest for DIET 1 (7940 ± 1160 pmol/L.min) and lowest for DIET 4 (800 pmol/L.min) with significant differences among the groups (p=0.005, Figure 38). In pairwise comparisons DIETS 3 and 4 were significantly different to DIET 1 (p=0.009, DIET 1 vs 3; p=0.001, DIET 1 vs 4).

Glycaemic load was significantly correlated with blood glucose AUC (r=0.35, p=0.022) and insulin AUC (r=0.35, p=0.021). Varying the GI had a stronger effect (p=0.026) on glycaemia than varying the protein/carbohydrate content (p=0.046), whereas the latter had a greater effect on insulinaemia (p=0.002).
Figure 35: Plasma GLUCOSE excursions over 10 hours for each of the four diets (n=11). Circles represent the high carbohydrate diets; squares the higher protein diets; solid lines the high-GI diets; and dashed lines the low-GI diets.

Figure 36: Incremental glucose AUC over 10 hours for each of the four diets (n=11). Values expressed as mean ± s.e.m. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
Figure 37: Plasma INSULIN excursions over 10 hours for each of the four diets (n=11). Circles represent the high carbohydrate diets; squares the higher protein diets; solid lines the high-GI diets; and dashed lines the low-GI diets.

Figure 38: Incremental insulin AUC over 10 hours for each of the four diets (n=11). Values expressed as mean ± s.e.m. DIET 1 = high CHO, high-GI; DIET 2 = high CHO, low-GI; DIET 3 = high protein, high-GI; DIET 4 = high protein, low-GI.
4.1 Weight loss and body composition

To our knowledge, this study is the only randomised controlled trial that has simultaneously compared high protein and low GI diets for weight loss and metabolic responses. All four diets resulted in weight loss (-4.2 to -6.2% of initial body weight) of the magnitude that would be expected with reduced-fat diets, with no significant differences among the groups. However, subjects adhering to the high-carbohydrate, low-GI diet (DIET 2) or the high-protein, high-GI (DIET 3) diet were twice as likely to achieve the clinical goal of >5% weight loss. Our results therefore support the use of reducing fat intake as a successful strategy for weight loss, but also suggest that the efficacy of reduced-fat diets can be improved by lowering dietary GL.

Men and women responded differently to the intervention diets and the removal of the men from the analysis gave clearer and more significant results. Unfortunately there were insufficient numbers to carry out a meaningful sub-analysis on men alone, but on the whole they tended to do better on the high-protein, low-GI diet (DIET 4), while responding similarly to the other three diets. In contrast women, who comprised 76% of the total group, lost significantly more weight and 80% more body fat on the two intermediate reduced-GL diets (DIETS 2 and 3) than those on the conventional reduced-fat diet with the highest GL (DIET 1, p=0.007).
These results suggest that dietary glycaemic load may be more relevant to women than men, at least during weight loss. Women generally lose weight more slowly and display differences in postprandial glucose and fat oxidation which might influence rate of fat loss (175). This may be due in part to body composition differences; men have on the whole more muscle and less fat than women. More active muscle tissue allows for greater glucose disposal. This may make men less susceptible to the metabolic consequences of a high-GI diet, particularly when total food intake has been reduced to achieve weight loss - the relative differences in GL between the diets is greater in energy restricted diets. There is some evidence that GI and/or GL may also have a bigger impact on women’s cardiovascular disease risk and this may explain in part the differences reported between studies. In meta-analyses, the relative risk (RR) of CVD associated with high 120-min post-load glucose concentration, is higher in women than men (RR 1.56 versus 1.23 respectively) (69). In American women, average dietary GI and/or glycaemic load were independent predictors of 10-yr prospective CVD risk (68), TAG (75), HDL-cholesterol (75) and CRP concentrations (176). While in elderly Dutch men, no relationship was found between GI and risk of CHD (77).

In the diet group as a whole, or in women only, our hypothesis that the lowest GL approach (DIET 4) would produce the greatest outcomes was not supported and in fact women assigned to this diet did no better than those following the conventional approach. One explanation might be that this diet was more complicated than the other approaches; this group had to focus on consuming both more protein and low-GI foods. One of the reasons cited elsewhere for the success of high-protein, low-carbohydrate diets has been the simplicity of the dietary advice and forced energy
restriction due to the greater limitations on food choice (105). Similarly it may be that more ‘extreme’ diets require greater discipline that declines over time. There was no evidence from the food diaries to support either explanation; however it is highly likely that dietary adherence was greatly improved in all groups during recording periods, making it difficult to accurately ascertain energy intakes over the full 12-weeks. Alternately genetic or metabolic predisposition may need to be taken into account to determine the effectiveness of one diet over another (177). However, we must also consider that this effect may be real and not due to dietary compliance factors. In essence our results showed that the optimum weight loss diet is not the one with the lowest GL. Perhaps there is an optimal range of GL that promotes the most favourable fuel oxidation mix for weight control and the promotion of spontaneous and planned activity, while stimulating the correct balance of hormones involved in metabolism. For example while it is generally accepted that lowering insulin secretion will promote fat oxidation and reduce fat storage, over the long term a very low secretion of insulin may be counter-productive to weight control; insulin has been shown to positively influence the synthesis and secretion of leptin, independently of changes in adiposity (178), and leptin appears to be involved in long-term energy balance, altering appetite, nutrient flux and energy expenditure (179).

The high-protein, low-GI diet (DIET 4) was the most effective, producing the greatest fat losses and improvement in the total-HDL cholesterol ratio, in subjects with hypertriglyceridaemia at baseline. A similar result was obtained in a CSIRO study: subjects with high TAGs at baseline lost more weight on a high-protein compared to high-carbohydrate diet, despite no differences in the group overall
These results suggest that dietary GL may be of greater importance in this vulnerable sub-group. Interestingly a criticism of low-fat, high-carbohydrate diets has been that they often (but not always) increase TAGs (180, 181). It is likely that there are genetic differences that account for an individual’s response to a high-carbohydrate diet, such that those with high TAGs may respond best to a low GL diet.

We did not find that subjects with hyperinsulinaemia at baseline responded differently to those with normal fasting insulin, as has been found previously (102, 119). Hyperinsulinaemic subjects showed a similar pattern of weight and fat loss to the group overall, with the two intermediate GL diets producing the best outcomes. The findings imply that conventional low-fat diets are not the best approach for the treatment of insulin resistance, and reduced GL diets may improve outcomes.

Neither did we find, as has been previously reported (101, 104, 120), that a high-protein diet better preserves lean mass during weight loss than a high-carbohydrate diet. Indeed lean mass losses were small across all groups. This is likely to be due to the relatively modest energy restriction and weight loss each week over the course of the intervention in all groups. Interestingly, while the differences among the four diet groups were not significant, both high-GI diets (DIET 1 and 3) showed statistically significant declines in lean mass over 12-weeks in contrast to non-significant changes in the two low-GI groups. This finding supports previous studies suggesting improved nitrogen balance (39, 60) and higher resting metabolic rate after weight loss (53) on low-GI diets. Beneficial changes in body composition, despite no differences in body weight, have been previously demonstrated with low- compared
to high-GI diets in both animals (54) and humans (63). Further studies employing
greater energy restriction might clarify the issue.

Central obesity was the most prevalent metabolic syndrome risk factor in all groups,
with ≥94% of each group having a waist circumference ≥94cm for men or ≥80cm for
women at baseline. While all groups achieved a significant reduction in waist
circumference with no statistical significance among groups, the trend was similar to
both weight and fat loss, with the high-carbohydrate, low-GI (DIET 2) and the high-
protein, high-GI groups (DIET 3) achieving the greatest losses, particularly in
women. Furthermore, all three reduced-GL diet groups had better reductions,
compared to the conventional diet group, in the percentage of subjects with a waist
circumference above the cut-offs on completion of the 12-week intervention.

4.2 Blood Lipids

Despite similar reductions in fat mass on DIETS 2 and 3, there were very different
effects on blood lipids. The low-GI, high-carbohydrate diet (DIET 2) was the most
efficient at reducing total and LDL-cholesterol. In contrast, the high-protein, high-
GI diet (DIET 3) produced an elevation of total and LDL-cholesterol (+8% overall,
+10% in women). There were apparent differences in fat intake between these two
groups, however this cannot explain the results: DIET 4 also ate more fat but did not
show any rise in LDL-cholesterol and DIET 1 reduced fat intake to a similar extent
as DIET 2 but did not show the same fall in LDL-cholesterol. Energy-restricted,
high-protein, low-fat diets have generally not been associated with undesirable
changes in serum lipids (61, 113, 143). Nonetheless, there is the potential for high
intake of animal products and dietary cholesterol to have adverse effects on plasma lipids (182) and any rise in LDL-cholesterol is of concern. Foster et al. also reported a significant rise in LDL-cholesterol after three months on a high-protein-low carbohydrate (Atkins) diet that diminished after 12 months (106).

