Alterations in the macronutrient content of the diet and the effects on body composition, cardiovascular disease risk and the control of energy metabolism in obese patients with type 2 diabetes mellitus

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Declaration

I declare that the work presented in this thesis is my own, except where otherwise acknowledged, and has not been submitted in any form for another degree or qualification at any other academic institution.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Anna Gryka

Abstract

Alterations in the macronutrient content of the diet and the effects on body composition, cardiovascular disease risk and the control of energy metabolism in obese patients with type 2 diabetes mellitus

Anna Gryka for the degree of Doctor of Philosophy

Background/Objective: Several studies have shown that a low carbohydrate diet (LCHOD) can improve glycaemic control in type 2 diabetes (T2DM). The objective of the current study was to compare two ways of administration of a LCHOD: self-prepared meals versus ready-made meals, and their effects on weight loss, glycaemic control, body composition, cardiovascular risk and resting metabolic rate over 12 months.

Research design and methods: Forty-one volunteers with the mean body mass index of 38.8 kg/m^2 and poorly controlled T2DM (glycosylated haemoglobin, HbA_{1c} > 7.5%) were randomized to either protein sparing modified fast (< 40g of carbohydrate daily, self-cooked; PSMF) or Go Lower (ready-made meals; GL) diet. Both groups received multivitamin supplementation and attended monthly visits. The main outcome was weight loss and its composition.

Results: Fourteen (34 %) participants completed 12 months of the intervention. There were no differences in the weight or any other changes between the diet groups at 12 months. Overall, body mass and fat mass decreased (-5.5 ± 7.3 kg, $P < 0.001$ and -5.1 ± 6.7 kg, $P < 0.001$ respectively) but fat free mass did not change. There was an overall reduction in HbA_{1c} (-0.4 ± 1.1 %, $P < 0.001$), increase in HDL-cholesterol (+0.07 ± 0.18 mmol/L, $P < 0.001$) and decrease in triacylglycerol (-0.6 ± 2.4 mmol/L, $P = 0.014$). Resting metabolic rate significantly decreased (-137 ± 265 kcal/d, $P < 0.001$).

Conclusion: LCHOD, independently of the approach taken, led to weight loss and improvements in glycaemic control in obese volunteers with poorly controlled T2DM. The results confirm that lifestyle modification using LCHOD is effective for improving T2DM and suggest that the type of approach to the diet can be matched to an individual's preferences.

Keywords: obesity, type 2 diabetes, carbohydrate, protein, diet, body composition

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List of Abbreviations

ACSM	American College of Sports Medicine
ACTH	adrenocorticotropic hormone
ADA	American Diabetes Association
ADDQoL	Audit of Diabetes Dependent quality of Life
AHA	American Heart Association
ALKP	alkaline phosphatase
ALT	alanine amino transferase
BMI	body mass index
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CHO	carbohydrate
CNP	cardiac natriuretic peptide
CRP	C-reactive protein
CVD	cardiovascular disease
DM	diabetes mellitus
DTSQ	Diabetes Treatment Satisfaction Questionnaire
ESS	Epworth Sleepiness Scale
F	fat
FG	fasting glucose
GGT	gamma-glutamyl transferase
GL	Go Lower
GLP-1	glucagon-like peptide 1
GPPAQ	General Practice Physical Activity Questionnaire
HbA_{1c}	glycosylated haemoglobin
HDL	high-density lipoprotein
HL	hepatic lipase
HOMA	Homeostasis Model Assessment
IDF	International Diabetes Federation
IL-1, 6, or 8	interleukin-1, 6 or 8
LCHOD	low-carbohydrate diet
LCKD	low-carbohydrate ketogenic diet

LDL	low-density lipoprotein
LPH	lipotropin
MDI	Major Depression Inventory
MDRD	Modification of Diet in Renal Disease
MHRA	Medicines and Healthcare Products Regulatory Agency
MSH	melanocyte stimulating hormones
NEFA	non-estrified fatty acids
NO	nitric oxide
ORWELL	Obesity Related Wellbeing Questionnaire
P	protein
PAI-1	plasminogen activator inhibitor 1
POMC	proopiomelanocortin
PSMF	protein sparing modified fast
QoL	quality of life
RAAS	rennin-angiotensin-aldosterone system
RBP4	retinol binding protein 4
RDA	recommended daily allowance
REE	resting energy expenditure
RMR	resting metabolic rate
ROS	reactive oxygen species
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TEE	total energy expenditure
TEF	thermic effect of food
TG	triacylglycerol
TNF-α	tumour necrosis factor alpha
TSH	thyroid stimulating hormone
VAS-F	visual analogue scales to assess fatigue
VLDL	very low density lipoprotein
WAT	white adipose tissue
WDB	Weight Decisional Balance
WHR	waist hip ratio
WHO	World Health Organization

List of conference communications

1st International Diabetes and Obesity Forum, 2010, Athens:

Gryka, A., Rolland, C., Broom, I., 2010. Assessment of the approach to diet, quality of life and treatment satisfaction in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, **12** (Suppl 1), p. 68.

XI International Congress on Obesity, 2010, Stockholm:

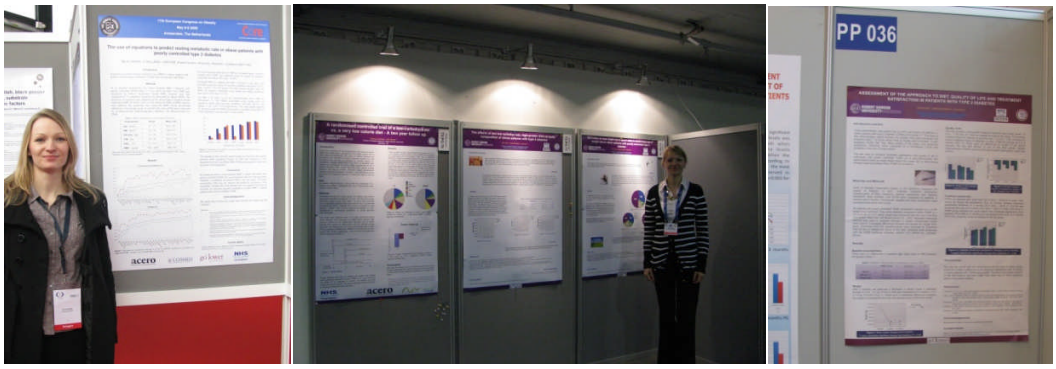
Gryka, A., Rolland, C., Broom, I., 2010. The effects of two low-carbohydrate, high-protein diets on body composition of obese patients with type 2 diabetes. *Obesity Reviews*, **11** (Suppl 1), p. 246.

Gryka, A., Rolland, C., Broom, I., 2010. Motivation to lose weight and dropout rates in randomized trial of weight loss in obese patients with poorly controlled type 2 diabetes. *Obesity Reviews*, **11** (Suppl 1), p. 246.

Gryka, A., Rolland, C., Broom, I., 2010. Climate change, obesity and type 2 diabetes. *Obesity Reviews*, **11** (Suppl 1), p. 334.

17th European Congress on Obesity, 2009, Amsterdam

Gryka, A., Rolland, C., Broom, I., 2010. The use of equations to predict resting metabolic rate in obese patients with poorly controlled type 2 diabetes. *Obesity Facts*, **3**(2), p. 159.



1 Introduction

"Today the biggest killers in the world are not infectious diseases; rather it is chronic afflictions such as cardiovascular disease, obesity and type 2 diabetes that affect populations across the globe".

(Rana et al. 2007)

1.1 Background

1.1.1 Epidemiology and definitions of obesity and diabetes mellitus

The prevalence of obesity and type 2 diabetes mellitus (T2DM) is rising globally. The risks of developing T2DM, cardiovascular disease (CVD), musculoskeletal and respiratory diseases grow progressively with an increasing body mass index (BMI, defined as body mass[1] [kg] /stature [m^2]). Obesity is the second preventable cause of cancer after smoking (World Health Organization, 2003). As obesity is implicated in the development of chronic diseases, it has become a major public health problem. It is estimated that in 2008 more than 500 million people were obese globally (Finucane et al. 2011), and by 2015 this number is projected to increase up to 700 million (Amasyali et al. 2008, World Health Organization 2011b). The problem is serious in Great Britain, with one in 4 adults being obese in 2009 (Bromley et al. 2010, Craig et al. 2010).

Obesity is usually diagnosed when the BMI (also named Quetelet's index) is higher than 30 kg/m^2. Obesity has been divided into classes based on the BMI values: class I (30-34.99), class II (35-39.99) and class III (over 40) (World Health Organization 2000). A BMI cut-off value for obesity was reported to be a poor tool to estimate body fat because of low sensitivity as it failed to identify about a half of people with excess body fat (Jumean et al. 2008). However, BMI has high specificity which

[1] The mass of a body is a measure of how much matter it contains. Weight is a degree of attraction between two bodies (gravity) that depends on the mass of those bodies and distance between them (altitude). 'Weight' is wrongly but very widely used in the general health context and it is used in this work in place of the correct 'body mass'.

means that individuals with a BMI over 30 kg/m^2 usually have a high percentage of body fat (Jumean et al. 2008). Moreover, the above quoted BMI cut off values are not applicable to all populations because for some ethnic groups metabolic complications may develop already when BMI is over 22 kg/m^2 (World Health Organization, 2000).

Due to the fact that there has been no systematic evaluation in large populations, there are no norms for body fat percentage for the general population and precise values for body composition cannot be recommended. The present classification of average body fat was based on healthy US population: men 12-15% and women 25-28% (McArdle et al. 2007). Also, the American Council on Exercise produced their classification in which there are values of essential fat, athletes, fitness, average and obesity; obesity being identified in men with over 25% and women with over 32% of body fat (Bryant et al. 2011).

Diabetes mellitus (DM) is a disorder where blood glucose levels are almost constantly elevated (hyperglycaemia) and the metabolism of carbohydrate, fat and protein is disturbed. These abnormalities are caused by either insulin deficiency or resistance to insulin, or both (World Health Organization 2000). Of all the metabolic pathologies associated with obesity, in T2DM the link is best established.

In the long term, elevated blood glucose concentrations can cause microvascular and macrovascular complications and affect wound healing. Macrovascular complications involve coronary disease, cerebrovascular disease or peripheral vascular disease, whereas microvascular complications include retinopathies, nephropathies and neuropathies. Hypertension in addition to being a disease in its own right is considered to be a complication of diabetes (Frayn 2003). There is also evidence that T2DM is independently associated with an increased cancer risk (LeRoith et al. 2008). DM is usually suspected when sugar in the urine is found and diagnosed when fasting plasma glucose concentration is over 7.0 mmol/l (Sims et al. 1973) or when plasma glucose concentration is over 11.1 mmol/l after 2 hours of oral administration of 75g of glucose (World Health Organization, International Diabetes Federation 2006).

T2DM accounts for 90% of all diabetic cases worldwide (World Health Organization 2011a). It is often a result of sedentary lifestyle and overweight and obesity. Increasing body mass, even in young men with no family history of diabetes or obesity, causes insulin resistance, rise in fasting concentrations of insulin, glucose, triacylglycerol and total cholesterol (Sims et al. 1973). Obesity is

considered to be the primary risk factor for T2DM (Colditz et al. 1995, World Health Organization 2006). Less frequent type 1 diabetes mellitus (T1DM) is a disease indicated by the presence of the pancreatic islet beta-cell antibodies, or by the idiopathic destruction of beta-cells (WHO, 1999).

The International Diabetes Federation (IDF) (2011) estimates the number of diabetes cases for 2010 to be 285 million globally. The WHO estimates the current number to be smaller, about 220 million (World Health Organization 2011a). This situation is foreseen to worsen: by the year 2030, the IDF estimated the global number of people with diabetes to be 439 million (International Diabetes Federation 2011b). Other sources predict less dramatic but still very serious increase to 366 million (Wild et al. 2004). The WHO (World Health Organization 2011a) approximated that in 2004 high blood sugar was the cause of 3.4 million deaths globally, while the IDF suggested a rough estimate of 4 million diabetes related deaths in 2010 (International Diabetes Federation 2011a). It is important to note that many of these deaths are a result of cardiovascular disease.

Metabolic disorders such as obesity, inflammation, diabetes and dyslipidaemia often overlap and they all have detrimental influence on the cardiovascular system. Cardiovascular disease affects a growing number of people worldwide and is one of the main causes of morbidity and mortality (Rana et al, 2007). It is characteristic that people with the cluster of these risk factors, called jointly metabolic syndrome (MS), have threefold increased risk for coronary heart disease and stroke (Isomaa et al, 2001). The WHO, in 1998, proposed a definition of metabolic syndrome, which identified individuals with increased cardiovascular morbidity and mortality (Isomaa et al, 2001). There are several definitions of MS. The most often used are definitions by the WHO (1999), National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (2001), IDF (2005), and American Heart Association / National Heart, Lung, and Blood Institute (AHA/NHLBI) (2005). NCEP ATP III (2001) and AHA/NHLBI (2005) definitions are very similar and name 5 criteria of which 3 must be fulfilled to diagnose MS. First four: waist circumference (≥102cm in men and ≥88cm in women), elevated triacylglycerols (TG) (≥1.7 mmol/l or drug treatment for elevated TG), reduced HDL-cholesterol (<1.03 mmol/l in men and <1.29 mmol/l in women or drug treatment for reduced HDL-C), and elevated blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic or drug treatment for hypertension) are the same for the two definitions. There is a difference in the fasting glucose concentration threshold: NCEP ATP III suggests slightly higher value than AHA/NHLBI (> 6.1 mmol/l vs. ≥ 5.5 mmol/l). A variation in the criteria used in different definitions of MS has lead to difficulty in comparing epidemiological studies (Baunduceau et al, 2008) and in diagnosing patients who according to some definitions would have MS and according to others would not. Balkau and

colleagues (2008), in their review of MS, suggested that "a score to evaluate the severity of the syndrome, using a combination of continuous variables would be more logical than a syndrome defined with arbitrary thresholds". However, a definition that would score severity of MS has not been yet developed.

1.1.2 Aetiology of obesity and T2DM

The estimations of a number of obese people worldwide over the next few years include an alarming figure of 700 million (World Health Organization 2011b). The challenge for science is to identify the causes and find solutions to this global epidemic. The large decrease in physical activity and the major changes in the food supply over the last few decades have been identified as drivers of this process. The term "obesogenic, toxic environment" was created to describe a combination of energy dense, nutrient insufficient food supplies and poor conditions for physical activity (James 2008). Apart from the environment, another reason for the global increase in obesity prevalence seems to be human physiology. Through evolution mechanisms to deal with starvation were favoured over mechanisms suppressing food intake. With the physiology set for survival for times of starvation, now in a time of a plentiful food supply, we are facing a dramatic increase in the risk of becoming obese (Appel 2003).

T2DM is caused by both environmental and genetic factors. Genetic components, resulting in metabolic disorders, play a bigger part in the development of T2DM than T1DM (World Health Organization 1999). However, due to the obesity epidemic, the percentages of diabetes attributable to weight gain in 2000 were about 90% in North America and over 80% in Western Europe (Rigby, James 2003).

1.1.2.1 Aetiology of obesity

Weight gain occurs when energy intake is higher than energy expenditure over a period of time. Common obesity is considered to be a result of an interaction between environmental and genetic influences. However, the reality is very complex and different between populations. The theories of the origin come from different fields (i.e. genetics, economics, sociology, psychology) and the lack of a unifying theory has been highlighted (Ulijaszek 2008). The Foresight report (Tackling Obesities – Future Choices, Butland et al. 2007) includes the famous "spaghetti plate" – an illustration of the interplay between the obesity causing factors (Figure 1).

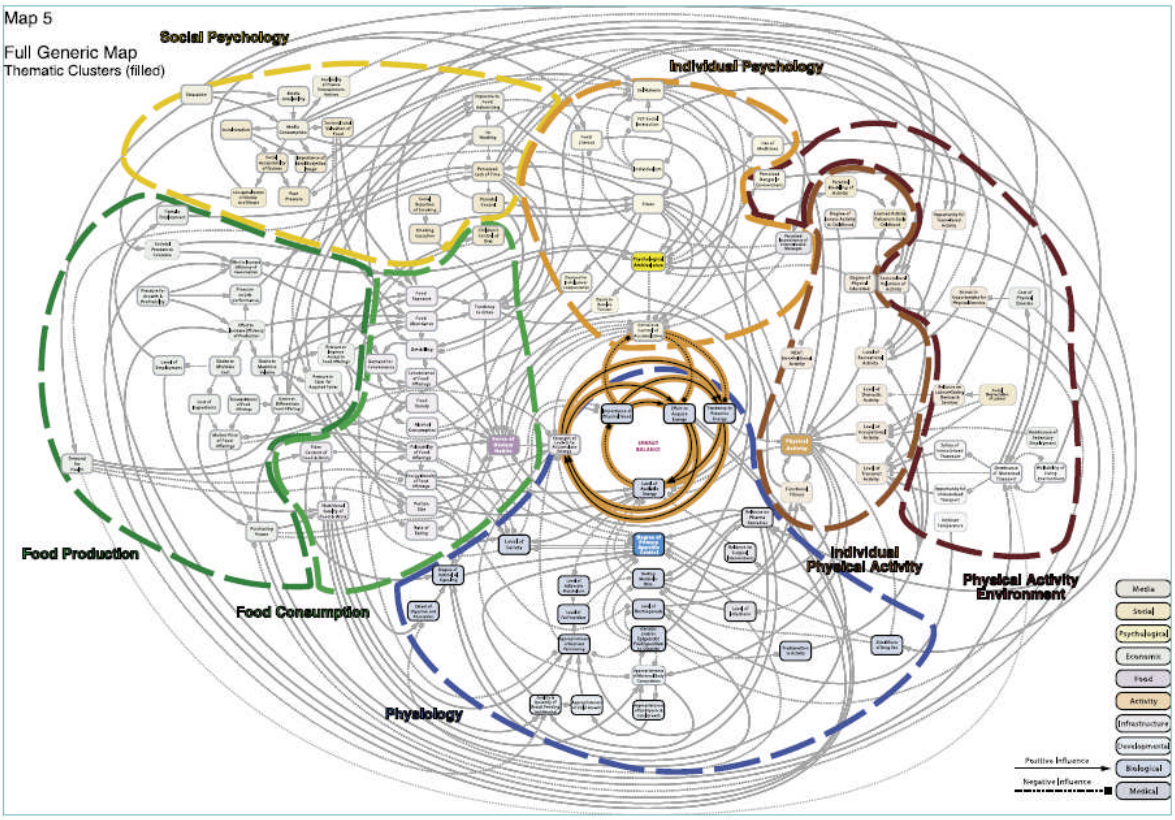


Figure 1. The obesity system map. (Source: Foresight, Tackling Obesities – Future Choices Project Report, Butland et al. 2007).

1.1.2.1.1 Hypotheses of the origin of obesity

As mentioned earlier, the prevalence of obesity has been dramatically rising over the last few decades. It is known that certain genotypes, when exposed to today's environment, predispose an individual to weight gain. There are several hypotheses why humans have this genetic predisposition.

Neel (1962) suggested the "thrifty genotype" hypothesis which stated that in the course of the human evolution there were periods of famine which were long enough to cause natural selection towards efficient fat storage in the times of plenty. This was the start of the search for the 'thrifty' genes. However, Neel in the same paper presented another possible hypothesis claiming that obesity and diabetes are a new phenomenon associated with the changes not in genes but in the environment (i.e. diet, physical activity, stress) (Neel 1962).

Later, Hales and Barker (1991) proposed the “thrifty phenotype” hypothesis in which the factor predisposing to obesity was not genetic but environmental. They claimed that thrifty factors, being obesity and T2DM, resulted from insufficient nutrition during prenatal development which programmed conservation of energy later in life. This hypothesis was supported by data demonstrating the greater prevalence of diabetes among individuals with low birth weight (Hales et al. 1991).

Speakman (2006) suggested that it might be the time to call off the search for the thrifty genes. He pointed out the flaws of the thrifty genotype hypothesis, saying that famines were relatively rare in human evolution, caused the death of less than 10% of populations and deaths were usually due to disease, not body weight. Speakman suggested that obesity could not have been the preferred phenotype because of the risk of being too attractive for predators (Speakman 2006). After humans invented tools and started using fire, the predation risk was eliminated and Speakman proposed that random genetic mutations and drift were the only factors that predisposed to body fatness (Speakman 2007). The hypothesis that obesity may be due to an absence of selection (lack of predation and insufficient influence of famines), and also due to genetic drift, was called the ‘drifty gene’ hypothesis.

Prentice and colleagues did not agree with Speakman’s drifty genotype hypothesis and modified the thrifty genotype hypothesis, stating that the natural selection in favour of fat storage occurred and was mediated through fertility (Prentice, Hennig et al. 2008). However, the counter-argument was proposed by Speakman who argued that reduced fertility during famines was usually followed by enhanced fertility, which compensated for the times of food deprivation (Speakman 2008a).

The knowledge of which genes predispose to weight gain and how they can interact with the environment will provide the key to understanding and tackling the obesity epidemic.

1.1.2.1.2 Genes

The main methods used to investigate genetic loci for obesity and T2DM during the last 15 years were candidate gene studies and genome-wide linkage studies. The progress of candidate gene studies has been slow until recently, when meta-analyses and large studies identified a few gene variants associated with obesity (Vimaleswaran et al. 2010). Genome-wide linkage studies were not successful at finding loci for obesity. Saunders and colleagues (Saunders et al. 2007) published a meta-analysis of genetic linkage data of BMI and BMI defined obesity from 37 studies. The data included 31,000 individuals from 10,000 families. However, despite sufficient statistical power the study did not

identify loci for obesity. The last published update (12^{th}) of the Human Obesity Gene Map (2005) has identified 127 candidate genes associated with obesity-related phenotypes (Rankinen et al. 2006). Methods used in the studies that contributed to building the map used mostly candidate gene and genome-wide linkage approaches.

The genome-wide association approach started to be used in 2005 and has now replaced genome linkage studies. It was proven to have better resolution, be more cost effective and easier as it does not require recruiting related individuals (Vimaleswaran et al. 2010). This method can identify genetic loci associated with the disease investigated. Usually, genome-wide association studies have a discovery stage followed by one or more replication stages that validate the initial finding (Vimaleswaran et al 2010). Up to February 2010, there have been 19 loci identified that are associated with obesity (Vimaleswaran et al. 2010). Among these loci, the first one found and also the one that has the largest effect on obesity susceptibility, was the fat mass and obesity associated (FTO) gene region (Fawcett et al. 2010). A number of studies investigated functions of FTO and it was summed up that the gene, in human, has a role in controlling energy intake but not energy expenditure (Fawcett et al. 2010, Speakman et al. 2008b).

Heritability of obesity has been found to explain 30-40% up to 80% of the variation in BMI (Martinez-Hernandez et al. 2007, McPherson 2007, Allison et al. 1996). The percentage is dependent on the ethnic group but has not yet been fully scientifically explained (Blakemore 2008).

Genes that increase the susceptibility to obesity have been divided into four major groups: (1) genes regulating food intake, (2) genes that affect adipocyte differentiation and fat storage, (3) genes involved in the regulation of spontaneous physical activity and finally (4) genes regulating basal and postprandial thermogenesis (McPherson 2007). Blakemore and Froguel (Blakemore 2008) in their review of genetics of obesity pointed out that the current evidence mainly supports the importance of genes regulating appetite (i.e. leptin, melanocortin). Degeneration of these genes such as disturbances of gene transcription or altered protein function may predispose to obesity. On the basis of the number of genes involved, obesity was divided into monogenic, syndromic or polygenic (Martinez-Hernandez et al. 2007).

Monogenic obesity, caused by a defect in a single gene is rarely seen in humans. Up to October 2005, the human obesity gene map reported the discovery of 11 genes in which mutations resulted in obesity (Rankinen et al. 2006). Examples of candidate genes for causing monogenic obesity are: Trp64Arg

variant in the beta-3 adrenergic receptor gene, Val103Ile variant in melanocortin-4 receptor (MC4R), single nucleotide polymorphisms (SNPs) in pro-opiomelanocortin (POMC) and also leptin (LEP), leptin receptor (LEPR), and neurotrophic tyrosine kinase receptor 2 (NTRK2) (Farooqi et al. 2007). The prevalence of single mutations in these genes in the general population is very low, which leads to the conclusion that the obesity epidemic is not caused by the single-gene mutations. The discovery of these rare mutations provides a basis for understanding pathways involved in the regulation of body weight, for example pathways regulating food intake and energy expenditure (Loos et al. 2003).

Syndromic obesity is one of the phenotypic features of rare genetic or chromosomal defects. Other features of these defects may include organ dysfunctions, mental retardation or dysmorphic features. There are about 30 syndromes that result in obesity (Martinez-Hernandez et al. 2007). However, they constitute a very small number of all obesity cases. The well known genetic endocrinopathies causing obesity are: the Prader-Willi syndrome (occurring 1 in 25000 cases); even less common are the Bardet-Biedl syndrome, Albright's hereditary osteodystrophy and Cohen's syndrome (Martinez-Hernandez et al. 2007).

Polygenic obesity is the most commonly observed case. It is characterized by several genes interacting and contributing to the obese phenotype; those effects can be additive or non-additive (Hinney et al. 2010). It is also not known whether obesity is caused mostly by common polymorphisms or by the varied combinations of different polymorphisms (Farooqi et al 2007)

Loos and Bouchard (2003) proposed four levels of genetic determination of obesity in relation to the environment. The levels were as follows: genetic obesity – when obesity occurs despite the environment; strong genetic predisposition – when an individual is overweight in a normal environment and obese in an obesogenic environment; slight genetic predisposition – when an individual is normal-weight in a normal environment and overweight in an obesogenic environment; and genetic resistance – when an individual is normal-weight even in an obesogenic environment.

The relationship between environment and genes can mean that the environmental factor modifies the activity of the protein coded by the investigated gene. Also, some genes can influence the effect of physical activity or food intake on the phenotype. If there is some degree of an additive effect of environmental and genetic factors, then this interaction can influence a trait like body weight or BMI (Martinez-Hernandez et al. 2007). At the population level, both the exposure to an obesogenic environment and a high genetic susceptibility increases the risk of obesity. It is important to remember

that environmental influences, as opposed to the genetic ones, can be modified (e.g. diet, physical exercise). Moreover, there is also the possibility of gene-gene interactions with genes attenuating or weakening each other's influences.

The complexity of the genetics of human obesity cannot be underestimated. The complete knowledge of human obesity genes would allow for the treatment of genetic obesity syndromes, the discovery of drugs that target molecular pathways that control human energy balance, and the classification of obese individuals into subgroups that respond to specific treatments like diets, drugs, exercise or surgery (Farooqi et al. 2007).

1.1.2.1.3 Environment

Although genes may predispose to becoming obese, it is highly unlikely that they have significantly changed since the 1980s when the obesity epidemic started. The most likely factors that influenced the huge rise in obesity prevalence were changes in the environment and society. The World Health Organization (2000) report on obesity points at modernisation, economic restructuring and transition to market economies, increasing urbanisation, changes in the role of women and social structures, and globalisation of world markets as the main environmental influences.

Swinburn and colleagues (1999) defined the obesogenic environment as the 'sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations'. These influences were grouped into 4 types: (1) food availability, (2) cost, (3) policies concerning food and (4) attitudes and beliefs relating to food. Each type of influence was proposed to have 2 sides: "micro-environmental" if it describes an effect on individual behaviour choices; and "macro-environmental" if it concerns food supply and lifestyle. The same authors in 2004 proposed a model of interactions between factors that promote obesity (movement inertia, mechanical dysfunction, psychological dysfunction, dieting, low socioeconomic status) and prevent obesity (social discrimination, personal physical discomfort, knowledge about weight gain causes, physiology). The model acknowledged that factors promoting obesity are stronger than those preventing it and apart from causing obesity they also prevent its management (Figure 2).

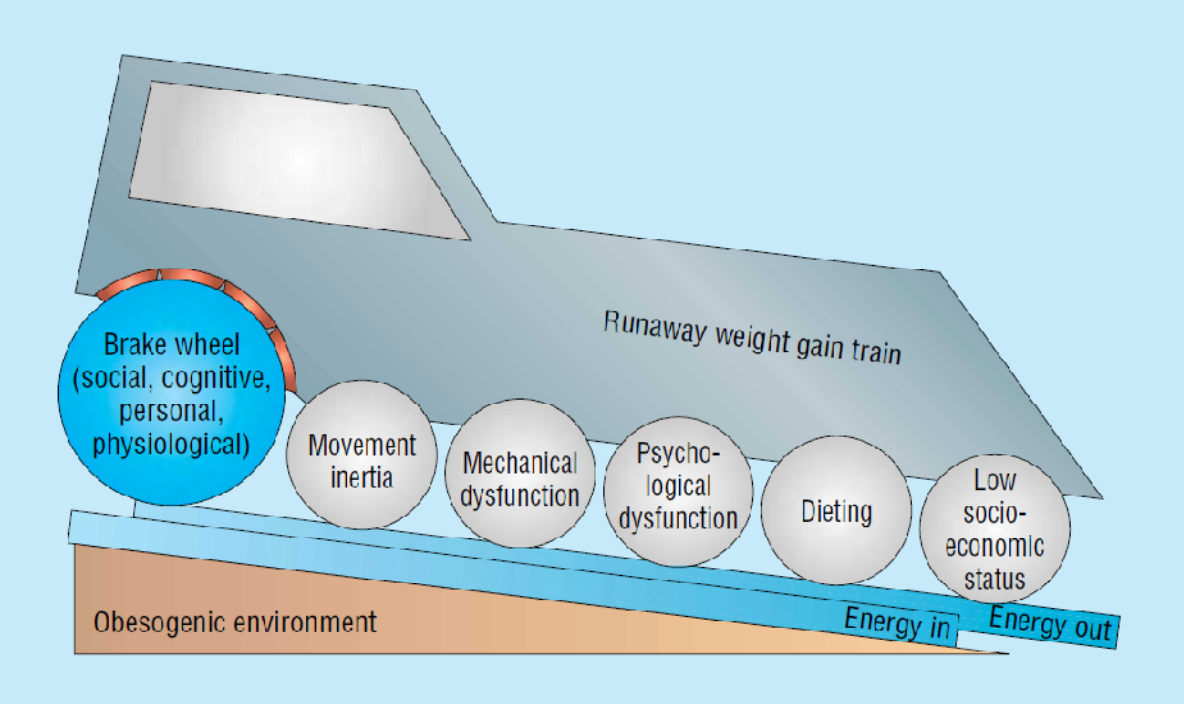


Figure 2. Model of interactions between factors that promote and prevent obesity (Source: Swinburn and Egger 2004).

The division of the obesogenic environment into "food environment" and "physical activity environment" suggested by Hill (2006) can be analogously generalised to an "energy intake environment" and "energy expenditure environment". The changes that have been recently occurring in these environments can be summarised as a tendency to overeat and to be sedentary. Changes in both energy intake and energy expenditure environments were happening simultaneously.

The changes in the food environment during the last few decades are a reflection of urbanisation and globalisation of world markets, which made the food more available and easily affordable (Ulijaszek 2008). The development of technology influenced the food industry which resulted in the creation of many new foods that were attractive and palatable and did not necessarily have appropriate nutritional value. The availability and palatability conspire to create an environment where passive overeating is likely. Therefore, there has been a shift towards the consumption of energy dense foods which are cheaper and more strongly advertised than the healthier options. Also, the world-wide trend for women to work full-time has added to the global change in the food environment. As women do not have time to cook, there has been an increasing demand for ready made meals, home deliveries and takeaways,

which usually are high in fat and/or sugar. Passive overeating over a longer period is likely to occur when such foods are consumed.

The physical activity environment (Hill 2006) has been changing along with the food environment. Advancing technology has allowed jobs, that in the past required hard physical work, to be done by machines. Nowadays, many jobs are being done sitting (e.g. using computers). The options of spending leisure time being physically inactive are also very broad (TV, internet, video games, etc.). Additionally, transportation modalities have changed and people walk less than they ever did. Moreover, the urbanisation and community design often promote the use of cars rather than bicycles or walking. All these factors result in decreased energy expenditure. Bell and co-workers (2004) showed that individuals with higher energy expenditure and intake are better at regulating energy balance than individuals with lower energy expenditure and intake. In other words, they showed that high energy flux helps to regulate energy balance more accurately. Therefore, physical inactivity does not only make it easier to gain weight because of proportionally too high energy consumption but also weakens the body's ability to regulate energy balance.

1.1.2.1.4 Behaviour and lifestyle

Humans, just like other mammals, have a tendency to overeat when food is plentiful, palatable and high in fat. However, as mentioned above, the availability of food for humans is different than for other non-domesticated mammals. There are social and cultural eating norms and, on an individual level, there are also personal feeding constraints (Ulijaszek 2008). All these influences make the behavioural model of human feeding very complex. The diagram from the Foresight report (on Figure 1) illustrates those relationships. There are various psychological theories for the causes of obesity. Generally, they associate obesity with behavioural, cognitive or emotional processes (British Nutrition Foundation 2000). The origin of those processes can be genetic, environmental, or the interaction of both.

Social differences also influence the risk of obesity. It has been established that higher socio-economic groups and those (with higher income) eat healthier foods and their physical activity is higher than subjects from lower groups (Ulijaszek 2008). In contrast with tradition of regular meals at set times, it has become culturally acceptable to have and consume food almost everywhere and at all times.

1.1.2.1.5 Physiology

Appetite and satiety are centrally regulated by the hypothalamus. The hypothalamic function is regulated by certain hormones: insulin, glucagon, gut-peptides, leptin, melatonin and cytokines. Within the hypothalamus there are specific peptides operating on numerous pathways that either stimulate or suppress appetite and satiety. These pathways often have very similar functions; therefore if one fails, others can compensate (British Nutrition Foundation 2000). The systems that regulate human feeding behaviour work in both the short term and long term (Frayn 2003). The full neuroendocrine map of human feeding control is very complex and there are still areas that need to be investigated. Figure 3 presents a simple model of negative-feedback regulation of food intake proposed by Morton et al. (2006) in their review on central nervous system control of food intake and body weight.

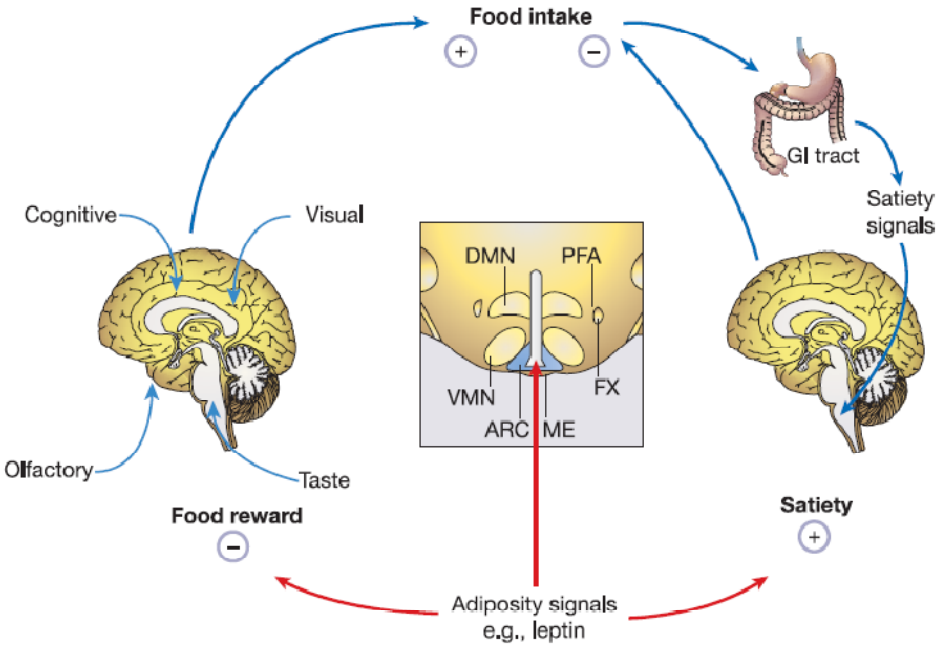


Figure 3. Regulation of food intake in response to adiposity signals (Source: Morton et al. 2006). ARC, arcuate nucleus; DMN, dorsomedial nucleus; FX, fornix; ME, median eminence; PFA, perifornical area; VMN, ventromedial nucleus.

The homeostasis of body weight maintenance, namely the mechanisms that prevent the body gaining or losing weight vary between individuals. Weight gain occurs when energy intake is greater than the body's ability to compensate with energy expenditure. The physiological control of the reduction of food intake is weak (Ulijaszek 2008) but the body does have the mechanisms to address this. Swinburn and Egger (2004), in their model of the obesity epidemic drivers (Figure 2) placed physiology as one of the factors that counterbalanced obesity. Among the physiological mechanisms that prevent weight

gain, they listed a decrease in appetite; increase in fat oxidation; increase in energy expenditure; occurrence of insulin resistance; increase in the activity of sympathetic nervous system and increase in leptin concentration.

1.1.2.2 Development of T2DM

There are two theories on the causes of hyperglycaemia; “glucocentric” and the more modern “lipocentric”. The classic glucocentric view on the causes of hyperglycaemia assumes that there is an impaired glucose metabolism. According to this view, the cause of hyperglycaemia is insulin resistance resulting from obesity and the loss of pancreatic beta-cells. Seen as a disease of glucose metabolism, T2DM should therefore be treated with glucose lowering drugs and insulin. The lipocentric view recognizes calorie surplus as the reason for hyperglycaemia in T2DM (Figure 4). The majority of type 2 diabetic patients are overweight and obese (World Health Organization 1999). Therefore, T2DM ought to be treated by eliminating excess calories and excess body weight. According to this view, insulin therapy should be a last resort in the treatment of T2DM.

There is a paradox of insulin resistance during T2DM. The body is resistant to insulin action on the glucose metabolism and at the same time sensitive to insulin action on lipogenesis. The paradox exists because these two actions have different molecular pathways. Resistance to insulin action on the glucose metabolism occurs because insulin down-regulates insulin receptor substrate 2 (IRS-2), which is the main effector of insulin signalling in the liver (Boissan et al. 2005). This results in liver resistance to suppress glucose production. On the other hand, sensitivity to insulin action on lipogenesis is observed because insulin stimulates production of sterol response binding element protein 1c (SREBP-1c), which stimulates lipolysis. Increased liver synthesis of fatty acids may in the long run lead to increased adiposity.

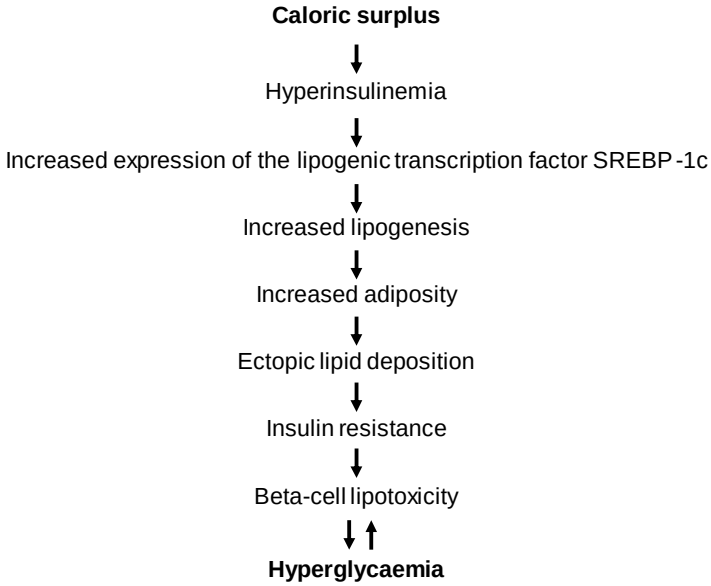


Figure 4. Pathway to hyperglycaemia according to the lipocentric view on T2DM (Unger et al. 2008).

Stefanović and co-workers (2008) reported that an increased oxidative stress and elevated levels of leptin which occur during obesity are likely to play a role in the development of T2DM (Figure 5). Oxidative stress causes insulin resistance and beta-cell dysfunction. Elevated levels of leptin are considered to be the main mediator of insulin resistance induced by oxidative stress. Although leptin concentrations depend on the adipose tissue excess and not on the presence of diabetes, these concentrations were higher in obese patients with T2DM than in obese non-diabetics (Stefanović et al. 2007).

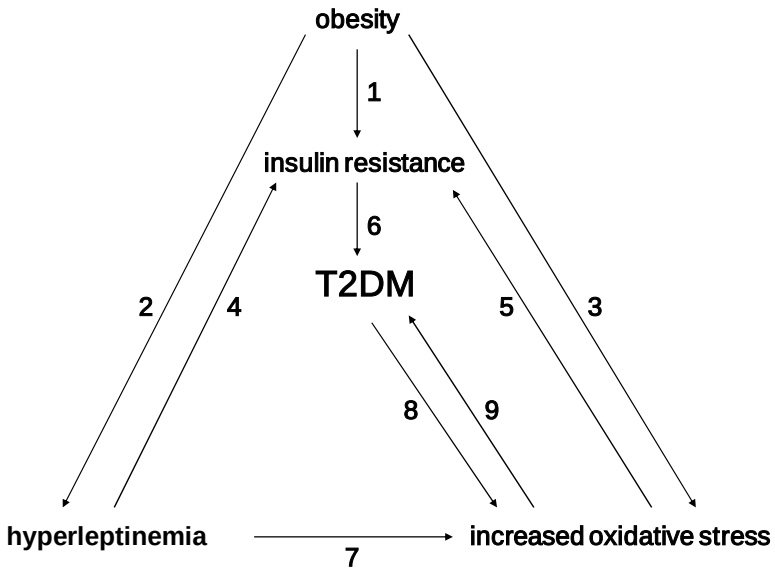


Figure 5. Interrelationship between levels of leptin, oxidative stress, obesity and T2DM (based on Stefanović et al. 2007). Obesity causes insulin resistance (1), higher levels of leptin (2) and increases oxidative stress (3). Both hyperleptinemia (4) and an increased oxidative stress (5) can cause insulin resistance which in the long run may cause T2DM (6). Elevated concentrations of leptin are said to induce oxidative stress (7). T2DM can cause oxidative stress (8) through an increased concentration of glucose and free fatty acids. Finally, oxidative stress can contribute to the development of T2DM (9) by causing pancreatic beta-cell dysfunction.

It was shown that circulating serum levels of leptin were more closely associated with subcutaneous than visceral adipose tissue (Wauters et al. 1998, Park et al. 2004). Montague et al (Montague et al. 1997) and Lefebvre et al (Lefebvre et al. 1998) also showed that leptin mRNA expression levels were higher in subcutaneous than in visceral adipocytes. Additionally, women tend to have higher leptin levels than men, due to more body fat and less visceral fat until menopause. Bennett et al (1997) observed that this was also the case after the correction for fat mass. They suggested that this difference can be explained by different partitioning between subcutaneous and visceral fat.

Genome-wide association studies identified up to date about 30 loci associated with risk of T2DM. It is possible to predict diabetes in a general population using risk scores developed from the identified loci. A study by Hivert et al (2011) on over 2800 participants from the United States, has shown that in high risk individuals, genetic risk score was associated with the higher risk of getting diabetes.

The difficulty in fighting the obesity epidemic lies in its complex and multifactorial etiology. It is equally hard to tackle T2DM with it being much related to obesity (90% in North America and 80% in Western Europe; Rigby and James, 2003). Therapies stemming from the glucocentric view of the disease development that aim to lower blood glucose levels, may ease the complications but cannot address its causes. Some of the newer drugs (i.e. incretin mimetics) along with lowering blood glucose can also induce a certain amount of weight loss. Finally, weight loss has been shown to be a very successful strategy for T2DM treatment, proving the validity of the lipocentric view of the obesity origin. Therefore, the recent Scottish Intercollegiate Guidelines Network (2010) guidelines on the management of diabetes state that obese patients with T2DM should be offered individualized obesity treatments to achieve metabolic control (SIGN, 2010). The interplay of genetic and environmental factors involved in T2DM etiology is complicated and the sets of genes involved and the environmental circumstances are different for each individual.

1.1.3 Tackling obesity and T2DM

There are numerous programmes, guidelines and recommendations that aim to tackle obesity and T2DM at the individual level. The majority of these suggest lifestyle changes, namely improvements in the quality of diet and increases in physical activity. This approach is not new. Hippocrates recognized that the diseases are caused naturally by environmental factors and lifestyle and therefore advised treatments that very often addressed diet and physical activity (Mantzoros 2006). In 1934, William Howard Hay wrote in 'Health via Food': "All we can do about disease after all is to stop creating it daily, as we do by our customary habits of living". Anand and Yosuf (2011) when commenting on the very recent world BMI trends report (Finucane et al. 2011) concluded that the global population should be a part of the solution to the global obesity problem, in other words, a problem that affects everyone should be corrected with everyone's effort.

Currently, the WHO suggests preventive measures of limiting energy from total fats, and consuming more unsaturated fats; increasing consumption of fruit, vegetables, legumes, whole grains and nuts, limiting sugar intake and increasing physical activity to 30 minutes of moderate intensity activity on most days (World Health Organization 2011b). The WHO leaves the implementation of the above recommendations to governments, public and individuals. The National Institute for Health and Clinical Excellence (NICE 2008) placed lifestyle and diet modifications as the first step in the treatment of obesity and T2DM. If these are not sufficient, then appropriate oral medications and perhaps insulin with the latter should be introduced (NICE 2008).

On the national level, there are many projects and actions undertaken to tackle both obesity and T2DM. The UK government Foresight Programme produced the 'Foresight Tackling Obesities: Future Choices' report that presents challenging visions of the future, to stimulate appropriate actions now. The report predicts that if no action is taken, in 2050 obesity will affect 60% of men and 50% of women (Butland et al. 2007). Recently, the Scottish government published a document 'Preventing Overweight and Obesity in Scotland: A Route Map Towards Healthy Weight'. The report was aimed at decision makers in local and central government. It outlined policies that are already in place and stressed that the scale of preventive activities has to increase. Finally, the report listed further government actions that have to be taken in order to address the obesity problem (The Scottish Government 2010). Among those actions were controlling exposure to energy consumption by intervening at the food supply level, increasing opportunities for energy expenditure by promoting and facilitating physical activity, delivering early life interventions to develop positive health behaviours, increasing responsibility of organizations for health of their employees, raising awareness among decision makers or changing public attitudes. The English government has produced the 'White Paper Healthy Lives, Healthy People: Our Strategy for Public Health in England', which described government's plans to improve various areas of public health (HM Government, Department of Health 2010). The key documents linked to this paper will follow; the obesity document is planned to be published in spring 2011.

