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Different Dietary Approaches for the Treatment of Obesity, and the Phenotypic Responses to these Diets

MICHELLE HESSION

A thesis submitted in partial fulfilment of the requirements of The Robert Gordon University for the degree of Doctor of Philosophy

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Different Dietary Approaches for the Treatment of Obesity, and the Phenotypic Responses to these Diets

By Michelle Hession
for the title of Doctor of Philosophy

ABSTRACT

Currents treatments for obesity have been unsuccessful. It is essential that a patient-centred approach for obesity management is developed and for this to be successful other diet and lifestyle approaches need to be considered.

A systematic review comparing low carbohydrate vs. low fat diets for the treatment of obesity was carried out. It found that low carbohydrate/high protein diets are as effective as, if not better, for treating obesity and cardiovascular disease risk factors.

A randomised controlled trial investigating dietary approaches for the treatment of obesity and its co morbidities was carried out. Variables including weight and body composition, cardiovascular risk factors, adipokines, liver and kidney function, and health and lifestyle factors were measured. Those with metabolic syndrome were also examined.

It was hypothesised that there are alternative ways of treating obese subjects depending on their phenotype. Those with a higher BMI tend to have a higher carbohydrate intake rather that a higher fat intake so may be better suited to a low carbohydrate/high protein diet rather than the conventional low fat/energy reduced diet. Subjects were initially treated with the standard dietary approach for obesity (health eating, HE) and if not successful after 3 months were randomised to either a very low calorie diet (Lighterlife, LL) or a protein sparing modified fast (PSMF).

All three groups showed a significant weight loss and reduced risk for CVD at 12 months. Significant improvements were seen for plasminogen-activated receptor-1, adiponectin, leptin and IL-6 on HE and LL, but only adiponectin significantly
improved on the PSMF. Neither diet showed any detrimental effects for those with a healthy liver and kidney function. Quality of life and levels of depression improved at 12 months.

Of the 54 subjects with metabolic syndrome at baseline, 12 remained on HE and 32 were randomised to LL and PSMF. This indicates that most subjects did not suit a low fat dietary approach. They were successful at losing weight on LL and PSMF and showed improvement in MS risk factors, and adipokine levels at 12 months.

In conclusion, the study demonstrates that a low fat diet may not necessarily be the first line of approach to treat obese subjects with a BMI over 35 kg/m$^2$, including those with MS. A very low calorie diet such as LL or a PSMF may be better suited to the subject.

*Keywords*: Obesity; Diet; Weight loss; Cardiovascular Risk; LighterLife; Protein Sparing Modified Fast; Randomised controlled clinical trial; Adipokines; metabolic syndrome.
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cardiovascular risk of morbidly obese patients. 10th International Congress on Obesity. Obesity Reviews. 7: S307.


ABBREVIATIONS

ACS American Cancer Society
AdipoQ Adipocyte complement-related protein
AKLP Alkaline phosphatase
ALT Alanine aminotransferase
AMPK AMP-activated protein kinase
ApoE Apolipoprotein E
ARC Arcuate nucleus
ASP Acylation-stimulating protein
ATP Adult Treatment Panel
BDI Beck Depression Inventory
BMI Body mass index
BMR Basal metabolic rate
Creat Creatinine
ALB Albumin
CBT Cognitive behavioural therapy
CHD Coronary heart disease
CPE Carboxypeptidase E
CT Computed tomography
CVD Cardiovascular disease
DASH Dietary Approaches to Stop Hypertension
DBP Diastolic Blood pressure
DEBQ Dutch Eating Behaviour Questionnaire
DEE Daily energy expenditure
DEXA Dual Energy X-Ray Absorptiometry
DLW Doubly labelled water
DPP Diabetes Prevention Program
EE Energy expenditure
EPW Epworth Sleepiness Scale
FFM Fat free mass
FIZZ Found in inflammatory zone
FPG Fasting plasma glucose
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<td>GB</td>
<td>Gallbladder</td>
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<tr>
<td>GGT</td>
<td>Gamma-glutamyl transferase</td>
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<tr>
<td>GHQ</td>
<td>General Health Questionnaire</td>
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<tr>
<td>HbA1c</td>
<td>Haemoglobin A1c</td>
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<tr>
<td>HC</td>
<td>Hip circumference</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HE</td>
<td>Healthy eating</td>
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<tr>
<td>HOMA</td>
<td>Homeostatic model assessment</td>
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<td>HR</td>
<td>Heart Rate</td>
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<tr>
<td>ICAM</td>
<td>Inflammatory cell adhesion molecule</td>
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<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth-factor-1</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IL-6 sr</td>
<td>Interleukin-6 soluble receptor</td>
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<tr>
<td>JBS2</td>
<td>Joint British Societies Guidelines 2</td>
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<tr>
<td>LCD</td>
<td>Low calorie diet</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>LighterLife</td>
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<tr>
<td>MCP-1</td>
<td>Monochemoattractant-protein 1</td>
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<tr>
<td>MIF</td>
<td>Macrophage inhibitory factor</td>
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<td>MRI</td>
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<tr>
<td>mRNA</td>
<td>Messenger ribonucleotide acid</td>
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<td>MS</td>
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<tr>
<td>NAFLD</td>
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<td>NCEP</td>
<td>The National Cholesterol Educational Program</td>
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<td>NEFA</td>
<td>Non-esterfied fatty acids</td>
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<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PA</td>
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<td>PCOS</td>
<td>Polycystic Ovarian Syndrome</td>
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PG  Prostaglandin
POMC  Proopiomelanocortin
PPARγ  Peroxisome proliferation-activated receptor-γ
PSMF  Protein sparing modified fast
PVN  Paraventricular nucleus
RCT  Randomised controlled trial
RDA  Recommended Daily Allowance
RELMs  Resistin-like molecules
RMR  Resting Metabolic Rate
SBP  Systolic blood pressure
SD  Standard deviation
SIGN  Scottish Intercollegiate Guidelines Network
T2D  Type 2 Diabetes
TAG  Triacylglycerols
TC/HDL  Total cholesterol vs. HDL cholesterol
TEE  Total energy expenditure
TGF-β  Transforming growth factor-β
TNFα  Tumour necrosis factor alpha
TNFα sR  Tumour-necrosis factor α soluble receptor
VAS  Visual Analogue Scale
VCAM  Vascular cell adhesion molecule
VLCD  Very low calorie diet
WC  Waist circumference
WHO  World Health Organisation
WHOQOL  World Health Organisation Quality of Life
WHR  Waist to hip ratio
CHAPTER 1: INTRODUCTION
1.1: Definition of Obesity

Obesity is defined as a condition in which “body fat stores (adipose tissue) are increased to an extent to which the individual’s health is impaired” (Garrow et al., 2000). It is a disease of positive energy balance, which arises as a result of dysregulation in the energy balance system (Gibney et al., 2002).

1.2: Aetiology of Obesity

1.2.1: Body Weight Regulation

Body weight regulation deals with the relationship between energy intake and expenditure, and includes the processes of energy transduction and storage (Garrow, 1978). The first law of thermodynamics states that energy can neither be created nor destroyed, but it can be interchanged between different forms (Bray, 1982). This also applies to the human body. It takes in chemical energy, converts it by oxidation of fuels into other forms of chemical energy (e.g. synthesis of storage compounds), and then into mechanical work and heat.

Energy intake is in the form of food, whereas energy expenditure includes resting energy expenditure, energy used during physical work and thermogenesis (Bray, 1982).

In the short term (hourly), energy intake may not equal energy expenditure, so the body uses its short term storage compound glycogen. However in the long term, the glycogen stores are not enough to buffer the mismatches between energy intake and energy expenditure. Energy balance varies from day to day, and the changes in energy intake and expenditure are not automatically matched. Positive energy balance on one day may not be suddenly compensated by negative energy balance the next day. The coefficient of variation of food intake within an individual is approximately twice as great as the coefficient of variation of total energy expenditure. The time required for eating is short (fast food can cause a rate of eating of 200-250 kcal/minute whereas the maximum rate of energy expenditure in aerobic conditions over a few minutes is of the order of 25 kcal/minute, or 8-10 times less (Garrow, 2000).

The capacity for storing protein in fat-free mass and storing carbohydrate as glycogen in the liver and muscle is limited. Short term day to day energy imbalances is mostly accommodated by swift changes in carbohydrate balance. Over a long period of time,
positive energy balance is mostly expressed as fat storage, as carbohydrate stores are limited (Garrow et al., 2000). Triacylglycerol, the long term energy store in adipose tissue, is used. If energy intake consistently exceeds energy expenditure, triacylglycerol accumulates in the adipose tissue, and obesity eventually occurs (Frayn, 2001).

1.2.2: Environmental Causes
The present epidemic of obesity is mainly caused by an environment that encourages excessive food intake and decreases in physical activity. Humans have evolved efficient physiological mechanisms to protect against weight loss, but they have weak physiological mechanisms to defend against weight gain. Small portion sizes, diets low in fat and energy density, and regular physical activity are behaviours which protect against body weight gain, but it is becoming more difficult to adopt and maintain these behaviours in the present environment (Hill and Peters, 1998). This is further discussed below.

1.2.2a: Physical Activity
A decrease in physical activity and an increase in the consumption of high-fat diets are two important environmental factors associated with the increase in overweight and obesity (Schrauwen and Westerterp, 2000).

Studies on obesity and physical activity are limited as physical activity is difficult to measure, particularly in large scale studies, and over long periods of time. One method which can be used is that of doubly-labelled water (DLW). This method is considered as the gold standard for measuring total energy expenditure (TEE) of free-living subjects. It has the advantage of providing an accurate measurement of TEE without changing the usual lifestyle of the person being studied (Keim et al., 2004). It is based on the principle that carbon dioxide production can be measured by the elimination rates of hydrogen and oxygen from the body (Hildreth et al., 1995). The DLW technique administers two forms of stable isotopically labelled water: $^2$H-labelled and $^{18}$O-labelled. The disappearance of the hydrogen-labelled water corresponds to the total water flux while the disappearance of the oxygen-labelled water represents the sum of water flux and CO$_2$ generated from respiration. The difference in disappearance rates between the two isotopes can be used to estimate the energy expended over a period of 1–3 half-lives of the labelled water in the body (Stifelman, 2007).
Energy expenditure from physical activity can be measured by subtracting the basal metabolic rate (BMR) from the average daily metabolic rate (Schrauwen and Westerterp, 2000).

Energy expenditure studies have suggested that physical activity results in a post-exercise increase in resting metabolic rate (RMR) (Schrauwen and Westerterp, 2000). Treuth et al. (1996) showed that the mean daily energy expenditure was raised by 0.6 MJ/d following a 60 minute high-intensity interval exercise session compared with an equal amount of work performed in a 60 minute low-intensity exercise session. These results indicate that increased physical activity could aid in the prevention of overweight and obesity by increasing RMR.

Physical activity can also be used as a way of increasing fat oxidation. It is known that high intensity exercise favours carbohydrate oxidation, whereas low intensity exercise favours fat oxidation (Schrauwen and Westerterp, 2000). Nevertheless, high intensity exercise has been thought to have a more evident impact on post-exercise fat oxidation, possibly due to low glycogen levels (Tremblay et al., 1994). Schrauwen et al. (1997, 1998) examined the hypothesis that acute lowering of the glycogen stores by high-intensity exercise, would lead to a rapid increase in fat oxidation. The study found that lean and obese subjects were able to adjust fat oxidation to equal fat intake within 1 day when glycogen stores were low. However, most people do not consume a high fat diet for several days consecutively, but have large day to day fluctuations in fat consumption. Therefore, most people will not adjust their fat oxidation to their fat intake and cumulative positive fat balances can occur. An increase in fat intake does not stimulate its own oxidation but the fat is stored, leading to obesity. When diet composition is isoenergetically switched from a low to a high-fat diet, fat oxidation slowly increases. Hence, lowering of the glycogen stores, with high intensity exercise, may prevent a build up of fat stores, and therefore prevent obesity (Schrauwen and Westerterp, 2000).

Physical activity may have an effect on food intake preferences and energy metabolism (Schrauwen and Westerterp, 2000). It was shown that subjects with a higher spontaneous activity level, consumed more carbohydrate, and physical activity caused a short term reduction in hunger and energy intake. These results show that regular exercise may be a useful way of preventing diet-induced obesity. Combining a low fat diet with physical activity may be effective in the treatment of obesity (Schrauwen and Westerterp, 2000).
Westerterp *et al.* (1999) however, showed that obese subjects are not less active than normal weight subjects. Decreased physical activity therefore cannot be the only explanation for the increasing prevalence of obesity worldwide (Schrauwen and Westerterp, 2000). Westerterp and Speakman (2007) collected data on daily energy expenditure (DEE) using the doubly labelled water technique, from the 1980s to the present, in both North America and Europe. Over this period, obesity rates have increased massively, but there has been no reduction in DEE. The study concluded that increased intake rather than reduced DEE is more likely to be the cause of the rise in obesity (Westerterp and Speakman, 2007).

1.2.2b: Overeating

In the last two decades, there has been an increase in the availability of high fat, energy dense foods. As dietary fat contains a higher proportion of energy per gram (37 kJ/g) compared to protein and carbohydrate (17 kJ/g), an increase in dietary fat intake can lead to a higher overall energy consumption (Schrauwen *et al.*, 2000). Lissner and Heitnamm (1995) reviewed data from 13 studies investigating the relationship between fat intake and obesity. They reported that in 11 out of 13 studies a significant relationship was found between energy-adjusted fat intake and one or more measures of obesity.

Sonne-Holm and Sorenson (1977) also showed that the increase in obesity in Danish men, between 1945 and 1975 correlated with an increase in fat intake. In epidemiological studies, increasing dietary fat is associated with increased prevalence of obesity, probably by increasing the intake of energy dense foods (Bray *et al.*, 2004). Blaak *et al.* (1994) found that the capacity of the human body to respond to an increase in dietary fat intake with a corresponding increase in fat oxidation is limited, thus leading to a deposition of fat in the adipose tissue. These results show that the consumption of high-fat diets is one reasonable explanation for the increased prevalence of obesity in westernized societies.

One possible reason for a higher energy intake on high-fat diets is the palatability of high fat foods, compared with low-fat foods, stimulating increased food consumption. Many studies have demonstrated that fat is less satiating than carbohydrate and protein and that fat is stored more efficiently. In a short-term study, van Amelsfort *et al.* (1989) achieved a reduction in energy from dietary fat from 48% to 28%, and an increase of 50% in polysaccharides, monosaccharides, disaccharides and dietary fibre by isoenergetic replacement of fat with
carbohydrate. The low-fat meal caused an increase in satiety and fullness and less desire to eat compared to the higher fat meal.

During a two-day dietary manipulation, Lawton et al. (1993) demonstrated that subjects who had high hunger levels overate when receiving high-fat foods but not when receiving high-carbohydrate foods. The different effects of macronutrients on appetite regulation are discussed in greater detail in section 1.9.

1.2.3: Genetic causes

Environmental factors such as decreased physical activity and overeating are not alone in the aetiology of obesity. The susceptibility to increase obesity due to changing environment is influenced by our genes. Many studies have concluded that 30-80% of weight variation might be determined by genetic factors (Clement, 2005). This has been shown in large scale epidemiological studies on different populations, including twins brought up together or separately, adopted children and nuclear families (Sorenson, 1995).

Twin studies have shown that monozygotic twins have a higher correlation for BMI than dizygotic twins, even though they were raised in similar family environments (Meyer and Stunkard, 1994). Adoption studies on both adults and children found a significant relationship between the BMI of adoptees and their biological parents (Stunkard, 1986).

In addition to this, there is evidence to show that obesity can be caused by gene-environment interactions. Heitmann et al. (1995) carried out a prospective study on dietary fat intake and BMI changes in women over a six year period, while accounting for genetic predisposition to obesity, as well as other factors including smoking status, physical activity, menopausal status and total energy intake. He divided them into four groups as follows: group 1 were overweight women with obese parents; group 2 were overweight women with non-obese parents; group 3 were normal weight women with obese parents; and group 4 were normal weight women with non-obese parents. The study suggested that a high fat diet promoted weight gain in women who had a genetic predisposition for obesity (defined by women who were already overweight, and had at least 1 obese parent).

Obesity is linked with various genetic syndromes, including Prader-Willi syndrome, Bardet-Biedl syndrome and Alstrom syndrome. These diseases were originally thought to be monogenic, but molecular research is leading to the idea that they are caused by a
contribution of different genes. There is a need for multicentre studies to gather the families affected together in order to characterize the genes responsible for these uncommon diseases (Clement, 2005).

Linkage studies, mouse model studies and studies on human mutations have given an insight into monogenic obesity, including mutations of leptin, the leptin receptor, proopiomelanocortin (POMC), proconvertase 1 (PC1), and melanocortin-4 (MC4) receptor defects, the latter affecting 5% of the obese population. These types of mutations lead to severe obesity.

Figure 1.1 gives an example of how these different mutations affect food intake and energy expenditure.

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Figure 1.1: Human mutation affecting the leptin and melanocortin axis that cause monogenic obesity. The asterisks indicate the genes that are mutated in the 6 identified human monogenic obesity syndromes and the spontaneous and transgenic forms of murine monogenic obesity are shown by the pink arrows. Mc4r – melanocortin-4 receptors; PVN – paraventricular nucleus; CPE – carboxypeptidase E; PC1 – prohormone convertase-1; POMC – proopiomelanocortin; ARC – arcuate nucleus; KO – knockout. Adapted from Cummings and Schwartz, 2003.
Children and adults with a leptin deficiency showed an increased weight loss, mainly of fat mass, decreased food intake and an improvement in other dysfunctions, including immunity, after leptin injection (Clement, 2005). Leptin will be discussed further in section 1.7.3a.

Finally, in relation to obesity and genetics, a study by Frayling identified a common variant in the FTO gene (fat mass and obesity associated) in a study of 2,000 diabetics when they were doing a genome-wide search for susceptibility to type 2 diabetes. They discovered that there was a strong link between the FTO variant and BMI. Subsequent to this, another study of 13 cohorts of 38,759 Britons, Italians and Finns aged 7 found a similar link between the FTO variant and body mass index was found. The authors do stress that environment and lifestyle are also significant factors (Frayling et al, 2007).

1.3: Psychological, Social and Behavioural factors in Obesity

1.3.1: Obesity and Mood Disorders
Research has suggested that obesity is significantly associated with mood disorders, such as depression and anxiety. Community studies in the United States and Canada have found associations between obesity and depressive symptoms (Faith et al., 2002; Stunkard et al., 2003). Sex differences have also been observed in this relationship with positive associations between obesity and depression among women and either negative or no associations among men (Palinkas et al., 1996; Carpenter et al., 2000; Onyike et al., 2003). Another US survey suggested a stronger relationship between obesity and depression in individuals less than 65 years of age (Heo et al., 2006). Longitudinal studies have found that depression predicts the onset of obesity (Goodman and Whitaker, 2002; Hasler et al., 2004), that obesity predicts the onset of depression (Roberts et al., 2003), that weight loss is associated with decreased depression (Dixon et al., 2003), and that depression predicts poorer success in weight loss (McGuire et al., 1999; Linde et al., 2004).

1.3.2: Social Factors
In the US, obesity prevalence is higher among middle-aged and older adults than younger adults, but childhood obesity is also growing at an alarming rate, faster than adult obesity. It is more common among Hispanics and African-Americans compared to other ethnic/racial groups (Flegal et al., 2002; Hedley et al., 2002) and racial/ethnic difference is greater
amongst women than men (Hedley et al., 2002). In developed economies, obesity is inversely associated with income and other indicators of socioeconomic status. The opposite is true in developing countries (Drewnowski and Specter, 2004; Zhang and Whang, 2004).

In England, the 1996 Health Survey showed that the prevalence of obesity increased from 14% in women from social class 1 to 25% in women from social class 5 (Prescott-Clarke and Primatesta, 1996). Similar results have also been found for men (Pietinen et al., 1996; Brunner et al., 1997).

The prevalence of feeling overweight and trying to lose weight are fewer in lower socioeconomic groups (Wardle and Griffith, 2001).

At the community level, obesity is related to easier access to energy-dense, inexpensive foods (Block et al., 2004; Drewnowski and Specter, 2004; Pereira et al., 2005), and with reduced opportunities for physical activity (Frank et al., 2004; Vandegrift and Yojed, 2004).

1.3.3: Behavioural Factors

Certain behavioural factors such as stress, speed of eating, emotions, diet, smoking, and physical activity are some behavioural factors which are related to overweight and obesity. The increasing prevalence of overweight and obesity in industrialized society may be accredited to a change in the pattern of physical activity due to increased mechanization, robotics, and computerization, which have reduced the need for even modest physical activity. The demand for heavy labour is rare. Increased car ownership and heavy road traffic result in less opportunity to travel on foot. Television watching now consumes a huge amount of leisure time and there are many gadgets which minimize (Garrow, 2000).

Smoking tends to be more prevalent in lower socioeconomic groups, and occupational physical activity is greater in those with manual occupations. Both of these factors would likely lead to a lower BMI in lower social classes. In contrast, involvement in leisure time physical activity is associated with those with a higher socioeconomic status, particularly for women (Wardle and Griffiths, 2001).

Associations between total dietary fat intake, or total energy intake and socioeconomic status are inconsistent (Bennet, 1995; Cox et al, 1993; Shimakawa et al, 1994; Poppit and Prentice
1996), though most studies find a higher level of fruit and vegetable consumption amongst higher socioeconomic groups (Gregory, 1990; Cox, 1993; Shimakawa et al, 1994).

Stress and emotions have been found to affect food intake and consequently body weight (Ganley, 1989; Macht and Simons, 2000; Greeno and Wing, 1994; Korkeila et al, 1998; Slochower et al, 1976). Stress related drinking and eating occur when people try to make themselves feel better by drinking or eating in stressful situations (Ganley, 1989; Slochower, 1976; Clair and Genest, 1987; Lowe and Fisher, 1983). It has been suggested that coping with stressful situations is associated with eating disorders (Macht and Simons, 2000) and, particularly with binges, and this can be related to obesity (French et al, 1999). Attempting to reduce stress by eating, drinking, or exercising can be considered so called “emotion-focused coping”, which means trying to avoid the problem which causes stress and the emotions connected to it (Laitinen et al, 2002).

Finally, speed of eating may contribute to obesity. A study at Osaka University investigated this concept, where 3,287 people were examined using a cross-sectional survey. BMI and dietary habits of eating until full (lifestyle questionnaire) and speed of eating (validated brief self administered questionnaire) were the main outcomes assessed. Just under half of the volunteers reported that they tended to eat quickly. Compared to those who did not eat quickly, fast eating men were 84 % more likely to be overweight, and women were twice as likely to be overweight. It was concluded that eating until full and eating quickly are associated with being overweight in Japanese men and women and combined may impact substantially on being overweight (Maruyama et al, 2008). Table 1.1 shows some exogenous behavioural factors, typically encountered in affluent societies, which contribute to poor control of food intake.
Table 1.1: Exogenous factors which contribute to poor control of food intake in humans.

1. Large food diversity and a high palatability diet
2. Profuse availability of food
3. Television watching (reduced activity, pressure of food advertising)
4. Snacking rather than meal eating
5. Fast rate of eating
6. High energy dense diets
7. Eating outside the home, and unsociable eating
8. Technological developments and less activity
9. Reduced physical activity
10. Urbanization, more access to energy dense foods, less need to walk

Adapted from Garrow, 2000.

1.4: Measurements of obesity

1.4.1: Body Mass Index (BMI)

BMI assesses whether an individual is an appropriate weight for their height, by dividing their weight in kilograms (kg) by their height in metres squared (m²) (WHO). It was invented between 1830 and 1850 by the Belgian polymath Adolphe Quetelet during the course of development of social physics. Quetelet concluded that other than the spurt of growth after birth, and during puberty, “the weight increases as the square of the height” known as the Quetelet Index, until it was changed to body mass index (Eknoyan, 2008).

The WHO has classified BMI into different categories as shown in Table 1.1. BMI values are age-independent and are the same for both males and females. BMI is a common method of measuring obesity, with the exception of situations of extreme height and age and in individuals who are very athletic and have a muscular build.
Recently there has been much debate as to whether different BMI categories should be developed for different ethnic groups. This is due to the growing evidence that the associations between BMI, body fat percentage, and distribution of body fat varies across different ethnic groups. The health risk increases below the 25 kg/m$^2$ cut-off which classifies overweight in the current WHO definitions. Several studies carried out in China, Japan, Taiwan, and Hong Kong have reported an association between a BMI $> 22.3$ kg/m$^2$ and increased atherogenic risk factors (Gallagher, 2004). In addition to this, Kim et al. (2004) showed from data collected in the 1998 Korea National Health and Nutrition Examination Survey that the prevalence of obesity, hypertension, and dyslipidemia had doubled at a BMI of 23 to 24 kg/m$^2$ and tripled at a BMI of 26 kg/m$^2$ in the Korean adult population.

The WHO Expert Consultation on BMI in Asian Population was established to examine this debate (WHO Expert Consultation on BMI in Asian Population, 2004). It was concluded that the proportion of Asian subjects with an increased risk of CVD and T2D is significant at BMIs lower that the present cut-off point for overweight (25 kg/m$^2$). However, the cut-off point for observed risk varies from 22 kg/m$^2$ to 25 kg/m$^2$ in different Asian populations and for high risk, it varies from 26 kg/m$^2$ to 31 kg/m$^2$. Consequently, the Consultation recommended that the current WHO BMI categories remain as the international classification.

Finally, the cut-off points of 23, 27.5, 32.5 and 37.5 kg/m$^2$ are to be added as points for public health action. It was, therefore, recommended that countries should use all categories (i.e. 18.5, 23, 25, 27.5, 30, 32.5 kg/m$^2$, and in many populations, 35, 37.5, and 40 kg/m$^2$) for reporting purposes, with a view to facilitating international comparisons.
Table 1.2: The International Classification of Adult underweight, overweight and obesity according to BMI.

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principle cut-off points</td>
</tr>
<tr>
<td><strong>Underweight</strong></td>
<td>&lt; 18.5</td>
</tr>
<tr>
<td>Severe thinness</td>
<td>&lt; 16.00</td>
</tr>
<tr>
<td>Moderate thinness</td>
<td>16.00 - 16.99</td>
</tr>
<tr>
<td>Mild thinness</td>
<td>17.00 - 18.49</td>
</tr>
<tr>
<td><strong>Normal range</strong></td>
<td>18.50 - 24.99</td>
</tr>
<tr>
<td></td>
<td>23.00 - 24.99</td>
</tr>
<tr>
<td><strong>Overweight</strong></td>
<td>≥ 25.00</td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00 - 29.99</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Obese</strong></td>
<td>≥ 30.00</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00 – 34.99</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00 - 39.99</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥ 40.00</td>
</tr>
</tbody>
</table>


### 1.4.2: Waist Circumference (WC)

Measuring waist circumference gives information on the distribution of body fat, and is also a measure of cardiometabolic risk. It is well known that subjects who have a large central fat distribution (abdominal/visceral adiposity), are more prone to suffer the consequences associated with being overweight. In 1947, a French physician named Jean Vague discovered
the importance of the “android” obesity phenotype, and the association with diabetes, atherosclerosis, gout and uric-acid calculous disease (Vague, 1947). Since this study was published, many epidemiological and physiological studies have documented the significance of “upper body” or “abdominal obesity” as a predictor of insulin resistance, T2D, hypertension, dyslipidemia, and cardiovascular morbidity and mortality (Sharma, 2004).

There has been general agreement that the ATP III criteria for defining waist circumference should not be used for Asians based on epidemiological and physiological data (Misra et al, 2003). Tan et al. (2004) used curve analysis to identify the waist circumference in Asians which is the best predictor of 2 other features of metabolic syndrome. They discovered that a waist circumference of 80 cm in men and 90 cm in women to be the best predictors of metabolic syndrome. Enkhmaa et al. (2005) developed comparable measurements in the Korean population and noted a prevalence of metabolic syndrome more in parallel with the prevalence of CVD and diabetes. Misra et al. (2005 and 2006) have suggested similar thresholds for Asian Indians. From these data, the IDF proposed new cut-offs for waist circumference in Asians, and these are being used in studies to determine the prevalence of metabolic syndrome.

The following table shows the ethnic specific risk values for increasing the risk of CVD including metabolic syndrome and T2D, for waist circumference as defined by the International Diabetes Federation (IDF).
Table 1.3: Ethnic specific cut-offs for waist circumference.

<table>
<thead>
<tr>
<th>Country/ Ethnic group</th>
<th>Waist Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Europids</strong></td>
<td>Male ≥ 94cm</td>
</tr>
<tr>
<td>In the USA, the ATP III values (102cm male; are likely to continue to be used for clinical purposes).</td>
<td>Female ≥ 80cm</td>
</tr>
<tr>
<td><strong>South Asians</strong></td>
<td>Male ≥ 90cm</td>
</tr>
<tr>
<td>Based on a Chinese, Malay and Asian-Indian population</td>
<td>Female ≥ 80cm</td>
</tr>
<tr>
<td><strong>Chinese</strong></td>
<td>Male ≥ 90cm</td>
</tr>
<tr>
<td></td>
<td>Female ≥ 80cm</td>
</tr>
<tr>
<td><strong>Japanese</strong></td>
<td>Male ≥ 90cm</td>
</tr>
<tr>
<td></td>
<td>Female ≥ 80cm</td>
</tr>
<tr>
<td><strong>Ethnic South and Central Americans</strong></td>
<td>Use South Asian recommendations until more specific data are available</td>
</tr>
<tr>
<td><strong>Sub-Saharan Africans</strong></td>
<td>Use European data until more specific data are available</td>
</tr>
<tr>
<td><strong>Eastern Mediterranean and Middle East (Arab) populations</strong></td>
<td>Use European data until more specific data are available</td>
</tr>
</tbody>
</table>

Adapted from Banerjee and Misra, 2007.

However, despite the fact that WC is thought to be a more effective method of assessing cardiometabolic risk, the National Health and Nutrition Examination Survey gave evidence from 15,000 subjects which showed a correlation coefficient of 0.9 or greater between measurements of BMI and WC irrespective of age, gender, and ethnicity of groups studied (Figure 1.2). Therefore, although it is agreed that obesity has adverse clinical impacts, there is a difference of opinion as to what measure of adiposity is the most useful in deciding which individuals are at most risk (Sung et al., 2007).
Figure 1.2: Relationship between BMI and WC in men and women. $r =$ correlation coefficient; $p =$ significance level (Sung et al., 2007).

1.4.3: Magnetic Resonance Imaging (MRI)

This technique was developed in the 1990s and is well established and validated for the measurement of visceral fat. It has an advantage in that the subjects are not exposed to ionizing radiation, as they are with computed tomography (CT). MRI scans can precisely measure specific adipose tissue depots such as total body adipose tissue mass, visceral adipose tissue mass, abdominal subcutaneous adipose tissue mass, and hepatic and intramuscular triacylglycerol content (Abate et al., 1995). However, as yet there is no standard protocol for using MRI for the measurement of visceral adiposity. A single axial image measured between the 4th and 5th lumbar vertebrae (L4-L5) is most often chosen to estimate total visceral adiposity (Demerath et al., 2007).
1.4.4: Waist to Hip Ratio (WHR)

A common method of measuring abdominal adiposity and fat distribution is the ratio of circumference of waist and hips, known as the WHR (Garrow et al., 2000). A WHR in men of > 0.90 and in women > 0.80 is an increased risk for obesity related morbidities, independent of BMI (Gray and Fujioka, 1991; Solomon and Manson, 1997). Individuals with excess abdominal fat show a number of metabolic changes including insulin resistance and increased free-fatty acid release compared to those whose fat is distributed subcutaneously around the lower body (Bjorntorp, 1987). These metabolic differences provide a way of measuring the risk of disease in relation to adipose distributions (Lapidus et al., 1984).

1.4.5: Dual Energy X-Ray Absorptiometry (DEXA)

This method uses an x-ray beam with two energy peaks, both high and low, combined with a whole body scanner. It was originally used to measure bone mass, but nowadays it is also used to measure body composition. Its reproducibility and accuracy are very good, but its widespread use is limited because of its cost (Moyad, 2004), and radiation activity.

1.4.6: Skinfold Thickness

Apart from BMI, skinfold thickness is another common method of measuring body composition in epidemiological studies. It provides a direct measure of body fat. Durnin and Womersly (1974) developed the equations used to estimate body fat percentage using skinfold thickness measurements. A disadvantage of this method is that not all body fat is accessible to the callipers such as intra-abdominal and intramuscular fat, and the distribution of subcutaneous fat can differ significantly over the human body (Bellisari et al., 1993; Rosenbaum et al., 1997). There is a significant inter-observer variation in using this method, which limits its use to measure weight change and obesity over time (Bray et al., 1978).

1.4.7: Bioelectrical Impedance

This is a popular method used to measure body composition, including lean body mass, and body fat percentage. It is based on the fact that lean body mass, which contains ions dissolved in water, can conduct electricity to a greater degree than fat mass (Baumgartner, 1996). The resistance of the body to electrical current is inversely related to lean body mass, so the greater the electrical resistance, the lower the body composition of lean tissue, and vice versa. Electrical resistance measurements are simple and efficient to perform (Moyad, 2004). The
method has useful features which are similar to anthropometry, such as safety, cost-effectiveness, ease of use and minimal interference with the patient (Sun et al., 2003).

Bioelectrical impedance was used to measure weight and body composition as part of the present clinical trial. Its simplicity, convenience, safety, minimal time consumption, and minimum invasiveness to the subject, compared to other methods were all reasons why it was chosen. The subjects were always fasted before each measurement, and jewellery and footwear were removed. This was to prevent any interference with the measurement. Subjects were also told to limit their water intake before the measurement was carried out. Gray et al. (1989) examined the effect of obesity on bioelectrical impedance. 87 adults varying in body composition underwent both measurements. Fat-free mass determined from underwater weighing was compared to fat-free mass measured using bioelectrical impedance. It was shown that there was excellent agreement between body composition measured using bioelectrical impedance and underwater weighing (density).

**1.4.8: Underwater weighing**

Underwater weighing is based on a two-compartment model (fat and fat-free mass). It measures body density and calculates body fat percentage using an equation which assumes that fat-free mass and fat have constant densities (Behnke, 1959; Siri, 1961). This method is generally regarded as the gold standard for measuring body composition, though it does have several limitations. Underwater weighing can be demanding for subjects, as being submerged in water may be difficult and produce anxiety for the subject. Also, as it is generally used in university and research settings, it is not always assessable for the public.

**1.4.9: BodPod/Air Displacement**

The air displacement plethsmography used by the BodPod is similar in principle to underwater weighing. The main difference is that air is more convenient and comfortable than water, so air displacement plethsmography provides an easier and safer testing environment, better reliability, and improved repeatability and accuracy (Malavolti et al 2006; Minderico et al, 2006; Sardinha et al, 2006).

**1.5: Prevalence of Obesity**

Obesity is a worldwide epidemic and is increasing at an alarming rate. It has increased > 75 % since 1980 (Flegal et al., 1998). Over 1 billion people are now overweight or obese
(Froguel and Boutin, 2001) with the World Health Organisation (WHO) declaring this to be a global epidemic (Cummings and Schwartz, 2003).

In the period from 1980 to 2002 the prevalence of obesity has trebled from 8% to 23% and quadrupled from 6% to 22% in Scottish women and men, respectively. According to the Scottish Health Survey, 2003, 65% of men and 60% of women are either overweight or obese (Scottish Health Survey, 2003).

Figure 1.3: Prevalence of obesity and overweight in men in Scotland (Scottish Health Survey, 2003).

Figure 1.4: Prevalence of obesity and overweight in women in Scotland (Scottish Health Survey, 2003).
1.6: Obesity and Disease

1.6.1: Metabolic Syndrome

Reaven (1988) was the first to recognise that several risk factors, including dyslipidemia, hypertension and hyperglycaemia, commonly cluster together, and subsequently named this Syndrome X, now referred to as metabolic syndrome, (Grundy et al., 2004) and also called insulin resistance syndrome.

Though the National Cholesterol Education Program (NCEP) Adult Treatment Panel III identified CVD as been the main clinical outcome of metabolic syndrome, the majority of people with the syndrome also have insulin resistance, which leads to an increased risk of T2D. Once a subject presents with T2D the risk for CVD increases sharply. Other diseases associated with metabolic syndrome are polycystic ovary syndrome, cholesterol gallstones, fatty liver, and some forms of cancer (Grundy et al., 2004).

There are no well accepted criteria for diagnosing metabolic syndrome, with The National Cholesterol Educational Program (NCEP) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), World Health Organisation (WHO) and International Diabetes Federation (IDF) having different criteria (Table 1.3).

There seems to be three etiological factors which lead to metabolic syndrome: obesity and disorders of adipose tissue; insulin resistance, and a proinflammatory state. (Grundy et al., 2004).

Many researchers place a greater emphasis on insulin resistance than obesity in the pathogenesis of metabolic syndrome. They dispute that insulin resistance and hyperinsulinemia directly lead to other metabolic risk factors (Reaven, 1988; Ferranninni et al., 1991). However, the unique role for insulin resistance is difficult to identify because of its link to obesity (Grundy et al., 2004).
<table>
<thead>
<tr>
<th>WHO 1999</th>
<th>NCEP 2001</th>
<th>IDF 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes/ impaired glucose tolerance/impaired fasting glucose/ insulin resistance and 2 of the following:</td>
<td>3 of the following:</td>
<td>Central obesity, defined as WC &gt; 94 cm for Europid men and &gt; 80 cm for Europid women, with ethnicity specific values for other groups, and any 2 of the following:</td>
</tr>
<tr>
<td>Dyslipidemia: TAG &gt; 1.7 mmol/l and/or HDL cholesterol &lt; 0.9 mmol/l, men; &lt; 1.0 mmol/l females</td>
<td>TAG &gt; 1.7 mmol/l or HDL cholesterol – Men &lt; 1.0 mmol/l and Women-&lt; 1.3 mmol/l</td>
<td>Raised TAG levels ≥ 1.7 mmol/l, or specific treatment for this lipid abnormality or Reduced HDL &lt; 1.03 mmol/l in males; &lt; 1.29 mmol/l in females or specific treatment for this lipid abnormality.</td>
</tr>
<tr>
<td>Hypertension Blood pressure &gt; 140/90 mmHg and/or medication</td>
<td>Blood Pressure ≥ 130/85mmHg</td>
<td>Raised blood pressure Systolic ≥130 mmHg or diastolic ≥ 85 mmHg or treatment of previously diagnosed hypertension.</td>
</tr>
<tr>
<td>Obesity BMI &gt; 30 kg/m² and/or WHR &gt; 0.90 males/0.85 females</td>
<td>Abdominal Obesity: Waist Circumference- Men &gt; 102 cm and women &gt;88 cm</td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria FPG ≥ 6.0 mmol/l</td>
<td></td>
<td>Raised FPG ≥ 5.6 mmol/l, or previously diagnosed T2D. If above 5.6 mmol/l, OGTT is recommended but is not necessary to define the presence of the syndrome.</td>
</tr>
</tbody>
</table>

WHO = world health organisation; NCEP = national cholesterol education program; IDF = international diabetes federation; WC = waist circumference; TAG = triacylglycerol; HDL= high density lipoprotein; BMI = body mass index; FPG = fasting plasma glucose; OGTT = oral glucose tolerance test; mmol/l = millimoles/litre; mmHg = millimetres mercury. IDF criteria adapted from International Diabetes Federation; NCEP ATP III criteria and WHO criteria adapted from Grundy et al., 2004.
1.6.2: Diabetes Mellitus

Diabetes mellitus is the term used to describe a metabolic disease characterised by chronic hyperglycaemia with disturbances of carbohydrate, lipid and protein metabolism. Symptoms of the disease include excessive thirst, polyuria, blurring of vision and weight loss and effects of diabetes mellitus can include long term damage, organ dysfunction and failure. (WHO, 1999).

Many pathogenetic processes are implicated in the development of relative or absolute deficiency of insulin secretion leading to diabetes mellitus, and these include damage to the beta cells of the pancreas, leading to insulin deficiency or processes which lead to resistance to the action of insulin. It is these processes that lead to the abnormalities in carbohydrate, protein and lipid metabolism (WHO, 1999).

Excess fat accumulation, particularly around the waist (visceral adiposity), is strongly associated with T2D (Guerrero-Romero and Rodríguez-Morán, 2003; Nathan and Delahanty, 2005; Vitale et al., 2006). This can initiate 2 abnormalities: reduced biological effect of insulin on target tissues (insulin resistance), and a decreased ability for pancreatic β-cells to secrete insulin in response to glucose (Bril and Ktorza, 2006). This leads to a raised blood glucose, which then causes more insulin to be released from the pancreas (hyperinsulinemia), eventually resulting in glucose overload and consequently death of the beta cell, mainly due to endoplasmic reticulum stress.

Diagnosis of diabetes mellitus is made by measuring the fasting plasma glucose concentration or by the oral glucose tolerance test (OGTT). Glycated haemoglobin (HbA1c), which is a measure of average glycaemia over a period of weeks can also be examined, though it is not a direct measurement used to diagnose diabetes mellitus. A fasting plasma glucose of > 7 mmol/l is used for diagnostic purposes and for whole blood measurements > 6.1 mmol/l. Diabetes is said to be present if the 2h post load venous plasma glucose is > 11.1 mmol/l. Both these measurements are recommended in population studies examining diabetes mellitus (WHO, 1999).

The increasing development of obesity is accompanied by a rise in the incidence of T2D. In 2000, an estimated 150 million people worldwide had diabetes, which is predicted to double by 2025. T2D is the most common form, with 80-90 % of all diabetic patients affected (Vijgen et al., 2006), and is the form linked to increasing adiposity.
Primary prevention of diabetes requires interventions which focus on the underlying pattern of physical inactivity and unhealthy diets (Bassett, 2005).

A reduction in carbohydrate intake is well acknowledged to be the main factor in glycaemic control, but strategies to reduce carbohydrate intake have received little support (Accurso et al., 2008). Though recent guidelines have admitted that low carbohydrate diets may be an alternative approach for weight loss, they are sceptical about the use of such diets for those presenting with diabetes (American Diabetes Association, 2008). Also, the Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes, reported “no justification for the recommendation of very low carbohydrate diets in persons with diabetes” (Mann et al., 2004).

Garg et al. (1994) showed that even a modest reduction in carbohydrate intake from 55-60% to 40% of total daily energy intake showed improvements in glycaemic control in subjects with T2D. Many other studies have also shown benefits of using low carbohydrate diets on glycaemic control (Allick et al., 2004; Gannon and Nuttall, 2006).

An additional benefit found after subjects consumed a low carbohydrate diet is either a reduction or discontinuation of medication for T2D (Westman et al., 2005; Boden et al., 2005).

Low carbohydrate diets have been shown to be at least as effective as low fat diets for the treatment of obesity, including those with T2D and metabolic syndrome (Volek and Feinman, 2005; Feinman and Fine, 2003; Krieger et al., 2006).

Accurso et al. (2008) recently published a review on the use of low carbohydrate diets in the treatment of T2D and metabolic syndrome. It was recommended that while “some proponents of carbohydrate restriction for the management of diabetes favour sustained adherence to very low levels of carbohydrate intake, all options may be considered and therapeutic choices can be determined by individuals and their physicians”. Accurso et al. also stated that basic biochemistry, clinical experience and an increasing understanding of metabolic syndrome support the need for an evaluation into the efficacy and safety of low carbohydrate diets in the treatment of T2D. Carbohydrate restriction has been shown to improve markers of CVD, even without weight loss and this should encourage the use of such diets.
1.6.3: Cardiovascular Disease

Overweight and obesity is associated with a number of cardiovascular complications including coronary heart disease (CHD), hypertension, stroke, and heart failure (Poirier et al., 2006). Healthy adults with central abdominal obesity have a higher risk of cardiovascular mortality and development of diabetes (Stamatelopoulos et al., 2006).

A higher cardiovascular disease risk in subjects with increased fatness may be attributed to progressing atherosclerosis via endothelial cell dysfunction. Obesity may initiate endothelial cell dysfunction through development of central adiposity, insulin resistance, diabetes, hypertension, and dyslipidemia (Panza et al., 1990; McVeigh et al., 1992; Steinberg et al., 1996 and Woo et al., 2004)

Obesity may also be directly related to atherosclerosis by an increase in the expression of proinflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6), which are associated with endothelial cell dysfunction, an early stage of atherosclerosis. These also cause an upregulation in the synthesis of C-reactive protein and endothelial cell adhesion molecules (Stamatelopoulos et al., 2006), biomarkers of cardiovascular disease.

1.6.3a: Coronary Heart Disease (CHD)

Until recently the association between obesity and CHD was seen as indirect, through co-morbidities (Lew et al., 1979), including hypertension, dyslipidemia, impaired glucose tolerance and T2D. These comorbidities are associated with insulin resistance and hyperinsulinaemia (Reaven et al., 1988). While nearly all of these co-morbidities relating CHD to obesity increase as BMI increases, they are also related to body fat distribution. Prospective studies with a follow up of more than two decades including the Framingham Heart Study, the Manitoba Study, and the Harvard School of Public Health Nurses study have documented that obesity is an independent predictor of CHD (Rabkin et al., 1977; Hubert et al., 1983).

There is increasing evidence to show that inflammation plays a role in the development of CHD. Observations have been made linking the presence of infection in the vessel wall with atherosclerosis, and studies have also linked infections in remote sites to the development of CHD, with an increase in the presence of cytokines such as Il-6 and TNFα (Yudkin et al., 2000). Long term longitudinal studies have shown that obesity is not only related to CHD,
but independently predicts coronary atherosclerosis (Rabkin et al., 1977; Garrison and Castelli, 1985; Manson et al., 1995).

1.6.3b: Hypertension

Many patients with high blood pressure are overweight, and hypertension is more common in obese patients (Stamler et al., 1978). The relationship between obesity and hypertension is unclear. However, factors which are thought to link obesity to hypertension include 1) the direct effects of obesity on haemodynamics: increased blood volume, stroke volume, and cardiac output and 2) links between obesity and peripheral vascular resistance: endothelial cell dysfunction, insulin resistance, sympathetic nervous system, adipokines (including IL-6, TNFα etc) and sleep apnoea. Visceral adiposity and ectopic fat storage may be important in activating the sympathetic nervous and renin-angiotensin systems (Davy and Hall, 2004).

Risk estimates from population studies have suggested that ≥ 75 % of hypertension can be attributed to obesity directly (Krauss, 1998). An increase of 10 kg in body weight is associated with a 3.0 mm Hg higher systolic blood pressure and a 2.3 mm Hg higher diastolic blood pressure (Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, 1998). This causes an estimated 12 % increased risk for coronary heart disease and a 24 % increased risk for stroke (Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, 1998).

Weight loss is critical in the management of hypertension in obese individuals (Poirer et al., 2006). A weight loss of 10 % causes a 6.1 mmHg decrease in systolic blood pressure and a weight loss of 10 kg was associated with a decrease in diastolic blood pressure of 3.6 mmHg (Avenell et al., 2004).

1.6.3c: Stroke

Obesity is related to atherosclerosis, the accumulation of fatty deposits in arteries throughout the body, including arteries in the brain. If a blood clot forms in a narrowed artery in the brain, it can obstruct blood flow to an area of the brain. The result is an ischemic stroke.

Many studies have reported an association between BMI, WHR and stroke (Lapidus et al., 1984; Folsom et al., 1990; Shinton et al., 1991; Abbott et al., 1994; Walker et al., 1996; Rexrode et al., 1997; Pyorala et al., 1998). Every unit increase in BMI is associated with a multiple adjusted increase of 4 % in the risk of ischemic stroke and 6 % for hemorrhagic
stroke (Kurth et al., 2002). The increase in stroke with obesity can be predicted by the proinflammatory/ prothrombotic state that accompanies the adipose tissue accumulation (Rost et al., 2001).

1.6.3d: Heart Failure

Heart failure is becoming an important cause of morbidity and mortality. Obesity has been linked to an increase in the development of heart failure (Hubert et al., 1983; Willett et al., 1999). Obesity-associated changes include left ventricular hypertrophy, diastolic dysfunction, increased circulating blood volume, increased cardiac output and an increased stroke output to end-diastolic pressure index (Davitiss et al., 1981; Eckel et al., 1997). When 22 obese patients were examined post-mortem, dilated cardiomyopathy was most frequently associated with sudden death ($n = 10$), with severe coronary atherosclerosis ($n = 6$), concentric left ventricular hypertrophy without dilation ($n = 4$), pulmonary embolism ($n = 1$), and hypoplastic coronary arteries ($n = 1$). Therefore, dilated cardiomyopathies may be the most common form of sudden death in severely obese patients (Duflou et al., 1995).

Weight loss has been shown to reverse heart failure in morbidly obese individuals (Alexander, 1993; Alpert et al., 1997; Zuber et al., 1999).

1.6.4: Sleep Apnoea

Sleep apnoea is a common disorder associated with daytime sleepiness and fatigue, and significant morbidity and mortality due to accidents and cardiovascular disorders. Male gender, progressing age, anatomical abnormalities (including small pharyngeal size due to fatty tissue in the neck), heredity, instability of respiratory control during sleep, and obesity have been reported as risk factors for sleep apnoea development.

The occurrence of sleep disordered breathing and sleep disturbances increases considerably in obese individuals and obesity is the most important amendable risk factor for sleep disordered breathing (Poirer et al., 2006). Visceral adiposity is associated with obstructive sleep apnoea, and the sleep disordered breathing may lead to, or worsen, insulin resistance, diabetes mellitus and CVD (Alam et al., 2007). Sleep apnoea has now been suggested to be linked to metabolic syndrome.

Two thirds of middle-aged sleep apnoeic men are obese, particularly the android-central type, and one third have hypertension. Studies on the possible independent role of sleep apnoea in
the development of insulin resistance and/or vice versa have been inconsistent. Recent studies indicate that the effects of sleep apnoea on insulin dynamics and effects could be accounted for completely by obesity (Vgontzas et al., 2000).

Many treatments are available for sleep apnoea but weight loss in obese patients should always be encouraged (Poirer et al., 2006).

1.6.5: Cancer

Obesity is associated with a higher death rate due to cancer in many organs, including breast, colon, oesophagus, gallbladder, kidney, rectum, prostate, ovary and uterus. It has been predicted that the increasing prevalence of obesity could result in as many as 12,000 cases of weight related cancer diagnosed annually by 2010 (Cancer Research UK, 2006). Research from the American Cancer Society (ACS) suggests that, at least in the United States, obesity is responsible for 20% of all cancer deaths in women and 14% in men. The ACS further estimates that 90,000 people each year are dying from obesity-related cancers (Galloway, 2005). For people with a BMI ≥ 40 kg/m², the relative risk of dying from any type of cancer is 1.52 for men and 1.62 for women, compared to people of normal weight (Bordeaux et al., 2006). A study of non-smokers showed that men and women who had higher levels of obesity at baseline, measured by BMI, were at a greater risk for cancer. Increased risks for cancer of the kidney, uterus for women and prostate for men were remarkable (Calle et al., 2003). Recent data indicates that obesity is second only to smoking, as a cause of cancer (Cancer Research UK, 2006).

Wardle (2006) suggested 3 possible causes of increased cancer in obese patients: 1. excess weight itself could be a cancer cause; 2. unhealthy diet and increased energy intake leading to excess weight could be a cancer risk; 3. a decrease in physical activity causing an increase in weight could also be a cancer risk.

Studies have shown a relationship between breast cancer and low levels of physical activity, and an even stronger relationship between colorectal cancer and low levels of physical activity. It was concluded that highly active people are at a much less risk of developing cancer (McTiernan et al., 2003).

It has also been shown that low fruit and vegetable consumption, a high intake of saturated fats and a low fibre intake contribute to obesity and cancer risk. Animal research has shown that increased energy intake can increase the risk of tumour growth (Wardle, 2006).
1.6.6 Degenerative joint disease (osteoarthritis)

Osteoarthritis is a pro-inflammatory disease which affects joints, including bones, muscles, ligaments, and synovia. Although the aetiology is not well established, obesity is an important risk factor (Pottie et al., 2006).

The overload effect on joint cartilage is one reason for osteoarthritis, particularly of the knee, in overweight people. However, a recent discovery in cartilage biology is the presence of mechanoreceptors on the surface of chondrocytes, which are sensitive to pressure and link extracellular environment to intracellular signalling cascades. Compression and stretch stimulate the mechanoreceptors to activate the signalling pathways as well as the release of second messengers. Cytokines, growth factors and metalloproteinases may be expressed and mediators such as prostaglandins and nitric oxide may be produced. As studies have shown that under certain conditions overload may induce both inhibition of matrix synthesis and cartilage degradation, it can be concluded that obesity may induce cartilage damage through activation of these mechanoreceptors (Pottie et al., 2006).

In the Framingham study, it was reported that a decrease in BMI of at least 2 units from baseline was associated with a reduction of 50% in the risk of developing symptomatic knee osteoarthritis (Felson et al., 1992).

It has also been suggested that adipokines are involved in the development of degenerative breakdown of cartilage and osteophytes formation. These adipokines include leptin, resistin, and adiponectin. If weight loss can prevent osteoarthritis, loss of body fat is more closely related to an improvement of the symptoms of osteoarthritis than loss of body weight itself. This suggests a role for adipose tissue in the pathophysiology of osteoarthritis. Leptin expression is upregulated in articular tissues that undergo biochemical and structural changes in osteoarthritis when compared to normal tissue. Also, in cultured human chondrocytes, leptin was found to increase the proliferation and the extracellular matrix synthesis, suggesting leptin could have favourable effects on cartilage synthesis (Pottie, 2006).