Differences in fibre intake may partially account for these differences. The low-GI, high-carbohydrate group (DIET 2) appeared to be consuming approximately 9g/d more fibre than the high-protein, high-GI group (DIET 3), and were the only group to meet the fibre RDI of 30g/d. Soluble fibres that form a gel in the intestines, can bind cholesterol in the gut, reducing absorption, and thus reduce both total and LDL cholesterol (183). These gel-forming fibres also slow the access of digestive enzymes, thereby slowing the absorption of sugars from carbohydrate-containing foods. This means that foods rich in soluble fibre are almost always low-GI. Oats, legumes, psyllium-containing cereals (e.g. Kellogg’s Guardian®) and most fruits are all good examples of low-GI, high soluble fibre foods and all were included in the eating plans for DIETS 2 and 4. A meta-analysis of the effect of soluble fibre on cholesterol estimated that 3g of soluble fibre can decrease total and LDL-cholesterol by ~0.13 mmol/L (183). The observed reductions in the low-GI, high-carbohydrate group (DIET 2) were 0.18 mmol/L and 0.17 mmol/L for total and LDL-cholesterol respectively. If a large proportion of the increased fibre intake in this group came from soluble fibre, these changes are in accordance with the literature.

We specifically chose lean red meat rather than other protein sources because of the belief that it adversely affects plasma lipids (182). Our results do not however suggest that high-protein diets rich in red meat will necessarily be detrimental to
blood lipid profiles since our second high protein group (DIET 4) showed no unfavourable effects. Moreover, the rise in total and LDL-cholesterol was associated statistically with differences in GI rather than protein in the diet (p < 0.009). Since the quantity of fat, type of fat and fibre were similar on both high protein diets, the divergent effects might be explained by the soluble fibre component of the low GI foods included in DIET 4 (184). In other words choosing low-GI carbohydrate foods seems to be important, even at lower total carbohydrate intakes. These findings imply that it may not be sufficient to recommend simply ‘wholegrains’, but low GI-wholegrains, particularly in the context of high meat intake.

In contrast to previous studies (101, 106, 107, 113) we did not find that TAGs fell more on a high protein diet. In fact TAGs fell in all groups, including the conventional low-fat, high-carbohydrate group (DIET 1), with no differences among the four groups. This may be explained by the fact that all four diet groups reduced their total carbohydrate intake from their usual diet, albeit to a greater extent on the two high protein diets. Secondly we purposely chose to use moderate carbohydrate, high protein diets, whereas many of the published high protein trials to show a greater reduction in TAGs have used low carbohydrate diets. Neither did we find that TAGs decreased more on a low-GI, compared to high-GI diet as others have shown (53, 65). Alternately, the failure to see an effect of less carbohydrate or lower GI on TAG levels may have been due to the fact that 70% of our subjects had TAG levels within the normal range at baseline.

A number of studies have previously shown low-fat, high-carbohydrate diets reduce HDL-cholesterol levels (35). We did not find this to be the case and in fact all four
diet groups improved their HDL-cholesterol levels, with no differences among the groups. This may have simply been reflective of the overall improvement in diet quality in all groups.

4.3 Postprandial and day-long profiles

The results from the profile days clearly demonstrate that reducing dietary GL by either lowering the GI of carbohydrate-containing foods, or by partial substitution of carbohydrate with protein, reduces both post-prandial and day-long glucose and insulin excursions. Furthermore combining these two dietary strategies to produce the lowest GL, produced the lowest glucose and insulin AUCs as predicted. This is important as it provides evidence to support the key mechanisms thought to operate in both low-GI and high-protein diets.

Data from the weight loss trial showed no significant differences among the groups in fasting insulin levels. The trend, however, was for a greater fall in insulin in the two groups who lost the most body fat (DIETS 2 & 3). Similarly, there was no measurable effect of diet composition on HOMA insulin sensitivity. Previous studies have shown that high-GI diets decrease, while low-GI diets improve, insulin sensitivity (64, 79). Subjects in those studies were in weight maintenance, however. Since weight loss per se improves insulin sensitivity (185-189), this may have overridden any effect of GI or carbohydrate content on insulin sensitivity.

The fall in fasting insulin and improvements in insulin sensitivity correlated with the change in fat mass. What is not known is which comes first; does a lowering of
insulin ‘demand’ result in better fat oxidation, or does a reduction in body fat lead to an improvement in insulin sensitivity, which in turn reduces pancreatic insulin output? If we assume good dietary adherence in all four diet groups, our data supports the latter. Subjects in the group with the lowest GL (DIET 4) did not have the greatest fall in fasting insulin. However, fasting insulin may not be a good measure of ambient insulin levels. Previous studies have shown that despite no differences in fasting insulin, a low-GI diet reduces the insulin required to deal with a glucose challenge (64, 78, 190). It is also possible that dietary adherence may not have been as good (despite food diary records to the contrary) in those assigned to DIET 4 and this reflected in their blood biochemistry findings.

It was not surprising that there was no change in fasting glucose levels, since baseline values were all in the normal range. As with insulin, fasting values are not reflective of overall glucose excursions (64).

4.4 Other blood parameters

Diet composition had significant effects on changes in leptin (p<0.006), falling more on DIET 2 and least on DIET 4. While the differences might imply greater improvement in leptin resistance on a high-carbohydrate-low-GI diet, or less hunger in the high-protein-low-GI group, the finding should be interpreted conservatively. On an individual basis, the absolute fall in leptin correlated significantly with the change in body fat mass (r=0.27), with no additional effect of GI or carbohydrate content.
Several previous studies have reported a correlation between insulin and leptin levels (178, 191, 192), and suggested that the greater the fall in plasma insulin concentration following weight loss, the greater the fall in plasma leptin concentration (193). We found no correlation between fasting levels, or change, in levels of insulin and leptin at any time point. We found no relationship between fasting levels of the two hormones as reported previously. Insulin excursions over the course of day may be needed to show a relationship; Carantoni et al. (193) found day-long insulin to be the only variable significantly correlated to the leptin response to weight loss. Furthermore the GI of the carbohydrates in the diet has previously been shown to alter the diurnal pattern and amount of leptin secretion, which is not revealed by a simple morning fasting sample (194). Since leptin is thought to be involved in the long-term control of energy balance, any influence of GI on plasma leptin may help to explain effects on body weight and fat levels independently of energy intake. Further research to include more frequent monitoring of leptin in response to diet is warranted.

Plasma high-sensitivity C-reactive protein (hs-CRP), as a marker of chronic low-level systemic inflammation, has been shown to be a strong independent predictor of cardiovascular disease (195, 196). In the Women’s Health Study dietary glycaemic load was significantly and positively associated with plasma hs-CRP (82). The proposed mechanism is that the repeated glucose ‘spikes’ that occur following chronic intake of high GI carbohydrates, exacerbate the proinflammatory process, increasing the risk of cardiovascular damage leading to disease. We were therefore interested to see whether plasma hs-CRP levels would fall in line with the glycaemic load of the diet. We did not find this to be the case and there were no significant
differences among the groups. In fact we found that hs-CRP levels fell in all groups, and although there was a marginally significant correlation with the change in fat mass, the groups with the largest fat loss did not show a larger fall in hs-CRP. This may have been due to the fact that all groups reduced absolute carbohydrate intake, and thus the glycaemic load of their diet. Furthermore, all groups lost weight during the intervention period. Taken together, these factors may have masked any additional effect of GI or GL. Further research is needed to measure the effect of GI and GL on hs-CRP during weight maintenance.

4.5 Dietary adherence

Achieving and evaluating dietary compliance is notoriously difficult in free-living situations and there is as yet no fool-proof method. Food diaries are the most common means of estimating food intake but they have serious limitations; underreporting is a well-recognized phenomenon, particularly in overweight subjects. Nevertheless they provide valuable information, particularly with regard to highlighting any differences among the groups, since all are likely to underreport to the same extent.