At the world wide level, the WHO produced the WHO Global Strategy on Diet, Physical Activity and Health which outlines what has to be done to promote healthy diets and physical activity. The Department of Nutrition for Health and Development of the WHO additionally promotes healthy nutrition to address the nutrition-related health problems. This work is done by supporting countries in building and implementing nutrition policies. The work also aims to contribute to the achievement of the Millenium Development Goals (United Nations 2010). Finally, there are organizations and charities that address the issues of obesity and diabetes, promote research, encourage education and help to support affected individuals. Examples include the International Association for the Study of Obesity, Diabetes UK and the American Diabetes Association.

During the recent years, scientists have become interested in the use of high-protein, low-carbohydrate diets as supplementary treatment for T2DM, obesity and also CVD. Westman and colleagues (Westman et al. 2007) argued that before agriculture allowed access to starchy foods, people lived on low-carbohydrate, high-protein diets, and therefore such a nutritional approach should be well accepted

by humans. There is a counter argument that carbohydrate intake was high due to fruit and berry scavenging in some parts of the world. However, carbohydrate amounts and the nutritional value of berries and fruit (hand-picked) cannot be compared to those of processed foods available in unlimited quantities nowadays.

It was proposed that, as recent increases in the prevalence of obesity and diabetes occurred concomitantly with an increase of carbohydrate consumption, restriction of carbohydrate intake may prove a successful therapy for these diseases (Westman et al. 2007). The Hindu physician Sushruta, who lived in the 6th century BC, described a disease of sweet urine (Tattersall 2009). The physician observed that this disease was occurring in families, and people who had it, were often overweight. Sushruta recommended physical activity and diet consisting of vegetables as the treatment for obese people with this disease (Tattersall 2009). Before insulin therapy became available for diabetic patients in the 1920s, the main treatment for diabetes was a low-carbohydrate diet, normal or high in protein and very high in fat (Yancy et al. 2005, Westman et al. 2006). Such a diet resulted in the control of the glucose levels in the urine (Westman et al. 2007).

1.2 Cardiovascular risk in obesity and T2DM

1.2.1 Cardiovascular risk in obesity

Obesity increases the risk of coronary heart disease (Zalesin et al. 2008) and stroke (Chan et al. 1994). Coronary heart disease (CHD) is the main reason for mortality among the obese (British Nutrition Foundation 2000). Excess adipose tissue increases the risk of developing cardiovascular disease (CVD) by association with increased inflammation processes, hypertension, increase in triacylglycerol (TG) levels, and changes in high density lipoprotein cholesterol (HDL-cholesterol) and low density lipoprotein cholesterol (LDL-cholesterol) to smaller and denser particle sizes (Frayn 2003).

It has been established that tissues may become resistant to insulin action when obesity develops. Metabolic changes observed in obesity come mostly from insulin resistance (Frayn 2003). Typically, the level of HDL cholesterol decrease and the levels of both LDL-cholesterol and TG increase. If the LDL-cholesterol particles are smaller and denser than in the healthy conditions, this profile is described as an atherogenic lipoprotein phenotype (Frayn 2003).

Elevated total cholesterol levels are associated with atherosclerosis (Willeit et al. 2000). Atherosclerosis is a process in which fatty acid deposits build up in the arterial walls (Frayn 2003). These deposits start from the damage to the arterial wall (Ross 1999). The damage may be caused by oxidative stress, hypertension, hyperlipidaemia (especially high LDL cholesterol), chronic infections, smoking, frequent alcohol consumption (Willeit et al. 2000) and also by the elevated concentrations of glucose in blood (Massi-Benedetti et al. 1999). The damage causes endothelial dysfunction and results in inflammatory processes. The process of formation of atherosclerotic lesion that results from the damage to the endothelium is explained on Figure 6. Atheromateous plaque has a complex structure that comprises of lipids rich in cholesterol and inflamed, proliferating cells of smooth muscle and connective tissue. Atheromateous plaque narrows arteries, which may result in the insufficient supply of blood to the organs. Atherosclerosis of coronary arteries (coronary heart disease) is especially dangerous, as it can result in chest pains (angina) due to ischemia and even in death. The Framingham study has shown that both adiposity and coronary risk factors relate to the volume of coronary plaque (Takeshita et al. 2008).

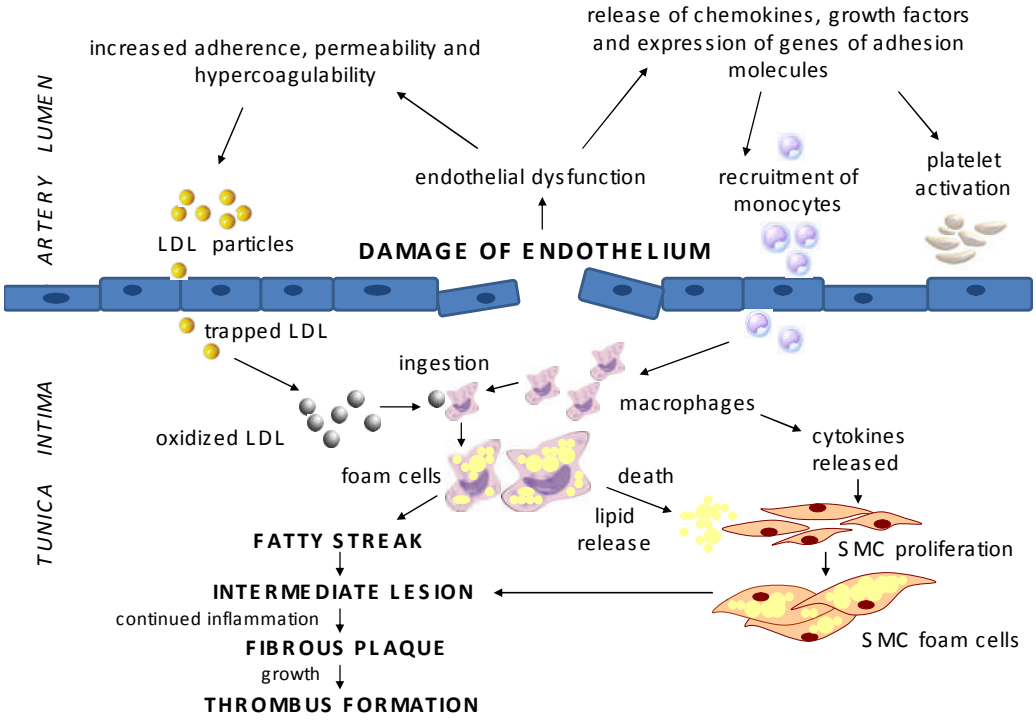
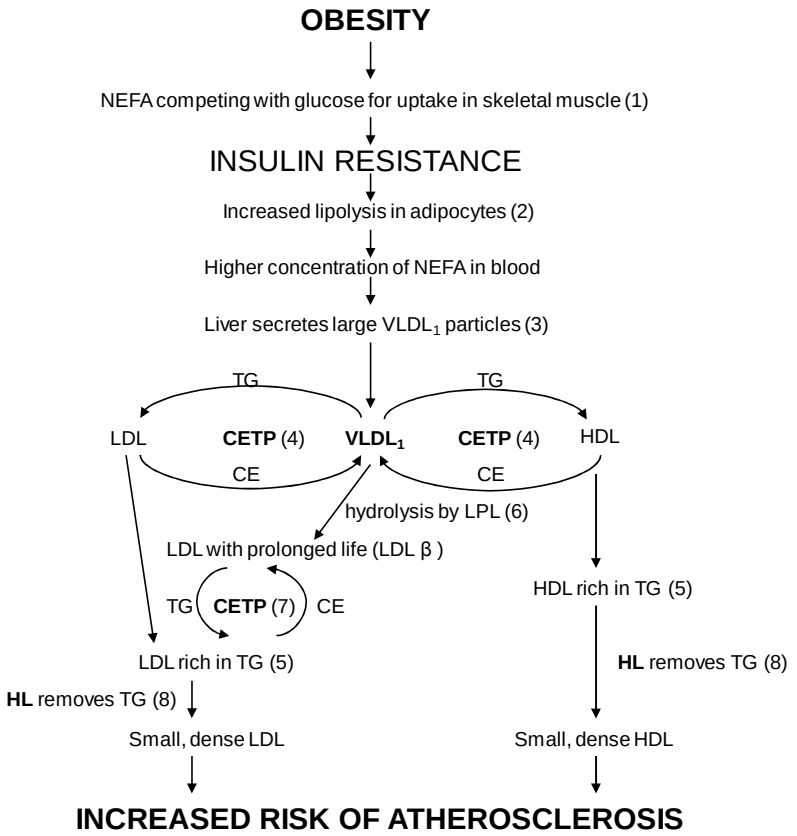


Figure 6. Damage to the vascular endothelium and formation of an atherosclerotic lesion (adapted from Arici and Walls, 2011). SMC – smooth muscle cells.

Obesity also has a lipotoxic effect on the heart itself. Increased concentrations of TG in non-adipose tissues (steatosis) are likely to increase intracellular acyl-CoA. Acyl-CoA is a substrate for ceramide synthesis which leads to cell dysfunction and apoptosis (Rana et al. 2007). Moreover, elevated non-estrified fatty acids (NEFA) concentrations can cause an irregular heartbeat and in severe cases, ventricular fibrillation which is an irregular fluttering of ventricles that can lead to death (Frayn 2003).

1.2.1.1 Lipid profile

The atherogenic lipoprotein phenotype characteristic for obesity, metabolic syndrome and T2DM (Packard 2003) increases the risk of coronary heart disease by predisposing to atherosclerosis. The mechanism for this process is associated with insulin resistance and illustrated in Figure 7.



KEY:

1. In obesity, NEFA are released from adipocytes into the blood and they compete with glucose for uptake into skeletal muscles which can be part of the reason for insulin resistance (Parker, Noakes et al. 2002, Miyashita, Koide et al. 2004).
2. During insulin resistance, the rates of lipolysis in adipocytes increase causing even more elevated NEFA levels (Volek, Sharman et al. 2004).
3. Increased concentrations of NEFA promote the secretion of $VLDL_1$.
4. An increase in $VLDL_1$ concentrations facilitates TG transfer from $VLDL_1$ to LDL and HDL and cholesteryl esters from HDL and LDL to $VLDL_1$ via CETP (Frayn 2003).
5. This results in $VLDL_1$ becoming richer in cholesterol and HDL and LDL richer in TG.
6. Additionally, LPL hydrolyses large $VLDL_1$ to LDLβ that has longer life in circulation than LDL hydrolysed from normal sized VLDL.
7. Longer life of LDLβ gives the particle more opportunities to be remodelled by CETP and to become rich in TG.
8. Particles of both LDL and HDL rich in TG are more prone to the action of HL which removes TG and makes them smaller and denser.

Figure 7. Stages in the linkage of insulin resistance and atherosclerosis (adapted from Packard, 2003).

NEFA – non-estrified fatty acids; $VLDL_1$ – large VLDL particles; CE – cholesteryl esters; CETP - cholesteryl ester transport protein; LDLβ – LDL with prolonged life; LPL – lipoprotein lipase; HL – hepatic lipase; TG, triacylglycerol.

HDL, when rich in TG, becomes susceptible to the lipolytic action of hepatic lipase (HL) (Volek et al. 2004). Hepatic lipase turns HDL into smaller, denser particles. Small HDL is less able to accept cholesterol from tissues for transfer to the liver and consequently levels of cholesterol in blood rise.

Cholesteryl ester transfer protein (CETP) transfers TG from VLDL cholesterol into LDL cholesterol. Lipolytic hydrolysis of TG-rich LDL particles by hepatic lipase (HL) results in the formation of small LDL particles (Packard 2003). Small, dense LDL cholesterol has a lower affinity to LDL receptors, therefore its life in circulation is longer and consequently chances of infiltration of arterial walls are higher. Additionally, smaller, denser LDL particles are more prone to oxidation (Packard 2003), which further increases the risk of developing atheromateous plaque.

1.2.1.2 Endocrine activity of adipose tissue

In adults, the majority of the fat tissue is composed of white adipose tissue (WAT). Brown adipose tissue is characteristic of neonates; the darker colour is a result of a high number of mitochondria in brown adipocytes. WAT includes not only adipocytes and pre-adipocytes, but also other types of cells like macrophages, endothelial cells, leukocytes and fibroblasts (Wozniak et al. 2008).

WAT has a significant role in energy homeostasis, immunity and inflammation (Antuna-Puente, Feve et al. 2008) because it produces specific molecules, named adipose derived hormones or adipokines. Adipokines are involved in autocrine and paracrine signalling and include leptin, adiponectin, resistin, and also adipsin, visfatin, apelin, vaspin, hepcidine, chemerin and omentin (Trayhurn 2005, Wozniak et al. 2008). WAT also has the capability of producing various inflammatory cytokines.

One of the features of obesity is a mild inflammatory state (Trayhurn 2005). An increase in the amount of fat tissue is related to the increase of inflammation-related adipokines. Therefore, levels of inflammatory markers are higher in obese subjects (Trayhurn 2005). Increased inflammation in obese individuals may result in the occurrence of T2DM or metabolic syndrome. For example, increased concentrations of leptin, resistin and TNF-α impair insulin sensitivity and trigger atherogenesis (Rana et al. 2007).

1.2.1.3 Inflammation

Typically, obesity and T2DM are characterized by a state of chronic low-grade inflammation that occurs in the liver, muscles and adipose tissue (Rana et al. 2007). It has been observed that in obese individuals there is an increased macrophage infiltration of WAT (Heilbronn 2008). Macrophages as well as WAT secrete various pro-inflammatory molecules.

Cytokines are molecules that facilitate cellular communication (proteins, peptides, glycoproteins). They are critical for the function of the immune system. Cytokines are secreted by immunity cells in response to pathogens. There are cytokines with pro- and anti-inflammatory properties. Cytokines secreted by adipose tissue are called adipocytokines and include interleukin (IL)-1beta, IL-6, IL-8, IL-10; tumour necrosis factor (TNF)-α; plasminogen activator inhibitor (PAI)-1; and retinol binding protein 4 (RBP4); monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor, nerve growth factor, vascular endothelial growth factor, and haptoglobin (Trayhurn 2005, Wozniak et al. 2008, Antuna-Puente et al. 2008).

Pro-inflammatory adipocytokines play a role in the pathogenesis of atherosclerotic cardiovascular disease (Amasyali et al. 2008). Interleukin-6 (IL-6) is a cytokine with both pro- and anti-inflammatory properties and is secreted by macrophages and T-cells. In obesity, and also in T2DM, IL-6 concentration is increased and the compound is thought to interfere with insulin action by suppressing insulin signalling transduction (Rana et al. 2007). IL-6 has the ability to inhibit TNF-α which is another pro-inflammatory cytokine, and one of the regulators of vascular homeostasis (Zhang et al. 2009). TNF-α can inhibit viral replication; induce apoptotic cell death and inflammation. If over expressed, TNF-α induces the production of reactive oxygen species (ROS) which results in endothelial dysfunction (Zhang et al. 2009). Another cytokine, PAI-1, is produced by endothelial cells and also by adipose tissue. It inhibits activators of fibrynolysis, and therefore inhibits the degradation of blood clots. IL-18 is a proinflammatory cytokine produced by macrophages. IL-18 induces severe inflammatory reactions.

Cytokines 1, 6, 8, and TNF-α all stimulate the liver to produce proteins of the acute-phase group. As their levels increase the response to inflammation, they are often used as markers to assess the state of inflammation. Acute-phase proteins have various functions during inflammation, for example opsonization[2] of antigens (C-reactive protein), inhibition of micronutrient uptake by microbes (ferritin,

[2] opsonization is the process by which an antigen is marked for destruction by a phagocyte.

haptoglobin), inhibition of coagulation or fibrynolysis (alpha-2-macroglobulin) or facilitation of immune cell migration to the sites of inflammation (serum amyloid A) (Gruys et al. 2005).

C-reactive protein (CRP) is a marker of inflammation that is produced in the liver. The protein is produced in response to high levels of IL-6 and it binds to microbes and enhances phagocytosis by macrophages. Elevated CRP levels are associated with an increased risk of cardiovascular disease. The risk is considered low when the concentration of CRP is lower than 1mg/L and is accepted as high when the concentration is higher than 3 mg/L (Shine et al. 1981, Seshadri et al. 2004). Levels higher than 10 mg/l are considered to be alarming in a biochemical setting (Shine et al. 1981). CRP levels were found to be decreased with weight loss only in insulin resistant obese subjects (McLaughlin et al. 2002). These effects were proportional to the amount of weight lost but independent of dietary macronutrient composition.

Apart from producing molecules that promote atherogenesis, WAT also produces a hormone which has anti-atherogenic actions: adiponectin. Unfortunately, in obesity, diabetes, atherosclerosis and hypertension, concentrations of this hormone are decreased (Han et al. 2007). Anti-atherogenic effects of adiponectin include the increase of secretion of nitric oxide (NO) and the inhibition of production of adhesion factors (Maia-Fernandes et al. 2008).

1.2.1.4 Blood pressure

Hypertension can cause vascular endothelial injury predisposing to atheroma formation (Turner et al. 2002). Systems involved in the aetiology of hypertension in obese patients are complex (Kurukulasuriya et al. 2008) and are still being investigated. The main mechanisms considered are the activation of the sympathetic nervous system and the dysregulation of the renin-angiotensin-aldosterone system (RAAS) and the cardiac natriuretic peptide (CNP) system.

In obesity, the sympathetic nervous system is constantly activated (Morse et al. 2005). The sympathetic nervous system is a part of the autonomic nervous system and mobilizes the body during activity. The sympathetic over-activity affects kidneys by increasing the reabsorption of sodium in the renal tubules (Rahmouni et al. 2005) and also causes peripheral vasoconstriction (Morse et al. 2005). Therefore, sympathetic activation leads to hypertension. Rahmouni and co-workers (2005) suggested that this activation is caused by hyperinsulinemia, hyperleptinemia, increased NEFA concentrations, and increased aldosterone concentrations.

The RAAS is dysregulated in obese patients (Sarzani et al. 2008). RAAS is an endogenous system that answers to a decrease in renal perfusion and promotes hypertension by increasing tubular active reabsorption of chloride and sodium, the secretion of potassium, and also water retention. In obesity, this system is activated and therefore blood pressure increases. RAAS influences the metabolism of adipocytes, because angiotensin II stimulates adipogenesis and adipocytes metabolism (Karlsson et al. 1998). Angiotensin II is produced by the cleavage of angiotensinogen by angiotensin converting enzyme and renin (Karlsson et al 1998). Angiotensionogen mRNA levels were found to be higher in the visceral fat than in the subcutaneous fat (Duserre et al. 2000). This relationship was more pronounced in obese individuals.

Another mechanism that is likely to cause hypertension in the obese is the dysregulated CNP system. Obese patients were found to have lower levels (Sarzani et al. 2008) and lower biological activity of CNP (Morse et al. 2005). CNP is involved in the sodium balance and metabolism, by inducing natriuresis and diuresis and also by inducing lipolysis and mobilization of fat stores (Sarzani et al. 2008).

1.2.2 Cardiovascular risk in T2DM

Diabetes is a major independent risk factor for CHD and other forms of cardiovascular disease (NHLBI 2002). The AHA (2007) considers T2DM along with overweight, obesity, high blood pressure and high blood total cholesterol as a risk factor for stroke (AHA 2007).

The widely used markers of cardiovascular risk factor are the blood lipid profile (TC, HDL, LDL and TG), blood pressure, waist circumference or waist to hip ratio (WHR) as well as the percentage of body fat and serum concentration of C-reactive protein. Hyperglycaemia is the main risk for adverse cardiovascular outcomes of T2DM and it is independent of other cardiovascular risks that are likely to occur during diabetes such as overweight, obesity and dyslipidaemia (National Heart, Lung and Blood Institute 2002). Hyperglycaemia causes the release of inflammatory cytokines, endothelial dysfunction and hypercoagulability (Golden et al. 2007).

Tight glycaemic control reduces the risk of microvascular complications in diabetes but only a trend towards reducing macrovascular complications has been reported (National Heart, Lung and Blood Institute 2002). Endothelial dysfunction and disturbances in vascular structure were proposed to be a

result of a series of processes activated by hyperglycaemia, like oxidative stress. Oxidative stress inhibits nitric oxide (NO) production. Instead of NO, peroxynitrite and nitrotyrosine are produced. Nitrotyrosine may cause cardiomyocyte apoptosis and vascular DNA damage (Rana et al. 2007).

Monitoring of lipid profile in patients with diabetes is of a high importance. Diabetics compared to non-diabetics have elevated TG and fibrinogen and decreased HDL-cholesterol concentrations (Kannel et al. 1990). Additionally, patients with T2DM also have elevated concentrations of non-estrified fatty acids (NEFA) (Frayn 2003). Patients with both metabolic syndrome and T2DM, have increased levels of small, dense LDL particles which are more atherogenic (Rana et al. 2007). The especially harmful lipid profile, also called the "lipid triad" is characterized by the elevated TG, decreased HDL-cholesterol and increased levels of small, dense LDL-cholesterol (Rana et al. 2007). Therefore one of the main aims of treatment for patients with T2DM should be improvement of their lipid profile.

It was shown that weight loss in obese type 2 diabetic patients improves risk factors for CHD and lowers glycaemia (Heilbronn et al. 1999). Moreover, the ATPIII report (National Heart, Lung and Blood Institute 2002) stated that weight reduction will achieve more overall risk reduction than will LDL lowering without an emphasis on weight control.

1.3 Treatments for obesity and T2DM

Treatment of obesity in subjects with T2DM is recommended to be a priority in the management of these patients (Gagliardi et al. 2007). A weight loss of 5-10% improves glycaemic control, often allowing the reduction or withdrawal of medication (Turner et al. 2002). Lifestyle change is the first line of the treatment with subsequent introduction of medications if the former is not successful. This section will focus on the treatments of obesity and T2DM.

1.3.1 Current guidelines for dietary treatment of obesity and T2DM

Dietary guidelines for type 2 diabetics are based on the knowledge of digestion and absorption processes and postprandial metabolism (Frayn 2003). Scottish Intercollegiate Guidelines Network new guideline on the management of diabetes stated that appropriate diet in T2DM should prevent macrovascular complications (SIGN 2010). Particular attention should be paid to the carbohydrates in the diet because they directly influence glycaemia. The content of simple sugars should be low,

because they rapidly increase blood glucose levels, and fibre content should be higher because it slows down glucose absorption (Frayn 2003). Additionally, overall calorie intake should also be monitored, as weight loss in overweight or obese individuals improves insulin sensitivity (American Diabetes Association et al. 2008) and is an integral part of diabetes care (SIGN 2010).

The Nutrition Subcommittee of the Diabetes Care Advisory Committee of Diabetes UK (Connor et al. 2003) based on the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) guidelines recommended the following nutritional composition of the diet for people with diabetes. Protein intake should be less than 1g per kg of body mass per day. Total energy from fat should not be higher than 35%. Saturated fatty acids (SFA) and trans unsaturated fatty acids should not constitute more than 10% of total energy intake. The n-6 polyunsaturated fatty acids (PUFA) should not constitute more than 10% of energy intake. The amount of PUFA n-3 was not specified but consumption of fish (especially oily) twice a week was suggested. Monounsaturated fatty acids (MUFA) should be from 10 to 20% of energy intake and together with carbohydrate should make up 60 to 70% of energy intake. Carbohydrate should constitute 45-60% of energy intake; the carbohydrate sources with low glycaemic index[3] were promoted.

Diabetes UK recently published a position statement about the use of LCHODs for people with T2DM (Diabetes UK 2011) based on the review of the available evidence from 1998 to 2009. The main recommendation stated that the type of the weight loss diet should be agreed on between the person with diabetes and their dietitian with the main objective of energy expenditure being higher than energy intake. Secondly, people who chose LCHOD should be aware of its possible side effects (i.e. headaches, constipation, lack of concentration, and risk of hypoglycaemia). Thirdly, blood glucose levels should be closely monitored and possible changes in medications considered. Fourthly, the diet should have appropriate amounts of vitamins, minerals and fibre. Finally, the amount of carbohydrate in the diet should be agreed on between the person with diabetes and their dietitian.

The ADA has published a position statement on nutrition recommendations for people with diabetes (2008). In the statement there are four goals of nutritional therapy distinguished: to achieve and maintain normal range of blood glucose levels, lipid and lipoprotein and blood pressure; to prevent or slow progression of diabetes complications; to address individual nutritional needs and to maintain the pleasure of eating. Moreover, the ADA (2008) did not give advice on the optimal macronutrient

[3] Glycaemic index is calculated as the area under the 2 h blood glucose response curve, plotted after the ingestion of a given amount of carbohydrate.

composition of the diet for diabetics, and stated that such composition probably does not exist because of individual circumstances of diabetic patients. On the contrary to such a flexible approach, the Joslin Diabetes Center currently advocates a diet composition of CHO, 40%; P, 30% and F, 30% of total energy for overweight and obese adults with T2DM or pre-diabetes or those at risk of developing T2DM (Acheson 2010).

Weight loss in overweight and obese diabetics is of particular importance. However, people with T2DM have more difficulty with losing weight than people without diabetes (Pi-Sunyer 2005). Low-carbohydrate or low-fat, low calorie diets are effective for weight loss for up to 1 year (Nordmann et al. 2006). It was advised for patients on low-carbohydrate diets to monitor their lipid profiles, kidney function and, in the presence of nephropathy, also protein intake (ADA 2008).

SIGN guidelines on diabetes management (2010) recognised that options of diet induced weight loss for patients with T2DM should be varied and allow individual preferences. The diet should also be able to improve glycaemic control. Choices included calorie restricted diets, low fat diets, low glycaemic index diets and low carbohydrate diets (with not less than 50 g of CHO per day for not longer than 6 months). Additionally, supplementation of vitamin E (500 mg/d) and PUFA n-3 was not generally recommended for T2DM. Finally, the guidelines recognized that alcohol is likely to contribute to the energy intake, and recommended that it should be taken in moderation as part of a healthy lifestyle. The target consumption levels recommended for people without diabetes should not be exceeded. Alcohol, as well as hypoglycaemia, negatively affects cognitive function. Moreover, effects of alcohol and hypoglycaemia on cognitive function are additive.

1.3.2 Low-carbohydrate, high-protein diet

The recommended carbohydrate intake for an adult is 130g/day (Food and Nutrition Board 2005). Long term safety of diets containing less carbohydrate for diabetic patients remains to be answered. According to the ADA (2008) there is insufficient evidence to modify recommended daily intake of protein (15-20% of total daily energy) and therefore high-protein diets are not currently recommended by the ADA.

1.3.2.1 Definition

The Reference Nutrient Intake (RNI) of protein for both men and women is 0.75 g of good quality protein/kg body weight/day (Department of Health 1991). To meet the body's nutritional needs and minimize the risk for chronic disease, protein should constitute between 10 and 35 % of total diet energy (Food and Nutrition Board 2005); Department of Health (1991) suggests avoiding intakes higher than twice the RNI (over 1.5 g/kg/d). The Australian National Survey (McLennan 1998) reported that the usual percentage of energy from protein was 18 % (90 g in 2000 kcal diet). According to the Food Agricultural Organization's FAOSTAT Foodbalance sheets, world's protein consumption in 2007 was 77.1 g per capita per day, out of which 47.3 g was from vegetal products and 29.8 g was from animal products (FAO, 2011). In the United Kingdom in 2007, protein consumption was higher than the world's mean and equated 104.5 g per capita per day. Specifically, vegetal protein consumption was very similar to the world's mean (44.9 g) but consumption of protein from animal products was twice as high (59.6 g) (FAO, 2011).

A standard protein diet contains about 15 % of energy content as protein, which is about 75 g of protein per day for 2000 kcal diet (St. Jeor et al. 2001). In high-protein and very high-protein diets, according to the AHA (St. Jeor et al. 2001), protein constitutes over 20 % and 30 % of total energy (100g/d and 150 g/d respectively for 2000 kcal diet). However, this classification has not been widely recognized. For example, the "high-protein" term was applied by Brinkworth and co-workers (Brinkworth et al. 2004b) to a diet in which protein accounted for 30 % of energy, whereas Muzio et al (2007) used it to describe a diet in which protein constituted only 19 % of total energy.

Although low-carbohydrate diets are also defined in many ways, there is a clear division on low-carbohydrate diets which do not induce ketogenesis and those that do. The latter are called low-carbohydrate ketogenic diets (LCKD) and contain 20 to 50 g of carbohydrate per day. During such low intake of carbohydrate (less than 60 g/d), availability of glucose is restricted and the body starts using substantially more fatty acids as a fuel source. This metabolic state is called ketosis and is detectable by blood or urine test for ketone bodies. Low-carbohydrate but non-ketogenic diets (LCHOD), on the other hand, contain more than 50 g but less than 200 g of carbohydrate per day. Westman and colleagues (2007) suggested that LCHODs are those containing 50 - 150 g of carbohydrate per day because they usually do not induce ketogenesis. Due to the lack of a standardized definition of a low-carbohydrate diet, Bravata and associates (2003), in their systematic review of low-carbohydrate diets, referred to diets containing 20g/d or less as lowest-carbohydrate, those containing 60g/d as lower-carbohydrate and those containing more than 60g/day of carbohydrate as higher-carbohydrate diets.

The restriction of carbohydrate during LCHOD and LCKD results in greater proportions of both fat and protein. Therefore, very often, diets in which the amount of carbohydrate is reduced are high in fat and/or high in protein.

The protein sparing modified fast (PSMF) is an example of a very high-protein LCKD. PSMF was defined as an *ad libitum* low calorie diet (1200 kcal/d) containing approximately 50 % of energy derived from protein, 40 % from fat and only 10 % from carbohydrate which should be no more than 40 g/day (Robertson et al. 2002). Although PSMF is a low calorie diet, it aims at preservation of lean body mass by providing an abundance of protein. This nutritional approach was used to treat a group of obese type 2 diabetic patients who were resistant to drug and standard diet therapy. At 12 months, this intervention was shown to reduce weight and improve glycaemic control in these subjects (Robertson et al. 2002).

Comparability of diet treatments could be improved if the macronutrient content of the diets investigated was expressed not only as a percentage of energy but also as grams per day. The expression of macronutrient content in absolute terms would help to avoid different interpretations of high, normal and low contents of a given macronutrient in the diet (Westerterp-Plantenga 2007). Such caution is especially advisable, when modifications of dietary proteins are reported.

Guidelines for evaluating high-protein diets are provided in the AHA's statement on Dietary Protein and Weight Reduction (St. Jeor et al. 2001). There are three main features evaluated: protein content of the diet (15 % of energy or 50-100 g/d is recommended), carbohydrate content of the diet (minimum of 100 g/d) and amount of fat, saturated fat and cholesterol in high-protein foods. When evaluated by these criteria, low-carbohydrate, very high-protein (protein accounting for over 30 % total energy) diets are not recommended. However, results of numerous studies published after 2001, with obese subjects with or without metabolic syndrome and T2DM, reported that low-carbohydrate, high-protein diets decreased body weight (Brehm et al. 2003), improved lipid profiles (Yancy et al. 2004) and lowered insulin (Seshadri et al. 2004, Foster et al. 2003) and glucose concentrations (Samaha et al. 2003) more than conventional low-fat diets for up to 6 months. It has to be noted that the above studies did not provide evidence that low-carbohydrate diets gave more favourable outcomes than low-fat diets for over 12 months. Bravata and co-workers (2003), in their systematic review, stated that low-carbohydrate diets are safe only for short-term use (up to 90 days) in non-diabetic patients. More recent reviews of low-carbohydrate diets suggested that on the condition of the weight loss occurrence, low-

carbohydrate diets are safe and effective when used for up to 6 months (Nordmann et al. 2006, Astrup et al. 2004, Hession et al. 2009). Nordmann and colleagues (2006) added that low-carbohydrate diets were at least as efficient as low-fat diets in inducing weight loss for up to 1 year. The agreement exists that there is a need for long term studies investigating overall safety and efficacy of high protein diets in healthy and at risk subjects.

It has been recognized that there is no nutritional strategy suitable for all patients. Different macronutrient compositions of the diets may prove to be beneficial in different groups of patients. Bloch (2005) proposed that the recognition of an individual's health condition and needs is crucial for the determination of the best nutritional strategy resulting in improved health and weight loss. Pros and cons of the diet should be considered before it is given to an individual. For example, it is known that diets low in carbohydrate can adversely affect exercise capability; therefore LCHOD or LCKD would not be the first choice for very active people. However, explained later on in this chapter, the majority of people with T2DM are not active (Morrato et al. 2007). Baba and co-workers (1999) reported favorable responses to high-protein, low-energy diets for individuals presenting with insulin resistance. Similarly, Robertson and colleagues (2002) reported that the PSMF resulted in weight loss and improved glycaemic control in patients with T2DM. Following these studies, Volek and Feinman in their review (2005a) concluded that LCHODs offer clinical benefits for insulin resistant patients. Finally, the study by Cornier and colleagues (Cornier et al. 2005) showed very clearly suitability of low carbohydrate diet for obese insulin resistant women. In this 16 week intervention non-diabetic insulin sensitive or insulin resistant women were randomised into low fat (CHO, 60%; P, 20%; F, 20%) or low carbohydrate diet (CHO, 40%; P, 20%; F, 40%). A low carbohydrate diet was more beneficial for insulin resistant women who lost about 13% of the initial body mass compared to 8% for insulin sensitive women. Low fat diet on the other hand, was better for insulin sensitive women who lost about 13% of the initial body weight on it compared to only 7% lost by insulin resistant women. All of the above results could lead to a global recommendation of a low-carbohydrate, high-protein, low calorie diet as a safe and efficient option for weight loss in obese subjects with T2DM.

1.3.2.2 Physiological response to low-carbohydrate diets

Starvation is a condition in which, as a result of lack of energy intake, muscle protein, glycogen, and fat stores are all used as energy supplies (Westman et al. 2007). This condition leads to both fat mass loss and lean body mass (LBM) loss. However, appropriate intake of protein can help to preserve LBM during negative energy balance (Krieger et al. 2006).

A decrease in dietary carbohydrate results in a decrease in blood glucose and insulin concentrations and increase in glucagon concentration. Additional observed hormonal changes include an increase in blood concentrations of epinephrine, norepinephrine, glucagon and growth hormone. All these trigger lipolysis in adipose tissue and induce the release of free fatty acids and glycerol into circulation (Food and Nutrition Board 2005).

When carbohydrate intake falls below a certain threshold, and the glycogen stores in the liver decrease slightly, the oxidation of fatty acids in the liver increases. The products of the oxidation, ketone bodies: aceto-acetate and 3-hydroxybutyrate, are released into the circulation. Most of the tissues and organs can use fatty acids and ketone bodies as a source of energy; the organs that need glucose are red blood cells, healing tissue and the brain; however the latter can use ketone bodies as a major part of its fuel (Food and Nutrition Board 2005). The rate of fatty acid oxidation is the main determinant of ketone body production. Ketogenesis takes place in the mitochondrion and is regulated at several steps. β-oxidation of fatty acids produces acetyl-CoA that can enter either the tricarboxylic acid cycle or the ketogenesis pathway. The ketogenesis pathway is presented in Figure 8.

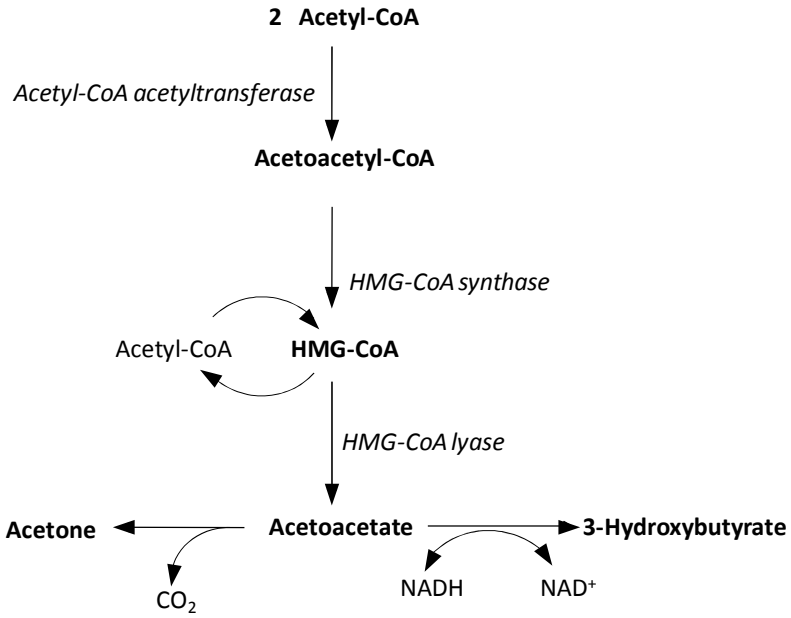


Figure 8. Ketogenesis pathway (adapted from Frayn 2003).

In humans, the threshold for ketosis has been reported to be between 50g and 100g of carbohydrate per day (Food and Nutrition Board 2005), which in terms of energy would constitute 10% of a 2000 kcal diet. The restriction of energy intake occurs naturally when carbohydrates account for 5–10% of the total energy of the diet (Westman et al. 2007). Yancy and co-workers (2004) suggested it to be a likely result of restricted food choices, the appetite suppressing properties of the diet, increased thermic effect of the diet or loss of energy through ketonuria. However, these still need to be proven and the extent of the effect is still being debated.

During ketosis, in contrast to starvation, glucose levels are sustained because the body produces it from the glycerol part of dietary fat and from protein through gluconeogenesis and glycogenolysis (Figure 9). The liver and kidney can deliver about 200g of glucose per day during dietary carbohydrate insufficiency (Westman et al. 2007). It is important to note that gluconeogenesis increases during severe carbohydrate restriction in an eucaloric diet (Bisschop et al. 2000) and it was proposed that it also increases during a hypocaloric LCKD (Krieger et al. 2006). Substrates for gluconeogenesis may be lactate, alanine or other amino acids and glycerol (Coffee 1998). As hepatic stores of glycogen depend on the amount of carbohydrate in the diet (Gannon et al 2004), in people on a low-carbohydrate diet, stores of hepatic glycogen are reduced (Figure 9). Much of the initial weight loss on LCHODs is glycogen and its bound compliment of water which is about 2kg in a typical adult.

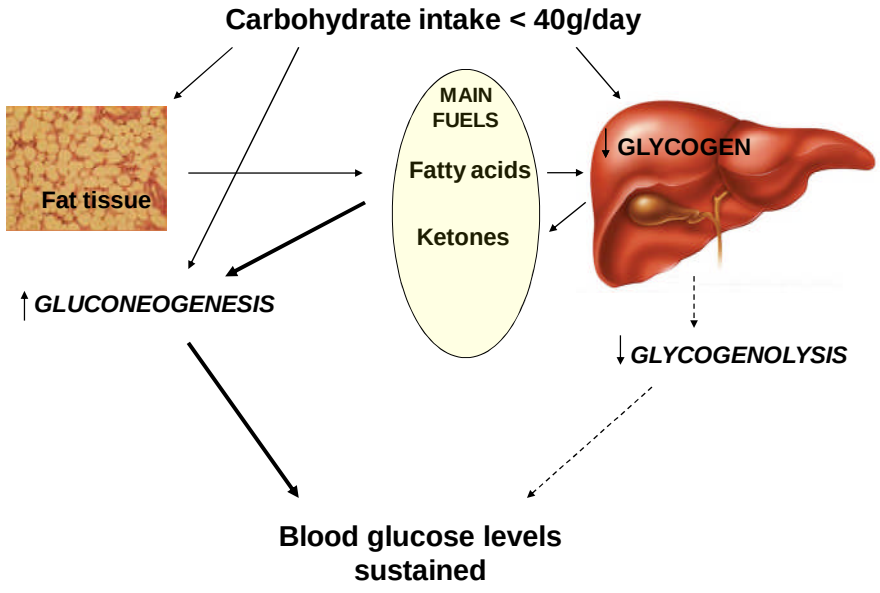


Figure 9. Fuels of the body during carbohydrate depletion. Arrows in bold signify enhanced effect; dashed arrows signify weakened effect (adapted from Frayn 2003).

1.3.2.3 Animal studies

Krieger and associates (2006) in their meta-analysis of studies investigating diets varying in carbohydrate and protein content, stated that low-carbohydrate, high-protein diets favourably affect body mass and composition independent of energy intake. They compared diets containing 41.4% of energy derived from carbohydrate with those containing a greater amount of carbohydrate. Additionally, they observed that protein intake over the recommended value of 0.75 g/kg/d (Department of Health 1991), namely over 1.05g/kg/d, resulted in the preservation of LBM. Moreover, recent animal studies showed that macronutrient composition influenced hypothalamic gene expression in ways that could affect overall food intake (Kinzig et al. 2005, Kinzig et al. 2007). Specifically, Kinzig and colleagues (2005, 2007) were measuring expression levels of POMC mRNA. POMC is a peptide precursor of the adrenocorticotrophic hormone (ACTH), lipotropin (LPH), melanocyte-stimulating hormones (MSH) and endorphins and is synthesised in the pituitary gland, hypothalamus and brainstem. Expression of POMC mRNA is elevated in obesity and during high-fat feeding and induces decreased food intake and body weight (Kinzig et al. 2007). In rats, low-carbohydrate, high-fat diets (CHO, 5 %; P, 15 %; F, 80 %) decreased the expression of POMC mRNA in the hypothalamus as compared to a standard chow diet (66:18:16 carbohydrate-protein-fat ratio) (Kinzig et al. 2005). However, more recently, Kinzig and co-workers (2007) showed that a diet with 5:65:30 carbohydrate-protein-fat ratio, significantly increased the expression of POMC mRNA in the arcuate hypothalamus as compared to the same chow diet (Kinzig et al. 2007). In summary, these data show that in rats, dietary macronutrient composition, independently of energy intake, influences feeding behaviours and body weight regulation.

1.3.2.4 Human studies

In contrast to animal studies, human studies show that observed positive effects of high-protein, low-carbohydrate diets were due to the lower energy intake and not due to the diet macronutrient composition (Heilbronn et al. 1999). Scientists in the AHA statement on Dietary Protein and Weight Reduction (St. Jeor et al. 2001), and many researchers after them (Farnsworth et al. 2003, Luscombe, et al. 2003, Sargrad et al. 2005, Foster et al. 2010) stated that weight loss is determined by total energy intake rather than macronutrient composition of the diet. They claimed that that there was no sufficient evidence that improved health, occurring as a result of high-protein diets, would take place without reduction in energy of the diet. Therefore, it was advised that while investigating LCKDs, particular attention should be put on the effects which are independent of weight loss. In 2007, Dashti and

colleagues published results of a one year long study that demonstrated that a LCKD is safe for use in longer term in obese individuals with T2DM (Dashti et al. 2007).

1.3.2.4.1 Glycaemia

Low-carbohydrate, high-protein diets, either ketogenic or not, are effective at lowering blood glucose levels. When a diabetic person is fasting, the rate of glycogenolysis is decreased due to depleted stores of glycogen, which results in a decrease in fasting blood glucose (Gannon et al 2004). The blood glucose lowering effect during the low-carbohydrate diet occurs mainly because postprandial glucose levels are determined by the amount of ingested carbohydrate (Boden et al. 2005) but also because dietary proteins stimulate insulin secretion (St. Jeor et al. 2001, Nuttall et al 2004, Nuttall et al. 2007).

The responses of patients with T2DM to different dietary macronutrient composition have been investigated. In 2003, Gannon and colleagues compared postprandial blood glucose responses of type 2 diabetics after 5 weeks on diets containing 30% fat and either 40% carbohydrate and 30% protein, or 55% carbohydrate and 15% protein. The higher protein diet significantly decreased posprandial glucose levels and improved overall glucose control (based on HbA_{1c} values). There was no difference in weight loss, therefore it was suggested that the observed differences in blood glucose responses were due to the variation in the protein: carbohydrate ratio. A year later, Nuttall and Gannon (2004) reported similar results in patients with T2DM, when they observed that replacing some of the dietary carbohydrates with protein reduced 24h integrated plasma glucose concentration. They also observed decreases in postprandial glucose concentration (Gannon et al 2004). In 2006, Nuttal and Gannon did a similar 5-week-long study on patients with untreated T2DM, this time examining the metabolic responses to LCHOD (CHO, 20 %; P, 30 %, F, 50%). As well as the improved glucose control, they reported improved nitrogen balance in these patients.

Miyashita and colleagues (2004) conducted a 4-week-long study in obese patients with T2DM. They reported that a low calorie LCHOD had more favourable outcomes on insulin sensitivity, visceral fat loss and HDL-cholesterol levels than an isocaloric low-fat diet. Amounts of protein in the two diets were the same. Similarly, Daly and associates (2006), on the basis of the results from their 3-month-long randomized controlled trial, reported similar results. In this trial, however, protein intake was significantly higher in the low-carbohydrate group than in the low-fat group (26.4% vs. 20.9% respectively). Results from the trials lasting a year and longer indicate either equal lowering blood

glucose effect of low-fat and low-carbohydrate diets (Davis et al. 2009, Larsen et al. 2011) or superiority of low-carbohydrate diets (Westman et al. 2008).

1.3.2.4.2 Energy expenditure

High-protein, low-carbohydrate diets have often appeared to be more efficient in enhancing weight loss than conventional diets in the short term. Advocates for these diets claim that this success is partly due to increased energy expenditure.

Normally, reduction in resting energy expenditure (REE) is expected to occur with the loss of fat mass and fat free mass (FFM) (Brehm et al. 2005). As REE depends on the amount of FFM, then preservation of FFM which can take place on energy restricted high-protein diets can reduce the fall in REE. This was observed by Baba and co-workers (Baba et al. 1999) who reported that patients on a high-protein diet (45% P, 25% CHO) had 12% higher REE than those on a high-carbohydrate diet (12% P, 58% CHO). They suggested that it may have been due to the formation of potentially thermogenic serotonin and dopamine in peripheral tissues. Luscombe and co-workers (2002) on the other hand, did not report REE to be increased by a high-protein diet (28% P, 42% CHO), but observed a blunting in the normal fall in energy expenditure seen during weight loss in T2DM patients. This disparity may be partly explained by the amounts of protein in the high-protein diets. For example, in the study by Baba et al (1999) it was 45 % of energy and in the study by Luscombe et al (2002) 28% of energy. Unfortunately, it is difficult to compare amount of protein in grams between these two studies because in study by Baba et al. (1999) subjects were receiving diet with an energy content equal 80% their resting energy expenditure and there was no mean values for the energy reported.