Further studies are needed to establish the effect of adipokines on articular tissues and, if their presence in arthritic joint is a cause or effect of osteoarthritis (Pottie, 2006).

Exercise is an effective treatment for osteoarthritis and an important component of primary, secondary and tertiary prevention. Prolonged inactivity due to osteoarthritis causes poor
aerobic capacity, and a higher risk for developing CVD, obesity and other inactivity related conditions (Minor, 1990; Ettinger and Afable, 1994; Ries et al., 1995).

1.6.7: Polycystic Ovarian Syndrome (PCOS)

PCOS is characterized by enlarged cystic ovaries, excess male hormones (androgens), irregular or absent menstrual cycles, infertility, acne, excess body and facial hair, obesity, particularly central obesity (Hunter and Sterrett, 2000), male-pattern baldness, and is most important in relation to obesity- insulin resistance. It affects 5 % to 10 % of women of childbearing age. PCOS is one of the most common hormonal disorders among women in this age group. Women with PCOS are insulin-resistant and have an increased risk of developing CHD and T2D (Kelly et al., 2002). Approximately 50% of women with PCOS are obese, and amongst these women, the syndrome’s severity, presentation, and treatment are influenced by the extent of obesity and consequently insulin resistance. Studies of weight loss by a combination of exercise and diet consistently show amelioration of both the biochemical and clinical markers of the disease, even with weight loss of only 5 %. This improvement seems to be additive to that achieved with insulin-sensitizing agents such as metformin (Lawrance and Kopelman, 2004).

1.7: Adipose Tissue and Adipokines

Adipokines are proteins produced primarily by adipocytes. Though adipose tissue secretes a variety of factors, only adiponectin and leptin, and possibly resistin, visfatin and adipsin, are certainly produced by adipocytes and can therefore be appropriately classified as adipokines (Fantuzzi et al., 2005).

White adipose tissue used to be regarded solely as an energy store which provided insulation to internal organs, but recently it has been recognised that adipose tissue has many diverse physiological and metabolic functions (Trayhurn and Wood, 2004). As well as release and deposition of fatty acids, adipocytes release a number of hormones termed adipokines. Over fifty adipokines, also known as adipocytokines have been discovered. They may be classified according to functional role: appetite and energy balance, insulin sensitivity, immunity, angiogenesis, lipid metabolism, blood pressure and haemostasis.
30

Figure 1.5: The multiple functions of white adipose tissue include the synthesis and secretion of adipokines, and the uptake, storage and synthesis of lipids. CCL, CC-chemokine ligand (CCL); CXCL, CXC-chemokine ligand (CXCL); IL, interleukin; IL1-RA, interleukin-1-receptor antagonist; NGF, nerve growth factor; PAI1, plasminogen activator inhibitor 1; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Adapted from Lago et al., 2007.

1.7.1: Adipose tissue and “inflammatory” adipokines

Obesity is characterized by a state of low-grade systemic inflammation (Fantuzzi, 2005). This is further linked to an increased risk for cardiovascular disease and T2D, particularly in the case of visceral adiposity (Wang et al., 2004), where the increased intra-abdominal fat produces a pro-inflammatory state.
Adipose tissue is composed of many cell types, mainly adipocytes. Other cell types include macrophages. Many factors involved in the inflammatory response are secreted by both preadipocytes and macrophages. The number of macrophages is related to the degree of adiposity and with adipocyte size in both humans and mice (Weisburg et al., 2003, Xu et al., 2003; Curat et al., 2004). Mouse studies have shown that white adipose tissue macrophages are bone marrow derived, demonstrating that the macrophages present in adipose tissue do not derive in situ from differentiation of preadipocytes but instead from circulating monocytes infiltrating adipose tissue (Weisburg et al., 2003).

Cytokines within adipose tissue originate from adipocyte, preadipocyte and other cell types such as macrophages. mRNA expression studies demonstrate that adipocytes can synthesize both TNFα and several interleukins, notably IL-1β and IL-6. It seems clear that TNFα is a powerful autocrine and paracrine regulator of adipose tissue. Other cytokines, notably leptin, and possibly IL-6, have lesser actions on adipose tissue. These cytokines act as hormones, reporting the state of adipose tissue stores throughout the body (Coppack, 2001).

1.7.1a: Tumour Necrosis Factor alpha (TNFα)

Tumour necrosis factor alpha (TNFα) is a cytokine implicated in the metabolic disturbances associated with chronic inflammation and malignancy. Biological functions of TNFα include induction of insulin resistance, anorexia and weight loss (Montague et al., 1997). Within adipose tissue, TNFα is expressed by adipocytes and stromovascular cells. The amount of TNFα mRNA is positively correlated with body fat and decreased in obese patients after weight loss (Montague et al., 1997).

TNFα can lead to insulin resistance by inducing serine phosphorylation of the insulin receptor substrate (IRS-1), which prevents insulin signalling (Hotamisligil et al., 1994). Hence, TNFα is regarded as a probable mediator of the insulin resistance and T2D associated with high visceral adiposity (Ofei et al., 1996). TNFα also impairs insulin signalling indirectly by increasing serum NEFAs, which have independently been shown to induce insulin resistance in multiple tissues (Ruan and Lodish, 2003).

In adipose tissue, TNFα represses genes involved in the uptake and storage of NEFAs and glucose, suppresses genes for transcription of factors involved in adipogenesis and lipogenesis, and changes expression of several adipocyte-secreted factors including IL-6 and adiponectin. In the liver, TNFα suppresses the expression of genes involved in glucose uptake
and metabolism and fatty acid oxidation and increases the expression of genes involved in *de novo* synthesis of cholesterol and fatty acids (Ruan *et al.*, 2002).

TNFα has also been shown to have effects on the regulation of adipose tissue mass. It impairs human pre-adipocyte differentiation *in vitro* (Petruschke and Hauner, 1993), and induces human preadipocyte and adipocyte apoptosis *in vitro* (Prins *et al.*, 1997). TNFα induces lipolysis *in vitro* and *in vivo* (van der Poll *et al.*, 1991). These results, when taken together, show that TNFα may be involved in limiting the ongoing increase in adipose tissue mass (Prins, 2002).

Triacylglycerols and NEFAs play an important role as physiological inducers of TNFα expression. High fat diets have been shown to produce significant increases in white adipose tissue TNFα mRNA. Mice carrying deletions of the TNFα receptors are more resistant to the development of diabetes, and immunoabsorption of TNFα in rodent models of obesity increases insulin sensitivity (Morin *et al.*, 1997). By contrast, treatment of obese and diabetic individuals with TNFα-neutralising antibodies over a 4 week period did not improve insulin sensitivity. This may be because TNFα acts at the local level within adipose tissue, and thus systemic inhibition might not affect insulin sensitivity (Nawrocki *et al.*, 2004).

1.7.1b: Interleukin 6 (IL-6)

It has been estimated that adipose tissue contributes approximately 30% of circulating II-6, with visceral adipose tissue producing higher levels than subcutaneous adipose tissue (Fain *et al.*, 2004; Fried *et al.*, 1998). Like TNFα, II-6 decreases with weight loss (Bastard *et al.*, 2000). Adipocytes and macrophages both contribute to the adipose tissue II-6.

Adipose tissue II-6 expression and circulating concentrations of the protein are positively correlated with obesity, impaired glucose tolerance and insulin resistance. In addition to this, plasma concentrations predict the development of T2D and cardiovascular disease (Fernandez-Real and Ricert, 2003).

The high levels of II-6 in obesity are responsible for the increase in acute-phase proteins, such as C-reactive protein (Fantuzzi, 2005). The acute phase reaction is associated with elevated levels of fibrinogen, a risk factor for coronary heart disease, with autocrine and paracrine activation of monocytes by II-6 in the vessel wall contributing to the deposition of fibrinogen. In fatty streaks and in the atheromatous “cap” and “shoulder” regions, macrophages, foam
cells and smooth muscle cells express IL-6, suggesting a role for the cytokine, with IL-1 and TNFα in the progression of atherosclerosis (Yudkin et al., 2000).

Peripheral administration of IL-6 induces hyperlipidaemia, hyperglycaemia, and insulin resistance in rodents and humans (Fernandez-Real and Ricart, 2003). IL-6 affects insulin signalling in peripheral tissues by reducing expression of insulin receptor signalling components and inducing the suppressor of cytokine signalling 3 (SOCS3), which is a negative regulator of insulin signalling (Senn et al., 2003).

In contrast to TNFα, studies of the IL-6 knockout mouse have confirmed that the cytokine is also produced in the hypothalamus, suggesting a role in central regulation of appetite and energy expenditure (Jones, 1994; Wallenius et al., 2002). Studies evaluating the role of IL-6 on energy homeostasis have suggested that IL-6 has a more complex involvement than originally thought. IL-6 levels in the central nervous system (CNS) are negatively correlated with body fat mass in overweight humans, suggesting a central deficiency in obesity. Central administration of IL-6 increases energy expenditure and decreases body fat in rodents (De Bendetti, 1997). Transgenic mice overexpressing IL-6 have a generalized defect in growth, including reduced body weight and decreased fat pad weights. In contrast, mice with a targeted deletion of IL-6 develop maturity-onset obesity and associated metabolic abnormalities, which are reversed by IL-6 replacement, suggesting that IL-6 prevents rather than causes these conditions (Wallenius et al., 2002). Thus, IL-6 has diverse effects on energy homeostasis in the periphery and CNS (Kershaw and Flier, 2004).

IL-6 has many effects on adipose tissue, including inhibition of lipoprotein lipase activity, induction of lipolysis and stimulation of basal glucose uptake (Mohammad-Ali, 1997; Mohammad-Ali et al., 1998). IL-6 also inhibits adipogenesis and decreases adiponectin secretion (Fernandez-Real and Ricart, 2003).

1.7.1c: Mono-chemoattractant-protein 1 (MCP-1)

MCP-1 is produced mainly by endothelial cells and macrophages. It is a strong chemotactic factor for monocytes (Yoshimura et al., 1989; Matsushima et al., 1989).

The presence of MCP-1 in white adipose tissue and plasma is increased in obese mice (Sartipy and Loskutoff, 2003). This indicates that MCP-1 may also be an adipokine which is increased on obesity.
MCP-1 is thought to play a role in atherogenesis. This is indicated from evidence showing that expression is increased in atherosclerotic lesions, and inhibition of its expression or of its receptor, decreases the amount of atheroma formation in hypercholesterolemic mice (Gu et al., 1998; Boring et al., 1998).

MCP-1 has previously been shown to be involved in insulin resistance in mice. Kanda et al. (2006) showed that there is an increase in the abundance of MCP-1 in both genetically obese diabetic (db/db) mice and in obese wild-type mice. It also found that the increase in expression of MCP-1 causes macrophage infiltration into adipose tissue, insulin resistance and hepatic steatosis linked to obesity in mice.

1.7.2: Adipose tissue and “cardiovascular” adipokines

As discussed in section 1.6, obesity has a strong relationship with cardiovascular disease, in particular visceral adiposity. This is proven by the evidence that weight loss decreases cardiovascular risk factors, including blood pressure (both systolic and diastolic), LDL and total cholesterol. As well as the general cardiovascular load imposed by obesity, other recognised contributors to the cardiovascular risk of obesity come from adipose tissue. Some of these will be discussed below.

1.7.2a: Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is an anti-fibrinolytic protein produced primarily by the liver, but also in adipose tissue. PAI-1 is an acute-phase response protein which inhibits the activation of plasminogen, the precursor of plasmin, which is involved in the breakdown of fibrin (Mutch et al., 2001).

Among the many mechanisms that are likely to explain the relationship of obesity with cardiovascular disease, disorders of the fibrinolytic system seem to play a role. Levels are increased with myocardial infarction and venous thrombosis. A close correlation with an abdominal pattern of adipose tissue distribution in both men and women as well as a positive association with other components of metabolic syndrome have been shown (Juhan-Vague and Alessi, 1997).

Increased levels of PAI-1 are found in obese subjects, and positive correlations have been found with components of insulin resistance (Vague et al., 1986; Shimomura et al., 1996; Juhan-Vague and Alessi, 1997). There is a higher production of PAI-1 from visceral adipose
tissue than subcutaneous adipose tissue, and plasma levels are correlated with visceral adiposity.

Support for its involvement in the metabolic syndrome is derived from studies showing that PAI-1 production by adipose tissue is increased by insulin and glucocorticoid.

Little is known about the regulation of the expression and secretion of PAI-1, particularly at the adipocyte level (Fruhbeck, 2001). Recently, increased levels of PAI-1 in obesity have been shown to result in part from insulin induced production, specifically by adipocytes in the fat itself. A study by Mavri et al. (1999) on dieting obese women showed that the decrease in PAI-1 after weight loss has a stronger correlation with changes in fat mass than with metabolic variables. This suggests that there is a significant role of adipose tissue in controlling plasma PAI-1 levels. However, another study found a correlation between circulating leptin levels and PAI-1 antigen, independent of BMI and body fat mass seems to suggest that leptin *per se* may possibly increase PAI-1 in obese subjects (Paz et al., 1997; De Mitrio et al., 1999).

### 1.7.3: Adipose Tissue and “Endocrine” Adipokines

#### 1.7.3a Leptin

Leptin is a 16-kD protein encoded by the *ob* gene (Zhang et al., 1994). Adipocytes are the main source and levels correlate with body fat (Maffei et al., 1995). Leptin expression and secretion is increased by insulin, TNFα, glucocorticoids, oestrogens and decreased by androgens, β3 adrenergic activity, free fatty acids, growth hormone, and peroxisome proliferation-activated receptor-γ (PPARγ) agonists (Margetic et al., 2002).

The main function of leptin is control of appetite. Mice and humans with a mutation in the leptin gene (*ob/ob* mice) or in the leptin receptor (*db/db* mice), are severely obese (Fantuzzi et al., 2005). Leptin/leptin receptor deficiency is rare in humans, and is associated with hyperphagic obesity and infertility, but when compared to mice is not linked to an alteration in metabolic rate (Montague et al., 1997). Many of leptin’s effects on energy intake and expenditure are mediated via hypothalamic pathways, whereas other effects are mediated via direct action on peripheral tissues including the pancreas and muscle (Bjorbaek and Kahn, 2004). Leptin levels decline with weight loss and energy restriction. Common obesity is characterized by increased leptin levels. Neither treatment with exogenous leptin or
endogenously high leptin levels are effective in ameliorating obesity, therefore some obese individuals are thought to be leptin resistant (Flier et al., 2004; Bjorbaek and Kahn, 2004).

Leptin was initially thought to be a satiety factor which regulates body weight by inhibiting food intake and increasing energy expenditure, but it is also involved in regulating endocrine function, reproduction, and immunity.

Leptin can be considered a pro-inflammatory cytokine (belongs to the family of long-chain helical cytokines), and has structural similarity with II-6, prolactin, II-12, II-15, granulocyte colony-stimulating factor and oncostatin M. Leptin production increases during infection and inflammation, which suggests that leptin is part of the cytokine network which controls the inflammatory-immune response and the host defence mechanism (Otero et al., 2004). Studies of rodents with genetic abnormalities in leptin or leptin receptors demonstrated obesity-related deficits in macrophage phagocytosis and expression of proinflammatory cytokines both in vitro and in vivo. Exogenous leptin up-regulated phagocytosis and production of proinflammatory cytokines. These results recognise an essential and novel function for leptin: up-regulation of inflammatory immune responses, which may offer a common pathogenetic mechanism which contributes to numerous complications of obesity (Loffreda et al., 1998).

Leptin protects T lymphocytes from apoptosis and alters T-cell proliferation, increasing the proliferation of naive T cells while decreasing the proliferation of memory T cells. Leptin modulates T-cell-derived cytokine production and increases the expression of the activation markers CD25 and CD71 in CD4+ and CD8+ cells. In monocytes leptin increases the expression of several activation markers and stimulates phagocytosis and cytokine production. In endothelial cells leptin upregulates the expression of adhesion molecules and induces oxidative stress.

Leptin can also be regarded as an angiogenic factor, as it has been shown to cause cultured endothelial cells to aggregate, form tubules, and show a similar arrangement to tissue vasculature (Sierra-Honigmann et al., 1998). Leptin has also been shown to increase wound healing, a process dependent on blood vessel growth (Fruhbeck et al., 2001).

Leptin appears to have a balancing effect regarding blood pressure homeostasis, with pressor action due to sympathetic activation and a depressor action due to nitric oxide release (Fruhbeck, 1999). Because of these actions leptin may be one of the factors underlying the relationship of obesity with hypertension and cardiovascular disease (Fruhbeck et al., 2001).
1.7.4: Adipose tissue and “Metabolic” Adipokines

Metabolic adipokines are adipokines that have, or are thought to have effects on energy homeostasis. In obesity, the flow of energy and nutrients from the gut to the tissues via the circulation is impaired and dysregulated. The changes in efficacy of endocrine action on adipose tissue (and liver and muscle) and alterations in the endocrine action of adipose tissue contribute to this dysregulation (Prins, 2002).

1.7.4a: Adiponectin

Adiponectin is a protein specifically secreted from adipose tissue. Its levels are reduced in insulin resistance and obesity (Hu et al., 1996; Arita et al., 1999). Hypo-adiponectinaemia is also associated with dyslipidaemia, hypertension, oxidative stress, and a carbohydrate-enriched diet (Pischon et al., 2005). Evidence is accumulating that adiponectin is involved in glucose and lipid homeostasis, and functions as an anti-atherogenic protein (Berg et al., 2001). Adiponectin enhances insulin action in the liver, therefore reduces hepatic glucose output (Berg et al., 2001), and it also reduces triacylglycerol levels in skeletal muscle. These observations indicate that adiponectin plays an important role in reducing obesity associated metabolic dysfunctions. Murine studies support this concept, as adiponectin significantly reduces the insulin resistance seen in lipoatrophy and obesity (Yamauchi et al., 2001). Thiazolidenediones have been shown to increase adiponectin, showing the possibility of adiponectin as an insulin sensitizer (Maeda et al., 2001). It is possible from these results for adiponectin to have a pharmacological role in “replacement” or “supplementation” therapy.

The metabolic and insulin-sensitizing effects of leptin and adiponectin can be somewhat explained by their direct activation of AMP-activated protein kinase (AMPK). They generate their action during the control of AMPK activity in muscle, liver and adipose tissue, which leads to increased fatty acid oxidation and the prevention of triacylglycerol accumulation and lipotoxicity in these tissues (Lafontan and Viguerie, 2006).

Two types of adiponectin receptor have been discovered. Overexpression and gene knockout rodent studies have shown the ability of these receptors to activate AMPK, p38 mitogen-activated protein kinase and peroxisome-proliferator-activated receptor (PPARα), and to stimulate fatty acid oxidation and glucose uptake in murine hepatocytes and C2C12 myocytes (Yamauchi et al., 2003).
1.7.4b: Resistin

Though there are many studies reporting a relationship between resistin and obesity in rodents, little work has been done on humans. Of the studies which have been carried out, results are conflicting. This study examines if there is a relationship between human obesity and resistin, and whether its levels are influenced by different dietary composition.

Resistin is secreted by adipocytes in the mouse (Prins, 2002). It belongs to the family of resistin-like molecules (RELMs), also known as “found in inflammatory zone” (FIZZ) (Fantuzzi, 2005). The initial report on resistin showed that resistin promotes insulin resistance and is increased in obese rodents and decreased by the thiazolidinedione drugs (Steppan et al., 2001). Further murine studies demonstrated that resistin administration caused insulin resistance, and anti-resistin methods improved insulin sensitivity. Other rodent studies have provided conflicting results (Way et al., 2001). Resistin expression is greater in visceral compared to subcutaneous adipose tissue in rodents (Benerjee and Lazar, 2003). However, the role of resistin in humans is unclear (Smith, 2002).

Human resistin shares only 64% homology with murine resistin (Banerjee and Lazar, 2003). Resistin expression in human adipocytes is insignificant, but is produced by pre-adipocytes and white cells (Savage et al., 2001). Macrophages appear to be the most important source of resistin in humans (Rea and Donnelly, 2004). Resistin expression in humans is not related to insulin resistance (Savage et al., 2001). Further studies are required to elucidate the function of resistin in human subjects (Prins, 2002).

Recent evidence has shown that stimulation of macrophages in vitro with endotoxin or proinflammatory cytokines leads to an increase in resistin production. In addition to this, administration of endotoxin to humans is associated with significant increases in circulating resistin levels (Lehrke et al., 2004). Therefore resistin seems to act as a mediator of the insulin resistance associated with sepsis and possibly other inflammatory situations (Fantuzzi, 2005).

There are few studies examining the effects of resistin in the modulation of inflammatory responses. Of these, resistin was shown to upregulate the expression of MCP-1, and also vascular cell adhesion molecule (VCAM) and inflammatory cell adhesion molecule (ICAM), in endothelial cells (Verma et al., 2003; Kawanami et al., 2004). The adhesion molecule-upregulating effects of resistin are antagonized by adiponectin (Kawanami et al., 2004).
Mice with a targeted deletion of resistin have given insight into its metabolic effects in rodents (Banerjee et al., 2004). Resistin-null mice have similar body weight and fat mass to wild-type mice, even when fed a high fat diet. Nonetheless, mice lacking resistin have improved fasting blood glucose levels on a chow diet and an improved glucose tolerance on high fat diet. Insulin sensitivity is unchanged. The improvement in glucose homeostasis in mice without resistin is due to decreased hepatic gluconeogenesis. Despite this evidence supporting the role of resistin in glucose homeostasis during fasting in rodents, a similar role has yet to be shown in humans (Kershaw and Flier, 2004).

1.8: Diets for Overweight and Obesity

Dietary patterns considerably influence the development of overweight and obesity. Even with an increased emphasis on nutrition, a widespread awareness of the fat and energy content of foods, and the availability of various low-fat, fat-free, and sugar-free foods and beverages, obesity continues to increase.

Today’s modern society has facilitated the increase in consumption of inexpensive, energy dense foods, with an abundance of conveniently located fast food restaurants, a high variety of foods at mealtimes (McCory et al., 1999) and larger portion sizes (Raynor and Epstein, 2001). Food manufacturers make every effort to improve the appearance and flavour of packaged foods and, through effective advertising, promoting overeating. Fast foods and convenience foods are generally high in energy and nutritionally poor, are easily available in most neighbourhoods, schools, hospitals, factories, and shopping centres. The effects of these factors are dramatic, as evidenced by the increasing waistline and adverse co-morbidities associated with obesity (Racette et al., 2003).

The goals of obesity treatment are to achieve and maintain a clinically significant weight loss, and to reduce the risk for, or severity of, obesity related co-morbidities. Weight losses of 5 % to 10 % of baseline body weight are considered by many research fellows to be a clinical success. However, long term success is dependent on maintaining 10 % weight loss for at least 1 year (Racette et al., 2003). Wing and Hill (2003) predicted that approximately 21 % of overweight or obese adults are successful at 1 year, but long term success is more difficult to maintain.
Most weight loss diet plans restrict energy intake in one way or another. However, weight maintenance requires long term changes in lifestyle. In the last decade there has been an increase in the use of alternative diets to low fat diets. These are discussed below.

1.8.1: Low-Fat Diets

This diet restricts the intake of fat to less than 35 % of energy intake. Low fat diets are popular because dietary fat is thought to contribute to obesity. However, according to The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults “Fat matters, but calories count”. In other words, a low fat diet is ineffective without a reduction in energy and won’t cause weight loss; the body will store carbohydrates as fat, as low carbohydrate diets tend to be higher in carbohydrate intake.

Although weight loss has many favourable effects on risk factors for various chronic diseases, low-fat (< 35 % of energy) or very-low-fat (< 20 % of energy) diets can increase triacylglycerol concentrations, decrease HDL-cholesterol concentrations, and, in some cases, increase plasma glucose concentrations. As dietary fat is decreased, the dietary carbohydrate content typically rises and the desired reduction in plasma cholesterol concentrations often occurs, but with an elevation of plasma triacylglycerols (Parks and Hellerstein, 2000). A moderate-fat diet (25–35 % of energy), particularly one that is higher in dietary fibre, results in lower triacylglycerol and higher HDL-cholesterol concentrations and, therefore, is not associated with the unfavourable effects mentioned above (Institute of Medicine of the National Academies, 2002).

A study by Pelkman et al. (2004) examined the effects of a low-fat compared to a moderate-fat, whole food diet on plasma lipids and lipoproteins in overweight and obese healthy subjects. Both diets contained recommended amounts of saturated fat and cholesterol. The moderate-fat diet resulted in favourable changes in the lipoprotein profile. Compared with baseline, HDL cholesterol was unchanged, whereas triacylglycerols and the ratios of total and non-HDL cholesterol to HDL cholesterol were lower at the end of the weight-maintenance period in the moderate-fat diet group. Despite similar weight loss, triacylglycerol rebounded, HDL cholesterol decreased, and the ratios of total and non-HDL cholesterol to HDL cholesterol did not change during the 10 week interval in the low-fat diet group. These results
suggest a moderate-fat weight-loss and weight-maintenance diet improves the cardiovascular disease risk profile on the basis of favourable changes in lipids and lipoproteins.

1.8.2: Low Calorie Diets and Very Low Calorie Diets (LCDs and VLCDs)

Weight reducing diets may be low calorie (LCDs, 800-1500 kcal/d) or very low calorie diets (VLCDs, < 800 kcal/d). VLCDs are very effective for reducing weight (Saris, 2001). However, maintenance of the reduced weight is inconsistent and generally unsuccessful. LCDs are more common as they are safer, have fewer side effects and are easier to adhere to (Rossner and Flaten, 1997).

Generally, LCDs are high in carbohydrates (55-60 % of total daily energy intake), low in fat (< 30 % of energy intake), and energy reduced (Freedman, 2001). LCDs should be high in fibre and have a low glycaemic index (Strychar, 2006). Diets of this kind include the National Cholesterol Education Program (NCEP) Step 1 diet with energy reduction, the DASH (Dietary Approaches to Stop Hypertension) diet, which is based on the US Department of Agriculture Food Guide Pyramid, and various commercial diets (Weightwatchers) (Freedman, 2001). The NCEP 1 and 11 diets have been found to reduce LDL-cholesterol by 12 % and 16 %, respectively. However, they have also been found to increase triacylglycerol levels, although not consistently (Yu-Poth et al., 1999; Parks and Hellerstein, 2000). Higher triacylglycerol levels are often associated with low HDL cholesterol levels and with small LDL-cholesterol particles which are more susceptible to oxidation. This metabolic state is said to be atherogenic (Austin and Hokanson, 1994; Hokanson and Austin, 1996). The 2001 NCEP revised guidelines recommend the Therapeutic Lifestyle Change Diet for reducing the risk of coronary artery disease. With this diet, 7 % of total daily energy intake comes from saturated fat and 25-35 % comes from total fat. This diet is aimed at reducing LDL-cholesterol and triacylglycerol levels and increase HDL-cholesterol levels (Third Report of the National Cholesterol Education Program Panel, 2001).

VLCDs are diets with a daily energy value of < 800 kcal/day. They must be prescribed and patients must be monitored regularly by a health professional, to avoid severe negative nitrogen balance and electrolyte changes associated with starvation (AACE Obesity Treatment, 1998). VLCDs are limited to individuals with a BMI of ≥ 30 kg/m², who face major health problems, and for whom other approaches have not worked (Position of the American Dietetic Association, 1998). VLCDs show an average weekly weight loss of 1.5-2.5 kg, compared with 0.4-0.5 kg with LCDs (National Task Force on the Prevention and
Treatment of Obesity, 1993). The average weight loss at 12-16 weeks on a VLCD is approximately 20 kg, but it is only 8 kg on the LCD (National Task force on the Prevention and Treatment of Obesity, 1993; National Heart, Lung and Blood Institute, 1998). VLCDs are thought to be associated with various side-effects, such as cholelithiasis, loss of lean body mass, ketosis and increased serum acid concentrations due to severe negative energy balance (AACE Obesity Treatment, 1998) and this needs to be further investigated.

1.8.3: Low Carbohydrate/ High Protein Diets

These diets usually allow for only 20 g to 90 g of carbohydrate per day with unrestricted amounts of protein and fat. Bravata et al. (2003) carried out a meta-analysis on low carbohydrate diets, and found that weight loss was associated with the lower energy intake and longer diet duration and not associated with reductions in carbohydrate (Bravata et al., 2003). However these diets have some complications including vitamin deficiencies, altered cognitive function and increased LDL-cholesterol (Denke, 2001).

When a very low carbohydrate diet is consumed, the body uses up its glycogen stores and gluconeogenesis is induced, so that lean tissue is used to produce glucose as an energy source for the brain. Free fatty acids are mobilized from adipose tissue, resulting in ketone body formation. Ketosis decreases appetite and the high protein content of the diet is thought to increase satiety and activates thermogenesis (Hill and Blundell, 1986; Johnston et al., 2002; Insel et al., 2004).

There have been many studies comparing low carbohydrate diets to low fat/ conventional diets, and this is further discussed in Chapter 3. Both diets have been shown to reduce weight, with a greater amount on low carbohydrate diets in the short term, but with no significant differences between the diets at 12 months (Foster et al., 2003; Stern et al., 2004).

With regards to cardiovascular risk factors, 3 separate studies by Stern et al. (2004), Yancy et al. (2004) and Foster et al. (2003) observed greater decreases in triacylglycerol levels on the low carbohydrate diet compared to the LCD after 6 months and 12 months. Foster et al. (2003) also reported an increase in LDL-cholesterol at 3 months compared with the LCD; however the difference was not significant at 1 year. Saturated fat on a low carbohydrate diet is generally high (Brehm et al., 2003). Saturated fat intake is associated with increased LDL-cholesterol levels, a risk factor for coronary heart disease. Insulin sensitivity improved on both diets (Brehm et al., 2003; Foster et al., 2003).
In the study by Brehm et al. (2003), leptin levels decreased on both the low carbohydrate diet and LCD at 3 months, although this may have a negative effect on weight loss, as decreases in leptin levels during periods of negative energy balance stimulate food intake and energy storage to restore homeostasis (Schwartz and Seeley, 1997; Woods et al., 1998; Schwartz et al., 2003).

Freedmen et al. (2001) studied the nutrient content of the low carbohydrate diets. They found the diet to have low levels of vitamins A, B6, and E, and of folate, calcium, magnesium, iron, potassium, and dietary fibre making the diet nutritionally inadequate. High protein diets could affect kidney and liver function by creating more of a demand on them for the metabolism and excretion of the excess urea and ammonia, although there is no evidence of adverse effects (St.Jeor, 2001).

Overall, low carbohydrate diets are not recommended, as more trials need to be performed to establish their efficacy and safety.

1.9: The effects of different macronutrients on appetite regulation

Macronutrients with the same energy content have different effects on satiety and satiation independent of their energy content, showing that not all sources of energy are treated equally by the body. Stubbs et al. (2000) reported in a review that under normal conditions in which fat contributes disproportionally to energy density, carbohydrate, protein and fat show an ordered effect on satiety in the following way: protein > carbohydrate > fat. There is a large amount of research about the satiating effect of protein; however there are not so many studies examining the effect of carbohydrate and fat on satiety.

Blundell et al. (1993) showed that fat has a weak satiating effect compared to sucrose. However different types of carbohydrate show different effects on satiety, e.g. fibre has been shown to have a higher satiety value compared to digestible complex carbohydrates and simple sugars (Pereira and Ludwig, 2001).

As high-fat foods are more energy dense, with a higher palatability and show a lower intrameal and intermeal satiety value, these factors can explain why people tend to overeat high-fat foods (passive overconsumption).

Raben et al. (2003) carried out a study on the effects of high protein, high carbohydrate, high fat, and high alcohol meals while controlling for energy density and fibre, and observed no
independent effect of macronutrient content, suggesting that energy density and fibre may explain the difference in the macronutrient’s differential effect on satiety.

1.10: Effect of diet on weight, cardiovascular disease risk and adipokine levels

Most dietary weight loss requires an enforced energy restriction. Recently there has been considerable public interest in the use of high protein/low carbohydrate ketogenic diets as well as very low calorie/meal replacement diets. These diets have proved popular because weight loss is achieved at the same time as hunger is satisfied. The need for enforced energy restriction and considerable self will are reduced. These dietary effects can be achieved by using either whole food intake, (protein sparing modified fast, PSMF) or by the use of constituted powder diets, i.e. meal replacement programmes (e.g. LighterLife, LL).

There have been many short term clinical trials investigating the effects of these low carbohydrate/high protein diets on weight loss and cardiovascular disease risk factors. However, they do not demonstrate if there are any long term health benefits/risks from these dietary approaches. Many of the trials have only been carried out for a maximum period of 6 months.

One such trial examined the effects of a low carbohydrate/high protein diet compared to a low fat/low energy diet (Brehm et al., 2003). The results showed that the low carbohydrate diet had greater participant retention and weight loss. During the active weight loss, there was a greater decrease in triacylglycerols and increase in HDL cholesterol on the low carbohydrate diet than with the low fat diet.

Another 6 month trial compared the effects of an ad-libitum, fat reduced diet (30 % of energy) either high in protein (25 % of energy, HP) or medium protein (12 % of energy, MP). The subjects included a total of 50 overweight and obese subjects with a BMI between 26-34 kg/m². The study concluded that a fat reduced diet high in protein seems to enhance weight loss and provide greater long term maintenance of reduced intra abdominal fat stores (Due et al., 2004).
A study by Samaha et al. (2003) examined the effects of a carbohydrate restricted (low carbohydrate) diet to a calorie and fat restricted (low fat) diet over 6 months. The subjects included 132 severely obese subjects with a prevalence of diabetes and metabolic syndrome. The study showed that the obese subjects with a high prevalence of diabetes or the metabolic syndrome lost more weight during the 6 months on a carbohydrate restricted diet than on a calorie and fat restricted diet, with an improvement in insulin sensitivity and triacylglycerol levels, even after adjustment for the amount of weight lost.

A year long trial by Foster et al. (2003) compared a low carbohydrate, high fat, high protein diet to a low calorie, high carbohydrate, low fat diet (conventional diet). The low carbohydrate diet showed a greater weight loss than the conventional diet for the first 6 months, but the difference was not significant at one year. The study found that the low carbohydrate diet was associated with a greater improvement in some risk factors for coronary heart disease.

Most of the literature on these low carbohydrate/high protein diets recommends that there is a need for future studies evaluating long term cardiovascular and weight loss outcomes before a carbohydrate restricted diet can be endorsed.

In relation to adipokines, it is now evident that adipose tissue not only stores excess triacylglycerols, but functions as an endocrine organ by releasing adipokines, including adiponectin, leptin, resistin, TNFα and Il-6. These adipokines have different roles in the body including appetite regulation, glucose and lipid metabolism, inflammation, and insulin resistance.

Obesity appears to be a state of chronic low-grade inflammation, with a rise in inflammatory adipokines, but also a decrease in the adipokine adiponectin. This is a predictor of cardiovascular disease risk and T2D. Monzillo et al. (2003) studied the effect of weight loss on adipokine levels in obese subjects with insulin resistance. Twenty four insulin resistant subjects were measured over a 6 month period and were assigned a hypocaloric diet and moderate physical activity. Leptin, Il-6 and TNFα decreased, whereas adiponectin was only increased in diabetic subjects. He concluded that further studies are needed to clarify the relationship between changes of adipokines and the health benefits of weight loss.
1.11: Aims and Objectives

With the current obesity epidemic in both adults and children, there is an urgent need for dietary regimens that not only will induce weight loss, but prevent weight gain. As obesity is associated with co-morbidities such as type 2 diabetes (T2D) and cardiovascular disease (CVD), it is vital that these dietary regimens also reduce the risk of such co-morbidities.

The use of conventional diets such as low fat diets is failing to stem the tide of population weight increase and alternative diets such as low carbohydrate diets have gained increased recognition.

Overfeeding with carbohydrate stimulates carbohydrate oxidation. If carbohydrate is consumed in excess, then oxidation of fat is inhibited, and dietary fat is stored in adipose tissue (Garrow, 2000). Aarsland et al. (1997) carried out a study in which volunteers were fed a high carbohydrate diet, by nasogastric infusion, and at a rate which was more than twice their energy consumption. This overfeeding was maintained for 4 days, and the rate of whole-body fat synthesis, and synthesis of fat in the liver, was measured by isotope labelling. On the first day of overfeeding net fat synthesis was 480 mg/kg and this increased to 2200 mg/kg per day on the fourth day. Only a small amount of this fat was synthesized in the liver, and it was concluded that the main site for fat synthesis during carbohydrate overfeeding is in the adipose tissue. While a high fat diet predisposes to obesity (mainly due to an increase in energy intake), a low fat diet taken in excess can increase body fat (Frayn and Whitley, 1997).

Low fat diets are viewed as the standard diet for weight control. However, though these diets can be effective for many people, there doesn’t seem to be any long term study where a low fat diet was instituted without confounding variables such as cessation of smoking and exercise (Volek and Feinman, 2005). Dansinger et al. (2005) compared 4 low-fat and high fat
diets and showed that weight change did not differ between diets at 1 year, and an 18 month comparison of a low-fat and Mediterranean style diet showed poor adherence to the low-fat diet (McManus et al., 2001). A total of 101 men and women (BMI 26.5-46 kg/m²) were recruited and randomised to either a moderate-fat (35% energy) or a low-fat (20% energy) diet for 18 months, with change in body weight the primary outcome. At 18 months, the low-fat group showed a mean increase in weight (2.9 kg), whereas the moderate-fat group showed a mean decrease of 4.1 kg, and the difference was significant between the groups. McManus et al. concluded that a moderate-fat Mediterranean style diet (high in monosaturated fat), controlled in energy, offers an alternative to a low-fat diet with greater adherence, and improvements in weight loss.

In addition to this, fat restriction per se has not been found to enhance long term weight loss, or weight maintenance (Kuczmarski et al., 1994; Poppitt et al., 2002). The trial by Poppitt et al. investigated the effects of 2 low fat, high carbohydrate diets on weight loss and metabolism on overweight subjects who were at high risk for CVD. It was hypothesized that if long term compliance could be achieved, and ad-lib low fat, high carbohydrate diet would reduce body weight and fatness and therefore improve the lipid profile and other factors associated with coronary disease risk. Forty-three subjects with > 3 risk factors for MS were recruited and randomly assigned to a low fat/complex carbohydrate diet, a low fat/simple carbohydrate diet, or a control group for 6 months. Neither diet showed significant improvements in weight loss. In relation to CVD risk, there were no significant improvements for LDL cholesterol. TAG was significantly higher in the low fat/simple carbohydrate group compared to the other groups, and HDL cholesterol decreased significantly in all groups. Poppit et al. concluded that a low-fat high-polysaccharide diet in overweight subjects with MS showed moderate, though insignificant weight loss, with some
improvement in serum cholesterol. Increasing simple carbohydrate did not lead to weight gain, but nor was there any improvement in weight or the lipid profile.

Most importantly, fat consumption has decreased and carbohydrate consumption has increased during the obesity and diabetes epidemic (Enns, 1997; Kennedy, 1999) The implies that other dietary approaches need to be investigated (Volek and Feinman, 2005), in particular low carbohydrate diets, which have shown to be as good as low fat diets for weight loss and CVD risk (Hession et al., 2008).

Mcquigg et al. (2008) examined the prevalence of CVD risk factors by BMI. The prevalence of low HDL and increased TAG in men increased with a higher BMI. They found that compared to a normal BMI, the prevalence of low HDL and high TAG increased significantly at a BMI of $32 \, \text{kg/m}^2 < 35 \, \text{kg/m}^2$ with an even higher prevalence at a BMI above $40 \, \text{kg/m}^2$. Similar results were seen in women. Both high TAG and low HDL are risk factors for MS.

Results from the study by McQuigg et al. (2008), found that the risk for developing T2D rapidly increases from a BMI of about 32.5 to 35 kg/m$^2$, and there is also a sudden increase in the risk for developing hypertension, dyslipidemia and CVD in this BMI category. The risk of developing T2D increases about 40-fold at a BMI above 35 kg/m$^2$ (McQuigg et al., 2008). This suggests that diet, in particular a high carbohydrate may influence this trend for development of obesity related co-morbidities above a BMI of 35 kg/m$^2$. This BMI category has not been extensively studied previously in relation to dietary therapy and is a reason why such a cut-off was chosen for the present clinical trial.
MS consists of risk factors which are related to increased carbohydrate consumption, including high TAG and low HDL-cholesterol, and this phenotype is investigated in the current trial. Studies of low carbohydrate diets have shown improvements in MS compared to low fat diets, even in the absence of weight loss (Sharman et al., 2002; Volek et al., 2003). Sharman et al. examined the effects of a ketogenic (very low carbohydrate diet) on fasting and post-prandial serum biomarkers in 20 normal weight-normolipidemic men over a 6 week period. Twelve men switched from their normal diet to a ketogenic (30 % protein, 8 % carbohydrate, and 61 % fat) and 8 controls remained on their habitual diet. TAG, postprandial lipemia and fasting insulin concentrations all decrease significantly in those consuming the ketogenic diet. Fasting serum total and LDL cholesterol, and oxidized LDL cholesterol showed no change and HDL cholesterol tended to increase on the ketogenic diet. In addition to this, in subjects with a predominance of small LDL particle size, there were significant increases in the mean and peak LDL particle diameter and large LDL cholesterol after the ketogenic diet. Whereas the control group showed no significant changes in the lipid profile. This was the first study to examine ketogenic diets on fasting and postprandial lipids independent of weight loss. Volek et al. carried out a similar study to Sharman et al. but investigated the effects of a very low carbohydrate and a low fat diet on fasting lipids, postprandial lipemia and inflammation markers in women. A crossover design was used where 10 healthy, normolipidemic women consumed both diets over a 4 week period each. Compared to the low fat diet, the very low carbohydrate diet increased total cholesterol, LDL cholesterol and HDL cholesterol, and decreased TAG. No changes in LDL particle size or markers of inflammation (C-reactive protein, IL-6 and TNFα) were seen. Volek et al. concluded that though there were modest increases in LDL cholesterol, there were favourable effects on CVD risk by the fact that there was a relatively larger increase in HDL cholesterol and a decrease in both postprandial and fasting TAG on the very low carbohydrate diet. Both
studies concluded that a low carbohydrate regimen improved MS compared to low fat even in the absence of weight loss.

Though there is weight loss with low fat diets, low carbohydrate diets have been shown to be more effective in terms of MS and weight loss (Brehm et al., 2003; Sondike et al., 2003; Samaha et al., 2003; Foster et al., 2003; Volek et al., 2004; Sharman et al., 2004; Meckling et al., 2004; Brehm et al., 2004; Stern et al., 2004; Yancy et al., 2004; Stern et al., 2004; Aude et al., 2004; Seshadri et al., 2004; McAuley et al., 2004; Dansinger et al., 2004). Many of these studies are discussed in Chapter 4.

Patients presenting with a high BMI as investigated in the present study, or a high waist circumference may have several options for weight loss. It can depend on factors such as physician experience, ethnic background, personal taste, and genetic profile. For example, a subject presenting with a high BMI and high TAG may be better suited to a low carbohydrate diet. This leads to the hypothesis in the present trial; that the dietary approach prescribed to the subject may depend on the phenotype at presentation.

The present clinical trial was a long term trial with a “screening period” of 3 months, after which subjects were randomised to 9 months of dietary treatment, for those who were “resistant” to the HE diet. The trial examined the effectiveness of a HE diet for obesity and its co-morbidities including CVD and T2D. It also compared a very low calorie (VLCD) (Lighterlife, LL) diet to a protein sparing modified fast (PSMF). The primary outcomes were weight loss, changes in CVD risk, and circulating adipokines. The secondary outcome investigated the presenting obese phenotypic response to the diets, including those with MS and T2D.
The study design was different to any study found in the literature examining dietary approaches for obesity, as only subjects with a BMI $\geq 35$ kg/m$^2$ were included. As discussed previously, McGuigg et al. showed that those with severe obesity demonstrated a higher TAG and low HDL levels, in addition to a higher risk for hypertension, dyslipidemia, and CVD. This leads to the hypothesis that those with severe obesity would be better suited to a low carbohydrate dietary approach.

The subjects recruited for the trial were healthy obese adults, and were representative of many obese individuals who embark on different diets for losing weight annually. The screening approach (first 3 months) was used to identify subjects who were non-responsive, in terms of appropriate weight loss to a HE programme, where they needed to lose $> 5\%$ to be randomised to either LL or the PSMF. A weight loss of 5-10\% is known to be clinically important in terms of improvements in lipids, blood pressure and glucose levels, in effect reducing the risk for CVD (Blackburn, 1995; Aucott et al., 2005).
The aim of this project was to examine different dietary approaches for the treatment of obesity, and to relate the phenotype at presentation to a suitable diet.

The objectives of this project include the following:

- To carry out a systematic review of low carbohydrate diets in relation to weight loss and cardiovascular risk factors and comparing them to low fat/energy dietary approaches over a minimum of 6 months.

- To conduct a clinical trial using the LighterLife weight loss programme and comparing to a protein sparing modified fast (low carbohydrate). A healthy eating, calorie deficit diet is also examined.

- To examine the outcomes of the trial including weight loss, cardiovascular risk factors (including LDL cholesterol, HDL cholesterol, total cholesterol, triacylglycerols, blood pressure) fasting plasma glucose, insulin resistance and adipokines.

- To relate the phenotype at presentation with the appropriate response to the different treatment modalities and to examine the adipokine response to these different treatments. The phenotype analysed was those with metabolic syndrome, and type 2 diabetes.

All of the above were carried out by the research student. However, the research fellow also met with the participants recruited for the clinical trial to give dietary advice. For lipid profile, fasting glucose, liver and kidney function, analysis was carried out by the Dept. of Clinical Biochemistry at Aberdeen Royal Infirmary, Aberdeen, UK.
CHAPTER 2: METHODS
2.1: Systematic Review

The first objective of the study was to carry out the systematic review of randomised controlled trials (RCTs) of low carbohydrate/high protein vs. low fat/low energy diets in the management and treatment of obesity and cardiovascular disease risk factors. This was done prior to commencing the clinical trial, and has recently been published both in abstract form and as a paper (Hession et al., 2008), and is discussed in greater depth in Chapter 3. The methods used for the systematic review are in detail below.

Inclusion Criteria

The protocol used for this systematic review follows the methods recommended by the Cochrane Collaboration (Avenell et al., 2004). Randomised controlled trials (RCTs) were included if they assessed the weight-loss effects of low carbohydrate/high protein diets (LC/HP) against low fat/high carbohydrate (LF/HC) diets. Only RCTs from January 2000 to March 2007 were evaluated, as this review is intended to assess the current literature in this field and update the NHS R&D Health Technology Assessment (HTA) systematic review of diet and lifestyle on weight loss and cardiovascular risk published by Avenell et al. Only studies conducted in an adult population were included, as defined by minimum age greater than 18 years. RCTs where the participants had a mean or median BMI of ≥ 28 kg/m² were included. A BMI cut off of ≥ 28 kg/m² was used to allow the inclusion of studies of ethnic groups where the classification of obesity is at a lower BMI cut off (Caterson and Broom, 2001). RCTs evaluated in this review had to be of at least 6 months duration, including the period of active intervention and follow up.

Types of Intervention

The focus of this review was to examine low carbohydrate/high protein diets against other types of diets designed to induce weight loss and/or prevent weight gain, and induce changes in cardiovascular risk factors. The types of dietary intervention evaluated were:

- High protein “ketogenic” diet, where the carbohydrate content was less than 40 g/day, irrespective of calorie content.
- Low carbohydrate diets (CHO ≤ 60 g/day).
- “Healthy eating” advice.
- Low fat (30 % or less daily energy from dietary fat)/600 kcal deficit diet
Outcome Measures

Weight loss or prevention of weight gain were the main outcomes assessed from the RCTs included in the review. With regard to cardiovascular disease risk factors, the following outcomes were also included:

- Serum lipids, including total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triacylglycerols.
- Systolic and diastolic blood pressure.
- Glycemic control.

Attrition rates were also analysed for each study to assess patient acceptability.

Search Strategy for the Identification of Included Studies

This systematic review was restricted to RCTs where the full study report was available. A wide search strategy was applied to identify as many RCTs evaluating dietary interventions as possible and which were relevant to the management of obesity and cardiovascular disease risk factors. Thirteen electronic databases were searched including MEDLINE, CAB abstracts and the Cochrane Central Register of Controlled Trials (CENTRAL). The search strategy incorporated weight loss, cardiovascular disease and obesity-related terms and text terms, specific to each database. Seven obesity and nutrition journals were hand-searched including the International Journal of Obesity and Obesity Research. Reference lists of included studies were searched and authors contacted for further details of their trials.

Quality Assessment of Studies

Full copies of studies were assessed by 2 researchers for methodological quality using a standard form. The researchers were not blinded to author, journal or institution. Differences of opinion were resolved by discussion. Trial quality was assessed, including whether or not the analysis was undertaken on an intention to treat basis.

Data Abstraction

A data abstraction form was created for this review based on a standard format (Avenell et al., 2004). For each study, data were abstracted and checked by different researchers prior to electronic data entry.
Data Analysis

The computer program Review Manager 4.2.2 was used for the analysis of the data from the reviews. If results from studies could be quantitatively combined, a statistical meta-analysis of the data was undertaken to determine the typical effect size of the intervention. For continuous data, a weighted mean difference (WMD) was calculated. The Chi-Square test was used to test for heterogeneity across the studies. The significance value was set at 0.05.

2.2: Clinical Trial and Study Design

The second objective of the study was to conduct a clinical trial using the LighterLife weight loss programme and comparing to a protein sparing modified fast (low carbohydrate). A healthy eating, calorie deficit diet is also examined. In addition to this, another objective was to examine the outcomes of the trial including weight loss, cardiovascular risk factors (including LDL cholesterol, HDL cholesterol, total cholesterol, triacylglycerols, blood pressure) fasting plasma glucose, insulin resistance and adipokines.

The methodology differed from previous clinical trials of diets for weight loss. The study examined only those with a BMI of 35 kg/m², and the design differed in that subjects were randomly allocated their diet at 3 months depending on whether they were successful in losing 5% of their baseline weight. This is discussed throughout the thesis, and in the aims and objectives section (p 45).

The study was approved by the Grampian Research Ethics Committee. Subjects were recruited from the waiting list at the obesity clinic, Woolmanhill hospital, Aberdeen and attended the outpatient clinic at Westburn House, Aberdeen Royal Infirmary for the duration of the trial. Figure 2.1 shows the outline of the study design.
Figure 2.1: Outline of subject treatments dependant on response to standard diet and lifestyle management

At baseline, subjects underwent a full physical examination by a physician. All subjects were assigned to a “healthy eating” (HE) low calorie diet for the first 3 months of the trial. 600 kcal were subtracted from each subject’s calculated total daily energy expenditure (TEE), using the Schoefield equation (Table 2.1) and were assigned the daily amount of energy to consume from this.

The subjects returned to the clinic at 2 weeks, 1 month, and 2 months for a weigh-in, in addition to dietary and lifestyle advice. At 3 months the subjects returned to the clinic. If subjects had lost > 5 % of their baseline body weight, they remained on the HE diet for at least the next 3 months of the trial. If subjects had not lost > 5 % of their baseline body weight, they were randomised to either 1 of the following diets; LighterLife (LL) or a protein sparing modified fast (PSMF) (Section 2.2.2) for the remainder of the trial.
Patients were randomised by simple randomization. Subjects were assigned a number relating to which diet they were going to be randomised to. This was placed in an envelope in the subject’s file, and opened at randomization.

After the 3 month visit, the subjects were seen on a monthly basis at the outpatient clinic (4 and 5 months).

Subjects who were on the HE diet until their 6 month visit and had lost ≥ 10 % of their baseline body weight stayed on this diet for the remainder of the trial. Otherwise the subjects who had not lost ≥ 10 % of their baseline body weight were randomised to either LL or PSMF which they continued for the remainder of the trial.

Following the 6 month visit, all subjects were seen at 8 and 10 months.

Subjects returned for their final visit at 12 months.

2.2.1: Clinical Trial Diets.

2.2.1 (a) HE diet
All subjects recruited onto the trial were assigned to HE for the first 3 months. The subject’s daily energy requirements were determined as described in section 2.2.3 and 600 kcal was subtracted from their calculated daily energy requirements to result in daily deficiency in energy intake. Subjects were advised the kcal/d to consume, taking into account the number of portions from each food group, as well as portion sizes. They were advised to consume a sufficient amount of water/day. They were given a 3-day food diary at their first visit in order to estimate compliance and to discuss any modifications at the next visit (2 weeks). The HE diet was a low energy and low fat (≤ 30 % total daily energy intake) diet.

2.2.1 (b): Protein Sparing Modified Fast (PSMF)
The PSMF was a low carbohydrate, high protein diet where the subject consumed conventional food while restricting carbohydrate intake to ≤ 40 g/day. Subjects were given an information booklet which included guidance about the macronutrient content of the diet, in addition to information on the carbohydrate content of common foods, a list of high protein
foods, and low carbohydrate recipes. The booklet also contained information on the side-effects the subject may experience while on the PSMF. The subjects were given a letter for their GP requesting a prescription for Forceval. Forceval replaces any mineral or vitamin deficiencies while on the diet. Subjects following the PSMF were given the 3 day food diary at 6 months to measure compliance.