It was interesting to note that both high-carbohydrate groups tended to consume far less fat than their dietary instructions advised. This probably reflects the widespread belief that high fat foods are ‘fattening’ and so despite advice to include a specified number of fat-rich foods, these were avoided. In contrast, those assigned to the high-protein diets consumed more fat intrinsic to protein-rich foods, so that any avoidance of fat-rich foods had less overall impact on their total fat intake. As a result both high
protein groups achieved a macronutrient profile closer to target than the high carbohydrate groups. Nevertheless, aside from the differences in fat, overall the food diaries reflected fairly accurately the targeted dietary changes and provided good evidence that the primary objectives of high carbohydrate or protein, and differences in GI and GL were indeed achieved.

According to the food diaries, all diet groups reduced their energy intake to a similar extent. Hence there is no evidence to suggest greater satiety leading to lower energy intake among the groups consuming a low glycaemic load. A direct effect of macronutrient composition on fuel partitioning might therefore be responsible for differences in weight and fat loss. However, because food diaries are a ‘blunt’ tool, we cannot rule out the possibility that they are not sufficiently accurate to detect differences of 100kJ/day between groups (the energy deficit that would explain a 2kg difference in weight over 12 weeks).

An important nutritional advantage of the two high meat diets was that iron and zinc intakes were significantly higher than on the high carbohydrate diets. While RDIs were met for both minerals on the high-protein diets, iron intake on the high carbohydrate diets fell short of the RDI (adult women aged 19-54yrs) by about 20%, and zinc by about 40%. Iron is the most common nutrient deficiency, affecting both developed and developing countries, and female ‘dieters’ are particularly at risk (197). Furthermore the bioavailability of haem iron from red meat is far greater than non-haem iron found in plant food sources. Similarly animal foods provide the richest source of zinc, and the avoidance of animal foods makes it extremely difficult to meet demands for this mineral. Dietary strategies to gain the health benefits of
eating a high proportion of animal foods, while reducing or eliminating any negative outcome, are needed. Consuming a large amount of fruits, vegetables and low-GI wholegrains alongside a relatively high animal food intake may prove to be one such strategy – coincidentally not too far removed from the diets of our ancestors (130).

Several recent studies, but not all (198), have shown that a high dairy and calcium intake can augment weight and fat loss during energy restriction (199-201), possibly through reducing fat absorption and increasing faecal fat (202). We purposefully therefore included the same number of dairy servings in the eating plan for each diet group. The subject food diaries confirmed a similar calcium intake for each group, ruling out any likely effect of calcium or dairy intake on the outcomes.

### 4.6 Study strengths & limitations

A major strength of the study design was that it compared four diets simultaneously, something that is atypical in nutrition research. This allowed us to evaluate the effects of low-GI and high-protein advice independently and additively, providing unique insight into these two increasingly popular weight loss strategies. A particular strength was extensive knowledge of the GI of individual Australian foods (169) and the fact that we were able to provide subjects with brand-name foods whose GI had been previously tested according to standardised methods. A major pitfall of many GI studies overseas has been the lack of reliable GI data for local foods and GI assignment has been based on extrapolation or in vitro methodology (66, 203). This may account in part for inconsistency in study outcomes. Importantly, the day-long profiles confirmed that the four diets produced differential postprandial responses as
predicted by their calculated glycaemic load, CHO content and GI. This confirmed that our eating plans, if adhered to correctly, would indeed elicit the metabolic responses hypothesised to assist weight and fat loss. The vast majority of studies have not tested their diets in this way. Dietary compliance was maximised by the provision of key foods, particularly red meat portions and low- or high-GI carbohydrates, and ready-made meals. A further strength was that the subjects were free-living, young adults who represent an important target for early intervention, and who were encouraged to ‘eat-to-appetite’ within the study design. Thus postulated differences in hunger and satiety responses to low GI carbohydrate and high protein foods could operate freely. Finally, the large sample size, high continuation rate, and the detailed and repeated ascertainment of dietary measurements increase the reliability and sensitivity of the data.

The study has limitations. Although a similar fat and fibre intake was targeted in all four groups, subjects on DIETS 3 and 4 consumed more fat, and those in DIET 2 more fibre. However these differences are interesting in themselves as they may reflect inherent characteristics of the diets that might also operate outside the research setting. At 12-weeks, the length of the study was relatively short and therefore provides no information about the sustainability or long-term effects of the diets on CVD function, exercise tolerance, renal function or bone health. However a longer timeframe would not have allowed us to achieve the same intensity of intervention, particularly in terms of weekly contact and the provision of foods (involving a high degree of organisation and comprising the biggest expense). Furthermore, from a medical treatment point of view, a three month period is a common timeframe for a ‘diet only’ intervention (204); insufficient weight loss at
this point in time might dictate pharmacological approaches with both expense and side effects. As a first trial examining the effects of glycaemic load therefore, three months was deemed appropriate before embarking on a more costly, longer term intervention.

4.7 Conclusions & further directions

The findings imply there is more effective advice than the conventional low fat diet. Both low GI and high protein diets can increase the rate of fat loss and improve CVD risk within a three month intervention. Women and individuals with high TAG may benefit most, while a high-carbohydrate, low-GI diet appears to be the most efficient way to reduce both body fat and LDL-cholesterol without compromising lean mass. Diet composition, not just overall energy intake, influences the rate of fat loss. The concept of ‘glycaemic load’ may increase our understanding of the determinants of weight loss. Reassuringly, advice to exchange high-GI for low-GI foods can optimise clinical outcomes within currently acceptable nutrition guidelines (55% E as carbohydrate, <30% E as fat) without the concerns that apply to higher protein diets. Multi-centre studies to evaluate weight reduction, weight maintenance and long-term outcomes, particularly in individuals with established risk factors, are clearly warranted.

Future studies should be adequately powered to distinguish effects in women versus men and in those with high versus normal TAG levels. Further research on mechanisms, other than energy restriction, that enhance the rate of fat oxidation and
reduce protein catabolism should be pursued. Finally, subjective and objective assessments of dieting, satiety, hunger and “emotional” eating are important, but often neglected, in obesity research.
Part 5: References


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Part 6: Publications and Presentations

6.1 Publications relating to this work


Popular Publications


6.2 Presentations relating to this work

February 2006 “GI and Health” USANA Health Sciences convention Asia Pacific. Sydney Convention Centre, Darling Harbour.

November 2005 “GI and Chronic Disease” Sydney Adventist Hospital, Wahroonga.

November 2005 “All About Protein”; “What’s hot in Diet Research?”; “Diets & Disease” Plenary Lecture; “Carbohydrate Confusion”. Network’05; Australian Fitness Network convention, Melbourne Convention Centre.

April 2005 “Carbohydrates, Health & Weight Control” FILEX ’05 The Fitness Industry Convention; Sydney Convention Centre, Darling Harbour.

April 2005 “The Glycaemic Index” General Practitioner workshop lecture series (Novodisc); Homebush, Sydney.

January 2005 “Low GI and High Protein Diets for Weight Loss – Applications for Athletes” Australian Institute of Sport Conference; Homebush, Sydney.

June 2004 “Carbohydrates – good or bad?”; “Putting the GI into Practice” FILEX ’04 The Fitness Industry Convention; Sydney Convention Centre, Darling Harbour.

April 2004 “Getting into the GI”; “High protein diets”; “Diet Wars” FitPro™ Convention; Loughborough University, UK.

October 2003 “High Protein or Low GI Diets for Weight Loss?”; “Nutrition for Performance” Re-Energise™, The Nutrition Convention; UK.

October 2003 “The Glycaemic Index” Energy and Excellence Conference; Loughborough University, UK.