The majority of the studies agree that a decrease in REE occurs with weight loss and is independent of diet macronutrient composition. For example, Brehm and co-workers (2005) observed a reduction in REE with weight loss after 2 and 4 months but there was no influence of macronutrient (low-fat diet 15% P, 55% CHO, 30% F or LKCD ad libitum diet with up to 20g of CHO for the first 2 weeks and 40-60g CHO afterwards). However, weight loss was significantly greater in the LCKD group in this study when compared to the low fat group (9.79 ± 0.71 kg vs. 6.14 ± 0.91 kg). Neither the thermic effect of food (TEF) nor the physical activity levels explained the differences in body weight loss. The difference in weight loss was suggested to be a result of underreporting of food consumption by subjects on the low-fat diet. Similarly, Luscombe and colleagues (2002 and 2003) reported that the amount of weight loss and the decrease in REE was dependent on energy restriction but not on the diet

composition. They observed that there was no difference in weight loss (7.9 ± 0.6kg) and REE in both the high-protein (27% P, 45% CHO) and the low-protein (16% P, 57% CHO) diet groups after 16 weeks of intervention (Luscombe et al. 2003).

In the study by Sargrad et al (2005) REE did not change and there was no difference in weight loss after 8 weeks between patients on either a high-protein (30% P, 40% CHO) or a high-carbohydrate diet (15% P, 55% CHO). Boden et al (2005) reported that total energy expenditure (TEE) of patients on a LCKD (ad libitum with up to 21g of CHO per day) did not change after 2 weeks apart from ~1000 kcal decrease in energy intake. In this study, patients lost 1.65 kg during 2 weeks and in the study by Sargrad et al (2005) weight loss was just above 2 kg after 8 weeks. The lack of decrease in REE in the study by Sargrad et al (2005) might have been due to a relatively small decrease in body weight (-2.2 kg in high-carbohydrate and -2.5 kg in high-protein group) as compared to the weight loss achieved in the study by Luscombe et al (2003) (-7.9 kg overall weight loss in both groups) in which REE fell (overall decrease 172 ± 25 kcal/d).

The rate of energy expenditure increases after a meal due to the costs of the digestion, absorption and the energy needed to store fuel (Frayn 2003). It was shown that a high-protein meal compared with a standard-protein meal, has a greater increase in the thermic effect of food (TEF) and can reduce the decrease in the TEF after weight loss (Luscombe et al. 2002, Luscombe et al. 2003, Luscombe-Marsh et al. 2005). In the short term, TEF in patients on a high-protein diet compared with a standard-protein diet did not improve weight loss. Although it can be theoretically calculated that in the long term TEF has the potential of enhancing weight loss, it was not observed (Luscombe-Marsh et al. 2005).

1.3.2.5 Appetite and satiety

Several studies have reported that low-carbohydrate, high-protein diets have better appetite suppressing effects compared to low-fat diets. In a single-meal study, Luscombe-Marsh and colleagues (2005) reported that the amount of food desired by obese hyperinsulinaemic subjects over a 3-h period following a test-meal was smaller after the high-protein low-fat meal than after an isocaloric standard-protein, high-fat meal. Similarly, Boden and colleagues (2005) showed that despite a 1000 kcal lower energy intake during 14 days on LCKD (ad libitum with up to 21g of CHO per day) hunger levels were similar to those of a low-fat diet in obese patients with T2DM.

It was proposed that potential factors influencing appetite during LCKD diets are ketosis, increased amount of protein, and lower serum insulin concentrations. Ketosis which occurs during LCKD has a potential anorectic effect (St. Jeor et al. 2001). Although ketone bodies in the blood may be appetite suppressing (Astrup et al. 2004), in a study by Foster et al (2003) there was no association between urinary ketones and weight loss. Likewise, Brehm et al (2003) reported that despite the fact that plasma levels of beta-hydroxybutyrate had returned to control values, subjects on a low-carbohydrate diet were losing weight for up to 6 months. Apart from ketosis, appetite could also be affected by the monotony of the diet and restricted food choices (Astrup et al. 2007).

Satiating effects of dietary protein could be related to the suppression of postprandial ghrelin secretion and satiety maintenance. Ghrelin concentrations were reported as total immunoreactive ghrelin (acylated and de-acylated forms) in some studies and as acylated ghrelin, which is the bioactive form of this hormone, in other studies. El Khoury et al (2006) observed in healthy men that a high-protein meal maintained lower acylated ghrelin concentrations 3 hours after ingestion than a high-carbohydrate or a high-fat meal. However, another study reported that satiating effects of protein was not related to the secretion of ghrelin after a meal (Moran et al. 2005).

1.3.2.6 Adverse events

Most of the adverse effects of LCKDs are usually reported during the first two weeks. These may be due to the loss of total body water and it may be attenuated by drinking more (Yancy et al. 2004). Other adverse effects that have been reported were constipation, headache, halitosis, muscle cramps, diarrhea, general weakness and rash (Yancy et al. 2004). Moreover, due to muscle glycogen depletion, fatigue was observed during exercise (St. Jeor et al. 2001). However, not all the studies report adverse effects. For example, Skov et al (1999) found no differences on high-protein or high-carbohydrate diets. Nevertheless, patients with diabetes or hypertension who go on low-carbohydrate high-protein diets are advised to be under the care of a clinician, because sudden changes in the proportions of macronutrients in the diet may influence their response to diabetic drugs (Westman et al. 2007).

Disturbances of renal function and bone metabolism are often reasons for rejecting high-protein diets as a weight loss therapy. According to the AHA a very high-protein diet is especially risky for patients with diabetes, because it can speed the progression, even for short lengths of time, of diabetic renal disease (St. Jeor et al. 2001). However, most of the studies examining high-protein diets did not report adverse effects on either kidney function or bone metabolism. In a study by Noakes et al (2005)

markers of renal function (urine urea: creatinine, serum urea and creatinine clearance) did not differ from baseline for neither high-carbohydrate, normo-protein (58 g/d) nor high-protein (104 g/d) diets. The study also did not report adverse effects on bone metabolism (Noakes et al. 2005).

One of the concerns about LCHODs is the limitation of fruits, vegetables, cereals, grains and low-fat milk. According to the AHA, these limitations are likely to cause deficiencies in vitamins and minerals and inadequate fibre intake (St. Jeor et al. 2001). The recommended intake of these nutrients was shown to lower blood pressure (St. Jeor et al. 2001); and reduced fiber intake could have long term adverse effects (Boden et al. 2005). However, there is no evidence that diabetic patients would benefit from increased amounts of fibre higher than the RDI (American Diabetes Association 2008). Additionally, according to our knowledge, none of the studies investigating LCHODs reported nutrient deficiencies. To address the problem of potential deficiencies in patients on LCHODs, vitamin and mineral supplements are recommended (Westman et al. 2007).

Trials that investigate the use of LCHODs have common limitations. Firstly, it may be difficult to distinguish the effects of a LCHOD and dietary supplements provided. Secondly, the state of health of participants - extrapolation of the results from healthy to diseased populations may be inadequate. Thirdly, in the case of studies with small numbers of participants / high dropout rates, results must be considered as preliminary (Sargrad et al. 2005). Fourthly, compliance to LCKD is often poor (Stern et al. 2004) and usually decreases after 6 months (St. Jeor et al. 2001, Robertson et al. 2002, Foster et al. 2003). Finally, the use of drugs confounds the contribution of the diet to the change of measures, biological markers, blood lipids, cardiovascular risk etc.

1.3.3 Very low calorie diets

Very low calorie diets (VLCD) containing up to 800 kcal/d have been used to achieve rapid weight loss since the 1970s (Tsai et al. 2006). The National Institute of Diabetes and Digestive and Kidney Diseases underlined that VLCDs should be delivered only under medical supervision (NIDDK 2008). VLCDs are intended for moderately to extremely obese patients and should be a part of a programme that includes behavioural therapy, nutrition counselling, physical activity and / or drug treatment.

Weight loss expected on VLCD is in the range of 1.5 to 2.5 kg per week resulting in about 20 kg in 3 months. This degree of weight loss is comparable to complete starvation (Howard 1981). Such a rapid weight loss has the potential to improve diabetic control, blood pressure and lipid profile (NIDDK

2008). VLCDs have been associated with substantial improvements in glycaemic control (Wing et al. 1991) and cardiovascular health in a 12 months period (Dhinsa et al. 2003). It has to be pointed out that the length of very low calorie intervention in the two above cited studies was 7 and 8 weeks respectively; the reported positive effects were observed at 1 year's time. Moreover, it is not uncommon that patients on VLCDs report fatigue, nausea, diarrhoea and constipation (NIDDK 2008). These adverse events are usually reported during the first two weeks due to the occurrence of ketosis (Rolland et al. 2009). The serious side effect that may occur is gallstones formation (NIDDK 2008).

The maintenance of weight loss achieved by means of VLCD may be difficult and weight regain is common. It has been suggested that weight maintenance and long-term glycaemic control in T2DM, are more likely to be achieved by combining VLCDs with increased physical activity, behavioural therapy and active follow up treatment (Hensrud 2001, NIDDK 2008).

Tsai and Wadden (2006) in a meta-analysis of VLCDs concluded that VLCDs did not produce greater long term weight losses than low calorie diets. It was suggested that this was the case due to poorer adherence on VLCDs than on less restrictive diets (NIDDK 2008). However, VLCDs may be beneficial for obese people with T2DM, as caloric restriction has substantial effects on glycaemic control even in the absence of weight loss (Wing 1995). Additionally, VLCDs induce greater improvements in glycaemic control than low calorie diets that achieved the same weight loss (Wing 1995). Monnier and associates in their review of VLCDs in the management of T2DM (2000) concluded that short term VLCDs may be useful for initiating weight loss and for introducing patient to the potential benefits of adhering to a dietary regimen. It was pointed out however, that overall improvements in glycaemic control in people with T2DM in the longer term were small (Monnier et al. 2000, Hensrud 2001).

1.3.4 Exercise

Physical activity was defined by the American College of Sports Medicine (ACSM) and American Diabetes Association (ADA) as "bodily movement produced by the contraction of skeletal muscle that substantially increases energy expenditure" (Colberg et al. 2010). While exercise was described as a "subset of physical activity done with the intention of developing physical fitness" (Colberg et al. 2010).

It has been recognized that the obesity epidemic and associated increase in T2DM are related to decreased physical activity levels (ADA 2002). Additionally, the majority of people with T2DM are not active (Morrato et al. 2007). Therefore, physical activity was suggested to be a crucial way of preventing as well as managing T2DM. The American Diabetic Association (2002) stated that in patients with T2DM regular exercise may improve insulin sensitivity and help to bring glucose levels to a normal range. This effect seems to be greater at the early stages of T2DM and insulin resistance. Both strength and aerobic exercise were beneficial for people with T2DM and a regular and varied exercise is recommended; other forms of exercise (i.e. yoga, tai-chi) have shown mixed results (Colberg et al. 2010).

The American College of Sports Medicine stated that, there is strong evidence that while glucose uptake by skeletal muscle during rest is impaired in T2DM, during exercise glucose is transported into cells by a different mechanism not impaired by T2DM or insulin resistance (Colberg et al. 2010). Acute improvements in insulin action facilitated by physical activities can last from 2 to 72 hours.

Chronic effects of physical activity on T2DM include improvements in insulin action, blood glucose control, fat oxidation, lipid profile, blood pressure, cardiovascular events and mortality (Colberg et al. 2010). Regular exercise can also reduce depression and improve health related quality of life in T2DM. About an hour per day of exercise may be needed to achieve weight loss, if relying on exercise alone. Additionally, it may be necessary to adjust the dose of hypoglycaemic medication according to the type and duration of exercise, and the type and progress of diabetes (ADA, 2002).

It has been recommended for people with T2DM to do aerobic exercise for at least 2.5 hours a week; the exercise should be moderate to vigorous and spread out over at least 3 days of the week. This should be supplemented with resistance training 2 to 3 times per week (Colberg et al. 2010). The presence of diabetes complications may make exercise more difficult; however, the specific physical activity recommendations were suggested for people with different complications (Colberg et al. 2010).

1.3.5 Medications

Medications which facilitate weight loss are targeted for obese individuals (BMI > 30kg/m^2). There is currently only one drug available, orlistat. Orlistat (Xenical, Roche) is a lipase inhibitor and therefore it reduces the digestion and absorption of dietary fat. Medications that reduce fat absorption are

considered to be safer than those influencing appetite, because they do not act on the nervous system and heart rate. The recommended dose is 120mg at meal times three times a day. Treatment with orlistat results in mean weight losses of around 3kg (Padwal and Majumdar, 2007). Orlistat was shown to improve total and LDL-cholesterol, blood pressure and glycaemic control in patients with diabetes (Rucker et al, 2008). Due to the fact that dietary fat is excreted, low-fat diets are advised during the treatment to avoid gastro-intestinal discomforts. The lower dose of Orlistsat (60mg) is now available over the counter under the name Alli (GlaxoSmithKline). It has been observed that in patient on orlistat HbA_{1c} and lipid profiles improve more than what would be expected with the weight loss that occurs (Davidson et al. 1999).

In the past, there were two other drugs available. Sibutramine (Reductil, Meridia) was a drug that suppressed appetite by the inhibition of the re-uptake of noradrenaline and serotonin in the brain. Apart from the inhibition of appetite it also displayed antidepressant qualities and could lower TG (Rucker et al. 2008). It was also reported that on average, sibutramine reduced weight by 4-5 kg (Padwal et al. 2007). Adverse effects of this drug included increased blood pressure and pulse rate. Sibutramine was suspended by the Medicines and Healthcare Products Regulatory Agency (MHRA) in January 2010 due to the risk of non-fatal heart attacks and strokes. Another anorectic drug, which was made available in the UK in 2006 but withdrawn due to serious adverse events shortly after, was rimonabant (Acomplia, Zimulti, Rimoslim, Slimona). It selectively blocked cannabinoid type 1 receptor (CB1). Cannabinoids stimulate hunger by binding to CB1 receptors. Rimonabant bound to CB1, blocked the access of cannabinoids to the receptor and prevented hunger stimulation which in the longer term resulted in weight loss. On average, rimonabant reduced weight by 4-5 kg (Padwal et al. 2007). Apart from the suppression of appetite, rimonabant was reported to improve waist circumference, concentrations of HDL-cholesterol and TG (Padwal et al. 2007), blood pressure and glycaemic control in diabetic patients (Rucker et al. 2008).

Oral antidiabetic drugs are used in the treatment of T2DM when diet and lifestyle modification for at least 3 months does not maintain glucose concentrations with desirable ranges. They should be used to support, and not replace the effect of diets and exercise (BNF 2006). Actions of these drugs vary from the stimulating of pancreatic beta-cells to synthesise insulin; sensitizing tissues for insulin actions; decreasing the rate of hepatic gluconeogenesis, to slowing down the absorption of carbohydrates from the intestine. Currently available drugs and their actions are presented in Table 1.

Table 1. Oral agents used in the treatment of diabetes and their actions (adapted from Diabetes UK, 2008; BNF 2006; Frayn 2003).

Group of drugs	Name of drugs	Action
Sulphonlyureas	glibenclamide, gliclazide, glipizide, glimepiride, tolbutamide, chloropropamide	Act on beta-cells to synthesize and secrete insulin; improve insulin sensitivity in the longer term.
Biguanide	metformin	Improves peripheral insulin sensitivity by increasing peripheral utilization of glucose and decreases the rate of gluconeogenesis.
Prandial glucose regulators	rapaglinide, neteglinide	Stimulate pancreas to synthesize insulin; should be taken only during the meals; action time is shorter than sulphonylureas.
Thiazolidinediones	rosiglitazone, pioglitazone	Improve peripheral insulin sensitivity.
Alpha glucosidase inhibitor	acarbose	Slows the absorption of starch and sucrose from the intestine, consequently slows the postprandial rise of glucose concentration.
Dipeptidyl peptidase-4 (DPP-4) inhibitors	sitagliptin	Increases levels of incretins, which stimulates insulin synthesis and decreases the rate of gluconeogenesis.

Insulin injections are used as a treatment in T2DM when oral therapy is not enough to keep glucose levels in the normal ranges. There are three groups of insulin available for subcutaneous injections: animal insulin, synthetic insulin which mimics human insulin and insulin analogues which are similar to human insulin but slightly vary in amino acid sequence (Diabetes UK, 2008). There is a wide selection of available insulin treatments ranging from those which act immediately or after some hours to those acting up to 30 hours (Diabetes UK, 2008).

Incretin mimetics is a new therapy which, similarly to insulin, is delivered in subcutaneous injections (Diabetes UK, 2008). The drugs available, Exenatide and Liraglutide, are glucagon-like peptide 1 (GLP-1) receptor agonists. GLP-receptor agonists act on different levels. Firstly, they stimulate insulin synthesis and slow down hepatic gluconeogenesis. They also delay stomach emptying and digestion at the same time slowing down the postprandial rise in glucose concentration. Finally, they reduce appetite and both Exenatide and Liraglutide treatments have been associated with weight loss (Diabetes UK, 2008).

Specific glucose-lowering strategies are being investigated at the moment and they include the use of insulin or one or more oral agents with or without revascularization (Golden et al, 2007). However, if a diet and lifestyle modification for obese patients with T2DM could achieve similar results or at least support the action of oral agents in lowering cardiovascular disease risk, that would be cheaper for health systems and would be of benefit to patients as they would reduce their risk of adverse effects induced by drugs.

1.3.6 Bariatric surgery

The NICE guideline on Obesity (2006) recommended bariatric surgery as a treatment option for adults whose BMI is over 40 kg/m^2 or between 35 and 40 kg/m^2 and accompanied by T2DM, hypertension or obstructive sleep apnoea. Bariatric surgery should only be performed when all other ways of addressing their obesity problem failed. Nevertheless, for patients with BMI over 50 kgm^2, bariatric surgery is recommended to be the first line treatment.

1.3.7 Cognitive behavioural therapy

Cognitive behavioural therapy (CBT) addresses difficulties with emotions and behaviours. It was developed in 1960s by Aaron Beck, to treat depression (Beck 1995). CBT is characterized by a systematic approach to achieve clearly defined goals. CBT deals with dysfunctional beliefs which results in improvements in mood and behaviour. The treatment is now used for a wide range of problems including anxiety, substance abuse, psychotic disorders, personality issues and eating disorders. CBT can be used to work with individuals and with groups in which individuals have similar goals.

CBT has proved successful in supporting obesity treatment (Cooper et al. 2001) and the approach has been employed by some of the commercially available weight loss programmes (i.e. Lighter Life). CBT has been used both, during weight loss as a supporting therapy and for the purpose of weight maintenance. In the context of obesity, CBT aims to change the habits that caused weight gain and promote behaviours that will help to lose weight and maintain it. There is a lack of evidence that psychological treatments of obesity, including CBT, work in the longer term without any other therapy (Cooper et al. 2010).

1.4 Resting metabolic rate prediction

Understanding of patophysiology of obesity was improved through measurements of energy expenditure and substate oxidation (Weyer et al. 1999). Energy expenditure has been first measured by Lavoisier who used calorimeter to measure heat production (Gibney at al. 2002). Direct measurement of heat production is technically demanding and currently is not frequently used. Indirect calorimetry provides an alternative way of resting metabolic rate (RMR) measurement. The method is based on the analysis of respiratory gases. Namely, the volumes of oxygen that individual consumes and carbon dioxide that individual produces during oxidation of carbohydrate, fat, protein and alcohol are measured over a period of time. Twenty four hour energy expenditure can be measured in the human whole body respiratory chambers. It can be also measured under a ventilated hood or with a face mask over a shorter period of time and then the results are extrapolated to 24 h. The measurement typically lasts about half an hour and is conducted when an individual is fasted and lies quietly at rest in the morning time (Gibney et al. 2002).

As the above measurements require considerable amounts of time, subject preparation, specialised equipment, and trained technicians, they are not always practical. Many equations that predict various aspects of energy expenditure were proposed: there are equations predicting the whole 24-h energy expenditure, sleeping metabolic rate, basal metabolic rate and finally resting metabolic rate. Development of an equation to predict RMR is based on the measurement of RMR which is assumed to be the true value.

It was shown before, that patients with T2DM had higher measured RMR than non-diabetic people (Huang et al. 2004, Bitz et al. 2004, Visockiene et al. 2006). Pi-Sunyer (2005) suggested this to be a result of deregulated energy metabolism and resulting from its inefficiency. The author also explained that this inefficiency was improved with weight loss and therefore resting metabolic rate decreases more in people with T2DM than expected with weight loss alone. Bitz et al (2004) suggested that a new predictive equation for people with T2DM should be developed. In the current work, twenty nine equations that predict RMR were tested for accuracy and bias when used for obese individuals with T2DM. These equations included variables such as age, gender, body mass, height, body composition and size.

1.5 Summary and conclusions

Obesity is a serious global problem and its linkage with T2DM is well established. Governments and nongovernmental organisations keep producing recommendations, guidelines and programs for actions to manage and prevent it. Low-carbohydrate, high-protein diets are an alternative option for obese individuals with T2DM who struggle with losing weight on standard Diabetes UK recommendations. The evidence supporting efficacy and safety of low-carbohydrate diets is growing and this alternative to the low fat diet has been recently acknowledged by the national and international guidelines for management of diabetes. The low-carbohydrate diet has been shown to be safe for up to two year period for obese subjects with T2DM.

Obesity and T2DM are related diseases and their aetiology is often common. Despite increasing knowledge about these conditions, their prevalence worldwide is increasing. Additionally, nutritional recommendations for those conditions do not always follow quickly expanding knowledge. A low-carbohydrate, high-protein diet is one of the alternative ways for obese individuals with T2DM to lose weight, improve diabetic control and reduce the risk of cardiovascular disease.

There have been numerous studies of the effects of low-carbohydrate diet on health of the people with T2DM. None of those studies compared different approaches to administering low-carbohydrate diet. Present study compares the effects of low-carbohydrate ketogenic diet administered either as commercially available ready-made meals or as self-prepared meals.

2 Aims

The current study aimed to investigate the efficiency of high-protein LCKD in the management of obesity and T2DM and to determine the effects on cardiovascular risk, body composition and energy metabolism.

There is no information in the current literature about the effect that the approach to a LCKD (i.e. ready made meals or self prepared meals) has on weight loss in obese individuals with T2DM. It is a novel idea to compare the two approaches to a diet.

The primary aim was to assess weight loss and its composition achieved after 12 months on two alternative hypoenergetic, high-protein LCKDs (the protein-sparing modified fast compared to commercial ready-made meals provided by Go Lower). The hypothesis was that the convenience of ready made meals would result in better adherence and consequently greater weight loss at 12 months. It was also assumed that the high protein component of the diet would result in losing fat mass and not fat free mass.

The secondary aims of the study included assessment of changes in cardiovascular risk associated with the diets; assessment of improvements in glycaemic control; assessment of changes in resting metabolic rate; assessment of improvements in quality of life, wellbeing, motivation to lose weight, physical activity, depression, fatigue, sleepiness and satisfaction with diabetes treatment using standardised and validated questionnaires; and testing of existing equations to predict resting metabolic rate in the studied population.

3 Methods

3.1 Study design

The study was a randomized clinical trial of weight management and diabetic control. It was conducted in a single centre located at the Aberdeen Royal Infirmary in Scotland. The trial was assigned ISRCTN number ISRCTN20400186.

The study was conducted as a dietary intervention for twelve months (Figure 10). Potential participants were invited to take part in the trial by a letter. If interested, they could contact the study centre by telephone, e-mail or post by returning their contact details in pre-stamped return envelope. If, after the screening visit, they fulfilled the inclusion criteria, were willing to participate and signed informed consent, they were randomizsed into one of the arms of the study. Randomisation method is described in the Statistics part of this chapter.

During the time of the programme patients visited the study centre on 14 occasions: at baseline, at 2 and 4 weeks and then monthly. The descriptions, timing and duration of all participants' visits are collated in the Patient Visits Table (Table 2). Participants were randomly assigned to either the protein-sparing modified fast (PSMF) diet or the Go Lower (GL) diet.

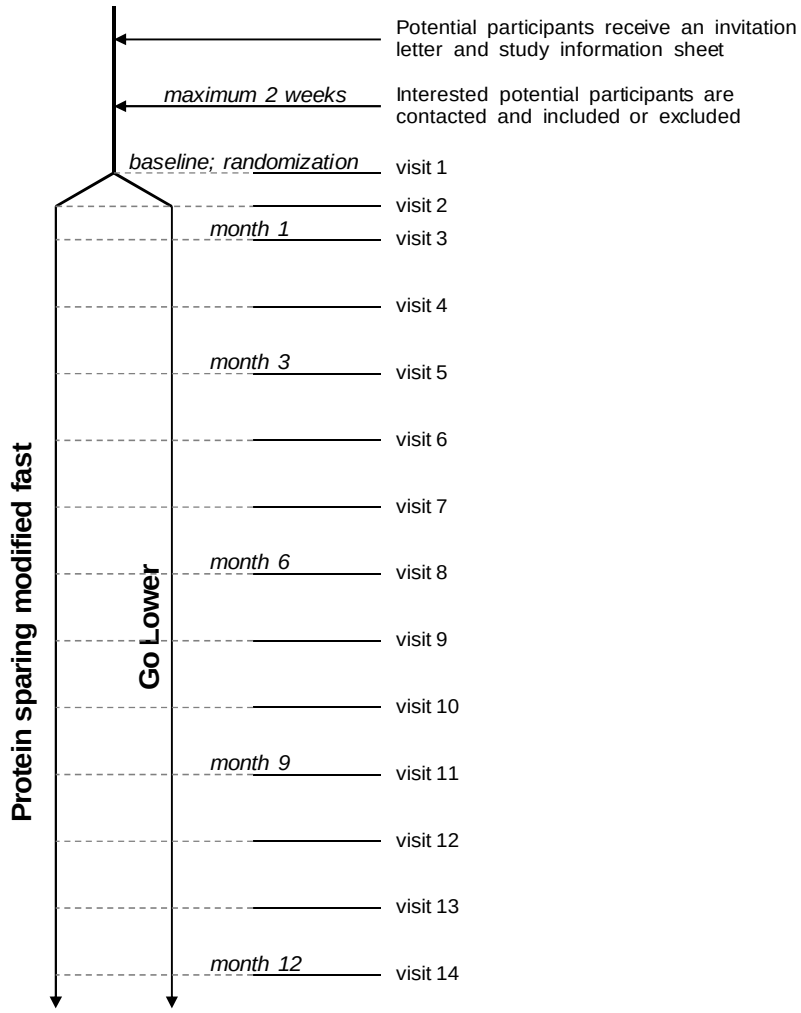


Figure 10. The timeline of the study.

The primary outcome measure was weight loss after 12 months of a dietary intervention. Secondary outcomes included improvements in glycaemic control as determined by HbA_{1c} and homeostasis model assessment – insulin resistance index (HOMA-IR) calculated from the following equation: fasting insulin × fasting glucose/22.5, (Matthews et al. 1985)); improvements in cardiovascular disease risk as determined by serum lipid profile, blood pressure and changes in visceral adiposity and also by the Framingham risk score; and improvements in quality of life using standardized and evaluated questionnaires. Fasting blood draws were performed in the morning on 5 occasions, at baseline and after 3, 6, 9 and 12 months into the dietary intervention. On these days, participants received breakfast (adjusted accordingly to their diet) at the study centre. Participants were seen by the research student who took all measurements and gave lifestyle advice. All measurements are summarised in Table 2;

the lifestyle advice consisted of a discussion of the practical side of dieting, weight related issues, and any problems arising during the programme that could potentially affect it (such as eating out or holiday).

Table 2. Visits and their descriptions.

Date	Visit No	Duration	Visit description + measurements
	1 Baseline	2 hrs	**Diet & Lifestyle instructions** Standard medical check, height, weight, waist & hips circumferences, blood pressure, blood tests, body composition, resting metabolic rate, diet composition, questionnaires, urine and blood ketones, 24h urine sample
	2 2nd week	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	3 4th week	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	4 2nd month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	5 3rd month	1.5 hrs	**Diet & Lifestyle advice** Weight, waist & hips circumferences, blood pressure, blood tests, body composition, diet composition, questionnaires, urine and blood ketones, 24h urine sample
	6 4th month	30min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	7 5th month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	8 6th month	2 hrs	**Diet & Lifestyle advice** Weight, waist & hips circumferences, blood pressure, blood tests, body composition, resting metabolic rate, diet composition, questionnaires, urine and blood ketones, 24h urine sample
	9 7th month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	10 8th month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	11 9th month	1.5 hrs	**Diet & Lifestyle advice** Weight, waist & hips circumferences, blood pressure, blood tests, body composition, diet composition, questionnaires, urine and blood ketones, 24h urine sample
	12 10th month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	13 11th month	30 min	**Diet & Lifestyle advice** Weight, urine and blood ketones
	14 Final visit 12th month	2 hrs	Weight, waist & hips circumferences, blood pressure, blood tests, body composition, resting metabolic rate, diet composition, questionnaires, urine and blood ketones, 24h urine sample

3.2 Participants

The participants were included if they were between 18 and 75 years old, obese (BMI > 30 kg/m2), and with poorly controlled T2DM (HbA1c ≥ 7.5%). All of the recruited patients had previously standard lifestyle approaches as part of their diabetes care (Diabetes UK guidelines). Participants with renal or hepatic disease, cancer, diet related illnesses, on antidepressant or obesity-related pharmacotherapy, pregnancy or lactation, and inability to monitor glucose levels were excluded. Patients with known coronary heart disease were included provided no major coronary event had taken place in the previous 6 months.

Participants were recruited from an outpatient Diabetes Centre in Aberdeen. They were under the care of a hospital based diabetes consultant. All participants provided written informed consent. All experimental procedures were approved by the North of Scotland Research Ethics Committee and the Robert Gordon University Ethics Committee.

3.3 Attrition

Of the 41 randomized participants, 25 were randomized to PSMF and 16 to GL (Figure 11). Twenty seven dropped out (66%) during various stages of the programme. There was no significant difference in the dropout rates between the two diet groups ($p = 0.747$). Dropout incidence was significant already at 3 months (10 participants from PSMF and 3 participants from GL) ($p<0.001$). The times and reasons for dropout are described in Table 3.

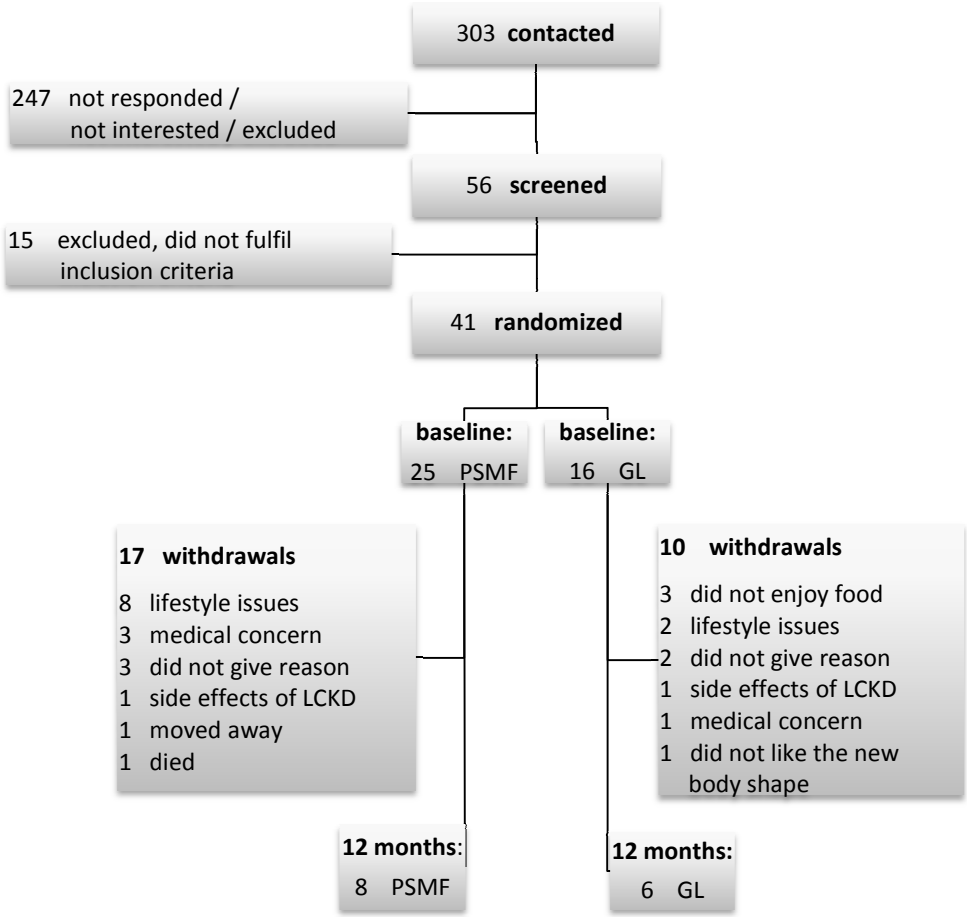


Figure 11. Participant flow chart. Abbreviations: PSMF, protein sparing modified fast; GL, Go Lower; LCKD, low-carbohydrate ketogenic diet.

Table 3. Times and reasons for dropout (total dropouts n = 27).

PSMF			Dropout time	GL		
Reasons for dropout	**Dropout No n = 17**	% of all dropouts		% of all dropouts	**Dropout No n = 10**	**Reasons for dropout**
side effects of LCKD medical concern	1 1	17.6	During the first 2 weeks	30.0		
did not give reason	1		1 month		1 1 1	side effects of LCKD did not enjoy the food medical concern
lifestyle issues did not give reason medical concern	5 1 1	41.1	2 months			
			3 months	10.0	1	did not give reason
			4 months			
lifestyle issues	1	5.9	5 months	10.0	1	did not enjoy the food
			6 months	20.0	1 1	did not like new body shape did not enjoy the food
			7 months			
lifestyle issues	1	5.9	8 months	20.0	2	lifestyle issues
medical concern moved away	1 1	11.8	9 months			
			10 months	10.0	1	did not give reason
death did not give reason lifestyle issues	1 1 1	17.6	11 months			

3.4 Dietary intervention

In this novel study, the two experimental high-protein LCKDs were the protein sparing modified fast (PSMF) and commercial Go Lower (GL) diet. Each approach investigated differed; Table 4 compares the two approaches.

For the PSMF, patients were provided with an information booklet (Appendix 1). Carbohydrate intake was restricted to a maximum of 40 g per day to induce ketosis. Patients were encouraged to include fruit and vegetables in their carbohydrate allowance. Foods encouraged during PSMF were lean meat, fish, eggs, green leafy vegetables, and at least 2 litres of fluids per day.

GL is a low-calorie (~1500 kcal/d) ketogenic diet. Participants who were randomized into GL diet were given their first food pack at the study centre. After that, according to their preferences, the foodpacks were available for collection at the study centre or delivered directly to their homes. Each food pack contained food for 2 weeks which included nut and seed granola for breakfast, choice of soups for lunch, main meals for dinner, and low carbohydrate snacks and treats such as nuts or seed and nut bars. Preparation of GL food typically involves heating up and adding selected vegetables, fruit or youghurt. The participants on GL were provided with an information booklet as well (Appendix 2). Additionally, all participants received multivitamin supplements.

Table 4. Comparison of what dieters were required to do when following protein sparing modified fast (PSMF) and Go Lower (GL) approaches.

Go Lower	PSMF
- receive ready-made meals (3 a day plus snacks) that can be supplemented with vegetables and certain fruit from the provided list; occasionally can have low-carbohydrate meals not produced by Go Lower	- shop themselves - prepare meals themselves
- do not pay for the Go Lower food	- pay for the food they buy
- are guided by the information booklet listing allowed amounts of certain vegetables and fruit (kitchen measures)	- are guided by the information booklet explaining how to count carbohydrate, read product labels, and informing about carbohydrate content in various food groups
- do not need to know carbohydrate content of the foods but have to adhere to the recommended portion sizes of non-Go	- need to learn how to weigh and calculate carbohydrate content of foods and drinks they consume

	Lower products (if eaten)	
-	should eat mostly Go Lower food; the information booklet contains the eating out guide	- can eat most of the foods provided they know their carbohydrate content and do not exceed 40g per day

Participants received initial instruction on how to follow their randomly assigned diet. They were then given assistance by phone or email at any time between the visits. Subjects were asked to complete 3-day food diaries at baseline and at every three months afterward. The food diaries were analyzed for energy and macronutrient content (WinDiets Research version) to confirm dietary compliance. Compliance to the experimental diets was mainly assessed by the occurrence of weight loss but also by testing urine and capillary blood for the presence of ketone bodies at each visit, and by recording levels of plasma urea and albumin every 3 months. All methods to measure compliance are described in detail later on in this chapter.

3.5 Measurements

3.5.1 Anthropometry

Body mass was measured at each visit, with participants wearing light clothing and no shoes. This measurement was done on the Tanita Body Composition Analyzer (type BC 410 MA III, Tanita Corporation of America, Inc., Arlington Heights, IL, USA) (Figure 12) and was mostly for the participant's information about their progress. Body mass reported in the results was recorded at baseline and then every 3 months based on the Bod Pod® Body Composition Tracking System measurements (Bod Pod, Life Measurement, Inc., Concord, CA, USA) as the weight measurement is a step in measuring body density. Conditions for this measurement are described below.

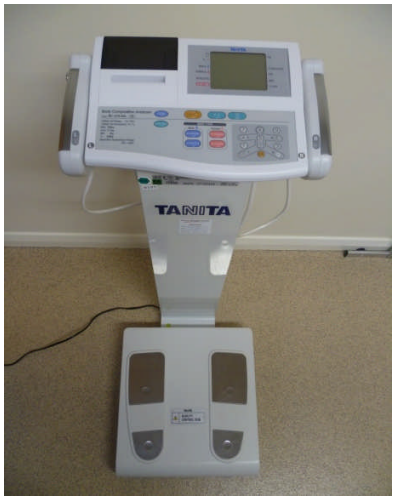


Figure 12. Tanita Body Composition Analyzer (BC 410 MA III, Tanita Corporation of America, Inc., Arlington Heights, IL, USA) used in the study.

Stature was measured at baseline only, with the use of SECA Leicester Height Measure (421S). Participants were measured with no shoes and their back to the vertical scale of the measure. When in the position and ready, the participant was asked to inhale; the measurer then gently applied upward pressure at the base of the jaw while instructing the participant to exhale. The reading was recorded to the nearest 0.5 cm.

Hip and waist circumferences were done using an anthropometric tape that was non-extensible but flexible. To keep participants as comfortable as possible, the measurer approached them from the front-side. Waist circumference was measured at the narrowest point between the lower costal (10^{th} rib) border and the top of the iliac crest, perpendicular to the long axis of the trunk. If there was no obvious narrowing, the measurement was taken at the mid-point between the lower costal rib and the iliac crest (ISAK, 2006). Hip circumference was taken at the level of greatest posterior protuberance of the buttocks, perpendicular to the long axis of the trunk, with feet together (ISAK, 2006).

Body composition was measured five times, at baseline and every 3 months afterwards, by using a densitometric method of air-displacement plethysmography (Bod Pod, Life Measurement Inc., USA; presented on Figure 13). The method is based on the determination of body density by measuring body mass and volume. The Bod Pod equipment was placed in a quiet room of its own, away from any direct source of heat, in a temperature between 21 and 32 ºC. Prior to testing, participants were asked to use the toilet. They were also asked not to exercise or eat for 12 h before the test but they could drink water up to 2 h before the test. Participants wore minimal, form-fitting clothing and removed any

additional elements of clothing (i.e. jewellery, socks, or glasses). During the volume measurement participants wore a Lycra swim cap.

Figure 13. Body composition measuring equipment, BodPod (by Life Measurements Inc, USA) in a clinic room in Westburn House.

To ensure accuracy, body composition was also determined by whole body impedance analysis (Body Composition analyzer BC-418 MA, Tanita Corporation of America, Inc., Arlington Heights, IL, USA). This measurement was done at the same times as the Bod Pod test, therefore participants were fasted. For this measurement, participants were asked to wear light clothing, take off any metal parts of clothing or jewellery and take out any heavy objects from their pockets. They were asked to stand bare-foot on the metal electrodes of the Tanita analyzer and hold the metal handles for a few seconds until the reading was obtained. The measurement, apart from weight and percentage of fat mass included, fat mass (kg), fat free mass (both kg and %), BMI (kg/m^2) and total body water (kg and %).

3.5.2 Blood pressure and metabolic rate

Blood pressure was measured with a digital manometer (Omron 705 IT, Omron Healthcare Co Ltd, Kyoto, Japan.). Participants were asked not to smoke, drink any coffee, tea or cola or participate in any strenuous activity 12 h before the test. Blood pressure readings were taken after at least 5 minutes of rest, on the non-dominant arm.

Resting metabolic rate (RMR) was measured using indirect calorimetry (Quark RMR, COSMED, Italy, presented on Figure 14) at baseline, 6 months and 12 months. The equipment was placed in the quiet room. Participants were asked not to exercise, eat, drink tea/coffee, alcohol or smoke for 12h or more before the measurement. RMR was measured after at least 20 minutes of rest in the study centre. During the measurement participants were resting in a supine position in thermoneutral conditions. For the first 5 to 10 minutes of the test participants were given time to relax. Once the RMR stabilized, an actual measurement was started and lasted 20 minutes.

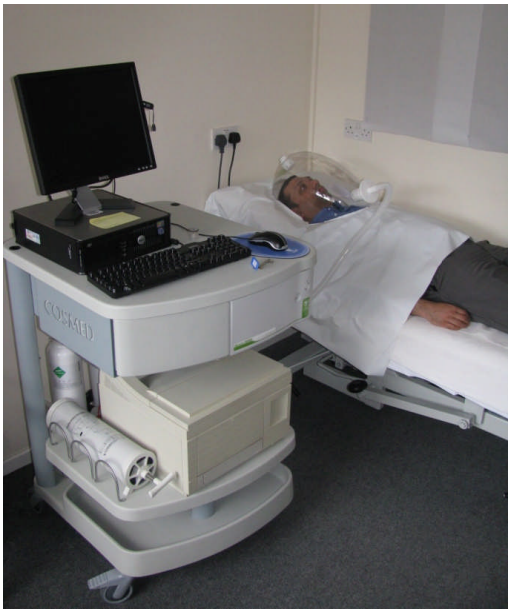


Figure 14. Resting metabolic rate measuring equipment, Quark RMR (by Cosmed, Italy) in a clinic room in Westburn House.

Twenty nine previously developed by others equations estimating RMR were compared using indirect calorimetry data from the present study, as described by Weijs (2008). The accuracy of the equations was assessed by the calculating the percentage of subjects whose estimated RMR fell within ± 10% of the measured RMR (mRMR). The equations were also assessed by bias (between the predicted and measured RMR) expressed as the mean percentage difference.

3.5.3 Cardiovascular risk

The cardiovascular risk (CVD) was determined from the information about the lipid profile, glycaemic control and blood pressure. The CVD risk points for men and women were calculated as proposed by

the group involved in the Framingham Heart Study (D'Agostino et al. 2008). The points were given for gender, age, total cholesterol, HDL-cholesterol, systolic blood pressure, treatment for hypertension, smoking and the presence of diabetes. The sum of the points could then be converted to a percentage of CVD risk and to heart age/vascular age. Above the limit of 21 points for women and 18 for men, CVD risk was over 30 %. This meant that if women had less than 18 points and men had less than 21 points, they were allocated a percent of the CVD risk (from 0 to 29.4 %). This also meant that women with 18 points and more and men with 21 points or more were all assumed to have CVD risk over 30 %. Apart from CVD risk, the points could also estimate heart age. Above the limit of 15 points for women and 17 points for men, heart age (or vascular age) was described to be 80 years.

3.5.4 Biochemistry

Fasting blood samples were drawn at baseline and every 3 months thereafter. Samples were delivered to the Aberdeen Royal Infirmary Clinical Biochemistry Department for analysis. The tests included lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerol), glycaemic control (HbA_{1c}, fasting glucose), thyroid function (thyroid stimulating hormone (TSH)), liver function (total bilirubin, alanine amino tansferase (ALT), alkaline phosphatase (ALKP), and gamma-glutamyl transferase (GGT), and kidney function (electrolytes, urea, creatinine and albumin). Most of the above (apart from glucose and HbA_{1c}) were determined from the samples collected into the vacutainer tubes with silicone coated interior for clot activation and polymer gel for serum separation (SST™II, serum separation tube, by Becton Dickinson). Samples for fasting plasma glucose and glycosylated haemoglobin were collected into the vacutainer tubes containing preservative fluoride (stops blood enzymes from working, so that glucose in the sample is not used) and oxalate (anticoagulant). Additionally, blood was collected into the purple top tube (with ethylenediaminetetraacetic acid, EDTA, which binds calcium in the blood and keeps the blood from clotting) and immediately spun at 4ºC to aliquot the plasma sample. Blood was also collected into the plain red top tube (containing clot activator, silica), kept at room temperature for 60 minutes, and then spun to aliquot serum. Both plasma and serum samples were stored in -80ºC until analysis. Fasting serum samples were used to measure insulin concentration. The analysis was done with the use of Human Insulin ELISA kit EZHI-14K by Millipore (Millipore, Billerica, MA, USA) in Robert Gordon University laboratories.

Blood samples that were collected into the gold top SST™II tubes and grey top tubes were sent to Clinical Biochemistry Department in the Aberdeen Royal Infirmary, Aberdeen. The laboratory was using ADVIA Chemistry systems (Bayer Healthcare LLC, Leverkunsen, Germany) for analysis.

3.5.4.1 Lipid profile

All the components of the lipid profile were determined from the serum samples in the Clinical Biochemistry Department in the Aberdeen Royal Infirmary, Aberdeen. This section summarizes methodology used to determine lipid profile.