2.2.1 (c): LighterLife

The LighterLife programme is a very low calorie diet (VLCD), in the form of energy deficient but otherwise nutritionally complete foodpacks, and is in parallel with weekly group sessions of cognitive behaviour therapy (CBT) (provided by a trained LighterLife counsellor) to discuss the underlying causes of the subject’s eating behaviour. The LL programme consists of 3 phases. Phase I is the weight loss stage, which is for a period 14 weeks; phase II which is the 14 week management program where subjects are slowly introduced to whole foods, and phase III which is a weight maintenance program. The LighterLife programme provides approximately 400-800 kcal/day, and is used as a total food replacement.

The idea of the foodpacks is so that the subject does not have to think about portion sizes, weighing out food, or look for healthier choices. After the subjects were randomised to LighterLife, they were given contact information for their LighterLife counsellor, who then arranged for them to join the counselling sessions (CBT). The subjects also visited the clinic at similar stages to the HE and PSMF subjects.
Figure 2.2: Comparison of dietary interventions in regards to contact and support sessions.

All subjects meet with research fellow/PhD student at baseline, 2 weeks, 1 month, and 2 months for 30 minutes for lifestyle support.

- LL group met with a LL trained counsellor once a week after randomisation for 2 hours until the end of the trial.
- HE group meet research fellow/PhD student at 5, 6, 8, 10 and 12 months for 30 minutes for lifestyle support.
- PSMF meet research fellow/PhD student at 5, 6, 8, 10 and 12 months for 30 minutes for lifestyle support.
2.2.2: Subjects

A total of 120 subjects were recruited from the new patient referrals to the obesity clinic, Woolmanhill hospital, Aberdeen. Subjects were contacted by post, and were given an information sheet, which gave details about the trial, including any risks of participating. They were given the option of taking part by signing a reply form attached to the information sheet and returning it by post.

To fit the inclusion criteria the subjects had to have a BMI $\geq 35$ kg/m$^2$, and be 18 years or over. Exclusion criteria included any subjects with a history of cardiovascular, kidney or liver disease, or cancer. Pregnant or lactating women were not included in the study, nor were subjects receiving medication for depression and mental illness. Subjects were asked to bring any medication they were taking to the clinic prior to commencing the trial.

All subjects gave written informed consent at their first visit. The study was not blinded.

After reviewing subject files for eligibility, 254 were contacted by letter and 187 replied. Of these subjects 120 were recruited for the study (Figure 2.2).

At 3 months, 30 subjects dropped out of the clinical trial. 18 subjects remained on the HE diet, and 72 were randomised either LL or the PSMF; 34 subjects were randomised to LL and 38 to the PSMF.

Of the 18 subjects who remained on HE at 3 months, 11 of these achieved the 10 % weight loss at 6 months and remained on HE. 5 or these 11 dropped out at 12 months. 7 subjects were randomised to LL and PSMF at 6 months.

As the data was analysed on an intention to treat basis, all 18 subjects were analysed on the HE diet at 12 months.

For subjects who missed appointments, or did not attend, they were followed up by phone, or email if possible. Alternative appointments were made for subjects who wanted to remain in the trial, but for those who did not want to remain, they were characterized as dropouts.

If subjects could not be contacted after several attempts, and did not show up for any appointments, they were also considered to be dropouts.
Figure 2.3: Flowchart and attrition rate for clinical trial.
M = males, F = females, D/O = dropouts, HE = Healthy eating, LL = Lighterlife, PSMF = Protein sparing modified fast.

2.2.3: Study Measurements

Baseline Measurements (0 Months)
Subjects were instructed to fast for 12 hours prior to their baseline visit. The Schofield equation with an activity factor of 1.5 was used to estimate daily energy expenditure.

The equation uses the following formulas depending on age and gender as shown in Table 2.1.
Men:

<table>
<thead>
<tr>
<th>Age Range</th>
<th>BMR Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 - 29 years</td>
<td>$15.0 \times W + 656$</td>
</tr>
<tr>
<td>30 - 60 years</td>
<td>$11.4 \times W + 870$</td>
</tr>
</tbody>
</table>

Women:

<table>
<thead>
<tr>
<th>Age Range</th>
<th>BMR Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 - 29 years</td>
<td>$14.8 \times W + 485$</td>
</tr>
<tr>
<td>30 - 59 years</td>
<td>$8.1 \times W + 842$</td>
</tr>
</tbody>
</table>


Height (cm) was measured using a stadiometer (The Leicester Height Measure, Seca Ltd, Birmingham, England), with each subject standing upright, after removal of footwear. Waist and hip circumference (cm) were measured using a tape measure, which was subsequently used to calculate waist to hip ratio (WHR).
Body composition was measured for all subjects using bioelectrical impedance (Body Composition Analyser BC-418MA, Tanita) (Figure 2.4). This also provided the subject’s weight (kg). Body composition measurements included BMI (kg/m²), body fat percentage (body fat %), total body water (kg), weight of fat (kg), and fat free weight (kg).
Blood pressure and pulse were measured after 15 minutes rest using an automated machine (Omron, Intelli-Sense, Omron Healthcare Co Ltd, Kyoto, Japan).

Blood samples were taken to measure fasting plasma glucose, insulin, insulin resistance, adipokines including leptin, resistin, adiponectin, PAI-1 (active), IL-6, MCP-1 and TNFα.

For measurement of insulin resistance, the homeostasis model assessment (HOMA) was applied using the following equation: \[ \frac{\text{fasting glucose (mmol/l) x fasting insulin (mIU/L)}}{22.5} \] (Katsuki et al., 2001).

Metabolic syndrome was defined using the National Cholesterol Education Program Adult Treatment Panel III (NCP ATP III) criteria. This set of criteria was chosen as it does not overestimate subjects presenting with MS, as other criteria may do, due to lower cut-off values.

A diagnosis of the metabolic syndrome requires the subject to have 3 or more risk factors outlined in Table 2.2.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Defining level</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG</td>
<td>≥ 6.0 mmol/l</td>
</tr>
<tr>
<td>BP</td>
<td>≥ 130/ ≥ 85 mmHg</td>
</tr>
<tr>
<td>TAG</td>
<td>≥ 1.7 mmol/l</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Men &lt; 1.0 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Women &lt; 1.3 mmol/l</td>
</tr>
<tr>
<td>WC</td>
<td>Men &gt; 102 cm</td>
</tr>
<tr>
<td></td>
<td>Women &gt; 88 cm</td>
</tr>
</tbody>
</table>

Type 2 diabetes was identified by using the World Health Organisation (WHO) values for diagnosis of diabetes mellitus as shown in Table 2.3.
Table 2.3: Diagnosis of Diabetes Mellitus using the WHO criteria

<table>
<thead>
<tr>
<th>Glucose concentration, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (Venous)</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus</strong></td>
</tr>
<tr>
<td>Fasting</td>
</tr>
<tr>
<td>Or</td>
</tr>
<tr>
<td>2h post glucose load</td>
</tr>
<tr>
<td>Or both</td>
</tr>
</tbody>
</table>

Lifestyle and health questionnaires were completed by all subjects at baseline. There were 7 questionnaires, investigating general health, mental health, physical activity, appetite, eating behaviour, quality of life and fatigue (section 2.2.6).

Finally, the subjects were given a 3 day food diary to complete and return to the clinic at their 2 week visit. They were asked to write down everything consumed, including portion sizes, brand of food item, and weight of food items if possible.

*Two week, 1, 2, 5, 7, 8 and 10 month measurements*

The subjects came to the clinic to be weighed, and discussed any issues regarding the diet. Any advice and encouragement needed regarding the diet were offered.

*Three, six and 12 (final) month measurements*

Similar measurements to baseline were performed at 3, 6 and 12 months on all subjects. At 3 and 6 months, subjects may have been randomised depending on weight loss achieved at these points. For the 6 and 12 months months visits, a urine sample was taken from the LighterLife and PSMF subjects to test for ketosis (Ketostix, Bayer Diagnostics Europe Ltd, Dublin, Ireland).

Finally at the 12 months visit the subjects discussed returning to the clinic for follow-up visits over the next 2 years.
2.2.4: Sample Collection and Storage

Blood samples were drawn by venepuncture.

The blood samples for measurement of the adipokines were first allowed to clot for 30 minutes, then centrifuged for 10 minutes at 1000 xg. The serum was removed and stored at -80 °C until assayed.

Other analytes including cardiovascular disease risk factors (fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol) were stored in vacutainers containing S.S.T (serum separating tube) and sent to the clinical biochemistry department (Aberdeen Royal Infirmary) for analysis. Laboratory technicians were blinded to the conditions of the trial.

2.2.5: Sample measurements

The adipokines and insulin were measured in duplicate by using commercially available immunoassay kits from Linco Research (St. Charles, MO) using the Lincoplex system (St.Charles, MO). The protocol for measurement of insulin and all the adipokines was as follows:

1. The human adipokine standard cocktail, controls, wash buffer, the antibody-immobilized beads, and serum matrix were all prepared prior to carrying out the assay.
2. A 96 well microtiter filter plate was used as part of the assay. Each well was labelled to account for 7 standards, 2 controls, and the samples.
3. The plate was blocked by pipetting 200 µl assay buffer into each well of the plate. The plate was then sealed and allowed to mix on a plate shaker for 10 minutes at room temperature.
4. The assay buffer was removed by vacuum, and the bottom of the plate was dried using paper towels.
5. 25 µl of assay buffer was added to the sample wells.
6. 25 µl of each standard, control, and samples were added into the appropriately labelled wells.
7. 25 µl of serum matrix was added to the background (blank), standard curve and control wells.
8. 25 µl of the mixed beads were added to each well.
9. The plate was sealed with aluminium foil, and incubated with agitation on a plate shaker overnight (16-18 hrs) at 2 - 8 ºC.
10. The fluid was removed gently by vacuum.
11. The plate was washed 3 times with 200 µl/ well of wash buffer. The wash buffer was removed by vacuum filtration between each wash.
12. 50 µl of the detection antibody cocktail was pipetted into each well
13. The plate was sealed, covered and incubated with agitation on the plate shaker for 30 minutes at room temperature.
14. 50 µl Streptavidin-Phycoerythrin (SAPE) was added to each well containing the 50 µl of detection antibody cocktail.
15. Again, the plate was sealed, covered and incubated with agitation on the plate shaker for 30 minutes at room temperature.
16. The contents were gently removed by vacuum.
17. The plate was washed 3 times by wash buffer, removing the wash buffer by vacuum filtration after each step.
18. 100 µl of sheath fluid was added to all wells. The plate was sealed, covered and the beads were resuspended on the plate shaker for 5 minutes.
19. Finally, the plate was read on the Luminex instrument.

The accuracy, sensitivity, and precision for the adipokine measurements is shown below.

The variables measured in the Clinical Biochemistry department of Aberdeen Royal Infirmary and the methods used to measure them are listed below:

**Total cholesterol**- Based on the enzymatic method using cholesterol esterase and cholesterol oxidase conversion followed by a Trinder endpoint. The cholesterol esters are hydrolysed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to cholesterol-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A coloured complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidise. The absorbance of the complex is measured as an endpoint reaction at 505/694nm.
**LDL cholesterol** - Estimated using the Friedewald formula, as follows: total cholesterol-HDL cholesterol- (TAG/5) (Friedewald et al., 1972).

**HDL cholesterol** - Elimination/catalase method. Cholesterol from non HDL particles is released and eliminated in the first step of the reaction. Cholesterol from HDL particles is released in the second step by detergent in reaction 2, and the HDL cholesterol is measured by a Trinder reaction.

**TAG** - The “TRIG” method is based on the Fossati three step enzymatic reaction with a Trinder endpoint. The single reagent procedure quantitates the total triacylglycerol including the mono and diacylglycerides and the free glycerol fractions. The triacylglycerols are converted to glycerol and free fatty acids by lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion by glycerol-3-phosphate-oxidase to hydrogen peroxide. A coloured complex is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidise. The absorbance of the complex is measured as an endpoint reaction at 505-694 nm.

**HbA1c** - ion-exchange high performance liquid chromatography.

**Fasting plasma glucose** - Glucose- Oxidase method. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidise with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

**Sodium, Potassium and Chloride** - The method is based on an indirect potentiometric procedure using an Ion Selective Electrode (ISO). The sodium ion selective electrode responds selectively to sodium ions according to the Nernst equation. The sample is mixed with ISE buffer, therefore providing a constant pH and a constant ionic strength solution. As the buffered sample is moved through the ion selective electrode, changes in the electrical potential take place. These electrical potential changes are measured against the potential of a reference electrode in order to derive the correct analog value for the sample.

**Creatinine** - The Creatinine_2 (Creat_2) method is based on the reaction of picric acid with creatinine in an alkaline medium. Creatinine reacts with picric acid in an alkaline medium to
produce a red coloured Creatinine-picrate complex. The rate of complex formation is measured at 505/571 nm and is proportional to the creatinine concentration.

**Albumin** – Serum albumin quantitatively binds to bromocresol green solution (BGS) to form an albumin-BCG complex that is measured as an endpoint reaction at 596/694 nm.

**ALKP**- Alkaline phosphatase hydrolyzes pNPP substrate to form p-nitrophenol. The reaction is followed by the colorimetric measurement of the rate of formation of p-nitrophenol at 410/478 nm, which is proportional to the ALKP activity.

**ALT**- The reaction is initiated by the addition of α-ketoglutarate as a second reagent. The concentration of NADH is measured by its absorbance at 340/410 nm and the rate of absorbance decrease is proportional to the ALT activity.

**GGT**- In the reaction with synthetic substrate (L-γ-glutamyl-3-carboxy-4-nitroanilide), glycylglycine acts as an acceptor for the γ-glutamyl residue and 5-amino-2-nitro-benzoate (ANB) is liberated. The liberated product has an absorption maximum near 400 nm. The rate of formation is measured photometrically at 410/478 nm as a zero-order kinetic assay.

**Urea**- Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase and NADH. The oxidation of NADH to NAD is measured as an inverse rate reaction at 340/410 nm.

### 2.2.6: Lifestyle and Health Questionnaires

All of the subjects were given the following questionnaires to complete at baseline, 3, 6 and 12 months. The questionnaires covered aspects of the subject’s general health, mental health, eating behaviour, physical activity, quality of life and sleeping activity.

#### 2.2.6a: General Health Questionnaire

The general health questionnaire (Goldberg, 1978) consists of 60 questions asking subjects about their recent general health and any medical complaints they may have experienced in the previous few weeks. It is designed to assess mental health, as opposed to only "general health". It was developed as a screening tool to assess those who have or who are at risk of
developing psychiatric disorders, it is a measure of the common mental health problems/domains of depression social withdrawal, anxiety and somatic disorders (Jackson, 2007).

The subjects were given 4 answers to choose from, and these were scored as 0-0-1-1 according to their response. The total score was calculated by combining the scores obtained for each answer. A total score of ≥ 39/60 identified a “positive” case (indicating poor health); with a total score of < 39/60 identified a “negative” case (indicating good health).

2.2.6b: Fatigue Questionnaire (Lee, 1981)
This questionnaire is a visual analogue scale (VAS) style questionnaire which asks the subject about fatigue and energy levels. There were 18 questions in which the subjects marked 100 mm VAS line to rate their feelings at that time. The answers ranged from “not at all” to “extremely” in severity.

To score the questionnaire, the mean of all the questions was obtained, out of a maximum of 100. A result from 0-33 was referred to as “low fatigue”; a score from 34-66 was referred to as “moderate fatigue” and a score from 67-100 was referred to as “high fatigue”.

2.2.6c: Physical Activity Level Questionnaire
Subjects were asked to complete a physical activity questionnaire that was modified from the Framingham Physical Activity Index. This questionnaire was short and instructions were easy to follow. It had the advantage of providing a quantitative as well as a qualitative value (low, medium or high) for the physical activity level. The questionnaire asked the subjects to estimate how many hours per day they spent in different activities, which were divided into sedentary, light, moderate and heavy activity categories. They were also asked to estimate how many hours they spent in bed per day.

The number or hours spent in bed and the other activity levels were calculated for a week and then divided by 7, to obtain the PAL for 1 day. The Physical Activity Level score was calculated as follows:
<table>
<thead>
<tr>
<th>Activity Type</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours in Bed / week</td>
<td>1.0</td>
</tr>
<tr>
<td>Hours of Sedentary Activity/week</td>
<td>1.1</td>
</tr>
<tr>
<td>Hours of Light Activity/week</td>
<td>1.5</td>
</tr>
<tr>
<td>Hours of Moderate Activity/week</td>
<td>2.4</td>
</tr>
<tr>
<td>Hours of Heavy Activity/week</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Physical Activity Level (PAL) for 1 day = PAL / 7
PAL for 1 hour = PAL / 24 = PAL/hr

<table>
<thead>
<tr>
<th>PAL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.4</td>
<td>(Low)</td>
</tr>
<tr>
<td>&gt; 1.4-1.7</td>
<td>(Medium)</td>
</tr>
<tr>
<td>&gt; 1.7</td>
<td>(High)</td>
</tr>
</tbody>
</table>

2.2.6d: **WHO Quality of Life Questionnaire**

The World Health Organisation Quality of Life (WHOQOL) questionnaire asks subjects about different dimensions on quality of life. Information on socio-demographic and health information is also obtained.

The questionnaire consists of 26 questions covering aspects of life such as health, finance, sleep, and relationships. The final scores are divided into 4 domains; physical, psychological, social, and environmental. SPSS (version 15.0) is used to calculate the scores. A maximum score of 100 is obtained for each domain: the higher the score, the better the quality of life.

The questionnaires, scoring materials and instructions were downloaded with permission from the University of Bath. The material in this publication is the result of use of the WHOQOL-UK and the assistance of the University of Bath and the World Health Organisation is acknowledged (Skevington and Lifford (forthcoming)).

2.2.6e: **Dutch Eating Behaviour Questionnaire**

The Dutch Eating Behaviour questionnaire (van Strien, 1986) uses questions which are based on different aspects of eating behaviour and are divided into 3 psychological theories of overeating: the psychosomatic theory, externality theory, and restraint theory.
The psychological theory focuses on emotional eating, i.e. eating in response to different emotional states such as anger, fear or anxiety, regardless of internal states of hunger and satiety. The externality theory focuses on external eating, in other words, eating in response to external food cues, such as the sight or smell of food, therefore neglecting internal states of hunger and satiety. The restraint theory focuses on dieting as the cause of overeating. It may be one of these theories or a combination which may contribute to the subject overeating. The DEBQ is easy to administrate and only takes about 10 minutes to complete.

The DEBQ is made up of 33 questions, with answers in a Likert scale format. A scoring template was drawn up. Each answer corresponded to a number ranging from 0-5, and was entered into the template, which then calculated the average score for each theory. There is no set interpretation for the calculated scores from this questionnaire. Scores are usually correlated with other variables such as weight.

2.2.6f: Epworth Sleepiness Scale
This questionnaire was designed to measure daytime sleep susceptibility in a simple and standardised way and attempts to cover the whole range of sleep propensities, from obstructive sleep apnea to hypersomnia (Johns, 1994). It was validated in a group of normal controls and sleep-disordered patients.

The subjects were asked questions based, retrospectively, on 8 situations of various soporific natures. This attempts to distinguish between dozing off and just feeling tired, with the answers ranging from 0, equalling never dozing to off to 3, equalling high chance of dozing, based on their feelings in the previous days. The scores for each question were then added up, with the total score out of 24.

2.2.6g: Beck Depression Inventory (BDI)
The BDI consists of 21 questions presented in a multiple choice format. It was designed to measure the presence and degree of depression in adolescents and adults. Each question corresponds to a certain category of depressive symptom and/or attitude. The statements are ranked and weighted to reflect the range of severity of the symptom from none to maximum severity. Values of 0, 1, 2, or 3 are assigned to each statement. The total score corresponds to a specific “depressive state” as follows:
0-4 - Possible Denial of Depression, faking good; this is below normal
05-09 - These up and downs are considered normal
10-18 - Mild to moderate depression
19-29 - Moderate to severe depression
30-63 - Severe Depression
Over 40 - This is significantly above even severely depressed persons, suggesting possible exaggeration of depression; possibly characteristic of histrionic or borderline personality disorders. Significant levels of depression are still possible.

2.2.7: Data Analysis
The power calculation was carried out using the mean and standard values from Wadden et al (1989) for a PSMF and a low calorie diet. It was calculated that 80 patients were needed for an 80% power using a 2-sided t-test at a 5% significance level. Assuming a 20% drop out rate, this was rounded up to 100 patients. But due to a high dropout rate we had to ask for permission to recruit an extra 20 patients, giving the final sample size as 120.

The primary outcome to be assessed was weight loss, with secondary outcomes including cardiovascular risk factors and adipokines. Further analysis included those presenting with T2D and metabolic syndrome at baseline.

Analysis was carried out on an intention to treat basis, with the last value carried forward in the case of missing data. Completer’s analysis was also carried out for weight change for those who completed the study.

For comparison of continuous variables between the LL and PSMF groups, the change from baseline to six and 12 months in each subject was calculated and the mean changes in the two diet groups using an unpaired t-test were compared. Paired t-tests were carried out for continuous variables from baseline to 3, 6 and 12 months for the HE group. Normality tests were carried out for all data, including weight and body composition, CVD risk factors, adipokines, liver and kidney analytes, and the questionnaire data. Any data which was skewed was log-transformed before t-tests were performed.
Statistical tests were carried out using SPSS 15.0 for Windows software program (SPSS, Chicago, IL). A p-value of < 0.05 was considered statistically significant.
CHAPTER 3: SYSTEMATIC REVIEW
3.1: INTRODUCTION

The prevalence of overweight and obesity is already high and continues to increase in both the developed and developing world (Caballero, 2007). Obesity has been implicated as the second most preventable cause of death in the US. After remaining reasonably constant in the 1960s and 1970s, the prevalence of obesity among adults in the United States increased by around 50% per decade throughout the 1980s and 1990s. Two thirds of adults in the United States today are obese or overweight. In the United States, 28% of men, 34% of women, and nearly 50% of non-Hispanic black women are at present obese (Olshansky et al., 2005). At any time, approximately 45% of women and 30% of men in the UK are trying to lose weight (Ware, 2003). Most adults in England are now overweight, and nearly 1 in 4 are obese (http://www.foresight.gov.uk/Obesity/17.pdf). Obesity has been shown to be associated with increased risk of type 2 diabetes mellitus, hypertension, dyslipidemia and consequent cardiovascular disease. Obesity ranks second only to smoking in the aetiology of cancer and is an important factor in osteoarthritis and obstructive sleep apnoea (Wilding 1997).

Recently, low carbohydrate/high protein diets have become popular as an aid to weight loss. Significant weight loss on a low carbohydrate/high protein diet without significant elevations of serum cholesterol has been reported. Studies comparing the “Atkins” diet to the classical low fat diet have appeared in the literature recently and are the subject of increasing public interest (Veech, 2004) due to the beneficial improvements in cardiovascular risk and weight loss achieved with this type of dietary approach (Layman et al., 2008).

This systematic review focused on randomised control trials of low carbohydrate/high protein diets compared to low fat/high carbohydrate conventional diets. The systematic review also examined the outcomes of such trials in relation to effects on cardiovascular disease risk. This systematic review attempts to update the literary evidence from randomised control trials of low carbohydrate/high protein diets compared to low fat/high carbohydrate diets to assess their impact on weight loss and cardiovascular risk. In addition, it demonstrates lower attrition rates in the low carbohydrate/high protein groups compared to the low fat/high carbohydrate groups suggesting patient preference for the former approach.

3.2 METHODS

The methods for the systematic review are detailed in Chapter 2.1.
3.3 RESULTS

Identified Studies:
A total of 13 (Brehm et al., 2003; Samaha et al., 2003; Foster et al., 2003; Seshadri et al., 2003; Due et al., 2004; Yancy et al., 2004; Brinkworth et al., 2004; Dansinger et al., 2005; Stern et al., 2004; Tsai et al., 2005; Truby et al., 2006; Cardillo et al., 2006; Gardner et al., 2007) out of 124 articles met the inclusion criteria and were included in the systematic review.

Study Characteristics
All the included studies were RCTs ranging from 6 to 36 months duration. Five of the trials were of 6 months’ duration; six of 12 months’. One trial lasted 17 months and another lasted 36 months. As there was only one study lasting 17 months (Brinkworth et al., 2004) and one lasting 36 months (Cardillo et al., 2006) data reported at that time point in that study were not included in the analysis. All of the studies were designed to reduce or prevent weight gain and also examined cardiovascular disease risk factors.

Ten of the studies compared low carbohydrate/high protein diets with low fat/high carbohydrate diets, and two studies compared medium protein diets with high protein diets.

Participant Characteristics
A total of 1222 volunteers were recruited between the 13 studies. Figure 3.1 shows the percentage attrition rates. Out of the 1222 participants assigned to the diets, there were 441 (36 %) attritions during the interventions. There was a higher attrition rate in the conventional/low fat/medium protein groups compared to the low carbohydrate/high protein intervention groups. The difference in attrition rates between the 2 groups was significant ($p = 0.001$) after performing a chi-squared test.
For the following variables, the LC/HP refers to the low carbohydrate/high protein intervention groups and the LF/HC refers to the low fat/high carbohydrate comparison/control groups.

Weight
The weighted mean difference (WMD) in weight change was -4.02 kg in favour of the LC/HP group at 6 months (Figure 3.2a) \((p < 0.00001)\). At 12 months this difference had fallen to only -1.05 kg \((p < 0.05)\) (Figure 3.2b). There were differences \((p < 0.0001)\) amongst the studies at 6 months, but agreement shown by lack of heterogeneity at 12 months.

Total Cholesterol
The WMD in total cholesterol change was 0.19 mmol/l at 6 months \((p < 0.0001)\) with the LC/HP group demonstrating the increased cholesterol (Figure 3.3a). This was also the case at 12 months, though the difference between the groups was smaller and not significant (0.10 mmol/l, \(p = 0.31\)) (Figure 3.3b). There were no differences amongst the studies at 6 \((p = 0.84)\) and 12 \((p = 0.14)\) months.
LDL-Cholesterol

The WMD in LDL cholesterol change was 0.14 mmol/l at 6 months ($p < 0.00001$) with the LC/HP group demonstrating the increased LDL cholesterol (Figure 3.4a). The difference between the groups was greater at 12 months (0.37 mmol/l) ($p < 0.00001$) with the LC/HP group again demonstrating the increased LDL cholesterol (Figure 3.4b). There were no differences amongst the studies at 6 months ($p = 0.65$), but there were differences found between the studies at 12 months ($p < 0.00001$).

HDL-Cholesterol

The WMD in HDL cholesterol change was 0.04 mmol/l at 6 months ($p = 0.03$) favouring the LC/HP group (Figure 3.5a). There was a slightly greater increase in the WMD in HDL cholesterol at 12 months (0.06 mmol/l) favouring the LC/HP group ($p < 0.05$) (Figure 3.5b). There were no differences found between the studies at 6 months ($p = 0.46$) or 12 months ($p = 0.49$).
Figure 3.2 (a) and (b): Weight loss at 6 and 12 months

**Review:** Systematic Review April 2008  
**Comparison:** 01 Weight change at 6 months  
**Outcome:** 01 Weight change at 6 months

### a) Weight change at 6 months

<table>
<thead>
<tr>
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<th>Treatment Mean (SD)</th>
<th>N</th>
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<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
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<td>20</td>
<td>-3.90 (1.00)</td>
<td>74.27 [-5.21, -3.99]</td>
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<td></td>
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<tr>
<td>Brinkworth</td>
<td>21</td>
<td>-8.10 (8.20)</td>
<td>22</td>
<td>-8.50 (6.10)</td>
<td>1.45 [0.40, 4.74]</td>
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<td>-3.20 (6.90)</td>
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<td>-3.50 (5.60)</td>
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<tr>
<td>Due</td>
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<td>23</td>
<td>-5.90 (7.50)</td>
<td>1.27 [-3.50, 1.13]</td>
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<tr>
<td>Foster</td>
<td>33</td>
<td>-8.90 (6.50)</td>
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<td>-3.10 (5.60)</td>
<td>3.05 [-3.80, -0.81]</td>
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<tr>
<td>Samaha</td>
<td>64</td>
<td>-5.80 (8.60)</td>
<td>68</td>
<td>-1.90 (4.20)</td>
<td>5.01 [-3.90, -1.57]</td>
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<tr>
<td>Seshadri</td>
<td>43</td>
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<td>-3.50 (4.90)</td>
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<td>-6.60 (5.40)</td>
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<tr>
<td>Yancy</td>
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<td>-6.50 (7.70)</td>
<td>2.89 [-5.50, -2.43]</td>
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Total (95% CI) 345 345  
Test for heterogeneity: $\chi^2 = 35.31, df = 8, (P = 0.0001), I^2 = 77.3$
Test for overall effect: $Z = 15.08, (P < 0.00001)$

### b) Weight change at 12 months

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<th>Control Mean (SD)</th>
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<th>Weight %</th>
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<td>-2.10 (6.80)</td>
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<td>-3.00 (4.90)</td>
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<tr>
<td>Due</td>
<td>23</td>
<td>-6.20 (7.60)</td>
<td>18</td>
<td>-4.30 (7.10)</td>
<td>5.32 [-1.00, -6.42]</td>
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<tr>
<td>Foster</td>
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<td>-4.20 (6.76)</td>
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<td>-2.45 (6.31)</td>
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<tr>
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<td>23.38 [-2.50, -4.65]</td>
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<td>Stein</td>
<td>44</td>
<td>-5.10 (8.70)</td>
<td>43</td>
<td>-3.10 (8.40)</td>
<td>8.40 [-2.00, -5.59]</td>
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<tr>
<td>Truby</td>
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<td>-9.10 (6.20)</td>
<td>16.00 [-2.50, 2.70]</td>
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<tr>
<td>Tsai</td>
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<td>65</td>
<td>-3.10 (8.40)</td>
<td>12.45 [-2.00, -4.95]</td>
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Total (95% CI) 309 308  
Test for heterogeneity: $\chi^2 = 6.71, df = 6, (P = 0.39), I^2 = 10.5$
Test for overall effect: $Z = 1.98, (P = 0.05)$
Figure 3.3 (a) and (b) Total cholesterol at 6 and 12 months

Review: Systematic Review April 2008
Comparison: 03 Total cholesterol change at 6 months
Outcome: 01 Total cholesterol change at 6 months

<table>
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<th>Study or sub-category</th>
<th>N</th>
<th>Treatment Mean (SD)</th>
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<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
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<td>22</td>
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<td>20 -0.04 (1.08)</td>
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<td>0.18</td>
<td>[-0.04, 0.40]</td>
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<td>23 0.03 (1.08)</td>
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<tr>
<td>Yancy</td>
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<td>-0.21 (1.08)</td>
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<td>Total (95% CI)</td>
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<td>345</td>
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Test for heterogeneity: Chi² = 4.17, df = 8 (P = 0.84), I² = 0%
Test for overall effect: Z = 4.23 (P < 0.0001)

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<tr>
<th>Study or sub-category</th>
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<td>[-0.17, 0.37]</td>
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<tr>
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<td>18 0.68 (1.08)</td>
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<td>0.58</td>
<td>[-1.25, 0.09]</td>
</tr>
<tr>
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<td>0.10 (1.08)</td>
<td>30 -0.03 (1.08)</td>
<td>13.76</td>
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<td>[-0.40, 0.66]</td>
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<td>Stern</td>
<td>44</td>
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<td>Total (95% CI)</td>
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Test for heterogeneity: Chi² = 5.56, df = 3 (P = 0.14), I² = 46.0%
Test for overall effect: Z = 1.01 (P = 0.31)
Figure 3.4 (a) and (b) LDL cholesterol at 6 and 12 months

Review: Systematic Review April 2008
Comparison: 05 LDL cholesterol change at 6 months
Outcome: 01 LDL cholesterol change at 6 months

<table>
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<tr>
<td>Brinkworth</td>
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<td>-0.20(0.29)</td>
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<tr>
<td>Dansinger</td>
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<td>-0.06(0.36)</td>
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<td>Foster</td>
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<td>0.08(0.33)</td>
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<tr>
<td>Gardner</td>
<td>70</td>
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<td>Samahia</td>
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<td>Seshadri</td>
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<td>Yancy</td>
<td>59</td>
<td>0.04(0.29)</td>
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Total (95% CI) 352                         338

Test for heterogeneity: Chi² = 5.42, df = 7 (P = 0.61), I² = 0%
Test for overall effect: Z = 4.53 (P < 0.00001)

Review: Systematic Review April 2008
Comparison: 06 LDL cholesterol change at 12 months
Outcome: 01 LDL cholesterol change at 12 months

<table>
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<tr>
<th>Study or sub-category</th>
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<tr>
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<td>Tsai</td>
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Total (95% CI) 211                         201

Test for heterogeneity: Chi² = 41.66, df = 3 (P = 0.00001), I² = 92.8%
Test for overall effect: Z = 8.44 (P < 0.00001)
Figure 3.5 (a) and (b): HDL cholesterol at 6 and 12 months

**Review:** Systematic Review April 2008  
**Comparison:** 07 HDL cholesterol change at 6 months  
**Outcome:** 01 HDL cholesterol change at 6 months

### Study or sub-category

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<td>0.05 (0.17)</td>
<td>24.65</td>
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<td>45.04</td>
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<td>Seshadri</td>
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<td>-0.04 (0.74)</td>
<td>1.80</td>
<td>0.18 [-0.08, 0.44]</td>
</tr>
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<td>Yancy</td>
<td>59</td>
<td>0.18 (0.74)</td>
<td>0.10 (0.74)</td>
<td>0.60</td>
<td>0.08 [-0.37, 0.53]</td>
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</table>

Total (95% CI): 375 [0.00, 0.07]

Test for heterogeneity: Chi² = 6.28, df = 8 (P = 0.62), I² = 0%
Test for overall effect: Z = 2.20 (P = 0.03)

### Study or sub-category

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<tr>
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<td>-0.13 (0.16)</td>
<td>36.78</td>
<td>0.10 [0.03, 0.17]</td>
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</table>

Total (95% CI): 210 [0.02, 0.07]

Test for heterogeneity: Chi² = 3.44, df = 4 (P = 0.49), I² = 0%
Test for overall effect: Z = 2.82 (P = 0.005)
Triacylglycerol
The WMD in triacylglycerol was -0.17 mmol/l at 6 months ($p = 0.0001$) favouring the LC/HP group (Figure 3.6a). At 12 months the WMD between the groups was -0.19 mmol/l favouring the LC/HP group ($p = 0.04$) (Figure 3.6b). Again, there was evidence of heterogeneity across the groups ($p = 0.01$).

Systolic Blood Pressure
The WMD drop in systolic blood pressure of -1.35 mmHg at 6 months favouring the LC/HP group was not significant (Figure 3.7a). At 12 months the WMD between the groups was a decrease of 2.19 mmHg favouring the LC/HP group ($p = 0.05$) (Figure 3.7b). There was no difference between the studies at either time.

Diastolic Blood Pressure
The WMD decrease in diastolic blood pressure of -0.49 mmHg at 6 months favouring the LC/HP group was not significant (Figure 3.8a). At 12 months, the WMD between the 2 groups of -0.81 mmHg favouring the LC/HP group was greater, but was also not significant (Figure 3.8b). There was no evidence of statistical heterogeneity across the studies at either time.

Fasting Plasma Glucose
The WMD between the groups in fasting plasma glucose was not significant and there was no evidence of statistical heterogeneity at either time (Figure 3.9a and 3.9b).
Figure 3.6 (a) and (b) Triacylglycerols at 6 and 12 months

Review: Systematic Review April 2008
Comparison: 09 Triacylglycerol change at 6 months
Outcome: 01 Triacylglycerol change at 6 months

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment</th>
<th>N</th>
<th>Control</th>
<th>N</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>-3.93(0.96)</td>
<td>22</td>
<td>0.19(0.96)</td>
<td>20</td>
<td>2.06</td>
<td>-6.12</td>
<td>[-4.70, -3.56]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>-0.50 (0.96)</td>
<td>21</td>
<td>-0.10 (0.96)</td>
<td>22</td>
<td>2.12</td>
<td>-0.40</td>
<td>[-0.97, 0.17]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>-0.11 (0.45)</td>
<td>40</td>
<td>-0.11 (0.62)</td>
<td>40</td>
<td>12.37</td>
<td>0.00</td>
<td>[-0.26, 0.24]</td>
</tr>
<tr>
<td>Due</td>
<td>-0.15 (0.96)</td>
<td>23</td>
<td>0.11 (0.96)</td>
<td>23</td>
<td>2.27</td>
<td>-0.26</td>
<td>[-0.81, 0.29]</td>
</tr>
<tr>
<td>Foster</td>
<td>0.50 (3.30)</td>
<td>33</td>
<td>-0.10 (2.10)</td>
<td>32</td>
<td>14.66</td>
<td>-0.22</td>
<td>[-0.44, 0.00]</td>
</tr>
<tr>
<td>Gardner</td>
<td>-0.40 (0.72)</td>
<td>70</td>
<td>-0.11 (0.62)</td>
<td>70</td>
<td>14.66</td>
<td>-0.22</td>
<td>[-0.44, 0.00]</td>
</tr>
<tr>
<td>Gardner</td>
<td>0.01 (0.96)</td>
<td>64</td>
<td>-0.33 (9.00)</td>
<td>68</td>
<td>6.00</td>
<td>-3.50</td>
<td>[-0.81, 0.29]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>-0.15 (0.96)</td>
<td>23</td>
<td>-0.11 (0.96)</td>
<td>23</td>
<td>2.27</td>
<td>-0.26</td>
<td>[-0.81, 0.29]</td>
</tr>
<tr>
<td>Tancy</td>
<td>-0.32 (0.96)</td>
<td>59</td>
<td>-0.31 (0.96)</td>
<td>60</td>
<td>5.86</td>
<td>-0.53</td>
<td>[-0.81, 0.29]</td>
</tr>
</tbody>
</table>

Total (95% CI) 375                         361 100.00 -0.16 | [-0.24, -0.08] |

Test for heterogeneity: Chi² = 222.85, df = 8 (P < 0.00001), I² = 96.4%
Test for overall effect: Z = 3.76 (P = 0.0002)

-1 -0.5 0 0.5 1
Favours treatment Favours control

Review: Systematic Review April 2008
Comparison: 09 Triacylglycerol change at 12 months
Outcome: 01 Triacylglycerol change at 12 months

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment</th>
<th>N</th>
<th>Control</th>
<th>N</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>-0.01(0.96)</td>
<td>40</td>
<td>-0.14(0.68)</td>
<td>40</td>
<td>23.51</td>
<td>0.13</td>
<td>[-0.23, 0.49]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>-0.05 (0.96)</td>
<td>23</td>
<td>0.33 (0.96)</td>
<td>23</td>
<td>8.47</td>
<td>-0.38</td>
<td>[-0.70, 0.02]</td>
</tr>
<tr>
<td>Due</td>
<td>-2.50 (2.50)</td>
<td>33</td>
<td>-0.09(4.20)</td>
<td>33</td>
<td>3.02</td>
<td>-0.41</td>
<td>[-1.84, -0.58]</td>
</tr>
<tr>
<td>Gardner</td>
<td>-0.26 (0.66)</td>
<td>70</td>
<td>-0.16 (0.68)</td>
<td>70</td>
<td>58.34</td>
<td>-0.17</td>
<td>[-0.40, 0.06]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>-0.05 (1.78)</td>
<td>44</td>
<td>0.05 (0.96)</td>
<td>43</td>
<td>8.47</td>
<td>-0.70</td>
<td>[-1.30, -0.10]</td>
</tr>
</tbody>
</table>

Total (95% CI) 210                         194 100.00 -0.19 | [-0.36, -0.01] |

Test for heterogeneity: Chi² = 12.59, df = 4 (P = 0.01), I² = 68.2%
Test for overall effect: Z = 2.08 (P = 0.04)
Figure 3.7 (a) and (b): Systolic Blood Pressure at 6 and 12 months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (Mean (SD))</td>
<td>N (Mean (SD))</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Brehm</td>
<td>22 (4.00 (12.70))</td>
<td>20 (2.00 (12.70))</td>
<td>6.12</td>
<td>2.00 [-5.69, 9.69]</td>
<td></td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21 (6.50 (12.70))</td>
<td>22 (3.60 (12.70))</td>
<td>6.28</td>
<td>-1.30 [-8.89, 6.29]</td>
<td></td>
</tr>
<tr>
<td>Dansinger</td>
<td>60 (-0.70 (10.00))</td>
<td>40 (-4.80 (14.00))</td>
<td>12.73</td>
<td>6.10 [-1.23, 9.43]</td>
<td></td>
</tr>
<tr>
<td>Foster</td>
<td>33 (-2.70 (12.70))</td>
<td>30 (1.20 (12.20))</td>
<td>10.34</td>
<td>-3.90 [-9.82, 2.02]</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>77 (-6.40 (9.50))</td>
<td>79 (-3.60 (7.60))</td>
<td>49.51</td>
<td>-2.10 [-6.80, 0.60]</td>
<td></td>
</tr>
<tr>
<td>Truby</td>
<td>40 (-7.20 (11.60))</td>
<td>47 (-4.0 (11.70))</td>
<td>15.01</td>
<td>-3.10 [-8.01, 1.81]</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>233 (100.00)</td>
<td>238 (233.36)</td>
<td>100.00</td>
<td>-1.35 [-3.25, 0.56]</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: $\chi^2 = 6.24$, df = 5 (P = 0.28), $I^2 = 19.9\%$
Test for overall effect: Z = 1.39 (P = 0.17)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (Mean (SD))</td>
<td>N (Mean (SD))</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40 (0.20 (12.00))</td>
<td>40 (-2.70 (13.00))</td>
<td>15.51</td>
<td>2.90 [-2.58, 8.38]</td>
<td></td>
</tr>
<tr>
<td>Foster</td>
<td>33 (-0.21 (9.40))</td>
<td>30 (2.00 (11.80))</td>
<td>16.59</td>
<td>-1.90 [-7.51, 3.09]</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>77 (-7.60 (11.00))</td>
<td>79 (-3.60 (9.30))</td>
<td>45.53</td>
<td>-4.50 [-7.70, -1.30]</td>
<td></td>
</tr>
<tr>
<td>Stern</td>
<td>44 (1.00 (19.00))</td>
<td>43 (2.00 (15.00))</td>
<td>9.03</td>
<td>-1.00 [-8.18, 6.18]</td>
<td></td>
</tr>
<tr>
<td>Tsai</td>
<td>64 (1.00 (19.00))</td>
<td>65 (2.00 (15.00))</td>
<td>13.34</td>
<td>-1.00 [-6.91, 4.91]</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>258 (100.00)</td>
<td>257 (100.00)</td>
<td>100.00</td>
<td>-2.19 [-4.35, -0.03]</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: $\chi^2 = 5.57$, df = 4 (P = 0.23), $I^2 = 28.2\%$
Test for overall effect: Z = 1.99 (P = 0.05)
Figure 3.8 (a) and (b): Diastolic Blood Pressure at 6 and 12 months

### 6 Months

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>N</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>5.00 (8.30)</td>
<td>1.00 (8.30)</td>
<td>7.24</td>
<td>4.00</td>
<td>[-1.03, 9.03]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>-1.70 (8.30)</td>
<td>-1.60 (8.30)</td>
<td>7.43</td>
<td>7.43</td>
<td>[-5.06, 6.86]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-4.00 (6.50)</td>
<td>-1.80 (6.90)</td>
<td>21.19</td>
<td>21.19</td>
<td>[-5.14, 0.74]</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>2.00 (12.70)</td>
<td>-1.10 (16.20)</td>
<td>4.10</td>
<td>3.10</td>
<td>[-3.58, 9.78]</td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>-3.30 (6.90)</td>
<td>-2.50 (5.80)</td>
<td>45.60</td>
<td>45.60</td>
<td>[-2.80, 1.20]</td>
</tr>
<tr>
<td>Truby</td>
<td>40</td>
<td>-4.90 (8.30)</td>
<td>-4.40 (8.60)</td>
<td>14.45</td>
<td>14.45</td>
<td>[-4.06, 3.06]</td>
</tr>
</tbody>
</table>

Total (95% CI): 233 vs. 238

Test for heterogeneity: Chi² = 5.59, df = 5 (P = 0.35), I² = 10.6%
Test for overall effect: Z = 0.72 (P = 0.47)

### 12 Months

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>N</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-1.40 (7.50)</td>
<td>-1.70 (6.60)</td>
<td>29.78</td>
<td>29.78</td>
<td>[-2.76, 3.36]</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>-2.70 (12.40)</td>
<td>-2.90 (6.70)</td>
<td>11.76</td>
<td>11.76</td>
<td>[-4.66, 5.06]</td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>-4.40 (8.40)</td>
<td>-2.20 (6.70)</td>
<td>44.74</td>
<td>44.74</td>
<td>[-4.59, 0.19]</td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>3.00 (15.00)</td>
<td>1.00 (10.00)</td>
<td>9.73</td>
<td>9.73</td>
<td>[2.00, -3.35, 7.35]</td>
</tr>
</tbody>
</table>

Total (95% CI): 194 vs. 192

Test for heterogeneity: Chi² = 3.03, df = 3 (P = 0.39), I² = 1.1%
Test for overall effect: Z = 0.90 (P = 0.37)
Figure 3.9 (a) and (b): Fasting plasma glucose at 6 and 12 months
3.4 DISCUSSION

The results of the present review show that weight loss was significantly greater in the LC/HP (treatment) group after 6 and 12 months compared to the LF/HC group. The difference was greater at 6 months and at that time there was significant heterogeneity amongst the studies, probably due to the different study designs but at 12 months the heterogeneity was no longer significant. The 36 month follow up by Cardillo et al., (2006) reported that mean weight change between baseline and 36 months was not different between the LC/HP and the LF/HC group. However, they do report that between 6 and 36 months, weight was unchanged for the LF/HC group but that subjects on the LC/HP approach regained weight, but this change was not significant.

Avenell et al. (2004) examined the effects of a protein sparing modified fast (PSMF) compared to a low calorie diet and a very low calorie diet. A PSMF is a low carbohydrate diet, which allows a maximum of 40 g of carbohydrate/day. The review examined weight loss comparing the PSMF to low calorie diets after 12, 18, 24, 36, and 60 months. There was a greater weight loss favouring the PSMF group compared to the control after 12, 24 and 36 months, but only seven RCTs were included in this analysis, which included a total of 480 participants. These results are consistent with the results of the present systematic review.

A review by Nordmann et al. (2006) comparing low carbohydrate diets vs. low fat diets showed significant weight loss with the low carbohydrate group at 6 months, but not at 12 months. The meta-regression by Krieger et al., (2006) also reported a greater weight loss in addition to a greater body fat and percentage body fat loss in studies lasting more than 3 months. Bravata et al., (2003) however, showed no significant differences in weight loss for both groups at either 6 or 12 months, but this review included studies with dietary approaches that are not considered low carbohydrate, which may have affected their outcomes/findings.

The present review showed that there was a significant improvement in HDL cholesterol and triacylglycerols at 6 and 12 months favouring the LC/HP group, but this was not significant at 17 months. The lack of significance at 17 months may be caused by the reintroduction of carbohydrates in the LC/HP group. There was heterogeneity between the studies for triacylglycerols, but this may have been due to differences in study design.

Low HDL cholesterol and raised triacylglycerol levels are risk factors for cardiovascular disease and impact on the atherogenicity of the LDL particle and these results indicate a LC/HP diet may be a better approach to weight loss and lowering the risk of cardiovascular
disease. These results are consistent with the review carried out by Nordmann et al. (2006). However Bravata et al (2003) did not show any significant improvement in these parameters, which again, may have been affected by their choice of studies.

The present review showed a significant improvement in total cholesterol and LDL cholesterol favouring the LF/HC group at 6 months, at which point total cholesterol and LDL cholesterol increased more in the LC/HP group but not at 12 months or 17 months. Nordmann et al. (2006), in a meta-analysis of low carbohydrate vs. low-fat diets found reports on 4 groups of patients demonstrating an improvement in total and LDL cholesterol favouring low-fat diets rather than low-carbohydrate diets. This finding is consistent with the studies included in the present review. An elevated total cholesterol could in part be explained by an increase in HDL cholesterol observed in the LC/HP group. Also, although an elevated LDL cholesterol increases the risk of acute cardiovascular events, we have just shown evidence that LC/HP increase HDL and decrease triacylglycerol which impacts on the atherogenicity of the LDL particle. These studies failed to investigate changes in LDL particle size. Furthermore, evidence from Sharman et al. (2002) suggest that on a LC/HLP LDL particle sizes change from small to large and therefore resulting in a less atherogenic profile.

There was a trend towards improvement in diastolic and systolic blood pressure at 6, 12 and 17 months favouring the LC/HP group. The difference was significant at 12 months favouring the LC/HP group for systolic blood pressure. Bravata et al. (2003) reported no change in systolic blood pressure after the low and very low carbohydrate diets. Nordmann et al., (2006) showed no significant difference in blood pressure at any timepoint.

At 6 months there was a trend towards improvement in fasting plasma glucose only slightly favouring the LF/HC group in which there was a greater decrease in fasting plasma glucose in the LF/HC group. This was surprising when compared to the review by Layman et al. (2008) where there is clear evidence of improvements in fasting glucose, postprandial glucose and insulin responses and HbA1c for individuals on a low carbohydrate/high protein diet. At 12 months, the opposite occurred in which there was a greater decrease in fasting plasma glucose, favouring the LC/HP group. The difference was not significant at 6, 12 and 17 months. Bravata et al. (2003) reported no change in fasting serum glucose among recipients of the low and very low carbohydrate diets. Nordmann et al. (2006) showed a greater improvement in fasting plasma glucose favouring the low carbohydrate group at 6 months, but this was no longer significant at 12 months.
Fasting glucose provides a limited assessment of overall glycaemic status; therefore, future studies should use HbA\(_1c\) values or more direct measurements of insulin sensitivity.

There was a higher attrition rate in the LF/HC compared to the LC/HP groups (Figure 1). Reasons for attrition included difficulty in complying with the diet or disliking the diet, difficulty in maintaining the scheduled visits, and significant events such as pregnancy and surgery.

Limitations

It is important to take account of attrition rates in the interpretation of outcomes as high attrition rates lead to a smaller statistical power. An intention to treat approach is commonly used to overcome attrition rates and possible bias in the outcomes. There are, however, limitations when using this approach in lifestyle trials as the intention to treat approach has been derived from drug trials and may not yield robust outcomes. This results in the need for higher retention rates to assess for real changes in response to the dietary interventions.

In addition, the use of a randomised controlled trial design in dietary interventions may not be appropriate. In general, any weight loss strategy has a maximum weight loss at 6 months followed by a return to initial weight. It is clear that patients are changing their treatment by their own accord, perhaps subconsciously or perhaps due to a metabolic response of the body aiming to return to its initial weight. The current thinking within the field of obesity suggests the use of continuous improvement methodology may be more appropriate for weight loss management McQuigg et al. (2008).

Also there was some evidence of heterogeneity between the studies included in this analysis. This calls for the use of more consistent and robust study designs for which we have to establish a clear definition of a low carbohydrate diet/high protein diet.

3.5 CONCLUSION

This systematic review included all known randomised control trials of low carbohydrate diets versus the low fat/high carbohydrate diet, from 2000 to 2007. Factors including weight, cholesterol, blood pressure and glycemic control were evaluated, as these are important in weight loss and cardiovascular disease risk.

Evidence from this systematic review demonstrates that low carbohydrate/high protein diets are more effective at 6 months and are as, if not more, effective than low fat diets in reducing
weight and cardiovascular disease risk up to 1 year. As there were only 13 studies included, and several of them allowed the reintroduction of carbohydrates in the low carbohydrate/high protein diet, the evidence of the long term efficacy of these diets is not complete. Certainly at 6 months, the evidence is in favour of the use of low carbohydrate/high protein diet. It may not be appropriate to return to a high carbohydrate intake for weight maintenance (Stubbs et al., 1998; Mazlan, 2001). A gradual reintroduction while still limiting the intake of carbohydrate may be more appropriate.

With the prevalence of obesity increasing there is a need for larger and long term RCTs of low/very low carbohydrate diets compared to the low fat/high carbohydrate diets to be carried out. The influence of behavioural therapy and exercise interventions needs to be evaluated, as well as, lifestyle, appetite, and mood questionnaires.

It is not known with certainty which aspect of low carbohydrate diets causes the weight loss and cardiovascular disease risk factor changes. Whether it is the low carbohydrate, the high protein or calorie restriction needs to be examined. In addition, there is a need to assess if the greater weight loss achieved at 6 months on a low carbohydrate/high protein diet results in more important long term improvements of cardiovascular disease.

There is a need for trials to include a follow-up period, to examine adherence to the low carbohydrate diets, and whether participants maintain their weight loss and CVD risk factor change when there is minimum contact with the study investigators. Finally, taking account of high attrition rates when using randomised controlled trials for dietary and lifestyle interventions, perhaps we will witness a move towards a continuous improvement methodology in the future.
CHAPTER 4: WEIGHT AND BODY COMPOSITION
One of the objectives of the trial was to examine weight loss using different dietary approaches according to response to a HE diet at 3 months. Weight loss was examined at 3 months for all the subjects before randomisation, and then 3 and 9 months post randomisation. Analysis was done on an intention to treat basis, but completers analysis was also carried out for weight loss in the HE, LL and PSMF at 12 months.

Weight and body composition was tested for normality, using the Kolmogorov-Smirnov Test on SPSS, and all data was normally distributed.

The following chapter shows weight and body composition levels at baseline, and changes pre-randomisation (3 months) and post randomisation at 6 and 12 months.

### 4.1 Pre-randomization analysis

(i) Baseline data

Table 4.1 shows the subjects baseline weight and body composition. Weight at 0 months was significantly associated with FFM (r = 0.798, p < 0.01), WC (r = 0.761, p < 0.01), HC (r = 0.581, p < 0.01), and WHR (r = 0.313, p < 0.01) at 0 months (Figures 4.1(a-d)).