YELLOW DIET = DIET 1 (high carbohydrate, high-GI)

<table>
<thead>
<tr>
<th>Protein-rich foods</th>
<th>Carbohydrate-rich foods</th>
<th>Fat-rich foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Choose 1 from the following:</strong></td>
<td><strong>Choose 5 of the following:</strong></td>
<td><strong>Choose 2-3 of the following:</strong></td>
</tr>
<tr>
<td>75g red meat (provided)</td>
<td>¼ cup Sultana Bran or Branflakes</td>
<td>½ Tbsp oil</td>
</tr>
<tr>
<td>75g chicken breast or other lean meat</td>
<td>2 Weet-Bix</td>
<td>2 tsp butter or margarine</td>
</tr>
<tr>
<td>75g fish or seafood</td>
<td>½ cup Sustain</td>
<td>¼ avocado (1Tbsp mashed)</td>
</tr>
<tr>
<td><strong>OR</strong></td>
<td>2 slices bread – choose from:</td>
<td>1 Tbsp mayonnaise</td>
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<tr>
<td></td>
<td>- Wholemeal</td>
<td>1 Tbsp salad dressing</td>
</tr>
<tr>
<td></td>
<td>- Hyfibe (fibre-enriched white)</td>
<td>20g raw nuts</td>
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<tr>
<td><strong>Choose 2 from:</strong></td>
<td>½ bagel</td>
<td>3 tsp peanut butter or tahini</td>
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<tr>
<td>1 egg</td>
<td>1 crumpet</td>
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<tr>
<td>2 slices (40g) deli lean meat</td>
<td>4 Ryvita crackers</td>
<td></td>
</tr>
<tr>
<td>40g tuna (1/2 small can)</td>
<td>1 K-Time bar</td>
<td></td>
</tr>
<tr>
<td>1 slice (20g) reduced fat cheese</td>
<td>1 medium baking potato (150g)</td>
<td></td>
</tr>
<tr>
<td>100g tofu</td>
<td>¼ cup mashed potato</td>
<td></td>
</tr>
<tr>
<td>2 Tbsp cottage cheese</td>
<td>30g raw jasmine rice (¼ cup cooked)</td>
<td></td>
</tr>
<tr>
<td>150g carton low fat yoghurt</td>
<td>1/3 cup (65g) gnocchi</td>
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</tr>
<tr>
<td><strong>PLUS</strong></td>
<td>30g pretzels</td>
<td></td>
</tr>
<tr>
<td>1 cup low fat milk or soy alternative</td>
<td>15 small rice crackers</td>
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<tr>
<td><strong>PLUS</strong></td>
<td><strong>PLUS</strong></td>
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<td></td>
<td>2 fruits: (fresh or canned in own juice)</td>
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<tr>
<td></td>
<td>¼ Melon, 1 cup diced pineapple, banana, ½ papaya,</td>
<td></td>
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<tr>
<td></td>
<td>½ mango or any other fruit</td>
<td></td>
</tr>
</tbody>
</table>

**PLUS**
The following vegetables are unrestricted – ensure you have ≥ 2 cups daily:

lettuce, spinach, broccoli, tomatoes, Asian greens, onions, cauliflower, zucchini, capsicum, carrots, beetroot, sprouts, celery, asparagus, cabbage, cucumber, green beans/peas, pumpkin, rocket, squash, mushrooms, leeks, eggplant, endive, fennel.
YELLOW (DIET 1) MEAL IDEAS

Breakfasts

1. ⅛ cup Sultana Bran or Bran Flakes with low fat milk – top with a serve of fruit if you wish.
2. 2 Weet-Bix with low fat milk and a large wedge of melon.
3. ½ cup Sustain with low fat milk and a sliced banana.
4. K-Time breakfast cereal bar and a piece of fruit.
5. 1 or 2 slices of Hyfibe toasted and topped with 2 tsp butter/margarine and vegemite.
6. 1 or 2 slices of wholemeal bread toasted and topped with ⅛ avocado and a sliced tomato.
7. 1 or 2 slices of Hyfibe toasted and topped with 1 poached egg, a handful of wilted spinach (30secs in the microwave), a tomato and a few dry-fried mushrooms.
8. Mango & pineapple salad topped with 150g low fat fruit yoghurt – option: use a fat serve and sprinkle with 20g raw nuts and seeds.
9. 1 or 2 slices of wholemeal toast topped with 3 tsp peanut butter.
10. A toasted bagel or crumpets (2 carb serves) with 2 tsp butter/margarine and jam.

Light Meals

1. Sandwich made with wholemeal or Hyfibe bread and filled with plenty of salad vegetables eg lettuce, tomato, cucumber, beetroot, grated carrot, sprouts, plus:
   a. ⅛ avocado
   b. 2 Tbsp hummus
   c. 2 Tbsp cottage cheese
   d. 1 slice of reduced-fat cheese
   e. ½ small can of tuna
   f. 2 slices ham or other lean deli meat
   g. 1 hard boiled egg
2. Baked potato served with a mixed salad and filled with:
   a. 40g tuna
   b. 2 Tbsp cottage cheese
   c. 1 Tbsp coleslaw with low fat dressing
3. Serve of vegetable-based soup (add bread if you wish from carb serves)
   a. Potato & Leek Soup*
   b. Herb & Veggie Soup*
   c. Tomato & Basil Soup*
4. 4 Ryvita crackers topped with hummus or lentil spread & tomato.
5. Large bowl of salad veg - choose from spinach, rocket, lettuce, tomatoes, cucumber, capsicum, carrot, mushrooms, sprouts, blanched green beans etc – and use a protein and/or fat serve by adding an egg, ½ small can of tuna, 2 slices of deli meat, 30g feta cheese or ¼ avocado. Bread from allowance.
6. 1 cup of cooked rice with stir-fried veggies and tofu.
Main Meals

1. Veggie Pizza* with a large mixed salad.
2. Bolognaise* served with ½ cup cooked jasmine rice or a baked potato and a green salad.
3. Stir-fry with tofu, chicken, seafood, lamb or beef (using 75g pack of provided meat) – add a variety of chopped veggies and flavour with soy, oyster, teriyaki and/or chilli sauce. Serve with jasmine rice from allowance.
4. Small serve of Beef Casserole* with baked potato wedges or mash and mixed steamed veggies.
5. Grilled/BBQ/Roasted 75g serve of meat with roast potato, beetroot and carrot (drizzle with olive oil and balsamic vinegar and bake for 20-30mins in a hot oven).
6. 75g fillet of fish grilled, baked or pan-fried in a little olive oil and served with rice salad and a green salad. NB make your own rice salad by mixing a cup of cooked rice with sliced spring onion, a finely chopped chilli, a handful of chopped coriander leaves, handful of halved cherry tomatoes, chopped capsicum, cucumber and 1 Tbsp olive oil salad dressing.
7. Small portion of Red or Green Thai Chicken Curry* with jasmine rice from allowance.
8. Mashed potato (use butter and milk from allowance) with a grilled 75g portion of meat or chicken and a large salad.
9. Gnocchi with tomato-based sauce and a green salad.
10. Mushroom & Spinach Risotto* - or make your own (watch fat content).

Snacks

1. Fruit
2. 150g carton low fat yoghurt
3. K-Time cereal bar
4. ½ bagel with low fat cream cheese
5. 1 row of rice crackers
6. Packet of rice/corn low fat chips
7. 20g raw nuts
8. Low fat dip with carrot sticks or Ryvita crackers
9. Slice of bread toasted and topped with a skim peanut butter or tahini
10. Cup of vegetable soup
Pre-Prepared Foods

The following can be counted as a carbohydrate-rich food serve:

- Potato & Leek Soup
- Herb & Vegetable Soup

The following can be counted as 2 carbohydrate-rich food serves:

- Veggie Pizza
- Mushroom & Spinach Risotto

The following can be counted as a protein-rich food serve:

- Thai Chicken Green or Red Curry
- Bolognaise
- Beef Casserole

What do I do if I am eating out?

You should try to eat as much home-prepared meals as possible as this will help you to best stick with your eating plan. However this is a ‘real life’ trial and eating out is a part of normal life. Try not to abandon your eating plan for such meals, but instead choose wisely and you will usually find you can order something in line with your dietary goals.

The following meals would be good choices for you:

- Stir-fries with steamed rice (skip the fried rice)
- Risotto with stock-based or tomato sauce (skip the creamy ones)
- Baked potato with a low fat filling eg cottage cheese or tuna
- Gnocchi with tomato sauce
- Grilled fish, meat or chicken (watch serve sizes) with boiled potatoes or rice & steamed veggies or a side salad
- Turkish sandwich with lean meat & loads of salad veggies
- Veggie soup with a bread roll
- Breakfast meals
  - Toast or a bagel with jam and use only a skim of butter
  - pancakes with fresh fruit
  - Field mushrooms with tomato on toast

Other tips:

- Asian style restaurants are ideal for your plan - choose stir-fries rather than the curries to keep the fat and energy content down
- Watch serve sizes – these are usually larger than you would serve at home.
- Ask for sauces and salad dressings on the side and add a little yourself
Can I drink alcohol?

Alcohol contains a significant amount of energy and can seriously hinder your weight loss attempts if you are drinking too much. However we do not expect you to be saintly for 12 weeks! Your eating plan should be achievable and realistic and if you normally enjoy a drink you may do so within reasonable limits – we suggest no more than 6 drinks a week. If you do overindulge try not to let it upset your resolve to stick with your eating plan – often when we drink it is the food that goes with it that is the problem rather than the alcohol.