3.5.4.1.1 Total cholesterol

Total cholesterol was determined by an enzymatic method with colorimetric (Trinder) endpoint. Firstly cholesterol esters were hydrolysed to free cholesterol and free fatty acids using cholesterol esterase (Varley 1969). Then the reactions were as follows:

[1] $\text{Cholesterol} + O_2 \rightarrow \text{cholest-4en-3one} + H_2O_2$ (cholesterol oxidase)

[2] $H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \rightarrow \text{quinoneimine dye} + 2\ H_2O$ (peroxidase)

The action of cholesterol oxidase in reaction [1], resulted in the production of hydrogen peroxide which was then measured colorimetrically in the Trinder reaction [2]. Phenol and 4-aminoantipyrine, called jointly Trinder reagent, react with hydrogen peroxide under influence of peroxidase and a red-violet quinone complex was formed which was read at 500nm (Varley 1969).

3.5.4.1.2 LDL-cholesterol

LDL cholesterol was calculated from the following Friedewald formula (1972):

LDL cholesterol = total cholesterol – HDL cholesterol – (0.46 * TG) [mmol/L]

or

LDL cholesterol = total cholesterol – HDL cholesterol – (0.2 * TG) [mg/dL].

3.5.4.1.3 HDL-cholesterol

To leave only HDL-cholesterol in a sample, LDL, VLDL and chylomicrons were eliminated by cholesterol esterase and cholesterol oxidase. Peroxide produced in this step was removed by catalase. In the second step, reagent containing sodium azide was added. Sodium azide inhibited the catalase and

Trinder reagent. Therefore, HDL was determined by an enzymatic method similar to that for total cholesterol.

3.5.4.1.4 Triacylglycerol

The method combined a three step enzymatic reaction with a Trinder reaction. Firstly, lipase from *C.viscosum* was used to hydrolyze the triacylglycerol [1] (Fossati et al, 1982). The released glycerol was measured with use of an enzymatic method. It was converted to glycerol-3-phosphate under the influence of glycerol kinase [2]. Glycerol-3-phosphate was then oxidized to dihydroxyacetone and hydrogen peroxide [3]. The latter was measured with the use of Trinder reaction [4].

[1] triacylglycerol → glycerol + free fatty acids (lipase)

[2] glycerol + phosphate → glycerol-3-phosphate (glycerol kinase)

[3] Glycerol-3-phosphate + O2 → dihydroxyacetone + H2O2 (glycerol-3-phosphate oxidase)

[4] H_2O_2 + phenol + 4-aminoantipyrene → quinoneimine + 4 H_2O (peroxidase)

3.5.4.2 Glycaemic control

3.5.4.2.1 HbA_{1c}

The method required a whole blood sample; therefore the blood was collected into a fluoride and oxalate tube. The concentration of HbA_{1c} and the concentration of total haemoglobin were measured separately. The ratio was reported as % HbA_{1c}.

For the measurement of total haemoglobin, there were two reagents needed: Haemoglobin Denaturant Reagent and Total Haemoglobin Reagent. Firstly, the whole blood sample was mixed with the first reagent and incubated in the room temperature for 5 min. The red blood cells were lysed and the haemoglobin chain was hydrolysed by the protease from the reagent. Then the second reagent was used. The method was based on the conversion of all haemoglobin derivatives into alkaline hematin in an alkaline solution of a nonionic detergent.

HbA_{1c} was measured by a latex agglutination inhibition method. It required the use of HbA_{1c} Agglutinator Reagent (R1), and HbA_{1c} Antibody Reagent (R2). The HbA_{1c} from the sample competed with the agglutinator for the anti-HbA_{1c} antibody, therefore reducing the rate of agglutination. A

concentration curve was obtained by monitoring the change in scattered light as a change of absorbance. The actual change in absorbance was inversely proportional to the concentration of HbA_{1c} in the sample.

3.5.4.2.2 Fasting plasma glucose

Glucose was measured with an enzymatic method. Glucose in a sample was oxidized to gluconic acid and hydrogen peroxide due to the catalytic action of glucose oxidase [1] (Varley 1969). The formed hydrogen peroxide was measured with the use of the Trinder reaction [2].

[1] β-D-glucose + O_2 → gluconic acid + H_2O_2 (glucose oxidase)

[2] 2 H_2O_2 + phenol + 4-aminoantipyrene → quinoneimine + 4 H_2O (peroxidase)

3.5.4.2.3 Fasting plasma insulin

Blood for the measurement of fasting plasma insulin was collected into the purple top tubes (with EDTA). Once drawn, a tube was kept on the ice on the way from the clinic room to the laboratory (which took no more that 1-2 minutes) and immediately spun at 4ºC to aliquot the plasma sample. Plasma samples were stored in -80ºC until analysis.

The analysis was done with the use of Human Insulin enzyme linked immunosorbent assay (ELISA) kit EZHI-14K by Millipore (Millipore, Billerica, MA, USA) in Robert Gordon University laboratories. After thawing, the plasma was pipetted into the wells of a microtiter plate and insulin from the samples bound to the monoclonal mouse anti-human insulin antibodies that coated the inside of the wells. Then, a second biotinylated monoclonal mouse anti-human antibody bound to the captured insulin and unbound materials were washed away from the sample. Following this, horseradish peroxidase conjugated to the immobilized biotinylated antibodies and free enzyme conjugates were washed away. The immobilized antibody-enzyme conjugates were quantified by monitoring horseradish peroxidase activities in the presence of the 3,3',5,5'-tetramethylbenzidine. The enzyme activity was measured spectrophotometrically by the increased absorbency at 450 nm after acidification of the formed products. The amount of captured insulin in an unknown sample is proportional to the increase in absorbency. Therefore the insulin concentration was derived by interpolation from a reference curve generated from the same assay with reference standards of known concentrations of human insulin.

3.5.4.3 Kidney function

3.5.4.3.1 Sodium, Potassium and Chloride

For the measurement of sodium, potassium and chloride concentrations in samples, an ion-selective electrode (ISE) method was used. Serum samples were mixed with ISE buffer that has a constant pH and a constant ionic strength solution. ISE is a sensor that converts the activity *a* (concentration) of an ion in the sampled electrolyte to electrical potential. The potential of the electrode was compared against a standard electrode potential and measured by voltmeter. The voltage, according to the Nernst equation [1], depends on the log of ionic activity.

$$[1] \qquad E = E_0 + ((2.303 * RT) / nF) * \log a_x$$

where E, electrolyte potential recorded; E_0, constant for the electrode system; R the universal or molar gas constant, 8.31 J/K; T, temperature (k); F, the Faraday, 96487 coulombs; n, the number of charges on the ion, including sign; a, activity of the ion x to which the electrode responds (Varley 1969).

3.5.4.3.2 Creatinine

The method to determine the concentration of serum creatinine was based on the Jaffe reaction (Jaffe 1886). In this reaction, creatinine reacted with picric acid in an alkaline solution. A red coloured creatinine-picrate complex was formed. The sample was read at 505/571 nm.

3.5.4.3.3 Urea

The urease/glutamate dehydrogenase method was used to measure serum urea concentration. Firstly, urea was hydrolysed to carbamic acid and ammonia in the presence of urease. Carbamic acid then decomposed to a molecule of ammonia and carbon dioxide [1]. The produced ammonia then reacted with 2-oxoglutarate in the presence of glutamate dehydrogenase and NADH [2]. The oxidation of NADH to NAD^+ was measured at 340 nm (Varley 1969).

$$[1] \quad \text{urea} + H_2O \rightarrow NH_3 + \text{carbamic acid} \rightarrow 2NH_3 + CO_2 \quad \text{(urease)}$$

$$[2] \quad \text{ammonia} + \text{2-oxoglutarate} + \text{NADH} \rightarrow \text{glutamate} + NAD^+ \quad \text{(glutamate dehydrogenase)}$$

3.5.4.3.4 Albumin

Serum albumin was quantified by the method that uses bromocresol green solution (BSG) as a binding dye (Doumas et al. 1971). An albumin-BCG complex was formed and then measured as an endpoint reaction at 596/694 nm.

3.5.4.3.5 Creatinine clearance

Creatinine clearance is an approximation of glomerular filtration rate (GFR) and is expressed in milliliters per minute. Renal clearance is a volume of plasma cleared of a given substance (here creatinine) in 1 minute (Pocock et al. 2006). Creatinine clearance was calculated by multiplying the creatinine concentration in the urine by the amount of urine excreted in one minute (24h sample urine volume / 1440) and dividing it by serum creatinine concentration (Pocock et al. 2006). 24-hour urine samples were collected from the subset of participants only (n = 21) because some of participants found collecting urine for 24 hours and keeping it chilled in the fridge unpleasant and refused collection. Consequently, changes in creatinine clearance were only assessed in 21 participants (last observation carried forward (LOCF) analysis).

Urine creatinine concentration was measured with creatinine assay KGE005 by R&D Systems (R&D Systems, Inc., Minneapolis, MN, USA) in Robert Gordon University laboratories. The urine creatinine assay was, just like serum creatinine assay, based on Jaffe reaction. In this reaction creatinine reacted with alkaline picrate solution to create a bright orange-red complex. Samples were diluted and pipetted into the wells on a microplate. Then, alkaline picrate reagent was added and incubated for 30 minutes in a room temperature. Intensity of the orange-red colour at 490 nm corresponded to the concentration of creatinine in the sample. The investigated samples were compared to the standard curve.

GFR can also be estimated from prediction equations. In the present study the CKD-EPI equation was used because it was shown to be more accurate than the Modification of Diet in Renal Disease Study equation (MDRD) (Levey et al. 2009). The equation required information about serum creatinine (Scr) concentration, age, gender and ethnicity. For the white race the CKD-EPI equations were as follows:

Female	Scr (μmol/l) ≤ 62	$GFR = 144 \times (Scr/0.7)^{-0.329} \times (0.993)^{Age}$
	Scr (μmol/l) > 62	$GFR = 144 \times (Scr/0.7)^{-1.209} \times (0.993)^{Age}$

Male	Scr (μmol/l) ≤ 80	$GFR = 141 \times (Scr/0.9)^{-0.411} \times (0.993)^{Age}$
	Scr (μmol/l) > 80	$GFR = 141 \times (Scr/0.9)^{-1.209} \times (0.993)^{Age}$

3.5.4.4 Liver function

3.5.4.4.1 ALKP

Alkaline phosphatase was determined from serum samples. The enzyme hydrolyzed *p*NPP substrate in 2amino-2methyl-1propanol (AMP) buffer to form *p*-nitrophenol [1]. The zinc and magnesium ions were added to the AMP to activate and stabilize the enzyme. The reaction was followed by the colorimetric measurement of the rate of formation of *p*-nitrophenol at 410/478 nm, which was proportional to the ALKP activity.

[1] pNPP + AMP → P-Nitrophenol + P-AMP (ALP, Mg2+, Zn2+)

3.5.4.4.2 ALT

The reaction was initiated by the addition of α-ketoglutarate [1]. The concentration of NADH [2] was measured by its absorbance at 340/410 nm and the rate of absorbance decrease was proportional to the ALT activity.

[1] L-Alanine + α-Ketoglutarate → Pyruvate + L-Glutamate (ALT)

[2] Pyruvate + NADH → Lactate + NAD (lactate dehydrogenase)

3.5.4.4.3 GGT

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

[1] L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine → 5-amino-2-nitrobenzoate + L-γ-glutamyl-glycylglycine (GGT)

3.5.4.4.4 Total bilirubin

The method used to measure total bilirubin was an endpoint reaction based on chemical oxidation by vanadate (Tokuda and Tanimoto 1993). Vanadate oxidizes bilirubin at pH 2.9 to produce biliverdin. Oxidation, in the presence of detergent, decreased bilirubin's (both conjugated and unconjugated) yellow colour. The decrease in optical density at 451/545 nm is proportional to the total bilirubin concentration.

3.5.4.5 Thyroid function

3.5.4.5.1 Thyroid stimulating hormone (TSH)

The assay is a two-site sandwich assay using chemiluminescence technology and two antibodies (immunochemiluminometric assay, ICMA) (Weeks et al. 1984). The capture antibody is a polyclonal sheep anti-TSH bound to a paramagnetic particle. The signal antibody is a monoclonal mouse anti-TSH labelled with chemiluminescent acridinium ester. The total reaction time is 2 h and bound labelled antibody is quantified luminometrically (RLU, relative light units). Siemens Advia Centaur XP system was used for this assay.

3.5.4.6 Ketone bodies monitoring

To ensure that carbohydrates were restricted throughout the diets, ketone bodies were measured both in urine and in capillary blood at each visit. Urine was tested using reagent strips (Ketostix® Reagent Strips, Bayer HealthCare LLC, Mishawaka, IN, USA). The procedure was delivered according to the instructions provided with the strips. Participants were given a disposable container and asked to provide a urine sample. Then, researcher took the sample to the laboratory, dipped the reagent strip in the sample and immediately removed it. At this point, timing begun; and at exactly 15 seconds the reagent area of the strip was matched to the ketone colour chart (on the bottle of strips). The results were read and recorded. Each color block on the colour chart represented a range of values. The reagents in the strip were 7.1% w/w sodium nitroprusside and 92.9% w/w buffer. The reaction involved acetoacetic acid in the urine but not acetone or beta-hydroxybutyric acid. The test was based

on the development of colours ranging from buff pink to maroon when acetoacetic acid reacted with nitroprusside. The threshold of acetoacetic acid detection by Ketostix reagent trips was at 0.3 to 0.6 mmol/l.

Concentration of ketone bodies in capillary blood was determined using an electrochemical method (Optium Xceed Sensor and Optium β-Ketone Test Strips, Abbott Diabetes Care Ltd., Witney, UK). The procedure was delivered according to the instructions provided (Abbot, 2006). The sensor was calibrated for every new batch of test strips. Before obtaining a drop of blood finger tip was cleaned with an alcohol swab and dried. A finger prick was done by a single use sterile lancet; the lancet was disposed of after use. A drop of blood was applied to the test strip immediately. The sample needed was 1.5µl of capillary blood. The sensor displayed blood β-ketone concentration in 10 seconds. The test measures beta-hydroxybutyrate concentration in the range between 0.0 and 8.0 mmol/l. If present in capillary blood, β-hydroxybutyrate reacts with the chemicals on the strip producing a small electrical current. The current is measured and the result is displayed on a sensor. The size of the current depends on the β-hydroxybutyrate concentration in the sample. The test strip contains β-hydroksybutyrate dehydrogenase (≥ 0.03 U), NAD (free acid form, ≥ 1.67µg), phenanthroline quinone (≥ 0.29 µg), and non-reactive ingredients (≥ 19.51 µg). The β-Ketone tests strips were calibrated to reflect plasma β-hydroxybutyrate using the Randox assay kit (RB1007).

3.5.5 Questionnaires

Questionnaires were administered at baseline and every 3 months afterwards to assess quality of life (Audit of Diabetes-Dependent Quality of Life and The Obesity-Related Wellbeing Questionnaire 97), satisfaction with diabetes treatment (Diabetes Treatment Satisfaction Questionnaire status version), physical activity (General Practice Physical Activity Questionnaire), motivation to lose weight based on transtheoretical model (Decisional Balance Description), depression (Major Depression Inventory), sleepiness (Epworth Sleepiness Scale) and fatigue (Lee Fatigue VAS Scale). Questionnaires were given to participants by the end of the long visit (see Table 2) after all the planned measurements were done and participants received breakfast.

3.5.5.1 Audit of Diabetes-Dependent Quality of Life

Audit of Diabetes-Dependent Quality of Life (ADDQoL) was developed for measurement of quality of life (QoL) per se (single item) and for measurement of impact of diabetes on quality of life (Bradley

2005) (Appendix 3). ADDQoL, among other uses, was recommended for use as an assessment tool with groups of patients and as an outcome measure for clinical research trials evaluating new treatments (Bradley 2005). The license to use this questionnaire in the present study was obtained from Prof Clare Bradley, Health Psychology Research, Department of Psychology, Royal Holloway, University of London.

ADDQoL consisted of two items asking about present QoL and impact of diabetes on present QoL, and of18 items assessing impact of diabetes on specific areas of life. The first two items were scored individually on a 7-point Likert scale as follows:

I. In general, my present quality of life is: excellent = 3, very good = 2, good = 1, neither good nor bad = 0, bad = -1, very bad = -2, extremely bad = -3.

II. If I did not have diabetes my quality of life would be: very much better = -3, much better = -2, a little better = -1, the same = 0, a little worse = 1, much worse = 2, very much worse = 3.

The 18 areas of life included working life and work-related opportunities, family life, friendships and social life, sex life, physical appearance, things participants could do physically, holidays and leisure activities, ease of travelling, confidence in ability to do things, motivation to achieve things, they way the society at large reacts to participants, worries about the future, finances, need to depend on others for things the participants would like to do themselves, living conditions, freedom to eat as they wish, enjoyment of food and freedom to drink as they wish (Bradley et al. 1999). Each of the 18 items consisted of two questions asking about the impact of diabetes on an aspect of life and the importance of this aspect. The impact part ("If I did not have diabetes my … would be") was scored on a 7-point Likert scale as follows: very much better = -3, much better = -2, a little better = -1, the same = 0, a little worse = 1, much worse = 2, very much worse = 3. The importance part was scored on a 4-point Likert scale as follows: very important = 3, important = 2, somewhat important = 1, not at all important = 0. A score for each item was obtained by multiplying impact and importance. Therefore, -9 meant maximum negative impact of diabetes and +9 meant maximum positive impact of diabetes. Each of the eighteen aspects can be interpreted separately; however, an average weighted impact (AWI) can also be calculated by summing up all applicable item scores and dividing the result by the number of them. Again, -9 meant maximum negative impact of diabetes and +9 meant maximum positive impact of diabetes.

3.5.5.2 Obesity Related Wellbeing Questionnaire

The Obesity-Related Well-Being questionnaire (ORWELL-97) is a validated and reliable tool (Duval, Marceau et al. 2006) specifically built for measuring health related QoL in obese people following treatment (Mannucci et al. 1999). It has been developed by an interdisciplinary team of psychiatrists, endocrinologists, nurses, and dietitians and has been used for research purposes in randomised controlled trials (Mazzoni et al. 1999). ORWELL-97 measures the intensity and the subjective relevance of physical and psychological distress generated by overweight (Mannucci et al. 2010). ORWELL-97 questionnaire has been published and made freely available for the research purposes (Mannucci et al. 1999).

The questionnaire consists of 18 items related to the obesity-caused somatic symptoms and physical functioning, impact on obesity-related worries and emotional status, and on relationships and social network (Mannucci et al. 1999). Each item of the ORWELL-97 questionnaire contains two questions addressing occurrence of a symptom and its relevance (Appendix 4). Both occurrence and relevance questions are scored on a 4-point Likert scale with the following scoring: not at all = 0, just a little = 1, not so much = 3, much = 4. The score of each item was calculated by multiplying occurrence and relevance. The score of the whole questionnaire was calculated by adding up all items. Lower scores of ORWELL-97 meant higher the quality of life.

3.5.5.3 Diabetes Treatment Satisfaction Questionnaire

Diabetes Treatment Satisfaction Questionnaire in its status form (DTSQs) was used to measure patients' satisfaction with the programme as a diabetes treatment (Bradley 1994) (Appendix 5). The questionnaire included six items assessing treatment satisfaction and two items assessing perceived frequency of hyperglycaemia and hypoglycaemia (Bradley 2006). DTSQs has been used in numerous studies to measure diabetes treatment satisfaction (Jennings et al. 1991, Witthaus et al. 2001, DAFNE Study Group 2002, Ashwell et al. 2008) and was proved sensitive to the changes in treatment (Bradley 1994).

Each of the items of the questionnaire was rated on a 7-point scale (0 to 6), where 6 meant a high level of satisfaction and 0 meant a high level of dissatisfaction. The treatment satisfaction Scale Total was computed by summing up the six items assessing treatment satisfaction. Then, the sum was divided by

the number of existing scores and finally multiplied by 6 (the number of items in the subscale). The scale had a minimum of 0 and a maximum of 36.

The DTSQ change version (DTSQc) was developed to overcome the ceiling effects[4] often seen with DTSQs (Howorka et al. 2000). DTSQc has the same items as DTSQs but requires comparison between the current and the previous treatment. The license to use DTSQs and DTSQc questionnaires in the present study was obtained from Prof Clare Bradley, Health Psychology Research, Department of Psychology, Royal Holloway, University of London.

3.5.5.4 General Practice Physical Activity Questionnaire

General Practice Physical Activity Questionnaire (GPPAQ) provides a 4-level physical activity index (inactive, moderately inactive, moderately active and active) that reflects participants' current activity level (Appendix 6). It is a validated tool intended for use in adults in routine general practice (National Health Service 2006). GPPAQ was not designed for use in physical activity interventions but provides a simple tool for assessing participant's physical activity. It takes only 30 seconds to complete, therefore, it was selected to be used with seven other questionnaires in the present study. GPPAQ consists of three items. It is scored with the use of the provided algorithm (National Health Service 2006). The questionnaire is available for free.

3.5.5.5 Weight Decisional Balance Scales

The Weight Decisional Balance (WDB) questionnaire is a valid and reliable measure suitable for use in research (O'Connell et al. 1988). The questionnaire consists of 20 items: 10 Pros and 10 Cons for the decision to lose weight (O'Connell et al. 1988). The importance of each of the items in making a decision about losing weight was scored by patients on a 5 point scale: not at all = 1, slightly = 2, moderately = 3, very = 4, extremely = 5 (Appendix 7).

In order to score the questionnaire, the scores for Pros were added up and divided by 10, and the same procedure was applied to Cons. The difference score was obtained by subtraction of Cons from the Pros. A positive value indicated more positive attitude towards weight loss, while negative value suggested negative attitude towards weight loss.

[4] Ceiling effect may occur when a lot of scores are in the upper level of the instrument's scale. When this happens, variance of an independent variable is not estimated/measured above a certain level.

3.5.5.6 Major Depression Inventory

The Major Depression Inventory (MDI) was designed to measure the severity of major depression according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) published by the American Psychiatric Association and also according to the International Statistical Classification of Disease and Health Related Problems , 10^{th} version (ICD-10) (Bech et al. 2001). MDI has been validated and it can be used as a screening instrument for detecting depression (Olsen et al. 2003).

MDI can be scored according to DSM-IV and ICD-10 algorithms for symptoms of depression but also it can be scored as a depression rating scale by adding up the individual items (Bech et al. 2001). In the current study MDI was scored as a depression rating scale. The scale had a minimum of 0 and maximum of 50. Total scores below 20 did not indicate depression. The scores of 20 to 24 indicated mild depression, the scores of 25 to 29 indicate moderate depression and the scores of 30 or more indicated severe depression (Olsen et al. 2003)

MDI consists of ten items (Appendix 8). Two of those items contain two questions 8a, 8b and 10a, 10b. Only the higher ones of the two choices in items 8 or 10 are used to calculate the overall score. The items refer to the occurrence of different symptoms of depression two weeks prior to completing the questionnaire. There are 6 possible responses for each item which, when treated as a depression rating scale, were scored as follows: all the time = 5, most of the time = 4, slightly more than half time = 3, slightly less than half time = 2, some of the time = 1, at no time = 0. MDI is free for use and available to download from the dedicated website of Psychiatric Research Unit at the Mental Health Centre in Denmark (Bech et al. 2001).

3.5.5.7 Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) contains eight items that assess daytime sleepiness in adults (Johns 1991). Each item requires patients to rate the chance of dozing in a specific situation (Appendix 9). There are four possible scores to rate the chance of dozing: 0 = no chance of dozing, 1 = slight chance of dozing, 2 = moderate chance of dozing, and 3 = high chance of dozing. The ESS score is calculated by summing up all eight items. Therefore, the minimum score is 0 and the maximum score is 24.

The ESS was originally developed to distinguish between normal patients and patients with a range of sleep disorders (Johns 1991). It has been confirmed to be a reliable method for measuring daytime sleepiness in adults (Johns 1992).

3.5.5.8 Visual Analogue Scales to assess fatigue

The fatigue and energy levels were assessed by the visual analogue scales (VAS-F) developed by Lee and colleagues (1991). VAS-F is a simple validated tool that is suitable for research purposes (Lee et al. 1991). The questionnaire consists of 18 items, of which five relate to energy levels and thirteen relate to fatigue levels (Appendix 10). Each of the items has a 100 mm line with two extremes defined (i.e. not at all tired on 0 mm end and extremely tired on 100 mm end). Participants were asked to place a mark on the line to indicate how they felt at the moment of completing the questionnaire. The scores of VAS-F were calculated for the fatigue and for the energy subscales independently. The scores from 13 fatigue items were summed and divided by 13 to get an average fatigue rating. The same procedure was applied to five energy items.

3.6 Statistical analysis

The target sample size of 84 subjects (42 in each group) was calculated for the study to have 80% power to demonstrate mean (± SD) weight loss of 5 ± 8 kg difference between the diets (nQuery Advisor 4.0, Statistical Solutions Ltd.). A standard deviation of 8 kg was taken from study by Wadden et al. (1989). Given an anticipated dropout rate of 30 % (based on Samaha et al. 2003) the enrollment target was set at 120 subjects. Unfortunately, only 41 volunteers were recruited before the study was stopped (25 on PSMF and 16 on GL) due to problems with funding.

Simple randomisation was used to assign individuals to the two dietary groups (Durham et al. 2008).
It was done using Microsoft Excel software. First column was created and contained sixty “1” (coding GL group) and sixty “2” (coding PSMF group). Then, a parallel column of RAND functions were created (using command =RAND()). Finally, RAND functions were sorted from smallest to largest with selection expanded to the first column with “1”s and “2”s. The order of two groups (1 for GL and 2 for PSMF) was saved. One hundred and twenty envelopes were prepared, each given a number between 1 and 120. A person not involved in the study put into the envelopes pieces of paper with the numbers coding dietary groups (1 or 2) in the saved order, and the sealed the envelopes. If a volunteer fulfilled inclusion criteria after screening, an envelope with the smallest available number on the cover

(between 1 and 120) was opened and acoording to the code inside it, the participant was being assigned to one of the dietary groups.

All variables were assessed for normality before entry into the analysis using the Kolmogorov-Smirnov test. The distribution was normal for most of the variables analyzed except fasting plasma glucose and insulin, HOMA-IR, reported dietary fat and protein content (g/d), triacylglycerol, LDL cholesterol, creatinine, ALKP and GGT. These were log transformed before the analyses. FFM, ALT and total bilirubin were reciprocally transformed as logging did not correct non-normality. Finally, there were a number of variables that were dichotomous, ordinal or were not corrected by any of the tried transformations (log, reciprocal, square root): questionnaire data, medication data, HbA_{1c}, HDL-cholesterol, TSH, and reported dietary carbohydrate intake both as percent of energy and expressed as grams per day. These were analysed using non-parametric tests.

All p-values were 2-sided, and we assumed a p-value of 0.05 as statistically significant. Analyses were performed with SPSS statistical software, version 17.0 (SPSS Inc., Chicago, Illinois).

There are two analyses presented in the results. First is the last observation carried forward analysis (LOCF) in which the last available measurement was carried forward through the missing time points to the final assessment. LOCF approach allowed using data of all participants who started the programme (n = 41). Second is the completers' analysis, using only the data of participants who completed the study (n = 14).

In the results section two dietary arms (GL and PSMF) were compared to investigate whether trends for clinically important differences between the diets could exist. The study did not have sufficient power to detect significant differences; however, it could indicate the direction of changes. Additionally, to check whether the LOCF approach biases the results, outcomes for completers and non-completers were compared. Outcomes of comparisons were reported as either significant differences, non-significant trends or lack of difference.

The following statistical methods were used:

- To compare baseline values between the dietary groups and also changes between the groups, an independent t-test was used (non-parametric, Mann-Whitney test).
- To compare baseline values between the dietary groups for categorical and nominal outcomes, a Chi squared analysis was performed (non-parametric).

- To compare differences from baseline within the groups, a paired t-test was used (non-parametric, Wilcoxon signed ranks test).
- To assess time effect on changes in variables, a repeated measures ANOVA was used (non-parametric, Friedman ANOVA).
- To assess time effect on the proportions of participants (i.e. changes in adverse events over time) a Cochran Q test was used; this test is a Friedman ANOVA for dichotomous data (Field 2009)
- To correlate variables or changes between variables, a Pearson correlation coefficient was used (Spearman's correlation coefficient for ordinal data)
- Stepwise linear regression models were used to identify the determinants of RMR. The adjusted coefficients of determination (R^2), derived from this analysis for each step, represent the percentage of variance in RMR, explained by the variables entered into the model (Field 2009). Measured RMR was entered as a dependent variable and age, body mass, height, BMI, waist circumference, FM, FFM, fasting glucose and glycosylated haemoglobin as independent variables. The models provided by the software, that explained the most variability in RMR were selected and described.

3.6.1 Funding source

The Go Lower company provided food for the Go Lower group, vitamin supplement for both groups and funded a studentship of the research student. Go Lower had no role in the design, conduct or reporting of the study.

4 Results

4.1 Baseline characteristics

LOCF

The physical characteristics of subjects at baseline are shown in Table 5a. Fat mass, fat free mass and medication use are described later on in this chapter. Most of the participants were Caucasians (n = 39), two were Asians. At baseline age, stature, body mass, BMI, waist circumference and RMR were not different between the two diet groups. Females were shorter (mean ± SD) (161.4 ± 6.1 vs. 175.7 ± 9.3 cm, $p < 0.001$), had smaller waists (118.6 ± 12.3 vs. 127.6 ± 10.9 cm, $p = 0.021$) and lower RMR than males (1860 ± 413 vs. 2165 ± 418 kcal/d, $p = 0.026$). However, when controlled for FFM, RMR (kcal/d) was significantly higher in women ($p = 0.015$). Age, weight and BMI were not different between the two genders. There was a difference in the proportion of males and females between the two diet groups; there were more males in the PSMF group (n = 14) than in the GL group (n = 3) (56.0% vs. 18.8%, $p = 0.025$). Also, in the PSMF group men had lower mean BMI than women (36.1 ± 4.0 vs. 42.4 ± 6.1, $p = 0.005$).

Table 5a. Baseline characteristics of participants (LOCF; n = 41).

	All (n = 41)	**GL (n = 16)** *m*, (n = 3)	**PSMF (n = 25)** *m*, (n = 14)	**P** †
Age (years)				
m	57.1 ± 9.4	56.7 ± 8.0	55.4 ± 9.0	0.830
f	55.6 ± 8.6	58.1 ± 7.9	55.9 ± 11.3	0.574
Total	**56.5 ± 9.0**	**57.9 ± 7.7**	**55.6 ± 9.9**	**0.447**
Stature (cm)				
m	175.7 ± 9.3*	176.0 ± 7.8	175.6 ± 9.9	0.954
f	161.4 ± 6.1*	162.1 ± 5.9	160.6 ± 6.5	0.556
Total	**167.4 ± 10.3**	**164.7 ± 8.2**	**169.0 ± 11.3**	**0.199**
Body mass (kg)				
m	115.6 ± 18.4	133.2 ± 6.4	111.8 ± 18.0	0.066
f	103.9 ± 19.8	98.8 ± 18.8	109.9 ± 20.0	0.177
Total	**108.8 ± 19.9**	**105.3 ± 21.9**	**111.0 ± 18.5**	**0.376**
BMI (kg/m^2)				
m	37.3 ± 4.5	43.0 ± 1.9	36.1 ± 4.0	0.011
f	39.9 ± 6.4	37.7 ± 6.1	42.4 ± 6.1	0.072
Total	**38.8 ± 5.8**	**38.7 ± 5.9**	**38.9 ± 5.9**	**0.918**
Waist (cm)				
m	127.6 ± 10.9*	138.3 ± 6.4	125.3 ± 10.4	0.057
f	118.6 ± 12.3*	117.6 ± 14.8	119.9 ± 9.0	0.665
Total	**122.3 ± 12.4**	**121.5 ± 15.8**	**122.9 ± 10.0**	**0.755**
RMR (kcal/d)				
m	2165 ± 418*	2585 ± 688	2075 ± 305	0.052
f	1860 ± 413*	1834 ± 430	1891 ± 411	0.746
Total	**1987 ± 437**	**1975 ± 550**	**1994 ± 360**	**0.894**

Presented values are means ± standard deviation. All variables were normally distributed. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; BMI, body mass index; RMR, resting metabolic rate; m, male; f, female.
† Significance level of the difference between the diet groups at baseline, calculated using an independent t-test
* marks the significant difference between the genders at baseline, $p < 0.05$, calculated using independent t-test

Completers

Completers' analysis gave similar results to the LOCF analysis. The physical characteristics of completers (n = 14) at baseline are shown in Table 5b. All completers were Caucasians. At baseline age, stature, body mass, waist circumference and RMR were not different between the two diet groups and genders. Men on GL (n = 2) had higher mean BMI than men on PSMF (n = 6) (44.0 ± 1.2 vs. 33.8 ± 3.4 kg/m^2). There were more males in the PSMF group (n = 6) than in the GL group (n = 2) but the trend was not significant (75.0% vs. 33.3%, p = 0.277). Finally, there were no differences between completers and dropouts at baseline in age, stature, body mass, BMI, and waist circumference.

Table 5b. Baseline characteristics of participants (completers, n = 14).

	All (n = 14)	**GL (n = 6)** *m*, (n = 2)	**PSMF (n = 8)** *m*, (n = 6)	**P** †
Age (years)				
m	57.4 ± 6.8	60.5 ± 6.4	56.3 ± 7.2	0.498
f	58.7 ± 5.5	60.2 ± 6.4	55.5 ± 0.7	0.379
Total	**57.9 ± 6.1**	**60.3 ± 5.7**	**56.1 ± 6.1**	**0.215**
Stature (cm)				
m	177.9 ± 11.0	171.5 ± 0.7	180.0 ± 12.2	0.386
f	163.7 ± 8.3	162.9 ± 8.2	165.5 ± 11.3	0.756
Total	**171.8 ± 12.0**	**165.7 ± 7.9**	**176.4 ± 13.0**	**0.103**
Body mass (kg)				
m	115.0 ± 18.2	129.6 ± 2.4	110.1 ± 18.7	0.213
f	108.3 ± 25.8	97.2 ± 18.6	130.6 ± 28.3	0.146
Total	**112.2 ± 21.1**	**108.0 ± 22.1**	**115.3 ± 21.3**	**0.547**
BMI (kg/m^2)				
m	36.4 ± 5.5	44.0 ± 1.2	33.8 ± 3.4	0.007
f	40.1 ± 6.9	36.4 ± 4.4	47.3 ± 3.9	0.042
Total	**38.0 ± 6.1**	**39.0 ± 5.2**	**37.2 ± 7.0**	**0.613**
Waist (cm)				
m	127.6 ± 13.4	142.0 ± 1.4	122.8 ± 11.9	0.075
f	113.3 ± 9.9	112.2 ± 11.0	115.5 ± 10.6	0.748
Total	**121.5 ± 13.7**	**122.2 ± 17.6**	**121.0 ± 11.4**	**0.882**
RMR (kcal/d)				
m	2156 ± 354	2205 ± 286	2140 ± 398	0.842
f	1769 ± 338	1669 ± 388	1967 ± 297	0.365
Total	**1990 ± 389**	**1848 ± 428**	**2097 ± 346**	**0.251**

Presented values are means ± standard deviation. All variables were normally distributed. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; BMI, body mass index; RMR, resting metabolic rate; m, male; f, female.
† Significance level of the difference between the diet groups at baseline, calculated using an independent t-test
* marks the significant difference between the genders at baseline, $p < 0.05$, calculated using independent t-test

4.2 Body mass and body composition

LOCF

At baseline weight was similar in both diet groups and genders (Table 6a). However, men had significantly greater fat free mass (65.3 ± 10.4 kg vs. 50.6 ± 7.0 kg, $p < 0.001$) than women, and women had significantly greater percentage of body fat (50.6 ± 6.1 vs. 43.1 ± 6.1 %, $p < 0.001$).

Table 6a. Changes in weight, fat mass, and fat free mass (LOCF, n = 41).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Weight (kg) [a]						
GL (n=16)	105.3 ± 21.9	97.3 ± 22.2*	98.3 ± 21.8*	-6.9 ± 6.2 (-6.7 ± 6.2 %) §	<0.001	0.326
PSMF (n=25)	111.0 ± 18.5	105.1 ± 18.7*	106.4 ± 19.4*	-4.6 ± 7.9 (-4.2 ± 6.7 %) §	0.001	
Total (n=41)	**108.8 ± 19.9**	**102.1 ± 20.2***	**103.2 ± 20.5***	**-5.5 ± 7.3**	**<0.001**	
m (n=17)	115.6 ± 18.4	108.7 ± 19.2*	110.1 ± 19.1*	-5.5 ± 6.7	0.001	0.979
f (n=24)	103.9 ± 19.8	97.3 ± 19.9*	98.3 ± 20.3*	-5.6 ± 7.8	<0.001	
FM (kg) [a]						
GL	51.7 ± 14.9	44.6 ± 15.2*	45.8 ± 14.9*	-5.9 ± 5.8	<0.001	0.565
PSMF	52.3 ± 14.6	47.4 ± 15.8*	47.6 ± 14.1*	-4.7 ± 7.3	0.001	
Total	**52.1 ± 14.6**	**46.3 ± 15.5***	**46.9 ± 14.3***	**-5.1 ± 6.7**	**<0.001**	
m	50.3 ± 12.7	43.7 ± 13.3*	45.1 ± 12.7*	-5.2 ± 6.4	<0.001	0.981
f	53.4 ± 15.9	48.1 ± 16.9*	48.2 ± 15.4*	-5.1 ± 7.0	<0.001	
FM (%) [a]						
GL	48.7 ± 7.1	45.1 ± 7.8*	45.9 ± 7.7*	-2.8 ± 3.4	<0.001	0.705
PSMF	46.7 ± 7.1	43.9 ± 9.3*	44.3 ± 7.8*	-2.4 ± 3.8	0.001	
Total	**47.5 ± 7.1**	**44.4 ± 8.6***	**44.9 ± 7.7***	**-2.6 ± 3.6**	**<0.001**	
m	43.1 ± 6.1	39.6 ± 7.1*	40.4 ± 6.3*	-2.7 ± 3.2	<0.001	0.805
f	50.6 ± 6.1	47.8 ± 8.0*	48.1 ± 7.0*	-2.4 ± 3.2	0.001	
FFM (kg) [b]						
GL	53.6 ± 12.9	52.8 ± 12.6*	52.6 ± 12.3*	-1.0 ± 1.5	0.029	0.333
PSMF	58.6 ± 9.7	58.2 ± 10.3	58.7 ± 10.7	+0.1 ± 4.5	0.565	
Total	**56.6 ± 11.1**	**56.1 ± 11.4***	**56.3 ± 11.6**	**-0.3 ± 3.7**	**0.350**	
m	65.3 ± 10.4	65.1 ± 10.6	65.0 ± 10.3	-0.2 ± 1.7	0.789	0.863
f	50.6 ± 7.0	49.7 ± 6.7*	50.1 ± 8.1	-0.4 ± 4.6	0.342	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; FM, fat mass; FFM, fat free mass; m, male; f, female. There were no differences between dietary groups at baseline.
† Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA
‡ Group effect – significance level of the difference in changes between the diet groups at 12 months, calculated using Mann-Whitney test
§ percent of base line body weight
* significant difference from the baseline, $p < 0.05$, calculated using paired t-test
[a] normal distribution
[b] positive skew, reciprocal transformation

At the end of the programme there were no differences in weight loss and body composition changes between the two dietary groups (Figure 15a). Overall, weight was significantly reduced (-5.5 ± 7.3 kg, $p < 0.001$), fat mass decreased (-5.1 ± 6.7 kg, $p < 0.001$ and -2.6 ± 3.6 %, $p < 0.001$), and fat free mass in kg did not change (-0.3 ± 3.7 kg, $p = 0.350$) but fat free mass expressed as a percentage of body mass significantly increased (from 52.5 ± 7.1 % to 55.1 ± 7.7 %, $p < 0.001$). Fat mass (%) measured by the whole body impedance analysis showed very similar decrease as detected by the air displacement plethysmography (-2.3 ± 4.1 %, $p = 0.001$). However, at all time points FM (%) measurements were significantly lower (42.7 ± 7.1 vs. 47.5 ± 7.1 % at baseline; 39.9 ± 8.0 vs. 44.4 ± 8.6 % at 6 months; 40.4 ± 8.0 vs. 44.9 ± 7.7 % at 12 months) than the ones delivered by the densitometric method ($p < 0.001$).

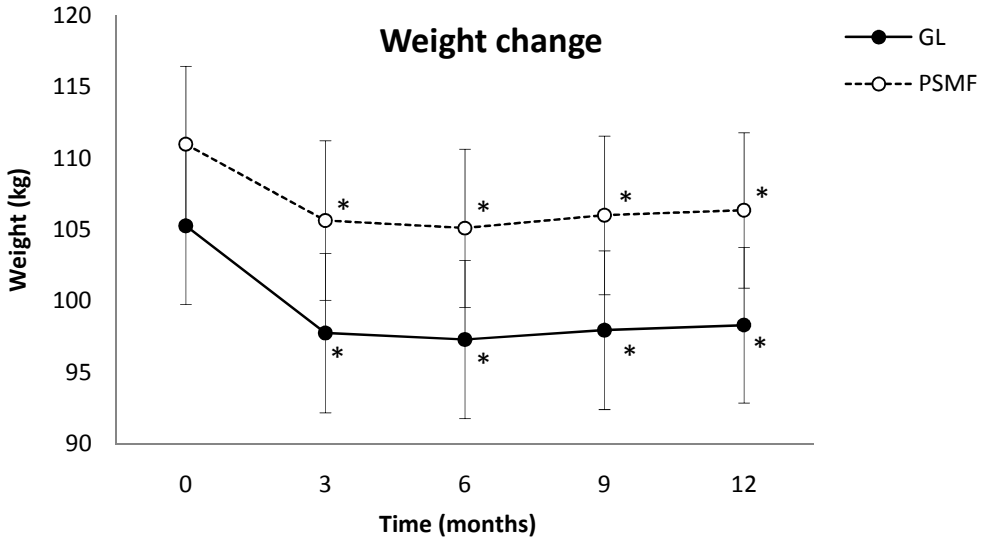


Figure 15a. Body weight changes (LOCF; GL n = 16, PSMF n = 25). There was no difference between the diets. Both diets were significantly different at each time point when compared to baseline ($p < 0.001$) (time effect). Values are means +/- 1 SE.
* marks significant difference from baseline, $p < 0.05$

Completers

Completers' analysis gave similar results to the LOCF analysis (Table 6b, Figure 15b). Namely, weight was significantly reduced (-5.0 ± 7.2 kg, $p = 0.001$), fat mass decreased (-6.1 ± 7.8 kg, $p = 0.002$; and -3.3 ± 4.2 %, $p < 0.001$), and fat free mass did not change (+1.1 ± 5.4 kg, $p = 0.497$). There was an effect of time on the changes in weight and fat mass; fat free mass was affected by neither time, diet group nor by the dropout status. There were no differences in weight changes between the GL and PSMF groups at any of the time points.