<table>
<thead>
<tr>
<th>Units</th>
<th>Weight</th>
<th>BMI</th>
<th>Bodyfat</th>
<th>WC</th>
<th>HC</th>
<th>WHR</th>
<th>FFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td>kg/m²</td>
<td>%</td>
<td>cm</td>
<td>cm</td>
<td></td>
<td>kg</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>120</td>
<td>120</td>
<td>118</td>
<td>117</td>
<td>117</td>
<td>116</td>
<td>118</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>119.3</td>
<td>44.2</td>
<td>48.2</td>
<td>127.2</td>
<td>139.4</td>
<td>0.9</td>
<td>61.4</td>
</tr>
<tr>
<td></td>
<td>(20.1)</td>
<td>(6.8)</td>
<td>(5.5)</td>
<td>(14.8)</td>
<td>(14.6)</td>
<td>(0.09)</td>
<td>(12.1)</td>
</tr>
</tbody>
</table>

*a Values are mean (SD). n = number of subjects; BMI = body mass index; WC = waist circumference; HC = hip circumference; WHR = waist to hip ratio; FFM = fat free mass.*
(ii) Three month data

Table 4.2 shows weight and body composition for subjects at 3 months.

Weight decreased significantly by 2.3 (SD 4.1) kg, $p < 0.05$, at 3 months. BMI and proportion of body fat also showed a significant decrease by 0.8 (SD 1.8) kg/m$^2$, $p < 0.05$, and 0.4 (SD 1.7) %, $p = 0.016$, respectively. WC and HC decreased significantly by 1.8 (SD 7.7) cm, $p = 0.010$ and 2.0 (SD 7.0) cm, $p = 0.002$ respectively. Finally, FFM decreased significantly by 0.8 (SD 2.2) kg, $p < 0.05$. 
Table 4.2: Weight and body composition at 3 months.

<table>
<thead>
<tr>
<th>Units</th>
<th>Weight</th>
<th>BMI</th>
<th>Bodyfat</th>
<th>WC</th>
<th>HC</th>
<th>WHR</th>
<th>FFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>120</td>
<td>120</td>
<td>118</td>
<td>117</td>
<td>117</td>
<td>116</td>
<td>118</td>
</tr>
<tr>
<td>Mean</td>
<td>117.0</td>
<td>44.3</td>
<td>47.8</td>
<td>125.3</td>
<td>137.3</td>
<td>0.91</td>
<td>60.5</td>
</tr>
<tr>
<td>(19.9)</td>
<td>(6.9)</td>
<td>(5.9)</td>
<td>(14.4)</td>
<td>(14.1)</td>
<td>(0.09)</td>
<td>(11.7)</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>-2.3*</td>
<td>-0.8*</td>
<td>-0.4*</td>
<td>-1.8*</td>
<td>-2.0*</td>
<td>0.009</td>
<td>-0.8*</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.016</td>
<td>0.010</td>
<td>0.002</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Values are mean (SD). n= number of subjects, BMI = body mass index, WC = waist circumference, HC = hip circumference, WHR = waist to hip ratio, FFM = fat free mass.

(iii) Comparisons of groups who lost > 5% weight and ≤ 5% weight at 3 months.

At 3 months, 18 subjects lost >5 % of their baseline weight and continued on HE. Figure 3(a-f) compares changes in body composition at 3 months for both groups.

The > 5% group lost significantly more weight than the ≤ 5 % group (-10.0 (SD 4.7) kg vs. -0.9 (SD 2.2) kg, p < 0.05). There was also a significant difference favouring the >5 % group in changes in bodyfat (-2.1  (SD 1.9) % vs. -0.09 (SD 1.5) %, p < 0.05), BMI (-4.7 (SD 2.2) kg/m² vs. -0.3 (SD 1.1) kg/m² p < 0.05), WC (-6.8 (SD 10.0) cm vs. -0.9 (SD 7.0) cm, p = 0.003), HC (-6.1 (SD 4.3) cm vs. -1.3 (SD 7.3) cm and FFM (-2.8 (SD 2.2) kg vs. -0.4 (SD 2.1) kg, p < 0.05) (Figures 4.2a-f).

Figure 4.2a: Weight change at 3 months for groups who lost > 5 % and ≤ 5 % weight.
* significant difference between groups, p < 0.05

Figure 4.2b: BMI change at 3 months for groups who lost > 5 % and ≤ 5 % weight.
(iv) Dropouts vs. non-dropouts at 3 months

There were 30 dropouts at 3 months. Baseline characteristics for the dropout group and the non-dropout groups are shown in Table 4.3. No significant differences were found between the groups for weight and body composition at baseline.
Table 4.3: Weight and body composition for dropout and non-dropout groups at baseline

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dropout</strong></td>
<td>121.2</td>
<td>45.2</td>
<td>49.1</td>
<td>60.7</td>
<td>129.2</td>
<td>142.5</td>
<td>0.90</td>
</tr>
<tr>
<td>(n = 30)</td>
<td>(24.1)</td>
<td>(8.0)</td>
<td>(4.0)</td>
<td>(11.8)</td>
<td>(16.3)</td>
<td>(14.1)</td>
<td>(0.08)</td>
</tr>
<tr>
<td><strong>Non-dropout</strong></td>
<td>118.7</td>
<td>NS</td>
<td>47.9</td>
<td>NS</td>
<td>126.6</td>
<td>NS 138.4</td>
<td>NS</td>
</tr>
<tr>
<td>(n = 90)</td>
<td>(19.0)</td>
<td>(6.3)</td>
<td>(5.8)</td>
<td>(12.2)</td>
<td>(14.4)</td>
<td>(14.4)</td>
<td>(0.09)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

4.2 Post randomization analysis

At 6 months the approach to data analysis changes. As a result of some subjects having been randomised (LL and PSMF) and some not (HE), these 2 groups cannot be compared to each other. Therefore the HE group is analysed independently from LL and PSMF, and LL and PSMF are compared to each other.

Data for the HE group will be compared to baseline, whereas data for the LL and PSMF groups will be compared to 3 months, as this is when they were randomised.

(i) HE group

Weight and body composition for the HE group at baseline, 3, 6, and 12 months are shown in Table 4.3.

At 6 months, mean weight (-12.5 (SD 7.2) kg, p < 0.05), BMI (- 4.7 (SD 4.0) kg/m², p = 0.002), bodyfat (- 2.8 (SD 4.2) %, p = 0.002), WC (- 7.0 (SD 11.5) cm, p = 0.020), HC (- 8.5 (SD 5.7) cm, p < 0.05), and FFM (-4.6 (SD 0.9) kg, p = 0.001) decreased significantly from baseline.

At 12 months, there were significant differences for weight (-20.1 (SD 12.1) kg, p < 0.05), BMI (-7.3 (SD 4.5) kg/m², p < 0.05), bodyfat (-5.8 % (SD 7.7), p = 0.006), FFM (-4.8 kg (SD 5.5), p = 0.001), WC (-16.9 (SD 11.7) cm, p < 0.05) and HC (-14.1 (SD 7.1) cm, p < 0.05) from baseline. Figure 4.4 shows percentage changes at 12 months for the HE group.
Table 4.4: Weight and body composition for HE group at baseline, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>0 months (n = 18)</th>
<th>3 months (n = 18)</th>
<th>6 months (n = 18)</th>
<th>12 months (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>121.0 (24.4)</td>
<td>111.0* (24.0)</td>
<td>108.5* (24.1)</td>
<td>100.9* (24.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>44.4 (6.8)</td>
<td>39.6* (6.4)</td>
<td>38.6 (6.7)</td>
<td>36.1* (7.8)</td>
</tr>
<tr>
<td>Bodyfat %</td>
<td>46.2 (6.1)</td>
<td>44.8* (6.7)</td>
<td>44.3* (7.4)</td>
<td>40.4* (10.9)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>130.3 (19.2)</td>
<td>124.3* (12.5)</td>
<td>124.1* (14.0)</td>
<td>114.2* (15.9)</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>135.5 (12.9)</td>
<td>129.3* (14.6)</td>
<td>126.9* (12.0)</td>
<td>122.3* (16.1)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.08)</td>
<td>0.9 (0.0)</td>
<td>0.9 (0.07)</td>
</tr>
<tr>
<td>FFM</td>
<td>65.1 (16.4)</td>
<td>62.3* (16.1)</td>
<td>61.4* (15.8)</td>
<td>60.1* (14.5)</td>
</tr>
</tbody>
</table>

* significant difference from baseline, $p < 0.05$. Data are reported as mean (SD).

Figure 4.3: HE group percentage changes at 12 months for weight and body composition.

Pearson correlations were calculated for weight and body composition changes at 12 months. Weight change was significantly associated with bodyfat change ($r = 0.709$, $p = 0.001$), FFM change ($r = 0.608$, $p = 0.007$), WC change ($r = 0.751$, $p < 0.05$), and HC change ($r = 0.784$, $p < 0.05$) (Figures 4.4(a-d)).
There were 6 subjects who completed the HE diet at 12 months. Weight and body composition were analysed for those who completed the study on the HE diet. They were as follows: Weight = 94.0 kg (SD 24.6); BMI = 34.8 kg/m² (SD 9.4); bodyfat = 40.3 % (SD 10.4); WC = 107.9 cm (SD 20.9); HC = 122.0 cm (SD 18.4); WHR = 0.88 (SD 0.07).

These subjects showed significant decreases for weight (-18.7 (SD 9.2) kg, \( p = 0.004 \)), BMI (-8.0 (SD 2.8) kg/m², \( p = 0.001 \)), bodyfat (-9.1 (SD 7.5) %, \( p = 0.032 \)), WC (14.6 (SD 6.3) cm, \( p = 0.002 \)), and HC (15.2 (SD 7.9), \( p = 0.005 \)).
(ii) LL vs. PSMF groups

There were 19 dropouts in the LL group between 3 and 12 months. Table 4.5 shows weight and body composition for the LL group who dropped out and the LL group who did not dropout between 3 and 12 months. There were no significant differences between the groups at 3 months.

Table 4.5: Weight and body composition for LL group who dropped out between 3 and 12 months and those who did not

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropout (n = 19)</td>
<td>115.8 (14.1)</td>
<td>45.0 (5.3)</td>
<td>48.0 (6.3)</td>
<td>59.8 (9.7)</td>
<td>124.5 (12.4)</td>
<td>139.9 (9.9)</td>
<td>0.88 (0.09)</td>
</tr>
<tr>
<td>Non-dropout (n = 15)</td>
<td>129.6 (22.9)</td>
<td>NS (8.7)</td>
<td>NS (8.2)</td>
<td>NS (14.7)</td>
<td>NS (17.6)</td>
<td>NS (15.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).

There were 15 dropouts in the PSMF group between 3 and 12 months. Table 4.6 shows weight and body composition for the PSMF group who dropped out and the PSMF group who did not dropout between 3 and 12 months. There were no significant differences between the groups at 3 months.

Table 4.6: Weight and body composition for PSMF group who dropped out between 3 and 12 months and those who did not

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropout (n = 15)</td>
<td>114.8 (16.1)</td>
<td>44.0 (5.6)</td>
<td>49.9 (4.8)</td>
<td>56.9 (9.4)</td>
<td>122.7 (10.3)</td>
<td>136.6 (10.7)</td>
<td>0.90 (0.07)</td>
</tr>
<tr>
<td>Non-dropout (n = 23)</td>
<td>110.0 (12.6)</td>
<td>NS (4.0)</td>
<td>NS (5.3)</td>
<td>NS (8.1)</td>
<td>NS (9.8)</td>
<td>NS (10.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).

Figures 4.5(a-b) shows the subjects who were randomised to LL and the PSMF who lost between 0-5 % weight at 3 months, and who gained weight at 3 months. 23 (67.6 %) subjects
lost between 0-5% and 11 (32.4 %) subjects gained weight at 3 months in the LL group. 28 (74.6 %) subjects randomised to the PSMF lost between 0-5 % weight at 3 months and 10 (26.3 %) gained weight at 3 months.

Figure 4.5a: Weight change at 3 months for the LL group. Black = Subjects who lost 0-5 % weight; white = subjects who gained weight.

Figure 4.5b: Weight change at 3 months for the PSMF group. Black = Subjects who lost 0-5 % weight; white = subjects who gained weight.

Weight and body composition for LL and PSMF at baseline, 3, 6 and 12 months are shown in Table 4.7.

At 6 months (3 months post-randomization) there were significant differences in changes between LL and PSMF, where LL group showed a greater decrease in weight (LL -11.9 (SD 12.9) kg vs. PSMF -2.8 (SD 4.3) kg, $p < 0.05$), BMI (LL -4.2 (SD 4.6) kg/m$^2$ vs. PSMF -1.0
(SD 1.6) kg/m², p < 0.05), bodyfat (LL -4.1 (SD 4.8) % vs. PSMF -0.9 (SD 2.5) %, p = 0.027), HC (LL -7.3 (SD 7.8) cm vs. PSMF -4.4 (SD 6.4) cm, p = 0.002), and FFM (LL -2.7 (SD 4.4) kg vs. PSMF -0.6 (SD 2.1) kg, p = 0.017).

At 12 months (9 months post randomization), there were significant differences between LL and PSMF, where LL showed greater improvements in weight (LL -15.5 (SD 21.2) kg vs. PSMF -4.3 (SD 6.1) kg, p = 0.002), bodyfat (LL -6.0 (SD 8.6) % vs. PSMF -1.2 (SD 4.0) %, p = 0.006), FFM (LL -2.5 (SD 4.1) kg vs. PSMF -0.7 (SD 2.4) kg, p = 0.033), WC (LL -21.7 (SD 34.7) cm vs. PSMF -7.8 (SD 15.6) cm, p = 0.044), and HC (LL -11.4 (SD 15.7) cm vs. PSMF -4.1 (SD 4.5) cm, p = 0.006). Figure 4.6 shows percentage changes for the LL and PSMF at 12 months for weight and body composition.
Table 4.7: Weight and body composition for LL and PSMF groups at baseline, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m^2)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>124.5</td>
<td>46.2</td>
<td>48.2</td>
<td>64.8</td>
<td>127.7</td>
<td>142.9</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>(n = 34)</td>
<td>(20.1)</td>
<td>(7.2)</td>
<td>(6.9)</td>
<td>(12.8)</td>
<td>(15.2)</td>
<td>(16.2)</td>
<td></td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>114.1</td>
<td>42.2</td>
<td>48.5</td>
<td>NS</td>
<td>58.1</td>
<td>124.7</td>
<td>NS</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(14.3)</td>
<td>(4.4)</td>
<td>(4.4)</td>
<td>(8.2)</td>
<td>(10.7)</td>
<td>(10.0)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><strong>3 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>122.1</td>
<td>45.9</td>
<td>47.8</td>
<td>64.1</td>
<td>125.9</td>
<td>140.1</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>(n = 34)</td>
<td>(19.3)</td>
<td>(7.0)</td>
<td>(7.1)</td>
<td>(12.1)</td>
<td>(15.0)</td>
<td>(12.4)</td>
<td></td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>111.5</td>
<td>41.6</td>
<td>48.3</td>
<td>NS</td>
<td>57.4</td>
<td>122.5</td>
<td>NS</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(14.0)</td>
<td>(4.6)</td>
<td>(5.2)</td>
<td>(8.7)</td>
<td>(10.4)</td>
<td>(10.8)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>110.1*</td>
<td>41.5*</td>
<td>44.6*</td>
<td>60.4*</td>
<td>118.5*</td>
<td>132.7*</td>
<td>0.8</td>
</tr>
<tr>
<td>(n = 34)</td>
<td>(18.0)</td>
<td>(7.3)</td>
<td>(8.1)</td>
<td>(11.1)</td>
<td>(16.3)</td>
<td>(14.1)</td>
<td>(1.1)</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>108.7*</td>
<td>40.5*</td>
<td>47.6*</td>
<td>NS</td>
<td>56.7</td>
<td>119.0*</td>
<td>0.05</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(15.5)</td>
<td>(5.3)</td>
<td>(5.8)</td>
<td>(9.0)</td>
<td>(9.9)</td>
<td>(10.2)</td>
<td>(0.6)</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>106.5*</td>
<td>39.7*</td>
<td>41.9*</td>
<td>60.6*</td>
<td>112.4*</td>
<td>130.9*</td>
<td>0.8</td>
</tr>
<tr>
<td>(n = 34)</td>
<td>(19.6)</td>
<td>(8.9)</td>
<td>(11.6)</td>
<td>(11.5)</td>
<td>(15.3)</td>
<td>(16.0)</td>
<td>(0.08)</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>109.7*</td>
<td>40.9*</td>
<td>47.3</td>
<td>NS</td>
<td>56.6</td>
<td>119.1*</td>
<td>0.05</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(16.3)</td>
<td>(4.2)</td>
<td>(6.4)</td>
<td>(8.8)</td>
<td>(10.9)</td>
<td>(11.3)</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

* significant difference from 3 months. P-value represents differences between LL and PSMF. Values are mean (SD). $p < 0.05$
Figure 4.6: Percent changes in weight and body composition for LL and PSMF at 12 months.

**LL group**

Weight change at 12 months was significantly associated with FFM change \((r = 0.836, p < 0.01)\), HC change \((r = 0.595, p < 0.01)\) and WHR change \((r = 0.447, p < 0.01)\).

**PSMF group**

Weight change at 12 months was significantly associated with FFM change \((r = 0.425, p < 0.01)\) and HC change \((r = 0.811, p < 0.01)\).

Analysis was carried out for those who completed the study on the LL and PSMF diets. 15 subjects completed the LL diet at 12 months. Weight and body composition at 12 months were as follows: Weight = 98.0 kg (SD 20.3); FFM = 62.1 kg (SD 14.1); BMI = 34.8 kg/m\(^2\) (SD 9.1); bodyfat = 35.7 % (SD 14.5); WC = 104.2 cm (SD 10.4); HC = 120.9 cm (SD 16.7) and WHR = 0.8 (SD 0.08).
Significant decreases were seen for all anthropometric measures including FFM (-5.1 (SD 5.9 kg, \( p = 0.002 \)), weight (-31.5 (SD 22.0) kg, \( p \leq 0.0001 \)), BMI (-12.0 (SD 9.6) kg/m\(^2\), \( p \leq 0.0001 \)), bodyfat (-11.6 (SD 9.4) %, \( p \leq 0.0001 \)), WC (-25.3 (SD 17.1) cm, \( p \leq 0.0001 \)), HC (-18.0 (SD 11.8) \( p \leq 0.0001 \)), and WHR (-0.06 (SD 0.06), \( p = 0.002 \)).

23 subjects completed the PSMF at 12 months. Weight and body composition at 12 months were as follows: Weight = 108.3 kg (SD 16.2); FFM = 56.8 kg (SD 8.4); BMI = 40.0 kg/m\(^2\) (SD 4.8); bodyfat = 45.8 % (SD 6.8); WC = 118.8 cm (SD 11.1); HC = 132.1 cm (SD 12.2) and WHR = 0.9 (SD 0.06).

There were no significant decreases seen in this group at 12 months.
4.3: DISCUSSION

Though there was a significant weight loss at 3 months, only 18 subjects met the 5 % weight loss target, with 72 subjects then being randomised to LL or the PSMF diet. In other words, the majority of the population at 3 months had to be randomised due to the HE approach not been suitable for weight loss in this population (BMI > 35 kg/m$^2$). Those who were unsuccessful on the HE approach only lost an average of 1.4 kg at 3 months. It was hypothesized that this would be the case due to the study design. It is also important to note that those who remained on the HE diet were not randomised, and therefore they essentially had a choice, compared to those who were randomised to LL or the PSMF and therefore had no choice as to what treatment they were to undergo. RCTs were originally designed for drug testing interventions, but are now used for dietary interventions also. The disadvantage to these is that the subject does not have the choice of which diet they are assigned to. Therefore the subject does not have choice of food type, and sometimes there are also physical activity restraints. In other words, the diet the subject is given may not suit their lifestyle.

80 % of the population at 3 months were unsuccessful using a HE approach, despite major input into subject contact and clear guidelines in following the diet. This failure rate implies that “one diet does not fit all” and that these subjects may present with a phenotype that is better suited to an alternative diet.

After carrying out a literature search, it seems that this is the first study to use a design where all subjects were assigned to 1 diet at the beginning of the trial. The HE diet is the standard dietary treatment used in primary care, and follows public health guidelines, to treat obesity,
and it was interesting to see that most of the subjects did not lose weight using this approach, which is significant in terms of the study design.

As well as a significant decrease in weight, the > 5 % group showed significant decreases in bodyfat, fat-free mass, BMI, waist circumference and hip circumference at 3 months, indicating that a weight loss of > 5 % has beneficial effects on health related anthropometric measurements.

There was no difference between those who dropped out between baseline and 3 months and those who completed the first 3 months, in baseline weight and body composition. As discussed previously, this suggests that it may have been some other issue which impacted on the subject’s ability to lose weight on the HE approach, other than increased weight, which provoked the subject to drop out. The failure to lose weight under the circumstances may be the major reason for dropout.

The results of this study suggest that both a HE diet and a very low calorie/meal replacement diet such as LL are effective for weight loss and improvements in body composition over a 9 (LL) and 12 (HE) month period. The PSMF also showed a significant improvement in weight at 12 months, though the change was smaller than that seen on the HE and LL diets. This individual response to the different dietary approaches is important in the overall design of clinical pathways for obesity management.

Those who followed the HE diet, which conforms to the currently recommended daily amount of macronutrient intake, lost a significant amount of weight at 3, 6 and 12 months, both when analysing those who completed the diet and using intention to treat analysis.
Previous studies comparing low carbohydrate diets to conventional HE diets showed mean weight losses on the HE diet at 6 months ranging from -1.9 kg to -10.1 kg (Brehm et al., 2003; Samaha et al., 2003; Due et al., 2004; Tay et al., 2008), whereas mean weight loss for subjects on the HE diet in the present trial was -12.1 kg for subjects on the HE diet at 6 months. At 12 months, the HE group showed a mean weight loss of -20.1 kg (-17 %). This is in contrast to similar studies examining HE diets, where weight loss at 12 months ranged from -2.6 kg to -4.1 kg (Stern et al., 2004; Dansinger et al., 2005; Gardner et al., 2007). Though, the subjects on these low fat diets were randomised, and this may be a reason as to why they were not as successful as the subjects following the HE diet in the present study. This is a huge success in terms of weight and anthropometric changes. However it is important to state that subjects on the HE diet in the present trial were in effect “preselected” to follow this diet because they reached the weight loss target at 3 months. Therefore it would be expected that they would lose a significant amount of weight over the course of the trial, as evidently this diet suited them in terms of lifestyle and weight loss.

Also, the HE group were not randomised compared to the other 2 groups. The HE group also lost a significant amount of bodyfat, but on the other hand, they also showed a significant decrease in fat-free mass (-4.8 kg). However, it is expected that there would be a certain amount of loss of fat-free mass when there is a decrease in weight. It is also possible that some of this fat-free mass consisted of body water, from the depletion of stored glycogen (Bortz et al., 1967; Yang et al., 1984). Also most diets that have a significant reduction in energy cause a sodium diuresis that occurs at the beginning of the diet (Brehm et al., 2002). The HE group had most of their weight loss in the first 3 months of the trial, and a similar pattern occurred in the LL group. Chaston et al. (2006) carried out a systematic review looking at the proportion of weight lost as fat free mass by different weight loss interventions.
He concluded that it is the amount of caloric restriction, exercise and the rate of weight loss that influences the amount of weight lost as fat free mass after non-surgical interventions. A significant relationship was found for fat free mass change and weight change at 12 months.

All subjects completed a 3 day food diary at the beginning of the study as a rough measure of compliance. However, those on the HE and LL diets did not complete these diaries again throughout the trial, and this should be done in future studies to ensure the subjects are consistently complying with the diet. The subjects were seen on a regular basis and any issues they had with their assigned diets were discussed. Clearly, the subjects who were successful on the HE diet were able to comply to this approach, which is evident with the large weight loss. The successful weight loss in this group could in part be due to the regular visits to the clinic for follow up. It has been shown before in overweight, non-diabetic subjects that dietary counselling on a regular, individualised basis carried out in the long term, showed greater changes in positive dietary practice and weight loss in comparison to subjects given no regular dietary advice (Knowler et al., 2002; Foster et al., 2003). More importantly, the large weight loss may be due to the fact that these subjects were “selected” to remain on the HE diet, and this may prove that the hypothesis is true, in that different phenotypes at presentation may suit a certain diet for weight loss.

VLCDs are known to cause rapid weight loss similar to weight loss seen during starvation (Genuth et al., 1974). Most VLCD programs allow the subjects to remain on the diet for a period of 12 weeks, with the average weight loss approximately 20 kg, and for programs up to 6 months weight loss can be up to 35 kg (Wadden et al., 1983). Subjects on the LL diet lost an average of 11.9 kg at 6 months and by 12 months they had lost an average of 15.5 kg.
When analysing the completers alone, they showed a mean weight loss of 31.5 kg at 12 months.

Long term maintenance of weight loss is difficult to achieve through diet alone (Flinn et al., 1988). The success of the weight loss on LL could in part be due to the strict design of the diet including the combination of the supplied food packages and cognitive behavioural therapy (CBT). However, these subjects were not successful at losing weight on the HE diet, and therefore, metabolically they could be more suited to this approach. CBT aims to prevent the subject from reverting back to their original eating habits which caused them to become obese initially. CBT is the most comprehensive means of medically treating obesity, though there is little published data concerning its long-term effectiveness (Melchionda et al., 2003). It is a psychotherapy method based on cognitions, assumptions, beliefs, and behaviors, with the aim of influencing negative emotions that relate to, in this case, obesity.

The majority of the weight lost on LL was from 3 to 6 months (-11.9 kg), which is likely, as this is when all subjects are on Stage 1 of the LL programme. In parallel to this, most of the fat free mass loss was from 3-6 months. Interestingly, there was a slight increase in fat free mass from 6 to 12 months. This stage could coincide with the LL subjects been weaned back on to a whole-food diet. The LL programme is designed to allow individuals to enter a weight maintenance phase once they have achieved their goal weight, an approach which was used in the present study. Of the 14 patients who completed the study, 2 patients were on the weight loss phase for 4 months, 4 were on the weight loss phase for 5 months and 2 (14%) patients were on the weight loss phase for 7 months. Six patients remained on the weight loss phase for the complete 9 months.
Bodyfat decreased significantly at 12 months on the LL diet. Measurements of bodyfat depots were not examined, however it is long acknowledged that visceral fat (abdominal obesity) plays a strong role in the development of metabolic risk factors. (Despres et al., 1990; Matsuzawa et al., 1995). Because of this, visceral fat may be a more useful indicator of CVD and T2D as opposed to total fat. Waist circumference and waist to hip ratio are used as measures of central obesity, whereas the BMI is more generally used as a measure of general obesity (Molarius, 1998). In the present study, waist circumference was highly correlated with waist to hip ratio for the LL group, and this was also the case for the HE and PSMF groups. However, from the present trial, it is uncertain whether waist to hip ratio should be used alone in predicting metabolic risk factors. Despite the large weight loss and decrease in waist circumference on both the HE and LL diets, the waist to hip ratio remained the same for the HE group and decreased from 0.9 to 0.8 for the LL group. Therefore the waist to hip ratio measurement should be used with caution. Similar studies have found waist circumference to be a better measurement of central obesity as it is a better predictor of abdominal visceral fat obtained with computed tomography than is waist to hip ratio. Waist circumference is also easy to measure and interpret (Pouliot et al., 1994; Molaris et al., 1998; Rankinen et al., 1999). BMI was highly correlated with waist circumference in the trial, and therefore may be a reliable indicator of abdominal adiposity where waist circumference is not measured.

The small weight loss seen in the PSMF group was surprising. One obvious explanation may be that the subjects did not comply fully with the diet. It has been reported that such diets produce approximately a 20 kg weight loss in 3 months, however subjects following the PSMF in the present trial only lost 2.8 kg after 3 months following the diet, and only 4.3 kg overall. The diet should be able to teach the subjects how to manage conventional foods, and helps the smooth transition from the PSMF to a normal maintenance diet (Wadden et al.,
1983). It is essentially a low carbohydrate diet, as the subjects are only allowed a maximum of 40 g of carbohydrate a day. This should lead to the presence of ketosis, which is thought to reduce appetite. However, many of the subjects measured were not in ketosis during consumption of the PSMF. Studies have shown that calorie for calorie, protein is more satiating than carbohydrate and fat (Barkeling et al., 1990). Therefore, if the diet was complied with, the high protein intake should induce an overall limited food intake. Another aspect of the PSMF is the limited food choice, due to the low carbohydrate allowance, which should therefore reduce the overall calorie consumption. If the subjects complied with the PSMF, these factors would have most likely aided with weight loss, however the low weight loss indicates that this was not the case in this trial. High protein diets tend to be more costly than conventional diets, and this could also contribute to non-compliance to the PSMF. Lack of use of the diet may suggest a low income and educational issues in regards to the population studied.

As discussed in Chapter 3, the systematic reviews showed that there are many studies comparing conventional HE diets, similar to the one examined in the present trial, to low carbohydrate/high protein diets. However, these studies randomised their subjects at baseline, which was not done in the present trial. At mentioned previously, RCTs are not always the best approach for lifestyle interventions. The subject is left with no choice as to what diet they are assigned to, and this may affect weight loss, and subsequently CVD risk and related morbidities. This was evident in the present trial. The few subjects who remained on the HE diet were highly successful in losing weight throughout the trial, compared to weight loss seen on previous dietary intervention studies. These subjects were preselected as they lost > 5% of their baseline weight at 3 months, and therefore did not need to be randomised. The study design in the present study gives added support to this point about RCTs, and also proves that a HE approach may not be suited to all obese subjects with a BMI > 35 kg/m².
CHAPTER 5: CVD RISK FACTORS
Another objective of the trial was to examine CVD risk factor changes using different dietary approaches. It was hypothesised that those who remained on the HE diet at 3 months “suited” this type of dietary approach, and would therefore show decreases in the risk of CVD. The same hypothesis applies for the LL and PSMF groups.

CVD risk factors changes were examined at 3 months for all the subjects before randomisation, and then in the HE, LL and PSMF groups 3 and 9 months post randomisation. Analysis was done on an intention to treat basis.

CVD risk factors was tested for normality using the Kolmogorov-Smirnov Test on SPSS and all data was normally distributed.

5.1: Pre-randomization analysis

(i) Baseline data

Table 5.1 shows cardiovascular disease (CVD) risk factors at baseline. Weight was significantly inversely associated with HDL cholesterol \((r = -0.280, p < 0.01)\), and significantly positively associated with systolic blood pressure \((r = 0.396, p < 0.01)\) and diastolic blood pressure \((r = 0.396, p < 0.01)\) (Figures 5.1(a-c)).

Table 5.1: Cardiovascular disease risk factor levels of subjects at baseline

<table>
<thead>
<tr>
<th>Units</th>
<th>Total chol</th>
<th>HDL chol</th>
<th>LDL chol</th>
<th>TAG</th>
<th>SBP</th>
<th>DBP</th>
<th>TC/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmHg</td>
<td>mmHg</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.1</td>
<td>1.2</td>
<td>3.1</td>
<td>1.5</td>
<td>131.8</td>
<td>86.1</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(0.2)</td>
<td>(0.8)</td>
<td>(1.1)</td>
<td>(18.4)</td>
<td>(11.6)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

\(a = \text{Mean and SD.}\)
Figure 5.1a: Weight vs. HDL cholesterol at 0 months.

Figure 5.1b: Weight vs. SBP at 0 months.

Figure 5.1c: Weight vs. DBP at 0 months.

WC was also correlated with CVD risk factors at baseline. Significant inverse associations were found for WC and HDL cholesterol \( (r = -0.384, p < 0.01) \), and significant positive associations for WC and SBP \( (r = 0.320, p < 0.01) \), DBP \( (r = 0.306, p = 0.001) \), and TC/HDL cholesterol and \( (r = 0.230, p = 0.014) \) (Figures 5.2a-d).
Figure 5.2a: WC vs. HDL cholesterol at 0 months.

Figure 5.2b: WC vs. SBP at 0 months.

Figure 5.2c: WC vs. DBP at 0 months.

Figure 5.2d: WC vs. TC/HDL cholesterol at 0 months.

(ii) Three month data

Table 5.2 shows cardiovascular risk factor levels at 3 months. HDL cholesterol showed a significant increase at 3 months (0.03 (SD 0.01) mmol/l, \( p = 0.018 \)) and TC/HDL showed a significant decrease at 3 months (-0.1 (SD 0.6), \( p = 0.021 \)).

Table 5.2: CVD risk factor levels at 3 months.

<table>
<thead>
<tr>
<th>Units</th>
<th>Total chol</th>
<th>HDL chol</th>
<th>LDL chol</th>
<th>TAG</th>
<th>SBP</th>
<th>DBP</th>
<th>TC/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td>mmol/l</td>
</tr>
<tr>
<td>( n )</td>
<td>117</td>
<td>117</td>
<td>117</td>
<td>117</td>
<td>119</td>
<td>119</td>
<td>117</td>
</tr>
<tr>
<td>Mean(^a)</td>
<td>5.2</td>
<td>1.3</td>
<td>3.2</td>
<td>1.5</td>
<td>132.5</td>
<td>86.0</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(0.2)</td>
<td>(0.8)</td>
<td>(0.9)</td>
<td>(19.8)</td>
<td>(11.4)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Difference from baseline(^a)</td>
<td>0.06</td>
<td>0.03</td>
<td>0.02</td>
<td>-0.07</td>
<td>0.6</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(0.1)</td>
<td>(0.4)</td>
<td>(1.0)</td>
<td>(12.4)</td>
<td>(8.0)</td>
<td>(0.6)</td>
</tr>
<tr>
<td>( p )</td>
<td>NS</td>
<td>0.018</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.021</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean (SD), \( p < 0.05 \)
(iii) Comparisons of groups who lost > 5 % weight and ≤ 5 % weight at 3 months.

Figures 5.3(a-d) shows that there were significant differences in changes in CVD risk between the groups for HDL cholesterol (-0.03 (SD 0.1) mmol/l vs. 0.04 (SD 0.1) mmol/l, \( p = 0.031 \)) favouring the ≤ 5 % group, LDL cholesterol (-0.1 (SD 0.3) mmol/l, vs. 0.06 (SD 0.4) mmol/l, \( p = 0.030 \)) favouring the > 5 % group, blood pressure (-5.8 (SD 15.3) mmHg vs. 1.7 (SD 11.8) mmHg, \( p = 0.017 \) (SBP) and -5.4 (SD 6.6) mmHg vs. 0.6 (SD 8.0) mmHg, \( p = 0.013 \) (DBP)) favouring the > 5% group, and total cholesterol (-0.2 (SD 0.4) mmol/l vs. 0.1 (SD 0.5) mmol/l, \( p = 0.008 \)) favouring the > 5% group.

Figure 5.3a: HDL cholesterol, LDL cholesterol, TAG, total cholesterol and FPG changes at 3 months for those who lost > 5% and ≤5% weight.
5.2: Post-randomization analysis

(i) HE group

Results for baseline, 3, 6 and 12 months are shown in Table 5.3.

At 6 months there were significant improvements for total cholesterol (-0.35 (SD 0.4) mmol/l, \( p = 0.003 \)), TAG (-0.35 (SD 0.58), mmol/l, \( p = 0.018 \)), and DBP (-5.2 (SD 8.2) mmHg, \( p = 0.015 \)).

At 12 months there were significant improvements in HDL cholesterol (0.1 (SD 0.1) mmol/l, \( p = 0.003 \)), TAG (-0.4 (SD 0.7) mmol/l, \( p = 0.015 \)), DBP (-7.0 (SD 8.8) mmHg, \( p = 0.004 \)) and TC/HDL cholesterol (-0.4 (SD 0.8), \( p = 0.018 \)).
Figure 5.4 shows percent changes in CVD risk at 12 months for the HE group.

Table 5.3: CVD risk factors for HE group at baseline, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>0 mths (n = 18)</th>
<th>3 mths (n = 18)</th>
<th>6 mths (n = 18)</th>
<th>12 mths (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chol (mmol/l)</td>
<td>5.5 (0.7)</td>
<td>5.2* (0.8)</td>
<td>5.1* (0.7)</td>
<td>5.2 (0.9)</td>
</tr>
<tr>
<td>LDL chol (mmol/l)</td>
<td>3.3 (0.6)</td>
<td>3.2 (0.7)</td>
<td>3.2 (0.7)</td>
<td>3.2 (0.8)</td>
</tr>
<tr>
<td>HDL chol (mmol/l)</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.2)</td>
<td>1.3* (0.3)</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>2.0 (0.9)</td>
<td>1.7* (0.8)</td>
<td>1.6* (0.5)</td>
<td>1.5* (0.6)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>136.5 (16.0)</td>
<td>130.7 (16.9)</td>
<td>130.1 (15.2)</td>
<td>128.5 (15.0)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87.3 (11.6)</td>
<td>82.5* (8.8)</td>
<td>81.4 * (9.0)</td>
<td>80.3* (9.0)</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>5.6 (0.8)</td>
<td>5.5 (0.9)</td>
<td>5.3 (0.7)</td>
<td>5.1* (0.9)</td>
</tr>
</tbody>
</table>

* significant difference from baseline. Data are reported as mean (SD), $p < 0.05$

Analysis was also carried out for the completers group. HDL cholesterol significantly increased by 0.15 (SD 0.13), $p = 0.045$.

Figure 5.4: Percent change in CVD risk for the HE group at 12 months.
Pearson correlations were calculated for weight change vs. CVD risk factor changes at 12 months for the HE group. Significant associations were found for weight change and blood pressure change (SBP: \( r = 0.502, p = 0.034 \) and DBP: \( r = 0.523, p = 0.026 \)).

(ii) LL vs. PSMF

CVD risk factors for LL and PSMF at baseline, 3, 6 and 12 months are shown in Table 5.4. At 6 months there was a significant difference between the groups for changes in LDL cholesterol \( (p = 0.05) \). LDL cholesterol decreased by 0.4 (SD 0.6) mmol/l for the LL group compared to no change for the PSMF group. There were no differences in changes in CVD risk factors between the groups at 12 months. Figure 5.5 shows percentage changes at 12 months for the LL and PSMF groups.

Analysis was also carried out for the completers in both the LL group and the PSMF group. For the LL group, HDL cholesterol increased significantly by 0.20 (SD 0.20) mmol/l, \( p = 0.002 \); TAG decreased significantly by 0.39 (SD 0.48) mmol/l, \( p = 0.007 \); SBP decreased significantly by 13.9 (SD 17.0) mmHg, \( p = 0.007 \) and DBP decreased significantly by 11.1 (SD 15.5) mmHg, \( p = 0.010 \).

There were no significant differences for the PSMF group completers.
Table 5.4: CVD risk factor changes for LL and PSMF groups at baseline, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>Total chol (mmol/l)</th>
<th>LDL chol (mmol/l)</th>
<th>HDL chol (mmol/l)</th>
<th>TAG (mmol/l)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>TC/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>5.9 (1.0)</td>
<td>3.1 (0.80)</td>
<td>1.2 (0.2)</td>
<td>1.4 (0.6)</td>
<td>133.6 (17.6)</td>
<td>89.5 (12.0)</td>
<td>5.0 (0.7)</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>5.2 (0.9)</td>
<td>NS</td>
<td>1.3 (0.3)</td>
<td>NS</td>
<td>132.6 (18.7)</td>
<td>NS</td>
<td>5.0 (0.9)</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>5.0 (0.9)</td>
<td>3.2 (0.7)</td>
<td>1.30 (0.2)</td>
<td>1.3 (0.7)</td>
<td>135.8 (18.5)</td>
<td>86.5 (11.9)</td>
<td>3.9 (0.8)</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>5.5 (0.9)</td>
<td>NS</td>
<td>3.3 (0.7)</td>
<td>NS</td>
<td>136.7 (22.0)</td>
<td>NS</td>
<td>3.8 (1.0)</td>
</tr>
<tr>
<td><strong>6 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>5.7* (1.1)</td>
<td>2.8* (0.9)</td>
<td>1.2* (0.1)</td>
<td>1.1* (0.6)</td>
<td>127.9* (15.0)</td>
<td>81.4* (10.7)</td>
<td>3.7 (0.8)</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>5.2 (0.9)</td>
<td>3.3 (0.7)</td>
<td>0.022</td>
<td>0.005</td>
<td>131.8 (18.6)</td>
<td>87.6 (8.2)</td>
<td>3.8 (0.9)</td>
</tr>
<tr>
<td><strong>12mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>5.7* (0.9)</td>
<td>2.9* (0.8)</td>
<td>1.37* (0.2)</td>
<td>1.1* (0.7)</td>
<td>128.2* (17.6)</td>
<td>82.8 (12.3)</td>
<td>3.6*</td>
</tr>
<tr>
<td>PSMF (n = 38)</td>
<td>5.2 (1.0)</td>
<td>3.2 (0.8)</td>
<td>1.442 (0.3)</td>
<td>1.5 (0.8)</td>
<td>132.9 (16.6)</td>
<td>86.5 (8.4)</td>
<td>3.7 (1.0)</td>
</tr>
</tbody>
</table>

*a* significant difference from baseline. * significant difference from 3 months. P-value represents differences between LL and PSMF. Values are mean (SD); *p* < 0.05.
Figure 5.5: Percent changes from 3 to 12 months in CVD risk for PSMF and LL groups.

**LL group**

Significant associations were found between weight change at 12 months and HDL cholesterol change ($r = -0.536, p = 0.002$), TAG change ($r = 0.657, p < 0.001$), systolic blood pressure ($r = 0.491, p = 0.006$), diastolic blood pressure ($r = 0.506, p = 0.004$) and TC/HDL cholesterol ($r = 0.608, p < 0.001$)

**PSMF group.**

There was a significant association between weight change and TAG change at 12 months ($r = 0.424, p = 0.008$), and TC/HDL cholesterol ($r = 0.332, p = 0.048$).
5.3: DISCUSSION

It is important that dietary approaches for weight loss are also beneficial for reducing the risk of CVD. Results from the present study show that there was a trend for improvement in CVD risk in all diet groups.

There are no internationally recognised healthy ranges for CVD risk factors. However, there are target values for different populations at risk for CVD or who already have CVD risk factors, such as hypertension or T2D. In the UK, organisations such as SIGN (Scottish Intercollegiate Guidelines Network), and the Joint British Societies Guidelines (JBS2) have target values for those at risk of developing CVD. At risk populations are estimated using the “10-year risk for coronary heart disease Framingham Point Scores” (NCEP ATP III). Globally, the NCEP ATP III has devised target values (National Cholesterol Education Program Adult Treatment Panel III) for these “at risk” populations.

In the present trial mean CVD risk factors at baseline were within the target values according to the ATP III classification. However both systolic and diastolic blood pressure were elevated, though mean blood pressure in the present population did not reach diagnosis for hypertension according to the ATP III (140/90 mm Hg).

Weight at baseline was significantly associated with blood pressure and there was an inverse relationship with weight and HDL cholesterol. Both low HDL cholesterol and hypertension are risk factors for CVD (Boden and Pearson, 2000; Szapary and Rader, 2001). This emphasises the importance of weight and its involvement with CVD risk. It has been suggested that an increase of 1 % HDL cholesterol could lead to a 3 % decrease in the risk of
heart disease (Boden and Pearson, 2000). Perhaps more importantly, as discussed in Chapter 4, waist circumference could be a more reliable predictor of CVD risk than weight, due to its relationship with visceral abdominal obesity as strong evidence from previous studies has shown. Results from this study show that waist circumference is as good a predictor as weight is, though it was no better at predicting CVD risk than weight or BMI alone. There was a strong relationship found between waist circumference and blood pressure, TC/HDL cholesterol and an inverse relationship with HDL cholesterol.

When comparing the groups who lost and did not lose 5 % baseline weight, there were differences in changes in CVD risk factors. Surprisingly, the ≤ 5 % weight loss group had a greater improvement in HDL cholesterol at 3 months compared to the > 5 % group. On the other hand, there were significantly greater improvements in LDL cholesterol, total cholesterol and blood pressure for the > 5 % group at 3 months compared to the ≤ 5 % group. This supports the idea that a weight loss of 5 % improves cardiovascular risk.

The amount of weight lost at 12 months for each dietary approach ranged from 3 – 17 %. Modest reductions in weight have been shown to have favourable effects on risk factors including TAG and blood pressure (Stevens 2001; Knowler et al., 2002).

Data from the 1940s found that there was a relationship between high fat diets and high cholesterol levels, which suggested that those with a high risk for heart disease should follow a low fat diet. Two decades later, low fat diets are the standard treatment for weight loss (La Berge, 2008). Currently, the standards for a HE diet are to reduce total fat intake to < 30 % total daily energy intake and decreasing saturated fat to < 10 % total daily energy intake. This
is based on a large amount of epidemiological data and is intended to reduce total cholesterol (National Institute of Health NH, Lung and Blood Institute, 1998).

Low carbohydrate, high fat diets have been shown to decrease triacylglycerol and increase HDL cholesterol (Lewis et al., 1977; Golay et al., 1996), whereas high carbohydrate, low fat diets decrease HDL cholesterol and increase TAG concentrations (Lewis et al., 1977; Garg et al., 1992). In contrast to this, significant improvements were seen in the HE group for HDL cholesterol, triacylglycerols, diastolic blood pressure and the ratio of TC/HDL cholesterol at 12 months in the present study. There was also a trend for the remaining CVD risk factors (total cholesterol, LDL cholesterol, and systolic blood pressure) to improve for the group following the HE diet, suggesting that this diet is appropriate for losing weight, while also reducing the risk for CVD, for those who remained on this diet. After 12 months of treatment, HDL cholesterol increased by an average of 8% and triacylglycerol decreased by an average of 25% on the HE diet, which is comparable with the level of change seen with pharmacological treatment, such as nicotinic acid and fibrates (Szapary et al., 2001).

For the LL group, there were significant improvements at 12 months in all CVD risk factors except diastolic blood pressure, and total cholesterol which significantly increased. This is despite these subjects only following the LL programme for 9 months, as opposed to the HE group who underwent the HE diet for 12 months. Weight loss at 12 months was significantly associated with an increase in HDL cholesterol, and decreases in triacylglycerol, systolic and diastolic blood pressure and TC/HDL cholesterol. The LL diet seemed to be protective of HDL cholesterol, as it increased significantly at 12 months, possibly due to the low carbohydrate content of the diet. The favourable effects of a VLCD, such as LL, are usually reported at the end of energy restriction (Pekkarinen et al., 1998). However these effects, in
particular on blood pressure, may not remain the same during weight maintenance, as seen in the present trial. From 6 to 12 months, when 8 subjects were slowly introduced to a whole food diet, there was a trend for a small increase in total cholesterol and blood pressure. Therefore it is important to monitor CVD risk factors in subjects who lose weight using LL and similar weight loss approaches, in particular when they are being introduced to whole foods.

There were no significant improvements seen in the PSMF group at either 6 or 12 months of the trial. This was probably due to the small weight change in the group. On a positive note, there was a trend for an improvement in CVD risk, as all CVD risk factors except HDL cholesterol tended to improve. Numerous short term RCTs in which carbohydrate was substituted for protein in low fat diets enhances weight loss with improved changes on CVD risk factors including blood lipid profile (Wolfe and Giovannetti, 1991; Parker et al., 2002; Layman et al., 2003). Weight change in the PSMF group was associated with both decreases in triacylglycerols and the ratio of TC/HDL at 12 months.

The PSMF group had a mean loss of < 5 % weight during the 9 months treatment. The results for the PSMF group highlight the need for obese subjects to aim to lose 5 % of their baseline weight, when undergoing a weight reducing diet for there to be beneficial effects on CVD risk.

In conclusion, all 3 diets tended to show improvements in CVD risk, though it is important to monitor these changes in particular for subjects following a VLCD similar to LL, as they may possibly revert back to their previous CVD levels when returning to a whole-food diet. It is also noteworthy that the small changes in CVD risk factors seen in the PSMF group is most likely due to the small weight loss, and that many similar studies of high protein diets show a
large weight loss in parallel with significantly improved CVD risk. In terms of weight loss and CVD risk reduction, it is difficult to decide which dietary approach is best, but it may be that “one diet does not fit all”, in terms of metabolic risk.

Nordmann et al. (2006) compared RCTs of low carbohydrate diets, without restriction of energy intake, to low fat diets with a follow up of at least 6 months. Five trials from 1980 to 2005 filled the inclusion criteria.

Subjects on the low carbohydrate diet lost 3.3 kg more weight than those following the low fat diet at 6 months, but the difference was no longer obvious at 12 months. Both systolic and diastolic tended to decrease more on the low carbohydrate diet at 6 months (WMD in systolic blood pressure, −2.4 mm Hg; WMD in diastolic blood pressure, −1.8 mm Hg). However, this trend was no longer shown at 12 months.

TAG (WMD, -0.25 mmol/l) and HDL cholesterol (WMD, 0.12 mmol/l) changed more favourably in those following the low carbohydrate diet at 6 months, and this was the same for 12 months (WMD for HDL cholesterol, 0.08 mmol/l and for TAG – 0.35 mmol/l). Total cholesterol (WMD, 0.23 mmol/l) and LDL cholesterol (WMD, 0.14 mmol/l) changed more favourably in those following the low fat diet at 6 months, and this was unchanged at 12 months.

In the systematic review by Hession et al. (2008) the WMD in total cholesterol change was 0.19 mmol/l at 6 months (p < 0.0001) with the low carbohydrate group demonstrating the increased cholesterol. This is comparable to the study by Nordmann et al. This was also the case at 12 months, though the difference between the groups was smaller and not significant (0.10 mmol/l, p = 0.31).
The WMD in LDL cholesterol change was 0.14 mmol/l at 6 months ($p < 0.00001$) with the low carbohydrate group demonstrating the increased LDL cholesterol. The difference between the groups was greater at 12 months (0.37 mmol/l) ($p < 0.00001$) with the low carbohydrate group again demonstrating the increased LDL cholesterol.

The WMD in HDL cholesterol change was 0.04 mmol/l at 6 months ($p = 0.03$) favouring the low carbohydrate group. There was a slightly greater increase in the WMD in HDL cholesterol at 12 months (0.06 mmol/l) favouring the LC/HP group ($p < 0.05$).

The WMD in triacylglycerol was -0.17 mmol/l at 6 months ($p = 0.0001$) favouring the low carbohydrate group. At 12 months the WMD between the groups was -0.19 mmol/l favouring the LC/HP group ($p = 0.04$).

The only significant difference in blood pressure was for systolic blood pressure at 12 months, where the WMD was 2.19 mmHg favouring the low carbohydrate group.

Except for total cholesterol and LDL cholesterol, these results tend to favour the use of low carbohydrate/high protein diets for decreasing CVD risk. However, it must be emphasized that the low fat groups in these trials were randomised, whereas this was not the case in the present trial. All CVD risk factors tended to improve at 12 months for those following the HE diet in the present trial, and there were significant improvements for TAG, HDL cholesterol, diastolic blood pressure and the ratio of total cholesterol vs. HDL cholesterol. These subjects were not randomised and responded extremely well in terms of weight loss, and CVD risk. This study suggests that certain subjects are suited to a HE approach, as demonstrated by the significant weight loss, in reduced CVD risk.
CHAPTER 6: ADIPOKINES
Another objective of the trial was to examine adipokine changes using different dietary approaches. It was hypothesised that those who remained on the HE diet at 3 months “suited” this type of dietary approach, and would therefore show decreases in adipokines levels, except for adiponectin which should increase. The same hypothesis applies for the LL and PSMF groups, who were not successful at losing weight using the standard HE approach.

Adipokine changes were examined at 3 months for all the subjects before randomisation, and then in the HE, LL and PSMF groups 3 and 9 months post randomisation. Analysis was done on an intention to treat basis.

All adipokines were tested for normality using the Kolmogorov-Smirnov Test on SPSS and were normally distributed.

### 6.1: Pre-randomization analysis

(i) Baseline data

Seven adipokines (Plasminogen-activator-inhibitor-1 (PAI-1), adiponectin, resistin, interleukin-6 (IL-6), leptin, monochemoattractant-protein-1 (MCP-1) and tumour-necrosis-factor-α (TNFα) were measured at baseline, 3, 6 and 12 months.

Baseline data is shown in Table 6.1. Leptin, resistin and IL-6 were not normally distributed, and therefore both the median and mean are reported for these. Only subjects who had samples taken at baseline and 3 months were analysed. Pearson correlations were calculated for weight at 0 months vs. adipokines at 0 months. There were no significant correlations found for weight at 0 months vs. adipokines at 0 months. There were no significant correlations found for weight at 0 months and any of the measured adipokines.

<table>
<thead>
<tr>
<th>Units</th>
<th>PAI-1</th>
<th>Adiponectin</th>
<th>Resistin</th>
<th>IL-6</th>
<th>Leptin</th>
<th>MCP-1</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>ng/ml</td>
<td>µg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
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<td>(20.4)</td>
<td>(126.7)</td>
<td>(2.7)</td>
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</tr>
<tr>
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<td>2.6</td>
<td>44.0</td>
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<td>-</td>
</tr>
</tbody>
</table>

* Values are mean (SD).
(ii) Three month data

Mean values for adipokines at 3 months are shown in Table 6.2. All adipokines were not normally distributed at 3 months, and therefore the median value is also shown for 3 months. There was a significant difference from baseline for IL-6 (-1.3 (SD 7.1) pg/ml, \( p = 0.025 \)), leptin (-2.3 (SD 16.9) ng/ml, \( p = 0.041 \)), MCP-1 (-53.3 (SD 112.5) pg/ml, \( p \leq 0.001 \)) and TNFα (-0.3 (SD 2.0) pg/ml, \( p = 0.029 \)). Adipokines at 3 months were log-transformed to analyse differences between baseline and 3 months.