A final thought

It is not what you do on the occasion that counts but what happens regularly. The odd meal out with the plan or the odd treat will not affect your overall progress. Try not to allow small upsets to throw you off track – simply move on and continue to make the best choices you can at each and every meal and snack.
BLUE DIET = DIET 2 (high carbohydrate, low-GI)

<table>
<thead>
<tr>
<th>Protein-rich foods</th>
<th>Carbohydrate-rich foods</th>
<th>Fat-rich foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Choose 1 from the following:</strong></td>
<td><strong>Choose 5 of the following:</strong></td>
<td><strong>Choose 2-3 of the following:</strong></td>
</tr>
<tr>
<td>75g red meat (provided)</td>
<td>1 cup All-Bran varieties or Guardian</td>
<td>1/2 Tbsp oil</td>
</tr>
<tr>
<td>75g chicken breast or other lean meat</td>
<td>1/4 cup Komplete oven-baked muesli</td>
<td>2 tsp butter or margarine</td>
</tr>
<tr>
<td>75g fish or seafood</td>
<td>1/3 cup natural muesli</td>
<td>1/4 avocado (1 Tbsp mashed)</td>
</tr>
<tr>
<td><strong>OR</strong></td>
<td>1/3 cup rolled oats</td>
<td>1 Tbsp mayonnaise</td>
</tr>
<tr>
<td><strong>Choose 2 small protein serves from:</strong></td>
<td>1 Up&amp;Go carton</td>
<td>1 Tbsp salad dressing</td>
</tr>
<tr>
<td>1 egg</td>
<td>2 slices bread – choose from:</td>
<td>20g raw nuts</td>
</tr>
<tr>
<td>2 slices (40g) deli lean meat</td>
<td>- Bürgen Soy-Lin or Honey &amp; Oat bran</td>
<td>2 Tbsp hummus</td>
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<tr>
<td>40g tuna (1/2 small can)</td>
<td>- Performax or Ploughman’s Wholegrain</td>
<td>3 tsp peanut butter or tahini</td>
</tr>
<tr>
<td>1 slice (20g) reduced fat cheese</td>
<td>4 Vita-Wheat crackers</td>
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</tr>
<tr>
<td>100g tofu</td>
<td>1/2 cup cooked pasta or noodles</td>
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</tr>
<tr>
<td>2 Tbsp cottage cheese</td>
<td>30g raw Doongara/basmati rice (1/2 cup cooked)</td>
<td></td>
</tr>
<tr>
<td>150g carton low fat yoghurt</td>
<td>1/4 cup raw (1/2 cup cooked) bulgur/barley</td>
<td></td>
</tr>
<tr>
<td><strong>The following foods can be counted as either a carb or a small protein serve:</strong></td>
<td>1 medium ear of corn or 1/2 cup kernels</td>
<td></td>
</tr>
<tr>
<td>1/2 cup beans/chickpeas</td>
<td>1 med sweet potato (100g)</td>
<td></td>
</tr>
<tr>
<td>1/4 cup dry (1/2 cup cooked) lentils</td>
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<tr>
<td><strong>PLUS</strong></td>
<td><strong>PLUS</strong></td>
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</tr>
<tr>
<td>1 cup low fat milk or soy alternative</td>
<td>2 of the following fruits: (fresh or canned in own juice)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple, pear, orange, mandarin, 2 plums,</td>
<td></td>
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<tr>
<td></td>
<td>2 apricots, nectarine, peach, grapefruit,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/2 cup strawberries</td>
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</tr>
</tbody>
</table>

**PLUS**

The following vegetables are unrestricted – ensure you have ≥ 2 cups daily:

lettuce, spinach, broccoli, tomatoes, Asian greens, onions, cauliflower, zucchini, capsicum, carrots, beetroot, sprouts, celery, asparagus, cabbage, cucumber, green beans/peas, pumpkin, rocket, squash, mushrooms, leeks, eggplant, endive, fennel.
BLUE (DIET 2) MEAL IDEAS

Breakfasts

11. 1 cup All-Bran or Guardian with low fat milk – top with a serve of fruit if you wish eg sliced strawberries or a spoonful of canned peaches.
12. ¼ cup Komplete oven-baked muesli with low fat milk – top with a serve of fruit if you wish eg sliced apple or grapefruit segments.
13. 1/3 cup natural muesli with low fat milk and ½ grapefruit.
14. Cover 1/3 cup rolled with water and cook in the microwave for 2 minutes. Stir through a little low fat milk and cook again for a further minute. Sprinkle with 1tsp brown sugar and serve. Options:
   a. use a fruit serve and add chopped or grated apple
   b. use a fat serve and add a Tbsp chopped nuts &/or seeds.
15. Carton of Up & Go and a piece of fruit.
16. 1 or 2 slices of Bürgen toasted and topped with a small can of baked beans.
17. 1 or 2 slices of Bürgen toasted and topped with ¼ avocado and a sliced tomato.
18. 1 or 2 slices of Performax toasted and topped with 1 poached egg, a handful of wilted spinach (30secs in the microwave), a tomato and a few dry-fried mushrooms.
19. 1 cup of fruit salad topped with 150g low fat natural yoghurt – option: use a fat serve and sprinkle with 20g raw nuts and seeds.
20. 1 or 2 slices of Ploughman’s Wholegrain toasted and topped with 3 tsp peanut butter.

Light Meals

7. Sandwich made with allowed breads and filled with plenty of salad vegetables eg lettuce, tomato, cucumber, beetroot, grated carrot, sprouts, plus:
   a. ¼ avocado
   b. 2 Tbsp hummus
   c. 2 Tbsp cottage cheese
   d. 1 slice of reduced-fat cheese
   e. ½ small can of tuna
   f. 2 slices ham or other lean deli meat
   g. 1 hard boiled egg
   h. lentil spread
8. Serve of bean or lentil soup (add bread if you wish from carb serves)
   a. Tomato & Barley Soup*
   b. Pumpkin, Sweet Potato & Cumin Dhal Soup*
   c. Sweet Potato, Carrot & Ginger Soup*
   d. Tasty Tomato, Sweet Potato & Basil Soup*
   e. Minestrone
   f. Lentil soup
9. 4 Vita-Wheat crackers topped with hummus or lentil spread & tomato.
10. Large bowl of salad veg - choose from spinach, rocket, lettuce, tomatoes, cucumber, capsicum, carrot, mushrooms, sprouts, blanched green beans etc – and use a protein and/or fat serve by adding an egg, ½ small can of tuna, 2 slices of deli meat, 30g feta cheese, ¼ avocado or a cup of mixed beans.
11. 1 cup of cooked pasta (2 carb serves) with tomato-based sauce and a large salad.
12. 2 sushi rolls or 1 mixed box of sushi.

**Main Meals**

11. 1 cup of cooked pasta (2 carb serves) topped with Bacon, Beans & Tomato Sauce* (or any other tomato-based sauce) and a large mixed salad.
12. Spicy Vegetable Chickpeas* served with ½ cup cooked Doongara/basmati rice and a green salad.
13. 1 cup (2 carb serves) cooked spaghetti topped with Bolognais* and served with a green salad.
14. Stir-fry with tofu, chicken, seafood, lamb or beef (using 75g pack of provided meat) – add a variety of chopped veggies and flavour with soy, oyster, teriyaki and/or chilli sauce. Serve with noodles or Doongara/basmati rice from allowance.
15. Chickpea Curry* served with Doongara/basmati rice or bread from allowance.
16. Tuscan Bean Stew*
17. Chilli Con Carne* with Doongara/basmati rice from allowance and a large mixed salad.
18. Small serve of Beef Casserole* with baked sweet potato wedges and mixed steamed veggies.
19. Grilled/BBQ/ Roasted 75g serve of meat with a steamed ear of corn and roast sweet potato, beetroot and carrot (drizzle with olive oil and balsamic vinegar and bake for 20-30mins in a hot oven).
20. 75g fillet of fish grilled, baked or pan-fried in a little olive oil and served with bean salsa and a green salad. *NB make your own bean salsa by mixing a can of mixed beans with sliced spring onion, a finely chopped chilli, a handful of chopped coriander leaves, handful of halved cherry tomatoes, juice of 1 lime and 1 Tbsp olive oil. You can also add olives, capers or anchovies for flavour.
21. Small portion of Red or Green Thai Chicken Curry* with Doongara/basmati rice from allowance.
22. Lentil casserole with a grilled 75g portion of meat or chicken and a large salad.