Table 6b. Changes in weight, fat mass, and fat free mass (completers, n = 14).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Weight (kg) [a]						
GL (n=6)	108.0 ± 22.1	97.4 ± 19.5*	99.6 ± 16.5*	-8.4 ± 7.7 (-7.0 ± 6.3 %) [§]	<0.001	0.228
PSMF (n=8)	115.3 ± 21.3	108.8 ± 24.3*	112.8 ± 24.5	-2.5 ± 5.9 (-2.6 ± 5.8 %) [§]	0.001	
Total (n=14)	**112.2 ± 21.1**	**103.9 ± 22.3***	**107.1 ± 21.7***	**-5.0 ± 7.2 (-4.5 ± 7.2)**	**0.001**	
m (n=8)	115.0 ± 18.2	106.2 ± 21.4*	108.5 ± 20.4*	-6.5 ± 7.1	<0.001	0.345
f (n=6)	108.4 ± 25.8	100.9 ± 25.2*	105.3 ± 25.3*	-3.0 ± 8.3	0.076	
FM (kg) [a]						
GL	55.3 ± 14.5	45.6 ± 12.5*	48.0 ± 10.8	-7.4 ± 7.2	<0.001	0.491
PSMF	52.5 ± 19.9	46.2 ± 22.0*	47.3 ± 18.2	-5.2 ± 8.6	0.009	
Total	**53.7 ± 17.2**	**45.9 ± 17.9***	**47.6 ± 15.0***	**-6.1 ± 7.8**	**<0.001**	
m	50.3 ± 14.2	41.4 ± 14.8*	43.6 ± 14.0*	-6.7 ± 6.4	<0.001	0.755
f	58.2 ± 21.1	51.9 ± 21.3	52.9 ± 15.7	-5.3 ± 10.0	0.173	
FM (%) [a]						
GL	50.7 ± 4.5	46.3 ± 5.4*	47.9 ± 5.7	-2.8 ± 3.2	0.001	0.755
PSMF	44.6 ± 9.5	40.8 ± 12.1*	40.8 ± 8.9	-3.8 ± 5.0	0.021	
Total	**47.2 ± 8.2**	**43.1 ± 9.9***	**43.8 ± 8.2***	**-3.4 ± 4.2**	**<0.001**	
m	43.2 ± 6.9	38.1 ± 7.7*	39.4 ± 6.8*	-3.7 ± 3.0	<0.001	0.362
f	52.5 ± 6.9	49.9 ± 8.8	49.7 ± 6.3	-2.8 ± 8.3	0.446	
FFM (kg) [b]						
GL	52.7 ± 10.7	51.8 ± 8.4	51.6 ± 8.4	-1.1 ± 1.8	0.160	0.108
PSMF	62.8 ± 10.7	62.8 ± 12.1	65.5 ± 10.9	+2.7 ± 6.7	0.592	
Total	**58.4 ± 10.8**	**58.1 ± 11.8**	**59.5 ± 11.9**	**+1.1 ± 5.4**	**0.135**	
m	64.7 ± 9.1	64.9 ± 10.4	64.9 ± 9.3	+0.2 ± 1.8	0.413	0.662
f	50.1 ± 6.3	49.0 ± 5.9	52.4 ± 11.9	+2.3 ± 8.3	0.154	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; FM, fat mass; FFM, fat free mass; m, male; f, female. There were no differences between dietary groups at baseline.
[†] Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA
[‡] Group effect – significance level of the difference in changes between the diet groups at 12 months, calculated using Mann-Whitney test
[§] percent of base line body weight
* significant difference from the baseline, $p < 0.05$, calculated using paired t-test
[a] normal distribution
[b] positive skew, reciprocal transformation

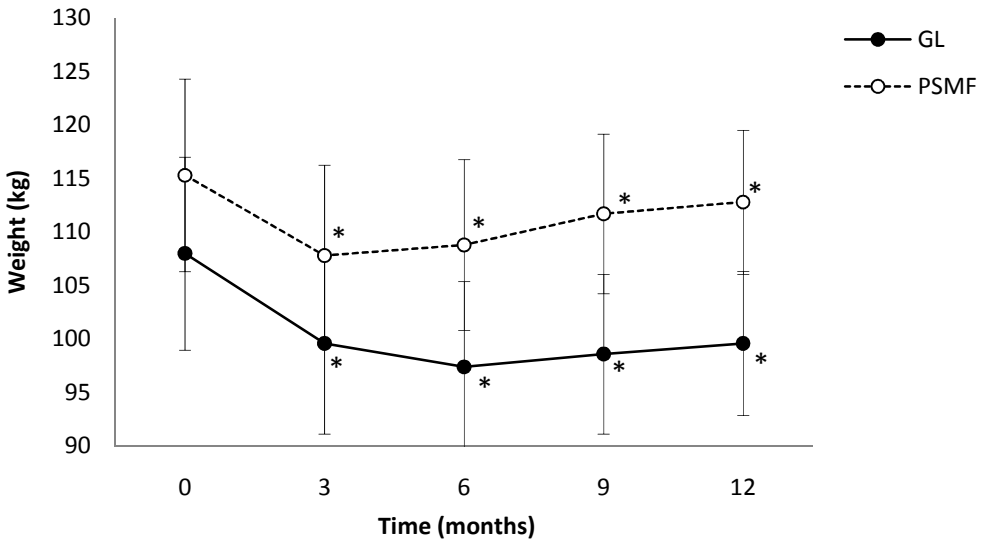


Figure 15b. Body weight changes (completers; GL n = 6, PSMF n = 8). There was no difference between the diets. Both diets were significantly different at each time point when compared to baseline ($p < 0.001$) (time effect). Values are means +/- 1 SE.
* marks significant difference from baseline, $p < 0.05$

4.3 Medication

LOCF

At baseline, there were no differences between the two diet groups in the reported medication doses (Table 7a). Overall, among patients taking metformin (32 out of 41) the dose was 1506 ± 835 mg/d, and among patients on insulin (13 out of 41) the dose was 130 ± 70 U/d. There were no significant associations of medications with weight apart from metformin. Participants on metformin (n = 32) were heavier than those not on metformin (n = 9) (112.2 ± 19.0 vs. 96.6 ± 19.0, p = 0.036). As explained in the methods section, patients who were on sulphonylureas at baseline were taken off these medications before starting the programme. At baseline, 14 (87.5%) of the GL group were on hypoglycaemic medications (insulin only n = 1, oral agents only n = 8, insulin plus oral agents n = 5), and 20 (80 %) of the PSMF group were on hypoglycaemic medications (insulin only n = 1, oral agents only n = 13, insulin plus oral agents n = 6). The proportion of participants on hypo-glycaemic medications was not different between two groups (p = 0.066).

Table 7a. Baseline medication and smoking status (LOCF, n = 41).

	All (n=41)	GL (n=16)	PSMF (n=25)	P †
Diabetic medication				
metformin only n (%)	21 (51.2)	8 (50.0)	13 (52.0)	1.000
insulin only n (%)	2 (4.9)	1 (6.3)	1 (3.0)	1.000
metformin + insulin n (%)	11 (26.8)	5 (31.3)	6 (24.0)	0.723
no metformin or insulin n (%)	7 (17.1)	2 (12.5)	5 (20.0)	0.685
Anti-hypertensive medication n (%)	32 (78.0)	15 (93.8)	17 (68.0)	0.066
Lipid-lowering medication n (%)	36 (87.8)	13 (81.3)	23 (92.0)	0.362
Smoking n (%)	4 (9.8)	0 (0)	4 (16.0)	0.143

Presented values are numbers, with percentages within the group in brackets. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast. † Significance level of the between-group proportions of subjects on medications or smoking at baseline, calculated using a chi-squared test.

Completers

At baseline, there were no differences between the two diet groups in the reported medication doses (Table 7b). Proportions of participants on medications were similar to LOCF analysis (Tables 7a and 7b). Overall, at baseline among patients taking metformin (12 out of 14) the dose was 1750 ± 965 mg/d, and among patients on insulin (4 out of 14) the dose was 85 ± 55 U/d. There were no significant associations of medications with weight at baseline.

Table 7b. Baseline medication and smoking status (completers, n = 14).

	All (n=14)	GL (n=6)	PSMF (n=8)	P †
Diabetic medication				
metformin only n (%)	8 (57.1)	2 (33.3)	6 (75.0)	0.277
insulin only n (%)	0 (0)	0 (0)	0 (0)	-
metformin + insulin n (%)	4 (28.6)	3 (50.0)	1 (12.5)	0.245
no metformin or insulin n (%)	2 (14.3)	1 (6.7)	1 (12.5)	1.000
Anti-hypertensive medication n (%)	10 (71.4)	6 (100.0)	4 (50.0)	0.085
Lipid-lowering medication n (%)	12 (85.7)	4 (66.7)	8 (100.0)	0.165
Smoking n (%)	3 (21.4)	0 (0)	3 (37.5)	0.209

Presented values are numbers, with percentages within the group in brackets. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast. † Significance level of the between-group proportions of subjects on medications or smoking at baseline, calculated using a chi-squared test.

Lipid-lowering and anti-hypertensive medication doses were not significantly changed in any of the groups during the study. Table 8 shows changes in hypoglycaemic medication for participants who completed the study (n = 14). Participants on insulin had the mean dose decreased from 130 ± 70 U/d to 89 ± 65 U/d ($p < 0.001$); metformin dose did not change. Three of 6 GL group participants (50%) had a reduction or removal of hypoglycaemic medication; in the PSMF group 3 participants had glimepiride discontinued.

Table 8. Changes in hypoglycaemic medications* among completers (n=14).

Participant	Baseline: total daily dose	12 months: total daily dose
Go Lower group (total n = 6)		
1	metformin 1000 mg rosiglitazone 8 mg	metformin 1000 mg rosiglitazone 8mg
2	metformin 3000 mg	metformin 3000 mg
3	insulin 68 units metformin 3000 mg	insulin 36 units metformin 2000 mg
4	insulin 164 units metformin 3000 mg	insulin 53 units metformin 1000 mg
5	insulin 26 units metformin 3000mg glimepiride 3.0 mg	metformin 2000 mg
6	none	none
Protein sparing modified fast group (total n = 8)		
1	metformin 3000 mg rosiglitazone 8 mg glimepiride 4 mg	metformin 3000 mg rosiglitazone 8 mg
2	metformin 1000 mg pioglitazone 30 mg	metformin 3000 mg pioglitazone 30 mg
3		metformin 500 mg
4	metformin 2000 mg glimepiride 4.0 mg	metformin 2000 mg
5	metformin 1000 mg	metformin 1000 mg
6	metformin 1000 mg glimepiride 4.5 mg	metformin 1000 mg
7	metformin 2000 mg	metformin 2000 mg
8	metformin 1000 mg pioglitazone 15 mg	metformin 1000 mg pioglitazone 15 mg

* medications were adjusted as advised by participants' physician

4.4 Dietary composition

LOCF

At baseline, dietary groups did not differ with respect to calorie or macronutrient intake (Table 9a). Total energy intake for the GL group was 1892 ± 627 kcal/d, and for the PSMF group was 1812 ± 716 kcal/d. About 41% of calories were from carbohydrate, 39 % were from fat, and 20% were from protein. Total energy intake was similar in both groups across all stages of the programme (Figure 17a).

Table 9a. Macronutrient composition changes of the diet in both treatment groups (LOCF, n = 41).

					P-value	
	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** †	**Group effect** ‡
Energy (kcal/d) [a]						
GL (n=16)	1892 ± 627	1400 ± 556*	1360 ± 558*	-532 ± 430	<0.001	0.103
PSMF (n=25)	1812 ± 716	1450 ± 590*	1539 ± 687*	-253 ± 561	0.024	
Total (n=41)	**1845 ± 673**	**1430 ± 570***	**1467 ± 637***	**-368 ± 524**	**<0.001**	
Carbohydrate (g/d) [c]						
GL	195 ± 71	105 ± 94*	113 ± 82*	-83 ± 78	<0.001	0.318
PSMF	194 ± 78	128 ± 90*	135 ± 97*	-57 ± 77	0.001	
Total	**195 ± 74**	**119 ± 91***	**126 ± 91***	**-67 ± 78**	**<0.001**	
Carbohydrate (% of energy) [c]						
GL	40.8 ± 10.6	26.3 ± 14.6*	28.7 ± 12.6*	-12.1 ± 14.5	0.001	0.503
PSMF	41.9 ± 8.6	32.1 ± 14.2*	32.4 ± 15.0*	-9.1 ± 12.9	<0.001	
Total	**41.4 ± 9.4**	**29.8 ± 14.5***	**30.9 ± 14.1***	**-10.3 ± 13.5**	**<0.001**	
Protein (g/d) [b]						
GL	91.3 ± 35.5	78.1 ± 14.6	85.2 ± 34.9	-6.1 ± 34.6	0.700	0.763
PSMF	83.5 ± 30.7	78.4 ± 27.9	77.0 ± 31.3	-5.6 ± 23.7	0.253	
Total	**86.7 ± 32.6**	**78.3 ± 23.2**	**80.3 ± 32.6**	**-5.8 ± 28.3**	**0.458**	
Protein (% of energy) [a]						
GL	19.6 ± 3.9	25.8 ± 8.8*	26.2 ± 8.7*	+6.6 ± 8.6	0.005	0.081
PSMF	19.8 ± 6.4	23.6 ± 8.8*	22.1 ± 6.7	+2.2 ± 6.4	0.015	
Total	**19.8 ± 5.5**	**24.5 ± 8.8***	**23.8 ± 7.7***	**+4.0 ± 7.6**	**0.001**	
Fat (g/day) [b]						
GL	86.3 ± 41.6	75.3 ± 26.3	76.8 ± 32.9	-9.5 ± 31.5	0.756	0.656
PSMF	80.5 ± 38.9	71.8 ± 32.0	76.6 ± 36.8	-3.4 ± 26.6	0.377	
Total	**82.9 ± 39.6**	**73.2 ± 29.5**	**76.7 ± 34.8**	**-5.9 ± 28.5**	**0.303**	
Fat (% of energy) [a]						
GL	38.7 ± 10.0	51.2 ± 13.2*	49.6 ± 12.4*	+10.8 ± 16.4	0.004	0.328
PSMF	38.4 ± 6.5	44.2 ± 10.4*	45.4 ± 11.6*	+6.7 ± 9.2	0.003	
Total	**38.5 ± 8.0**	**47.0 ± 11.9***	**47.1 ± 12.0***	**+8.4 ± 12.6**	**<0.001**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast. There were no differences at baseline between the diet groups.

† Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using independent t-test

* significant difference from the baseline within the groups, $p < 0.05$, calculated using paired t-test

[a] normal distribution

[b] positive skew, log transformation

[c] non-normal distribution, untransformed, non-parametric tests used

There were no differences in 12 months changes in energy or macronutrient content of the diet between the diet groups (Table 9a). Participants on the GL diet reported a slightly greater decrease in caloric consumption (GL, -532 ± 430 kcal vs. PSMF, -253 ± 561 kcal, p = 0.103), however, this difference was not significant (Figure 17a). Overall, LOCF analysis showed that energy restriction and weight loss were correlated at 6 months (r = 0.372, P = 0.020) (Figure 16) but not at 12 months (r = 0.079, p = 0.632). In other words, at 6 months, energy restriction could account for 13.9% of the variation in weight loss.

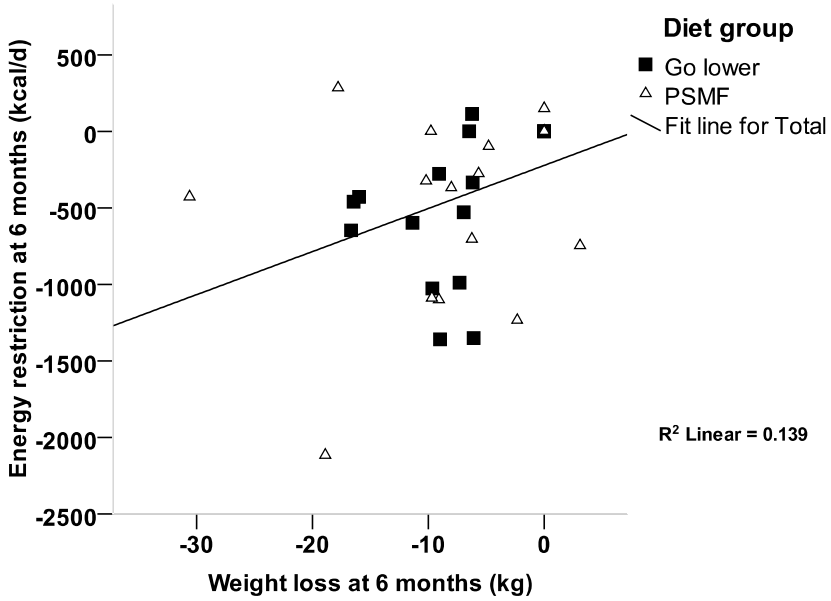


Figure 16. Scatter plot of weight loss and energy restriction at 6 months (LOCF, n = 41) (r = 0.372, p = 0.020). R^2 Linear, coefficient of determination.

From baseline, overall carbohydrate intake decreased from 195 ± 74 g/d to 134 ± 99 (p < 0.001) at 3 months, to 119 ± 91 g/d (p < 0.001) at 6 months, to 126 ± 91 g/d (p < 0.001) at 12 months. The mean reported carbohydrate intakes did not reach the target level of 40 g/d and was over 25 % of total energy at all time points (Figure 18a).

Participants reported slightly decreased intake of protein when expressed as grams per day; at 12 months protein intake was on average 5.8 g/d less than at baseline. However, this did not reach significance (p = 0.458). When expressed as a percentage of energy, protein intake increased from 19.8% at baseline to 23.8% at 12 months (p < 0.001) (Table 9a, Figure 19a). Finally, when expressed as grams per day per kilogram of body mass, protein intake did not change in any of the groups

throughout the programme (overall from 0.81 ± 0.29 g/d/kg at baseline to 0.80 ± 0.33 at 12 months, p = 0.865). These values are in agreement with the Reference Nutrient Intakes (Department of Health 1991).

The absolute fat content of the diet did not significantly change (overall -5.9 ± 28.5 g/d, p = 0.303). When expressed as a percentage of energy, fat content increased overall by 8.4 % at 12 months (p < 0.001) (Table 9a, Figure 20a).

Participants on the GL diet showed trend for reporting slightly lower energy and carbohydrate intakes and higher protein and fat intakes than participants on PSMF diet; however, these differences were not significant.

Completers

There was no difference between completers (n = 14) and non-completers (n = 27) in energy or macronutrient intakes at baseline (Figures 17b, 18b, 19b, 20b). The participants who completed the study had a mean weight loss of 8.2 ± 6.2 kg at 6 months and 5.0 ± 7.1 kg at 12 months, which corresponded to the mean reduction in daily energy intake of approximately 315 kcal/d at 6 months, and 96 kcal/d at 12 months. However, weight loss and energy restriction were not correlated at any of the time points in this group.

Completers tended to report slightly higher protein and lower carbohydrate intakes throughout the study than non-completers (Table 9b). At 6 months, completers reported lower carbohydrate intake (78 ± 64 vs. 140 ± 98 g/d, p = 0.038) and at 9 months higher protein intake than non-completers (98 ± 40 vs. 75 ± 30 g/d, p = 0.041). At 12 months protein intake was still 14 g/d higher in completers, but the difference was no longer significant (p = 0.195). Completers' fat consumption did not differ from non-completers at any of the time-points.

Table 9b. Macronutrient composition changes of the diet in both treatment groups (completers, n= 14).

					P-value	
	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** †	**Group effect** ‡
Energy (kcal/d) [a]						
GL (n=6)	1890 ± 757	1395 ± 522	1291 ± 520*	-559 ± 309	0.004	0.897
PSMF (n=8)	2041 ± 795	1263 ± 244*	1401 ± 542*	-640 ± 711	0.007	
Total (n=14)	**1976 ± 753**	**1319 ± 376***	**1354 ± 515***	**-622 ± 556**	**0.002**	
Carbohydrate (g/d) [c]						
GL	208 ± 75	77 ± 87*	98 ± 47*	-110 ± 84	0.016	0.959
PSMF	209 ± 87	80 ± 46*	102 ± 89*	-108 ± 104	0.011	
Total	**209 ± 79**	**78 ± 63***	**100 ± 72***	**-109 ± 93**	**<0.001**	
Carbohydrate (% of energy) [c]						
GL	43.0 ± 7.1	19.1 ± 15.0	25.3 ± 11.8	-17.7 ± 14.8	0.144	0.672
PSMF	39.5 ± 8.6	23.7 ± 11.7*	25.6 ± 15.9	-13.9 ± 17.4	0.060	
Total	**41.0 ± 7.9**	**21.7 ± 12.9***	**25.5 ± 13.8***	**-15.5 ± 15.8**	**0.007**	
Protein (g/d) [b]						
GL	91.3 ± 35.5	78.1 ± 14.6	85.2 ± 34.9	-6.1 ± 34.6	0.700	0.763
PSMF	83.5 ± 30.7	78.4 ± 27.9	77.0 ± 31.3	-5.6 ± 23.7	0.253	
Total	**86.7 ± 32.6**	**78.3 ± 23.2**	**80.3 ± 32.6**	**-5.8 ± 28.3**	**0.458**	
Protein (% of energy) [a]						
GL	17.4 ± 3.5	25.5 ± 8.8	26.6 ± 8.7*	+9.2 ± 7.7	0.042	0.377
PSMF	20.2 ± 7.6	28.3 ± 4.7*	25.0 ± 6.9	+4.9 ± 9.4	0.041	
Total	**19.0 ± 6.2**	**27.1 ± 6.6***	**25.7 ± 7.4***	**+6.7 ± 8.7**	**0.004**	
Fat (g/day) [b]						
GL	84.8 ± 38.5	85.4 ± 21.0	89.3 ± 38.7	+4.5 ± 25.0	0.908	0.289
PSMF	89.5 ± 40.7	65.7 ± 14.9	73.5 ± 32.3	-16.0 ± 39.6	0.720	
Total	**87.5 ± 38.3**	**74.1 ± 19.8**	**80.3 ± 34.7**	**-7.2 ± 34.6**	**0.881**	
Fat (% of energy) [a]						
GL	38.8 ± 8.5	56.9 ± 12.4	52.4 ± 11.9	+13.6 ± 18.2	0.009	0.606
PSMF	38.1 ± 4.4	46.3 ± 18.4*	47.6 ± 12.3*	+9.5 ± 11.0	0.170	
Total	**38.4 ± 6.2**	**50.8 ± 11.3***	**49.7 ± 11.9***	**+11.2 ± 14.0**	**0.001**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast. There were no differences at baseline between the diet groups.

† Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using independent t-test

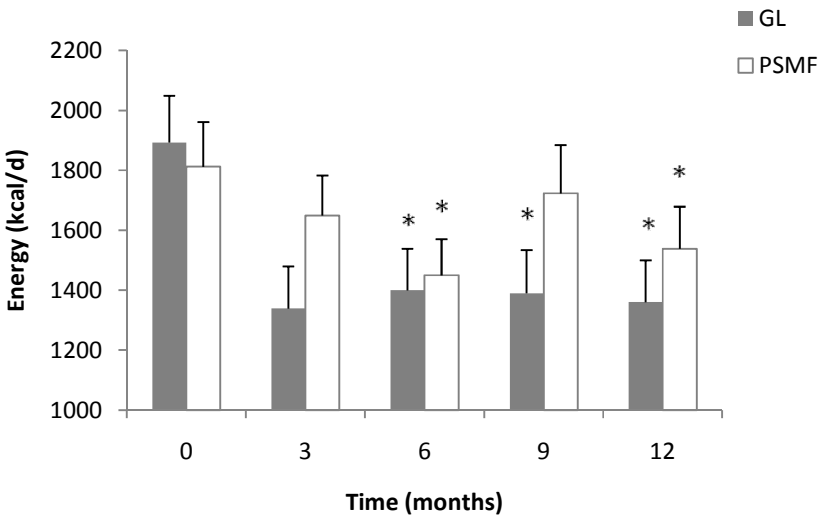
* significant difference from the baseline within the groups, $p < 0.05$, calculated using paired t-test

[a] normal distribution

[b] positive skew, log transformation

[c] non-normal distribution, untransformed, non-parametric tests used

a)



b)

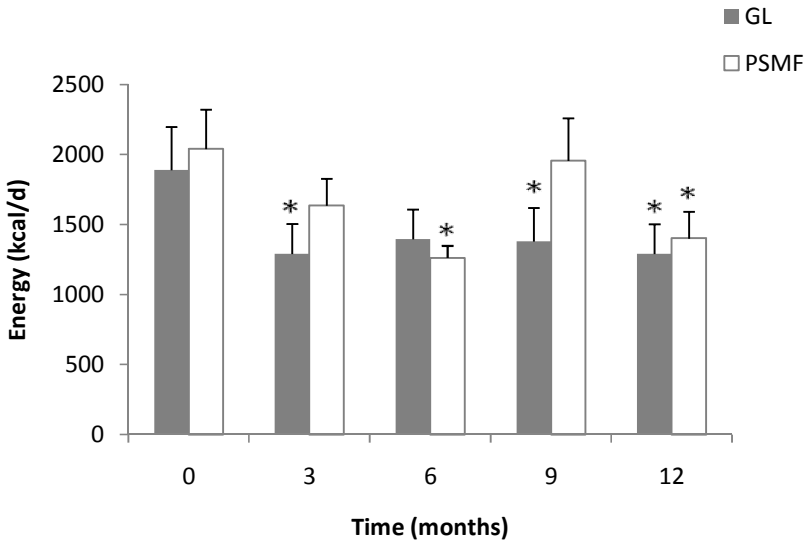
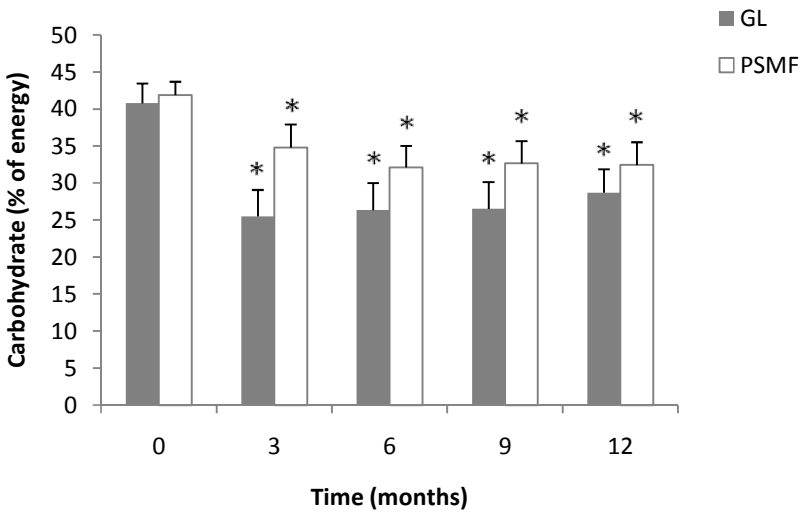


Figure 17. Changes in reported energy content of the diet. Presented values are means ± 1 SE. The two graphs show: a, LOCF analysis (GL, n = 16; PSMF, n = 25); b, completers' analysis (GL, n = 6; PSMF, n = 8).
* marks significant difference from baseline ($p < 0.05$)

a)



b)

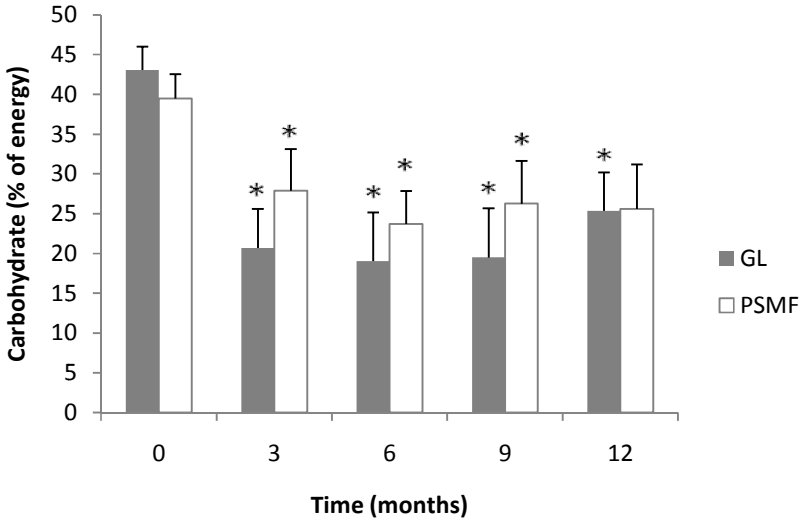
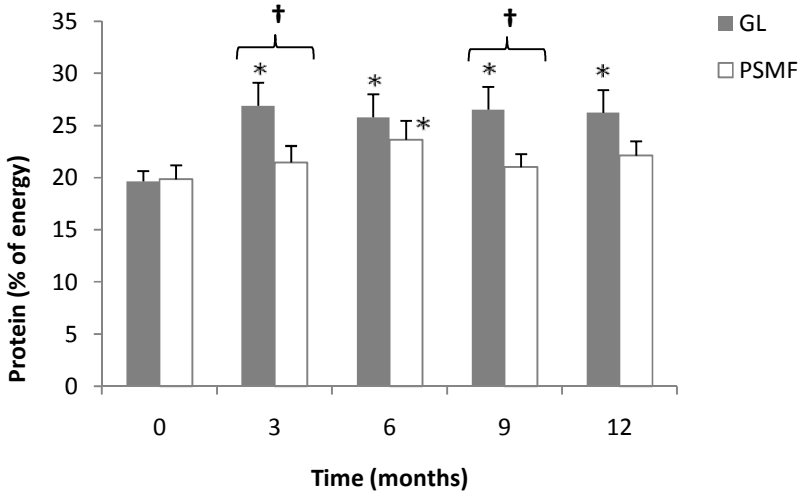


Figure 18. Changes in the reported carbohydrate content of the diet. Presented values are means ± 1 SE. The two graphs show: a, LOCF analysis (GL, n = 16; PSMF, n = 25); b, completers' analysis (GL, n = 6; PSMF, n = 8).
* marks significant difference from baseline ($p < 0.05$)

a)



b)

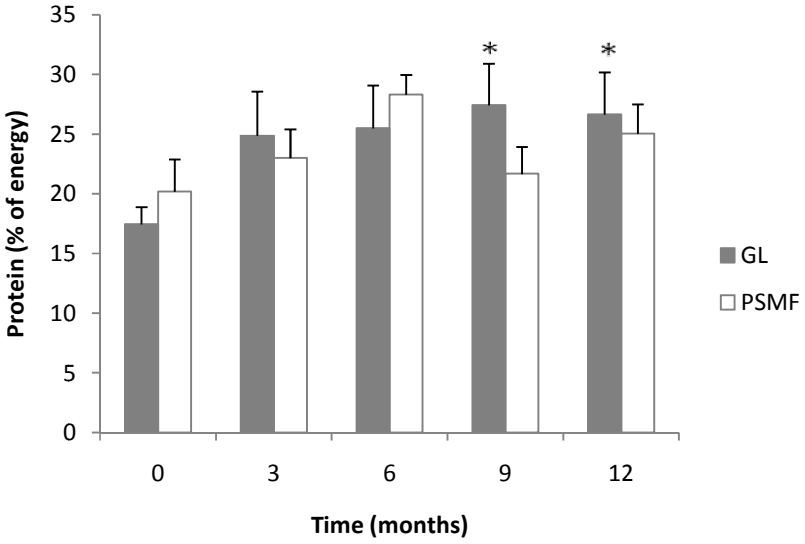
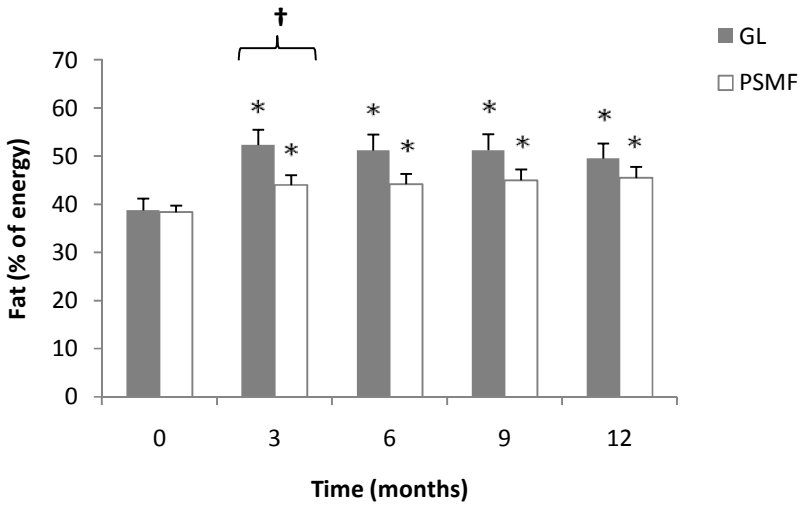


Figure 19. Changes in the reported protein content of the diet. Presented values are means ± 1 SE. The two graphs show: a, LOCF analysis (GL, n = 16; PSMF, n = 25); b, completers' analysis (GL, n = 6; PSMF, n = 8).

† marks significant difference between the two diets at a given time point ($p < 0.05$)

* marks significant difference from baseline ($p < 0.05$)

a)



b)

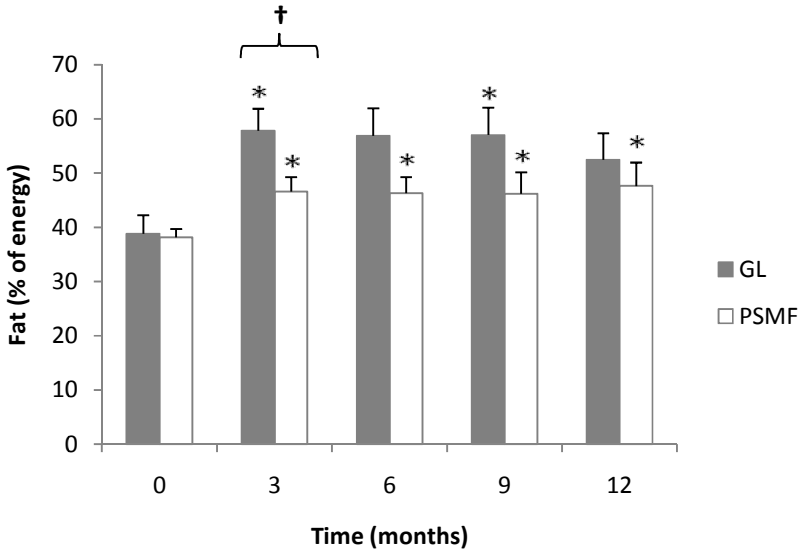


Figure 20. Changes in the reported fat content of the diet. Presented values are means ± 1 SE. The two graphs show: a, LOCF analysis (GL, n = 16; PSMF, n = 25); b, completers' analysis (GL, n = 6; PSMF, n = 8).
† marks significant difference between the two diets at a given time point ($p < 0.05$)
* marks significant difference from baseline ($p < 0.05$)

4.5 Compliance

Compliance to the programme was measured not only by the direct weight loss and indirect reported dietary data assessment but also by the evaluation of the effects of the macronutrient composition of the diet; namely concentrations of ketone bodies in urine and in capillary blood to assess carbohydrate intake, and fasting serum urea and albumin concentrations to assess protein intake.

LOCF

Both urine and capillary blood ketone concentrations did not differ between the GL and PSMF groups at baseline. The presence of ketone bodies in the urine did not change throughout the study ($p = 0.921$), remaining detectable in about 10% of the participants. Similarly, concentration of ketone bodies in capillary blood was low at baseline (0.1 ± 0.1 mmol/l) and did not change during the programme ($p = 0.405$).

Serum urea levels were similar at baseline in both groups (GL, 6.5 ± 1.5 mmol/l; PSMF, 5.9 ± 1.9 mmol/l, $p = 0.315$) (Table 10a). Overall, urea concentration increased significantly at 3 months ($p = 0.014$) and remained elevated until the end of the programme ($p < 0.001$). Serum albumin concentrations were also not different between the diet groups at baseline ($P = 0.886$) and increased overall at 3 months ($p < 0.001$), to remain significantly higher until the end of the programme ($p < 0.001$) (Table 10a).

Table 10a. Changes in serum albumin and urea levels (LOCF, n = 41).

	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** †	**Group effect** ‡
Albumin (g/l) [a]						
GL	45 ± 2	47 ± 2*	47 ± 3*	+2 ± 2	0.001	0.017
PSMF	45 ± 2	45 ± 3*	45 ± 3	0 ± 1	0.001	
Total	**45 ± 2**	**46 ± 3***	**46 ± 3***	**+1 ± 2**	**< 0.001**	
Urea (mmol/l) [a]						
GL	6.5 ± 1.5	7.3 ± 1.6*	7.5 ± 1.8*	+1.0 ± 1.1	0.025	0.247
PSMF	5.9 ± 1.9	6.5 ± 2.8	6.4 ± 2.3	+0.5 ± 1.4	0.036	
Total	**6.1 ± 1.8**	**6.8 ± 2.4***	**6.8 ± 2.2***	**+0.7 ± 1.3**	**< 0.001**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast. There were no differences between the dietary groups at baseline.

† Time effect – significance level of changes over one year time, calculated using repeated measures ANOVA

‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using Mann-Whitney test

* significant difference from baseline, $p < 0.05$, calculated using paired t-test

[a] normal distribution

Completers

Completers' analysis gave similar findings in compliance as the LOCF analysis (Table 10b). Completers were not different from non-completers for the measures of compliance.

Table 10b. Changes in serum albumin and urea levels (completers, n = 14).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Albumin (g/l) [a]						
GL (n=6)	44 ± 2	47 ± 2*	46 ± 2*	+2 ± 1	0.019	0.043
PSMF (n=8)	46 ± 2	47 ± 2*	46 ± 3	0 ± 1	0.019	
Total (n=14)	**45 ± 2**	**47 ± 2***	**46 ± 3***	**+1 ± 1**	**0.001**	
Urea (mmol/l) [a §]						
GL	7.4 ± 0.9	7.7 ± 0.8	8.2 ± 1.5	+0.8 ± 1.0	0.035	0.852
PSMF	5.3 ± 1.0	6.3 ± 1.6*	6.2 ± 1.9	+0.9 ± 1.6	0.013	
Total	**6.2 ± 1.4**	**6.9 ± 1.5***	**7.0 ± 2.0***	**+0.8 ± 1.3**	**0.003**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast.
† Time effect – significance level of changes over one year time, calculated using repeated measures ANOVA
‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using Mann-Whitney test
* significant difference from baseline, $p < 0.05$, calculated using paired t-test
§ significant difference between the two diets at baseline
[a] normal distribution

4.6 Resting metabolic rate

4.6.1 Changes during the programme

LOCF

At baseline, resting metabolic rate (RMR, kcal/d) was similar in both diet groups, however, it was significantly higher in men (2165 ± 418 vs. 1860 ± 413 kcal/d, $p = 0.026$) who had more fat free mass than women (65.3 ± 10.4 vs. 50.6 ± 7.0, $p < 0.001$). There was still a significant effect of gender on RMR (kcal/d) after controlling for FFM ($p = 0.015$). There was no difference in RMR (kcal/d) changes between the genders or dietary groups during the programme (Table 11a).

Due to the fact that RMR (kcal/d) when corrected for FFM was significantly higher in women ($p=0.015$), the RMR was also expressed as kilocalories per kilogram of fat free mass per day (kcal/kg FFM/d). Females had significantly higher RMR (kcal/kg FFM/d) than men at all time points (Table 11a). However, just like in the RMR expressed by kcal/d, RMR (kcal/kg FFM/d) decreased throughout

the study. It decreased on average from 35 ± 5 kcal/kg/d at baseline to 33 ± 6 kcal/kg/d at 6 months ($p < 0.001$) and was still different from baseline at the end of the programme ($p = 0.004$). There was no difference in RMR (kcal/kg FFM/d) changes between the genders, diet groups or completers/non-completers during the programme (Table 11a).

Table 11a. Changes in resting metabolic rate (LOCF, n = 41).

	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** †	**Group effect** ‡
RMR (kcal/d) [a]						
GL (n=16)	1975 ± 550	1767 ± 499*	1773 ± 487*	-202 ± 287	0.004	0.212
PSMF (n=25)	1994 ± 360	1872 ± 342*	1899 ± 330	-95 ± 248	0.032	
Total (n=41)	**1986 ± 437**	**1831 ± 408***	**1850 ± 398***	**-137 ± 265**	**<0.001**	
m (n=17)	2165 ± 418	2001 ± 364*	1989 ± 372*	-176 ± 267	0.007	0.436
f (n=24)	1860 ± 413	1712 ± 401*	1751 ± 393	-109 ± 265	0.019	
RMR (kcal/kg FFM/d) [a]						
GL	36.7 ± 5.4	33.4 ± 5.7*	33.8 ± 6.3*	-2.9 ± 4.8	0.007	0.283
PSMF	34.2 ± 4.5	32.5 ± 5.3*	32.7 ± 5.1	-1.4 ± 3.8	0.054	
Total	**35.2 ± 5.0**	**32.9 ± 5.4***	**33.1 ± 5.6***	**-2.0 ± 4.2**	**<0.001**	
m	33.2 ± 3.5	30.7 ± 2.6*	30.6 ± 2.9*	-2.6 ± 3.9	0.036	0.446
f	36.6 ± 5.4	34.4 ± 6.3*	35.0 ± 6.3	-1.6 ± 4.4	0.006	

Presented values are means ± standard deviation. Abbreviations: RMR, resting metabolic rate; GL, Go Lower; PSMF, protein sparing modified fast; m, male; f, female; FFM, fat free mass. There were no differences between dietary groups at baseline.
† Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA
‡ Group effect – significance level of the difference in 12 months changes between the diet groups, Mann-Whitney test used
* significant difference from the baseline, $p < 0.05$, calculated using paired t-test
[a] normal distribution

There was no correlation between RMR and energy intake. There were positive correlations between change in RMR (kcal/d) and changes in FM ($r = 0.621$, $p < 0.001$) and FFM ($r = 0.386$, $p = 0.013$). Change in RMR (kcal/kg FFM/d) was strongly correlated to change in FM ($r = 0.715$, $p < 0.001$) but not correlated to change in FFM.

Completers

Completers' analysis confirmed most of the above results (Table 11b). However, in completers there was no difference between men and women in RMR (kcal/kg FFM/d) at any of the time points. There were positive correlations between change in RMR (kcal/d) and changes in FM ($r = 0.562$, $p = 0.037$) but not fat free mass. Change in RMR (kcal/kg FFM/d) was also strongly correlated with change in FM ($r = 0.834$, $p < 0.001$).

Table 11b. Changes in resting metabolic rate (completers, n = 14).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
RMR (kcal/d) [a]						
GL (n=6)	1848 ± 428	1638 ± 329*	1653 ± 273	-195 ± 380	0.198	0.573
PSMF (n=8)	2097 ± 346	1978 ± 399	2061 ± 328	-35 ± 149	0.0112	
Total (n=14)	**1990 ± 389**	**1833 ± 397***	**1886 ± 361**	**-104 ± 273**	**0.05**	
m (n=8)	2156 ± 354	1995 ± 368*	1972 ± 385	-184 ± 331	0.138	0.228
f (n=6)	1769 ± 338	1616 ± 349	1771 ± 323	+3 ± 125	0.052	
RMR (kcal/kg FFM/d) [a]						
GL	34.9 ± 4.5	31.6 ± 3.8*	32.5 ± 6.2	-2.3 ± 6.3	0.251	0.755
PSMF	33.7 ± 4.4	31.9 ± 5.7	31.9 ± 5.5	-1.8 ± 3.7	0.170	
Total	**34.2 ± 4.3**	**31.7 ± 4.8***	**32.2 ± 5.5**	**-2.0 ± 4.8**	**0.064**	
m	33.2 ± 3.5	30.7 ± 2.6*	30.4 ± 3.9	-3.1 ± 4.9	0.099	0.108
f	36.6 ± 5.4	34.4 ± 6.3	34.5 ± 6.9	-0.6 ± 4.6	0.379	

Presented values are means ± standard deviation. Abbreviations: RMR, resting metabolic rate; GL, Go Lower; PSMF, protein sparing modified fast; m, male; f, female; FFM, fat free mass. There were no differences between dietary groups at baseline.
† Time effect – significance level of changes over 12 months, calculated using repeated measures ANOVA
‡ Group effect – significance level of the difference in 12 months changes between the diet groups, Mann-Whitney test used
* significant difference from the baseline, $p < 0.05$, calculated using paired t-test
[a] normal distribution

4.6.2 RMR prediction equations for patients with T2DM

Twenty nine equations that predict RMR were tested for their accuracy (Figure 21) and bias (Figure 22). The most accurate estimation of RMR for the obese class I, II and III subjects with T2DM, was obtained using the Lazzer equation that used FFM (Lazzer et al. 2007a, Lazzer et al. 2007b) (prediction accuracy: 64.3 %; bias: -3.3 %) (Figure 21). This equation was derived from a sample of 346 volunteers (164 males, 182 females). Men were between 20 and 65 years of age and with the mean BMI of 45 kg/m^2. Women were between 19 and 60 years of age and had mean BMI of 45 kg/m^2.

Predicted RMR for subjects with BMI > 35 kg/m^2 (n = 35) was most accurate using the DeLorenzo (DeLorenzo et al. 2001) equation (prediction accuracy: 65.7 %; bias: -3.4 %). De Lorenzo equation was based on a sample of 127 males and 193 females aged between 18 and 59 years, and with mean BMI 27 kg/m^2 (range 17 – 40 kg/m^2). For the group of volunteers with BMI > 35 kg/m^2, the least biased equation was the Müller equation (Müller et al. 2004) calculated using BMI (prediction

accuracy 60.0%; bias -2.3%). Müller equation was derived from a sample of 388 males and 658 females, aged 5 – 80 years, and with the mean BMI of 27 kg/m^2.

For BMI ≤ 35 kg/m^2 (n = 21) the Korth equation (Korth et al. 2007) with FFM, and Johnstone equation (Johnstone et al. 2006) with FFM were most accurate (prediction accuracy: 71.4 %; and biases: -0.02 % and -1.9 % respectively). The least biased equation for this group was the Korth equation (2007) calculated using FFM (Figure 22). Korth equation was derived from a sample of 50 males and 54 females, with the mean age of 37 years (21–68), and the mean BMI of 26 kg/m^2 (18 – 41 kg/m^2). The equation by Johnstone et al (2006) was based on a sample of 43 males and 107 females, with the age ranging 21 to 64 and BMI between 17 and 49 kg/m^2 (54 % of the sample were overweight or obese).

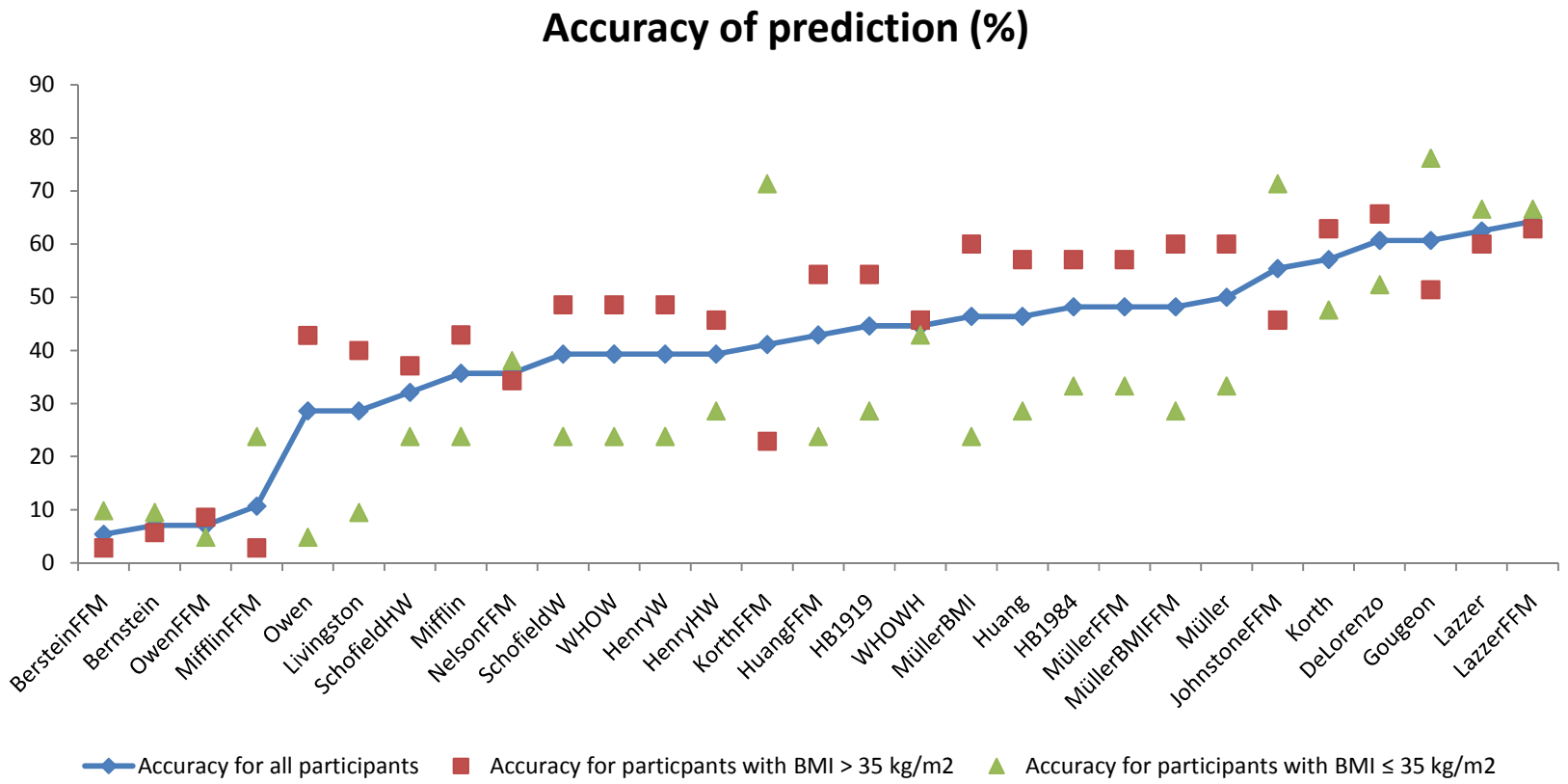


Figure 21. Accuracy of prediction of the 29 prediction equations (in order from the least accurate to the most accurate) for all participants (♦), participants with BMI > 35 kg/m^2 (■) (n = 35), and with BMI ≤ 35 kg/m^2 (▲) (n = 21). The accuracy of the equations was assessed by the calculating the percentage of subjects whose estimated RMR fell within ± 10% of the measured RMR (mRMR).