<table>
<thead>
<tr>
<th>Units</th>
<th>PAI-1</th>
<th>Adiponectin</th>
<th>Resistin</th>
<th>IL-6</th>
<th>Leptin</th>
<th>MCP-1</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>ng/ml</td>
<td>μg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
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<td>pg/ml</td>
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<td>(114.6)</td>
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<td>μg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
<tr>
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<td>-1.3</td>
<td>-2.3</td>
<td>-53.3</td>
<td>-0.3</td>
</tr>
<tr>
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<td>(112.5)</td>
<td>(2.0)</td>
</tr>
<tr>
<td>Median</td>
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<td>μg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
<tr>
<td></td>
<td>44.1</td>
<td>18.5</td>
<td>16.9</td>
<td>2.7</td>
<td>40.4</td>
<td>221.2</td>
<td>4.5</td>
</tr>
<tr>
<td>( p )</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.025</td>
<td>0.041</td>
<td>( \leq 0.001 )</td>
<td>0.029</td>
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</tbody>
</table>

*Values are mean (SD). \( p < 0.05 \)

(iii) Comparisons of groups who lost > 5% and ≤ 5% weight at 3 months

Figure 6.1(a-d) compares mean changes in adipokine levels for the groups who lost > 5% and ≤ 5% weight. There was a significant difference between the groups for changes in leptin at 3 months (-11.2 (SD 26.2) ng/ml vs. -0.2 (SD 11.6) ng/ml, \( p = 0.016 \)), where the > 5% group showed a greater decrease from baseline.
Figure 6.1a: Changes in PAI-1, resistin, and leptin at 3 months for groups who lost > 5 % and ≤ 5 % weight.

Figure 6.1b: MCP-1 changes at 3 mths for groups who lost > 5 % and ≤ 5 % weight.

Figure 6.1c: Adiponectin changes at 3 mths for groups who lost > 5 % and ≤ 5 % weight.

Figure 6.1d: TNFα and IL-6 changes at 3 months for groups who lost >5% and ≤ 5 % weight.
6.2: Post-randomization analysis

(i) HE GROUP

An inverse correlation was found between weight and adiponectin \( r = -0.663, p = 0.003 \) at baseline, but there was no significant relationships found for any of the remaining adipokines and weight.

There were no correlations found for weight change and adipokine changes at 12 months in this group.

Figure 6.2 shows percent change from baseline to 12 months for the HE group. Adipokine levels at baseline, 3, 6 and 12 months are shown in Table 6.3.

At baseline, 3, 6, and 12 months, IL-6 was not normally distributed. Median values for IL-6 at these timepoints were as follows:

Baseline: 3.3 pg/ml
3 months: 2.8 pg/ml
6 months: 2.2 pg/ml
12 months: 2.3 pg/ml

At 12 months, there was a significant differences from baseline for PAI-1 (-16.7 (SD 22.5) ng/ml, \( p = 0.011 \)), and leptin (-12.7 (SD 16.4) ng/ml, \( p = 0.010 \)).

| Table 6.3: Adipokine levels at baseline, 3, 6 and 12 months for HE group. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | 0 mths (n = 17) | 3 mths (n = 17) | 6 mths (n = 17) | 12 mths (n = 17) |
| PAI-1 (ng/ml)                   | 53.1 (27.8)     | 41.5 (29.9)     | 40.0* (28.3)    | 37.0* (20.4)     |
| Leptin (ng/ml)                  | 33.1 (22.2)     | 26.6 (16.1)     | 32.5 (26.6)     | 21.8* (14.7)     |
| Adiponectin (µg/ml)             | 20.0 (16.8)     | 19.4 (14.4)     | 19.9 (13.3)     | 22.7 (17.8)      |
| Resistin (ng/ml)                | 18.5 (13.0)     | 17.5 (11.1)     | 18.0 (10.3)     | 19.8 (9.5)       |
| IL-6 (pg/ml)                    | 4.6 (4.7)       | 4.5 (6.2)       | 4.2 (6.6)       | 4.0 (6.6)        |
| TNFα (pg/ml)                    | 6.0 (3.8)       | 6.5 (3.4)       | 6.8 (4.3)       | 6.9 (3.4)        |
| MCP-1 (pg/ml)                   | 227.9 (116.0)   | 198.4 (114.7)   | 221.5 (114.6)   | 236.1 (141.7)    |

* significant difference from baseline. Data are reported as mean (SD). \( p < 0.05 \)
Analysis was also carried out for those from the HE group who completed the study. Data is shown in Table 6.4. There were significant differences at 12 months for PAI-1 (-29.5 (SD 17.1) ng/ml, $p = 0.018$) and IL-6 (-1.0 (SD 0.7) pg/ml, $p = 0.041$).

There were significant associations for weight change and PAI-1 change ($r = 0.826$) and weight change and resistin change ($r = 0.829$).
Table 6.4: Adipokine levels for those who completed the study at baseline, 3, 6 and 12 months for HE group.

<table>
<thead>
<tr>
<th></th>
<th>0 mths (n = 6)</th>
<th>3 mths (n = 6)</th>
<th>6 mths (n = 6)</th>
<th>12 mths (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>60.4 (26.1)</td>
<td>26.0 (11.5)</td>
<td>36.3 (21.5)</td>
<td>30.8* (16.0)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>19.8 (18.4)</td>
<td>36.0 (17.8)</td>
<td>33.0 (16.6)</td>
<td>14.7 (16.1)</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>30.3 (20.4)</td>
<td>26.5 (17.4)</td>
<td>26.7 (13.4)</td>
<td>34.2 (29.9)</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>19.9 (18.4)</td>
<td>14.5 (7.0)</td>
<td>16.6 (8.6)</td>
<td>16.9 (8.3)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.1 (0.9)</td>
<td>3.3 (1.5)</td>
<td>2.1 (1.1)</td>
<td>2.0* (0.9)</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>8.6 (4.0)</td>
<td>7.7 (2.1)</td>
<td>9.1 (4.6)</td>
<td>7.8 (3.2)</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>152.3 (67.1)</td>
<td>202.9 (82.7)</td>
<td>221.8 (108.5)</td>
<td>216.2 (116.8)</td>
</tr>
</tbody>
</table>

* significant difference from baseline. Data are reported as mean (SD). $p < 0.05$

(ii) LL vs. PSMF

There were no significant correlations found for adipokines and weight at baseline for both the LL group and the PSMF group.

Adipokine levels at baseline, 3, 6 and 12 months for the LL and PSMF groups are shown in Table 6.6. Similar to the HE group, IL-6 was not normally distributed at any timepoint throughout the trial in both the LL and PSMF groups. Therefore the median values is shown for all timepoints as follow:

Baseline: LL – 2.7 pg/ml; PSMF – 2.5 pg/ml

3 months: LL – 3.2 pg/ml; PSMF – 2.1 pg/ml

6 months: LL – 2.7 pg/ml; PSMF - 1.7 pg/ml

12 months: LL – 2.7 pg/ml; PSMF – 2.1 pg/ml

For the LL group, there was a significant improvements for PAI-1 (-24.1 (SD 32.3) ng/ml, $p \leq 0.001$) and leptin (-7.9 (SD 18.9) ng/ml, $p = 0.034$) at 6 months. At 12 months, significant differences were seen for PAI-1 (-22.1 (SD 34.6) ng/ml, $p = 0.002$), adiponectin (6.8 (SD 14.3) µg/ml, $p = 0.013$) and leptin (-9.1 (SD 18.9) ng/ml, $p = 0.016$).

For the PSMF group there was a significant decrease at 12 months for adiponectin (-3.8 (SD 9.9) µg/ml, $p = 0.042$), but no differences seen at 6 months for any adipokines in this group.

Figure 6.3 shows percent change in adipokines at 12 months for the LL and PSMF groups.
Table 6.5: Adipokine levels for LL and PSMF groups at 0, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>PAI-1 ng/ml</th>
<th>Adiponectin μg/ml</th>
<th>Resistin ng/ml</th>
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<th>Leptin ng/ml</th>
<th>MCP-1 pg/ml</th>
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<td>LL (n = 31)</td>
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<td>(6.4)</td>
<td>(26.0)</td>
<td>(122.9)</td>
<td>(2.2)</td>
</tr>
<tr>
<td>PSMF (n = 36)</td>
<td>52.3 NS</td>
<td>17.8* 0.046</td>
<td>17.6 NS</td>
<td>3.3 NS</td>
<td>37.6 NS</td>
<td>242.5 NS</td>
<td>6.2 NS</td>
</tr>
<tr>
<td></td>
<td>(49.5)</td>
<td>(13.5)</td>
<td>(12.3)</td>
<td>(4.6)</td>
<td>(18.2)</td>
<td>(100.1)</td>
<td>(2.3)</td>
</tr>
</tbody>
</table>

* significant difference from baseline. * significant difference from 3 months. P-value represents differences between LL and PSMF. Values are mean (SD); p < 0.05.
LL group.

Significant associations were found for weight change vs. PAI-1 change ($r = 0.584, p < 0.01$), adiponectin change ($r = -0.627, p < 0.01$), and leptin change ($r = 0.646, p < 0.01$) at 12 months.

PSMF group.

There were no significant associations found between weight change at 12 months and adipokine changes for the PSMF group.

Completer’s analysis was also carried out for the LL and PSMF groups.

The LL group showed significant changes for PAI-1 at both 6 (-42.0 (SD 31.1) ng/ml, $p \leq 0.001$) and 12 months (-39.0 (SD 37.3) ng/ml, $p = 0.002$), adiponectin at 12 months (+14.2 (SD 19.1) μg/ml, $p = 0.020$), and leptin at both 6 (-16.4 (SD 26.5) ng/ml, $p = 0.038$) and 12 months (-19.0 (SD 24.5) ng/ml, $p = 0.016$). The PSMF group did not show any significant changes. Results are shown in Table 6.6.
For the LL group, weight change at 12 months was significantly correlated with leptin change at 12 months ($r = 0.632$). For the PSMF group, there were no significant correlations found for weight change at 12 months, and adipokine changes at 12 months.
Table 6.6: Adipokine levels for LL and PSMF groups who completed the study at 0, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>PAI-1 ng/ml</th>
<th>Adiponectin μg/ml</th>
<th>Resistin ng/ml</th>
<th>IL-6 pg/ml</th>
<th>Leptin ng/ml</th>
<th>MCP-1 pg/ml</th>
<th>TNFα</th>
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<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 14)</td>
<td>48.9</td>
<td>23.8</td>
<td>19.2</td>
<td>6.2</td>
<td>37.8</td>
<td>262.4</td>
<td>4.3</td>
</tr>
<tr>
<td>(n = 22) PSMF</td>
<td>48.8</td>
<td>NS</td>
<td>23.6</td>
<td>NS</td>
<td>19.9</td>
<td>47.4</td>
<td>319.4 NS</td>
</tr>
<tr>
<td></td>
<td>(34.5)</td>
<td>(14.4)</td>
<td>(17.0)</td>
<td>(2.8)</td>
<td>(22.2)</td>
<td>(140.4)</td>
<td>(1.7) NS</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 14)</td>
<td>59.5</td>
<td>19.3</td>
<td>21.0</td>
<td>4.3</td>
<td>41.8</td>
<td>249.3</td>
<td>4.2</td>
</tr>
<tr>
<td>(n = 22) PSMF</td>
<td>60.1</td>
<td>NS</td>
<td>24.6</td>
<td>NS</td>
<td>16.7</td>
<td>46.0</td>
<td>219.0 NS</td>
</tr>
<tr>
<td></td>
<td>(51.7)</td>
<td>(14.9)</td>
<td>(13.4)</td>
<td>(2.2)</td>
<td>(19.2)</td>
<td>(109.3)</td>
<td>(2.0) NS</td>
</tr>
<tr>
<td><strong>6 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 14)</td>
<td>17.5*</td>
<td>22.4</td>
<td>16.2</td>
<td>4.2</td>
<td>26.1*</td>
<td>236.6</td>
<td>4.0</td>
</tr>
<tr>
<td>(n = 22) PSMF</td>
<td>46.7</td>
<td>0.007</td>
<td>21.8</td>
<td>NS</td>
<td>16.1</td>
<td>43.2</td>
<td>224.2 NS</td>
</tr>
<tr>
<td></td>
<td>(43.0)</td>
<td>(13.3)</td>
<td>(10.6)</td>
<td>(3.3)</td>
<td>(20.5)</td>
<td>(86.3)</td>
<td>(2.5) NS</td>
</tr>
<tr>
<td><strong>12 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL (n = 14)</td>
<td>20.4*</td>
<td>31.8*</td>
<td>13.8</td>
<td>4.1</td>
<td>24.7*</td>
<td>280.4</td>
<td>4.5</td>
</tr>
<tr>
<td>(n = 22) PSMF</td>
<td>49.3</td>
<td>0.029</td>
<td>19.7</td>
<td>0.029</td>
<td>16.0</td>
<td>38.3</td>
<td>233.2 NS</td>
</tr>
<tr>
<td></td>
<td>(44.5)</td>
<td>(14.0)</td>
<td>(17.7)</td>
<td>(1.7)</td>
<td>(18.7)</td>
<td>(79.9)</td>
<td>(2.1) NS</td>
</tr>
</tbody>
</table>

* significant difference from baseline. * significant difference from 3 months. P-value represents differences between LL and PSMF. Values are mean (SD); p < 0.06.
6.3: DISCUSSION

Little work has been done on the changes in adipokine levels on different dietary approaches for the treatment of obesity, in particular those with a BMI ≥ 35 kg/m². This study aimed to examine whether the adipokines respond differently according to the specific diet, in this case, a HE diet, a very low calorie diet, and a PSMF.

Many studies have shown positive changes in these adipokines after weight loss. This study takes it a step further, and investigates whether diet composition can influence these changes. These particular adipokines were chosen as they are the adipokines most commonly associated with obesity and its co-morbidities, and changes in their levels after weight loss and different dietary composition may give us more insight into how they operate in the obese human body.

**Plasminogen-activated inhibitor-1 (PAI-1)**

PAI-1 is an adipokine which plays an important role in the haemostatic system. It is produced by many types of cells, including adipocytes, and it is increased in obesity (Mutch et al., 2001; Correia and Haynes, 2006).

Obese subjects suffer from many atherothrombotic disorders, and PAI-1 plays a key role in this, acting as an anti-fibrinolytic factor. Impaired fibrinolysis has long been associated with obesity and its link to CVD and atherosclerosis (Fearnley et al. 1963; Bennett et al., 1966).

The average value for concentration of PAI-1 in healthy non-obese humans is approximately 20 ng/ml (Giltay et al., 1998). In the present study population, the mean PAI-1 concentration at baseline was 55 (SD 39.5) ng/ml. Furthermore, BMI and bodyfat % were correlated with levels of PAI-1, suggesting that obesity is involved in increasing levels of PAI-1. This is
similar to findings in a previous study investigating the role of PAI-1 in obesity. Both visceral and subcutaneous PAI-1 mRNA in adipose tissue were positively associated with BMI (Alessi et al., 2000). In support of this, in a study by Skurk and Hauner, (2004), plasma levels of PAI-1 activity were also related to BMI.

Weight loss has been found to reduce the concentration of PAI-1 (Sundell et al., 1989; Folsom et al., 1993; Marckmann et al., 1998), indicating the influence of adipose tissue on the production of this protein. In the present study, the HE group showed a consistent decrease in PAI-1 with weight loss throughout the trial, and at both 6 and 12 months, mean PAI-1 concentration showed a significant decrease from baseline. This was also the case for the HE group completers, who showed a significant decrease in PAI-1 at 12 months. Similar to previous studies, this decrease in PAI-1 was significantly associated with weight loss. A similar pattern was seen in the LL group. Mean concentration of PAI-1 decreased significantly at both 6 and 12 months. Previous studies have supported the use of VLCDs for weight loss and subsequent decreases in PAI-1 levels (Folsom et al., 1993; Svendsen et al., 1996; Bastard et al., 2000).

Unsurprisingly, mean concentration of PAI-1 did not decrease significantly in the PSMF groups at any timepoint, though there was a trend for a decrease at 12 months, indicating that even a small weight loss may positively influence PAI-1 levels.

Haemostatic abnormalities are also associated with MS. PAI-1 is elevated in MS, and is associated with increased insulin, triacylglycerols and as already mentioned, BMI (Juhan-Vague et al., 1996). This was further supported in the present study, where PAI-1 at baseline was positively correlated with waist circumference and insulin, and inversely correlated with
HDL-cholesterol, variables which are associated with MS. Many other studies have shown an association between PAI-1 and components of MS (Vague et al., 1986; Juhan-Vague et al., 1989; Juhan-Vague et al., 1991; Kohler and Grant, 2000). When comparing those with MS in the present study to those without MS, mean PAI-1 tended to be higher in MS group, though this did not reach significance. This may be explained by the fact that both groups were severely obese at baseline, and PAI-1 levels were exceptionally high in both groups.

Those following the HE diet with MS achieved significant decreases in weight, BMI, waist circumference, TAG, and diastolic blood pressure, and a significant increase in HDL cholesterol, all related to MS. PAI-1 also tended to decrease in these subjects, though this did not reach significance. This decrease in PAI-1 was significantly correlated with weight change in this group at 12 months.

The LL group also showed significant improvements in waist circumference, and HDL cholesterol, both components of MS. PAI-1 showed a significant decrease in this group at 12 months, and this was strongly associated with a significant increase in HDL cholesterol, and a decrease in triacylglycerols, both associated with MS.

The group with MS following the PSMF did not show any significant decreases in components of MS, and hence, as expected, there was no significant decrease in PAI-1 levels at 12 months.

The results from the present study support the role of PAI-1 in the pathogenesis of MS, and shows that weight loss using both a HE and the LL approach can also lead to a decrease in the mean concentration of this adipokine.
Adiponectin

Adiponectin was discovered by four independent groups using different approaches (Scherer et al., 1995; Maeda et al., 1996; Hu et al., 1996). It is the most abundant plasma adipokine in humans (Fang and Sweeney, 2006), accounting for 0.01% total plasma protein (Arita et al., 1999).

Adiponectin expression is specific to adipose tissue (Arita et al., 1999), however numerous human studies have shown that plasma adiponectin is reduced in obese and diabetic subjects and shows an inverse association with insulin resistance (Hotta et al., 2000). In the present study population, mean adiponectin concentration 20.6 (SD 13.6) μg/ml, and this did not change significantly at 3 months, even for those who lost 5 % of their baseline weight. On the other hand, there was a trend for adiponectin to be inversely correlated with weight and BMI, and there was a significant negative correlation between adiponectin and waist circumference (abdominal/visceral obesity) at baseline. As insulin resistance is associated with waist circumference, this suggests a possible indirect role for adiponectin and insulin resistance and MS, which are associated with an increased abdominal obesity.

When analysing the 3 dietary approaches, the HE group showed a small insignificant increase in adiponectin, whereas the LL group had a significant increase in adiponectin at 12 months. On the other hand, the PSMF group showed a significant decrease in adiponectin at 12 months. Changes in weight and bodyfat % were inversely associated with adiponectin changes at 12 months in the LL group. The increase in adiponectin, particularly in the LL group supports previous studies where adiponectin increased after weight loss (Hotta et al., 2000; Yang et al., 2001; Stefan and Stumvoll, 2002; Faraj et al., 2003; Esposito et al., 2003).
Previous studies have shown that adiponectin is inversely correlated with insulin resistance (Arita et al., 1999; Yatagai et al., 2003; Ryo et al., 2004; Yamamoto et al., 2004). This is supported in the present study, where there was a strong inverse correlation at baseline between adiponectin and fasting insulin concentration. In addition to this, the increase in adiponectin in the LL group at 12 months was associated with a decrease in fasting plasma glucose, and HbA1c which are also associated with T2D.

Finally, it has already been mentioned that adiponectin was strongly associated with waist circumference at baseline. Previous studies have shown that hypoadiponectinemia is associated independently with MS, more strongly than other inflammatory markers (Matsushita et al., 2006). Decreased plasma adiponectin has also been observed in CVD and hypertension. Indeed, this was supported in the present study, where baseline adiponectin levels were strongly correlated with mean HDL cholesterol and as already mentioned, inversely to fasting insulin and waist circumference. Also, adiponectin was significantly lower in the group with MS at baseline compared to the group without MS, and this remained at 3 months. The group with MS in this group showed a significant increase in adiponectin at 12 months, supporting the use of VLCDs such as LL as an approach for the treatment of hypoadiponectinemia.

Resistin

Resistin was discovered by three different groups in 2001 (Steppan et al., 2001; Kim et al., 2001), and is expressed only in adipose tissue (Fehmann and Heyn, 2002).
Early rodent studies showed an elevated level of resistin in obesity and insulin resistance, and it was down-regulated after the use of the insulin sensitizer, rosiglitazone. Immunoneutralization of resistin showed a decrease in hyperglycaemia and an increased insulin sensitivity (Steppan et al., 2001). These studies raised the hypothesis that resistin could be a potential link between obesity, insulin resistance and T2D.

Silha et al (2003) were the first to examine resistin levels in normal men and women. Mean plasma concentration in the normal population was 21.5 µg/l, and the obese population (mean BMI 33 kg/m$^2$) did not differ significantly, with a mean concentration of 28.8 µg/l (Silha et al., 2003). In the present study, mean concentration of plasma resistin at baseline was 20.7 (SD 16.5) ng/ml (median concentration 16.6 ng/ml), which is lower than the normal population in the study by Silha et al (2003).

No significant correlations were found between resistin and weight or BMI in the present study. Also, the group who lost 5 % baseline body weight did not show any greater decrease in resistin at 3 months compared to the group who did not achieve this weight loss target.

Those who completed the HE diet showed a trend for a decrease in mean concentration of resistin at 12 months, and although not reaching significance, the change in resistin correlated with weight change. Neither the LL nor the PSMF group showed significant decreases for resistin at 12 months, despite the large weight loss seen in the LL group.

There was no evidence from the present study to support a strong link between resistin levels and weight, BMI, components of MS including insulin resistance. Yet out of all the adipokines examined in this study, the literature has shown resistin to be the most controversial. Though there is a great deal of evidence demonstrating a link between resistin,
weight, insulin resistance and T2D, there is also mounting evidence to oppose this. These studies found a decreased level of resistin with increased adiposity, and not only was resistin downregulated in obesity, but it goes against the idea that resistin is the link between obesity and T2D. There have also been studies showing increases in resistin levels with weight loss, contradicting the idea that weight loss is associated with a reduced level of resistin (Nagaev and Smith, 2001; Fain et al., 2003; Lee et al., 2003). Further human studies need to be carried out to confirm whether there is a relationship between resistin and obesity, including insulin resistance and T2D.

**Interleukin 6 (IL-6)**

Interleukin 6 is produced by many different types of cells, including those of adipose tissue. It is increased in obesity (Fried et al., 1998; Bastard et al., 2002). IL-6 has many functions, with its main role being the induction of hepatic C-reactive protein (CRP) production. CRP is known to be a major risk factor for CVD (Rexrode et al., 2003).

IL-6 is thought to play a role in obesity, coronary heart disease and inflammation (Yudkin and Kumari, 2000). There is an increased release of the cytokine from visceral compared to subcutaneous fat, and this could to some extent explain the relationship between cardiovascular complications and abdominal obesity in humans (Bastard et al., 2006). Recent studies have also suggested that IL-6 could be related to insulin resistance (Bastard et al., 2000; Bastard et al., 2002).

Kern et al. (2001) examined levels of IL-6 concentration in subjects in different BMI categories, from normal BMI to a morbidly obese BMI. Plasma concentration of IL-6 differed significantly between those in the normal BMI category (< 25) and those in both the
BMI 30-40 and BMI > 40 categories. There was also a significant difference between those in the overweight (BMI 25-30) category and the BMI > 40 category. In lean subjects (BMI < 25), plasma IL-6 was 0.73 pg/ml and increased about fourfold to 2.86 pg/ml in the morbidly obese subjects (BMI > 40). In the present study, plasma concentration was even higher than that reported in the morbidly obese subjects in the study by Kern et al (2001), with IL-6 showing a mean concentration of 5.5 (10.1) pg/ml (median concentration 2.6 pg/ml). This study supports previous findings that IL-6 is elevated in obese subjects.

The proposal that IL-6 is involved in insulin resistance came from studies showing an increased IL-6 concentration in both obese and T2D subjects (Kern et al., 2001). There were only 9 subjects with T2D analysed in the present study, so it is difficult to make conclusions. IL-6 did not show any significant difference for those who had T2D compared to those who did not, in each diet group. Mean concentrations of IL-6 in all groups were high.

IL-6 did not change significantly in any diet group in the present trial when measured using intention to treat analysis. However, the HE completers did show a significant improvement. Another study examined the changes in IL-6 after a VLCD (Bastard et al., 2000). Adipose tissue IL-6 decreased significantly after subjects consumed a VLCD for 3 weeks. Bruun et al. (2006) also reported a decrease in IL-6 after a 15 week lifestyle intervention, consisting of a low energy diet and increased exercise.

The results from the present trial suggest that those who were successful on the HE diet demonstrated decreased IL-6 levels. Though there were no significant improvements for the LL and PSMF groups, IL-6 tended to remain the same or decrease, which can be seen as positive as it did not increase on either diet.
Leptin

Leptin was discovered in 1994, by Freidman and colleagues, when the gene that was responsible for obesity in the \textit{ob/ob} mouse was positionally cloned (Zhang \textit{et al.}, 1994), and subsequently named leptin. It is expressed principally in adipose tissue (Havel, 2001).

Administration of leptin in rodents caused a decrease in energy intake and weight loss (Caro \textit{et al.}, 1996). Leptin also increases energy expenditure, as its administration causes weight loss, that cannot be explained by the reduction in food intake alone (Levin \textit{et al.}, 1996; Scarpace \textit{et al.}, 1997). Humans with defects in the leptin receptor (db/db) or mutations causing leptin deficiency are hyperphagic and severely obese. Leptin was shown to reduce hyperphagia and cause weight loss in leptin deficient subjects (Farooqi \textit{et al.}, 1999).

The findings that leptin levels are elevated in many obese individuals led to the idea that most obese subjects are resistant to the actions of leptin (Caro \textit{et al.}, 1996). This resistance to leptin may result from decreased leptin transport into the central nervous system (Caro \textit{et al.}, 1996) or from impaired signalling downstream of the leptin receptor (Bjorbaek \textit{et al.}, 1999; El-Haschimi \textit{et al.}, 2000).

In the present study, mean leptin levels at baseline were high (42.2 (SD 20.4) ng/ml; median concentration 44.0 ng.ml) compared to data reported in lean individuals from previous studies. In a study by Muscelli \textit{et al.} (2008), leptin levels were significantly higher in obese compared to lean individuals (26.6 vs.6.4 ng/ml). Leptin was also highly correlated body weight and % fat mass. This was supported in the present study where leptin was significantly associated with BMI, body fat %, and hip circumference. A significant inverse correlation was also found between leptin and fat free mass. Various other studies have found a relationship between leptin and adipose tissue mass (Hamilton \textit{et al.}, 1995; Considine \textit{et al.}, 1996). There was no significant correlation found between leptin and waist circumference in
the present study. This suggests that there may be a greater release of leptin from subcutaneous adipose tissue compared to visceral adipose tissue. Studies have shown that there are regional variations in leptin gene expression (Montague et al., 1997). In addition to this, a study by Van Harmelen et al. (1998) also found a higher secretion of leptin from subcutaneous adipose tissue compared to visceral adipose tissue.

The present study also found a positive correlation between mean leptin levels and fasting insulin, insulin resistance and also the inflammatory cytokine, TNFα. Silha et al. (2003) also found a strong correlation between leptin and insulin resistance and this remained significant after controlling for BMI. Previous reports which found a relationship between leptin and insulin resistance have concluded that it is due to the increased fat mass (Cnop et al., 2002) and the present study supports this conclusion. However, other studies on human subjects and animal models have demonstrated that the association between leptin and insulin resistance is independent of fat mass (Fischer et al., 2002; Appleton et al., 2002). In the present study, when adjusted for BMI and fat mass, the relationship between leptin and insulin resistance did not remain significant, supporting the idea that this relationship is due to the fat mass.

With regards to the relationship found between leptin and TNFα, this was reported in a previous study by Kirchgessner et al (1997), where TNFα was found to influence the production of leptin in mice and cultured adipocytes.

Mean leptin levels decreased significantly at 3 months in the present study, and the group who lost > 5% of their baseline weight showed a significantly greater decrease in leptin compared to those who did not lose > 5%. The decrease in leptin was positively correlated with the changes in weight and BMI at 3 months. These results show how strongly leptin levels are related to weight, and that a 5% weight loss can significantly decrease leptin levels in an obese population.
At 12 months, all diet groups showed a trend for a decrease in leptin, with the LL and HE groups showing significant decreases in mean leptin levels. The decrease in leptin in the LL group was strongly correlated with the decrease in weight, body fat %, waist circumference, and also fasting insulin change at 12 months. The influence of weight reduction on leptin levels was also seen in a study by Arvidsson et al. (2004). Two diets were compared in their study, as mentioned previously. One diet had moderate fat/ carbohydrate content and the other diet had low fat/high carbohydrate content. Both diets have similar weight loss, and similar effects on leptin. The evidence from the present study agrees with the study by Arvidsson et al. (2004), where diet composition did not seem to influence leptin changes. The present study supports that idea that weight loss is the main contributing factor, and not macronutrient composition, in the decrease in leptin levels in an obese population.

**Tumour necrosis Factor α (TNFα)**

TNFα is a cytokine which, in addition to its role in the immune response (Hotamisligil et al., 1997), is involved in energy metabolism, in particular lipid and glucose metabolism, and appetite (Kirchgessner et al., 1997).

TNFα is produced in adipose tissue and its expression is elevated in obesity. The increased expression of TNFα in adipose tissue is thought to contribute to insulin resistance. Neutralization of TNFα in obese and insulin resistant rodents results in an increase in insulin sensitivity (Hotamisligil et al., 1994).

In the present study, mean concentration of TNFα did not significantly correlate with any of the anthropometric factors measured, including weight and body fat %, or insulin resistance at baseline. These results were unexpected, as it is widely reported that TNFα is linked to insulin resistance and obesity (Zinman et al., 1999). However, the subjects in the present
study were already obese, and hence there were no normal subjects to use for comparison to examine the relationship between TNFα and anthropometric factors.

Dandona et al. (1998) showed a significant difference in the concentration of serum TNFα between obese and lean individuals (3.45 pg/ml vs. 0.72 pg/ml). The mean concentration of TNFα was 6.3 (SD 2.7) pg/ml in the present study, supporting the idea that TNFα is increased in obese subjects.

In addition to this, TNFα did not show any significant decreases in any of the diet groups at 12 months, despite the large weight loss seen in both the LL and HE groups. However, in the study mentioned previously by Arvidsson et al. (2004), circulating levels of TNFα did not show a significant difference after a mean weight loss of 7.5 % at 10 weeks. They concluded that adipose tissue only has a minor effect on the regulation of circulating TNFα levels and the present study shows evidence which supports this. TNFα seems to be produced and act locally in human fat tissue (Lofgren et al., 2000), and there is no in situ release from adipose tissue into the blood (Mohamed-Ali, 1997). Serum and subcutaneous adipose tissue levels of IL-6 decreased significantly after a reduction of 2.1 kg/m², after 3 weeks of a VLCD, whereas TNFα did not change in serum or adipose tissue (Bastard et al., 2000), similar to findings in the present study.

A number of studies have observed no changes in adiponectin, IL-6, or TNF-α after significant reductions in weight (5–9 kg) and insulin in dietary and exercise interventions (Dvorakova-Lorenzova et al, 2006; Xydakis et al, 2004).
Monochemoattractant Protein-1 (MCP-1)

MCP-1 is a relatively new protein to be added to the list of adipokines. It is a member of the chemokine family, and is increased in obese individuals (Sartipy and Loskutoff, 2003; Weisberg et al., 2003; Xu et al., 2003; Takahashi et al., 2003). It plays a role in the recruitment of monocytes and memory T lymphocytes into tissues, and is involved in the pathogenesis of atherosclerosis (Baggiolini, 1998; Christiansen et al., 2004). MCP-1 is also involved in insulin resistance (Sartipy and Loskutoff, 2003), as well as lipid and carbohydrate metabolism.

MCP-1 showed no significant correlations with anthropometric factors, and components of MS, including insulin resistance at baseline.

Results from the present study were disappointing as MCP-1 did not show any significant changes in either diet group throughout the trial. In contrast to this, Christiansen et al. (2005) showed a decrease in MCP-1 after weight loss (Christiansen et al., 2005).
CHAPTER 7: TYPE 2 DIABETES AND
METABOLIC SYNDROME
Another objective of the trial was to relate the phenotype at presentation with the appropriate response to the different treatment modalities. It was hypothesised that different phenotypes would “suit” alternative dietary approaches, and not always the standard HE approach. The phenotype analysed were those with metabolic syndrome and T2D. Only 13 subjects had T2D so are not reported as the sample size was insignificant. There were 54 subjects with MS at baseline and results for this phenotype are reported in this chapter. Analysis was done on an intention to treat basis. The groups were tested for normality using the Kolmogorov-Smirnov Test on SPSS. All data was normally distributed. The following chapter shows weight and body composition, CVD risk factor, and adipokine levels at baseline, and changes at 3, 6 and 12 months for the groups with MS, and the groups without MS for comparison.

At baseline 13 subjects (15%) were diagnosed with type 2 diabetes, using the oral glucose tolerance test (OGTT) results at 120 min. 12 of these subjects had not been previously diagnosed. The OGTT was carried out on 87/120 subjects. There were only 9 subjects with T2D remaining in the trial at 3 months, and therefore results are not shown in detail. There were only 3 subjects on each diet.

54 subjects (62%) were defined as having metabolic syndrome at baseline, as identified using the NCEP ATP III criteria (Chapter 2).

7.1 Pre-randomization analysis

(i) Weight and body composition
Weight and body composition at baseline and 3 months for the groups with and without MS are shown in Table 7.1.

At 3 months the MS group had a significantly greater decrease in weight (-3.4 (SD 4.8) kg vs. -1.4 (SD 3.1) kg, \( p = 0.009 \)), BMI (-1.3 (SD 2.1) kg/m\(^2\) vs. -0.4 (SD 1.5) kg/m\(^2\), \( p = 0.004 \)) and WC (-3.7 (SD 7.8) cm vs. -0.3 (SD 7.4) cm, \( p = 0.017 \)) than the group without MS.
Table 7.1: Weight and body composition for groups with and without MS at baseline and 3 months

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>With MS (n = 54)</td>
<td>127.9 (20.3)</td>
<td>47.2 (5.0)</td>
<td>49.1 (5.9)</td>
<td>64.4 (12.4)</td>
<td>133.9 (15.8)</td>
<td>143.4 (14.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Without MS (n = 66)</td>
<td>112.3 (17.1)</td>
<td>41.9 (5.5)</td>
<td>47.4 (5.0)</td>
<td>NS</td>
<td>121.4 (11.3)</td>
<td>135.9 (12.5)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS (n = 54)</td>
<td>124.4* (20.5)</td>
<td>45.8* (7.4)</td>
<td>48.6 (7.5)</td>
<td>63.1* (12.1)</td>
<td>130.2* (14.8)</td>
<td>141.9* (14.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>Without MS (n = 66)</td>
<td>110.9* (17.4)</td>
<td>41.5* (5.8)</td>
<td>47.2 (5.3)</td>
<td>NS</td>
<td>121.1 (10.8)</td>
<td>133.4* (10.5)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* significant difference from baseline. $p < 0.05$. P-value represents differences between groups with and without MS. Values are mean (SD).
(iii) CVD risk factors

CVD risk factors at baseline for the groups with and without MS are shown in Table 7.2. At 3 months the MS group had a significantly greater decrease in diastolic blood pressure (-2.2 (SD 7.7) mmHg vs. 1.6 (SD 7.9) mmHg, $p = 0.009$).
Table 7.2: CVD risk for groups with and without MS at baseline and 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Total chol (mmol/l)</th>
<th>LDL chol (mmol/l)</th>
<th>HDL chol (mmol/l)</th>
<th>TAG (mmol/l)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>TC/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS (n = 54)</td>
<td>5.0 (1.0)</td>
<td>3.1 (3.1)</td>
<td>1.1 (0.2)</td>
<td>1.9</td>
<td>140.7</td>
<td>92.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Without MS (n = 66)</td>
<td>5.2 (0.9)</td>
<td>NS</td>
<td>1.3 (0.2)</td>
<td>1.2</td>
<td>124.5</td>
<td>81.2</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS (n = 54)</td>
<td>5.1 (1.0)</td>
<td>3.0 (0.7)</td>
<td>1.2* (1.2)</td>
<td>1.7</td>
<td>139.0</td>
<td>89.8*</td>
<td>4.2*</td>
</tr>
<tr>
<td>Without MS (n = 66)</td>
<td>5.2 (1.0)</td>
<td>NS</td>
<td>1.4 (0.6)</td>
<td>1.3</td>
<td>127.0</td>
<td>82.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* significant difference from baseline. P-value represent differences between groups with and without MS. Values are mean (SD); $p < 0.05$. 
(iii) Adipokines

Table 7.3 shows adipokine levels for the groups with and without MS at baseline and 3 months. For the group without MS, all adipokines except MCP-1 and TNFα were not normally distributed at baseline, and there the median is shown in addition to the mean. For the MS group, IL-6 was not normally distributed at baseline and the median is also shown. For differences between groups, the data was log-transformed and t-tests were performed.

At 3 months, for the non-MS group, only leptin and MCP-1 were normally distributed. Therefore, the median is also shown for the remaining adipokines. For the MS group PAI-1, resistin, IL-6 and TNFα were not normally distributed and were therefore log-transformed.

There was a significant difference between the groups for adiponectin at 3 months (MS group 17.2 (SD 9.8) μg/ml vs. non MS group 23.8 (SD 13.5) μg/ml, $p = 0.016$).
Table 7.3: Adipokine levels for groups with and without MS at baseline and 3 months.

<table>
<thead>
<tr>
<th></th>
<th>PAI-1 ng/ml</th>
<th>Adiponectin μg/ml</th>
<th>Resistin ng/ml</th>
<th>IL-6 pg/ml</th>
<th>Leptin ng/ml</th>
<th>MCP-1 pg/ml</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS (n = 40)</td>
<td>61.9 (44.9)</td>
<td>17.8 (10.6)</td>
<td>20.1 (15.0)</td>
<td>4.5 (4.2)</td>
<td>39.7 (20.9)</td>
<td>298.9 (127.2)</td>
<td>5.5</td>
</tr>
<tr>
<td>Median Without MS (n = 44)</td>
<td>48.2 (32.5)</td>
<td>NS (15.4)</td>
<td>21.3 (18.0)</td>
<td>7.3 (13.0)</td>
<td>44.4 (20.1)</td>
<td>273.1 (127.4)</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS (n = 41)</td>
<td>57.1 (54.8)</td>
<td>17.2 (9.8)</td>
<td>17.6 (12.8)</td>
<td>4.0 (4.6)</td>
<td>38.4 (23.6)</td>
<td>232.4 (102.1)</td>
<td>5.2</td>
</tr>
<tr>
<td>Median Without MS (n = 44)</td>
<td>54.3 (40.8)</td>
<td>NS (13.5)</td>
<td>23.8 (14.2)</td>
<td>4.4 (5.8)</td>
<td>41.9 (17.2)</td>
<td>227.7 (125.3)</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* significant difference from 3 months. P-value represents differences between groups with and without MS. Values are mean (SD); p < 0.05.
(iv) Insulin resistance, HbA1c and FPG

Insulin resistance, HbA1c and FPG at baseline and 3 months are shown for the groups with and without MS in Table 7.4. The MS group had a higher level for FPG at both baseline and 3 months.

Table 7.4: Insulin resistance, HbA1c and FPG for groups with and without MS at baseline and 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOMA-IR</td>
<td>HbA1c</td>
</tr>
<tr>
<td>With MS</td>
<td>3.6 (2.3)</td>
<td>5.7 (0.4)</td>
</tr>
<tr>
<td>(n = 54)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Without MS</td>
<td>3.1 (2.1)</td>
<td>5.5 (0.3)</td>
</tr>
<tr>
<td>(n = 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With MS</td>
<td>3.7 (2.2)</td>
<td>5.7 (0.4)</td>
</tr>
<tr>
<td>(n = 54)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Without MS</td>
<td>3.3 (2.4)</td>
<td>5.5 (0.3)</td>
</tr>
<tr>
<td>(n = 66)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value represent differences between groups with and without T2D. Values are mean (SD). $p < 0.05$. 
7.2 Post-randomization analysis

(a) HE GROUP

(i) Weight and body composition

There were 12 subjects from the HE group diagnosed with MS at baseline. Table 7.5 shows weight and body composition for the HE group with and without MS at baseline, 3, 6 and 12 months.

A significant difference was found between the groups at 12 months for change in WC (-9.0 (9.0) cm vs. -20.8 (SD 11.1) cm, $p = 0.038$, where the group with MS showed a greater decrease in WC.
Table 7.5: Weight and body composition for HE groups with and without MS at baseline, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Bodyfat (%)</th>
<th>FFM (kg)</th>
<th>WC (cm)</th>
<th>HC (cm)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 12)</td>
<td>129.0</td>
<td>45.8</td>
<td>47.3</td>
<td>68.1</td>
<td>137.7</td>
<td>140.2</td>
<td>0.97</td>
</tr>
<tr>
<td>p = 23.1</td>
<td>7.7</td>
<td>7.1</td>
<td>17.3</td>
<td>18.1</td>
<td>13.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Non MS (n = 6)</td>
<td>105.1</td>
<td>38.8</td>
<td>44.0</td>
<td>59.0</td>
<td>117.4</td>
<td>125.9</td>
<td>0.9</td>
</tr>
<tr>
<td>0.047</td>
<td>0.035</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.040</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 12)</td>
<td>118.2*</td>
<td>41.5*</td>
<td>45.1*</td>
<td>65.0*</td>
<td>127.5*</td>
<td>134.0*</td>
<td>0.9</td>
</tr>
<tr>
<td>p = 21.8</td>
<td>7.4</td>
<td>7.6</td>
<td>17.5</td>
<td>12.8</td>
<td>14.3</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Non MS (n = 6)</td>
<td>97.6</td>
<td>NS</td>
<td>41.3</td>
<td>55.8</td>
<td>117.2</td>
<td>120.0</td>
<td>0.9</td>
</tr>
<tr>
<td>NS</td>
<td>4.6</td>
<td>2.6</td>
<td>14.3</td>
<td>10.3</td>
<td>5.9</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td><strong>6 mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 12)</td>
<td>115.1*</td>
<td>40.4*</td>
<td>44.9*</td>
<td>63.6</td>
<td>127.5*</td>
<td>131.6*</td>
<td>0.9</td>
</tr>
<tr>
<td>p = 22.7</td>
<td>7.0</td>
<td>8.4</td>
<td>17.7</td>
<td>14.1</td>
<td>12.7</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Non MS (n = 6)</td>
<td>95.2*</td>
<td>NS</td>
<td>40.3*</td>
<td>49.0</td>
<td>117.8</td>
<td>117.4*</td>
<td>0.9</td>
</tr>
<tr>
<td>NS</td>
<td>4.6</td>
<td>4.1</td>
<td>3.0</td>
<td>9.8</td>
<td>7.6</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td><strong>12mths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 12)</td>
<td>107.9*</td>
<td>37.7*</td>
<td>40.2*</td>
<td>63.0*</td>
<td>115.8*</td>
<td>127.7*</td>
<td>0.95</td>
</tr>
<tr>
<td>p = 15.5</td>
<td>8.8</td>
<td>11.4</td>
<td>15.5</td>
<td>17.9</td>
<td>17.8</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Non MS (n = 6)</td>
<td>88.8*</td>
<td>NS</td>
<td>38.5</td>
<td>54.5*</td>
<td>108.4</td>
<td>113.6*</td>
<td>0.91</td>
</tr>
<tr>
<td>NS</td>
<td>11.6</td>
<td>7.4</td>
<td>11.6</td>
<td>10.3</td>
<td>7.0</td>
<td>0.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

* significant difference from 3 months. P-value represents differences between groups. Values are mean (SD). p < 0.05
(ii) Cardiovascular disease risk factors

Table 7.6 shows CVD risk at baseline, 3, 6 and 12 months for the HE groups with and without MS.

No significant differences were found between the groups for changes in CVD risk at 6 and 12 months.

The MS group showed significant improvements at 12 months for HDL cholesterol (0.1(SD 0.1) mmol/l, \( p = 0.003 \)), TAG (-0.4 mmol/l (SD 0.7), \( p = 0.015 \)), DBP (-7.0 mmHg (SD 8.8), \( p = 0.004 \)) and TC/HDL cholesterol (-0.4 (SD 0.8), \( p = 0.018 \)).
Table 7.6: CVD risk for HE group with and without MS at baseline, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>Total chol (mmol/l)</th>
<th>LDL chol (mmol/l)</th>
<th>HDL chol (mmol/l)</th>
<th>TAG (mmol/l)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>TC/HDL</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
<tr>
<td>MS</td>
<td>5.4 (0.6)</td>
<td>3.3 (0.5)</td>
<td>1.1 (0.2)</td>
<td>2.1 (0.9)</td>
<td>142.4 (11.2)</td>
<td>90.7 (9.5)</td>
<td>4.6 (0.9)</td>
</tr>
<tr>
<td>(n = 12)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non MS</td>
<td>5.7 (0.9) NS</td>
<td>3.5 (0.8) NS</td>
<td>1.4 (0.3) NS</td>
<td>1.6 NS</td>
<td>124.6 (18.5)</td>
<td>80.6 NS</td>
<td>4.2 NS</td>
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<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3 mths</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>5.0* (0.6)</td>
<td>3.0* (0.6)</td>
<td>1.1 (0.2)</td>
<td>1.9</td>
<td>133.8 (18.4)</td>
<td>84.2* (8.5)</td>
<td>4.6</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non MS</td>
<td>5.5 (1.0) NS</td>
<td>3.5 (0.8) NS</td>
<td>1.3 (0.2) NS</td>
<td>1.3 NS</td>
<td>125.0 NS</td>
<td>79.4 NS</td>
<td>4.2 NS</td>
</tr>
<tr>
<td>(n = 6)</td>
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<td></td>
</tr>
<tr>
<td>6 mths</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>5.1 (0.4)</td>
<td>3.2 (0.5)</td>
<td>1.1 (0.2)</td>
<td>1.8</td>
<td>131.9* (13.2)</td>
<td>83.0* (8.5)</td>
<td>4.6</td>
</tr>
<tr>
<td>(n = 12)</td>
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<td></td>
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</tr>
<tr>
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<td>3.3 (1.07) NS</td>
<td>1.3* (0.2) NS</td>
<td>1.2 0.034</td>
<td>127.8 NS</td>
<td>80.3 NS</td>
<td>3.9 NS</td>
</tr>
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<td>(n = 6)</td>
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</tr>
<tr>
<td>12mths</td>
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</tr>
<tr>
<td>MS</td>
<td>5.2 (0.9)</td>
<td>3.3 (0.7)</td>
<td>1.2* (0.2)</td>
<td>1.6*</td>
<td>129.9 (17.3)</td>
<td>81.0* (9.1)</td>
<td>4.3*</td>
</tr>
<tr>
<td>(n = 12)</td>
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</tr>
<tr>
<td>Non MS</td>
<td>5.3* (1.0) NS</td>
<td>3.2 (1.0) NS</td>
<td>1.5 (0.4) NS</td>
<td>1.1 NS</td>
<td>125.8 NS</td>
<td>79.1 NS</td>
<td>3.6* NS</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

* significant difference from 3 months. P-value represent differences between groups with and without MS. Values are mean (SD). $p < 0.05$
(iii) Adipokines

Table 7.7 shows adipokines levels at baseline, 3, 6 and 12 months for the HE groups with and without MS.

At 6 months there was a significant difference between the groups for changes in adiponectin (MS 2.2 μg/ml (SD 7.7) vs. non-MS group -4.9 μg/ml (SD 3.9), $p = 0.050$).

There were no significant differences between the groups for changes in adipokines from baseline to 12 months.
Table 7.7: Adipokine levels for HE groups with and without MS at 0, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 mths</th>
<th>6 mths</th>
<th>12 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAI-1 ng/ml</td>
<td>Adiponectin μg/ml</td>
<td>Resistin ng/ml</td>
<td>IL-6 pg/ml</td>
</tr>
<tr>
<td>MS</td>
<td>51.6 (28.1)</td>
<td>13.0 (9.2)</td>
<td>17.2 (9.3)</td>
<td>4.3 (3.9)</td>
</tr>
<tr>
<td>Non MS</td>
<td>47.4 (31.7)</td>
<td>NS (20.6)</td>
<td>NS (18.6)</td>
<td>NS (7.3)</td>
</tr>
<tr>
<td><strong>Non MS</strong></td>
<td><strong>47.2 (31.7)</strong></td>
<td><strong>NS (18.7)</strong></td>
<td><strong>NS (17.9)</strong></td>
<td><strong>NS (7.3)</strong></td>
</tr>
<tr>
<td><strong>Non MS</strong></td>
<td><strong>43.2 (27.7)</strong></td>
<td><strong>NS (14.2)</strong></td>
<td><strong>NS (12.3)</strong></td>
<td><strong>NS (6.2)</strong></td>
</tr>
</tbody>
</table>

Values are mean (SD). * Sig diff from 3 months. P-values represent difference between groups. $p < 0.05$
(iv) **Insulin Resistance, HbA1c and FPG**

Table 7.8 shows insulin resistance, HbA1c and FPG levels for the HE group with and without MS. There were no significant differences in the changes between the groups at 6 or 12 months.

Table 7.8: Insulin resistance, HbA1c and FPG for HE groups with and without the MS at baseline, 3, 6 and 12 months.

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR</th>
<th>HbA1c (%)</th>
<th>FPG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 6)</td>
<td>3.6</td>
<td>5.7</td>
<td>5.5</td>
</tr>
<tr>
<td>(2.0)</td>
<td>(0.4)</td>
<td>(0.8)</td>
<td><strong>p =</strong></td>
</tr>
<tr>
<td>Non MS (n = 12)</td>
<td>2.4</td>
<td>NS</td>
<td>5.5</td>
</tr>
<tr>
<td>(2.1)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td></td>
</tr>
<tr>
<td><strong>3 mths</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 6)</td>
<td>0.97</td>
<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>(0.9)</td>
<td>(0.4)</td>
<td>(0.6)</td>
<td><strong>p =</strong></td>
</tr>
<tr>
<td>Non MS (n = 12)</td>
<td>2.8</td>
<td>0.009</td>
<td>5.3</td>
</tr>
<tr>
<td>(1.0)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td></td>
</tr>
<tr>
<td><strong>6 mths</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 6)</td>
<td>2.5</td>
<td>5.6</td>
<td>5.3</td>
</tr>
<tr>
<td>(1.3)</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td><strong>p =</strong></td>
</tr>
<tr>
<td>Non MS (n = 12)</td>
<td>1.6</td>
<td>NS</td>
<td>5.3</td>
</tr>
<tr>
<td>(0.8)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td></td>
</tr>
<tr>
<td><strong>12mths</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS (n = 6)</td>
<td>2.0</td>
<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>(1.6)</td>
<td>(0.4)</td>
<td>(0.9)</td>
<td></td>
</tr>
<tr>
<td>Non MS (n = 12)</td>
<td>2.2</td>
<td>NS</td>
<td>5.4</td>
</tr>
<tr>
<td>(0.7)</td>
<td>(0.3)</td>
<td>(0.5)</td>
<td></td>
</tr>
</tbody>
</table>

* significant difference from baseline. P-value represents differences between groups with and without MS. Values are mean (SD). 

$p < 0.05$
(b) LL vs. PSMF

(i) Weight and body composition

Table 7.9 shows weight and body composition at baseline, 3, 6 and 12 months for the LL group with and without MS at baseline, and the PSMF group with and without MS at baseline.

Significant differences were found at 6 months between the LL and PSMF groups with MS for changes in weight (LL -13.0 (SD 13.9) kg vs. PSMF -3.5 (SD 4.9) kg, p = 0.013) and BMI (LL -4.8 (SD 5.3) kg/m^2 vs. PSMF -1.3 (SD 1.8) kg/m^2, p = 0.031).