**Snacks**

11. Fruit
12. 150g carton low fat yoghurt
13. 20g raw nuts
14. 2 Tbsp hummus with carrot sticks or Vita-Wheat crackers
15. Slice of allowed bread toasted and topped with a skim peanut butter or tahini
16. Up & Go
17. Small can of baked beans
18. Cup of vegetable soup

**Pre-Prepared Foods**

The following can be counted as a carbohydrate-rich food serve:

- Tomato, Lentil & Barley Soup
- Pumpkin, Sweet Potato & Cumin Dhal Soup
- Sweet Potato, Carrot & Ginger Soup
• Tasty Tomato & Basil Soup

The following can be counted as a protein-rich food serve:
• Thai Chicken Green or Red Curry
• Bolognaisne
• Chilli con carne
• Beef Casserole

The following can be counted as either a carbohydrate-rich food serve OR a small protein-rich food serve:
• Lentil Casserole
• Tuscan Bean Stew
• Chickpea Curry
• Spicy Vegetable Chickpeas

The following can be counted as indicated:
• Hoummos – 2Tbsp = 1 fat serve
• Bacon, Beans & Tomato Sauce = 1 small protein serve
• Curried Lentil Spread = use freely

What do I do if I am eating out?

You should try to eat as much home-prepared meals as possible as this will help you to best stick with your eating plan. However this is a ‘real life’ trial and eating out is a part of normal life. Try not to abandon your eating plan for such meals, but instead choose wisely and you will usually find you can order something in line with your dietary goals.

The following meals would be good choices for you:

• Dishes based on lentils or beans eg Dahl, fish with bean salsa or bean burittos
• Stir-fries with noodles (skip the rice – usually the wrong type)
• In Indian restaurant the rice is usually basmati and therefore a better option – tandoori dishes with steamed basmati rice, Dahl & a side salad are good options
• Pasta with stock-based or tomato sauce (skip the creamy ones)
• Grilled fish, meat or chicken (watch serve sizes) with sweet potato (kumera) & steamed veggies or a side salad
• Sandwich on wholegrain (not wholemeal) or sourdough with lean meat & loads of salad veggies
• Minestrone or veggie soup with multigrain or sourdough bread
• Breakfast meals
  o Natural muesli or Bircher with fresh fruit
  o Field mushrooms and/or avocado with tomato on sourdough toast
  o Fruit toast with a little cream cheese
  o Skim milk fruit smoothie
Other tips:

- In Asian style restaurants choose stir-fries rather than the curries to keep the fat and energy content down and noodles rather than rice
- Watch serve sizes – these are usually larger than you would serve at home.
- Ask for sauces and salad dressings on the side and add a little yourself

Can I drink alcohol?

Alcohol contains a significant amount of energy and can seriously hinder your weight loss attempts if you are drinking too much. However we do not expect you to be saintly for 12 weeks! Your eating plan should be achievable and realistic and if you normally enjoy a drink you may do so within reasonable limits – we suggest no more than 6 drinks a week. If you do overindulge try not to let it upset your resolve to stick with your eating plan – often when we drink it is the food that goes with it that is the problem rather than the alcohol.

A final thought

It is not what you do on the occasion that counts but what happens regularly. The odd meal out with the plan or the odd treat will not affect your overall progress. Try not to allow small upsets to throw you off track – simply move on and continue to make the best choices you can at each and every meal and snack.
RED DIET = DIET 3 (High protein, high-GI)

<table>
<thead>
<tr>
<th>Protein-rich foods</th>
<th>Carbohydrate-rich foods</th>
<th>Fat-rich foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main meal choose from:</strong></td>
<td><strong>Choose 3 of the following:</strong></td>
<td><strong>Choose 2 of the following:</strong></td>
</tr>
<tr>
<td>180g red meat (provided)</td>
<td>¼ cup Sultana Bran or Branflakes</td>
<td>1/2 Tbsp oil</td>
</tr>
<tr>
<td><em>4 meals/wk</em></td>
<td>2 Weet-Bix</td>
<td>2 tsp butter or margarine</td>
</tr>
<tr>
<td>Or</td>
<td>½ cup Sustain</td>
<td>¼ avocado (1Tbsp mashed)</td>
</tr>
<tr>
<td>180g chicken breast or other lean meat</td>
<td>1 slice bread – choose from:</td>
<td>1 Tbsp mayonnaise</td>
</tr>
<tr>
<td>180g fish or seafood</td>
<td>▪ Wholemeal</td>
<td>1 Tbsp salad dressing</td>
</tr>
<tr>
<td><strong>PLUS</strong></td>
<td>▪ Hyfibe (fibre-enriched white)</td>
<td>3 tsp peanut butter or tahini</td>
</tr>
<tr>
<td><strong>At light meal or breakfast choose 1 from:</strong></td>
<td>1 med potato (150g)</td>
<td>20g raw nuts</td>
</tr>
<tr>
<td>2 eggs</td>
<td>¼ cup mashed potato</td>
<td>30g cheese</td>
</tr>
<tr>
<td>4 slices (80g) deli lean meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75g tuna</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 slices (40g) reduced fat cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100g tofu</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PLUS</strong></td>
<td><strong>PLUS</strong></td>
<td></td>
</tr>
<tr>
<td>1 cup low fat milk or soy alternative</td>
<td>2 fruits: (fresh or canned in own juice)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>¼ Melon, 1 cup diced pineapple,</td>
<td></td>
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<td></td>
<td>banana, ½ papaya,</td>
<td></td>
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<tr>
<td></td>
<td>½ mango or any other fruit</td>
<td></td>
</tr>
<tr>
<td>200g (3/4 cup) natural or diet yoghurt</td>
<td></td>
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</tr>
</tbody>
</table>

**PLUS**

The following vegetables are unrestricted – ensure you have ≥ 2 cups daily:

lettuce, spinach, broccoli, tomatoes, Asian greens, onions, cauliflower, zucchini, capsicum, carrots, beetroot, sprouts, celery, asparagus, cabbage, cucumber, green beans/peas, pumpkin, rocket, squash, mushrooms, leeks, eggplant, endive, fennel.
RED (DIET 3) MEAL IDEAS

Breakfasts

1. 2 poached eggs with 1 slice of wholemeal/Hyfibe toast, a handful of wilted spinach (microwave for 30 secs), a grilled tomato and a handful of dry-fried mushrooms.
2. 2 boiled eggs with 1 slice of toast spread with 1 tsp butter/margarine and ½ grapefruit.
3. 2-egg omelette with spinach and mushroom (use milk and butter from allowance).
4. ¾ cup Sultana Bran or Branflakes with low fat milk.
5. ½ cup Sustain with low fat milk.
6. 2 Weet-Bix with low fat milk.
   a. options for breakfasts 4-6: add a serve of sliced fruit or a spoonful of natural yoghurt.
7. Glass of low fat flavoured milk and a piece of fruit.
8. 2 grilled rashers of lean bacon (1/2 protein serve) with 1 poached egg (1/2 protein serve), tomato and 1 slice of wholemeal toast.
9. 2 slices of wholemeal/Hyfibe toast with 3 tsp peanut butter.

Light Meals

1. 4 slices of lean ham/chicken with a large mixed salad, dressed with 1 Tbsp salad dressing & 1 slice of wholemeal bread.
2. Tuna Nicoise salad – mix shredded lettuce, half of a 75g packet tuna (1/2 protein serve), olives, blanched green beans, quartered tomatoes and 1 hard-boiled egg. Dress with 1Tbsp olive oil dressing.
3. Open sandwich – top 1 slice of bread with plenty of salad vegetables and your choice of 75g tuna/2 hard boiled eggs/4 slices meat/2 slices reduced fat cheese.
4. Baked potato filled with a serve of Bolognaise* or tuna mayonnaise. Serve with a side salad.
5. Sandwich made with 2 slices Hyfibe/wholemeal bread (2 carb serves) filled with 4 slices of meat & lots of salad.
6. Stir-fried veggies with tofu/lean meat/chicken/seafood (no rice or noodles).
7. Chicken soup with bread from allowance.

Main Meals

You must choose the red meat meals 4 times a week – the meat is vacuum-packed and provided for you each week. You can add bread or a potato if you have carb serves left from your daily allowance.