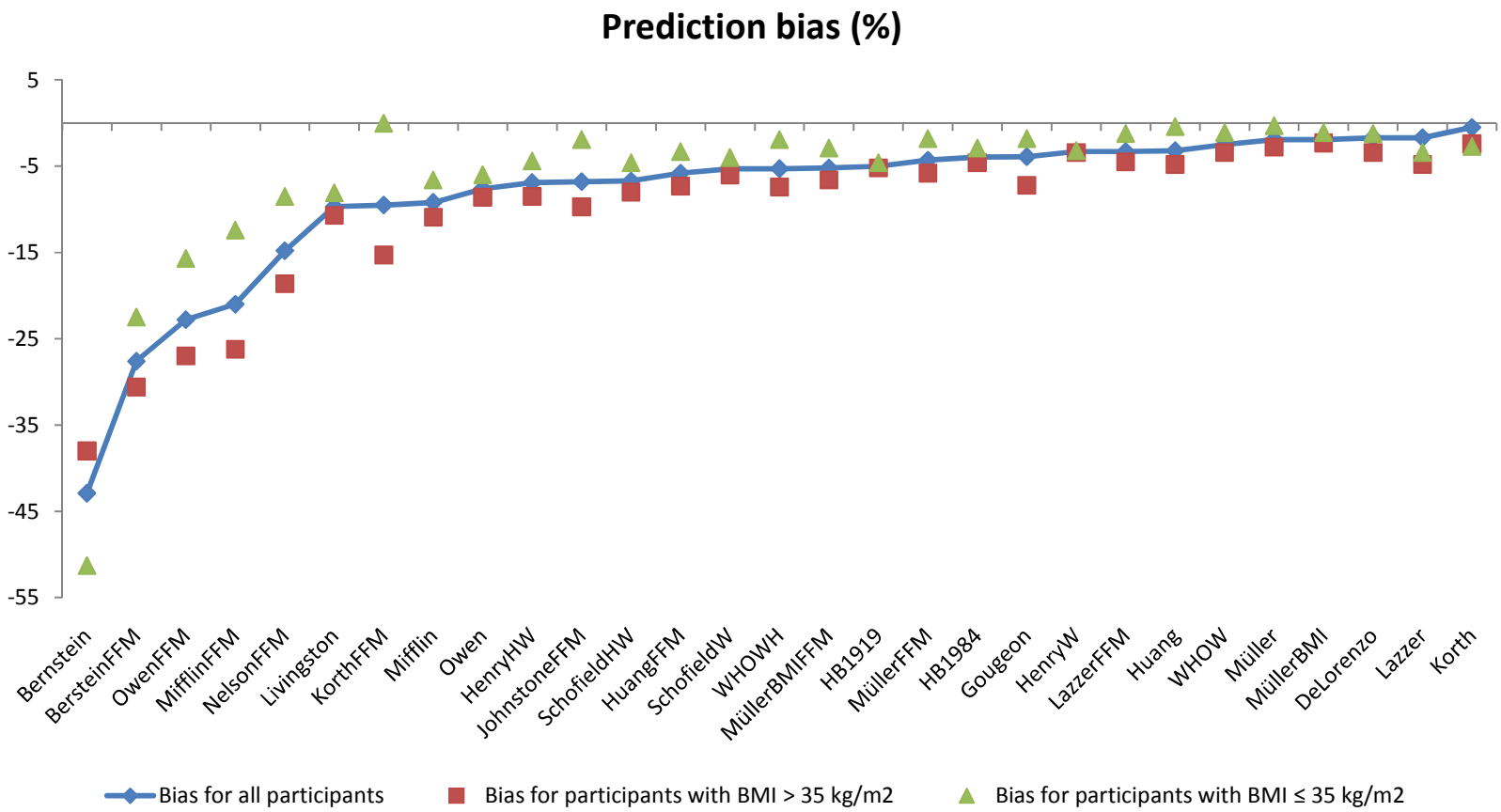


Figure 22. Prediction Bias of the 29 prediction equations (in order of the highest to the lowest) for all participants (♦), participants with BMI > 35 kg/m^2 (■) (n = 35), and with BMI ≤ 35 kg/m^2 (▲) (n = 21). Bias was expressed as the mean percentage difference between the predicted and measured RMR.

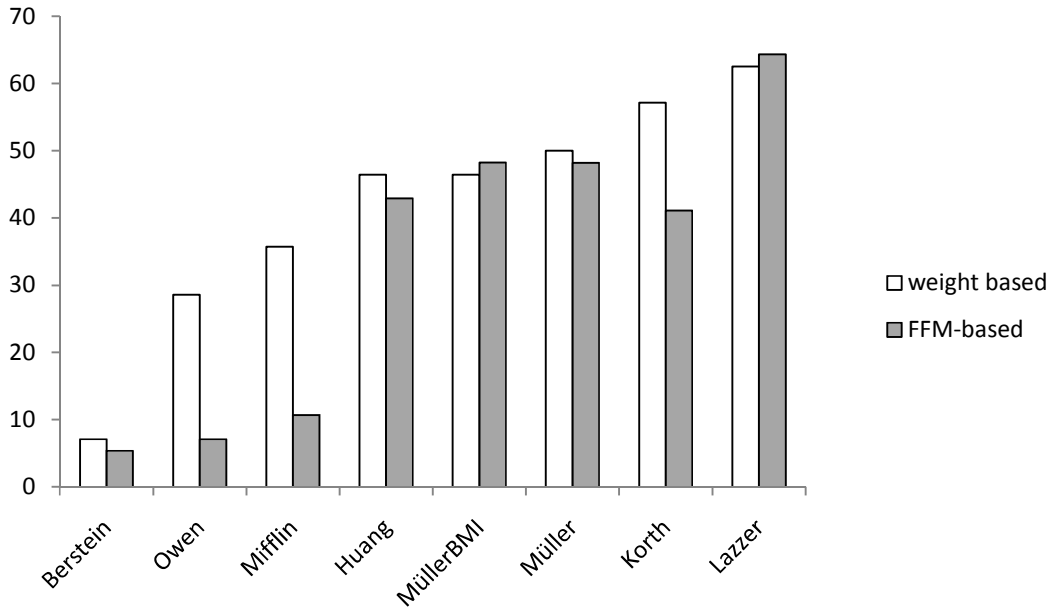


Figure 23. Comparison of the prediction accuracy of weight-based and fat free mass based equations to predict RMR. Equations are presented in the order of the increasing accuracy of the weight based version of equation.

The equations that had both weight-based and FFM-based versions were compared (Figure 23). With the exception of the equations by Müller with BMI (Muller et al. 2004) and Lazzer (Lazzer et al. 2007a, Lazzer et al. 2007b), FFM-based equations seemed to predict RMR less accurately in this population.

4.6.3 RMR prediction equation

The baseline data from an additional 15 participants obtained during patient screening were used (total n = 56) to propose a predictive equation for the estimation of RMR in an obese population with T2DM. The 15 subjects were excluded before randomisation because they no longer wanted to participate or they did not fulfil the inclusion criteria. However, all of those 15 subjects had T2DM and were obese, and therefore their data was used for this analysis.

The independent variables, used to predict RMR were age, gender, body mass, stature, BMI, waist circumference, FM, FFM, fasting glucose (logged) and HbA_{1c} (Table 12). RMR varied

between the subjects with a range from 1128 to 3345 kcal/d and a standard deviation of 394 kcal/d. Table 12 presents three models to predict RMR in obese people with T2DM. In the stepwise multiple regression analysis, FFM (kg) was the strongest determinant of RMR and explained 58% of its variability (Figure 24). Other determinants of RMR were waist circumference (cm), gender, and HbA_{1c} (%):

(Model 1) **RMR (kcal/d)** = - 901 + 28.6 * FFM (kg) + 16.7 * waist (cm) – 270 * gender (male = 0 and female = 1) – 41.7 * HbA_{1c} (%)

The four variables included in the above equation explained 81% of the variability in RMR. Model 2 that explained a total of 79.4 % of RMR variability did not include HbA_{1c} (%):

(Model 2) **RMR (kcal/d)** = - 1072 + 29.6 * FFM (kg) + 14.6 * waist (cm) – 269 * gender (male = 0 and female = 1)

Additionally, Model 3 was also constructed. It included FFM, waist circumference, BMI and gender; and explained 79.5 % of variability in RMR.

(Model 3) **RMR (kcal/d)** = -1079 + 28.9 * FFM (kg) + 11.6 waist (cm) + 8.6 * BMI (kg/m^2) – 212 * gender (male = 0 and female = 1)

Two further predictive equations were developed separately for men and women. The equations explaining the most variability in RMR in men (84.8%) included FFM, waist circumference and glycosylated haemoglobin:

(Model 4) **RMR (kcal/d)** = -1072.2 + 24.9 * FFM (kg) + 20.6 * waist (cm) – 86.1 * HbA_{1c} (%)

The proposed equation for women included FFM and waist circumference only and explained 77.1 % of variability in RMR:

(Model 5) **RMR (kcal/d)** = -1201.0 + 28.8 * FFM (kg) + 11.7 * waist (cm)

Table 12. Multiple regression models to predict resting metabolic rate in obese persons with T2DM, showing regression coefficients.

Independent variables	Model 1 (R^2 = 0.811)			Model 2 (R^2 = 0.794)			Model 3 (R^2 = 0.795)		
	regression coefficient	± SE	P	regression coefficient	± SE	P	regression coefficient	± SE	P
Constant	-900.7	256.5	0.001	-1072.1	256.4	<0.001	-1079.1	255.6	<0.001
Age (years)									
Gender	-270.4	63.1	<0.001	-268.7	65.7	<0.001	-212.2	82.1	0.013
Weight (kg)									
Height (cm)									
BMI (kg/m^2)							8.6	7.5	0.258
Waist (cm)	16.7	2.5	<0.001	14.6	2.4	<0.001	11.6	3.5	0.002
FM (kg)									
FFM (kg)	28.6	3.4	<0.001	29.6	3.5	<0.001	28.9	3.5	<0.001
log FG (mmol/l)									
HbA_{1c} (%)	-41.7	17.9	0.024						

Abbreviations: R^2, coefficient of determination; SE, standard error; Gender, male = 0, female = 1; BMI, body mass index; FM, fat mass; FFM, fat free mass; log FG, logarithm to the base of 10 of fasting plasma glucose; HbA_{1c}, glycosylated haemoglobin.

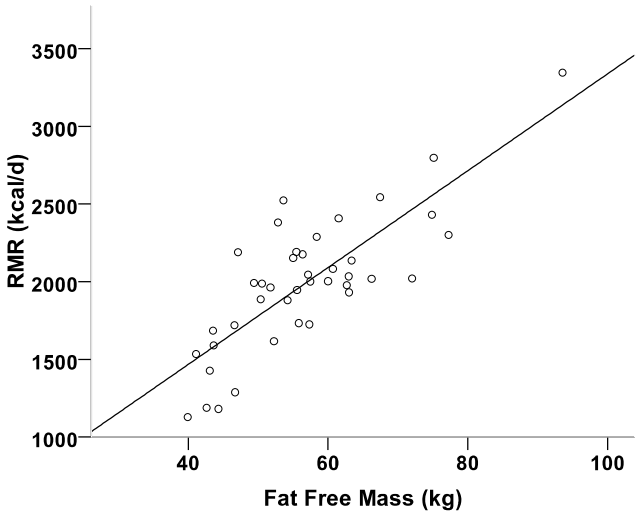


Figure 24. Scatter plot of RMR and fat free mass in 56 obese subjects. RMR was significantly related to fat free mass ($r = 0.763$, $R^2 = 0.580$, $p < 0.001$).

4.7 Glycaemic control

LOCF

At baseline, there were no differences between the two diets in fasting plasma glucose, fasting plasma insulin, and glycosylated haemoglobin concentrations, and in HOMA index. Proportions of subjects on insulin and on metformin treatment were the same between GL and PSMF groups. At baseline there were no differences in any of the glycaemic measures between participants on insulin or not, and those on metformin or not.

There were no differences in changes in any of the glycaemic measures between the two groups (Table 13a). There was an overall significant decrease in HBA_{1c} (-0.4 ± 1.1 %, $p < 0.001$), fasting plasma insulin (-1.9 ± 9.0 μU/ml, $p = 0.005$) and HOMA-IR index (-1.2 ± 5.6, $p = 0.018$). Change in HbA_{1c} was correlated with the change in weight ($r = 0.427$, $p = 0.005$) (Figure 25).

Table 13a. Changes in glycaemic control (LOCF, n = 41).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Fasting plasma glucose (mmol/l) [a]						
GL (n=15)	10.4 ± 2.9	9.4 ± 3.0	10.4 ± 4.5	0.0 ± 4.0	0.335	0.805
PSMF (n=25)	10.1 ± 3.8	9.4 ± 3.8	9.6 ± 3.3	-0.4 ± 3.0	0.253	
Total (n=40)	**10.2 ± 3.5**	**9.4 ± 3.5**	**9.9 ± 3.8**	**-0.2 ± 3.4**	**0.164**	
HbA_{1c} (%) [b]						
GL (n=16)	8.9 ± 1.5	8.1 ± 1.4*	8.5 ± 1.5*	-0.4 ± 0.7	<0.001	0.710
PSMF (n=25)	9.1 ± 1.5	8.5 ± 1.9	8.7 ± 1.6	-0.5 ± 1.3	0.001	
Total (n=41)	**9.0 ± 1.5**	**8.3 ± 1.7***	**8.6 ± 1.6***	**-0.4 ± 1.1**	**<0.001**	
Fasting plasma insulin (μU/ml) [a]						
GL (n=16)	17.1 ± 12.5	15.3 ± 14.2	15.4 ± 14.1	-1.7 ± 12.8	0.241	0.994
PSMF (n=24)	12.1 ± 11.0	10.1 ± 9.3*	10.1 ± 8.9*	-2.0 ± 5.4	0.018	
Total (n=40)	**14.1 ± 11.7**	**12.2 ± 11.6***	**12.2 ± 11.4***	**-1.9 ± 9.0**	**0.005**	
HOMA-IR [a]						
GL (n=15)	7.6 ± 6.7	6.0 ± 5.9	6.3 ± 5.8	-1.3 ± 7.3	0.438	0.594
PSMF (n=24)	5.3 ± 5.3	4.2 ± 4.7	4.0 ± 3.4	-1.2 ± 4.5	0.013	
Total (n=39)	**6.2 ± 5.9**	**4.9 ± 5.2**	**4.9 ± 4.5**	**-1.2 ± 5.6**	**0.018**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; m, male; f, female; HBA_{1c}, glycosylated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance. There were no differences between the dietary groups at baseline.

† Time effect – significance level of changes over one year time, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using Mann-Whitney test

* significant difference from the baseline, $p < 0.05$, calculated using Wilcoxon signed ranks test

[a] positive skew, log transformation

[b] non-normal distribution, untransformed

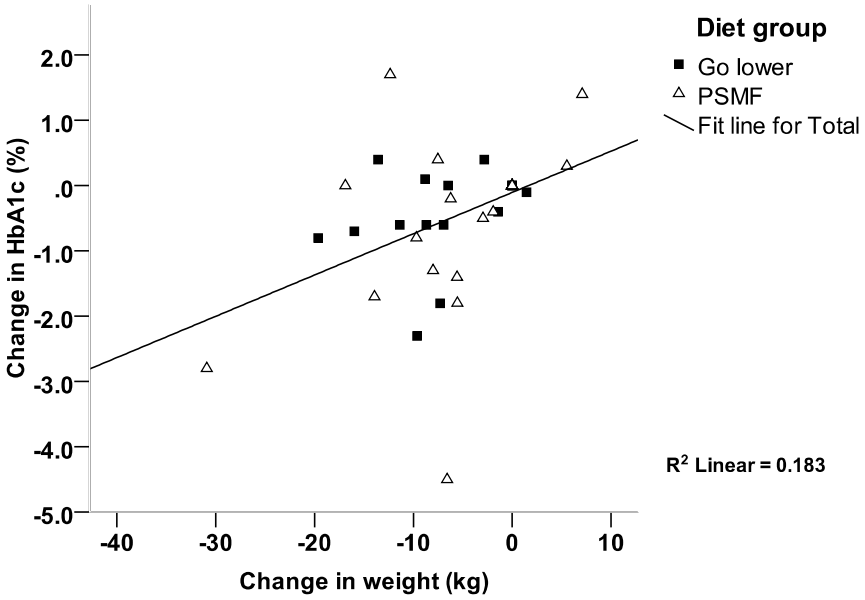


Figure 25. Scatter plot of change in glycosylated haemoglobin (HbA_{1c}) and change in weight at 12 months (n = 41). Presented data are for each individual (overall, r = 0.427, p = 0.005). The Go Lower group is shown as squares; the protein sparing modified fast (PSMF) group is shown as triangles. R^2 Linear coefficient of determination.

Of the participants using insulin (n = 13), the dose was reduced from baseline to 12 months by 40 ± 58 U/d (p = 0.028). Change in insulin dose was positively correlated with the decrease in weight (r = 0.654, p = 0.015) and fat mass (r = 0.681, p = 0.010). There were no changes in metformin and other hypoglycaemic medications throughout the programme.

Completers

At baseline, there were no differences between the two diets in fasting plasma glucose, fasting plasma insulin, and glycosylated haemoglobin concentrations, and in HOMA index. Proportions of subjects on insulin and on metformin treatment were the same between GL and PSMF groups. At baseline there were also no differences in any of the glycaemic measures between participants on insulin or not.

Completers' analysis showed similar results to LOCF analysis; however changes in glycaemic control although more pronounced, were not always significant (Table 13b). Completers, in contrast to dropouts, displayed a nonsignificant trend for a decrease in fasting insulin and HOMA during the programme (Table 13b). Completers' analysis showed that there was an overall significant decrease in HBA_{1c} (-0.65 ± 1.36 %, p < 0.001) and fasting insulin (-4.0 ± 9.0 μU/ml, p = 0.041) but not HOMA

index or fasting glucose. Change in HOMA index for the completers was actually more pronounced than in all participants (LOCF) (-2.1 ± 6.6 vs. -1.2 ± 5.6). The fact that this did not reach significance is probably due to the small sample size (n = 14) and high standard deviation. Additionally, the change in HbA_{1c} was not correlated with changes in weight or fat mass but with the change in fasting glucose (r = 0.600, p = 0.023).

Table 13b. Changes in glycaemic control (completers, n = 14).

	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** †	**Group effect** ‡
Fasting plasma glucose (mmol/l) [a]						
GL (n=6)	11.2 ± 2.4	9.4 ± 2.3	12.3 ± 5.3	+1.0 ± 6.0	0.730	0.572
PSMF (n=8)	11.1 ± 4.3	9.4 ± 4.1	9.5 ± 3.0	-1.6 ± 3.9	0.745	
Total (n=14)	**11.2 ± 3.5**	**9.4 ± 3.3**	**10.7 ± 4.2**	**-0.5 ± 4.9**	**0.559**	
HbA_{1c} (%) [b]						
GL	8.7 ± 1.3	7.4 ± 0.6*	8.4 ± 1.5	-0.2 ± 0.4	<0.001	0.298
PSMF	9.4 ± 1.8	8.1 ± 2.2	8.4 ± 1.6	-1.0 ± 1.7	0.029	
Total	**9.1 ± 1.6**	**7.8 ± 1.7***	**8.4 ± 1.6***	**-0.6 ± 1.4**	**<0.001**	
Fasting plasma insulin (μU/ml) [a]						
GL	14.8 ± 14.3	7.1 ± 3.3	7.3 ± 2.4	-7.5 ± 12.5	0.553	0.662
PSMF	10.6 ± 6.6	10.6 ± 10.3	9.1 ± 8.8	-1.4 ± 4.6	0.022	
Total	**12.4 ± 10.4**	**9.1 ± 8.0**	**8.3 ± 6.7**	**-4.0 ± 9.0**	**0.041**	
HOMA-IR [a]						
GL	8.1 ± 9.5	3.0 ± 1.8	3.8 ± 1.4	-4.2 ± 9.2	0.699	0.755
PSMF	4.5 ± 2.0	5.1 ± 7.1	4.0 ± 4.2	-0.5 ± 3.7	0.080	
Total	**6.0 ± 6.3**	**4.2 ± 5.5**	**3.9 ± 3.2**	**-2.1 ± 6.6**	**0.159**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; m, male; f, female; HBA_{1c}, glycosylated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance. There were no differences between the dietary groups at baseline.

† Time effect – significance level of changes over one year time, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 months changes between the diet groups, calculated using Mann-Whitney test

* significant difference from the baseline, $p < 0.05$, calculated using Wilcoxon signed ranks test

[a] positive skew, log transformation

[b] non-normal distribution, untransformed

4.8 Cardiovascular disease risk

LOCF

At baseline, GL and PSMF groups had different total cholesterol concentration ($p = 0.025$) and systolic blood pressure ($p = 0.012$). There were no baseline differences between the groups in any other variables presented in this sub-chapter. Lipid profile change at 12 months was similar in both diet groups (Table 14a). Overall, triacylglycerol decreased (-0.6 ± 2.4 mmol/l, $p = 0.006$), total cholesterol and LDL cholesterol did not change and HDL cholesterol increased ($+0.07 \pm 0.18$ mmol/l, $P < 0.001$) (Figure 26). In addition, TC/HDL ratio decreased at 12 months (-0.3 ± 0.8, $p = 0.009$). Systolic blood pressure decreased (-6 ± 14 mmHg, $p = 0.014$) and diastolic blood pressure did not change during the programme.

Table 14a. Changes in lipid profile (LOCF, n = 41).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Total cholesterol (mmol/l) [a]						
GL (n=16)	5.4 ± 1.6 §	5.7 ± 1.7	5.1 ± 1.3	-0.4 ± 1.2	0.165	0.331
PSMF (n=25)	4.3 ± 1.0 §	4.3 ± 0.9	4.3 ± 1.0	-0.1 ± 0.5	0.621	
Total (n=41)	**4.8 ± 1.4**	**4.8 ± 1.4**	**4.6 ± 1.2**	**-0.2 ± 0.9**	**0.222**	
Triacylglycerol (mmol/l) [b]						
GL	3.7 ± 4.1	2.6 ± 1.7	2.5 ± 1.6	-1.2 ± 3.8	0.153	0.947
PSMF	2.0 ± 0.7	1.6 ± 0.7*	1.7 ± 0.7	-0.2 ± 0.7	0.038	
Total	**2.7 ± 2.7**	**2.0 ± 1.2***	**2.1 ± 1.2***	**-0.6 ± 2.4**	**0.006**	
HDL cholesterol (mmol/l) [c]						
GL	1.24 ± 0.30	1.37 ± 0.37*	1.32 ± 0.38	0.08 ± 0.18	0.023	0.554
PSMF	1.10 ± 0.19	1.18 ± 0.23*	1.17 ± 0.73	0.07 ± 0.18	0.006	
Total	**1.16 ± 0.24**	**1.26 ± 0.30***	**1.23 ± 0.30***	**0.07 ± 0.18**	**<0.001**	
LDL cholesterol (mmol/l) [b]						
GL	2.9 ± 1.5	3.3 ± 1.6	2.6 ± 1.3	-0.3 ± 1.0	0.265	0.414
PSMF	2.3 ± 0.8	2.4 ± 0.7	2.3 ± 0.8	-0.0 ± 0.4	0.598	
Total	**2.5 ± 1.1**	**2.7 ± 1.2**	**2.4 ± 1.0**	**-0.1 ± 0.7**	**0.147**	
TC/HDL [b]						
GL	4.6 ± 1.8	4.5 ± 1.7	4.2 ± 1.6	-0.5 ± 1.1	0.295	0.908
PSMF	4.0 ± 1.0	3.7 ± 1.0*	3.8 ± 1.0*	-0.2 ± 0.6	0.018	
Total	**4.2 ± 1.4**	**4.0 ± 1.3***	**3.9 ± 1.3***	**-0.3 ± 0.8**	**0.009**	
Systolic BP (mmHg) [a]						
GL	161 ± 19 §	153 ± 23*	152 ± 23*	-9 ± 16	0.031	0.385
PSMF	145 ± 17 §	144 ± 18	143 ± 18	-3 ± 13	0.384	
Total	**152 ± 19**	**147 ± 21***	**147 ± 20***	**-6 ± 14**	**0.014**	
Diastolic BP (mmHg) [a]						
GL	86 ± 9	82 ± 11	83 ± 12	-3 ± 12	0.323	0.414
PSMF	85 ± 10	83 ± 9	83 ± 9	-1 ± 8	0.723	
Total	**85 ± 10**	**83 ± 10**	**83 ± 10**	**-2 ± 10**	**0.234**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC/HDL, ratio of total cholesterol and high-density lipoprotein cholesterol; BP, blood pressure.

† Time effect – significance level of changes over 12 m, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 m changes between the diet groups, Mann-Whitney test used

* significant difference from baseline, $p < 0.05$, calculated using paired t-test or Wilcoxon signed ranks test

§ significant difference between the two diets at baseline

[a] normal distribution

[b] positive skew, log transformation

[c] non-normal distribution, untransformed, non-parametric tests used

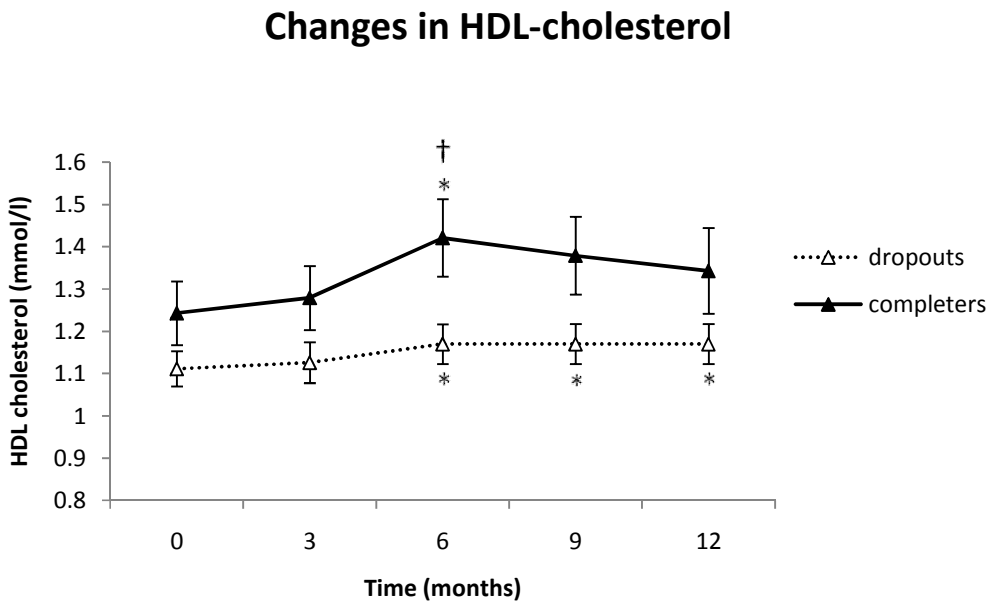


Figure 26. Changes in the high-density lipoprotein (HDL) cholesterol; comparison of completers and dropouts.
* significant difference from baseline, $p < 0.05$
† significant difference between the completers and dropouts at a single time-point, $p < 0.05$

Although the number of participants whose CVD risk over 30%, according to the Framingham score (D'Agostino et al. 2008), decreased from 19 at baseline to 14 at 12 months ($p = 0.250$) and the number of participants with heart age classified as 80 or over decreased from 33 at baseline to 28 at 12 months ($p = 0.180$), these changes were not significant. The differences in changes between the diet groups were not significant.

At baseline there was no difference in blood pressure between the participants who took antihypertensive medications and those who did not. Also, participants on lipid lowering medications had no different lipid profiles than participants not on lipid lowering medications, with the exception of total cholesterol. Participants who were on lipid lowering medications had lower total cholesterol than participants not on it (4.5 ± 1.2 vs. 6.4 ±1.7 mmol/l, $p = 0.003$). Neither the antihypertensive medications nor the lipid lowering medication doses changed during the study.

Completers

Completers' analysis revealed that there were no baseline differences between the diet groups for any of the cardiovascular risk factors (Table 14b).

Table 14b. Changes in lipid profile (completers, n = 14).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Total cholesterol (mmol/l) [a]						
GL (n=6)	5.3 ± 2.1	6.0 ± 2.2	4.4 ± 0.7	-0.9 ± 1.2	0.163	0.345
PSMF (n=8)	4.2 ± 0.6	4.0 ± 0.6	4.0 ± 0.8	-0.1 ± 0.8	0.791	
Total (n=14)	**4.7 ± 1.5**	**4.9 ± 1.7**	**4.2 ± 0.8**	**-0.5 ± 1.4**	**0.285**	
Triacylglycerol (mmol/l) [b]						
GL	2.6 ± 1.0	2.2 ± 1.4	2.2 ± 0.8	-0.4 ± 1.4	0.656	0.852
PSMF	1.9 ± 0.7	1.2 ± 0.4*	1.4 ± 0.5	-0.5 ± 0.7	0.117	
Total	**2.2 ± 0.9**	**1.6 ± 1.0***	**1.7 ± 0.7**	**-0.4 ± 1.0**	**0.088**	
HDL cholesterol (mmol/l) [c]						
GL	1.38 ± 0.34	1.56 ± 0.41	1.47 ± 0.46	0.08 ± 0.24	0.122	0.980
PSMF	1.14 ± 0.19	1.31 ± 0.25*	1.25 ± 0.30	0.11 ± 0.29	0.099	
Total	**1.24 ± 0.28**	**1.42 ± 0.34***	**1.34 ± 0.38**	**0.10 ± 0.26**	**0.009**	
LDL cholesterol (mmol/l) [b]						
GL	2.7 ± 1.6	3.4 ± 2.0	2.0 ± 0.5	-0.8 ± 1.3	0.557	0.468
PSMF	2.2 ± 0.7	2.3 ± 0.5	2.2 ± 0.7	-0.0 ± 0.7	0.625	
Total	**2.4 ± 1.2**	**2.7 ± 1.4**	**2.1 ± 0.6**	**-0.4 ± 1.0**	**0.351**	
TC/HDL [b]						
GL	3.8 ± 1.3	4.0 ± 1.3	3.2 ± 0.8	-0.6 ± 1.3	0.448	0.968
PSMF	3.7 ± 0.4	3.2 ± 0.6	3.4 ± 0.9	-0.3 ± 1.0	0.148	
Total	**3.8 ± 0.9**	**3.5 ± 1.0**	**3.3 ± 0.8**	**-0.4 ± 1.1**	**0.161**	
Systolic BP (mmHg) [a]						
GL	161 ± 10	139 ± 15*	137 ± 7*	-24 ± 11	0.002	0.018
PSMF	144 ± 19	142 ± 16	141 ± 13	-3 ± 17	0.909	
Total	**152 ± 17**	**141 ± 15***	**140 ± 11***	**-12 ± 18**	**0.035**	
Diastolic BP (mmHg) [a]						
GL	89 ± 7	77 ± 15*	83 ± 17	-6 ± 17	0.106	0.432
PSMF	87 ± 13	83 ± 10	85 ± 10	-2 ± 12	0.717	
Total	**88 ± 11**	**81 ± 12***	**84 ± 13**	**-3 ± 14**	**0.055**	

Presented values are means ± standard deviation. Abbreviations: GL, Go Lower; PSMF, protein sparing modified fast; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC/HDL, ratio of total cholesterol and high-density lipoprotein cholesterol; BP, blood pressure. There were no differences between the dietary groups at baseline.
† Time effect – significance level of changes over 12 m, calculated using repeated measures ANOVA or Friedman ANOVA
‡ Group effect – significance level of the difference in 12 m changes between the diet groups, Mann-Whitney test used
* significant difference from baseline, $p < 0.05$, calculated using paired t-test or Wilcoxon signed ranks test
[a] normal distribution
[b] positive skew, log transformation
[c] non-normal distribution, untransformed, non-parametric tests used

Lipid profile and blood pressure changes at 12 months were not significantly different in both diet groups within the completers, and also between completers and dropouts (Figure 27).

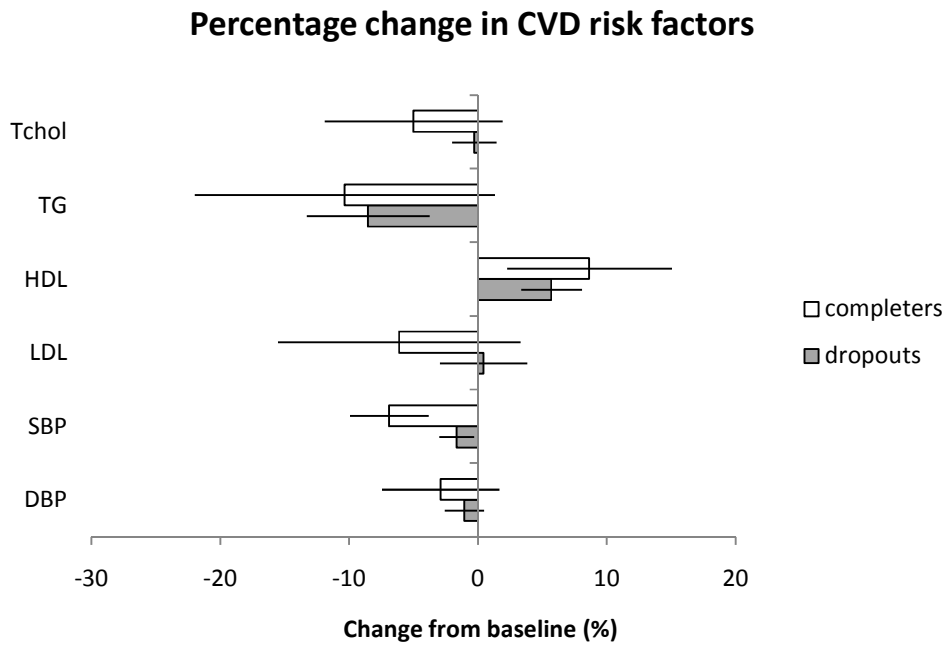


Figure 27. Percentage change of cardiovascular risk factors (mean ± SE): comparison of completers and dropouts. Tchol, total cholesterol; TG, triacylglycerols; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

The changes from baseline for completers were very similar or even of greater magnitude than those obtained in the LOCF analysis, however, perhaps due to the smaller participant numbers, some of the changes were no longer significant. For example, decreases in TG and TC/HDL ratio were no longer significant. Total cholesterol, LDL cholesterol did not change but HDL cholesterol increased (+0.10 ± 0.26 mmol/l, p = 0.009) and systolic blood pressure decreased (-12 ± 18 mmHg, p = 0.035). The number of participants whose CVD risk was over 30% decreased from 5 at baseline to 3 at 12 months and the number of participants whose heart age was estimated to be over 80 years, decreased from 11 at baseline to only 8 at 12 months but none of these changes were significant. The differences in changes in CVD risk or heart age by Framingham score between completers and dropouts were not significant.

4.9 Liver, kidney and thyroid function

LOCF

Participants on both diets showed similar changes in liver, kidney and thyroid function (Table 15a). Overall through 12 months of the intervention, kidney function remained unchanged (serum creatinine, $p = 0.081$; glomerular filtration rate, $p = 0.341$). Concentration of thyroid stimulating hormone did not change either ($p = 0.535$). Liver function improved, with significant decreases in alanine aminotransferase (ALT) (-5 ± 14 U/l, $p < 0.001$) and gamma-glutamyl transferase (GGT) (-9 ± 37 U/l, $p < 0.001$) levels. Furthermore, there were no changes in total bilirubin ($p = 0.072$) and alkaline phosphatase (ALKP) ($p = 0.183$) concentrations.

Table 15a. Changes in liver, kidney and thyroid function (LOCF, n = 41).

	Baseline	6 months	12 months	Total Change	Time effect †	Group effect ‡
Creatinine (µmol/l) [b]						
GL (n=16)	72 ± 20	76 ± 17	76 ± 18	+4 ± 10	0.165	0.741
PSMF (n=25)	75 ± 20	76 ± 19	76 ± 20	+1 ± 6	0.414	
Total (n=25)	**74 ± 20**	**76 ± 18**	**76 ± 19**	**+2 ± 8**	**0.081**	
Cr. clearance (ml/min) [b]						
GL (n=7)	86 ± 64	81 ± 52	92 ± 46	+6 ± 34	0.538	0.145
PSMF (=14)	79 ± 36	79 ± 42	68 ± 33	-11 ± 21	0.195	
Total(n=21)	**82 ± 46**	**80 ± 46**	**76 ± 40**	**-6 ± 8**	**0.571**	
eGFR (ml/min/1.73m^2) [a]						
GL (n=16)	84 ± 20	81 ± 19	83 ± 20	-1 ± 9	0.626	0.307
PSMF (n=25)	91 ± 21	91 ± 21	89 ± 22	-2 ± 8	0.399	
Total (n=41)	**89 ± 20**	**87 ± 21**	**87 ± 21**	**-2 ± 8**	**0.341**	
TSH (µmol/l) [d]						
GL	1.9 ± 1.0	2.0 ± 0.8	2.0 ± 0.9	+0.1 ± 0.6	0.164	0.170
PSMF	1.7 ± 0.9	1.7 ± 1.1	1.8 ± 1.1	+0.1 ± 0.7	0.749	
Total	**1.8 ± 1.0**	**1.8 ± 1.0**	**1.9 ± 1.0**	**+0.1 ± 0.7**	**0.535**	
ALT (U/l) [c]						
GL	40 ± 21	31 ± 17*	33 ± 19	-7 ± 12	0.001	0.643
PSMF	34 ± 23	28 ± 21*	30 ± 24*	-4 ± 15	0.044	
Total	**36 ± 22**	**29 ± 20***	**31 ± 22***	**-5 ± 14**	**< 0.001**	
ALKP (U/l) [b]						
GL	76 ± 28	71 ± 26	70 ± 27	-6 ± 15	0.175	0.183
PSMF	73 ± 25	74 ± 29	74 ± 31	+1 ± 12	0.810	
Total	**74 ± 26**	**71 ± 28**	**72 ± 30**	**-2 ± 13**	**0.183**	
GGT (U/l) [b]						
GL	75 ± 60	57 ± 43	53 ± 38*	-22 ± 43	0.020	0.771
PSMF	53 ± 37	46 ± 42*	52 ± 53*	-1 ± 32	0.021	
Total	**61 ± 1.4**	**50 ± 42***	**52 ± 47***	**-9 ± 37**	**< 0.001**	
Total bilirubin (µmol/l) [c]						
GL	9.0 ± 4.6	9.2 ± 3.9	9.7 ± 4.1	+0.7 ± 2.4	0.138	0.151
PSMF	9.4 ± 3.9	9.7 ± 4.4	9.4 ± 4.1	-0.0 ± 2.4	0.291	
Total	**9.2 ± 4.1**	**9.5 ± 4.2**	**9.5 ± 4.1**	**+0.3 ± 1.9**	**0.072**	

Presented values are means ± standard deviation. GL, Go Lower; PSMF, protein sparing modified fast; eGFR, estimated glomerular filtration rate, calculated from CKD-EPI equation; Cr. Clearance, creatinine clearance adjusted for body surface area; ALT, alanine amino transferase; ALKP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TSH, thyroid stimulating hormone. There were no differences between the dietary groups at baseline for any of the variables in the table.

† Time effect – significance level of changes over 12 m, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 m changes between the diet groups, Mann-Whitney test used

* significant difference from baseline, $p < 0.05$, calculated using paired t-test or Wilcoxon signed ranks test

[a] normal distribution

[b] positive skew, log transformation
[c] positive skew, reciprocal transformation
[d] non-normal distribution, untransformed, non-parametric tests used

Completers

Completers' analysis showed similar results to LOCF analysis (Table 15b). Overall, kidney and thyroid functions remained unchanged. Liver function improved, with significant decreases in ALT (-8 ± 21 U/l, p = 0.006) and GGT (-19 ± 51 U/l, p = 0.017) levels. Also, there were no changes in total bilirubin and ALKP concentrations.

Table 15b. Changes in liver, kidney and thyroid function (completers, n = 14).

	Baseline	**6 months**	**12 months**	**Total Change**	**Time effect** [†]	**Group effect** [‡]
Creatinine (µmol/l) [b]						
GL (n=6)	75 ± 16	78 ± 16	76 ± 18	+1 ± 10	0.250	0.923
PSMF (n=8)	77 ± 21	80 ± 17	78 ± 20	+1 ± 9	0.458	
Total (n=14)	**76 ± 19**	**79 ± 16**	**77± 18**	**+1 ± 9**	**0.186**	
Cr. clearance (ml/min) [b]						
GL (n=2)	29 ± 14	59 ± 33	76 ± 21	+24 ± 7	0.706	0.190
PSMF (n=5)	83 ± 40	76 ± 34	61 ± 28	-24 ± 31	0.480	
Total (n=7)	**67 ± 43**	**67 ± 33**	**68 ± 25**	**-10 ± 34**	**0.752**	
eGFR (ml/min/1.73m^2) [a]						
GL (n=6)	81 ± 18	79 ± 18	79 ± 18	-2 ± 8	0.718	0.774
PSMF (n=8)	88 ± 18	84 ± 22	87 ± 21	-1 ± 8	0.446	
Total (n=14)	**85 ± 17**	**82 ± 20**	**84 ± 19**	**-1 ± 7**	**0.480**	
TSH (µmol/l) [d]						
GL	2.5 ± 1.1	2.3 ± 0.6	2.5 ± 0.8	0.0 ± 0.8	0.818	0.852
PSMF	1.8 ± 1.0	2.0 ± 1.3	2.3 ± 1.3	+0.4 ± 1.2	0.834	
Total	**2.1 ± 1.1**	**2.2 ± 1.0**	**2.4 ± 1.0**	**+0.3 ± 1.1**	**0.616**	
ALT (U/l) [c]						
GL	41 ± 19	26 ± 9*	30 ± 8	-12 ± 16	0.008	0.662
PSMF	38 ± 18	24 ± 6*	33 ± 20	-5 ± 25	0.184	
Total	**40 ± 18**	**25 ± 7***	**32 ± 15**	**-8 ± 21**	**0.006**	
ALKP (U/l) [b]						
GL	70 ± 24	67 ± 33	65 ± 35	-5 ± 16	0.234	0.362
PSMF	74 ± 23	70 ± 20	76 ± 31	+2 ± 12	0.830	
Total	**72 ± 23**	**69 ± 25**	**71 ± 32**	**-1 ± 13**	**0.383**	

GGT (U/l) [b]						
GL	87 ± 82	59 ± 51	48 ± 37	-40 ± 53	0.097	0.662
PSMF	67 ± 48	44 ± 28	63 ± 64	-4 ± 46	0.155	
Total	**76 ± 63**	**51 ± 39***	**56 ± 53***	**-19 ± 51**	**0.017**	
Total bilirubin (µmol/l) [c]						
GL	10.8 ± 6.5	10.7 ± 4.5	11.7 ± 4.8	+0.8 ± 3.2	0.306	0.148
PSMF	9.7 ± 3.0	10.7 ± 4.6	8.7 ± 3.8	-1.0 ± 1.3	0.049	
Total	**10.2 ± 4.6**	**10.7 ± 4.4**	**10.0 ± 4.1**	**+0.2 ± 2.4**	**0.169**	

Presented values are means ± standard deviation. GL, Go Lower; PSMF, protein sparing modified fast; eGFR, estimated glomerular filtration rate, calculated from CKD-EPI equation; Cr. Clearance, creatinine clearance adjusted for body surface area; ALT, alanine amino transferase; ALKP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TSH, thyroid stimulating hormone.

† Time effect – significance level of changes over 12 m, calculated using repeated measures ANOVA or Friedman ANOVA

‡ Group effect – significance level of the difference in 12 m changes between the diet groups, Mann-Whitney test used

* significant difference from baseline, $p < 0.05$, calculated using paired t-test or Wilcoxon signed ranks test

§ significant difference between the two diets at baseline

[a] normal distribution

[b] positive skew, log transformation

[c] positive skew, reciprocal transformation

[d] non-normal distribution, untransformed, non-parametric tests used

4.10 Questionnaires

4.10.1 Physical activity

Physical activity was assessed by the General Practice Physical Activity Questionnaire (GPPAQ) that categorizes subjects into one of the four categories: inactive, moderately inactive, moderately active and active.

LOCF

There were no differences between the diet groups at baseline and at any of the recorded time points. LOCF analysis showed that physical activity did not change during the study ($p = 0.285$). Most participants were inactive and moderately inactive throughout the programme (Figure 28). There was no association between physical activity and weight, FFM or BMI.