At 12 months was a significant difference in weight change at 12 months between the groups (LL -19.8 (SD 27.3) kg vs. PSMF -4.3 (SD 7.7) kg, p = 0.049).
Table 7.9: Weight and body composition for LL and PSMF groups with and without MS at 0, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>LighterLife With MS</th>
<th>LighterLife Without MS</th>
<th>PSMF With MS</th>
<th>PSMF Without MS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>15</td>
<td>19</td>
<td>17</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>131.0 (19.0)</td>
<td>117.9 (19.5)</td>
<td>117.8 (13.7)</td>
<td>110.2 (12.8)</td>
<td>0.028</td>
</tr>
<tr>
<td>3 mths</td>
<td>128.6(a) (18.9)</td>
<td>117.2 (18.2)</td>
<td>114.9 (14.2)</td>
<td>108.8 (13.6)</td>
<td>0.021</td>
</tr>
<tr>
<td>6 mths</td>
<td>115.6* (17.0)</td>
<td>107.1* (18.0)</td>
<td>111.4* (17.7)</td>
<td>107.5* (14.6)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>102.9* (22.2)</td>
<td>104.5* (17.8)</td>
<td>112.4 (18.0)</td>
<td>107.4 (14.8)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>49.9 (7.7)</td>
<td>43.2 (7.4)</td>
<td>43.0 (4.9)</td>
<td>41.6 (4.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>3 mths</td>
<td>49.2(a) (7.8)</td>
<td>43.4 (7.2)</td>
<td>42.3 (5.0)</td>
<td>41.0 (4.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>6 mths</td>
<td>44.3* (7.1)</td>
<td>39.4* (7.9)</td>
<td>40.9* (5.5)</td>
<td>40.2* (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>40.9* (11.3)</td>
<td>38.9* (7.0)</td>
<td>41.3 (7.0)</td>
<td>40.5 (4.8)</td>
<td>NS</td>
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<td><strong>Bodyfat (%)</strong></td>
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<tr>
<td>Baseline</td>
<td>51.8 (3.9)</td>
<td>45.1 (7.5)</td>
<td>47.0 (7.1)</td>
<td>49.7 (2.4)</td>
<td>0.014</td>
</tr>
<tr>
<td>3 mths</td>
<td>51.9 (4.0)</td>
<td>44.6 (7.5)</td>
<td>47.6 (7.8)</td>
<td>49.8 (2.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>6 mths</td>
<td>48.3* (7.1)</td>
<td>42.0* (8.6)</td>
<td>45.5* (7.7)</td>
<td>49.2(2.9)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>45.0* (10.8)</td>
<td>39.5* (11.9)</td>
<td>45.0 (8.8)</td>
<td>49.1* (2.5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>132.5 (18.0)</td>
<td>124.0 (11.8)</td>
<td>129.4 (10.4)</td>
<td>119.3 (8.9)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>131.0 (18.0)</td>
<td>122.1 (11.3)</td>
<td>125.5 (9.6)</td>
<td>120.1 (9.6)</td>
<td>NS</td>
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<tr>
<td>6 mths</td>
<td>125.4 (17.8)</td>
<td>113.5* (14.3)</td>
<td>122.5 (10.3)</td>
<td>117.2* (8.9)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>112.3* (18.4)</td>
<td>112.5* (13.4)</td>
<td>122.6 (12.5)</td>
<td>117.4* (8.8)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>HC (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>147.6 (10.8)</td>
<td>135.2 (11.5)</td>
<td>135.6 (11.6)</td>
<td>137.0 (8.8)</td>
<td>0.012</td>
</tr>
<tr>
<td>3 mths</td>
<td>147.6 (10.8)</td>
<td>135.2 (11.5)</td>
<td>134.7 (12.0)</td>
<td>134.1 (9.3)</td>
<td>0.008</td>
</tr>
<tr>
<td>6 mths</td>
<td>138.2* (13.3)</td>
<td>128.6* (13.6)</td>
<td>131.6* (12.2)</td>
<td>132.1* (8.7)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>135.6* (17.6)</td>
<td>127.4* (14.2)</td>
<td>132.5 (14.0)</td>
<td>132.8 (8.9)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.08)</td>
<td>0.9 (0.07)</td>
<td>0.8 (0.07)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>0.8 (0.1)</td>
<td>0.9 (0.08)</td>
<td>0.9 (0.07)</td>
<td>0.9 (0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>6 mths</td>
<td>0.90 (0.1)</td>
<td>0.88 (0.09)</td>
<td>0.9 (0.07)</td>
<td>0.8 (0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>0.8* (0.02)</td>
<td>0.8 (0.01)</td>
<td>0.9 (0.06)</td>
<td>0.8 (0.05)</td>
<td>0.026</td>
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<td><strong>FFM (kg)</strong></td>
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<tr>
<td>Baseline</td>
<td>62.7 (8.7)</td>
<td>64.8 (15.5)</td>
<td>61.7 (9.9)</td>
<td>55.2 (5.1)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>61.1 (7.5)</td>
<td>64.9 (15.0)</td>
<td>61.2 (10.5)</td>
<td>54.3 (4.8)</td>
<td>NS</td>
</tr>
<tr>
<td>6 mths</td>
<td>59.2 (8.4)</td>
<td>61.5* (13.1)</td>
<td>60.3 (11.6)</td>
<td>53.8* (4.8)</td>
<td>NS</td>
</tr>
<tr>
<td>12 mths</td>
<td>58.3 (7.3)</td>
<td>62.6* (14.0)</td>
<td>59.4* (5.4)</td>
<td>54.3* (5.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD). * significant difference from baseline.* Sig diff from 3 months. P-values represent difference between LL and PSMF with MS. p < 0.05
(ii) CVD risk factors

Table 7.10 shows CVD risk at baseline, 3, 6 and 12 months for the LL group with and without MS at baseline, and the PSMF group with and without MS at baseline.

At 6 months there were significant differences between the LL and PSMF groups with MS for changes in total cholesterol (LL 0.1 (SD 0.7) mmol/l vs. PSMF -0.5 (SD 0.9) mmol/l, \( p = 0.037 \)).

At 12 months, there were significant differences between the groups for changes in HDL cholesterol (LL 0.1 (SD 0.1) mmol/l vs. PSMF -0.03 (SD 0.1) mmol/l, \( p = 0.035 \)).
Table 7.10: CVD risk for LL and PSMF groups with and without MS at 0, 3, 6 and 12 months

<table>
<thead>
<tr>
<th>LighterLife</th>
<th>PSMF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With MS</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>15</td>
</tr>
<tr>
<td><strong>Total chol (mmol/l)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.8 (1.0)</td>
</tr>
<tr>
<td>3 mths</td>
<td>4.8 (0.9)</td>
</tr>
<tr>
<td>6 mths</td>
<td>5.0 (1.3)</td>
</tr>
<tr>
<td>12 mths</td>
<td>4.8 (1.0)</td>
</tr>
<tr>
<td><strong>LDL chol (mmol/l)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.0 (0.9)</td>
</tr>
<tr>
<td>3 mths</td>
<td>3.0 (0.7)</td>
</tr>
<tr>
<td>6 mths</td>
<td>2.9 (0.8)</td>
</tr>
<tr>
<td>12 mths</td>
<td>2.9 (0.8)</td>
</tr>
<tr>
<td><strong>HDL chol (mmol/l)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>3 mths</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>6 mths</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>12 mths</td>
<td>1.3* (0.2)</td>
</tr>
<tr>
<td><strong>TAG (mmol/l)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.9 (2.4)</td>
</tr>
<tr>
<td>3 mths</td>
<td>1.3 (0.9)</td>
</tr>
<tr>
<td>6 mths</td>
<td>1.3 (0.9)</td>
</tr>
<tr>
<td>12 mths</td>
<td>1.2 (1.0)</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>140.3 (13.9)</td>
</tr>
<tr>
<td>3 mths</td>
<td>138.0 (17.7)</td>
</tr>
<tr>
<td>6 mths</td>
<td>130.4* (17.6)</td>
</tr>
<tr>
<td>12 mths</td>
<td>130.3 (18.8)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>95.4 (11.0)</td>
</tr>
<tr>
<td>3 mths</td>
<td>88.9* (11.9)</td>
</tr>
<tr>
<td>6 mths</td>
<td>83.0 (11.6)</td>
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<tr>
<td>12 mths</td>
<td>85.0 (14.2)</td>
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<tr>
<td><strong>TC/HDL</strong></td>
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</tr>
<tr>
<td>Baseline</td>
<td>4.2 (0.9)</td>
</tr>
<tr>
<td>3 mths</td>
<td>4.0 (1.0)</td>
</tr>
<tr>
<td>6 mths</td>
<td>3.9 (0.9)</td>
</tr>
<tr>
<td>12 mths</td>
<td>3.8 (1.1)</td>
</tr>
</tbody>
</table>

Values are mean (SD). * significant difference from baseline. * Sig diff from 3 months. P-values represent difference between LL and PSMF with MS, *p < 0.05

(iii) Adipokines

Table 7.11 shows adipokine levels for LL and PSMF groups with and without MS at...
0, 3, 6 and 12 months. There was a significant difference between the LL group with MS compared to the PSMF group with MS for changes in adiponectin at 12 months (LL 11.3 μg/ml (SD 14.9) and PSMF -2.3 μg/ml (SD 13.2), $p = 0.013$).
Table 7.11: Adipokines for LL and PSMF groups with and without MS at 0, 3, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>LighterLife</th>
<th></th>
<th>PSMF</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With MS</td>
<td>Without MS</td>
<td>With MS</td>
<td>Without MS</td>
<td>With MS</td>
<td>Without MS</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>13</td>
<td>18</td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>PAI-1 (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>67.4 (42.8)</td>
<td>47.6 (28.1)</td>
<td>67.4 (58.7)</td>
<td>49.0 (37.5)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>44.1 (22.0)</td>
<td>62.4 (40.0)</td>
<td>79.6 (79.8)</td>
<td>48.3 (44.9)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>6 mths</td>
<td>24.0* (17.6)</td>
<td>35.3 (29.1)</td>
<td>65.4 (61.1)</td>
<td>40.9 (33.9)</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>12 mths</td>
<td>23.2* (17.3)</td>
<td>38.2 (30.8)</td>
<td>64.6 (65.0)</td>
<td>41.4 (31.8)</td>
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<tr>
<td><strong>Adiponectin (μg/ml)</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>20.0 (9.8)</td>
<td>22.1 (14.0)</td>
<td>20.1 (12.2)</td>
<td>23.4 (14.6)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>18.0 (9.5)</td>
<td>20.0 (11.0)</td>
<td>20.2 (11.6)</td>
<td>25.5 (13.5)</td>
<td>NS</td>
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</tr>
<tr>
<td>6 mths</td>
<td>19.6 (9.9)</td>
<td>21.4 (8.7)</td>
<td>17.5 (12.1)</td>
<td>22.6 (13.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>12 mths</td>
<td>28.7* (15.6)</td>
<td>22.1 (14.2)</td>
<td>15.1 (10.9)</td>
<td>21.5 (14.6)</td>
<td>0.006</td>
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<td><strong>Resistin (ng/ml)</strong></td>
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<tr>
<td>Baseline</td>
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<td>18.8 (13.9)</td>
<td>24.5 (9.9)</td>
<td>24.2 (18.7)</td>
<td>17.6 (17.2)</td>
<td>NS</td>
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<tr>
<td>3 mths</td>
<td>17.8 (10.0)</td>
<td>22.6 (12.6)</td>
<td>19.8 (11.2)</td>
<td>18.7 (14.9)</td>
<td>NS</td>
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</tr>
<tr>
<td>6 mths</td>
<td>17.0 (9.9)</td>
<td>18.7 (13.1)</td>
<td>18.7 (17.2)</td>
<td>17.7 (11.1)</td>
<td>NS</td>
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<tr>
<td>12 mths</td>
<td>18.0 (11.0)</td>
<td>17.9 (13.9)</td>
<td>20.1 (15.3)</td>
<td>15.3 (8.8)</td>
<td>NS</td>
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<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
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<tr>
<td>Baseline</td>
<td></td>
<td>7.0 (5.7)</td>
<td>8.7 (19.0)</td>
<td>3.4 (3.2)</td>
<td>4.7 (7.2)</td>
<td>NS</td>
</tr>
<tr>
<td>3 mths</td>
<td>4.9 (3.1)</td>
<td>4.4 (5.4)</td>
<td>2.6 (2.2)</td>
<td>4.6 (7.6)</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>6 mths</td>
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<td>4.3 (5.3)</td>
<td>2.3 (1.6)</td>
<td>4.7 (7.3)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
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<td>3.1 (2.6)</td>
<td>1.9 (0.8)</td>
<td>2.5 (2.0)</td>
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<tr>
<td><strong>Leptin (ng/ml)</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>50.0 (23.5)</td>
<td>40.0 (19.0)</td>
<td>37.9 (17.6)</td>
<td>50.8 (17.4)</td>
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</tr>
<tr>
<td>3 mths</td>
<td>51.6 (31.9)</td>
<td>42.1 (15.1)</td>
<td>37.0 (17.1)</td>
<td>48.0 (12.5)</td>
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<tr>
<td><strong>MCP-1 (pg/ml)</strong></td>
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<td></td>
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<td></td>
<td>325.0(117.1)</td>
<td>254.8(113.0)</td>
<td>323.4(137.9)</td>
<td>298.3(133.6)</td>
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<tr>
<td>3 mths</td>
<td>251.1*87.9)</td>
<td>223.8(124.8)</td>
<td>232.0*105.2</td>
<td>245.9(130.6)</td>
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<td></td>
</tr>
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<td>6 mths</td>
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<td>224.1(123.8)</td>
<td>231.8(100.5)</td>
<td>247.1(110.1)</td>
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<td>233.7(127.5)</td>
<td>244.9(113.2)</td>
<td>241.6(94.1)</td>
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<td><strong>TNFa (pg/ml)</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td>4.5 (2.5)</td>
<td>5.4 (1.8)</td>
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<td>NS</td>
</tr>
<tr>
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<td>4.3 (2.5)</td>
<td>4.9 (2.2)</td>
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<td></td>
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<tr>
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<td>5.3 (2.3)</td>
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<td></td>
</tr>
<tr>
<td>12 mths</td>
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<td>4.8 (2.3)</td>
<td>5.2 (1.9)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD). * significant difference from baseline. * Sig diff from 3 months. P-values represent difference between LL and PSMF with MS. p < 0.05
Table 7.12 shows values for insulin resistance, HbA1c and FPG for LL and PSMF group with and without MS. The LL group had a significant decrease in HbA1c at 6 months, compared to no significant change for the PSMF group.

Table 7.12: Insulin Resistin, HbA1c and FPG at baseline, 3, 6 and 12 months for LL and PSMF with and without MS.

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<th>LighterLife</th>
<th>PSMF</th>
</tr>
</thead>
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<tr>
<td></td>
<td>With MS</td>
<td>Without MS</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.5 (1.8)</td>
<td>3.1 (2.4)</td>
</tr>
<tr>
<td>3 mths</td>
<td>4.3 (3.1)</td>
<td>3.7 (2.9)</td>
</tr>
<tr>
<td>6 mths</td>
<td>4.1 (3.1)</td>
<td>2.7 (2.7)</td>
</tr>
<tr>
<td>12 mths</td>
<td>3.1 (2.8)</td>
<td>3.1 (2.6)</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.5 (0.5)</td>
<td>5.5 (0.3)</td>
</tr>
<tr>
<td>3 mths</td>
<td>5.6 (0.4)</td>
<td>5.5 (0.2)</td>
</tr>
<tr>
<td>6 mths</td>
<td>5.3 (0.3)</td>
<td>5.4 (0.3)</td>
</tr>
<tr>
<td>12 mths</td>
<td>5.3* (0.3)</td>
<td>5.5 (0.3)</td>
</tr>
<tr>
<td><strong>FPG (mmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.4 (0.7)</td>
<td>5.0 (0.4)</td>
</tr>
<tr>
<td>3 mths</td>
<td>5.2 (0.8)</td>
<td>5.1 (0.3)</td>
</tr>
<tr>
<td>6 mths</td>
<td>4.8 (0.20)</td>
<td>4.8 (0.6)</td>
</tr>
<tr>
<td>12 mths</td>
<td>4.7 (0.4)</td>
<td>5.0 (0.4)</td>
</tr>
</tbody>
</table>

* Significant difference from 3 months. Values are mean (SD). P-values represent difference between LL and PSMF with T2D. HOMA-IR = homeostatic model assessment for insulin resistance. $p < 0.05$
7.3: DISCUSSION

The prevalence of T2D in an obese population (BMI > 30 kg/m²) is approximately 20 % (Colditz et al., 1995). In the present study, there were 13 (15 %) subjects, out of 87 measured, diagnosed with T2D at baseline. Interestingly, 12 (13.8 %) of these subjects had not been previously diagnosed by their GP, despite being at high risk for development of the disease. Recent data from the Counterweight Study has shown that under diagnosis of T2D in obese populations is a major issue with prevalence between 9-14 %, similar to the findings in the present study. This data was taken from 23 GP practices in the UK, representing populations from all demographic areas (The Counterweight Project Team, 2001 and 2003). The evidence from the present study is of great concern, as both obesity and T2D greatly increase the risk of developing CVD. Screening for T2D in an obese patient should be carried out annually.

Chronic diseases such as diabetes, CVD and cancer represent a large proportion of human illness and combine to make up nearly 70 % of deaths in the US (Arias et al., 2003). One of the risk factors underlying these diseases is obesity. Lack of physical activity and unhealthy diets contribute to the global increase in the prevalence of obesity and MS (Esposito et al., 2006).

The ideal diet for the treatment of MS remains undecided, though there is an agreement that lifestyle changes, including dietary treatment, for weight loss should be the first line of approach (Grundy et al., 2005; Muzio et al., 2007). Recent studies evaluated treatments for MS include lifestyle interventions (Orchard et al., 2005; Azadbakht et al., 2005) and drug treatment studies (Orchard et al., 2005; Van Gaal et
al., 2005; Despres et al., 2005; Esposito et al., 2006). These were RCTs with a follow-up of 6 months or over, and none of the subjects had diabetes. The diets examined included the Mediterranean diet which is low in saturated fat, high in monounsaturated fat and dietary fibre, the Diabetes Prevention Program (DPP) which is low fat/calorie diet with exercise and behavioural advice and the DASH diet (Dietary Approach to Stop Hypertension) which is based on an eating plan rich in fruits and vegetables, and low-fat or non-fat dairy. The drugs examined included rimonabant, metformin, and rosiglitazone. The weighted mean resolution of MS was 24.4 % for the lifestyle interventions and 14.4 % for the drug therapies. However, metformin therapy alone did not show positive results, so after excluding the metformin study, the weighted mean resolution of MS was 24.4 % for lifestyle interventions and 27.9 % for the drug trials.

Though low-fat diets are considered by health experts to be the first line of approach for prevention of primary and secondary CVD (Parikh et al., 2005), the problem with this dietary approach is that it tends to promote unrestricted carbohydrate intake. This reduces HDL cholesterol and increases triacylglycerol concentration, both risk factors for MS (Grundy, 1999). Therefore, it is important to examine alternative dietary approaches for the treatment of MS, one of the objectives of the present study.

Fifty four subjects (45 %) were diagnosed with MS at baseline in the present study. Only 12 subjects responded to the HE diet at 3 months.

With regard to CVD risk factors, as expected the MS group had a higher mean serum concentration of triacylglycerol, fasting plasma glucose, and blood pressure compared to the non-MS group at baseline, and they also had a lower mean concentration of serum HDL.
cholesterol. These factors are all components of MS. At 3 months, the MS group showed a significantly greater decrease in diastolic blood pressure compared to those without MS. However there were no other significant improvements in CVD risk for either group at 3 months. It should be noted that diastolic blood pressure was significantly higher at baseline in the group with MS compared to the group without MS.

For weight and anthropometric factors, the 12 subjects who remained on the HE diet responded well to the diet at all stages of the trial. At 6 months, they showed significant improvements for weight, BMI, bodyfat, hip circumference and waist circumference and at 12 months they showed significant improvements for all anthropometric factors including weight, BMI, bodyfat, fat free mass, waist circumference, hip circumference and waist to hip ratio. For CVD risk, there were significant improvements at 6 months for blood pressure and at 12 months there were significant improvements for HDL cholesterol, triacylglycerol, diastolic blood pressure and TC/HDL cholesterol. There was also a trend for systolic blood pressure to improve at both 6 and 12 months. These factors are all components of MS, and were therefore particularly high at commencement of the trial, so it is interesting to see that at 12 months, blood pressure and triacylglycerol were below their target values according to the ATP III criteria. Similar to the present study, Muzio et al., (2005) showed a decrease in blood pressure and triacylglycerol, as well as an increase in HDL cholesterol using a healthy eating/low calorie diet.

However, in the present trial, there was a trend for total cholesterol and LDL cholesterol to increase between 3 and 12 months, though this was not significant. These results are in contrast to the group without MS who were following the HE diet, where total cholesterol decreased significantly and there was also a trend for a decrease in LDL cholesterol. As a
result of this, though the HE diet has been shown to be effective in terms of weight loss and CVD risk, total cholesterol and LDL cholesterol changes should be monitored, particularly in the long term. In contrast to this, in another study by Muzio et al. (2007), comparing a high carbohydrate/low fat diet to a low carbohydrate/high protein diet, both total cholesterol and LDL cholesterol showed improvements at 5 months on both diets.

The LL group with MS showed significant improvements in weight, BMI, bodyfat and hip circumference at 6 months, and at 12 months they showed significant improvements for weight, BMI, waist circumference, hip circumference and waist to hip ratio. Importantly, there was a significant decrease in bodyfat, which is important in terms of metabolic risk.

There was a trend for improvement in all CVD risk factors at both 6 and 12 months. However, despite the large weight loss, significant improvements were only seen for systolic blood pressure at 6 months, and HDL cholesterol at 12 months.

There are few studies examining the effects of a VLCD as a treatment strategy for MS. Due to the success of weight loss and improvement in CVD risk, the present study indicates that a very low calorie diet such as LL may be useful in the treatment of MS, for those who have not responded to a HE dietary approach.

Subjects with MS randomised to the PSMF showed significant decreases for weight, BMI, body fat and hip circumference at 6 months but did this not remain at 12 months. In regards to CVD risk, only total cholesterol decreased significantly at 6 months. However, there was a trend for all other CVD risk factors to improve at 6 and 12 months.
The study by Muzio et al. (2007) examined the effects of moderate variation in the macronutrient content of the diet on CVD risk in obese patients with MS. The first diet was reasonably high in carbohydrate (65 % energy) and low in fat, whereas the second diet was low in carbohydrate (48 % energy) and high in protein and monosaturated fat. At the end of the study all elements of MS (except HDL cholesterol which did not change) decreased on both diets after 5 months. The high carbohydrate diet also showed a significant decrease in LDL cholesterol. In conclusion, although both diets showed improvements in MS, the low carbohydrate diet had a greater decrease blood pressure and hypertriacylglycerolemia (Muzio et al. 2007), than the low fat diet.

The results from this study agree with previous recommendations that a low fat/high carbohydrate diet should be the first line or approach in the treatment of MS. However, for subjects in whom this treatment is not successful both a VLCD and a low carbohydrate diet may also be useful in the treatment of obese subjects with MS.

So far, the outcomes of those diagnosed with MS in the present trial have been discussed. However, there are strong arguments in the literature regarding the relevance of the MS as a clinical diagnosis (Kahn et al., 2005; Reaven, 2006). These arguments against the use of the syndrome are very convincing.

One strong point against the use of MS is that there is no conclusive evidence that the syndrome itself increase the risk of CVD above that of the existing individual CVD risk factors (Khunti and Davies, 2005; Farmer, 2006). The syndrome does predict T2D, but that is expected given the definitions involve abnormal glucose levels and/or insulin resistance. Reaven (2005) also makes the point that “it is not sure what led to the decision to select five criteria (why not four or six), and why satisfying any three of the five arbitrary criteria has
any clinical utility than two others”, based on the ATP III criteria. Interestingly, the
diagnostic criteria of the metabolic syndrome did not come from a prospective study, and do
not represent the outcome of any evidence-based process, but come from the best estimates of
a panel of experts (Reaven, 2005).

A group of factors can only be defined as a syndrome if it either predicts future events, or if it
identifies a unique pathological process. This is not clear in the case of metabolic syndrome,
and insulin resistance as the underlying process is not certain. However, Grundy et al. (2005)
states that that MS is indeed a syndrome, but probably has more than one cause. Grundy et al.
also states that magnitude of the increased risk for CVD varies according to which
components of the MS the subject presents with, and also the other, non-MS components in
the particular person.

Doctors are recommended to treat CVD risk factors regardless of whether the patient is
diagnosed with MS or not (Reaven, 2006; Kahn et al, 2005). This is also in compliance with
the JBS 2 guidelines and UK guidelines for clinical care. The American Diabetes
Association, the European Association for the Study of Obesity and Reaven (2006) all argue
that the use of MS is not clinically useful. However, Khunti and Davies (2005) and new
SIGN guidelines (SIGN, 2007) suggest that it is still a useful measure to identify people at
high risk for developing CVD and who need primary prevention and risk monitoring.
CHAPTER 8: LIVER AND KIDNEY FUNCTION
There are concerns about the effects of low carbohydrate/high protein diets on liver and kidney function. Therefore, as part of the clinical trial, sodium (NA), potassium (K), urea, creatinine (Creat), albumin (ALB), alanine aminotransferase (AAT), alkaline phosphatase (ALKP), and gamma glutamyl transferase (GGT) were analysed in the LL and PSMF groups at baseline 3, 6 and 12 months.

Data was tested for normality using the Kolmogorov-Smirnov Test, and data was normally distributed. Analysis was carried out on an intention to treat basis.

9.1: Effects of LL and PSMF diets on liver and kidney function.

Data is shown for the LL and PSMF groups in Table 8.1.

At 6 months there was a significant difference between the groups for changes in urea (LL -0.8 (SD 1.1) mmol/l vs. PSMF 0.6 (SD 1.4) mmol/l, $p < 0.05$).

There were significant differences in changes at 12 months between the groups for urea (LL -0.06 (SD 1.4) mmol/l vs. PSMF 0.7 (SD 1.8) mmol/l, $p < 0.05$), and ALT (LL -9.2 (SD 19.5) U/L vs. PSMF -0.07 (SD 13.9) U/L, $p = 0.037$).
Table 8.1: Kidney and liver function at baseline, 3, 6 and 12 months for the LL and PSMF groups.

<table>
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<tr>
<th></th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
<th>Urea mmol/l</th>
<th>Creat umol/L</th>
<th>ALB g/L</th>
<th>ALT U/L</th>
<th>ALKP U/L</th>
<th>GGT U/L</th>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
<td>139.2</td>
<td>4.8</td>
<td>4.3</td>
<td>79.6</td>
<td>42.6</td>
<td>26.4</td>
<td>79.9</td>
<td>36.3</td>
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<tr>
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<td>(1.0)</td>
<td>(11.7)</td>
<td>(3.4)</td>
<td>(10.7)</td>
<td>(21.4)</td>
<td>(35.5)</td>
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<td>139.0 NS</td>
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<td>4.7 NS</td>
<td>79.8 NS</td>
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<td>(10.2)</td>
<td>(2.3)</td>
<td>(37.9)</td>
<td>(34.7)</td>
<td>(62.4)</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LL (n = 34)</td>
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<td>4.4</td>
<td>82.8</td>
<td>43.0</td>
<td>29.9</td>
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<td>(17.9)</td>
<td>(19.8)</td>
<td>(34.1)</td>
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<td>4.6 NS</td>
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<td>45.0 0.001</td>
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<td>(9.7)</td>
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<td>(22.5)</td>
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<td>3.6</td>
<td>79.5</td>
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<td>(1.4)</td>
<td>(11.0)</td>
<td>(2.4)</td>
<td>(20.1)</td>
<td>(26.7)</td>
<td>(52.2)</td>
</tr>
<tr>
<td><strong>12 mths</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>139.1</td>
<td>4.4</td>
<td>4.2</td>
<td>79.6*</td>
<td>42.8</td>
<td>23.0*</td>
<td>75.7*</td>
<td>24.3</td>
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<td>(1.0)</td>
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<td>(2.1)</td>
<td>(9.1)</td>
<td>(22.2)</td>
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<td>5.1 0.005</td>
<td>83.0 NS</td>
<td>45.5 0.015</td>
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<td>(11.4)</td>
<td>(5.8)</td>
<td>(27.9)</td>
<td>(27.2)</td>
<td>(53.6)</td>
</tr>
</tbody>
</table>

Values are mean (SD). * Sig diff from 3 months. P-values represent difference between LL and PSMF groups; p > 0.05.
8.2: DISCUSSION

It is interesting to note that the typical Palaeolithic diet contained 3 to 4 times more protein than the average American diet (O’ Keefe and Cordain, 2004). Anatomically modern humans have remained biologically unchanged at least in the last 50,000 years and it was not until around 10,000 years ago that the shift from a roaming hunter and gatherer to a stationary farmer started (Manninen, 2004).

Though there have been various studies elucidating the benefits of high protein diets for weight and fat loss, and improvements in insulin sensitivity and lipid profiles, there are still concerns. The American Heart Association published a statement in 2001 as follows “Individuals who follow these (high protein) diets are at risk for…potential cardiac, renal, bone, liver abnormalities overall” (St Jeor et al., 2001). Yet, there is little evidence supporting this statement (Manninen, 2004).

Both renal and liver function were examined in the present study for the LL and PSMF diets, as these are the diets which there are concerns about. Neither diet seemed to show any adverse effects on either renal or liver function. As expected, there was a trend for increased urea serum concentration on the PSMF, responding to the higher protein content of the diet. Creatinine decreased significantly on LL. This is not surprising, as creatinine tends to decrease on a very low calorie diet. This is because the body tends to react in a similar way to starvation, where there is also a decrease in creatinine levels. Benedict (1907) was the first to report the elimination of creatine in the urine of starving men. In addition to this, the main sources of creatine (precursor of creatinine) in the diet are meat and fish, and these were not present in the LL diet.

There is no data in the literature showing that a healthy kidney will be damaged from a diet in which protein is increased above that of the RDA (0.8g/kg body weight). A study by Poortmans and Dellalieux (2000) examined the use of a moderate and high protein intake on bodybuilders, to investigative renal function of a high protein intake. Despite the higher concentrations of uric acid and calcium, the bodybuilders had renal clearances of creatinine, albumin and urea within the normal range. The study concluded that, at least in the short term, protein intake under 2.8g/kg does not seem to impair renal function in bodybuilders and well trained athletics.
Knight et al. (2003) examined protein intake and renal function changes in women over an 11 year period (Nurses Health Study). The study concluded that high protein intake was not associated with a decline in renal function in women with normal renal function.

The American Heart Association also raised concerns about the use of high protein diets and liver function. Though, there is no evidence from the literature to support this. In the present study, no detrimental effects were seen in either group at 12 months. On a positive note, the LL group showed a significant reduction in ALT, which is indicative of a healthy liver function. All analytes measured in relation to liver function were in a normal range at baseline, and this remained at 12 months. Therefore, the present study supports the use of very low calorie diets and high protein/low carbohydrate diets for weight loss, without unfavourable effects on kidney or liver function.
CHAPTER 9: LIFESTYLE AND HEALTH FACTORS
The 7 lifestyle and health questionnaires were completed at baseline, 3, 6, and 12 months. The questionnaires included the Physical Activity Level Questionnaire (PAL), the General Health Questionnaire (GHQ), the Epworth Sleepiness Scale (EPW) questionnaire, the World Health Organization Quality of Life (WHOQOL) questionnaire, the Dutch Eating Behaviour questionnaire (DEBQ), the Fatigue (FAT) questionnaire and the Beck Depression Inventory questionnaire (BDI). These questionnaires examined common health and lifestyle factors associated with being obese, such as physical activity, and quality of life. It is important that these are monitored during weight loss/gain. The questionnaires have been validated, and were applicable to the present population studied. They were easy to use for patients, and scoring was simple.

Data is reported on an intention to treat basis. Normality tests were carried out using SPSS and in this case were not normally distributed so are reported as median and interquartile range.

9.1: Pre-randomization analysis

(a) Baseline data

Results from the questionnaires at baseline are shown in Tables 9.1-4.

<table>
<thead>
<tr>
<th>WHOQOL</th>
<th>phy</th>
<th>psyc</th>
<th>env</th>
<th>soc</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>57.9</td>
<td>53.2</td>
<td>66.3</td>
<td>61.0</td>
</tr>
<tr>
<td>Median</td>
<td>59.5</td>
<td>54.2</td>
<td>65.6</td>
<td>66.7</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>46.4-67.9</td>
<td>45.8-62.5</td>
<td>56.3-75.0</td>
<td>41.7-75.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DEBQ</th>
<th>rest</th>
<th>emo</th>
<th>ext</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>2.8</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Median</td>
<td>2.9</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>2.5-3.3</td>
<td>2.9-3.8</td>
<td>2.5-3.3</td>
</tr>
</tbody>
</table>
Table 9.3: PAL, GHQ and BDI scores at baseline.

<table>
<thead>
<tr>
<th></th>
<th>PAL</th>
<th>GHQ</th>
<th>BDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>1.2</td>
<td>11.4</td>
<td>13.0</td>
</tr>
<tr>
<td>Median</td>
<td>1.2</td>
<td>7.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>1.1-1.4</td>
<td>1.0-18.0</td>
<td>6.0-17.0</td>
</tr>
</tbody>
</table>

Table 9.4: EPW and FAT scores at baseline.

<table>
<thead>
<tr>
<th></th>
<th>EPW</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>7.9</td>
<td>35.1</td>
</tr>
<tr>
<td>Median</td>
<td>8.0</td>
<td>33.5</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>5.0-10.0</td>
<td>25.0-44.7</td>
</tr>
</tbody>
</table>

Pearson correlations were calculated to observe if there were any relationships between subjects’ questionnaire scores and weight at baseline. There was a significant inverse correlation between weight and the DEBQ “restrained” ($r = -0.200, p = 0.029$), DEBQ "emotional” eating ($r = -0.232, p = 0.011$) and weight and fatigue at baseline ($r = -0.213, p = 0.020$) (Figures 9.1(a-c)).

Figure 9.1a: Weight vs. DEBQ (res) at baseline.

Figure 9.1b: Weight vs. DEBQ (emo) at baseline.

Figure 9.1c: Weight vs. fatigue at baseline.
(b) Three month data

Results at 3 months are shown in Table 9.5-9.8. There were significant differences from baseline for the Epworth sleepiness scale ($p = 0.02$), the Beck Depression Inventory ($p = 0.03$), the WHO “physical” questionnaire ($p = 0.08$), the Dutch Eating Behaviour questionnaire (emotional), and ($p = 0.030$).

Table 9.5: WHOQOL scores at 3 months.

<table>
<thead>
<tr>
<th>WHOQOL</th>
<th>phy</th>
<th>psyc</th>
<th>env</th>
<th>soc</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>61.5</td>
<td>59.9</td>
<td>66.8</td>
<td>63.3</td>
</tr>
<tr>
<td>Median</td>
<td>61.6</td>
<td>58.3</td>
<td>68.8</td>
<td>66.7</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>50.0-71.4</td>
<td>45.8-66.7</td>
<td>58.2-78.1</td>
<td>50.0-83.3</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>0.009</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$p > 0.05$

Table 9.6: DEBQ scores at 3 months.

<table>
<thead>
<tr>
<th>DEBQ</th>
<th>rest</th>
<th>emo</th>
<th>ext</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>2.8</td>
<td>3.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Median</td>
<td>2.9</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>2.6-3.2</td>
<td>2.8-3.6</td>
<td>2.6-3.3</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>0.030</td>
<td>NS</td>
</tr>
</tbody>
</table>

$p > 0.05$

Table 9.7: PAL, GHQ and BDI scores at 3 months.

<table>
<thead>
<tr>
<th>PAL</th>
<th>GHQ</th>
<th>BDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>1.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Median</td>
<td>1.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>1.1-1.4</td>
<td>0-4.0</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$p > 0.05$

Table 9.8: EPW and FAT scores at 3 months.

<table>
<thead>
<tr>
<th>EPW</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>7.4</td>
</tr>
<tr>
<td>Median</td>
<td>8.0</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>4.0-25.2</td>
</tr>
<tr>
<td>$p$</td>
<td>0.021</td>
</tr>
</tbody>
</table>

$p > 0.05$
9.2: Post-randomization analysis

(c) 6 and 12 month data

Six and 12 month data is analysed according to diet. As the results for many of the questionnaires were not normally distributed, boxplots are used to display the changes over time i.e. (from baseline to 12 months) for the groups. Boxplots are used to display quartiles as well as the minimum and maximum scores in a distribution, and identify outliers and extreme scores. These are shown in the following boxplots.

(i) HE group

Figure 9.2(a-l) shows boxplots displaying the ranges, median, interquartile range and outliers for the HE group for the different timepoints in which the questionnaires were measured throughout the trial. There was a significant difference at 12 months from baseline for depression (BDI questionnaire) \( (p = 0.001) \), quality of life (physical) \( (p = 0.013) \), quality of life (psychological) \( (p = 0.031) \), eating behaviour (emotional) \( (p = 0.046) \), and daytime sleepiness \( (p = 0.025) \) where there were improvements in all responses.
Figure 9.2c: Changes over time for EPW in the HE group.

Figure 9.2d: Changes over time for BDI in the HE group.

Figure 9.2e: Changes over time for WHOQOL (phy) in the HE group.

Figure 9.2f: Changes over time for WHOQOL (psy) in the HE group.

Figure 9.2g: Changes over time for WHOQOL (soc) in the HE group.

Figure 9.2h: Changes over time for WHOQOL (env) in the HE group.
Pearson correlations were calculated for weight change at 12 months and changes in questionnaire scores at 12 months. No significant correlations were found.

(ii) LL vs. PSMF groups.

Figures 9.3(a-k) compare responses to lifestyle and health questionnaires at baseline, 3, 6 and 12 months for the LL and PSMF groups.

The LL group showed a significant improvement for physical activity ($p = 0.003$) and depression ($p = 0.050$) at 12 months, whereas the PSMF group showed a significant improvement at 12 months for physical activity ($p = 0.049$), “restrained” ($p = 0.047$) and “emotional” ($p = 0.001$) eating behaviour.
Figure 9.3a: Changes in GHQ baseline to 12 months for LL and PSMF groups.

Figure 9.3b: Changes in PAL from baseline to 12 months for LL and PSMF groups.

Figure 9.3c: Changes in WHOQOL (physical) from baseline to 12 months for the LL and PSMF.

Figure 9.3d: Changes in WHOQOL (psychological) from baseline to 12 months for the LL and PSMF groups.
Figure 9.3e: Changes in WHOQOL (social) from baseline to 12 months for the LL and PSMF.

Figure 9.3f: Changes in WHOQOL (environmental) from baseline to 12 months for the LL and PSMF groups.

Figure 9.3g: Changes in Fatigue from baseline to 12 months for the PSMF and LL groups.

Figure 9.3h: Changes in BDI from baseline to 12 months for the LL and PSMF groups.
Figure 9.3i: Changes in DEBQ (restrained) from 0 to 12 months for the LL and PSMF groups.

Figure 9.3j: Changes in DEBQ (emotional) from 0 to 12 months for the LL and PSMF groups.

Figure 9.3k: Changes in DEBQ (external) from 0 to 12 months for the LL and PSMF groups.

Figure 9.3k: Changes in EPW from 0 to 12 months for the LL and PSMF groups.
9.3: DISCUSSION

*Dutch Eating Behaviour Questionnaire (DEBQ)*

After performing a literature search, no studies were found which examined eating behaviour, using the DEBQ, in a RCT of different dietary approaches for the treatment of obesity. Hence, the present study is possibly the first to analyse this.

The three psychological aspects of eating behaviour are emotional, restrained and external eating and these were measured using the Dutch Eating Behaviour Questionnaire (van Strien *et al.*, 1986). Emotional eating describes a tendency to eat in response to negative emotions including depression, disappointments and loneliness. External eating refers to eating in response to external food cues such as the sight, smell and taste. Finally, restrained eating refers to the conscious determination and efforts to restrict food intake to control weight (Elfhag *et al.*, 2008). These types of eating behaviours are of particular interest in terms of obese and overweight individuals.

Emotional and external eating are considered the most challenging eating styles as they are associated with an increased body weight (Elfhag and Linne, 2005). Nevertheless, this was not supported in the present trial, where at baseline, there was a significant inverse association between weight and emotional eating behaviour, and also weight and restrained eating behaviour.

Emotional eating is based on psychosomatic theories that consider overeating as a compensation and response to negative emotions, having psychological roots in
inadequate relationships during childhood. Therefore emotional eating would be expected to be related to depression. In the present study, emotional eating was strongly associated with depression, suggesting that there is a relationship between depression and this eating characteristic. At 3 months, mean scores for emotional eating showed a significant improvement.

Restrained eating has been associated with higher (Elfhag and Linne, 2005) and (Provencher et al., 2003) but also lower body weights (Boschi et al., 2001) and healthier food intake (Beiseigel and Nickols-Richardson, 2004; Elfhag et al., unpublished). Interestingly, this is similar to the present study, where there was an inverse relationship between weight and “restrained eating” at baseline. The restraint tends to increase over time as weight decreases in successful weight loss treatments. This suggests that restraint is a favourable and an essential eating strategy for controlling body weight (Bjorvell et al., 1994; Karlsson et l, 1994). However, the theory of restraint has also be a problem as restraint may create a risk for eventual cravings and overeating (Stunkard and Messick, 1985; Tuschl, 1990).

HE group

The theory behind the use of the DEBQ is that obesity is an individual problem and that if a subject’s eating behaviour is diagnosed (i.e. emotional, external or restrained), treatment can be made to fit the individual based on dealing with this eating behaviour.

At 12 months, there was a significant improvement from baseline for the “emotional” aspect of eating behaviour. Despite the large weight loss seen in this group there were no significant relationships found between weight change and eating behaviour change at 12 months. However, it may be noteworthy to see that all aspects of eating
behaviour changes were strongly correlated with each other. This suggests that there may be an interrelationship between emotional, external and restrained eating behaviour, and when there is an improvement in one, this can also lead to an improvement in overall eating behaviour, in an obese population.

**LL vs. PSMF**

There was a significant difference between these groups at baseline and at randomization for weight, where the LL group had a significantly higher weight. However, despite this, there were no significant differences in scores for all questionnaires between the groups at baseline or at randomization.

When comparing these groups at 12 months, there were no differences between the groups for changes in eating behaviour, despite the difference in weight loss between these groups. Though there was a small weight loss at 12 months for the PSMF group, it is interesting to see that there was a significant decrease in a propensity to eat due to “emotional” and “restrained” eating.

Though there was a trend for improvements for emotional and external aspects of eating behaviour for the LL group, neither of these were significant at 12 months. Weight loss at 12 months was significantly associated with an increase in “restrained” eating, and this agrees with the previous finding mentioned earlier that “restrained eating” can increase with weight loss. This is interesting, as the LL group were on a strictly controlled diet, where they were only allowed to consume the food packages assigned to them each week. This may be where the association between this weight loss and “restrained eating” developed.
Of note, the LL group were also undergoing CBT, and this could positively influence eating behaviour. This was difficult to measure when these subjects were restricted to the controlled diet for the majority of the clinical trial. Future studies should use the DEBQ to examine eating behaviour after these subjects are permanently weaned back onto a whole food diet.

In addition to this, it may be possible that the ketogenic aspect of the LL diet may influence eating behaviour, though this was not directly examined in this trial.

**Beck Depression Inventory (BDI) questionnaire**

Mean scores for depression were particularly high in the study population at baseline, and equated to a “mild to moderate” level of depression according to the BDI questionnaire. At 3 months, there was already a significant improvement in depression scores, despite only 18 subjects achieving a 5% weight loss.

According to a study by Markowitz et al. (2008) depression is one of the most serious mental health problems in America, and has serious consequences of human suffering and sometimes loss of life (Klerman and Weisman, 1992). Subjects who suffer from both obesity and depression face particular risks to their health, and these can also affect employment, physical and social functioning (Keitner and Miller, 1990). In addition to this, these conditions may propagate one another, depression could increase the risk for obesity, or obesity can cause depression (Markowitz et al., 2008).

It was interesting to see in the present study, that a high depression score was strongly associated with decreased general health (GHQ), sleepiness (EPW), fatigue and all aspects of eating behaviour (DEBQ). In addition to this, depression was associated
with a low quality of life (environmental) measured using the WHOQOL. This shows the strong impact depression has on other aspects of life.

Markowitz et al. (2008) presented a comprehensive review about the reciprocal relationship between obesity and depression and summarizes recent studies showing that increases in weight, measured either categorically or continuously, are associated with greater levels of depression (Carpenter et al., 2000; Dong et al., 2004; Onyike et al., 2003; Simon et al., 2006). On the other hand, it should be noted that Prof Andrew Hill stated in an article for the ASO (Association for the Study of Obesity), that obesity per se does not necessarily contribute to a state of poor psychological health. He stated that the “variation in psychological adjustment among the obese is broadly comparable to that in the population at large” and that “there is certainly no major psychiatric disorder or specific personality disorder associated preferentially with obesity”. He did note however, that there is emerging literature linking obesity with depression.

**HE group**

Dr Aronson, an endocrinologist based in Toronto carried out a study exploring the association between weight loss and depression. 61 % of the patients achieved 5 % weight loss at 6 months. Among the successful patients, mean depression scores improved by 57.2%, going from an overall rating of mild depression to minimal depression using the Beck Depression Inventory. Aronson believed this improvement in depression and mood to be greater than results from the use of antidepressants and avoids the need for medication (http://annecollins.net/weight-loss-depression.htm). This study is similar to results found in the present trial where depression scores were
reduced significantly from a “mild to moderate” depression score to a “low” depression score. The present study supports the idea that depression associated with obesity could be reduced when using a low calorie/HE diet for weight loss.

**LL vs. PSMF group**

In addition to the HE group, the LL group also showed a significant decrease in mean depression scores at 12 months. However, this was not the case for the PSMF group possibly due to the small weight loss at 12 month.

The decrease in depression in the LL group was significantly associated with the decrease in weight at 12 months. Similar to the HE diet, this suggests that weight loss using this type of dietary approach can be successful in treating depression associated with obesity.

The improvement in depression scores in the LL group was also strongly associated with a decrease in fatigue, daytime sleepiness, general/mental health scores, and the physical aspect of quality of life, suggesting that a decrease in depression and weight loss can lead to improvements in all these other aspects related to obesity. It is noteworthy that this group also underwent cognitive behavioural therapy (CBT), and it is possible that this treatment, in parallel with weight loss, together contributed to the improvement in the above lifestyle factors.

*Physical Activity Level questionnaire (PAL)*

Overweight and a sedentary lifestyle are serious public health, clinical, and economic problems in modern societies (Lakka *et al.*, 2005). In the present study, mean PAL scores at baseline were low, supporting the lack of physical activity for obese subjects.
Data from the HSE (Health Survey England, 2003) report, 2003, showed that physical activity levels were related to BMI. The proportion of men who had high activity levels fell from 44% in those with a normal BMI, to 31% in those who were obese, and only 16% in those who were morbidly obese. For women, the proportion who had a high physical level fell from 30% in the lean individuals, to 18% in those who were obese and morbidly obese (HSE, 2003).

Regression analysis from the HSE, 2006 showed a link between physical activity and waist circumference. Men and women with low activity levels were twice as likely to have raised waist circumference compared to those with high activity levels (HSE, 2006).

HE group
Despite continued advice on physical activity, there was no significant improvement at 12 months. Regular exercise can reduce body weight and fat mass without dietary caloric restriction in overweight individuals. Nevertheless, the optimal approach for losing weight seems to be the combination of increased physical activity and a decreased caloric intake. This is also an effective way of treating and reducing the risk for CVD (Lakka et al., 2005), which is increased in obese subjects.

LL vs. PSMF groups
Those following the LL diet showed a significant improvement at 12 months in physical activity levels possibly due to the CBT effect. However, this was not the case for those following the PSMF diet.
It is important to note that subjects in all 3 groups remained in the obese category at 12 months, and therefore, this may explain why PAL was not particularly high at this timepoint. These subjects were yet to lose a considerable amount of weight for them to be classified as a normal weight/lean (BMI 20 – 25 kg/m²). However, it is positive to see that there was an improvement in PAL at 12 months, in particular for those following the HE and LL diets. For the LL group, the increase in PAL was significantly associated with a decrease in fat-free mass, weight and bodyfat percentage.

Although there may be advantages to modifying protein and carbohydrate intake, the optimal doses of these macronutrients for weight loss have not been determined (Jakicic et al., 2001). The data in the present study suggests that it is not the type of diet, but the overall weight loss that improves the physical activity level, and may also be influence by other factors such as CBT.

**General Health Questionnaire (GHQ)**

The QHQ was designed primarily as a screening instrument for clinical and preclinical assessment of psychosomatic disorders and mental illness (Hobi et al., 1989). The questionnaire is able to pick up aspects of mental stress and exhaustion, depression and suicidal behaviour and a general disturbed feeling (Hobi et al., 1989). It attempts to identify states of mental health disorders, and consequently possible referral to a psychiatrist (Makowska and Merecz, 2000).

There are few studies where the GHQ was used to assess mental/general health in an obese population. There were 7 subjects who were identified as being a “positive
case”, with a score above 39. At the end of the trial, all these subjects were identified as “negative cases”. Interestingly, weight change at 12 months was strongly associated with the decrease in GHQ scores for these 7 subjects.

There was a trend for GHQ scores to improve in all groups at 12 months, though none of these were significant. It is noteworthy that most subjects were identified as negative cases at the commencement of the trial, and this may explain the lack of a significant change at 12 months. It was interesting to see that the improvement in GHQ scores was associated with a decrease in BMI, and hence this suggests that there is a relationship between weight loss and improvement in general/mental health.

The present trial has shown that the GHQ can be a useful way of identifying unique cases in an obese population which may need to be further examined.

**Fatigue questionnaire**

Fatigue can be defined “either as a progressive impairment of generating capacity of muscle (peripheral or muscle fatigue) or a lessened capacity for work and reduced efficiency of accomplishment, usually accompanied by a feeling of weariness, sleepiness and irritability” (Goldenberg, 1995). It is a general sensation of feeling tired or exhausted (Dernis-Labous *et al.*, 2003).

The prevalence of fatigue in a healthy adult population is in the range of 14 to 25 % (Goldenberg, 1995). It is found in a broad variety of illnesses including, thyroid diseases, renal failure, clinical depression, anaemia, and liver, pulmonary or cardiovascular disturbances (Prince *et al.*, 2000), and it is associated with psychological factors such as depression, and sleep disturbance (Goldenberg, 1995).
This was similar to results found in the present study. Fatigue was strongly associated with higher scores for depression (BDI), and general/mental health (GHQ). Fatigue was also associated with a high level of restrained, emotional, and external eating, and also expectedly, daytime sleepiness (EPW).

Fatigue is a common complaint in mood disorders. It has been reported that 24 to 80% of subjects with chronic fatigue syndrome may suffer from depression (Ax et al., 2001), and also Judge et al. (2000) found that over two thirds of subjects with depression show signs of fatigue.

Fatigue is difficult to classify by etiology; as a subjective occurrence, it can only be diagnosed by patients’ self-reports (Barofsky, 1991). There are no cut-offs for defining fatigue using the VAS Fatigue questionnaire which was used in the present study. Instead, the higher the score (which ranges from 0-100), the more the subject suffers from fatigue.

Few studies have looked at the relationship between obesity, fatigue and also depression (Lim et al., 2005). The mean score for fatigue at baseline in the present study was relatively low, and surprisingly, levels of fatigue were significantly inversely associated with both weight and BMI. There was no significant improvement in fatigue levels at 3 months.

None of the dietary approaches showed a significant improvement in fatigue levels at 12 months.
However, it is noteworthy that fatigue levels were relatively low at baseline, and therefore it would not be expected that there would be a great improvement at 12 months in any of the groups.

Fatigue did not seem to be associated with being obese in the present study, and it seemed to be more related to other lifestyle factors in the obese population. However, studies are limited where this questionnaire was used to assess fatigue in an obese population, and therefore this needs to be further examined.

*Epworth Sleepiness Scale (EPW) questionnaire*

This questionnaire was designed to examine daytime sleep propensity in a standardised way and tries to cover a range of sleep propensities from sleep apnoea to hypersomnia. It was validated in a group of normal controls and those with sleep disorders (Johns 1991 and 1992). The questionnaire is based on the idea that knowing how often or for how long subjects sleep during the day does not give an assessment of their sleepiness as such, as healthy subjects often take a nap during the day. Instead, a description of how subjects unintentionally “nod off” while carrying out normal day to day activities that do not involve high levels of stimulation, little mobility, and relaxation, can provide a better indicator of levels of fatigue. In summary, a distinction is made between just feeling tired and daytime sleepiness.

Daytime sleepiness is a common complaint in obese subjects, even in those who do not suffer from sleep apnoea or any form of sleep disordered breathing (Vgontzas *et al.*, 1994).
Johns and Hocking (1997) showed the distribution of EPW scores for 72 non-obese subjects. They showed a mean score of 4.6 (SD 2.8), which gave a reference range of 0-10. This is in contrast to the present study, where the mean scores at baseline for the total population was 7.9 (SD 4.2), indicating that daytime sleepiness is higher than in non-obese subjects.

There was a trend for scores to improve for all 3 groups at 12 months in the present study. There were significant improvements for the HE group at 12 months. It was interesting to see that improvements in daytime sleepiness scores in the LL group were associated with improvement in scores for general/mental health (GHQ), quality of life (environmental), and the emotional aspect of eating behaviour in the LL group. This was not seen in the HE group, or the PSMF and could be due to the CBT received by these subjects.

*World Health Organisation Quality of Life questionnaire (WHOQOL)*

In the present study, scores at baseline were generally good for all aspects of quality of life measured by the WHOQOL (environmental (env), physical (phy), social (soc), and psychological (pys))

At 3 months there was a significant improvement in the “physical” aspect of quality of life.

**HE group**

The quality of life of overweight and obese subjects is an extensively discussed topic. From several studies, it emerges that obese people suffer significant damage as a result of their overweight in terms of quality of life concerning their physical and
psychosocial well-being, with greater incidence associated with the degree of obesity (Allegri et al., 2008). Though scores in the present study for all aspects of quality of life were satisfactory at baseline, the “social” and “psychological” aspect of quality of life showed a significant improvement at 12 months for the HE group. Weight loss has been shown to improve quality of life in obese persons undergoing a variety of treatments (Kolotkin et al., 2001), and the present study supports the use of low calorie/HE diets as a method of weight loss while improving quality of life.