1. Grilled/BBQ beef fillet served with 2 cups of stir-fried mixed veggies in soy/oyster sauce or a large mixed salad.
2. Grilled/BBQ lamb fillet with tzatziki and a large mixed salad.
3. Stir-fried beef or lamb (provided) with heels of veggies and flavour with soy/oyster/teriyaki/chilli sauce.
4. Large portion of Bolognaise* with a baked potato or a slice of bread and a side salad, or simply pour over steamed veggies.
5. Beef Casserole* with veggies.
6. Large portion of Green or Red Thai Chicken Curry* with steamed green beans and bok choy.
7. 180g fillet of fish grilled/baked/BBQ with ¼ cup mashed potato (1 carb serve) and salad or veggies.
8. 180g chicken breast grilled/BBQ/baked & served with a tomato-based sauce and steamed veggies.

Snacks

1. Small carton of nuts (20g)
2. 200g carton of natural or diet yoghurt
3. A pear or an apple with 40g reduced-fat cheese
4. 1 cup of diced pineapple
5. ¼ melon
6. Carrot sticks (or other raw veg) with tzatziki dip
7. Glass of low fat milk or soy drink
8. Hard boiled egg

Pre-Prepared Foods

The following can be counted as a main meal protein-rich food serve:
- Thai Chicken Green or Red Curry
- Bolognaisae
- Beef Casserole

The following can be counted as a light meal protein-rich food serve:
- Roast Chicken, Garlic & Borlotti Bean Soup

What do I do if I am eating out?

You should try to eat as much home-prepared meals as possible as this will help you to best stick with your eating plan. However this is a ‘real life’ trial and eating out is a part of normal life. Try not to abandon your eating plan for such meals, but instead choose wisely and you will usually find you can order something in line with your dietary goals.

The following meals would be good choices for you:
- Grilled, baked, BBQ or poached meat, chicken or fish with steamed veggies (ask for no butter) or a large mixed salad (ask for the dressing on the side and add a little yourself) eg grilled steak & salad
- Seafood eg steamed mussels, grilled prawns or BBQ octopus salad
- Stir-fries with beef, pork, chicken or prawns with extra veggies (no rice or noodles)
- Veggie soups with 1 slice of bread from your daily allowance
- Asian style clear soups
• Breakfast meals opt for poached eggs with spinach & tomato or an omelette – toast from allowance

Other tips:

• Skip the rice, pasta and potatoes – the portions are usually too large for your plan
• Keep your carb portions for when you can control the amounts

Can I drink alcohol?

Alcohol contains a significant amount of energy and can seriously hinder your weight loss attempts if you are drinking too much. However we do not expect you to be saintly for 12 weeks! Your eating plan should be achievable and realistic and if you normally enjoy a drink you may do so within reasonable limits – we suggest no more than 6 drinks a week. If you do overindulge try not to let it upset your resolve to stick with your eating plan – often when we drink it is the food that goes with it that is the problem rather than the alcohol.

A final thought

It is not what you do on the occasion that counts but what happens regularly. The odd meal out with the plan or the odd treat will not affect your overall progress. Try not to allow small upsets to throw you off track – simply move on and continue to make the best choices you can at each and every meal and snack.
GREEN DIET = DIET 4 (high protein, low-GI)

<table>
<thead>
<tr>
<th>Protein-rich foods</th>
<th>Carbohydrate-rich foods</th>
<th>Fat-rich foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main meal choose from:</strong></td>
<td><strong>Choose 3 of the following:</strong></td>
<td><strong>Choose 2 of the following:</strong></td>
</tr>
<tr>
<td>180g red meat (provided)</td>
<td>1 cup All-Bran varieties or Guardian</td>
<td>½ Tbsp oil</td>
</tr>
<tr>
<td>4 meals/wk</td>
<td>1/4 cup Komplete oven-baked muesli</td>
<td>2 tsp butter or margarine</td>
</tr>
<tr>
<td>Or</td>
<td>1/3 cup natural muesli</td>
<td>¼ avocado (1 Tbsp mashed)</td>
</tr>
<tr>
<td>180g chicken breast or other lean meat</td>
<td>1/3 cup rolled oats</td>
<td>1 Tbsp mayonnaise</td>
</tr>
<tr>
<td>180g fish or seafood</td>
<td>1 Up&amp;Go carton</td>
<td>2 Tbsp hummus</td>
</tr>
<tr>
<td></td>
<td>1 slice bread – choose from:</td>
<td>3 tsp peanut butter or tahini</td>
</tr>
<tr>
<td>PLUS</td>
<td>▪ Bürgen Soy-Lin or Honey &amp; Oatbran</td>
<td>20g raw nuts</td>
</tr>
<tr>
<td><strong>At a light meal or breakfast choose 1 from:</strong></td>
<td>▪ Performax</td>
<td>30g cheese</td>
</tr>
<tr>
<td>2 eggs</td>
<td>▪ Ploughman’s Wholegrain</td>
<td>1 Tbsp salad dressing</td>
</tr>
<tr>
<td>4 slices (80g) deli lean meat</td>
<td>1/4 cup raw (1/2 cup cooked) bulgur/ barley</td>
<td></td>
</tr>
<tr>
<td>75g tuna</td>
<td>1 medium ear of corn or 1/2 cup kernels</td>
<td></td>
</tr>
<tr>
<td>2 slices (40g) reduced fat cheese</td>
<td>1 med sweet potato (100g)</td>
<td></td>
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<tr>
<td>100g tofu</td>
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</tbody>
</table>

The following foods can be counted as either your light meal protein serve or 1 carb serve:

- 1/4 cup dry (1/2 cup cooked) lentils
- 1/2 cup beans/chickpeas
- 140g baked beans
- 1 serve lentil or bean soup

PLUS

- 1 cup low fat milk or soy alternative +
- 200g (3/4 cup) natural or diet yoghurt

PLUS

- 2 of the following fruits: (fresh or canned in own juice)
  - Apple, pear, orange, mandarin, 2 plums,
  - 2 apricots, nectarine, peach, grapefruit,
  - 1/2 cup strawberries

PLUS

The following vegetables are unrestricted – ensure you have ≥2 cups daily:

- lettuce, spinach, broccoli, tomatoes, Asian greens, onions, cauliflower, zucchini, capsicum, carrots, beetroot, sprouts, celery, asparagus, cabbage, cucumber, green beans/peas, pumpkin, rocket, squash, mushrooms, leeks, eggplant, endive, fennel.
GREEN (DIET 4) MEAL IDEAS

Breakfasts

10. 2 poached eggs with 1 slice of Bürgen toast, a handful of wilted spinach (microwave for 30 secs), a grilled tomato and a handful of dry-fried mushrooms.
11. 2 boiled eggs with 1 slice of Bürgen toast spread with 1 tsp butter/margarine and ½ grapefruit.
12. 2-egg omelette with spinach and mushroom (use milk and butter from allowance).
13. 1 cup All Bran or Guardian with low fat milk.
14. ¼ cup Komplete with low fat milk.
15. Cover 1/3 cup rolled oats with water and cook in the microwave for 2 minutes. Add a little low fat milk and cook for a further minute. Serve with a tsp honey or brown sugar if wished.
16. 1/3 cup natural muesli with low fat milk.
    a. options for breakfasts 4-7: add a serve of sliced fruit or a spoonful of natural yoghurt.
17. Carton of Up & Go and a piece of fruit.
18. 2 grilled rashers of lean bacon (1/2 protein serve) with 1 poached egg (1/2 protein serve), tomato and 1 slice of Bürgen toast.
19. A slice of Bürgen toast topped with a small can of baked beans.

Light Meals

8. 4 slices of lean ham/chicken with a large mixed salad, dressed with 1 Tbsp salad dressing & 1 slice of Bürgen bread.
9. Tuna Nicoise salad – mix shredded lettuce, half of a 75g packet tuna (1/2 protein serve), olives, blanched green beans, quartered tomatoes and 1 hard-boiled egg. Dress with 1Tbsp olive oil dressing.
10. Chicken salad with loads of salad veggies and 1Tbsp olive oil & vinegar dressing.
11. Open sandwich – top 1 slice of Bürgen bread with plenty of salad vegetables and your choice of 75g tuna/2 hard boiled eggs/4 slices meat/2 slices reduced fat cheese.
12. A serve of wholesome bean, barley or lentil soup.
13. Sandwich made with 2 slices Hyfibe/wholemeal bread (2 carb serves) filled with 4 slices of meat & lots of salad.
14. Stir-fried veggies with tofu/lean meat/chicken/seafood (no rice or noodles).
15. Chicken soup with Bürgen bread from carb allowance.