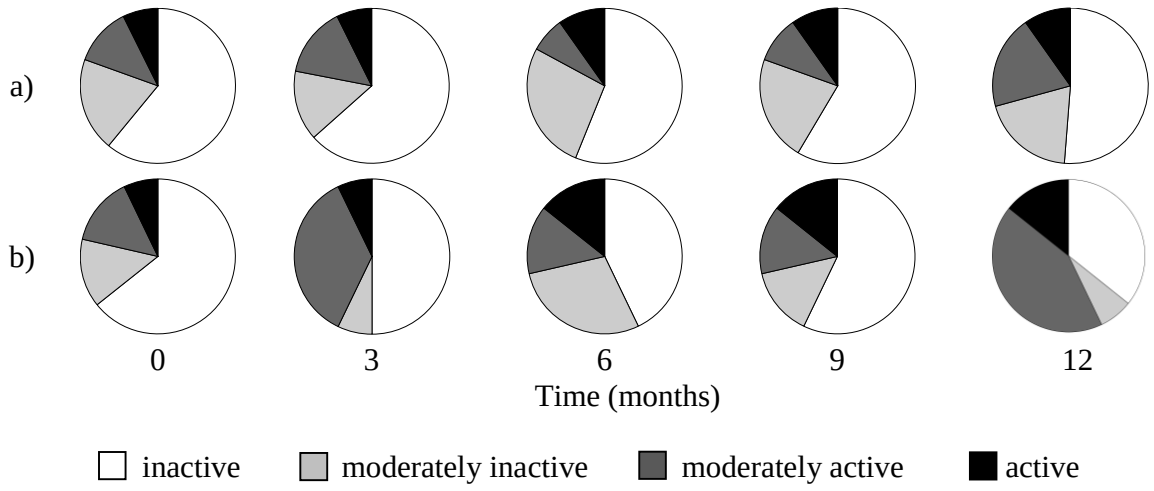


Figure 28. Physical activity changes in all participants with last observations carried forward (n = 41) (a) and in completers only (n = 14) (b).

Completers

Completers' analysis seems to show slightly higher physical activity during the program than LOCF analysis. There were no differences between the diet groups at baseline and at any of the recorded time points. Also, at baseline there was no difference in physical activity between completers and dropouts. Although it appears that more participants were moderately active at 12 months (Figure 28b), physical activity did not significantly change during the study (p = 0.161). Most participants were inactive and moderately inactive throughout the programme. There was no association between physical activity and weight, FFM or BMI.

4.10.2 Attitude towards weight loss

LOCF

At baseline there were no significant differences in motivation to lose weight between GL and PSMF groups. The attitudes towards weight loss did not change from baseline to 12 months (LOCF, p = 0.959) with the majority of participants being positive. Despite the fact that the general attitude towards weight loss remained positive throughout the programme (Table 16a), the mean weight decisional balance (WDB) scores decreased (LOCF, p = 0.007). Finally, there was a positive association between the change in weight and change in the attitude towards weight loss (r = 0.340, p = 0.03).

Table 16a. Weight decisional balance scale rating changes (LOCF, n = 41)

	Time (months)				
	0	**3**	**6**	**9**	**12**
Attitude towards weight loss					
Positive n (%)	40 (98)	39 (95)	39 (95)	37 (90)	36 (88)
Negative n (%)	1 (2)	2 (5)	2 (5)	2 (5)	3 (7)
Undecided n (%)	0 (0)	0 (0)	0 (0)	2 (5)	2 (5)
WDB score (± SD)	1.42 ± 0.69	1.35 ± 0.76	1.18 ± 0.68	1.13 ± 0.74	1.11 ± 0.76

Abbreviation: WDB, weight decisional balance.

Completers

Completers' analysis showed similar results to LOCF analysis (Tables 16a and 16b). At baseline there were no significant differences in motivation to lose weight between GL and PSMF groups. There were also no differences between completers and dropouts. The attitudes towards weight loss did not change from baseline to 12 months (LOCF, p = 0.959; completers, p = 0.564) with the majority of participants being positive. Despite the fact that the general attitude towards weight loss remained positive throughout the programme (Table 16b), the mean weight decisional balance (WDB) scores decreased (completers, p = 0.03). The association between the change in weight and change in the attitude towards weight loss in the completers' analysis was no longer significant.

Table 16b. Weight decisional balance scale rating changes (completers, n = 14)

	Time (months)				
	0	**3**	**6**	**9**	**12**
Attitude towards weight loss					
Positive n (%)	14 (100)	14 (100)	14 (100)	12 (86)	11 (79)
Negative n (%)	0 (0)	0 (0)	0 (0)	1 (7)	2 (14)
Undecided n (%)	0 (0)	0 (0)	0 (0)	1 (7)	1 (7)
WDB score (± SD)	1.21 ± 0.54	1.11 ± 0.57	0.97 ± 0.53	0.81 ± 0.67	0.77 ± 0.71

Abbreviation: WDB, weight decisional balance.

4.10.3 Obesity related wellbeing

LOCF

There were no differences in ORWELL-97 scores between diet groups at baseline and at any of the recorded time points (Figure 29a). There was a significant decrease in the overall scores ($p = 0.018$) indicating an improvement in quality of life. There was a positive association between the change in weight and the change in obesity related quality of life ($r = 0.392$, $p = 0.011$).

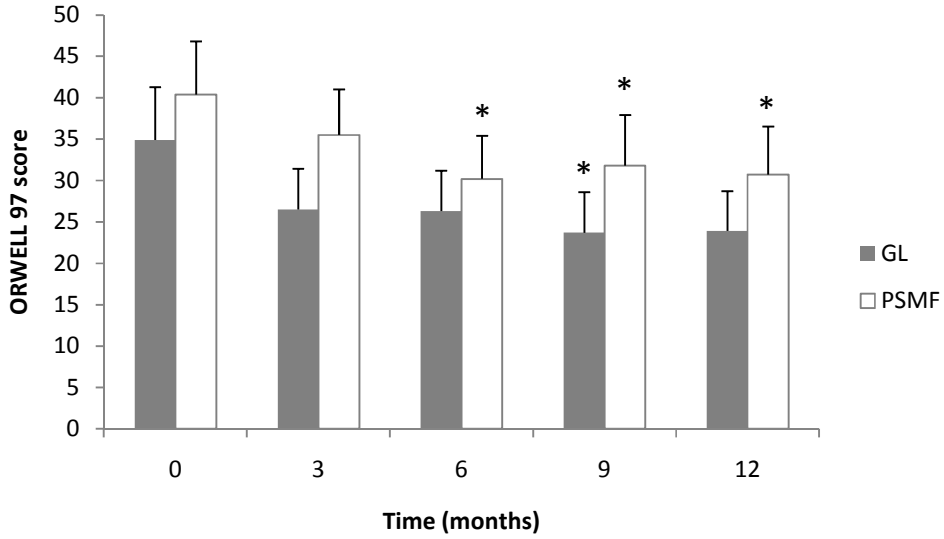


Figure 29a. Obesity related wellbeing changes (LOCF; GL n = 16, PSMF n = 25).
* significant difference from baseline ($p < 0.05$)

Completers

There were no differences in ORWELL-97 scores between diet groups (Figure 29b) or between completers and dropouts at baseline and at any of the recorded time points. Although it seems that changes in LOCF and completers' analyses are similar (Figures 29a and 29b), according to the completers' analysis, the overall scores of the questionnaire did not significantly change ($p = 0.337$). The association between the change in weight and the change in obesity related quality of life was no longer significant for the completers' analysis.

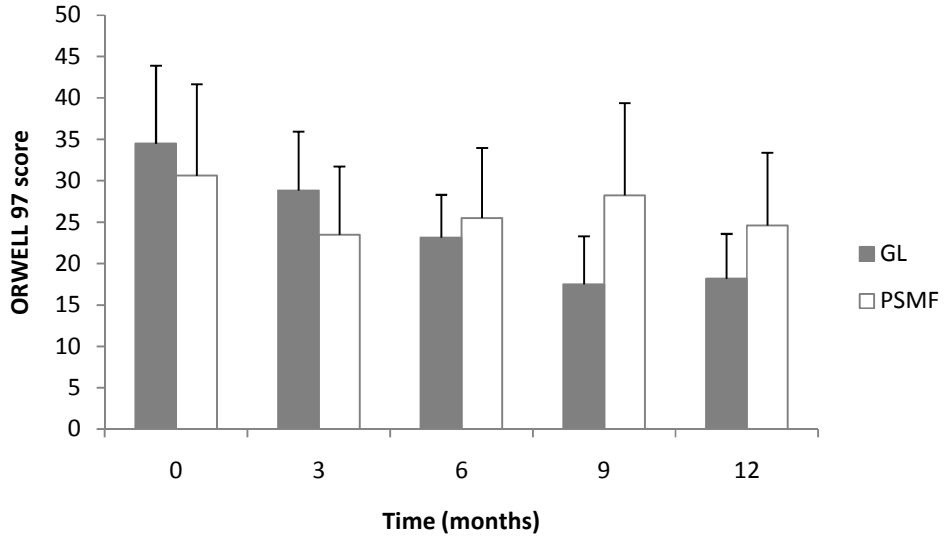


Figure 29b. Obesity related wellbeing changes (completers; GL n = 6, PSMF n = 8).

4.10.4 Diabetes related quality of life

LOCF

At baseline there was no difference in the average weighted impact (AWI) and the 18 items between GL and PSMF. According to the LOCF analysis (n = 41), AWI of diabetes on quality of life significantly decreased (from -1.6 ± 1.4 to -1.4 ± 1.3, p = 0.049). Overall, there were significant decreases in the diabetes negative impact on holidays and leisure activities and worries about the future. However, the enjoyment of food decreased (Figure 30a).

ADDQoL also has two items rating present quality of life (per se) and the potential quality of life without diabetes (impact of diabetes on quality of life). Both LOCF and completers analyses showed that the present quality of life significantly increased indicating a shift from 'good' towards 'very good'; and the potential quality of life without diabetes did not change.

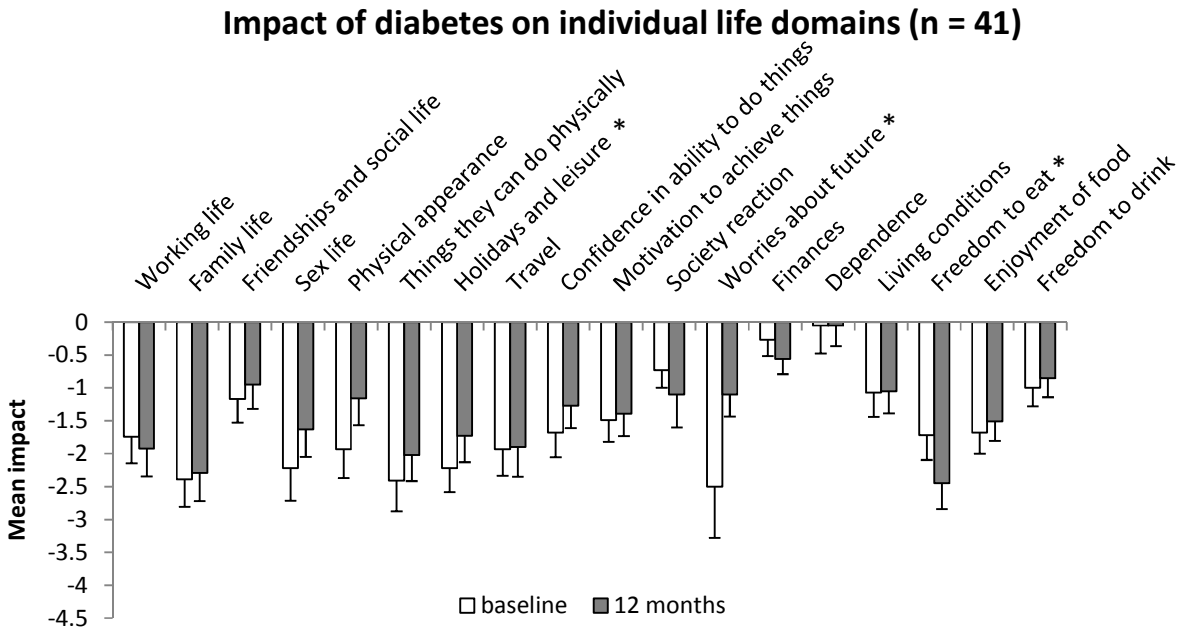


Figure 30a. Impact of diabetes on individual life domains: baseline and 12 month scores for LOCF analysis (n = 41). * marks significant change from baseline to 12 months ($p < 0.05$). Error bars refer to 1 standard deviation.

Completers

At baseline there was no difference in the average weighted impact (AWI) and the 18 items between GL and PSMF. At baseline, completers (n = 14) rated the impact of diabetes on their enjoyment of food more negative than the dropouts (-2.5 ± 0.5 vs. -1.3 ± 0.4. p = 0.011) but the impact of diabetes on their confidence to do things was smaller than for dropouts (-0.9 ± 0.6 vs. -2.1 vs. 0.4, p = 0.029).

For completers, the AWI of diabetes on quality of life showed a bigger change than in LOCF analysis, but it was not significant (decrease from -1.6 ± 0.4 at baseline to -1.1 ± 0.3 at 12 months). There was a significant change only in two life domains: holidays and leisure, and society's reaction to the participants (Figure 30b).

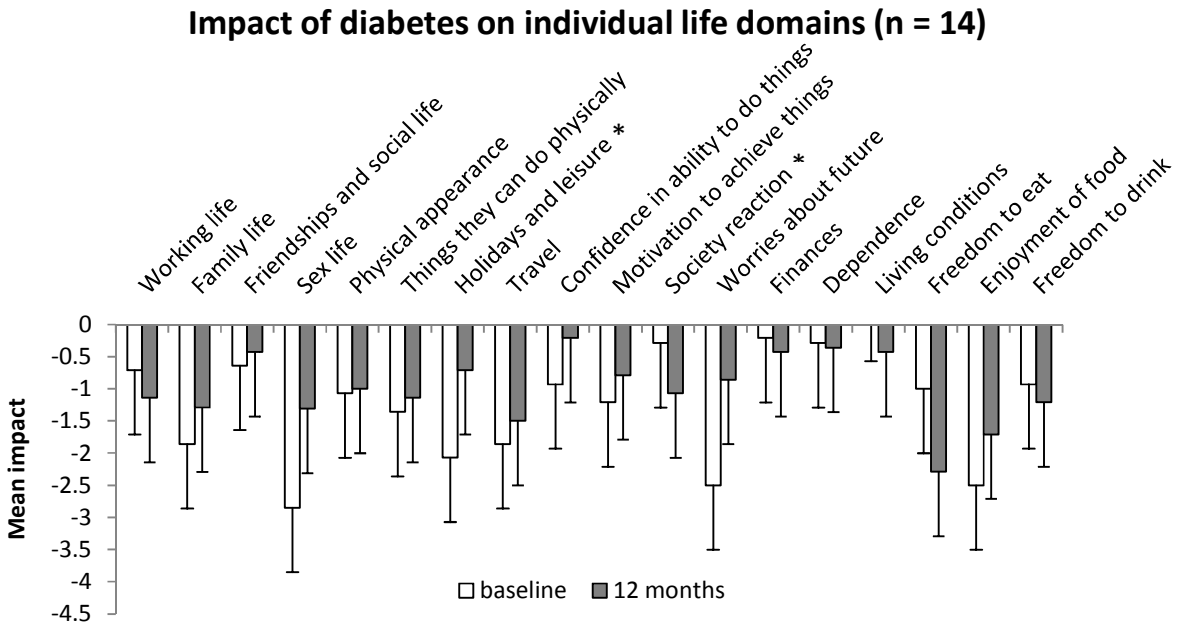


Figure 30b. Impact of diabetes on individual life domains: baseline and 12 month scores for completers (n = 14). * marks significant change from baseline to 12 months ($p < 0.05$). Error bars refer to 1 standard deviation.

4.10.5 Depression

Depression was monitored with the Major Depression Inventory (MDI) scored as a depression rating scale that ranged from 0 to 50.

LOCF

There were no differences between the dietary groups at baseline or during the programme. There was a significant overall decrease in the score during the programme (from 13.0 ± 8.7 at baseline to 10.4 ± 9.7 at 12 months, $p < 0.001$) suggesting an improvement in any depressive states. MDI scores were highest at baseline but even then they were still within the normal ranges and did not indicate depression. There was a positive association between change in weight and change in the MDI score in GL group ($r = 0.547$, $p = 0.028$) but not in PSMF group ($r = -0.040$, $p = 0.849$).

Completers

The results of completers' analysis were very similar to LOCF analysis. There were no baseline differences between the dietary groups or between completers and dropouts. There was a significant

overall decrease in the score during the programme (from 13.2 ± 8.2 at baseline to 8.9 ± 10.5 at 12 months, p = 0.007) suggesting an improvement in any depressive states. MDI scores were highest at baseline but did not indicate depression. There was a positive association between change in weight and change in the MDI score in GL group (r = 0.896, p = 0.016) but not in PSMF group (r = -0.098, p = 0.817).

4.10.6 Energy and Fatigue

A visual analogue scales (VAS-F) were used to evaluate subjective fatigue and energy level changes during the programme (Lee, Hicks et al. 1991).

LOCF

There were no baseline differences between dietary groups. No changes in the fatigue or energy levels were reported (Table 17a), however, there was a trend towards an increase in energy levels towards the end of the study. There was a negative correlation between change in weight and in energy ratings (r = -0.309, p = 0.049).

Table 17a. Changes in the fatigue and energy rating (LOCF, n = 41).

	Baseline	6 months	12 months	Total change	P
VAS-F					
Fatigue [b]	22.0 ± 17.1	22.9 ± 21.4	22.6 ± 19.2	+0.6 ± 13.0	0.799
Energy [a]	51.8 ± 25.8	54.1 ± 20.5	56.9 ± 23.0	+5.1 ± 18.2	0.487

Values presented are in mm on 100mm scale (means±SD). VAS-F, Visual Analogue Scale to measure Fatigue Severity
[a] normal distribution
[b] positive skew, log transformation

Completers

Completers' analysis showed very similar results to LOCF analysis. There were no baseline differences between dietary groups or between the completers and dropouts. No changes in the fatigue or energy levels were reported (Table 17b), however, there was a trend towards an increase in energy levels towards the end of the study. There was no correlation between changes in weight and fatigue ratings. The correlation between change in weight and energy ratings was no longer significant (r = -0.275, p = 0.341).

Table 17b. Changes in the fatigue and energy rating (completers, n = 14).

	Baseline	6 months	12 months	Total change	P
VAS-F					
Fatigue [b]	21.5 ± 14.4	22.1 ± 18.8	21.2 ± 12.7	-0.3 ± 16.3	0.799
Energy [a]	55.7 ± 25.8	57.1 ± 19.6	63.7 ± 23.6	+8.0 ± 24.9	0.487

Values presented are in mm on 100mm scale (means±SD). VAS-F, Visual Analogue Scale to measure Fatigue Severity
[a] normal distribution
[b] positive skew, log transformation

4.10.7 Sleepiness

The Epworth Sleepiness Scale (ESS), which measures the general level of daytime sleepiness, had possible scores between 0 and 24.

LOCF

Figure 31a shows changes in ESS rating throughout the programme. At all time points the scores were not different between PSMF and GL (data not shown). There was an overall decrease in the score ($p < 0.001$).

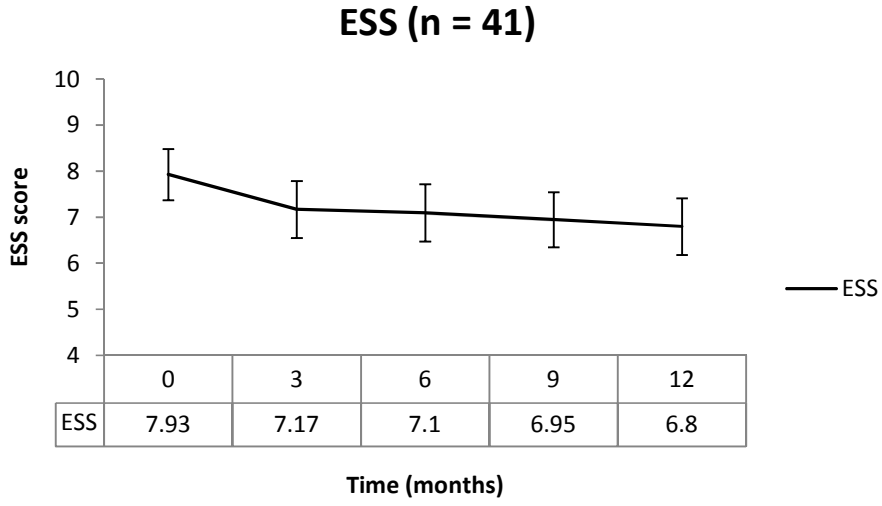


Figure 31a. Change in Epworth Sleepiness Scale scores (LOCF, n = 41).

Completers

At all time points the scores were not different between PSMF and GL, as well as between completers and dropouts. There was an overall decreasing trend (Figure 31b). Despite more pronounced decrease than with LOFC analysis (Figure 31a), the changes did not reach significance, most likely due to the smaller sample size.

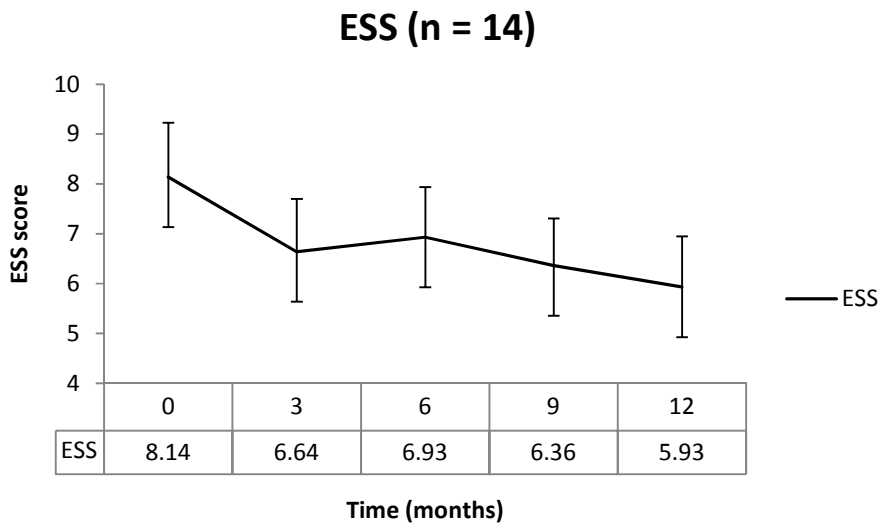


Figure 31b. Change in Epworth Sleepiness Scale scores (completers, n = 14).

4.10.8 Diabetes Treatment satisfaction

The Diabetes Treatment Satisfaction Questionnaire status (DTSQs) was rated on a scale from 0 (dissatisfaction with the treatment) to 36 (full satisfaction with the treatment).

LOCF

At baseline there was no difference between dietary groups in the DTSQs score. Overall, LOCF analysis showed that the diabetes treatment satisfaction increased (28.6 ± 6.9 at baseline to 29.7 ± 6.4 at 12 months, $p = 0.039$). This change was not correlated with change in weight.

Completers

Completers' analysis showed very similar results to LOCF analysis. At baseline there were no differences between dietary groups or between completers and dropouts in the DTSQs score. Overall,

completers' analysis showed that the diabetes treatment satisfaction increased (28.6 ± 7.3 at baseline to 29.5 ± 6.0 at 12 months, p = 0.045).

4.11 Adverse events

Most of the complaints recorded were characteristic for the switch of the main body energy fuel from glucose to fatty acids. During the first 2 weeks of the intervention participants reported headaches (n = 8, 20.0 %), hunger (n = 5, 12.5 %), sickness (n = 4, 10.0 %), diarrhoea (n = 4, 10.0 %), tiredness (n = 4, 10.0 %), nausea (n = 3, 7.5 %), irritated stomach (n = 1, 2.5 %), constipation (n = 1, 2.5 %), bloody stools (n = 1, 2.5 %), and mood swings (n = 1, 2.5 %). Three persons reported hypoglycaemia (Table 18). A subject who experienced constipation, bloody stools and sickness during the first week decided to discontinue the study. Another one, who had a bowel resection in the past, experienced both recurrent diarrhoea and bowel irritation; this subject dropped out after 7 months of participation.

There were no differences between the GL and PSMF groups in the proportion of the subjects with an adverse event (Table 18).

Table 18. Adverse events recorded from 2 weeks to 12 months of the intervention. Presented data are for as many volunteers as there were in the study at the reported times.

Event	Adverse events (n, %)								
	during the first 2 weeks			*at 3 months*			*at 12 months*		
	GL (n=16)	PSMF (n=25)	**Total (n=41)**	GL (n=13)	PSMF (n=15)	**Total (n=28)**	GL (n=6)	PSMF (n=8)	**Total (n=14)**
Mood swings		1 (4.2)	**1 (2.5)**						
Nausea	1 (6.3)	2 (8.3)	**3 (7.5)**		1 (6.7)	**1 (3.6)**			
Sickness	1 (6.3)	3 (12.5)	**4 (10.0)**	2 (15.4)		**2 (7.1)**			
Weakness					1 (6.7)	**1 (3.6)**			
Tiredness	2 (12.5)	2 (8.3)	**4 (10.0)**	1 (7.7)	1 (6.7)	**2 (7.1)**	1 (16.7)	1 (12.5)	**2 (14.3)**
Light-headedness					1 (6.7)	**1 (3.6)**			
Lack of appetite									
Hunger		5 (20.8)	**5 (12.5)**	2 (15.2)		**2 (7.1)**	1 (16.7)		**1 (7.1)**
Irritated stomach	1 (6.3)		**1 (2.5)**	1 (7.7)		**1 (3.6)**	1 (16.7)		**1 (7.1)**
Constipation		1 (4.2)	**1 (2.5)**				1 (16.7)		**1 (7.1)**
Bloody stools		1 (4.2)	**1 (2.5)**						
Diarrhoea	2 (12.5)	2 (8.3)	**4 (10.0)**	3 (23.1)		**3 (10.7)**			
Sound stomach				1 (7.7)		**1 (3.6)**			
Flatulence					1 (6.7)	**1 (3.6)**			
Headache	5 (31.3)	3 (12.5)	**8 (20.0)**		1 (6.7)	**1 (3.6)**			
Halitosis				(7.7)	1 (6.7)	**1 (3.6)**			
Bad taste in mouth					1 (6.7)	**1 (3.6)**			
Hypoglycemia	2 (12.5)	1 (4.2)	**3 (7.5)**	2 (15.4)	1 (6.7)	**3 (10.7)**	1 (16.7)		**1 (7.1)**

GL, Go Lower; PSMF, protein sparing modified fast. Lack of appetite was recorded at 1 month in two participants (GL = 1 and PSMF = 1). Empty fields mean that no adverse events were recorded at a time.

There were five individual serious adverse events recorded during the trial (Table 19). None of them were likely to have been caused by the participation in the programme.

Table 19. Serious adverse events among 41 participants over 12 months (n = 5).

Recorded	Dietary group	Serious adverse event	Related
2nd day	Protein sparing modified fast	Septicaemia; hospitalization	No
first week	Go Lower	Suspected blockage in the bile duct	Very unlikely
first week	Protein sparing modified fast	Kidney stones; hospitalization	Possibly, but unlikely; occurred very early in the trial
10th month	Protein sparing modified fast	Breast cancer; hospitalization	No
11th month	Protein sparing modified fast	Death	No; external post-mortem showed that the cause of death was the coexistence of cardiovascular disease, diabetes, hypertension and chronic obstructive pulmonary disease

5 Discussion

The results from this study showed that for obese individuals with poorly controlled T2DM, the two approaches to high-protein LCKD, PSMF and GL, had similar effects on weight loss, diabetic control and CVD risk after 12 months. The hypothesis that convenience of ready made meals would results in better adherence and consequently greater weight loss at 12 months was not proved. The similar effects of PSMF and GL could be the evidence of matching nutritional composition of the participants' diet in both groups. It could also be an indication that the outcomes of being randomly assigned to either of the investigated approaches for 12 months would be very similar in this population. Overall, the LCHOD improved glycaemia, HDL-cholesterol, TG concentrations, systolic blood pressure, and was safe for obese people with T2DM when used for 12 months.

Due to problems with funding, only n = 41 (and not planned n = 120) volunteers started the programme. Apart from the funding issue, there also was a problem with participant recruitment. Initially, volunteers were identified from the Woolmanhill Diabetic Clinic. As most of the available potential volunteers were approached, new way of recruiting had to be planned. We have applied for and were grated ethical approval to recruit volunteers from GP surgeries in Aberdeen. This was supported by both the MCN lead for Diabetes and the Diabetes lead for Aberdeen City. The study was advertised by the means of posters in GP surgeries (Appendix 11). The participants were planend to be recruited by the PhD student who was responsible for discussing the study with each volunteer individually and for obtaining a signed consent. Unfortunately, the effectiveness of this new approach was not evaluated as the funding for the study did not allow continuation of the recruitment (i.e. the posters were taken down).

Simple randomization, used in this study, is not an ideal approach when recruitment target is not reached. Smaller than planned sample size resulted in uneven group numbers (n = 16 in GL group and n = 25 in PSMF group). Additionally, groups were not matched for gender, which in turn resulted in different proportion of men and women between the groups at baseline (see Results).

The above problem could have been prevented by the appropriate choice of randomisation method. For example, block randomization would help to ensure equal group numbers (Julious et al. 2010). In this approach blocks of *n* participants have equal numbers. Blocked randomisation would minimise the possibility of a long sequence of assignments to the same dietary group. Moreover, to ensure equal

proportions of genders within the groups, stratification into genders should have been conducted (Durham et al. 2008). Alternatively, equal proportions of genders could be assured by the matched-group design – matching for gender between the diet groups of equal numbers (Polgar and Thomas 2008). Finally, a more sophisticated but also more accurate method of minisation could be used. Minimization is an adaptive stratified sampling, recommended when there are a few stratification factors. This method has been given a status of "a platinum standard" (Treasure and MacRae 1998).

The differences between GL and PSMF approaches may appeal to individuals with different lifestyles. Randomisation of participants in our study did not match a given approach to a patient's lifestyle. This explained some of the dropouts we have seen (e.g. inability to adhere to the Go Lower diet when on holiday/business trips; unwillingness to learn about PSMF). Additionally, the fact that Go Lower food was provided for free and foods for the PSMF group were not may have affected decision to drop out from the study, as protein is the most expensive source of energy in the diet (St. Jeor 2000). However, no difference in dropout rates between the two diets was noted at any of the time points. Thus, each diet had advantages and disadvantages which were counterpoised against one another. Personal cost and preparation time may elicit greater engagement with the PSMF, balancing the convenience of GL.

The dropout rate in the study was quite high (66 %) compared to other studies. The anticipated dropout (30%) based on the study by Samaha et al. (2003) unfortunately underestimated the real dropout. Participants did not have to give reasons for discontinuing the present study, however some of them did. Table 3 outlines the times when most of the participants dropped out and, where available, gives reasons for dropout. The reason given most often was the mismatch between lifestyle and the restrictions of the diet. In other words, those who dropped out were not willing to make the changes in their lives that would allow success in the programme. This is an important finding, as it reflects the participants' dedication to the programme and their readiness for change. Lifestyle interventions, unlike drugs, require substantial involvement and work both on the side of a patient as well as a health professional. Perhaps some of the participants did not realize the challenge they took when they joined this one-year-long lifestyle intervention programme. On the other hand, participants were recruited from the secondary care and had poorly controlled diabetes. Most of them were struggling with diabetes for years, had various complications and were recommended lifestyle interventions before. The fact that diabetes and obesity treatments were not successful until the start of the present study was perhaps a proof that this specific population was less likely to succeed in the programme. Among other reasons for dropout were medical concerns. Those were mostly cases when a participant was hospitalized and could not adhere to the diet for a longer time. Also, there was a case when a

participant dropped out due to dissatisfaction with the new body image. Again, this shows lack of readiness to change. In the GL group only, four participants said the reason they dropped out was the fact that they did not enjoy the food. The ready-meals approach has got the disadvatange of restricted choices. The lack of food enjoyment was never the reason for dropout in the PSMF group.

The times of dropout were different between the two approaches. Participans seemed to drop out from the PSMF group at the beginning and at the end of the programme, while dropouts in the GL group were distributed throughout the duration of the study (Table 3). It was clear however, that attrition was high during the first month of the study in both groups (17.6 % dropped out from PSMF and 30 % from the GL). This was in agreement with Larsen et al. (2010) who noticed that most people drop out at the beginning of trials. Interestingly, during the second month none dropped out from the GL group but over 41 % (n = 7) dropped out from the PSMF group. Out of these seven, one dropped out because of the medical concern, one did not give any reason, and the rest blamed lifestyle issues. It can be speculated that after one month of dieting, some of the participants realised that they need to make a permanent change to their diet, food shopping, cooking and eating habits. Perhaps, ready made meals approach of the GL diet made this phase easier by eliminating element of planning, shopping and cooking. Additionally, participants of the GL group by receiving ready meals were being educated about portion sizes and kinds of food that they should be having on this diet. Learning through experience of food and its quantities could have made this stange of the programme easier.

Further investigation of the dropout reasons was planned and ethical approval was obtained. A simple questionnaire was developed asking participants anonymously for the resons of dropping out or completing the programme, and the study arm they were randomized into. The questionnaire was prepared in a written form (Appendix 12). It was planned to be posted to participants after they completed the study or dropped out. The posted materials would also include an addressed and stamped return envelope. The written answers were supposed to undergo thematic analysis to identify what factors affected dropping out from the study or completing the programme. This investigation, however, did not take place because, in light of the lack of funding, the research team decided against it.

The main analyses performed in this study were on 41 participants using the last observation carried forward (LOCF) method to deal with the dropout cases. The completers' analysis was performed as well, however, the sample size in this case was small (n = 14). In the LOCF method, the last recorded measurement was carried forward through the remaining time points to the finish. The approach

allowed keeping the sample size at baseline level and examining trends over time. LOCF is also more widely used than the baseline observation carried forward method or other ways of addressing dropout in clinical trials of dietary intervention. The arguments proposed against using this method include biased estimate of the treatment effect and underestimation of the sample's variability (Liu-Seifert et al. 2010). Last observation carried forward can be a conservative approach (underestimating the effect of a treatment) when considered from the point of view that participants no longer receive any treatment and therefore cannot benefit from it (i.e. lose weight). However, it can overestimate the effect in cases when the intervention is not effective and completers gain weight towards the end of the programme. Based on the available literature it was concluded that the use of a high protein LCHOD is an effective way of addressing excess weight in this population and therefore overestimating the effect in dropouts when using LOCF should not be an issue. Finally, there were different approaches reported to be used to classify dropouts. For example, Larsen et al (2011) did not treat a participant as a dropout when they discontinued the programme and included those cases in the analysis (intention to treat). Therefore, only those that were not available for measurements were treated as dropouts. Contrastingly, in the current study when a patient declared inability to comply with the programme, he or she was excluded. The latter approach provided evidence of the diet's effect in participants who declared adhering to the programme.

5.1 Participants at baseline

The baseline characteristics of the participants in the present study were similar to those reported by the Diabetes Outcome Progression Trial (ADOPT) and The Anglo–Danish–Dutch study of intensive treatment in people with screen-detected diabetes in primary care (ADDITION). ADOPT reported baseline data for Caucasians with T2DM from Europe (n = 2037) and Caucasians from North America (n = 1815) (Viberti et al. 2006). In the ADDITION study, 94.7% of about 3000 participants were Caucasians (Sandbaek et al. 2008). Unlike our study, obesity was not an inclusion criterion in ADOPT or ADDITION, therefore BMIs, waist circumferences and blood pressures were slightly lower (Table 20). The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study reported baseline characteristics in ranges (e.g. 39.4 % of 9795 participants were overweight and 48.4 % were obese) so it was not included in Table 20. The participants for the FIELD study were recruited from Australia, New Zealand and Finland. Caucasians were 92.8 % of the whole sample, the percent of men was 62.7% of the whole sample, and only 56.6 % of the whole sample had hypertension (Scott et al. 2005). The other two large multicentre trials (The Action to Control Cardiovascular Risk in Diabetes, ACCORD and United Kingdom Prospective Diabetes Study, UKPDS) had slightly different

populations to the one from which we have taken our sample. The ACCORD trial recruited only 64.5 % of Caucasians, the remainder being Black and Hispanic (Action to Control Cardiovascular Risk in Diabetes Study Group 2008). The UKPDS included cases of only newly diagnosed diabetes and recruitment age was up to 65 years (UKPDS Group 1991). The similarities between our sample's, ADOPT's and ADDITION's baseline characteristics, suggest that apart from the small number of participants, our sample was comparable to the population of obese Caucasians with T2DM.

Table 20. Baseline characteristics of ADOPT and ADDITION cohorts compared to our sample.

	ADOPT North America **(n = 1815)**	**ADOPT** Europe **(n = 2037)**	**ADDITION** NL, UK, DK **(n 3000)**	**Current study** Scotland **(n = 41)**
Age (years)	56 ± 10	58 ± 10	60 ± 7	56 ± 9
Men (%)	56.5	60.6	57.9	41.5
BMI (kg/m^2)	33.4 ± 6.8	31.0 ± 5.5	31.6 ± 5.6	38.8 ± 5.8
Waist (cm)	108 ± 15	104 ± 14	107 ± 13	122 ± 12
SBP (mmHg)	130 ± 14	137 ± 16	151 ± 23	152 ± 19
DBP (mmHg)	79 ± 9	82 ± 9	87 ± 12	85 ± 10
Antihypertensive therapy (%)	74.2	84.2	41.0	78.0

Values represent mean and 1 standard deviation.

Baseline characteristics of participants could influence the response to the diet (Noakes et al. 2005). For example, Noakes and co-workers (2005) found no difference in the effects of high-protein and high-carbohydrate diets in women with low TG concentrations at 3 months but reported greater fat mass loss (6.4 ± 0.7 kg vs. 3.4 ± 0.7 kg) in high-protein compared to high-carbohydrate diet in women with baseline TG concentrations >1.5mmol/L. They chose TG as a marker of insulin resistance syndrome. The group proposed that an interaction of phenotype and diet can influence weight loss and suggested a mechanism of higher weight loss in the high TG group. We have observed no such relationship in our study. However, all of the participants in our study had T2DM, and hence insulin resistance. The reason could be the fact that people with T2DM find it more difficult to lose weight that those without diabetes (Pi-Sunnyer 2005). The mean fat mass loss in women was -8.1 ± 4.5 kg at 3 months (n = 15; those who completed 3 months) and -3.0 ± 7.3 kg at 12 months (n = 6; those who completed 12 months). This fat mass loss was therefore similar to the one reported by Noakes et al (2005) in women with TG > 1.5 mmol/l. If the high TG levels in the Noakes et al (2005) study indicated insulin resistance, then what the current study have observed seems to confirm their

suggestion that women who are insulin resistant lose more fat mass on a high-protein diet at 3 months. It has to be noted that firstly, the present study had a smaller number of participants; and secondly the nutritional composition of the diet in these two studies was slightly different; the diet in the Noakes study had more protein (34% vs. 24%) and carbohydrate (46% vs. 31%) but less fat (20% vs. 47%).

5.2 Weight loss and body composition

Participants on both diets achieved mean weight loss of about 5.5 kg which was just over 5% of their body mass. This significant decrease at 12 months was in agreement with findings from the six previous trials investigating effects of LCHODs on weight loss in obese patients with T2DM or insulin resistance (Robertson et al. 2002, McAuley et al. 2006, Dashti et al. 2007, Davis et al. 2009, Elhayany et al. 2010, Larsen et al. 2011).

The body mass loss in the current study equated to the median response in the above studies. As described before, the amount of carbohydrate in the diet throughout the study was higher than recommended (126 ± 91 g/d vs. recommended 40g/d or less). Also, with carbohydrate restriction it was expected for the energy intake to be about 1200 kcal/d, in the current study at 12 month 1467 ± 637 kcal/d were reported. However, the reported dietary intake had very similar gross composition to the low-carbohydrate Mediterranean diet described by Elhayany et al (2010) (CHO, 35%; P, 20%; F, 45 %). In that study participants lost over 10 kg of body mass over the 12 months of the programme. Apart from the similar reported macronutrient composition of the diet and the fact that participants reported slightly higher energy intakes in that study, their weight loss was almost double the one observed in the current study. Elhayany et al (2010) reported that the low carbohydrate Mediterranean diet contained 23% of energy from monounsaturated fatty acids (MUFA) and only 7% from saturated fatty acids (SFA). We did not investigate this aspect of the diet. It is not certain whether fatty acid composition could account for a difference in weight loss. The population in both studies was similar (overweight and obese with T2DM); however, Elhayany et al (2010) conducted their trial in Israel where the Mediterranean diet is a more habitual way of eating than the LCKD is in Scotland. Greater familiarity with the diet, along with more varied fresh products may have resulted in better adherence and more accurate dietary reporting in their study. Elhayany and associates used 24-h food recall questionnaire and a food frequency questionnaire to collect dietary data, while we have used 3-day food diaries (2 week days and one weekend day). The method chosen in the current study was shown to be representative of seven day habitual intake in free living subjects (Fyfe, Stewart et al. 2010).

Finally, in the Elhayany et al study, participants attended 24 scheduled meetings as opposed to our 14 meetings. Interestingly, in a study by Larsen and colleagues (2011), where weight loss on a high-protein diet was only -2.2 kg, there was only 9 individual and 4 group visits conducted. This suggests that the main difference observed was due to more intensive support in the Elhayany study.

Due to the small numbers, the current study did not have the statistical power to ascertain any difference in weight loss between the two approaches. As a result, it cannot be assumed the weight loss would be similar in both diets in a much larger sample.

Fat mass accounted for the most of the weight lost (-5.1 ± 6.7 kg, $p < 0.001$) while reduction in fat free mass was not significant (-0.3 ± 3.7, $p = 0.350$). This confirms our hypothesis that high protein component of the diet would result in losing fat mass and not fat free mass regardless of the approach (ready-made meals vs. self-prepared meals). Preservation of fat free mass during weight loss is beneficial as it increases chances of successful weight loss maintenance and, in those with T2DM, improves glucose control.

Out of the five randomised trials mentioned earlier, only one reported changes in body composition. McAuley and associates (2006) used bioelectrical impedance and showed that on high-fat LCHOD (CHO, 33 %; P, 21 %; F, 41 %) women lost 3.4 kg of fat mass and 2.0 kg of fat free mass. Fat mass loss could have been underestimated due to the choice of measurement method. The bioelectrical impedance method assumes that hydration of FFM is constant and not different between normal weight and obese individuals (Deurenberg 1996). However, body water distribution changes with the fluctuating weight and glycogen depletion (that may occur during restriction of dietary carbohydrate intake). As prediction formulas are developed in normal-weight subjects, fat mass in obese individuals may be underestimated (Deurenberg 1996). This seemed to be confirmed in the present study, because at all time points FM (%) measurements were about 5% lower than the ones delivered by the air displacement plethysmography ($p < 0.001$). In the current study most of the body mass lost was fat and there was no difference in body composition changes between men and women. The fact that most of the weight lost was FM was perhaps a result of a higher reported protein content of the diet. Another factor that may preserve fat free mass during weight loss is exercise, but it was not a part of the current study.

There were also four other 12 month long trials of LCHOD that reported body composition changes; however participants in those studies did not have T2DM or insulin resistance. All of the four studies

used dual-energy X-ray absorptiometry (DXA). Gardner et al (2007), Clifton et al (2008), and Due et al (2004) reported CHO intakes being 35 % of total energy intake or higher and weight loss about 5 kg with FM constituting about three quarters of it. The reported macronutrient proportions in the study by Brinkworth and colleagues (2009) were different (CHO, 8.9%; P, 32.3 %; F, 54.9 %) and weight loss was greater (-14.5 ± 1.7 kg). Out of 14.5 kg of body mass lost, 11.3 kg was fat mass. Based on those results, it seems that weight loss was more pronounced and FFM loss was smaller with very low carbohydrate intake suggesting that protein sparing enhances weight loss. It has to be noted, however, that DXA interprets glycogen as FFM and water as about 8 % fat. Some of the apparent FFM loss will be due to these.

5.3 Dietary intake and adherence

The total energy intake and macronutrient composition of the diet (carbohydrate, fat and protein content) were analysed. Alcohol, if consumed, was included into the total calorie count. Alcohol was consumed in very small quantities by only some participants; therefore it was not distinguished along carbohydrate, fat and protein as a source of energy.

The baseline dietary macronutrient composition of the participants in the current study was not different between the ready-made meals and self-prepared meals, and had slightly higher fat and lower carbohydrate contents (CHO, 41 %; P, 20 % (less than 1g/kg body mass); F, 39 %) than the levels recommended by Diabetes UK (CHO, 45-60%, P not more than 1g/kg body mass; F less than 35 %) (Connor et al. 2003). Ma and associates (2006) have found the similar trend in the population of obese patients with poorly controlled T2DM (HbA_{1c} > 7%) in the United States (CHO, 35%; P, 20%; F, 45%). The authors expressed concern for cardiovascular implications of this macronutrient composition in the vulnerable group of type 2 diabetics.

It is known that a standard energy restricted, low-fat diet may be difficult to adhere to (Hession et al. 2009) and the high-protein LCHOD is an alternative method for weight loss. The only restriction put on patients on a high-protein LCHOD is in the amount of carbohydrate they ingest. The recommended carbohydrate intake in the current intervention was 40g/d. At this, or a lower intake, it is likely that participants develop ketosis.

Efficiency of detection of ketone bodies by electrochemical sensor was shown to be better than the measurement in the urine by using a reagent strip (Guerci et al. 2003). In the present study, ketone

body concentrations both in urine (acetoacetic acid) and capillary blood (β-hydroxybutyrate) did not change through the study in neither of the dietary groups, remaining at their baseline levels. This could be an indication of poor adherence or diluted urine if a lot of liquids were consumed. The analysis of food diaries confirmed that the intake of carbohydrate at 6 months and at 12 months was about 120 g/d (Table 8), which was 80 g/d higher than recommended and too high to induce ketosis. Diets with more than 60 g/d of CHO are unlikely to have a ketotic effect. The LCKD was advised as it was shown to be a more successful way of reducing body mass than a low-fat diet at 6 months and as successful at 12 months (Hession et al. 2009). In the present study participants did not achieve ketosis. Therefore, the expected physiological effects (hormonal changes that trigger lipolysis) could not have taken place. The participants lost on average 5.5 ± 7.3 kg of body mass at 12 months, however, this was rather modest compared to the weight loss reported by Dashti et al (2007) in which obese patients with T2DM on LCKD for a year decreased their body mass from 108.1 ± 21.21 to 83.5 ± 18.0 kg. It can be only speculated that if the ketosis was achieved, weight loss would have been much higher.