**LL vs. PSMF groups**

Though there was a trend for improvement in all aspects of quality of life, none of these were significant in either group.

It is surprising that there wasn’t a greater improvement seen in the LL group, despite the large weight loss and CBT of which these subjects underwent. However, for both groups, weight loss at 12 months was highly correlated with improvements in quality of life, even for the PSMF group where weight loss was low.

Few studies have examined non-surgical interventions in the treatment of obesity and quality of life (Kushner and Foster, 2000). The present study indicates that it is possibly weight loss, and not the type of dietary treatment which has a positive impact on the subject’s quality of life.
CHAPTER 10: CONCLUSION
10.1: Systematic Review and Clinical Trial

As described in Chapter 1, the aims of this project included the following:

- To carry out a systematic review of low carbohydrate/high protein diets in relation to weight loss and cardiovascular disease risk factors and comparing them to low fat/energy dietary approaches over a minimum of 6 months.

- To conduct a clinical trial using the LighterLife weight loss programme and comparing it to a protein sparing modified fast (low carbohydrate). A healthy eating, calorie deficit diet is also examined.

- To examine the outcomes of the trial including weight loss, cardiovascular risk factors (including LDL cholesterol, HDL cholesterol, total cholesterol, triacylglycerols, blood pressure) fasting plasma glucose, insulin resistance and adipokines.

- To relate the phenotype at presentation with the appropriate response to the different treatment modalities.

Systematic Review

The systematic review comparing low carbohydrate/high protein diets to conventional/low fat diets for weight loss, and CVD risk was carried out prior to the clinical trial. A total of 13 long term (> 6 months) RCTs published after the year 2000, were included in the review. Low carbohydrate diets were shown to be superior for weight loss at 6 months, and also at 12 months, though the difference in weight loss was not as large. In regards to CVD risk, HDL cholesterol, TAG, and SBP (at 12 months) all showed significant improvements favouring the low carbohydrate/high protein group. However, both LDL cholesterol and total cholesterol showed greater increases in those following the low carbohydrate/high protein diet. As both HDL cholesterol and TAG showed greater improvements on the low carbohydrate diet, this may affect the atherogenicity of the LDL particle, which was not investigated in the systematic review, and needs to be monitored in future studies.
Currently, low fat diets are the usual approach recommended by GPs and clinicians to treat obesity, and its comorbidities. Though, evidence from the systematic review suggests that low carbohydrate/high protein diets certainly should not be disregarded. At present, there is conflicting results regarding the use of these diets, however, it seems clear from this review that they are as good as, if not better in promoting weight loss and decreasing the risk for CVD (Hession et al., 2008). More long term studies urgently need to be carried out to elucidate this, as the obesity epidemic continues to rise at an alarming rate.

**The overall clinical trial analysis and obesity related co-morbidities**

Three diets were analysed as part of the trial, the HE, LL and PSMF. The HE diet was not compared to the LL and PSMF. The reason for this was because those following the HE diet were not randomised, whereas the other 2 groups were.

Only 18 subjects remained on the HE diet, whereas there were 72 subjects randomised to either the LL or the PSMF at 3 months. However, the 18 subjects who followed the HE diet were successful in losing weight at 12 months. These subjects were morbidly obese at baseline and this may explain the large weight loss. In addition to this, the HE group showed significant improvements in body composition, including bodyfat, BMI, waist circumference, and hip circumference. For CVD risk, there were significant improvements for HDL cholesterol, TAG, DBP and TC/HDL cholesterol.

In addition to this, all remaining CVD risk factors tended to improve. However, the subjects following the LL diet were highly successful in losing weight after 9 months of following the diet. There were also significant improvements for all other measurements of body composition. CVD risk also decreased significantly in this group. These results suggest that very low calorie diets such as LL may be used as an alternative approach to the conventional low fat diet in treating obese subjects above a BMI of $\geq 35$ kg/m$^2$.

It was surprising to see that the PSMF diet did not produce a large weight loss, similar to previous studies of low carbohydrate/high protein diets (Yancy et al., 2002; Foster et al., 2003; Samaha et al., 2003). However, it is important to note that they did achieve a significant weight loss after following the diet for 9 months. Possible
reasons for not achieving the anticipated large weight loss possibly were lack of motivation, and insufficient knowledge of the diet. High protein diets also tend to be more expensive than high carbohydrate/low fat diets and this may also have affected the outcomes. In conclusion, both the HE diet and the LL diet were highly successful in losing weight and decreasing CVD risk.

Health and lifestyle factors including eating behaviour, physical activity, sleepiness and fatigue, quality of life, depression and general/mental health were also examined throughout the trial.

Mean depression scores were particularly high for the groups at baseline. Studies examining whether obesity predicts depression and vica versa have been inconsistent (Onyike et al., 2003). At 12 months, mean depression scores showed significant decreases for the HE and LL groups, possibly due to the large weight losses seen in these groups at 12 months. The present clinical trial suggests that weight loss may contribute to a decrease in depression scores for those following a HE and a very low calorie dietary approach. Future studies should examine whether macronutrient composition influences this change in depression.

Matching phenotype at presentation
The main objective in the present clinical trial was to identify a suitable diet to match the obese phenotype at presentation. Not all obese subjects are similar, with some presenting with T2D, MS or other lipid abnormalities such as dyslipidemia. On the other hand, some obese subjects may be considered healthy, as apart from being obese, they do not present with any additional comorbidities. An important aspect in the study design was that only subjects who had a BMI $\geq 35$ kg/m$^2$ were chosen as part of the inclusion criteria. Evidence has shown that the more obese the subject, it is their carbohydrate intake as opposed to fat intake which is the problem (Accurso et al., 2008). In other words, these subjects have a degree of carbohydrate intolerance, and may be better suited to an alternative dietary approach, such as a low carbohydrate diet. This was investigated in the current trial.

The study design in the present clinical trial differs from other RCTs in the literature examining low carbohydrate/high protein diets (Brehm et al., 2003; Dansinger et al., 2005; Gardner et al, 2007). These studies did not have a cut-off for an obese BMI,
except for Samaha et al. (2003), which also used a BMI ≥ 35 kg/m². These studies also differed from the present clinical trial in that they randomised their subjects at baseline to the diets been examined. The reason for such a study design for the present trial was that it was hypothesised that subjects would not be successful in achieving a > 5 % weight loss using the conventional HE approach. This was certainly the case, as only 18 subjects, out of 90 subjects who remained on the trial after 3 months, were able lose > 5 % of their baseline body weight. This supports the hypothesis that subjects with a BMI ≥ 35 kg/m² may be more suitable to a low carbohydrate/high protein dietary approach.

In addition to this, those subjects with MS and T2D were examined throughout the trial. 54 subjects were diagnosed with MS at baseline, and 13 subjects were diagnosed with T2D. Worryingly, 12 out of the 13 subjects had not been previously diagnosed by their clinician with T2D. Proper screening measures need to be put in place to prevent this from occurring. Due to the small sample for subjects with T2D, they were not investigated further after baseline.

MS as discussed previously is a cluster of abnormalities including high blood pressure, low HDL cholesterol and high TAG levels. These can all be caused by a higher carbohydrate intake, and is one of the reasons why this group was examined. It has previously been suggested that a low carbohydrate approach may be better for treating obese individuals with MS, as opposed to a low fat approach (Volek and Feinman, 2005). Out of the 54 subjects with MS at baseline, 12 of these lost > 5 % baseline weight at 3 months, and continued on the HE diet.

At 12 months, the MS group following the HE diet showed significant improvements in weight and body composition. In addition to this they showed significant improvements in HDL cholesterol, TAG, DBP and TC/HDL cholesterol. In regards to adipokines, PAI-1, adiponectin and leptin tended to decrease in this group at 12 months. In conclusion, those with MS were successful on the HE diet.

There were 15 subjects and 17 subjects following the LL and PSMF diets, respectively. The LL group showed greater weight loss at 12 months compared to the PSMF group, and this was similar for those who did not have MS. The group also showed significant improvements in all other measures of body composition at 12
months. In relation to CVD risk, the LL group did not show any significant improvement except for an increase in HDL cholesterol. This was unexpected due to the large weight loss and is possibly is due to the small sample size (n = 15). Though on a positive note, there was a tendency for CVD risk to decrease after 12 months of following the LL diet.

Surprisingly, this was not the case for those with MS following the PSMF diet. On the other hand, the group without MS following the PSMF were also not successful in losing weight or improving body composition. This diet was a low carbohydrate/high protein dietary approach, and as found in the systematic review and other published studies, has previously been successful for weight loss and body composition. It is unknown why this particular sample did not do well on the PSMF.

There were no significant differences for adipokines in the HE group with and without MS. For the LL group, both PAI-1 and adiponectin showed significant improvements at 12 months, but there were no significant changes seen in the PSMF group as expected, due to the small weight loss. On a positive note, there were no detrimental changes in adipokines for all groups at 12 months, suggesting that all three diets are effective in terms of the adipokine profile for subjects with MS.

In conclusion, the majority of the subjects diagnosed with MS at baseline were not successful in losing > 5 % baseline weight. This is thought to be due to a possible “carbohydrate intolerance”, and was the key hypothesis of the trial. Although the subjects who remained on the HE diet were successful in terms of weight, body composition and risk of CVD disease, they were morbidly obese at baseline and this may be a reason why they did so well.

On the other hand, 32 subjects with MS did not achieve the target weight loss. Those randomised to the LL diet did particularly well in terms of weight loss and improvement in body composition, and also decreasing the risk for CVD. In terms of risk factors for MS, mean HDL cholesterol, TAG and blood pressure were in the normal range at 12 months. The study agrees with the hypothesis that a HE approach should not necessarily be the first line of approach for MS subjects with a BMI over 35 kg/m², and that an alternative diet such as a very low calorie diet may be a preferred method of treatment.
Though the PSMF did not show any significant changes, it should not be ignored and further studies should be carried out to investigate whether they are more beneficial for MS subjects, over a BMI of 35 kg/m$^2$.

As a final conclusion, this study agreed with the hypothesis that not all obese subjects are suited to the standard HE dietary approach. Only 18 subjects were successful at 3 months in regards to weight loss and were randomised to alternative dietary approaches. These subjects showed improvements in weight, CVD risk, and adipokines levels after 9 months. In addition to this, to detrimental effects were seen in the LL and PSMF groups in regards to kidney and liver function. The MS phenotype also showed improvements in all variables on whichever diet they remained on at 12 months, though greater improvements were seen using the LL approach, possibly due to non-compliance to the PSMF.

10.2 Limitations of study
As with the majority of RCTs of comparable studies, this clinical trial had some limitations. Attrition rates were high, in particular in the first 3 months of the trial, where 25% of the subjects dropped out. This was despite the subjects visiting the clinic on a regular basis in particular in the early stages of the trial, and also they were not on a strict dietary regime at this stage. After randomization, 20 subjects dropped out of LL and 18 dropped out of the PSMF. The subjects on LL were seen on a weekly basis for cognitive behavioural therapy (CBT) by a LL counsellor, and also by a trained research fellow on a monthly basis after randomization. The PSMF group visited the clinic on a monthly basis between 3 months and the final visit at 12 months.

Analysis was carried out on an intention to treat basis, with the last value carried forward. This method can sometimes be problematic, as it cannot always be assumed that those who dropped out would return to their weight achieved at the time of dropout. Intention to treat gives a pragmatic estimate of the advantage of a change in treatment policy rather than of possible benefit in patients who receive treatment exactly as planned. Clinical effectiveness may be overestimated if an intention to treat analysis is not done.
However, although the analysis was carried out on an intention to treat basis in the present trial, weight change was also reported for the completers alone at 12 months for the 3 groups.

In addition to this, t-tests were used to compare differences between timepoints. In the case of data which was not normally distributed, the data was log-transformed and then t-tests were carried out comparing differences between the groups, and differences for each group from different timepoints. For similar studies in the future ANOVA may be a better approach for testing differences between the groups, and also a repeated measures analysis of variance for testing differences in variables over time.

The small weight loss seen in the PSMF group was unforeseen, as these diets are normally successful in inducing a high weight loss. This may have been due to the little contact the subjects had with a health professional, and the fact that these subjects did not receive CBT similar to the LL subjects.

The design of the study was quite atypical, and a disadvantage was that the healthy eating/low calorie diet could not be compared to LL or the PSMF diets. However, there was a valid reason for this and it reverts back to the hypothesis discussed earlier. Essentially, the first 3 months of the trial was a “screening process”, and the reason behind this was so that those subjects who responded well to, or did not respond well to a low calorie/high carbohydrate diet would be separated at randomization.

Finally, it is well known that RCTs are the “gold standard” method for clinical trials. However, this method can also be a cause of high attrition rates. Subjects are randomly assigned to a diet which may not suit them in terms of their lifestyle, eating preferences or family and this can cause lack of motivation and lead to dropout.

10.3 Future Research

There is insufficient evidence from the study to develop clinical guidelines. However, the study design differs from previous RCTs of dietary approaches for obesity, and proved that a low fat (HE) diet is not necessarily the first line of approach for obese subjects with a BMI over 35 kg/m², due to a possible carbohydrate intolerance. Matching phenotype at presentation is important in terms of weight loss success and
reducing the risk for CVD. Larger sample sizes and longer term trials, and different dietary approaches are needed to evaluate healthy and at-risk populations. Also, motivation and compliance issues need to be well monitored in particular for those following a low carbohydrate/high protein diet.


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Appendix 1
SYSTEMATIC REVIEW OF RANDOMIZED CONTROL TRIALS OF LOW CARBOHYDRATE VS LOW-FAT OR LOW-CALORIE DIETS IN THE MANAGEMENT OF OBESITY AND ITS COMORBIDITIES

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Key words: Obesity, meta-analysis, low carbohydrate, cardiovascular risk.

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Author Contributions: Ms Hession, Dr Rolland, Dr Kulkarni, Dr Wise, and Prof. Broom had full access to all of the data in the study and take responsibility for the integrity of the data analysis.

Study concept and design: Hession, Rolland, Kulkarni, Wise, Broom.

Acquisition of data: Hession, Rolland.

Analysis and interpretation of data: Hession, Rolland.

Drafting of the manuscript: Hession, Rolland, Kulkarni.

Critical revision of the manuscript for important intellectual content: Wise, Broom.
Statistical Analysis: Hession

Administrative, technical, or material support: Hession, Rolland, Kulkarni, Wise, Broom.

Study supervision: Broom.

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There are few studies comparing the effects of low carbohydrate/high protein diets against low fat/high carbohydrate diets for obesity and cardiovascular disease risk. This systematic review focuses on randomized controlled trials of low carbohydrate diets compared to low fat or low calorie diets. Studies conducted in adult populations with mean or median BMI of ≥28 kg/m² were included.

Thirteen electronic databases were searched and randomized controlled trials from January 2000 to March 2007 were evaluated. Trials were included if they lasted at least 6 months and assessed the weight-loss effects of low carbohydrate diets against low fat/low calorie diets.

For each study, data were abstracted and checked by two researchers prior to electronic data entry. The computer program Review Manager 4.2.2 was used for the data analysis.

Twelve articles met the inclusion criteria. There were significant differences between the groups for weight, HDL cholesterol, triacylglycerols, and systolic blood pressure, favouring the low carbohydrate diet. There was a higher attrition rate in the low fat compared to the low carbohydrate groups suggesting a patient preference for a low carbohydrate/high protein approach as opposed to the Public Health preference of a low fat/high carbohydrate diet.

Evidence from this systematic review demonstrates that low carbohydrate/high protein diets are more effective at 6 months and are as, if not more, effective than low fat diets in reducing weight and cardiovascular disease risk up to 1 year. More evidence and longer term studies are needed to assess the long term cardiovascular benefits from the weight loss achieved using these diets.
INTRODUCTION

The prevalence of overweight and obesity is already high and continues to increase in both the developed and developing world\(^1\). Obesity has been implicated as the second most preventable cause of death in the US. After remaining reasonably constant in the 1960s and 1970s, the prevalence of obesity among adults in the United States increased by around 50% per decade throughout the 1980s and 1990s. Two thirds of adults in the United States today are obese or overweight. In the United States, 28% of men, 34% of women, and nearly 50% of non-Hispanic black women are at present obese\(^2\). At any time, approximately 45% of women and 30% of men in the UK are trying to lose weight\(^3\). Most adults in England are now overweight, and nearly 1 in 4 are obese (http://www.foresight.gov.uk/Obesity/17.pdf). Obesity has been shown to be associated with increased risk of type 2 diabetes mellitus, hypertension, dyslipidemia and consequent cardiovascular disease. Obesity ranks second only to smoking in the aetiology of cancer and is an important factor in osteoarthritis and obstructive sleep apnoea\(^4\).

Recently, low carbohydrate/high protein diets have become popular as an aid to weight loss. Significant weight loss on a low carbohydrate/high protein diet without significant elevations of serum cholesterol has been reported. Studies comparing the “Atkins” diet to the classical low fat diet have appeared in the literature recently and are the subject of increasing public interest\(^5\) due to the beneficial improvements in cardiovascular risk and weight loss achieved with this type of dietary approach\(^6\).

This systematic review focuses on randomized control trials of low carbohydrate/high protein diets compared to low fat/high carbohydrate conventional diets. The
systematic review also examines the outcomes of such trials in relation to effects on cardiovascular disease risk. This systematic review focuses on updating the literary evidence from randomised control trials of low carbohydrate/high protein diets compared to lowfat/high carbohydrate diets to assess their impact on weight loss and cardiovascular risk. In addition, it demonstrates lower attrition rates in the low carbohydrate/high protein groups compared to the low fat/high carbohydrate groups suggesting patient preference for the former approach.

METHODS

Inclusion Criteria

The protocol used for this systematic review follows the methods recommended by the Cochrane Collaboration \(^7\). Randomized controlled trials (RCTs) were included if they assessed the weight-loss effects of low carbohydrate/high protein diets (LC/HP) against low fat/high carbohydrate (LF/HC) diets. Only RCTs from January 2000 to March 2007 were evaluated, as this review is intended to assess the current literature in this field and update the NHS R&D Health Technology Assessment (HTA) systematic review of diet and lifestyle on weight loss and cardiovascular risk published by Avenell et al\(^7\). Only studies conducted in an adult population were included, as defined by minimum age greater than 18 years. RCTs where the participants had a mean or median BMI of \(\geq 28\) kg/m\(^2\) were included. A BMI cut off of \(\geq 28\) kg/m\(^2\) was used to allow the inclusion of studies of ethnic groups where the classification of obesity is at a lower BMI cut off \(^8\). RCTs evaluated in this review had
to be of at least 6 months duration, including the period of active intervention and follow up.

Types of Intervention

The focus of this review was to examine low carbohydrate/high protein diets against other types of diets designed to induce weight loss and/or prevent weight gain, and induce changes in cardiovascular risk factors. The types of dietary intervention evaluated were:

- High protein “ketogenic” diet, where the carbohydrate content was less than 40 g/day, irrespective of calorie content.
- Low carbohydrate diets (CHO ≤ 60 g/day).
- “Healthy eating” advice.
- Low fat (30% or less daily energy from dietary fat)/600 kcal deficit diet

Outcome Measures

Weight loss or prevention of weight gain were the main outcomes assessed from the RCTs included in the review. With regard to cardiovascular disease risk factors, the following outcomes were also included:

- Serum lipids, including total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triacylglycerols.
- Systolic and diastolic blood pressure.
- Glycemic control.
Attrition rates were also analysed for each study to assess patient acceptability.

Search Strategy for the Identification of Included Studies

This systematic review was restricted to RCTs where the full study report was available. A wide search strategy was applied to identify as many RCTs evaluating dietary interventions as possible and which were relevant to the management of obesity and cardiovascular disease risk factors. Thirteen electronic databases were searched including MEDLINE, CAB abstracts and the Cochrane Central Register of Controlled Trials (CENTRAL). The search strategy incorporated weight loss, cardiovascular disease and obesity-related terms and text terms, specific to each database. Seven obesity and nutrition journals were hand-searched including the International Journal of Obesity and Obesity Research. Reference lists of included studies were searched and authors contacted for further details of their trials.

Quality Assessment of Studies

Full copies of studies were assessed by 2 researchers for methodological quality using a standard form. The researchers were not blinded to author, journal or institution. Differences of opinion were resolved by discussion. Trial quality was assessed, including whether or not the analysis was undertaken on an intention to treat basis.

Data Abstraction

A data abstraction form was created for this review based on a standard format. For each study, data were abstracted and checked by different researchers prior to electronic data entry.
Data Analysis

The computer program Review Manager 4.2.2 was used for the analysis of the data from the reviews. If results from studies could be quantitatively combined, a statistical meta-analysis of the data was undertaken to determine the typical effect size of the intervention. For continuous data, a weighted mean difference (WMD) was calculated. The Chi-Square test was used to test for heterogeneity across the studies. The significance value was set at 0.05.

Handling of Missing Data

Data processing for this review in Review Manager required the input of the mean and the standard deviation of the change between two time-points. Where weight or risk factors were reported as actual values instead of changes, the differences were calculated by subtracting the endpoint value from the baseline value. If standard deviations (SDs) for changes in weight and risk factors were missing, the following assumption was made: a previously published linear regression of the SD of the mean change in weight on the absolute mean change for weight\(^7\), derived from weight-loss RCTs, was used to supply missing SDs. Similar data was used to infer missing SDs for the other variables analysed in this review. The details of these equations are listed in Appendix 1.
RESULTS

Identified Studies:

A total of 13\textsuperscript{9,10,11,12,13,14,15,16,17,18,19,20,21} out of 124 articles met the inclusion criteria and were included in the systematic review. Those not included are listed in Appendix 2. Reasons for which they were not included are summarised in Table 1.

Study Characteristics

All the included studies were RCTs ranging from 6 to 36 months duration. Five of the trials were of 6 months’ duration; six of 12 months’. One trial lasted 17 months and another lasted 36 months. As there was only one study lasting 17 months\textsuperscript{10} and one lasting 36 months\textsuperscript{11} data reported at that time point in that study was not included in the analysis. All of the studies were designed to reduce or prevent weight gain and also examined cardiovascular disease risk factors.

Ten of the studies compared low carbohydrate/high protein diets with low fat/high carbohydrate diets, and two studies compared medium protein diets with high protein diets. Table 2 gives a summary of the diets and carbohydrate content for each of the studies.

Participant Characteristics

A total of 1222 volunteers were recruited between the 13 studies. Figure 1 shows the percentage attrition rates. Out of the 1222 participants assigned to the diets, there were 441 (36\%) attritions during the interventions. There was a higher attrition rate in
the conventional/low fat/medium protein groups compared to the low carbohydrate/high protein intervention groups. The difference in attrition rates between the 2 groups was significant ($p = 0.001$) after performing a chi-squared test.

Quality of Trials

For the following variables, the LC/HP refers to the low carbohydrate/high protein intervention groups and the LF/HC refers to the low fat/high carbohydrate comparison/control groups.

Weight

The weighted mean difference (WMD) in weight change was -4.02 kg in favour of the LC/HP group at 6 months (Fig 2a) ($p < 0.00001$). At 12 months this difference had fallen to only -1.05 kg ($p < 0.05$) (Fig 2b). There were differences ($p < 0.0001$) amongst the studies at 6 months, but agreement shown by lack of heterogeneity at 12 months.

Total Cholesterol

The WMD in total cholesterol change was 0.19 mmol/L at 6 months ($p < 0.0001$) with the LC/HP group demonstrating the increased cholesterol (Fig 3a). This was also the case at 12 months, though the difference between the groups was smaller and not significant (0.10 mmol/L, $p = 0.31$) (Fig 3b). There were no differences amongst the studies at 6 ($p = 0.84$) and 12 ($p = 0.14$) months.
LDL-Cholesterol

The WMD in LDL cholesterol change was 0.14 mmol/L at 6 months ($p < 0.00001$) with the LC/HP group demonstrating the increased LDL cholesterol (Fig 4a). The difference between the groups was greater at 12 months (0.37 mmol/L) ($p < 0.00001$) with the LC/HP group again demonstrating the increased LDL cholesterol (Fig 4b). There were no differences amongst the studies at 6 months ($p = 0.65$), but there were differences found between the studies at 12 months ($p < 0.00001$).

HDL-Cholesterol

The WMD in HDL cholesterol change was 0.04 mmol/l at 6 months ($p = 0.03$) favouring the LC/HP group (Fig 5a). There was a slightly greater increase in the WMD in HDL cholesterol at 12 months (0.06 mmol/L) favouring the LC/HP group ($p < 0.05$). There were no differences found between the studies at 6 months ($p = 0.46$) or 12 months ($p = 0.49$).

Triacylglycerol

The WMD in triacylglycerol was -0.17 mmol/L at 6 months ($p = 0.0001$) favouring the LC/HP group (Fig 6a). At 12 months the WMD between the groups was -0.19 mmol/L favouring the LC/HP group ($p = 0.04$). Again, there was evidence of heterogeneity across the groups ($p = 0.01$).
Systolic Blood Pressure

The WMD drop in systolic blood pressure of -1.35 mmHg at 6 months favouring the LC/HP group was not significant (Fig 7a). At 12 months the WMD between the groups was a decrease of 2.19 mmHg favouring the LC/HP group ($p =0.05$) (Fig 7b). There was no difference between the studies at either time.

Diastolic Blood Pressure

The WMD decrease in diastolic blood pressure of 0.49 mmHg at 6 months favouring the LC/HP group was not significant (Fig 8a). At 12 months, the WMD between the 2 groups of 0.81 mmHg lowering favouring the LC/HP group was greater, but was also not significant (Fig 8b). There was no evidence of statistical heterogeneity across the studies at either time.

Fasting Plasma Glucose

The WMD between the groups in fasting plasma glucose was not significant and there was no evidence of statistical heterogeneity at either time (Fig 9).

**DISCUSSION**

The results of the present review show that weight loss was significantly greater in the LC/HP (treatment) group after 6 and 12 months compared to the LF/HC group. The difference was greater at 6 months and at that time there was significant heterogeneity
amongst the studies, probably due to the different study designs but at 12 months the heterogeneity was no longer significant. The 36 month follow up by Cardillo et al \(^1\) reported that mean weight change between baseline and 36 months was not different between the LC/HP and the LF/HC group. However, they do report that between 6 and 36 months, weight was unchanged for the LF/HC group but that subjects on the LC/HP approach regained weight, but this change was not significant.

Avenell \textit{et al} \(^2\) examined the effects of a protein sparing modified fast (PSMF) compared to a low calorie diet and a very low calorie diet. A PSMF is a low carbohydrate diet, which allows a maximum of 40 g of carbohydrate/day. The review examined weight loss comparing the PSMF to low calorie diets after 12, 18, 24, 36, and 60 months. There was a greater weight loss favouring the PSMF group compared to the control after 12, 24 and 36 months, but only seven RCTs were included in this analysis, which included a total of 480 participants \(^2\). These results are consistent with the results of the present systematic review.

A review by Nordmann \textit{et al} \(^3\) comparing low carbohydrate diets vs. low fat diets showed significant weight loss with the low carbohydrate group at 6 months, but not at 12 months. The meta-regression by Krieger \textit{et al} \(^4\) also report a greater weight loss in addition to a greater body fat and percentage body fat loss in studies lasting more than 3 months. Bravata \textit{et al} \(^5\), however, showed no significant differences in weight loss for both groups at either 6 or 12 months, but this review included studies with dietary approaches that are not considered low carbohydrate, which may have affected their outcomes/findings).

The present review showed that there was a significant improvement in HDL cholesterol and triacylglycerols at 6 and 12 months favouring the LC/HP group, but this was not significant at 17 months. The lack of significance at 17 months may be
caused by the reintroduction of carbohydrates in the LC/HP group. There was heterogeneity between the studies for triacylglycerols, but this may have been due to differences in study design.

Low HDL cholesterol and raised triacylglycerol levels are risk factors for cardiovascular disease and impact on the atherogenicity of the LDL particle and these results indicate a LC/HP diet may be a better approach to weight loss and lowering the risk of cardiovascular disease. These results are consistent with the review carried out by Nordmann et al. However Bravata et al did not show any significant improvement in these parameters, which again, may have been affected by their choice of studies.

The present review showed a significant improvement in total cholesterol and LDL cholesterol favouring the LF/HC group at 6 months, at which point total cholesterol and LDL cholesterol increased more in the LC/HP group but not at 12 months or 17 months. Nordmann et al, in a meta-analysis of low carbohydrate vs. low-fat diets found reports on 4 groups of patients demonstrating an improvement in total and LDL cholesterol favouring low-fat diets rather than low-carbohydrate diets. This finding is consistent with the studies included in the present review. An elevated total cholesterol could in part be explained by an increase in HDL cholesterol observed in the LC/HP group. Also, although an elevated LDL cholesterol increases the risk of acute cardiovascular events, we have just shown evidence that LC/HP increase HDL and decrease triacylglycerol which impacts on the atherogenicity of the LDL particle. These studies failed to investigate changes in LDL particle size. Furthermore, evidence from Sharman et al suggest that on a LC/HP LDL particle sizes change from small to large and therefore resulting in a less atherogenic profile.
There was a trend towards improvement in diastolic and systolic blood pressure at 6, 12 and 17 months favouring the LC/HP group. The difference was significant at 12 months favouring the LC/HP group for systolic blood pressure. Bravata et al.\textsuperscript{25} reported no change in systolic blood pressure after the low and very low carbohydrate diets\textsuperscript{25}. Nordmann \textit{et al}.\textsuperscript{23} showed no significant difference in blood pressure at any timepoint.

At 6 months there was a trend towards improvement in fasting plasma glucose only slightly favouring the LF/HC group in which there was a greater decrease in fasting plasma glucose in the LF/HC group. This was surprising when compared to the review by Layman \textit{et al}. where there is clear evidence of improvements in fasting glucose, postprandial glucose and insulin responses and HbA1c for individuals on a low carbohydrate/high protein diet\textsuperscript{6}. At 12 months, the opposite occurred in which there was a greater decrease in fasting plasma glucose, favouring the LC/HP group. The difference was not significant at 6, 12 and 17 months. Bravata \textit{et al}.\textsuperscript{25} reported no change in fasting serum glucose among recipients of the low and very low carbohydrate diets. Nordmann \textit{et al}.\textsuperscript{23} showed a greater improvement in fasting plasma glucose favouring the low carbohydrate group at 6 months, but this was no longer significant at 12 months.

Fasting glucose provides a limited assessment of overall glycaemic status; therefore, future studies should use HbA1c values or more direct measurements of insulin sensitivity.

There was a higher attrition rate in the LF/HC compared to the LC/HP groups (Figure 1). Reasons for attrition included difficulty in complying with the diet or disliking the diet, difficulty in maintaining the scheduled visits, and significant events such as pregnancy and surgery.
Limitations

It is important to take account of attrition rates in the interpretation of outcomes as high attrition rates lead to a smaller statistical power. An intention to treat approach is commonly used to overcome attrition rates and possible bias in the outcomes. There are, however, limitations when using this approach in lifestyle trials as the intention to treat approach has been derived from drug trials and may not yield robust outcomes. This results in the need for higher retention rates to assess for real changes in response to the dietary interventions.

In addition, the use of a randomised controlled trial design in dietary interventions may not be appropriate. In general, any weight loss strategy has a maximum weight loss at 6 months followed by a return to initial weight. It is clear that patients are changing their treatment by their own accord, perhaps subconsciously or perhaps due to a metabolic response of the body aiming to return to its initial weight. The current thinking within the field of obesity suggests the use of continuous improvement methodology may be more appropriate for weight loss management.

Also there was some evidence of heterogeneity between the studies included in this analysis. This calls for the use of more consistent and robust study designs for which we have to establish a clear definition of a low carbohydrate diet/high protein diet.

CONCLUSION

This systematic review included all known randomized control trials of low carbohydrate diets versus the low fat/high carbohydrate diet, from 2000 to 2007.
Factors including weight, cholesterol, blood pressure and glycemic control were evaluated, as these are important in weight loss and cardiovascular disease risk.

Evidence from this systematic review demonstrates that low carbohydrate/high protein diets are more effective at 6 months and are as, if not more, effective than low fat diets in reducing weight and cardiovascular disease risk up to 1 year. As there were only 13 studies included, and several of them allowed the reintroduction of carbohydrates in the low carbohydrate/high protein diet, the evidence of the long term efficacy of these diets is not complete. Certainly at 6 months, the evidence is in favour of the use of low carbohydrate/high protein diet. It may not be appropriate to return to a high carbohydrate intake for weight maintenance. A gradual reintroduction while still limiting the intake of carbohydrate may be more appropriate.

With the prevalence of obesity increasing there is a need for larger and long term RCTs of low/very low carbohydrate diets compared to the low fat/high carbohydrate diets to be carried out. The influence of behavioural therapy and exercise interventions needs to be evaluated, as well as, lifestyle, appetite, and mood questionnaires.

It is not known with certainty which aspect of low carbohydrate diets causes the weight loss and cardiovascular disease risk factor changes. Whether it is the low carbohydrate, the high protein or calorie restriction needs to be examined. In addition, there is a need to assess if the greater weight loss achieved at 6 months on a low carbohydrate/high protein diet results in more important long term improvements of cardiovascular disease.

There is a need for trials to include a follow-up period, to examine adherence to the low carbohydrate diets, and whether participants maintain their weight loss and CVD.
risk factor change when there is minimum contact with the study investigators. Finally, taking account of high attrition rates when using randomised controlled trials for dietary and lifestyle interventions, perhaps we will witness a move towards a continuous improvement methodology in the future.
Figure 1

Percentage attrition rate in low-carbohydrate (white) and low-fat diets (black) reported in the literature.
Figure 2: Weight at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>-8.50 (1.00)</td>
<td>-3.90 (1.00)</td>
<td>-4.60 (3.99)</td>
<td>74.27</td>
<td>-4.60 [-5.21, -3.99]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>-8.10 (8.20)</td>
<td>-8.50 (6.10)</td>
<td>-0.40 (6.76)</td>
<td>1.45</td>
<td>0.40 [-3.94, 4.76]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>-3.20 (6.90)</td>
<td>-3.50 (5.60)</td>
<td>0.30 (2.61)</td>
<td>5.12</td>
<td>0.30 [-2.01, 2.61]</td>
</tr>
<tr>
<td>Due</td>
<td>-9.40 (8.50)</td>
<td>-5.90 (7.50)</td>
<td>-3.50 (1.13)</td>
<td>1.27</td>
<td>-3.50 [-8.13, 1.13]</td>
</tr>
<tr>
<td>Foster</td>
<td>-6.90 (6.50)</td>
<td>-3.10 (5.60)</td>
<td>-3.80 (0.81)</td>
<td>3.05</td>
<td>-3.80 [-6.79, -0.81]</td>
</tr>
<tr>
<td>Samaha</td>
<td>-5.80 (8.60)</td>
<td>-1.90 (4.20)</td>
<td>-3.90 (1.57)</td>
<td>5.01</td>
<td>-3.90 [-6.23, -1.57]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>-8.50 (9.30)</td>
<td>-3.50 (4.30)</td>
<td>-5.00 (-1.78)</td>
<td>2.63</td>
<td>-5.00 [-8.22, -1.78]</td>
</tr>
<tr>
<td>Truby</td>
<td>-6.00 (6.40)</td>
<td>-6.60 (5.40)</td>
<td>0.60 (3.11)</td>
<td>4.31</td>
<td>0.60 [-1.91, 3.11]</td>
</tr>
<tr>
<td>Yancy</td>
<td>-12.00 (9.30)</td>
<td>-6.50 (7.70)</td>
<td>-5.50 (-2.43)</td>
<td>2.89</td>
<td>-5.50 [-8.57, -2.43]</td>
</tr>
</tbody>
</table>

Total (95% CI) 345

Test for heterogeneity: Chi² = 35.31, df = 8 (P < 0.0001), I² = 77.3%
Test for overall effect: Z = 15.08 (P < 0.00001)

-10 -5 0 5 10
Favours treatment Favours control

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>-2.10 (4.80)</td>
<td>-3.00 (4.90)</td>
<td>0.90 (3.03)</td>
<td>24.02</td>
<td>0.90 [-1.23, 3.03]</td>
</tr>
<tr>
<td>Due</td>
<td>-6.20 (7.60)</td>
<td>-6.30 (7.10)</td>
<td>0.10 (2.62)</td>
<td>5.32</td>
<td>-1.90 [-6.42, 2.62]</td>
</tr>
<tr>
<td>Foster</td>
<td>-4.20 (6.76)</td>
<td>-2.45 (6.31)</td>
<td>-1.75 (-4.98, 1.48)</td>
<td>10.42</td>
<td>-1.75 [-4.98, 1.48]</td>
</tr>
<tr>
<td>Gardner</td>
<td>-4.70 (7.20)</td>
<td>-2.20 (6.50)</td>
<td>-2.50 (-4.65, -0.35)</td>
<td>23.38</td>
<td>-2.50 [-4.65, -0.35]</td>
</tr>
<tr>
<td>Stem</td>
<td>-5.10 (8.70)</td>
<td>-3.10 (8.40)</td>
<td>-2.00 (-5.59, 1.59)</td>
<td>8.40</td>
<td>-2.00 [-5.59, 1.59]</td>
</tr>
<tr>
<td>Truby</td>
<td>-9.00 (6.10)</td>
<td>-9.10 (6.20)</td>
<td>0.10 (2.70)</td>
<td>16.00</td>
<td>0.10 [-2.50, 2.70]</td>
</tr>
<tr>
<td>Total</td>
<td>-5.10 (8.70)</td>
<td>-3.10 (8.40)</td>
<td>-2.00 (0.95)</td>
<td>12.45</td>
<td>-2.00 [-4.95, 0.95]</td>
</tr>
</tbody>
</table>

Total (95% CI) 309

Test for heterogeneity: Chi² = 6.71, df = 6 (P = 0.35), I² = 10.5%
Test for overall effect: Z = 1.98 (P = 0.05)
<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22 -0.02 (1.08)</td>
<td>20 -0.04 (1.08)</td>
<td>1.84</td>
<td>0.02</td>
<td>[-0.63, 0.67]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21 0.00 (1.08)</td>
<td>22 -0.20 (1.08)</td>
<td>1.88</td>
<td>0.20</td>
<td>[-0.45, 0.85]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40 -0.02 (0.66)</td>
<td>40 -0.20 (0.56)</td>
<td>16.25</td>
<td>0.18</td>
<td>[-0.04, 0.40]</td>
</tr>
<tr>
<td>Due</td>
<td>23 -0.33 (1.08)</td>
<td>23 0.03 (1.08)</td>
<td>2.02</td>
<td>0.34</td>
<td>[-0.36, 0.28]</td>
</tr>
<tr>
<td>Foster</td>
<td>33 0.12 (0.24)</td>
<td>30 -0.11 (0.24)</td>
<td>55.76</td>
<td>0.23</td>
<td>[0.11, 0.35]</td>
</tr>
<tr>
<td>Samaha</td>
<td>64 0.05 (1.08)</td>
<td>68 -0.02 (1.08)</td>
<td>5.78</td>
<td>0.07</td>
<td>[-0.30, 0.44]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>43 0.07 (1.00)</td>
<td>35 -0.07 (1.10)</td>
<td>3.53</td>
<td>0.14</td>
<td>[-0.33, 0.61]</td>
</tr>
<tr>
<td>Truby</td>
<td>40 -0.29 (0.80)</td>
<td>47 -0.55 (0.70)</td>
<td>7.73</td>
<td>0.26</td>
<td>[-0.06, 0.58]</td>
</tr>
<tr>
<td>Yancy</td>
<td>59 -0.21 (1.08)</td>
<td>60 -0.35 (1.08)</td>
<td>5.21</td>
<td>0.14</td>
<td>[-0.25, 0.53]</td>
</tr>
</tbody>
</table>

Total (95% CI) 345 345 100.00 0.19 [0.10, 0.28]

Test for heterogeneity: Ch² = 4.17, df = 8 (P = 0.84), I² = 0%
Test for overall effect: Z = 4.23 (P < 0.0001)

-1 -0.5 0 0.5 1
Favours treatment Favours control

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40 -0.11 (0.59)</td>
<td>40 -0.21 (0.62)</td>
<td>55.78</td>
<td>0.10</td>
<td>[-0.17, 0.37]</td>
</tr>
<tr>
<td>Due</td>
<td>23 0.10 (1.08)</td>
<td>18 0.68 (1.08)</td>
<td>8.84</td>
<td>-0.58</td>
<td>[-1.25, 0.09]</td>
</tr>
<tr>
<td>Foster</td>
<td>33 0.10 (1.08)</td>
<td>30 -0.03 (1.08)</td>
<td>13.76</td>
<td>0.13</td>
<td>[-0.40, 0.66]</td>
</tr>
<tr>
<td>Stern</td>
<td>44 0.13 (1.11)</td>
<td>43 -0.21 (0.91)</td>
<td>21.61</td>
<td>0.37</td>
<td>[-0.06, 0.80]</td>
</tr>
</tbody>
</table>

Total (95% CI) 140 131 100.00 0.10 [-0.10, 0.30]

Test for heterogeneity: Ch² = 5.56, df = 3 (P = 0.14), I² = 46.0%
Test for overall effect: Z = 1.01 (P = 0.31)

-1 -0.5 0 0.5 1
Favours treatment Favours control
**Figure 4:** LDL cholesterol at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval.

### a) Treatment vs. Control at 6 Months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment N</th>
<th>Mean (SD)</th>
<th>Control N</th>
<th>Mean (SD)</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>-0.02 (0.29)</td>
<td>20</td>
<td>-0.15 (0.29)</td>
<td>11.48</td>
<td>0.13</td>
<td>[-0.05, 0.31]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>-0.20 (0.29)</td>
<td>22</td>
<td>-0.30 (0.29)</td>
<td>11.77</td>
<td>0.10</td>
<td>[-0.07, 0.27]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-0.06 (0.36)</td>
<td>40</td>
<td>-0.18 (0.62)</td>
<td>7.17</td>
<td>0.12</td>
<td>[-0.10, 0.34]</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>0.08 (0.33)</td>
<td>30</td>
<td>-0.04 (0.40)</td>
<td>10.67</td>
<td>0.12</td>
<td>[-0.06, 0.30]</td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>0.04 (0.57)</td>
<td>63</td>
<td>-0.04 (0.40)</td>
<td>12.83</td>
<td>0.08</td>
<td>[-0.09, 0.25]</td>
</tr>
<tr>
<td>Samaha</td>
<td>64</td>
<td>0.10 (0.59)</td>
<td>68</td>
<td>0.07 (0.46)</td>
<td>10.78</td>
<td>0.03</td>
<td>[-0.15, 0.21]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>63</td>
<td>0.18 (0.98)</td>
<td>35</td>
<td>0.15 (0.64)</td>
<td>2.71</td>
<td>0.03</td>
<td>[-0.33, 0.39]</td>
</tr>
<tr>
<td>Yancy</td>
<td>59</td>
<td>0.04 (0.29)</td>
<td>60</td>
<td>-0.19 (0.29)</td>
<td>32.59</td>
<td>0.23</td>
<td>[0.13, 0.33]</td>
</tr>
</tbody>
</table>

Total (95% CI): 352 vs. 338
Test for heterogeneity: $\chi^2 = 5.42, df = 7$ (P = 0.61), $I^2 = 0$
Test for overall effect: Z = 4.53 (P < 0.00001)

### b) Treatment vs. Control at 12 Months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment N</th>
<th>Mean (SD)</th>
<th>Control N</th>
<th>Mean (SD)</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foster</td>
<td>33</td>
<td>0.01 (0.42)</td>
<td>30</td>
<td>-0.09 (0.31)</td>
<td>22.49</td>
<td>0.10</td>
<td>[-0.08, 0.28]</td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>0.58 (0.02)</td>
<td>63</td>
<td>0.01 (0.44)</td>
<td>62.44</td>
<td>0.57</td>
<td>[0.46, 0.68]</td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>0.18 (0.91)</td>
<td>43</td>
<td>-0.10 (0.75)</td>
<td>6.03</td>
<td>0.28</td>
<td>[-0.07, 0.63]</td>
</tr>
<tr>
<td>Tsai</td>
<td>64</td>
<td>-0.10 (0.75)</td>
<td>65</td>
<td>0.18 (0.90)</td>
<td>9.05</td>
<td>-0.28</td>
<td>[-0.57, 0.01]</td>
</tr>
</tbody>
</table>

Total (95% CI): 211 vs. 201
Test for heterogeneity: $\chi^2 = 41.66, df = 3$ (P < 0.00001), $I^2 = 92.8$
Test for overall effect: Z = 8.44 (P < 0.00001)
Figure 5: HDL cholesterol 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Control N</th>
<th>Control Mean (SD)</th>
<th>Treatment N</th>
<th>Treatment Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>0.18 (0.74)</td>
<td>20</td>
<td>0.10 (0.74)</td>
<td>0.60</td>
<td>0.08</td>
<td>[-0.37, 0.53]</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>0.00 (0.74)</td>
<td>22</td>
<td>0.04 (0.74)</td>
<td>0.62</td>
<td>-0.04</td>
<td>[-0.48, 0.60]</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>0.09 (0.16)</td>
<td>40</td>
<td>0.06 (0.23)</td>
<td>16.10</td>
<td>0.03</td>
<td>[-0.06, 0.12]</td>
</tr>
<tr>
<td>Due</td>
<td>23</td>
<td>-0.03 (0.74)</td>
<td>23</td>
<td>0.23 (0.74)</td>
<td>0.66</td>
<td>-0.26</td>
<td>[-0.65, 0.17]</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>0.17 (0.53)</td>
<td>30</td>
<td>0.03 (0.31)</td>
<td>2.70</td>
<td>0.14</td>
<td>[-0.07, 0.35]</td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>0.13 (0.24)</td>
<td>63</td>
<td>0.05 (0.17)</td>
<td>24.65</td>
<td>0.08</td>
<td>[0.01, 0.15]</td>
</tr>
<tr>
<td>Samaha</td>
<td>64</td>
<td>0.00 (0.12)</td>
<td>66</td>
<td>-0.02 (0.18)</td>
<td>45.04</td>
<td>0.02</td>
<td>[-0.03, 0.07]</td>
</tr>
<tr>
<td>Seshadri</td>
<td>63</td>
<td>-0.02 (0.20)</td>
<td>35</td>
<td>-0.02 (0.33)</td>
<td>7.82</td>
<td>0.00</td>
<td>[-0.12, 0.12]</td>
</tr>
<tr>
<td>Yancy</td>
<td>59</td>
<td>0.14 (0.74)</td>
<td>66</td>
<td>-0.04 (0.74)</td>
<td>1.80</td>
<td>0.18</td>
<td>[-0.08, 0.44]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>375</td>
<td></td>
<td>367</td>
<td></td>
<td>100.00</td>
<td>0.04</td>
<td>[0.00, 0.07]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 6.28, df = 8 (P = 0.62), I² = 0%
Test for overall effect: Z = 2.20 (P = 0.03)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Control N</th>
<th>Control Mean (SD)</th>
<th>Treatment N</th>
<th>Treatment Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>0.08 (0.18)</td>
<td>40</td>
<td>0.08 (0.25)</td>
<td>20.65</td>
<td>0.00</td>
<td>[-0.10, 0.10]</td>
</tr>
<tr>
<td>Due</td>
<td>23</td>
<td>0.12 (0.74)</td>
<td>18</td>
<td>-0.09 (0.74)</td>
<td>0.90</td>
<td>0.21</td>
<td>[-0.25, 0.67]</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>0.13 (0.50)</td>
<td>30</td>
<td>0.02 (0.28)</td>
<td>4.81</td>
<td>0.11</td>
<td>[-0.09, 0.31]</td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>0.12 (0.23)</td>
<td>63</td>
<td>0.07 (0.19)</td>
<td>36.86</td>
<td>0.05</td>
<td>[-0.02, 0.12]</td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>-0.03 (0.18)</td>
<td>43</td>
<td>-0.13 (0.16)</td>
<td>36.78</td>
<td>0.10</td>
<td>[0.03, 0.17]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>210</td>
<td></td>
<td>194</td>
<td></td>
<td>100.00</td>
<td>0.06</td>
<td>[0.02, 0.11]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 3.44, df = 4 (P = 0.49), I² = 0%
Test for overall effect: Z = 2.82 (P = 0.005)
### a) Figure 6: Triacylglycerols at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment</th>
<th>N</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td>Mean (SD)</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Brehm</td>
<td>-3.93 (0.96)</td>
<td>22</td>
<td>0.19 (0.96)</td>
<td>2.06 [-6.12]</td>
<td>2.12 [-6.00]</td>
<td>-6.70, -3.54</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>-0.50 (0.96)</td>
<td>21</td>
<td>-0.10 (0.96)</td>
<td>2.12 [-6.00]</td>
<td>2.12 [-6.00]</td>
<td>-6.00, -0.17</td>
</tr>
<tr>
<td>Dansinger</td>
<td>-0.12 (0.65)</td>
<td>40</td>
<td>0.00 (0.65)</td>
<td>12.37 [0.00]</td>
<td>0.12 [-6.00]</td>
<td>-6.26, -0.26</td>
</tr>
<tr>
<td>Due</td>
<td>-0.15 (0.96)</td>
<td>23</td>
<td>0.11 (0.96)</td>
<td>2.27 [-0.26]</td>
<td>0.26 [-0.26]</td>
<td>-0.52, 0.29</td>
</tr>
<tr>
<td>Foster</td>
<td>2.20 (3.30)</td>
<td>33</td>
<td>-1.00 (2.10)</td>
<td>0.38 [3.20]</td>
<td>0.20 [-0.70, 0.20]</td>
<td></td>
</tr>
<tr>
<td>Gardiner</td>
<td>-0.40 (0.72)</td>
<td>70</td>
<td>0.18 (0.72)</td>
<td>14.66 [-0.66]</td>
<td>0.66 [-0.66, 0.00]</td>
<td></td>
</tr>
<tr>
<td>Samaha</td>
<td>-4.20 (9.00)</td>
<td>64</td>
<td>0.70 (9.00)</td>
<td>0.10 [-3.50]</td>
<td>-3.50 [-6.13, -0.87]</td>
<td></td>
</tr>
<tr>
<td>Seshadri</td>
<td>-0.01 (0.23)</td>
<td>43</td>
<td>0.00 (0.23)</td>
<td>60.19 [-0.01]</td>
<td>0.01 [-0.12, 0.10]</td>
<td></td>
</tr>
<tr>
<td>Yancy</td>
<td>-0.84 (0.96)</td>
<td>59</td>
<td>0.31 (0.96)</td>
<td>5.86 [-0.53]</td>
<td>0.53 [-0.87, -0.19]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>375</td>
<td>361</td>
<td>100.00</td>
<td>-0.16 [-0.24, -0.08]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: CH² = 222.85, df = 8 (P < 0.00001), I² = 96.4%
Test for overall effect: Z = 3.76 (P = 0.0002)

### b) Figure 6: Triacylglycerols at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment</th>
<th>N</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td>Mean (SD)</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Dansinger</td>
<td>-0.01 (0.94)</td>
<td>40</td>
<td>-0.14 (0.68)</td>
<td>23.51 [0.13]</td>
<td>0.13 [-0.23, 0.49]</td>
<td></td>
</tr>
<tr>
<td>Due</td>
<td>-0.05 (0.96)</td>
<td>23</td>
<td>0.33 (0.96)</td>
<td>8.67 [-0.38]</td>
<td>-0.38 [-0.97, 0.21]</td>
<td></td>
</tr>
<tr>
<td>Foster</td>
<td>-2.50 (2.50)</td>
<td>33</td>
<td>0.09 (4.20)</td>
<td>1.02 [-2.41]</td>
<td>-2.41 [-6.14, -0.68]</td>
<td></td>
</tr>
<tr>
<td>Gardiner</td>
<td>-0.33 (0.66)</td>
<td>70</td>
<td>0.16 (0.68)</td>
<td>5.84 [-0.17]</td>
<td>-0.17 [-0.40, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Stern</td>
<td>-0.65 (1.78)</td>
<td>44</td>
<td>0.05 (0.96)</td>
<td>8.47 [-0.70]</td>
<td>-0.70 [-1.30, -0.10]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>210</td>
<td>194</td>
<td>100.00</td>
<td>-0.19 [-0.36, -0.01]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: CH² = 12.59, df = 4 (P = 0.01), I² = 68.2%
Test for overall effect: Z = 2.08 (P = 0.04)
Figure 7: Systolic blood pressure at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval

### a) Systolic blood pressure change at 6 months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>20</td>
<td>6.12</td>
<td>2.00</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>22</td>
<td>6.28</td>
<td>-1.30</td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>40</td>
<td>12.73</td>
<td>6.10</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>30</td>
<td>10.34</td>
<td>-3.90</td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>79</td>
<td>49.51</td>
<td>-2.10</td>
</tr>
<tr>
<td>Truby</td>
<td>60</td>
<td>67</td>
<td>15.01</td>
<td>-3.10</td>
</tr>
</tbody>
</table>

Total (95% CI): 233 - 238, 100.00 - 1.35

Test for heterogeneity: Chi² = 6.24, df = 5 (P = 0.28), I² = 19.9%
Test for overall effect: Z = 1.39 (P = 0.17)

### b) Systolic blood pressure change at 12 months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>WMD (fixed)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>40</td>
<td>15.51</td>
<td>2.90</td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>30</td>
<td>16.59</td>
<td>-2.21</td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>79</td>
<td>45.53</td>
<td>-4.50</td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>43</td>
<td>9.03</td>
<td>-1.00</td>
</tr>
<tr>
<td>Tsai</td>
<td>64</td>
<td>65</td>
<td>13.34</td>
<td>-1.00</td>
</tr>
</tbody>
</table>

Total (95% CI): 258 - 257, 100.00 - 2.19

Test for heterogeneity: Chi² = 5.57, df = 5 (P = 0.26), I² = 28.2%
Test for overall effect: Z = 1.99 (P = 0.05)
Figure 8: Diastolic blood pressure at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval.