Main Meals

You must choose the red meat meals 4 times a week – the meat is vacuum-packed and provided for you each week. You can add bread, a sweet potato or an ear of corn if you have carb serves left from your daily allowance.

9. Grilled/BBQ beef fillet served with 2 cups of stir-fried mixed veggies in soy/oyster sauce or a large mixed salad.
10. Grilled/BBQ lamb fillet with tzatziki and a large mixed salad.
11. Grilled/BBQ fish fillet with bean salsa and a green salad. For bean salsa mix together a can of mixed beans, a small finely sliced onion, a handful of coriander, the juice of ½ lemon and a small chilli. Dress with 1tbsp olive oil and vinegar dressing. You can also add sliced olives, capers or cherry tomatoes.
12. Stir-fried beef or lamb (provided) with heaps of veggies and flavour with soy/oyster/teriyaki/chilli sauce.
13. Large portion of Bolognais* with a baked sweet potato or a slice of Bürgen bread and a side salad, or simply pour over steamed veggies.
15. Large portion of Green or Red Thai Chicken Curry* with steamed green beans and bok choy.
16. 180g fillet of fish grilled/baked/BBQ with a steamed ear of corn (1 carb serve) and salad or veggies.
17. 180g chicken breast grilled/BBQ/baked & served with a tomato-based sauce and steamed veggies.

**Snacks**

9. Small carton of nuts (20g)
10. 200g carton of natural or diet yoghurt
11. A pear or an apple with 40g reduced-fat cheese
12. ½ cup strawberries with a spoonful of natural yoghurt
13. A piece of fruit
14. Carrot sticks (or other raw veg) with tzatziki dip
15. Glass of low fat milk or soy drink
16. Hard boiled egg

**Pre-Prepared Foods**

The following can be counted as a main meal protein-rich food serve:
- Thai Chicken Green or Red Curry
- Bolognais
- Beef Casserole

The following can be counted as a light meal protein-rich food serve:
- Roast Chicken, Garlic & Borlotti Bean Soup

The following can be counted as a carbohydrate-rich food serve:
- Tomato, Lentil & Barley Soup
- Pumpkin, Sweet Potato & Cumin Dhal Soup

The following can be counted as a fat serve:
- Hoummos – 2Tbsp
What do I do if I am eating out?

You should try to eat as much home-prepared meals as possible as this will help you to best stick with your eating plan. However this is a ‘real life’ trial and eating out is a part of normal life. Try not to abandon your eating plan for such meals, but instead choose wisely and you will usually find you can order something in line with your dietary goals.

The following meals would be good choices for you:

- Grilled, baked, BBQ or poached meat, chicken or fish with steamed veggies (ask for no butter) or a large mixed salad (ask for the dressing on the side and add a little yourself) eg grilled steak & salad
- Seafood eg steamed mussels, grilled prawns or BBQ octopus salad
- Fish with bean salsa/salad or lentils
- Stir-fries with beef, pork, chicken or prawns with extra veggies (no rice or noodles)
- Minestrone soup
- Asian style clear soups
- Breakfast meals opt for natural (not toasted) or bircher muesli with fruit & yoghurt, or poached eggs on sourdough with spinach & tomato.

Other tips:

- Skip the bread – it is usually the wrong type for your plan
- Skip the rice, pasta and potatoes – the portions are usually too large for your plan
- Keep your carb portions for when you can control the amounts, with the exception of legumes – these are a good eating out option

Can I drink alcohol?

Alcohol contains a significant amount of energy and can seriously hinder your weight loss attempts if you are drinking too much. However we do not expect you to be saintly for 12 weeks! Your eating plan should be achievable and realistic and if you normally enjoy a drink you may do so within reasonable limits – we suggest no more than 6 drinks a week. If you do overindulge try not to let it upset your resolve to stick with your eating plan – often when we drink it is the food that goes with it that is the problem rather than the alcohol.

A final thought

It is not what you do on the occasion that counts but what happens regularly. The odd meal out with the plan or the odd treat will not affect your overall progress. Try not
to allow small upsets to throw you off track – simply move on and continue to make the best choices you can at each and every meal and snack.
Appendix 2:  Subject Information Sheet
A study comparing the effects of 4 different diets on weight loss, body composition and blood cholesterol

SUBJECT INFORMATION SHEET

We are currently seeking overweight, but otherwise healthy, non-vegetarians, aged between 18 and 40 years, to participate in a study comparing the effects of four diets on weight loss, body composition (body fat and muscle levels) and several risk factors for heart disease, including blood cholesterol levels. Overweight and obesity remain on the increase in Australia and are a major public health problem. Body fat stored around the mid-section, high blood pressure and high blood fat levels (including cholesterol) are all risk factors for heart disease that can be affected by diet. The study will evaluate the effectiveness of different diets on assisting weight loss and improving these heart disease risk factors, and forms part of the requirements for Ms McMillan’s PhD. You are not eligible to participate if you suffer from a medical condition such as diabetes, have suffered from an eating disorder or have lost or gained a significant amount of weight in the past two months.

Should you be eligible and choose to participate, you will be randomly prescribed one of the test diets which you will be required to follow for a period of 12 weeks. A qualified dietitian will give you comprehensive written and verbal instructions, including eating plans and meal preparation, designed to help you to lose weight. You will meet with the dietitian once a week when you will be weighed and have any questions regarding the diet answered. Each diet will be based on common palatable foods and no supplements or non-food items will be prescribed. A shop will be set up within the nutrition research unit where the bulk of foods will be provided free of charge. You will be required to visit the shop at least once a week
and all food items collected will be recorded. Any unused foods will be returned and also recorded in order to carry out a dietary analysis of the foods eaten.

Before commencing the diet and after completing 12 weeks of dietary intervention, you will undergo a scan at the Royal Prince Alfred Hospital to assess your levels of body fat, where the fat is stored and how much lean muscle you have. This takes only a few minutes and is of no risk to you. If you are, or could be, pregnant you must not undergo a scan. You must inform us as soon as is possible and you will be unable to take part in the trial.

Every 6 weeks (3 times in total) you will be required to provide a venous blood sample for the assessment of various factors in your blood including cholesterol. You are required to fast for at least 10 hours overnight before providing the blood sample the next morning. For example, if your appointment with us is at 8am Tuesday morning, you will need to stop eating by 10pm on Monday night. You are allowed to drink only water during the fasting period. No coffee, tea, juice or other beverages are allowed. You should report to the research unit at the arranged time in a fasting condition where a trained phlebotomist will take a small amount of blood from your arm. This involves only minor discomfort and strict health and hygiene protocols will be adhered to.

All personal information and results arising from the study will be used for research purposes only and will remain strictly confidential. You will not be personally identified in any publications or presentations arising from this research study. You are free to withdraw from the study at any time without repercussions.

If you need more information or would like to speak with one of the investigators, please contact Joanna McMillan-Price or Fiona Atkinson.

Ph: Joanna 9357 2795 or 0438 287072; Fiona 0412 686880
Email: jmrprice@bigpond.net.au

Any person with concerns or complaints about the conduct of a research study at Sydney University can contact the Human Ethics Committee (Ph: 9351 4811).
Appendix 3:  End of Study Questionnaire
End of Study Questionnaire

Name:
Diet:

Please read each question carefully and mark your response by placing a cross anywhere on the line. The category markers are there as guide only – do not circle a category.

Question 1: Did you find the diet difficult to follow?

not at all difficult    slightly difficult    moderately difficult    very difficult    extremely difficult

Question 2: Considering the diet as a whole, how much did you like the foods/meals?

dislike extremely    dislike slightly    like slightly    like a lot    like extremely

Question 3: Were the meals sufficiently filling?

not at all filling    slightly filling    moderately filling    very filling    extremely filling
Question 4: Rating the diet as a whole, how hungry were you between meals?

not at all hungry  slightly hungry  moderately hungry  very hungry  extremely hungry

Question 5: Please list below any benefits or side effects you have experienced from following your assigned diet.

<table>
<thead>
<tr>
<th>SIDE EFFECTS</th>
<th>BENEFITS</th>
</tr>
</thead>
</table>

Question 6: How difficult would you find it to follow this type of diet permanently?

not at all difficult  slightly difficult  moderately difficult  very difficult  extremely difficult
Appendix 3

Thank you for your time in providing us with this valuable feedback.