### a) Diastolic blood pressure at 6 months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment N</th>
<th>Treatment Mean (SD)</th>
<th>Control N</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>5.00 (8.30)</td>
<td>20</td>
<td>1.00 (8.30)</td>
<td>7.24 [-1.03, 9.03]</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>-1.70 (8.30)</td>
<td>22</td>
<td>-1.60 (8.30)</td>
<td>7.43 [-5.06, 4.86]</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-6.00 (6.50)</td>
<td>40</td>
<td>-1.80 (6.90)</td>
<td>21.19 [-5.18, 0.76]</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>2.00 (12.70)</td>
<td>30</td>
<td>-1.10 (14.20)</td>
<td>4.10 [-3.58, 9.78]</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>-3.30 (6.90)</td>
<td>79</td>
<td>-2.50 (5.80)</td>
<td>45.60 [-2.80, 1.20]</td>
<td>-0.80</td>
<td></td>
</tr>
<tr>
<td>Truby</td>
<td>40</td>
<td>-4.90 (8.30)</td>
<td>47</td>
<td>-6.40 (6.60)</td>
<td>14.45 [-4.06, 3.06]</td>
<td>-0.50</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>233</strong></td>
<td></td>
<td><strong>238</strong></td>
<td></td>
<td><strong>100.00</strong> [-0.49, 0.86]</td>
<td>1.1%</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 5.59, df = 5 (P = 0.35), I² = 10.6%
Test for overall effect: Z = 0.72 (P = 0.47)

### b) Diastolic blood pressure at 12 months

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment N</th>
<th>Treatment Mean (SD)</th>
<th>Control N</th>
<th>Control Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-1.40 (7.50)</td>
<td>40</td>
<td>-1.70 (6.40)</td>
<td>29.78 [-2.76, 3.36]</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Foster</td>
<td>33</td>
<td>-2.70 (12.40)</td>
<td>30</td>
<td>-2.90 (6.70)</td>
<td>11.76 [-4.66, 5.06]</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>77</td>
<td>-4.40 (8.40)</td>
<td>79</td>
<td>-2.20 (6.70)</td>
<td>48.74 [-4.59, 0.19]</td>
<td>-0.20</td>
<td></td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>3.00 (15.00)</td>
<td>43</td>
<td>1.00 (10.00)</td>
<td>9.73 [-3.35, 7.35]</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>194</strong></td>
<td></td>
<td><strong>192</strong></td>
<td></td>
<td><strong>100.00</strong> [-0.76, 0.90]</td>
<td>1.1%</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 3.03, df = 3 (P = 0.39), I² = 1.1%
Test for overall effect: Z = 0.90 (P = 0.37)
### Figure 9: Fasting plasma glucose at 6 (a) and 12 (b) months. WMD, weighted mean difference; CI, confidence interval

**Review:** Systematic Review April 2008  
**Comparison:** 15 Fasting plasma glucose change at 6 months  
**Outcome:** 01 Fasting plasma glucose change at 6 months

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment N</th>
<th>Mean (SD)</th>
<th>Control N</th>
<th>Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brehm</td>
<td>22</td>
<td>0.50 (1.35)</td>
<td>20</td>
<td>-0.19 (1.35)</td>
<td>3.00 [-0.13, 1.51]</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Brinkworth</td>
<td>21</td>
<td>0.10 (1.35)</td>
<td>22</td>
<td>-0.10 (1.35)</td>
<td>3.08 [-0.61, 1.01]</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>-0.63 (1.40)</td>
<td>40</td>
<td>-0.22 (1.20)</td>
<td>6.16 [-0.78, 0.35]</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Due</td>
<td>23</td>
<td>0.00 (1.35)</td>
<td>18</td>
<td>0.00 (1.35)</td>
<td>2.89 [-0.83, 0.83]</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>0.01 (0.42)</td>
<td>63</td>
<td>-0.04 (0.54)</td>
<td>73.06 [-0.12, 0.22]</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Samaha</td>
<td>66</td>
<td>-0.61 (1.30)</td>
<td>68</td>
<td>-0.11 (1.10)</td>
<td>11.82 [-0.91, -0.09]</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 240 231 100.00 -0.01 [-0.15, 0.13]

Test for heterogeneity: Ch² = 9.53, df = 5 (P = 0.09), I² = 47.5%
Test for overall effect: Z = 0.13 (P = 0.90)

---

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment N</th>
<th>Mean (SD)</th>
<th>Control N</th>
<th>Mean (SD)</th>
<th>WMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>WMD (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansinger</td>
<td>40</td>
<td>0.07 (1.66)</td>
<td>40</td>
<td>-0.26 (1.05)</td>
<td>6.80 [-0.28, 0.94]</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Due</td>
<td>23</td>
<td>0.10 (1.35)</td>
<td>18</td>
<td>0.30 (1.35)</td>
<td>3.63 [-1.03, 0.83]</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Gardner</td>
<td>70</td>
<td>-0.09 (0.74)</td>
<td>63</td>
<td>0.02 (0.51)</td>
<td>54.87 [-0.11, 0.10]</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Stern</td>
<td>44</td>
<td>0.17 (0.61)</td>
<td>43</td>
<td>0.17 (0.67)</td>
<td>34.70 [-0.27, 0.27]</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 177 164 100.00 -0.05 [-0.20, 0.11]

Test for heterogeneity: Ch² = 2.05, df = 3 (P = 0.56), I² = 0%
Test for overall effect: Z = 0.56 (P = 0.58)

---

**Review:** Systematic Review April 2008  
**Comparison:** 16 Fasting plasma glucose change at 12 months  
**Outcome:** 01 Fasting plasma glucose change at 12 months

---
Assumptions used for missing standard deviations.  

- SD of weight change in kg = 5.915 + (0.283 x mean change in weight)

Similar linear regression was also used for risk factors, but relationships were not found, so the means of reported SDs were used to supply missing SDs.

- SD for change in triacylglycerols (TGs) = 0.96 mmol L\(^{-1}\)
- SD for change in total cholesterol = 1.08 mmol L\(^{-1}\)
- SD for change in HDL cholesterol = 0.74 mmol L\(^{-1}\)
- SD for change in LDL cholesterol = 0.29 mmol L\(^{-1}\)
- SD for change in systolic blood pressure = 12.7 mmHg
- SD for change in diastolic blood pressure = 8.3 mmHg

The following equations were used for calculating missing SDs for fasting plasma glucose and HbA1c.

- If the initial fasting plasma glucose was <7 mmol L\(^{-1}\), the SD for change in fasting plasma glucose was 1.35 mmol L\(^{-1}\).
- If the initial fasting plasma glucose was ≥7 mmol L\(^{-1}\), the SD for change in fasting plasma glucose was 3.77 mmol L\(^{-1}\).
Systematic Review

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APPENDIX 2: CONFERENCE ABSTRACTS AND POSTERS
SYSTEMATIC REVIEW OF RANDOMIZED CONTROL TRIALS OF LOW CARBOHYDRATE VS LOW FAT DIETS IN THE MANAGEMENT OF OBESITY AND ITS COMORBIDITIES

M.Hession1, C.Rolland1, A.Avenell2, A.Wise1, I.Broom2

1School of Life Sciences, Robert Gordon University; 2University of Aberdeen, Aberdeen, Scotland.

INTRODUCTION:
Obesity has become a worldwide epidemic causing huge economic and health related problems. The focus of this review was to evaluate randomized control trials of low carbohydrate/high protein diets versus low fat/low calorie or very low calorie dietary treatments to prevent or manage obesity. We assessed the outcomes of these trials, in particular weight loss and cardiovascular risk factors.

PATIENTS AND METHODS:
Randomized control trials of at least 6 months were evaluated covering from 2000 to 2004. The mean age for all trials was 18 years or over and a mean or medium BMI of >28kg or over.

RESULTS:
Weight change and total cholesterol were analysed. Weight loss at 6 months resulted in all of the studies showing weight loss favouring treatment (low carbohydrate/high protein) over control (low calorie/low fat) of -5.80kg to -12kg. Weight loss after 12 months resulted in a greater weight loss favouring treatment (between -6.20kg and -7.20kg). Only 2 of the studies showed a beneficial effect on cholesterol level after 6 months (between 0.02mmol/l and 0.21mmol/l). Similar results were found for cholesterol level at 12 months with a greater decrease for the control groups (-0.27mmol/l to -0.68mmol/L).

CONCLUSION:
High protein/low carbohydrate diets favour weight loss but may have a negative effect on total cholesterol level, however, there is still a requirement for more randomized control trials to test this hypothesis.
INTRODUCTION

- Obesity has become a worldwide epidemic causing huge economic and health-related problems such as cardiovascular disease and type 2 diabetes.
- Most adults in England are now overweight, and 1 in 5 are obese. At any time, approximately 45% of women and 30% of men in the UK are trying to lose weight but most of them fail to do so.
- The focus of this review was to evaluate randomized controlled trials of low-carbohydrate-high-protein (LCHP) diets versus low-fat low-calorie (LFLC) diets.

METHOD

- The review followed the structure used by the Cochrane Collaboration. Randomized controlled trials of at least 6 months duration were evaluated.
- Inclusion criteria: age ≥18 years, BMI ≥28, and no serious medical condition.
- Outcomes examined: weight change, total, HDL, and LDL cholesterol, triglycerides, diastolic blood pressure, and fasting plasma glucose levels.
- 13 electronic databases were searched systematically. Nutrition journals, particularly those in the field of obesity, were hand-searched by two reviewers to locate RCTs.
- Full copies of studies were assessed by two reviewers for methodological quality using a standard format. The data was analysed using a computer program of the Cochrane Collaboration.

RESULTS

- There was a significant improvement at 6 months in weight favouring the treatment group (P < 0.001), but the difference was not significant at 12 months (Fig. 1, 2).
- There was also significant improvements in triglycerides, HDL cholesterol, and fasting plasma glucose levels at 6 months favouring the treatment group (P < 0.001)(Fig. 3, 4, 5).
- Systolic and diastolic blood pressure also improved at 6 months favouring the treatment groups but the difference was not significant (Fig. 6).
- Both total and LDL cholesterol decreased with low carbohydrate high-protein diets with low fat groups demonstrating more favourable changes (P = 0.001)(Fig. 4, 5).
- There was a higher dropout rate in the low fat diet group after 6 months compared to the low carbohydrate high-protein group (Fig. 6).

CONCLUSION

- Only 3 of the included studies were for more than 6 months duration, with 1 only being for a duration of 17 months, and not all of the outcomes were measured in each study.
- The results from this review suggest that low carbohydrate-high-protein diets have an overall favourable effect on cardiovascular risk disease because of the decrease in HDL cholesterol and triglycerides. But it also suggests that they do have adverse effects on CVD by increasing total and LDL cholesterol levels.
- In conclusion the use of low-carbohydrate diets results in improved weight loss at 6 months but with evidence of maintaining this advantage over low-fat diets at 12 months, although the numbers of such trials is small. Although there was a statistically significant increase in both total cholesterol and LDL cholesterol at 6 months this was offset by a marked rise in HDL cholesterol and fasting triglycerides. One paper demonstrated a change in LDL particle size to the less atherogenic type with low-carbohydrate diets.
The role of the LighterLife weight loss programme in lowering the cost of obesity

A Wise¹, C Rolland¹, J Cox², BH Hewlett², M Hession¹, I Broom¹
Department for Life Sciences, Robert Gordon University, Aberdeen, UK¹; LighterLife, Harlow, UK²

Introduction
Effective weight loss treatment is of importance as obesity has severe health and socioeconomic repercussions. The National Audit Office (2001) published evidence that due to its association with diseases such as hypertension and type 2 diabetes, obesity cost the NHS £0.5 billion in 1998 only. We used a section of the LighterLife client database to assess the different medical problems obese clients presented with at their starting date to determine the extent to which obesity affects all aspects of health and wellbeing.

Patients and methods
All subjects were either self or GP referred and underwent a medical examination before starting the Programme. Based on a group of 7377 subjects, ages ranged from 16-69 (average: 43.4, SD 11.2); BMI ranged from 30-83 (average: 38.3, SD 6.3); and starting weight ranged from 62-229kg (average: 103.1, SD 19.1).

Results
Out of 7377 subjects, the most common medical problem observed was hypertension (26.0%), followed by depression (17.8%), hypothyroidism (12.3%), and type 2 diabetes (4.1%). Other medical problems such as asthma, joint and back pain, arthritis, high cholesterol, cancer, water retention, hernias and polycystic ovarian syndrome were also reported. Less than 10% of the 7337 subjects had no existing medical problems.

Conclusion
Previous data on 2128 patients (Hewlett. Int J Obesity 2004; S136) demonstrated a 1.3kg weight loss per week using the LighterLife Programme. Such significant weight loss could greatly improve the medical problems subjects initially presented with, and consequently reduce the cost to the NHS.
Weight, Physical Activity, and General Health Changes after 3 and 6 Months of Dietary Interventions.

Hession M¹, Rolland C¹, Tuya C¹, Wise A¹, Murray S², Pirie I¹, Jarrett K², Broom J¹.

¹Robert Gordon University, Aberdeen, Scotland
²Lighterlife, Harlow, UK

Obesity is increasing at pandemic proportions. The use of low carbohydrate-high protein diets has become popular. A study is being carried out comparing a low fat healthy eating (HE) diet (600 kcal deficit) to either a ‘conventional’ food protein-sparing modified fast (PSMF) or a Very Low Calorie Nutritionally Complete Diet Formula (LighterLife, LL).

77 patients have been recruited. Weight was measured at baseline, 3 (n=31) and 6 (n=17) months. Patients completed a physical activity (PA), general health (GHQ) and Beck depression inventory questionnaire (BDI) at baseline, 3 and 6 months.

The difference in weight at 3 and 6 months was correlated against the 3 questionnaire scores. Significant correlations were found with weight change vs. PA at 6 months of the PSMF (r=0.92, P=0.029); weight change and GHQ difference at 6 months of PSMF (r=0.90, P=0.037).

There was a significant difference found with the BDI scores at 3 months after the HE diet (P=0.011), but no significant differences for the PA or GHQ.

No significant differences were found for the physical activity, general health or depression between the 3 diets at 6 months.

Although current data is limited this suggests greater improvement in general health and physical activity with the PSMF diet at 6 months. It can also be suggested that the healthy eating diet has a beneficial effect on BDI scores after 3 months, but again data is limited.
Weight, physical activity, and general health changes after 3 and 6 months of dietary interventions

Hession M1, Rolland C1, Tuya C1, Wise A1, Murray S2, Pirie I1, Jarrett K2, Broom J1.
1The Robert Gordon University, St Andrew St, Aberdeen AB25 1HG and 2LighterLife, Cavendish House, Harlow Business Park, Parkway, Harlow, Essex CM19 5QF.

INTRODUCTION

A study is being carried out comparing a low calorie healthy eating (HE) diet (600kcal deficit) to either a ‘conventional’ food protein-sparing modified fast (PSMF) or a very low calorie diet formula (LighterLife, LL).

Lifestyle questionnaires are completed by patients to find out if the diets have any effect on the questionnaire scores.

METHOD

Patients are assigned to HE for first 3 months. At 3 months those who have lost > 5% body weight remain on HE, and those who have lost < 5% are randomized to either LL or PSMF.

Weight was measured at baseline, 3 and 6 months.

Patients completed a physical activity (PA), general health (GHQ) and Beck depression inventory questionnaire (BDI) at baseline, 3 and 6 months.

Questionnaires were analysed to see if scores changed at 3 months from baseline.

The change in weight at 3 and 6 months was correlated against the changes in questionnaire scores.

Finally, questionnaire scores at 6 months were compared between the 3 diets.

RESULTS

Significant correlations were found with weight change vs.

PA change at 6 months of the PSMF ($r=0.92, P=0.029$) and weight change vs. GHQ change at 6 months of PSMF ($r=0.90, P=0.037$); Fig 1-3. Differences in weight and questionnaires were calculated from baseline to 6 months.

![Fig 1: PA vs. weight changes at 6 months of HE, LL and PSMF](image1)

![Fig 2: BDI vs. weight changes at 6 months of HE, LL and PSMF](image2)

![Fig 3: GHQ vs. weight changes at 6 months of HE, LL and PSMF](image3)

There were no significant differences found with the questionnaire scores 3 months after the HE diet (Table 1)

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th>BDI</th>
<th>GHQ</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.914</td>
<td>11.00</td>
<td>8.53</td>
</tr>
<tr>
<td>3</td>
<td>0.926</td>
<td>9.69</td>
<td>8.66</td>
</tr>
<tr>
<td>P</td>
<td>0.859</td>
<td>0.089</td>
<td>0.946</td>
</tr>
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</table>

Table 1: Differences in questionnaire scores after 3 months of HE

No significant differences were found for questionnaire scores between the 3 diets at 6 months (data not shown).

Despite greater weight loss observed with LL at 6 months, questionnaire scores did not differ from PSMF or HE, nor was LL weight change associated with LL questionnaire changes.

CONCLUSION

Current data is limited but this suggests greater improvement in general health and physical activity with the PSMF diet at 6 months.

There were no differences in questionnaire scores from baseline to 3 months, but again data is limited.
Physical activity, general health and depression analysis in relation to weight change after 3 months of a healthy eating dietary intervention

By Hession M¹, Rolland C¹, Wise A¹, Tuya C¹, Murray S², Broom J¹.
¹The Robert Gordon University, St Andrew St, Aberdeen AB25 1HG and ²LighterLife, Cavendish House, Harlow Business Park, Parkway, Harlow, Essex, CM19 5QF.

INTRODUCTION
High protein / low carbohydrate diets have become popular as an alternative to low fat diets in the treatment of obesity.

An ongoing randomised controlled trial comparing a healthy eating low calorie (HE) diet to two alternative diets (a- LighterLife (LL, very low calorie diet formula) and b- PSMF, Protein Sparing Modified Fast).

Patients are assigned initially to HE (600 kcal deficient) diet for the first 3 months.

METHOD
Questionnaires were used to analyse physical activity (PA), general health (general health questionnaire, GHQ) and depression level (Beck Depression Inventory, BDI).

A Pearson correlation was carried out to investigate the relation between initial questionnaire scores and weight change at 3 months.

An unpaired t-test was performed to determine if there was a difference between baseline and 3 month scores for patients who had achieved >5% compared to those who did not.

RESULTS
No significant association was found between baseline questionnaire scores and weight change at 3 months (Fig 1a, 1b and 1c). P > 0.05

No significant difference was found between the questionnaire score changes at 3 months for patients who lost >5% compared to those who did not ( BDI: t = 0.086, GHQ: t = 1.450, PAL: t = 0.728, p > 0.05)

CONCLUSION
These data suggest that weight loss at 3 months was not related to baseline scores for the three questionnaires.

There was no significant difference in questionnaire score changes between patients who achieved the weight target (>5%) compared to those who did not, suggesting degree of weight loss does not affect physical activity, general health, and depression levels after 3 months of HE diet.

![Fig 1a: Scatterplot of weight change at 3 months vs. baseline PA scores, P > 0.05](image)

![Fig 1b: Scatterplot of weight change at 3 months vs. baseline GHQ scores, P > 0.05](image)

![Fig 1c: Scatterplot of weight change at 3 months vs. baseline BDI scores, P > 0.05](image)

<table>
<thead>
<tr>
<th>GHQ (range-0-60)</th>
<th>&gt; 5% weight loss group</th>
<th>&lt; 5% weight loss group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-3.33</td>
<td>1.64</td>
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<table>
<thead>
<tr>
<th>PA (range 0.40-1.8 approx)</th>
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<th>&lt; 5% weight loss group</th>
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<tr>
<td>0.020</td>
<td>0.078</td>
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</table>

<table>
<thead>
<tr>
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<th>&gt; 5% weight loss group</th>
<th>&lt; 5% weight loss group</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.22</td>
<td>-1.09</td>
<td></td>
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</table>

Fig 2: Difference in 3 month questionnaire scores for the group who lost >5% weight and group who lost < 5% weight at 3 months. P > 0.05
Reported weight loss from a 1 yr randomised controlled trial examining 3 dietary interventions for obesity management – Initial findings

By C ROLLAND¹, M HESSION¹, C TUYA¹, S MURRAY², K JARRETT², A WISE¹ and J BROOM¹,
1 Robert Gordon University, Aberdeen, AB25 1HG; 2 LighterLife, Harlow, CM18 7BL

Obesity is a widespread disease both in the UK and worldwide and the prevalence is increasing in epidemic proportions. The increased occurrence of obesity has been accompanied by extra pressure to find novel and efficient treatments resulting in the development of different dietary weight loss approaches.

An ongoing 1yr randomised controlled trial is comparing a healthy eating 600 calorie deficit high carbohydrate (HC) diet with two high protein (HP) diets: a) a Protein Sparing Modified Fast (PSMF) using conventional food and b) a nutritionally complete formula Very Low Calorie Diet (VLCD) – (Lighterlife Programme).

The HC diet was a standard healthy eating, low fat (<30% total energy intake) approach where the patient’s energy requirements were determined and 600 calories were removed to result in daily calorific deficiency. The PSMF was a low fat, high protein diet where the patient ate conventional food while restricting their carbohydrate intake to 40g/day. The LighterLife Programme used a VLCD in parallel with weekly group sessions of cognitive behaviour therapy (CBT) to determine the underlying causes of the patient’s eating behaviour. The programme consisted of three stages; stage 1 - 100 days of weight loss and small group counselling; stage 2 – 4 week blocks of weight loss and small group counselling for clients who desire further weight loss; stage 3 – a 12 week weight management module where conventional food is reintroduced with ongoing group counselling.

Seventy-seven patients (10 males: 67 females) with a BMI ≥35 kg/m² were recruited; age range 18-68 (average: 41.4, SD: 11.3); BMI range 35-66 (average: 44.6, SD: 7); and starting weight range 85-173kg (average: 118.8, SD: 20.4). Patients were excluded if they were under 18 years of age, on weight reducing medication or anti-depressants, history of renal disease, evidence of active malignant disease, pregnant or lactating.

Patients entered a 3 to 12 month phase of HC with those who failed to achieve 5% weight loss at 3 months and 10% at 6 months were randomised to HP a or b.
An ANOVA was carried out to compare the changes in weight, between baseline and 6 months for the three dietary approaches.

At 6 months there was a significant difference in weight loss ($F= 16.75; P<0.001$) with greatest weight loss achieved on LighterLife followed by HC and then PSMF (mean weight loss= 24.3 kg, 13.2 kg and 3.6 kg respectively).

These initial results suggest a potential role of VLCDs and CBT in the effective treatment of obesity. However, further research is needed to examine the efficacy of VLCDs and CBT in achieving long-term weight maintenance in the British population.
The effects of a randomised control trial of low fat and high protein diets on cardiovascular risk of morbidly obese patients.

Rolland C¹, Hession M¹, Tuya C¹, Murray S², Wise A¹, Broom J¹.
¹ Robert Gordon University, Aberdeen, Scotland
² Lighterlife, Harlow, UK

Concerns have been expressed about the use of high protein ketogenic diets (HP) in the management of obesity. Several reviews comparing HP and ‘healthy eating’ high carbohydrate (HC) dietary approaches to obesity management have suggested the need for longer term randomised control trials (RCT) to evaluate both weight loss and cardiovascular risk.

A 1yr RCT comparing HC with two HP diets: a) ‘conventional’ food (PSMF) and b) Very Low Calorie Nutritionally Complete Formula Diet (Lighterlife). 77 patients with BMI ≥ 35 kg/m² entered a 3 to 12 month phase of HC with those who failed to achieve 5% weight loss at 3 months and 10% at 6 months were randomised to HP a or b. An ANOVA was carried out to compare the changes in weight, serum lipids, serum glucose and HbA1c between baseline and 6 months for the three dietary approaches. Paired t-tests were carried out to compare changes for these variables between baseline and 6 months within each diet.

At 6 months there was a significant difference in weight loss (p < 0.05) with greatest weight loss achieved on Lighterlife followed by HC and then PSMF. No significant difference for changes in total cholesterol (TC), HDL, triglyceride, TC/HDL, LDL, glucose, HbA1c (p>0.05) were observed. A significant weight loss between baseline and 6 months for the HC and Lighterlife diets but not for the PSMF were observed.

At this point in the study, benefits of weight loss were not reflected by a significant improvement in cardiovascular risk.

(word count 247)
Phenotypic presentation of an obese population and their response to a healthy eating energy deficient diet.

Rolland C¹, Hession M¹, Tuya C¹, Murray S², Wise A¹, Broom J¹.
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Obesity predisposes to and aggravates cardiovascular disease (CVD). In the obese, the metabolic syndrome (MS) is the main predisposing factor i.e. hypertension, insulin resistance, dyslipidaemia and increased waist circumference.

In an ongoing randomised controlled trial of high protein ketogenic diet (HP) versus high carbohydrate calorie-deficient diet (HC) in patients with a BMI ≥35kg/m², the presence of MS was investigated using both ATP III and IDF guidelines. Patients entered a 3 to 12 month phase of HC with those who failed to achieve 5% weight loss at 3 months and 10% at 6 months were randomised to a HP diet. 77 (10 males: 67 females) were recruited; age range 18-68 (average: 41.4, SD: 11.3); BMI range 35-66 (average: 44.6, SD: 7); and starting weight range 85-173kg (average: 118.8, SD: 20.4). Patients were excluded if they were under 18 years of age, on weight reducing medication or anti-depressants, history of problems with their kidneys, evidence of active malignant disease, pregnant or lactating.

Twelve (16%; ATP III) or 18 (23%; IDF) out of 77 patients presented with MS at baseline. Three month data were available for 5 (ATP III) and 7 (IDF) patients. One (ATP III) and 2 (IDF) patients achieved a 5% weight loss at 3 months.

The results suggest that fewer patients with a BMI ≥35kg/m² present with MS than might be expected (40%) from previous phenotypic characterisation of the obese population.
Occurrence and diagnosis of Type II Diabetes in an obese population

Rolland C\textsuperscript{1}, Hession M\textsuperscript{1}, Oomen J\textsuperscript{1}, Murray S\textsuperscript{2}, Wise A\textsuperscript{1}, Broom J\textsuperscript{1}.

\textsuperscript{1}Robert Gordon University, Aberdeen, Scotland
\textsuperscript{2}LighteLife, Harlow, UK

Obesity increases the risk of developing Type II Diabetes. The National Diabetes Audit\textsuperscript{1} (NDA) (2006) published that approximately 20\% of people are expected to have diabetes remain undiagnosed.

In an ongoing randomised controlled trial of high protein ketogenic diet (HP) versus high carbohydrate calorie-deficient diet (HC) in patients with a BMI $\geq$35kg/m\textsuperscript{2}, the occurrence of undiagnosed diabetes was investigated. Patients entered a 3 to 12 month phase of HC with those who failed to achieve 5\% weight loss at 3 months and 10\% at 6 months were randomised to a HP diet. 120 (20 males: 100 females) were recruited; age range 18-68 (average: 41.2, SD: 11.7); BMI range 35-66 (average: 44.6, SD: 6.8); and starting weight range 85.3-174.5kg (average: 119.4, SD: 20.1). Patients were excluded if they were under 18 years of age, on weight reducing medication or anti-depressants, history of problems with their kidneys, evidence of active malignant disease, pregnant or lactating.

An oral glucose tolerance test was carried out on 87 out of 120 patients. Of these 87 patients, 1 was diagnosed by the GP and 12 (13.8\%) were previously undiagnosed.

These results were lower than predicted by the NDA, but still demonstrate a discrepancy of diagnosis at the level of primary care.

\textsuperscript{1}National Diabetes Audit. Key findings about the quality of care for people with diabetes in England incorporating registrations from Wales. Abridged report for the audit period 2004/05. Leeds; The Information Centre, National Clinical Audit Support Programme, 2006:8.
Analysis of insulin sensitivity in response to weight loss in obese patients
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¹ Robert Gordon University, Aberdeen, Scotland
² LighterLife, Harlow, UK

INTRODUCTION: The increasing prevalence of type 2 diabetes mellitus (T2DM) has become a major public health problem and obesity is a major contributor to this increase.
OBJECTIVE: Identify the insulin resistance (IR) of patients at baseline and determine the effect of three different diets on IR in obese patients with the aim to use these diets to prevent and treat T2DM.
METHODS: Fourteen patients aged 18-68 years, BMI 43.7 ± 6.8 kg/m² were placed on a 600Kcal deficient diet. Those who did not achieve 5% weight loss at 3 months or 10% weight loss at 6 months following this diet were randomly allocated to a protein sparing modified fast (PSMF) or a very low calorie diet (VLCD). An oral glucose tolerance test (OGTT) was carried out at baseline and either at 6 or 12 months. HOMA-IR was calculated to determine changes in IR.
RESULTS: At baseline 3 patients had T2DM, and 6 patients had impaired glucose tolerance (IGT). At 12 months, 7 of these patients reversed their glucose tolerance. Patients following the VLCD lost 30.80kg ± 5.75, compared with 20.73kg ± 5.44 for the 600 calorie deficient diet and 3.99kg ± 0.35 for the PSMF.
Patients following the VLCD had the greatest decrease in IR with HOMA-IR reducing by 5.08 ± 5.47, 600Kcal deficient IR reduced by 2.97 ± 0.98, and PSMF reduced IR by 0.25 ± 5.11.
CONCLUSION: Greater weight loss resulted in greater insulin sensitivity, demonstrating that these dietary approaches can be used to reverse T2DM and IGT.
Comparison of kidney and liver changes after 3 months of a low carbohydrate vs. a very low calorie diet.

Hession, M1, Rolland, C1, John, O1, Murray, S2, Wise, A1, Broom, J1.

1Robert Gordon University, Aberdeen, Scotland.
2LighterLife, Harlow, UK.

Long term efficacy of low carbohydrate and very low calorie diets and their effects on liver and kidney function are still uncertain.

A randomized control trial is in progress to assess weight and cardiovascular risk factors with 3 diets: 1. a healthy eating/low calorie diet (HE). 2. a very low calorie diet (LighterLife-LL) and 3. a protein sparing modified fast (PSMF).

120 patients underwent a screening process for 3 months in which they consumed HE. Patients who achieved a 5% weight loss at 3 months remained on HE. Patients who did not achieve 5% weight loss were randomized to LL or PSMF. Data presented here are for 3 months post-randomization.

72 patients were randomized (LL, n= 35 and PSMF, n =37). From baseline to 3 months post-randomization, urea decreased (-0.53 mmol/L SD 1.4) on LL but increased on PSMF (+0.63 mmol/L SD 1.8), whereas albumin increased (+0.41 g/L SD 1.7) on LL but decreased on PSMF (-0.05 g/L SD 2.1). The changes over 3 months differed significantly between the diets. No differences were found for all other analytes: sodium, potassium, chlorine, creatinine, alanine aminotransferase, alkaline phosphatase and gamma glutyl transferase.

Although there were differences for urea and albumin 3 months post randomization between the diets, there were no differences found for all other analytes, this may indicate that neither diet causes adverse effects on liver and kidney function. More long term results need to be obtained before this can be concluded.
Baseline differences between achievers and non achievers of weight loss in a 3 month period with a healthy eating weight loss regimen

Rolland C¹, Hession M¹, John O¹, Murray S², Wise A¹, Broom J¹
¹Robert Gordon University, Aberdeen, Scotland
²LighterLife, Harlow, UK

Determining the factors that predict whether a person will achieve a 5% weight loss using a healthy eating diet (HE) could lead to a more effective, personalized treatment of their obesity.

In an ongoing randomized controlled trial, 120 obese patients underwent a screening period during which they were assigned to a HE for a period of 3 months. Patients aimed to achieve at least a 5% weight loss at the end of the 3 months. We investigated baseline differences between those who did and did not achieve the weight loss target. At baseline and 3 months patients underwent measurements for body composition, cardiovascular risk factors and lifestyle questionnaires.

Of the initial 120 patients, 18 (15%) achieved a 5% weight loss, 72 (60%) did not and 30 (25%) dropped out. At baseline, age (47.5 SD 11.0 vs. 41.4 SD 11.8, \( P=0.044 \)) fasting triglycerides (2.0 SD 1.0 vs. 1.5 SD 1.1, \( P=0.007 \)) and total cholesterol/high density lipoprotein (4.6 SD 0.9 vs. 4.0 SD 0.9, \( P=0.012 \)) were significantly different between the achievers and non-achievers, but they did not differ in quality of life, depression, fatigue or activity level.

Patients who dropped out during the 3 month screening period were significantly younger (37.5 SD 10.2 vs. 42.5 SD 12.0, \( P=0.042 \)) than the patients who remained in the study and they scored higher on the Beck Depression Inventory questionnaire (16.8 SD 10.8 vs. 11.9 SD 8.6, \( P=0.014 \)).

Age appears to be an important factor when determining the likelihood of an individual to adhere to a HE. Levels of depression also give an insight as to the likelihood of an individual’s success.

1. Conflict of Interest: None disclosed
2. Funding
Research relating to this abstract was funded by LighterLife
APPENDIX 3: LIFESTYLE AND HEALTH QUESTIONNAIRES
Dutch Eating Behaviour Questionnaire, Van Strien et al.,
Swets & Zeitlinger b.v., Lisse.

Please answer each question by ticking the most appropriate option.

1) Do you have the desire to eat when you are irritated?
   □ Never
   □ Seldom
   □ Sometimes
   □ Often
   □ Very often
   □ I’m never irritated

2) If food tastes good to you, do you eat more than usual?
   □ Never
   □ Seldom
   □ Sometimes
   □ Often
   □ Very often

3) Do you have a desire to eat when you have nothing to do?
   □ Never
   □ Seldom
   □ Sometimes
   □ Often
   □ Very often
   □ I always have something to do

4) If you have put on weight, do you eat less than you usually do?
   □ Never
   □ Seldom
   □ Sometimes
   □ Often
   □ Very often
   □ I never put on weight
5) Do you have a desire to eat when you are depressed or discouraged?
- Never
- Seldom
- Sometimes
- Often
- Very often
- I’m never depressed or discouraged

6) If food smells and looks good, do you eat more than usual?
- Never
- Seldom
- Sometimes
- Often
- Very often

7) How often do you refuse food or drink offered because you are concerned about your weight?
- Never
- Seldom
- Sometimes
- Often
- Very often

8) Do you have a desire to eat when you are feeling lonely?
- Never
- Seldom
- Sometimes
- Often
- Very often
- I never feel lonely

9) If you see or smell something delicious, do you have a desire to eat it?
- Never
- Seldom
- Sometimes
- Often
- Very often

10) Do you have a desire to eat when somebody lets you down?
- Never
- Seldom
- Sometimes
- Often
- Very often
- Nobody ever lets me down
11) Do you try to eat less at mealtimes than you would like to eat?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

12) If you have something delicious to eat, do you eat it straight away?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

13) Do you have a desire to eat when you are cross?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
☐ I’m never cross

14) Do you watch exactly what you eat?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

15) If you walk past the baker, do you have the desire to buy something delicious?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

16) Do you have a desire to eat when you are approaching something unpleasant to happen?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
17) Do you deliberately eat foods that are slimming?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

18) If you see others eating, do you also have the desire to eat?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

19) When you have eaten too much, do you eat less than usual the following days?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
☐ I never eat too much

20) Do you get the desire to eat when you are anxious, worried or tense?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
☐ I’m never anxious, worried or tense

21) Can you resist eating delicious foods?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

22) Do you deliberately eat less in order not to become heavier?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

312
23) Do you have a desire to eat when things are going against you or when things have gone wrong?

- Never
- Seldom
- Sometimes
- Often
- Very often

24) If you walk past a snackbar or a cafe, do you have the desire to buy something delicious?

- Never
- Seldom
- Sometimes
- Often
- Very often

25) Do you have a desire to eat when you are emotionally upset?

- Never
- Seldom
- Sometimes
- Often
- Very often
- I’m never emotionally upset

26) How often do you try not to eat between meals because you are watching your weight?

- Never
- Seldom
- Sometimes
- Often
- Very often

27) Do you eat more than usual when you see others eating?

- Never
- Seldom
- Sometimes
- Often
- Very often

28) Do you have a desire to eat when you are bored or restless?

- Never
- Seldom
- Sometimes
- Often
- Very often
- I’m never bored or restless
29) How often in the evening do you try not to eat because you are watching your weight?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

30) Do you have a desire to eat when you are frightened?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
☐ I'm never frightened

31) Do you take into account your weight with what you eat?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often

32) Do you have a desire to eat when you are disappointed?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
☐ I'm never disappointed

33) When preparing a meal, are you inclined to eat something?

☐ Never
☐ Seldom
☐ Sometimes
☐ Often
☐ Very often
Rowett Research Institute

Name: ________________  Date: ____________  Time: ______

Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the following situations in contrast to feeling just tired?

This refers to how you feel at the moment. Even if you have not done some of these things try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:

0 = Would Never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of Dozing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting inactive in a public place (e.g. theatre or meeting)</td>
<td></td>
</tr>
<tr>
<td>As a passenger in a car for an hour without a break</td>
<td></td>
</tr>
<tr>
<td>Lying down to rest in the afternoon when circumstances permit</td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after lunch without alcohol</td>
<td></td>
</tr>
<tr>
<td>In a car, when stopped for a few minutes in the traffic</td>
<td></td>
</tr>
</tbody>
</table>

Please check that you have answered all questions.
Thank you for your co-operation.
I am trying to find out about your level of energy at this moment. There are 18 items I would like you to respond to. This should only take about 1 minute of your time. Thank you.

DIRECTIONS: You are asked to place an 'X' through these lines to indicate how you are feeling RIGHT NOW.

PLEASE COMPLETE THE FOLLOWING ITEMS.

Not at all tired

Not at all sleepy

Not at all drowsy

Not at all fatigued

Not at all worn out

Not at all energetic

Not at all active

Extremely tired

Extremely sleepy

Extremely drowsy

Extremely fatigued

Extremely worn out

Extremely energetic

Extremely active
Not at all vigorous  Extremely vigorous

Not at all efficient  Extremely efficient

Not at all lively  Extremely lively

Not at all bushed  Totally bushed

Not at all exhausted  Totally exhausted

Keeping my eyes open is no effort at all  Keeping my eyes open is a tremendous chore

Moving my body is no effort at all  Moving my body is a tremendous chore

Concentrating is no effort at all  Concentrating is a tremendous chore

Carrying on a conversation is no effort at all  Carrying on a conversation is a tremendous chore

I have absolutely no desire to close my eyes  I have a tremendous desire to close my eyes
I have absolutely no desire to lie down

I have a tremendous desire to lie down

Now check that you have answered all the questions, and that you have only given one answer to each.
# GENERAL HEALTH QUESTIONNAIRE

**Study number ______ Subject number ______ Date ______**

Please read this carefully:

We should like to know if you have had any medical complaints, and how your health has been in general, over the past few weeks. Please answer ALL the questions on the following pages simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about present and recent complaints, not those that you had in the past.

It is important that you try to answer ALL the questions. Thank you very much for your co-operation.

## HAVE YOU RECENTLY:

<table>
<thead>
<tr>
<th></th>
<th>Better than usual</th>
<th>Same as usual</th>
<th>Worse than usual</th>
<th>Much worse than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>been feeling perfectly well and in good health?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>2</td>
<td>been feeling in need of a good rest?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>3</td>
<td>been feeling run down and out of sorts?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>4</td>
<td>felt that you are ill?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>5</td>
<td>been getting any pains in your head?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>6</td>
<td>been getting a feeling of tightness or pressure in your head?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>7</td>
<td>been able to concentrate on whatever you're doing?</td>
<td>Better than usual</td>
<td>Same as usual</td>
<td>Less than usual</td>
</tr>
<tr>
<td>8</td>
<td>been afraid that you were going to collapse in a public place?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>9</td>
<td>been having hot or cold spells?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>10</td>
<td>been perspiring (sweating) a lot?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>11</td>
<td>found yourself waking early and unable to get back to sleep?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>12</td>
<td>been getting up feeling your sleep hasn't refreshed you?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
<tr>
<td>13</td>
<td>been feeling too tired and exhausted even to eat?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
</tr>
</tbody>
</table>

**PLEASE TURN OVER**
### HAVE YOU RECENTLY:

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>No more than usual</th>
<th>Rather more than usual</th>
<th>Much more than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 - lost much sleep over worry?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 - been feeling mentally alert and wide awake?</td>
<td>Better</td>
<td>Same</td>
<td>Less alert</td>
<td>Much less alert</td>
</tr>
<tr>
<td>16 - been feeling full of energy?</td>
<td>Better</td>
<td>Same</td>
<td>Less energetic</td>
<td>Much less energetic</td>
</tr>
<tr>
<td>17 - had difficulty in getting off to sleep?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>18 - had difficulty in staying asleep once you are off?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>19 - been having frightening or unpleasant dreams?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>20 - been having restless, disturbed nights?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>21 - been managing to keep yourself busy and occupied?</td>
<td>More</td>
<td>Same</td>
<td>Rather less</td>
<td>Much less</td>
</tr>
<tr>
<td>22 - been taking longer over the things you do?</td>
<td>Quicker</td>
<td>Same</td>
<td>Longer</td>
<td>Much longer</td>
</tr>
<tr>
<td>23 - tended to lose interest in your ordinary activities?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>24 - been losing interest in your personal appearance?</td>
<td>No more</td>
<td>Rather more</td>
<td>Much more</td>
<td></td>
</tr>
<tr>
<td>25 - been taking less trouble with your clothes?</td>
<td>More</td>
<td>About same</td>
<td>Less trouble</td>
<td>Much less trouble</td>
</tr>
<tr>
<td>26 - been getting out of the house as much as usual?</td>
<td>More</td>
<td>Same</td>
<td>Less</td>
<td>Much less</td>
</tr>
<tr>
<td>27 - been managing as well as most people would in your shoes?</td>
<td>Better</td>
<td>About</td>
<td>Rather</td>
<td>Much</td>
</tr>
<tr>
<td>28 - felt on the whole you were doing things well?</td>
<td>Better</td>
<td>About</td>
<td>Less well</td>
<td>Much</td>
</tr>
<tr>
<td>29 - been late getting to work, or getting started on your housework?</td>
<td>No later</td>
<td>Rather later</td>
<td>Much later</td>
<td></td>
</tr>
<tr>
<td>30 - been satisfied with the way you've carried out your task?</td>
<td>More</td>
<td>About same</td>
<td>Less satisfied</td>
<td>Much less satisfied</td>
</tr>
<tr>
<td>31 - been able to feel warmth and affection for those near to you?</td>
<td>Better</td>
<td>About same</td>
<td>Less well</td>
<td>Much less well</td>
</tr>
<tr>
<td>32 - been finding it easy to get on with other people?</td>
<td>Better</td>
<td>About same</td>
<td>Less well</td>
<td>Much less well</td>
</tr>
<tr>
<td>33 - spent much time chatting with people?</td>
<td>More time</td>
<td>About same</td>
<td>Less</td>
<td>Much less</td>
</tr>
</tbody>
</table>

**GO ON TO THE NEXT PAGE**
<table>
<thead>
<tr>
<th>HAVE YOU RECENTLY:</th>
<th>Not at all</th>
<th>No more than usual</th>
<th>Rather more than usual</th>
<th>Much more than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>34. - kept feeling afraid to say anything to people in case you made a fool of yourself?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>35. - felt that you are playing a useful part in things?</td>
<td>More so than usual</td>
<td>Same as usual</td>
<td>Less useful than usual</td>
<td>Much less useful</td>
</tr>
<tr>
<td>36. - felt capable of making decisions about things?</td>
<td>More so than usual</td>
<td>Same as usual</td>
<td>Less so than usual</td>
<td>Much less capable</td>
</tr>
<tr>
<td>37. - felt you're just not able to make a start on anything?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>38. - felt yourself dreading everything that you have to do?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>39. - felt constantly under strain?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>40. - felt you couldn't overcome your difficulties?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>41. - been finding like a struggle all the time?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>42. - been able to enjoy your normal day-to-day activities?</td>
<td>More so than usual</td>
<td>Same as usual</td>
<td>Less so than usual</td>
<td>Much less than usual</td>
</tr>
<tr>
<td>43. - been taken things hard?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>44. - been getting edgy and bad-tempered?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>45. - been getting snubbed or panicky for no good reason?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>46. - been able to face up to your problems?</td>
<td>More so than usual</td>
<td>Same as usual</td>
<td>Less able than usual</td>
<td>Much less able</td>
</tr>
<tr>
<td>47. - found everything getting on top of you?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>48. - had the feeling that people were looking at you?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>49. - been feeling unhappy and depressed?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>50. - been losing confidence in yourself?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>51. - been thinking of yourself as a worthless person?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>52. - felt that life is entirely hopeless?</td>
<td>Not at all</td>
<td>No more than usual</td>
<td>Rather more than usual</td>
<td>Much more than usual</td>
</tr>
<tr>
<td>53. - been feeling hopeful about your own future?</td>
<td>More so than usual</td>
<td>About same as usual</td>
<td>Less so than usual</td>
<td>Much less hopeful</td>
</tr>
<tr>
<td>Question</td>
<td>More so than usual</td>
<td>About same as usual</td>
<td>Less so than usual</td>
<td>Much less than usual</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>54 - been feeling reasonably happy, all things considered?</td>
<td>Definately not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 - been feeling nervous and strung-up all the time?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 - felt that life isn't worth living?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 - thought of the possibility that you might make away with yourself?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58 - found at times you couldn't do anything because your nerves were</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59 - found yourself washing you were dead and away from it all?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 - found that the idea of taking your own life kept coming into your</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Goldberg & The Institute of Psychiatry 1978

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First Published 1978
# PHYSICAL ACTIVITY QUESTIONNAIRE

<table>
<thead>
<tr>
<th>Please describe your activity in a typical week</th>
<th>Number of hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) How many hours per day do you usually spend in bed?</td>
<td></td>
</tr>
<tr>
<td>If you do not do any paid work, please go to question 5</td>
<td></td>
</tr>
<tr>
<td>2) How many <em>days per week</em> do you usually do paid work?</td>
<td></td>
</tr>
<tr>
<td>3) How many <em>hours per day</em> do you usually spend in paid work?</td>
<td></td>
</tr>
<tr>
<td>4) In each day you do paid work, <em>how many hours</em> do you usually spend in</td>
<td></td>
</tr>
<tr>
<td>a) <em>Sedentary activities</em></td>
<td></td>
</tr>
<tr>
<td>(e.g. standing, sitting, reading, writing, calculating, working at a computer)</td>
<td></td>
</tr>
<tr>
<td>b) <em>Light activities</em></td>
<td></td>
</tr>
<tr>
<td>(e.g. general lab work, general office work, unhurried walking, driving)</td>
<td></td>
</tr>
<tr>
<td>c) <em>Moderate activities</em></td>
<td></td>
</tr>
<tr>
<td>(e.g. light lifting or carrying, moderate walking, painting, decorating)</td>
<td></td>
</tr>
<tr>
<td>d) <em>Heavy activities</em></td>
<td></td>
</tr>
<tr>
<td>(e.g. heavy lifting or carrying, hurried walking, going up stairs or ladders, digging, strenuous exercise)</td>
<td></td>
</tr>
</tbody>
</table>
5) In your other activities, e.g. housework and travelling (including travelling to work), please estimate how many hours per day you spend in each type of activity, and then indicate how many days each week do you usually do this type of activity.

Any time you do not list below will be assumed to be spent in sedentary activities

<table>
<thead>
<tr>
<th>Light activities</th>
<th>Hours per day</th>
<th>Hours per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>(e.g. washing, dressing, shaving, ironing, cooking, washing dishes, shopping, unhurried walking, driving)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Moderate activities | | |
|---------------------| | |
| (e.g. gardening, golf, cricket-bowling, playing pool, cycling, moderate walking, child care, light lifting or carrying, painting, decorating) | | |

| Heavy activities | | |
|------------------| | |
| (e.g. competitive sports, football, swimming, playing tennis, squash, hockey, running, aerobics, dancing, going up stairs or ladders, digging, strenuous exercise) | | |

Office use only

TOTAL HOURS =

PAL =
WHQOL

ABOUT YOU
Before you begin we would like to ask you to answer a few general questions about yourself: by circling the correct answer or by filling in the space provided.

What is your gender? Male Female

What is your date of birth? Day _____ Month _____ Year _____

What is the highest education you received? None at all Primary school Secondary school Tertiary

What is your marital status? Single Separated Married Divorced Living as married Widowed

Are you currently ill? Yes No

If something is wrong with your health what do you think it is?____________ illness/ problem

Instructions
This assessment asks how you feel about your quality of life, health, or other areas of your life. Please answer all the questions. If you are unsure about which response to give to a question, please choose the one that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life in the last two weeks. For example, thinking about the last two weeks, a question might ask:

<table>
<thead>
<tr>
<th>Do you get the kind of support from others that you need?</th>
<th>Not at all</th>
<th>Not much</th>
<th>Moderately</th>
<th>A great deal</th>
<th>Completely</th>
</tr>
</thead>
<tbody>
<tr>
<td>You should circle the number that best fits how much support you got from others over the last two weeks. So you would circle the number 4 if you got a great deal of support from others as follows,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you get the kind of support from others that you need?</th>
<th>Not at all</th>
<th>Not much</th>
<th>Moderately</th>
<th>A great deal</th>
<th>Completely</th>
</tr>
</thead>
<tbody>
<tr>
<td>You would circle number 1 if you did not get any of the support that you needed from others in the last two weeks. Please read each question, assess your feelings, and circle the number on the scale for each question that gives the best answer for you.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Very poor</th>
<th>Poor</th>
<th>Neither poor nor good</th>
<th>Good</th>
<th>Very good</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(G1) How would you rate your quality of life?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

|                                                                 | Very dissatisfied | Dissatisfied | Neither satisfied nor satisfied | Satisfied | Very satisfied |

|                                |               |             |                                   |          |               |

325
<table>
<thead>
<tr>
<th></th>
<th>How satisfied are you with your health?</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The following questions ask about how much you have experienced certain things in the last two weeks.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3(F1,4)</td>
<td>To what extent do you feel that physical pain prevents you from doing what you need to do?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>An extreme amount</td>
</tr>
<tr>
<td></td>
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<td>1</td>
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<td>5</td>
</tr>
<tr>
<td>4(F11.3)</td>
<td>How much do you need any medical treatment to function in your daily life?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>An extreme amount</td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5(F4,1)</td>
<td>How much do you enjoy life?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>An extreme amount</td>
</tr>
<tr>
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<td>2</td>
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<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6(F24.2)</td>
<td>To what extent do you feel your life to be meaningful?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>An extreme amount</td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7(F5,3)</td>
<td>How well are you able to concentrate?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>Extremely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
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<td>4</td>
<td>5</td>
</tr>
<tr>
<td>8(F16.1)</td>
<td>How safe do you feel in your daily life?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>Extremely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>9(F22.1)</td>
<td>How healthy is your physical environment?</td>
<td>Not at all</td>
<td>A little</td>
<td>A moderate amount</td>
<td>Very much</td>
<td>Extremely</td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10(F2,1)</td>
<td>Do you have enough energy for everyday life?</td>
<td>Not at all</td>
<td>A little</td>
<td>Moderately</td>
<td>Mostly</td>
<td>Completely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>11(F7,1)</td>
<td>Are you able to accept your bodily appearance?</td>
<td>Not at all</td>
<td>A little</td>
<td>Moderately</td>
<td>Mostly</td>
<td>Completely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>12(F18.1)</td>
<td>Have you enough money to meet your needs?</td>
<td>Not at all</td>
<td>A little</td>
<td>Moderately</td>
<td>Mostly</td>
<td>Completely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>13(F20.1)</td>
<td>How available to you is the information that you need in your day-to-day life?</td>
<td>Not at all</td>
<td>A little</td>
<td>Moderately</td>
<td>Mostly</td>
<td>Completely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>14(F21.1)</td>
<td>To what extent do you have the opportunity for leisure activities?</td>
<td>Very poor</td>
<td>Poor</td>
<td>Neither poor nor good</td>
<td>Good</td>
<td>Very good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>15(F9.1)</td>
<td>How well are you able to get around?</td>
<td>Very dissatisfied</td>
<td>Dissatisfied</td>
<td>Neither satisfied nor dissatisfied</td>
<td>Satisfied</td>
<td>Very satisfied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

The following questions ask you to say how good or satisfied you have felt about various aspects of your life over the last two weeks.
<table>
<thead>
<tr>
<th>25(F23.3)</th>
<th>How satisfied are you with your transport?</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

The following question refers to **how often** you have felt or experienced certain things in the last two weeks.

<table>
<thead>
<tr>
<th>26(F8.1)</th>
<th>How often do you have negative feelings such as blue mood, despair, anxiety, depression?</th>
<th>Never</th>
<th>Seldom</th>
<th>Quite often</th>
<th>Very often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
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<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Did someone help you to fill out this form?

........................................................................................................................................................................

How long did it take to fill this form out?

........................................................................................................................................................................

Do you have any comments about the assessment?

........................................................................................................................................................................
........................................................................................................................................................................
........................................................................................................................................................................

THANK YOU FOR YOUR HELP
The End