Poor adherence was not unique to the present study. McAuley and associates (2006) investigated effects of high carbohydrate, high-protein and high-fat (low-carbohydrate) diets on weight loss in overweight, insulin resistant women (n = 93) and at 12 months concluded that dietary compliance was poor in all groups. The high-fat diet was based on Atkins' idea and carbohydrate intake at 12 months was supposed to be between 20 and 100 g/d; carbohydrate intake was reported to be about 160g/d (33% of total energy intake). This was even higher than what we have seen in our study (less than 31%). Similarly, in another twelve month long study by Davis and associates (2009) where the LCKD was based on the Atkins diet, at 12 months reported carbohydrate intake was 33% of total energy intake. Davis et al (2009) suggested that a high percentage of Hispanics in their study may have been the reason, as diets of Hispanics traditionally had higher carbohydrate amounts than the general U.S. population's diet, and therefore switching to a low-carbohydrate regime could have been more difficult for the participants. In the current study, participant's reported baseline carbohydrate intake was actually lower than recommended by the Diabetes UK (41.4 ± 9.4 % of energy intake; the recommended range is 45-60 % of energy intake). This could have been caused by underreporting as participants at the time of filling in food diaries were aware of the low-carbohydrate nature of the intervention. The reason for the decrease in compliance with time could have been limited food choice. Carbohydrate foods are a principal part of many diets, and removing them may be difficult in a long term.

Serum urea increased significantly from baseline to 12 months (6.1 ± 1.8 to 6.8 ± 2.2 mmol/L, $p < 0.001$), as did albumin (45 ± 2 to 46 ± 3 g/l, $p < 0.001$). This effect cannot be associated with the fact that the diet was high in protein because the reported amount of dietary protein did not change when expressed as grams per day ($p = 0.458$) (it increased when expressed as percentage of total energy intake). It was discovered previously by Keys (1950) that albumin concentration did not change during 24 weeks of semi starvation (Energy, 1570 kcal/d; P, 12.7 %; CHO, 70 %; F, 17.2 %) during which mean weight loss was 16.9 kg (24 % of baseline body weight). Albumin concentration was also found to increase after a 3-day total starvation (Broom et al. 1986). This was related to albumin diffusion coefficient change rather than to the albumin production rates. Urea concentrations increased most likely because more protein was metabolized (Broom 1986) rather than as the effect of high-protein diet.

Most of the compliance measures (ketone bodies and plasma urea concentrations) in completers were no different to dropouts, and there were no differences in the reported energy or fat (g/d) intakes between these two groups. However, completers reported lower intakes of carbohydrate (g/d). This was only significant at 6 months (141 vs. 78 g/d, $p = 0.038$). Completers also reported higher protein intakes but this was only significant at 9 months (98 vs. 75 g/d, $p = 0.041$). Only one measure of compliance, serum albumin concentration, was higher in completers at 6 months (46.9 vs. 45.4 g/l, $p = 0.036$) which is in agreement with slightly higher reported intake of protein in this group (g/d) (Mutlu et al. 2006). It is not entirely clear why the completers were better at adhering to the programme. There were no differences in any of the measures that were taken at baseline between completers and those who dropped out. It could be that other than the weight loss effects of the diet (perhaps glycaemic control or improved wellbeing) encouraged completers to continue. The difference could have also been in factors not measured in the present study, such as participant's family or friends' support or participant's own goals.

5.4 Resting metabolic rate

In the present study RMR (kcal/d) significantly decreased (-137 ± 265 kcal/d, $p < 0.001$) at 12 months and there was no difference in changes between the genders or dietary groups. However, the range of individual changes was rather wide from -1040 kcal/d to +184 kcal/d. Therefore, following the idea of Senechal and colleagues (2010) the whole sample (n = 41) was divided into participants whose RMR at 12 months increased 5 % from baseline and into those whose RMR did not increase more than 5 % from baseline value. We have found that over 12 months, RMR increased in the "increasers" group (n

= 4) from 1597 ± 421 to 1713 ± 461 kcal/d ($p = 0.065$) and decreased in the "decreasers" group (n = 37) from 2029 ± 423 to 1864 ± 394 ($p < 0.001$). As a result, the difference in RMR between these two groups changed, from approaching significance ($p = 0.054$) at baseline to non significant ($p = 0.653$) at 12 months. Unlike Senechal et al (2010), there were no observed differences in baseline fat mass between these two groups. There could have been several possible reasons for this. Participants in Senechal et al (2010) study were only non-diabetic women who on average lost 9.5 kg of body mass (about 12% of baseline value) on a calorie restricted diet (CHO, 55%; P, 15%; F, 30%) with no information about body composition changes. Therefore, their intervention was shorter and much more intensive. It is possible that if participants in the current study lost 12% of their initial body mass, changes in RMR would classify them differently to the groups with increased or decreased RMR.

None of the 6- and 12-month-long trials of LCHOD in obese with T2DM, insulin resistance or obese but otherwise healthy participants reported RMR measurements. Trials investigating other dietary interventions however, showed results similar to ours. Those studies used mostly energy restriction with the general aim to keep macronutrient composition close to the standard recommendations (CHO, 55%; P, 15%; F, 30%). For example, in a 12 month long programme of 121 overweight sedentary men who achieved significant body and fat mass loss, RMR (both kcal/d and kcal/FFM/d) significantly decreased (Frey-Hewitt et al. 1990). Interestingly, in a parallel group that received the same dietary intervention but also undertook exercise (jogging), RMR did not change (Frey-Hewitt et al 1990). In the Minnesota experiment (Keys et al. 1950), change in basal metabolic rate was expressed as percentage of baseline oxygen volume uptake. It was reported that after 6 months of semi-starvation, oxygen consumption declined with weight loss, achieving about 60 % of the baseline value per average person but about 80 % of baseline value when expressed in kg of body mass and about 85 % when expressed in kg of active tissue (Keys et al. 1950). It should be noted that participants in this experiment were not overweight and they lost about 24% of body mass during the 6 months of semi-starvation.

LOCF analysis showed that females had significantly higher RMR (kcal/kg FFM/d) than men at all time points (Table 11). This was not the case in completers. In contrast, a study of 114 males and 121 females without diabetes found that RMR was 5-10 % lower in females than in males after adjusting for differences in body composition, age and activity (Ferraro et al. 1992). This theoretically should be applicable to the present study because there was no association found between insulin resistance and RMR (De Luis et al. 2006). The higher RMR (adjusted for FFM) in women in the current study was found probably due to the small sample.

Severely obese patients with T2DM have higher measured RMR than obese non-diabetic people (Huang et al. 2004, Bitz et al. 2004, Pi-Sunyer 2005). Therefore, Bitz et al. (2004) suggested that a new predictive equation for people with T2DM should be developed. Huang and associates (2004) proposed an equation that included age, gender, body mass, stature and information about the presence of diabetes. The equation proposed by Huang et al. (2004) explained 75% of the variation in RMR in their participants.

All twenty nine equations that were tested, underestimated RMR in obese people with poorly controlled T2DM. The equation by Lazzer et al. (2007) was the most accurate in estimating RMR; however its accuracy was only 62.5 %. There was no accurate equation available to predict RMR in obese patients with poorly controlled T2DM. Additionally, weight based and fat free mass based equations were compared. Fat free mass based equations did not seem to be more accurate than weight based ones. Therefore, RMR prediction accuracy in this population may not be improved by body composition data.

Based on the data from the current study, an RMR prediction equation was developed for obese people with T2DM. The equation included FFM, waist circumference, gender and glycosylated haemoglobin (section 1.6.2, Model 1), and explained 81% of the variability in RMR. Due to the fact that glycosylated haemoglobin explained only about 1% of the variability in RMR, another model was suggested that included only FFM, waist circumference and gender (section 1.6.2, Model 2). The second model explained 79.4 % of the variability in RMR, and it therefore could also be used when glycosylated haemoglobin data are not available. Moreover, two equations were developed separately for men (Model 4) and women (Model 5). Model 4 explaned as much as 84.8 % of variability in RMR and apart from FFM and waist circumference included glycosylated haemoglobin. Model 5 explained 77.1 % of variability in RMR and included only FFM and waist circumference. Based on this sample, glycosylated haemoglobin did not explain variability in RMR in women. Gougeon and colleagues (2002) reported that prediction of RMR in people with T2DM was improved by including glycaemic control into the equation. This therefore, was confirmed only in men in the current study. Prediction of RMR based on the larger sample of both men and women would be needed to confirm these results.

The equation from Model 1 did not explain 19 % of the variability in the RMR. Weyer and colleagues (1999) proposed that considerable variability in RMR that remains after adjusting for the variables described previously, is partly genetically determined. Moreover, it has been suggested that

sulphonylurea therapy was associated with reduction in RMR (Nair et al. 1984, Bogardus et al. 1986). It was not the case in the current study, as participants were instructed to discontinue sulphonylureas when included in the programme because some of the side effects (weight gain, diarrhoea, constipation or loss of appetite) could influence weight loss. However, insulin therapy could have been one of the factors explaining some of the remaining 19 % of variability in RMR. Welle and associates (1988) reported that insulin therapy was associated with the reduction in RMR. While Gougeon and colleagues (2002) reported that prediction of RMR in people with T2DM was improved by including glycaemic control into the equation, their equation had only 60.7 % accuracy when used for the current study's participants (see Figure 21). The equation proposed by these authors included weight, hip circumference and fasting plasma glucose and explained 81% of the variability in RMR (Gougeon et al. 2002). Both the current and the Gougeon et al (2002) studies had similar participant characteristics, sample sizes, and variables measured. The difference in the factors resulted perhaps from the body composition measurement method. In the study by Gougeon et al (2002) body composition was determined by the bioelectrical impedance analysis as opposed to the air displacement plethysmography method (BodPod) which was used in the present study. Air displacement plethysmography is currently one of the most accurate available methods of body composition assessment and often used as a standard (Marcias et al. 2007).

5.5 Glycaemic control

Significant improvement in insulin resistance and glycaemic control in the current study suggests a positive effect of the high-protein LCKD in the investigated population. The changes in hypoglycaemic medication were not likely to be influential in the improvements because only three of the fourteen participants that completed the study had their hypoglycaemic medication reduced or withdrawn and one had the dose of metformin increased.

The fasting plasma glucose concentration was 10.2 ± 3.5 mmol/l at baseline, which was comparable to the baseline values reported by Dashti et al. (2007) which was 10.5 ± 3mmol/l and Elhayany et al. (2010) which were 10.3 ± 1.7, 10.1 ± 1.8 and 10.5 ± 2.0 mmol/l for three randomised groups. Both Dashti et al (2007) and Elhayany et al (2010) reported significant decreases in fasting glucose concentrations. In the current study, there was a decreasing trend but it did not reach significance possibly due to the small sample size. Other 12 month long trials involving type 2 diabetics did not report fasting glucose but the HbA_{1c} changes. The HbA_{1c} is considered to be a better representative of

diabetic control as it identifies the average plasma glucose concentration over 2 to 3 months (American Diabetes Association 2008).

Glycosylated haemoglobin significantly decreased at 12 months (-0.4 ± 1.1 %, $p < 0.001$). This was in agreement with most of the other studies that reported this measure (Robertson et al. 2002, Elhayany et al. 2010, and Larsen et al. 2011) apart from the study by Davis et al. (2009). The fact that weight loss of 5 to 10% of body mass improves diabetic control (Turner et al. 2002) may explain why Davis et al. (2009) did not see HbA_{1c} decrease at 12 months; the average weight loss in that study was 3.3% of the initial body mass. Moreover, the duration of T2DM could have been a factor. Daly et al (2006) proposed that with an increasing duration of T2DM, the capacity for an improvement in glycaemic control diminishes. The positive relationship between the changes in HbA_{1c} and weight (Figure 25) in the current study confirms the importance of weight management in patients with T2DM.

There was a decrease in overall fasting plasma insulin concentration in the current study (-1.9 ± 9.0, $p = 0.005$). Both the participants with baseline insulin therapy and those without showed the decrease. McAuley et al (2006) who recruited insulin-resistant, but not diabetic, women reported a similar decrease. Elhayany et al (2010), however, saw a significant increase in fasting insulin concentrations. The patients who were on insulin treatment were not included in their trial. There were no striking baseline differences between participants in the present study and two others. Also insulin resistance measured by HOMA improved in the present study as well as in the Elhayany et al (2010) study. Reported dietary compositions were also similar, therefore it is unclear why the difference occurred.

Homeostatic model assessment (HOMA) assesses β-cell function and insulin resistance from fasting glucose and insulin concentrations (Matthews et al. 1985). It is a suitable method to assess the effects of a treatment (Wallace et al. 2004). Although change in fasting glucose in the current study was not significant, there was an overall significant decrease in the insulin resistance measurement by HOMA (-1.2 ± 5.6, $p = 0.018$). Improvement in HOMA index was also seen at 12 months by Elhayany and colleagues (2010). Other 12 month long trials did not report this measure probably due to the lack of both fasting glucose and insulin concentration data.

The fatty acid composition of the diet may play a role in improving glycaemic control in patients who are insulin resistant or have T2DM. Elhayany proposed that a low carbohydrate Mediterranean diet rich in MUFA (reported CHO, 35%; P, 20%; F, 45 %), was better at improving glycaemic control than traditional Mediterranean diet or diet recommended by the ADA (2003). In contrast, Larsen and

colleagues suggested that glycaemic control was improved by the reduction in energy content of the diet rather than by the nutritional composition (reported values at 12 months for high-protein diet were CHO, 42 %, P, 26 %; F, 30.7 % and for high-carbohydrate diet was CHO, 48 %; P, 19 %; F, 32 %). The main difference between the two above studies seems to be in the contents of fat. However, Davis and colleagues (2009) stated that the Atkins diet (reported at 12 months CHO, 33%; P, 23%; F, 44%) had effects on glycaemia similar to that of a low-fat diet (CHO, 50 %; P, 19 %; F, 31 %). Those seemingly contradictory results may be explained when fatty acid composition of dietary fat is taken into account. McAuley stressed that when recommending high-protein diet, an appropriate composition of dietary fat must be ensured. As expected, the diet in McAuley et al. (2006) and Larsen et al. (2011) studies had about twice as much SFA and half the amount of MUFA and PUFA by comparison. Unsurprisingly, consumption of MUFA was suggested to improve insulin sensitivity (Elhayany et al. 2010, Brunerova et al. 2007). It is possible that for successful longer term dietary intervention for obese patients with T2DM the recommended macronutrient proportion is not as important as the type of carbohydrate (low glycaemic index) and fat (proportion of SFA, MUFA and PUFA).

5.6 Cardiovascular disease risk

One of the major arguments against the use of high-protein diets, according to the AHA, was that the high intakes of saturated fat, cholesterol, and other associated dietary factors, may cause increased risk for coronary heart disease (St. Jeor et al. 2001). Lipid profile outcomes of LCHODs usually include decreases in serum TG levels and increases in HDL cholesterol (Yancy et al. 2004, Nordmann et al. 2006). These favourable changes are contrasted by elevated LDL-cholesterol levels and total cholesterol levels. In their review, Nordmann and colleagues (2006) noted that every 10% decrease in CHO intake decreased TG by 7.6 ± 0.6 % but highlighted that it was not known whether favourable effects of LCHODs on lipid profiles outweighed adverse effects. However, it has been observed that LCHOD was associated with an increase in LDL-cholesterol particle size, from small and dense to large and buoyant (Volek et al. 2005b, Westman et al. 2006, Morgan et al. 2009). This change is considered beneficial, as high levels of small, dense LDL cholesterol are associated with an increased CVD risk (Lamarche et al. 1997). Additionally, accumulation of large LDL-cholesterol sub fraction was not associated with an increased CVD risk (St-Pierre et al. 2005, Volek et al. 2005a). Moreover, since 2006 when Nordmann and associates published their review, several new reviews have been published. Accurso et al (2008) reviewed the use of LCHODs in T2DM and metabolic syndrome and stated that carbohydrate restriction, even without weight loss, improves markers of cardiovascular

health. Similar conclusions were presented by Volek et al (2008) and Acheson (2010). Boling and associates (2009) reviewed the use of LCHOD for obesity and related diseases and concluded that LCHODs improved dyslipidaemia associated with the metabolic syndrome better than diets higher in carbohydrate or lower in fat. The effect on LCHOD on LDL-cholesterol was found to be mostly neutral or beneficial but due to the wide range of LDL-cholesterol responses, Boling et al (2009) suggested monitoring lipid profiles of patients on LCHODs.

Confirming the beneficial effect of LCHOD in obese patients with T2DM, the lipid profile in the current study improved. HDL-cholesterol increased and TG decreased. LDL-cholesterol and total cholesterol did not change (Table 14), possibly because they were in the normal ranges at baseline. Systolic BP decreased while diastolic BP did not change (Table 14). The completers' analysis showed very similar outcomes.

It has been demonstrated that LCHODs are safe and beneficial for obese individuals with T2DM. McLaughlin and associates (2006) reported that although there was no difference in weight loss between the patients on the high protein diet (CHO, 15 %; P, 40%) compared to the standard diet (CHO, 60 %; P, 15%) after 4 months, obese hyperinsulinaemic patients had greater reduction in fasting TG, higher increase in HDL-cholesterol and an increase in LDL cholesterol particle size. It was concluded that high-protein, high-fat diets for the short term can be efficient in reducing markers for cardiovascular disease risk in obese hyperinsulinaemic people (McLaughlin et al. 2006). Similarly, replacement of dietary carbohydrate with protein, showed to have beneficial effects at 6 months by Stern and co-workers (2004). They reported that although weight loss was similar between LCKD (less than 30g CHO/day) and conventional diet with an energy deficit of 500kcal (less than 30% F), the effects on atherogenic dyslipidaemia and glycaemic control were more favourable with LCKD. It was suggested that in obese people, who may have high carbohydrate intake at baseline, the restriction of carbohydrates for 6 months may have beneficial metabolic effects (Stern et al. 2004, Volek et al. 2004). Brinkworth and co-workers (2004) proposed that high-protein diets in people with T2DM may, in the long term (over 1 year), decrease cardiovascular risk more than low-protein diets with similar weight loss. The group showed that high-protein diet (planned CHO, 40%; P, 30%; F, 30% but reported CHO, 46%; P, 20%; F, 32%) attenuated blood pressure increase with weight regain after weight loss. The finding supported reports from larger studies (Liu et al 2002, Appel 2003) that dietary protein intake may be negatively related to blood pressure. Finally, Dashti and colleagues (2007) reported that LCKD had beneficial effects in obese people with T2DM after 1 year. They compared the effect of LCKDs in obese subjects with either high or normal blood glucose levels. After 56 weeks of

the diet, body weight, BMI, blood glucose, total cholesterol, LDL and HDL cholesterol, and TG improved in both groups. However, this effect was more pronounced in subjects with high baseline glucose levels. The group concluded that LCKDs are safe and beneficial for long-term use by obese diabetic individuals.

The observed decrease in blood pressure in the current study (systolic: -6 ± 14 mmHg, p = 0.014; diastolic: -2 ± 10 mmHg, p = 0.234) could have been related to weight loss. The new SIGN guideline on obesity (2010) stated that a 5kg weight loss at 12 months is associated with systolic blood pressure decrease from 3.8 to 4.4 mmHg and diastolic blood pressure reduction from 3.0 to 3.6 mmHg. However, due to large individual variation and possible confounding variables, a specific effect cannot be implied. Changes in blood pressure in the current study were very similar to those reported at 12 months in people with T2DM by Davis et al (2009) and Larsen et al (2011).The decrease in blood pressure can also be facilitated by an increase in the dietary protein content. It has been previously shown that blood pressure may be inversely related to dietary protein intake (Appel 2003, Liu et al. 2002).

The Framingham CVD risk score (%) showed a decreasing trend but did not significantly change during the study remaining quite high. At 12 months, fourteen participants (3 in completers' analysis) had CVD risk over 30%. Similarly, at 12 months almost 70% participants had their heart age estimated to be over 80 years old (mean age was 55.6 ± 9.9 years). It is important to note that the population investigated came from secondary care, and therefore most of the participants in the study had diabetes and its complications long before joining the programme. The Framingham risk score (D'Agostino et al, 2008) seems to be better suited to estimate CVD risk in the general population or in newly diagnosed diabetics (D'Agostino RB et al. 2008).

Changes in CVD risk can also be estimated from the changes in concentration of plasma C-reactive protein (CRP) as elevated CRP levels are associated with an increased risk for cardiovascular disease. As described in the introduction, CRP is a marker of inflammation. CRP was initially planned to be analyzed, however, CRP assay provided by the Clinical Biochemistry reported specific values only if they were over 10 mg/l as those levels are considered alarming (Shine et al. 1981, Seshadri et al. 2004). Only highly sensitive assay of CRP (hsCRP) could detect differences that would be caused by the intervention like ours as changes would probably be in the range between 1 and 4 mg/l (Kip et al. 2004). The analysis of plasma samples in Robert Gordon University has been considered, however it was not delivered due to the funding issues.

5.7 Liver and kidney function

The data from the UK Prospective Diabetes Study stressed the role of blood pressure and glycaemic control in prevention of diabetic nephropathy (Bilous 2008). It has been agreed that the most effective strategies for preventing diabetic nephropathy is to achieve appropriate glycaemic control (HbA_{1c} < 7%), blood pressure (<130/80 mmHg) and normalize LDL-cholesterol level (<2.6 mmol/l). The strategies also include the use of the drugs that block the rennin-angiotensin-aldosterone system (Gross et al. 2005). Although the science, medicine and pharmacology are advancing, the prevalence of diabetes as a cause of chronic kidney disease is increasing (Eknoyan 2005). There are different reasons for renal lesions in T2DM and some of the kidney damage cannot be reversed, especially that related to ageing. However, pancreas transplant studies have shown that normo-glycaemia sustained for ten years can reverse kidney damage (Firetto et al. 2006). It has not been shown whether the improvement in blood pressure and other factors would induce the regeneration of the damaged kidneys in T2DM.

Impaired liver or kidney functions were exclusion criteria in the current study. After 12 months of the LCHOD kidney function was not altered and some of the markers of liver function tests improved (ALT, -5 ± 15 U/L, $P < 0.001$; GGT, -9 ± 37 U/l, $p < 0.0014$). There were no differences between the GL and PSMF in any kidney or liver function tests. Completers' results were not significantly different.

Creatinine concentration was in the low end of the normal range at baseline and showed an increasing trend but it did not significantly change (Table 15). Creatinine clearance adjusted for body surface area was in the low end of the normal range at baseline as well and did not change during the study (Table 15). Estimated glomerular filtration rate (eGFR) calculated from the CKD-EPI equation, which was shown to be more accurate than the Modification of Diet in Renal Disease Study equation (MDRD) (Levey et al. 2009), showed a decreasing trend but again the change was not significant (Table 15). Similarly, a recent randomised controlled trial demonstrated that renal function was not adversely affected by weight loss on a LCKD (planned CHO less than 4% energy) in obese but otherwise healthy individuals (Brinkworth et al. 2010). These results were not different from the outcomes of weight loss on a high-carbohydrate diet in the study by Brinkworth et al (2010).

The burden of liver disease in patients with T2DM is significant and the prevalence of elevated liver enzymes in T2DM is quite high. Kotronen et al. (Kotronen et al. 2008) have reported that people with

T2DM have 80% more fat in the liver than healthy people matched for age, weight and sex. Elevated levels of ALT (found in 16% of people with T2DM) and GGT (found in 23% of people with T2DM) were found to be related to BMI, metabolic control and the presence of metabolic syndrome (Forlani et al. 2008). The aetiologies of elevated liver enzymes vary, but a common one is non-alcoholic fatty liver disease (NAFLD). The presence of NAFLD was associated with abdominal obesity, elevated levels of TG and elevated but still normal ALT levels (Leite et al. 2008).

The significant decreases in two liver enzyme levels: alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) observed in this study would suggest an improvement in liver function when using LCHOD in poorly controlled T2DM (Table 15). Both the mean ALT and ALKP concentrations were above the normal ranges at baseline but at 12 months they decreased to be in the upper limits of the normal ranges. There were no changes in alkaline phosphatase (ALKP) and total bilirubin concentrations. These findings are in agreement with other trials. The 12 month long study involving reduced carbohydrate Mediterranean diet (Elhayany et al. 2010) showed that ALT levels dramatically improved (Fraser et al. 2008). Interestingly, this improvement was more pronounced on this diet than on a standard Mediterranean diet or on a diet recommended by the ADA (2003) (Fraser et al. 2008). Similar outcomes were reported in 2007 by Ryan and colleagues who wrote that for obese insulin resistant patients, diet moderately lower in carbohydrate improved ALT levels better than high carbohydrate diet, apart from equal weight loss. The authors suggested this to be an effect of overall decreased insulin levels (Ryan et al. 2007).

Albumin is a marker of nutritional status and is useful for monitoring liver function. Low albumin levels may be a sign of altered liver function and / or altered kidney function. In the current study albumin levels were quite high at baseline and a small but significant increase was seen (Table 9). It is therefore a further confirmation that liver and kidney functions were not negatively affected by the treatment. The change in albumin level, as mentioned before in this chapter, was probably related to the decreased energy content of the diet.

Such changes in liver function tests suggest a marked improvement. Hepatic steatosis, that is associated with the increased visceral fat, is now thought to be a part of the metabolic syndrome (Forlani et al. 2008). A large proportion of people with T2DM also present different characteristics of MS. Alexander and colleagues (2003) analysed NHANES III data and reported that 87% of US citizens (n = 3510) over 50 years old who had T2DM met criteria for metabolic syndrome (National Cholesterol Education Program ATP III). A similar observation was reported by an Italian group

(Bianchi et al. 2008), who found that 70% of people with T2DM (n = 1610) met the NCEP ATP III criteria for MS.

5.8 Quality of life and wellbeing

The questionnaires were secondary measures in the current study. The study was not powered to detect differences in quality of life (QoL), physical activity, depression, fatigue, sleepiness or treatment satisfaction.

The quality of life of people with T2DM is associated with even mild diabetes complications (Lloyd et al. 2001) and may be affected by hypoglycaemic episodes during insulin therapy (SIGN, 2010). Obesity has a negative impact on QoL as well (Gough et al. 2009). In the current study, the quality of life in diabetes was assessed with the Audit of Diabetes Dependent Quality of Life (ADDQoL). This tool consisted of two general items (to assess quality of life and impact of diabetes) and of 18 items focusing on varied aspects of life. The present quality of life (one item directly asking about the QoL) shifted from 'good' towards 'very good' over the duration of the study. It has been previously reported that people with obesity and people with T2DM suffer from reductions in health related QoL (Gough et al 2009). The effects of obesity and T2DM were found to be additive (i.e. individuals who are both obese and have T2DM, experience reduction in health related QoL equal to the sum of the two independent effects) (Gough et al. 2009). As participants of the current study experienced both weight loss and improvements in glycaemic control, improvement in QoL were expected.

The impact of diabetes on some of the eighteen life domains was different between completers and dropouts at baseline in the current study. Firstly, completers rated the impact of diabetes on their enjoyment of food higher than dropouts, possibly suggesting greater awareness of foods which are not recommended, or the lack of eating pleasure associated with recommended foods. Secondly, the completers reported significantly lower impact of diabetes on their confidence to do things. It is an interesting finding, as completers had no different attitude towards weight loss than dropouts. If the confidence to do things helped completers in finishing the programme, the question arises as to what would help dropouts gain more confidence to do things. Phrased differently, if confidence is just a character trait of completers, then it can be suggested that confident people respond better to this kind of intervention. And again a question follows as to, what kind of intervention would be suitable for less confident people.

The obesity related quality of life questionnaire (ORWELL-97) (Appendix 4) is a subjective measure of physical and psychological distress generated by overweight (Mannucci, Petroni et al. 2010). A score of 70 or more in the ORWELL 97 questionnaire was considered indicative of a clinically significant burden of obesity on health related QoL (Mannucci et al. 2010). In the present study, at baseline, the mean score was 32.3 ± 27, suggesting that the impact of obesity on QoL was not dramatic. ORWELL-97 scores in the completers' analysis showed a nonsignififcant decreasing trend over the duration of the study; however, LOCF analysis showed a significant decrease (Figure 29a) indicating improvement in QoL. The reasons for not reaching the statistical significance in completers' analysis could have been the fact that rating of wellbeing was quite positive already at baseline, or due to the smaller than in LOCF sample size. When interpreted in the perspective of the improvements in body mass and composition, cardiovascular risk factors and glycaemic control, increased quality of life and a trend towards an improved wellbeing could confirm a successful intervention.

5.9 Physical activity

According to the results from the General Practice Physical Activity Questionnaire (GPPAQ) (Appendix 6), physical activity did not change during the programme. Physical activity was not a part of the intervention, and information about its stable levels throughout the study eliminates the possibility of weight and body composition changes due to exercise and not diet. However, it is possible that the choice of GPPAQ for the assessment of physical activity, which has only 4 possible outcomes (inactive, moderately inactive, moderately active and active), was not the ideal choice. A measure that is more precise would perhaps be more sensitive (such as Scottish Physical Activity Questionnaire) and suit the present study better.

5.10 Depression

Prevalence of depression in patients with T2DM is more common in people with diabetes than in the general population (SIGN, 2010). Raval et al (2010) reported that 23% of patients in the tertiary care in India had major depression and 18% had moderate depression (diagnosed with Patient Health Questionnaire 9). The research group from the Netherlands (Pouwer et al. 2010) reported that about 40% of patients from an out-patient clinic had a depressive affect (assessed by the World Health Organization-5 Well Being Index). Pouwer and associates (2010) also reported, based on the results from the Composite International Diagnostic Interview, which 2% of men and 21% of women with T2DM suffered from a depressive disorder. The reported risk factors for depression in patients with

diabetes included obesity, central obesity, age, various diabetic complications, and increased pill burden (Raval et al. 2010; Pouwer et al. 2010).

In the present study, the Major Depression Inventory (MDI) scores at baseline were within the normal ranges and did not indicate depression. Although the numbers were not recorded, a substantial part of patients that otherwise fulfilled the inclusion criteria, could not be included in the study due to the antidepressant therapy (i.e. diagnosed with and treated for depression). Those patients were not included into the intervention because of the possible effects of antidepressants on body mass. In the current study, there was a significant overall decrease in MDI score during the programme, suggesting improvement in any depressive states. Although the participants in the current study did not have depression, this result seems to be in agreement with the evidence used in SIGN (2010) guideline, that an improvement in depression was associated with the improvement in glycaemic control.

5.11 Fatigue, energy and sleepiness

Fatigue, assessed with VAS-F, was at about 20 mm level on a visual analogue scale (see Table 17) during the whole programme; where the 0 mm end of the scale meant no fatigue at all and the 100 mm end meant absolute exhaustion. This was a rather low rating as compared to the rating of healthy controls (41.3 ± 23.2 mm) from Lachapelle's and Finalyson's study (1998). The study tested three tools to measure fatigue and compared results of healthy controls and patients with brain injury (LaChapelle, Finlayson 1998). Lachapelle and Finalyson (1998) found no significant rating on the VAS-F fatigue scale between the groups, which they explained by the scale's failure to distinguish between sleepiness and fatigue, as pointed out by Chambers and Docktor (1993). In the current study, the change in sleepiness assessed by the Epworth Sleepiness Scale was also not significant.

Baseline energy levels were 55.7 ± 25.8 mm (see Table 17) where 0% was 'no energy at all' and 100% was 'feeling extremely energetic'. Energy rating showed an increasing trend by the twelfth month (+8.0 ± 24.9 mm) but this was not significant (Table 17). Perhaps, the change would be more pronounced with the greater weight loss or larger number of participants.

The sleepiness levels evaluated by the Epworth Sleepiness Scale (ESS) (Appendix 9) did not change. There was a decrease from 8.1 to 5.9 on a 24-point scale but this was not significant. The ESS was originally developed to distinguish between normal patients and patients with a range of sleep disorders (Johns 1991). The control group of normal patients in Johns' study (1991) had a mean rating

of 5.9 ± 2.2 while the mean score for 104 medical students was 7.6 ± 3.9 (Johns 1992). Participants by the end of the current study rated their sleepiness 5.9 ± 3.8. These results suggest that participants in the investigated sample, on average, did not have sleep disorders at baseline and their sleepiness levels did not change during the study.

5.12 Attitude towards weight loss

The weight decisional balance (WDB) was constructed to assess baseline attitudes to weight loss and then, during weight loss, to alert about shifts in the attitudes that could undermine chances for success (O'Connell et al. 1988). It was expected that in the current study, there would be a baseline difference between completers and dropouts in the attitude toward weight loss that would at least partly explain reasons for dropout. No such difference was detected and all the participants were positive about weight loss at baseline. Although it was not expected that all dropouts would be positive about weight loss at baseline, it could be explained by a finding from the study by Green and associates (2007) who reported that people with T2DM and those with cardiometabolic risk had good awareness of healthy lifestyle (diet, exercise, benefits of weight loss) but did not translate it into healthy behaviour.

Although the majority of participants remained positive about losing weight throughout the programme, the mean WDB score decreased ($p = 0.03$). The fact that there was a positive association between the change in weight and change in the mean WDB score ($r = 0.340$, $p = 0.03$) in the LOCF analysis but not in the completers' analysis was probably due to the small sample size. Such an association would mean a diminishing motivation to lose weight with the progress of the program (or with weight loss). It would seem logical for the motivation to pursue something to decline when the goal is closer.

5.13 Diabetes treatment satisfaction

The diabetes treatment satisfaction status version (measured by DTSQs, Appendix 5) score was quite high at the beginning (28.6 ± 7.3 out of 36.0) and despite showing an increasing trend, it did not change in completers during the study. Although it was recommended to also administer DTSQ-change at the completion of the study or at the time of dropout, these data were collected only from a subset of participants were therefore not presented. DTSQc was designed to overcome possible ceiling effect of DTSQs.

5.14 Adverse events

The adverse events reported in the first two weeks of the intervention were characteristic for going into ketosis (e.g. headaches, sickness, tiredness and nausea) (Rolland et al. 2009). However, the numbers of participants that reported these events were small and apart from the trend for less adverse events as the study progressed, the frequency did not significantly change. Considering the fact that the majority of participants were not in ketosis during the study, these effects were more likely associated with the change in type of food consumed or calorie restriction.

A recent study from Aberdeen, Scotland suggested that LCHODs are high in protein and low in fibre may increase risk of colon cancer (Russell et al. 2011). The researchers reported that after 4 weeks on high protein and reduced in carbohydrate, weight maintaining diets, obese participants displayed decrease in fecal cancer-protective metabolites and increase in carcinogenic compounds. As fibre and resistant carbohydrate can counteract negative metabolic outcomes of high protein LCHODs, it was recommended to include sufficient amounts of them in the weight loss diets (Russell et al. 2011).

5.15 Limitations

We are aware of certain limitations to this study. First limitation was the small sample size as the recruitment for this study was stopped due to the cuts in funding. The chosen randomisation method did not allow for this event. This resulted in uneven numbers of participants in the two study arms and different gender proportions between the groups. Secondly, 66% of patients dropped out by 12 months which further reduced the sample. However, both last observation carried forward and completers analyses were performed and the outcomes of them were very similar. Thirdly, most of the participants were on diabetic, lipid-lowering and/or antihypertensive medications which could have affected the study outcome measures. It was reported that, at baseline, there were no differences between the two groups in use of these medications. The changes in medication doses were not significant but due to the small sample size, the effect of medication cannot be ruled out. Fourthly, some of the measures we have taken, like quality of life, depression rating, physical activity or motivation to lose weight, could have been influenced by factors other than the diet. Due to the nature of the trial, we were unable to control for those possible confounding factors. Fifthly, in real life, unlike in this trial, individuals need to pay for the Go Lower products which may either put them off choosing this option or make them responsible for their choice and motivate them to follow the programme more closely. Sixthly, the current study did not measure readiness to change before an intervention. It was found that a

substantial number of participants dropped out due to the lifestyle issues. Perhaps the use of readiness to change assessment before an intervention could help to differentiate between people who are ready to make a life style change and those who are not. Finally, patients in this study were randomised to their treatment, which did not reflect their preferences matched to life style. This could possibly influence the study outcomes, i.e. dropout rates.

6 Conclusions

The results presented in this work should be viewed with caution as the recruitment target was not reached and the study was not sufficiently powered to demonstrate differences in the outcomes.

The outcomes of the present study suggest that protein sparing modified fast and commercially available Go Lower diet, two different approaches to administering a high-protein LCKD, had similar effects on weight loss and related metabolic changes. This is a novel research, as different approaches to administration of low-carbohydrate diet have not been compared before. The hypothesis that convenience of ready made meals provided by Go Lower would produce greater weight loss at 12 months was not confirmed. Therefore, it seems that the two approaches could be used according to a patient's preferences or perhaps interchangeably. Secondly, the present study supports previous findings that a high-protein LCKD administered for 12 months is a safe and effective means for weight loss in obese people with T2DM. Thirdly, weight loss in the present study was similar to those reported in studies with similar dietary macronutrient composition. Studies in which adherence to the low carbohydrate requirement was good, achieved much better weight loss than the present study and other studies with lower adherence. Moreover, observed changes in body composition were anticipated – most of the body mass lost was fat mass. Fourthly, both completers and the last observation carried forward analyses gave very similar results, confirming that the last observation carried forward approach did not cause over- or underestimation of the diet effect. It was also clear that completers' analysis, apart from showing the same or even more pronounced changes, did not always give statistically significant results due to the smaller sample size. Fifthly, comparison of dietary intervention trials is difficult as often there is no consistency in reporting macronutrient composition (grams per day versus percentage of energy intake), dietary data (method used), frequency of support (number and frequency of visits and contact with the participant), or the extent of adherence to the programmes. Sixthly, it is possible that for a successful longer term dietary intervention for obese patients with T2DM the recommended macronutrient composition is not as important as the type of dietary carbohydrate (low glycaemic index) and fat (proportions of SFA, MUFA and PUFA). Finally, questionnaires together with the hard data outcomes from the present study show that the intervention was beneficial on many levels (such as physiology, quality of life and wellbeing).

7 Suggested future work

There are six suggestions for the future work in relation to the present study. Firstly, the target recruitment numbers were not reached; therefore the relationships in the defined population cannot be fully identified. The present study could act as a pilot for a bigger intervention which would be able to clarify conclusions about the effects of these approaches in a poorly controlled type 2 diabetic population. Secondly, a follow-up of the participants from the current study could provide an insight into the longer term effects of the two compared approaches. Thirdly, some of the trials that investigated diets with comparable nutritional composition on similar populations had very different weight loss outcomes. One apparent difference seemed to be the type and frequency of participant support. Perhaps an investigation into how the frequency and kind of professional support influences the adherence to the lifestyle interventions in different populations would provide useful suggestions for practice. Fourthly, the present study had a high dropout rate. Many participants who dropped out declared lifestyle issues as the reason for discontinuation. Perhaps a trial, in which participants would not be randomised into the dietary groups but would be able to choose the type of intervention themselves according to their preferences, would reduce those dropouts. Moreover, the participants' freedom to choose the type of diet or lifestyle modification would reflect a real-life situation where everyone is in charge of making these choices for themselves. The experiment designed in this way could deliver results that would be a better reflection of an intervention's effect in the non-experimental setting. Fifthly, a study investigating differences between approaches to a diet would be supplemented by an element of qualitative data exploring participant's experiences and views on the diets. Exploration of subjective experience of the diet could be taken further to investigate reasons for dropout and completion of the programme. These aspects would be very useful, acting as directions for new lifestyle interventions. Finally, the equation developed in the current study for predicting RMR explained 81% of the variability in RMR. It was hypothesized that some of the remaining 19 % could be explained by the type and doses of medications used. Future design of RMR prediction equations for this particular population should perhaps include medication. Additionally, to develop more accurate predictive equations in the future, bigger databases should be used.

8 References

ABBOTT DIABETES CARE Ltd., 2006. *Optium β-Ketone Test Strips.* Instructions leaflet. Witney: Abbott.

ACCURSO, A., BERNSTEIN, R.K., DAHLQVIST, A., DRAZNIN, B., FEINMAN, R.D., FINE, E.J., GLEED, A., JACOBS, D.B., LARSON, G., LUSTIG, R.H., MANNINEN, A.H., MCFARLANE, S.I., MORRISON, K., NIELSEN, J.V., RAVNSKOV, U., ROTH, K.S., SILVESTRE, R., SOWERS, J.R., SUNDBERG, R., VOLEK, J.S., WESTMAN, E.C., WOOD, R.J., WORTMAN, J. and VERNON, M.C., 2008. Dietary carbohydrate restriction in type 2 diabetes mellitus and metabolic syndrome: time for a critical appraisal. *Nutrition & metabolism*, **5**, p. 9, doi: 10.1186/1743-7075-5-9.

ACHESON, K.J., 2010. Carbohydrate for weight and metabolic control: where do we stand? *Nutrition*, **26**(2), pp. 141-145.

ACTION TO CONTROL CARDIOVASCULAR RISK IN DIABETES STUDY GROUP, GERSTEIN, H.C., MILLER, M.E., BYINGTON, R.P., GOFF, D.C.,JR, BIGGER, J.T., BUSE, J.B., CUSHMAN, W.C., GENUTH, S., ISMAIL-BEIGI, F., GRIMM, R.H.,JR, PROBSTFIELD, J.L., SIMONS-MORTON, D.G. and FRIEDEWALD, W.T., 2008. Effects of intensive glucose lowering in type 2 diabetes. *The New England Journal of Medicine*, **358**(24), pp. 2545-2559.

ALBERTI, K.G., ZIMMET, P. and SHAW, J., 2005. The Metabolic Syndrome - a new worldwide definition. *Lancet*, **366**(9491), pp. 1059-1062.

ALBERTI, K.G. and ZIMMET, P.Z., 2008. Should we dump the metabolic syndrome? No. *BMJ*, **336**(7645), p. 641.

ALLISON, D.B., KAPRIO, J., KORKEILA, M., KOSKENVUO, M., NEALE, M.C. and HAYAKAWA, K., 1996. The heritability of body mass index among an international sample of monozygotic twins reared apart. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, **20**(6), pp. 501-506.

AMASYALI, B., KILIC, A., CELIK, T. and IYISOY, A., 2010. A new frame in thromboembolic cardiovascular disease: Adipocytokine. *International Journal of Cardiology*, **139**(1), pp. 100-102.

AMERICAN DIABETES ASSOCIATION, BANTLE, J.P., WYLIE-ROSETT, J., ALBRIGHT, A.L., APOVIAN, C.M., CLARK, N.G., FRANZ, M.J., HOOGWERF, B.J., LICHTENSTEIN, A.H., MAYER-DAVIS, E., MOORADIAN, A.D. and WHEELER, M.L., 2008. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care*, **31(**Suppl 1), pp. S61-78.

AMERICAN DIABETES ASSOCIATION, 2002. Diabetes Mellitus and Exercise. *Diabetes Care*, **25** (Suppl 1), p. s64, doi: 10.2337/diacare.25.2007.s64.

AMERICAN HEART ASSOCIATION, 2007. *Heart Disease and Stroke Statistics - 2007 Update.* Dallas, Texas: American Heart Association.

ANAND, S.S. and YUSUF, S., 2011. Stemming the global tsunami of cardiovascular disease. *Lancet*, **377**(9765), pp. 529-532.

ANTUNA-PUENTE, B., FEVE, B., FELLAHI, S. and BASTARD, J.P., 2008. Adipokines: The missing link between insulin resistance and obesity. *Diabetes & Metabolism,* **34**(1), pp. 2-11.

APPEL, L.J., 2003. The effects of protein intake on blood pressure and cardiovascular disease. *Current Opinion in Lipidology,* **14**(1), pp. 55-59.

ARICI, M. and WALLS, J., 2001. End-stage renal disease, atherosclerosis, and cardiovascular mortality: is C-reactive protein the missing link? *Kidney International,* **59**(2), pp. 407-414.

ARORA, S.K. and MCFARLANE, S.I., 2005. The case for low carbohydrate diets in diabetes management. *Nutrition & metabolism,* **2**, pp. 16, doi: 10.1186/1743-7075-2-16.

ASHWELL, S.G., BRADLEY, C., STEPHENS, J.W., WITTHAUS, E. and HOME, P.D., 2008. Treatment satisfaction and quality of life with insulin glargine plus insulin lispro compared with NPH insulin plus unmodified human insulin in individuals with type 1 diabetes. *Diabetes Care,* **31**(6), pp. 1112-1117.

ASTRUP, A., MEINERT LARSEN, T. and HARPER, A., 2004. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet,* **364**(9437), pp. 897-899.

ASTRUP, A.S., NIELSEN, F.S., ROSSING, P., ALI, S., KASTRUP, J., SMIDT, U.M. and PARVING, H.H., 2007. Predictors of mortality in patients with type 2 diabetes with or without diabetic nephropathy: a follow-up study. *Journal of Hypertension,* **25**(12), pp. 2479-2485.

BABA, N.H., SAWAYA, S., TORBAY, N., HABBAL, Z., AZAR, S. and HASHIM, S.A., 1999. High protein vs. high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects. *International Journal of Obesity and Related Metabolic Disorders,* **23**(11), pp. 1202-1206.

BALKAU, B., VALENSI, P., ESCHWEGE, E. and SLAMA, G., 2007. A review of the metabolic syndrome. *Diabetes & Metabolism,* **33**(6), pp. 405-413.

BANJA, J., 2004. Obesity, responsibility, and empathy. *The Case Manager,* **15**(6), pp. 43-46.

BAUDUCEAU, B., VACHEY, E., MAYAUDON, H., BURNAT, P., DUPUY, O., GARCIA, C., CEPPA, F. and BORDIER, L., 2007. Should we have more definitions of metabolic syndrome or simply take waist measurement? *Diabetes & Metabolism,* **33**(5), pp. 333-339.

BECH, P., LINDBERG, L., REFSGAARD, E., LUNDE, M. and SONDERGAARD, S., 2001-last update, WHO Major Depression Inventory (MDI). Available: http://www.who-5.org/ [03/15, 2011].

BECH, P., RASMUSSEN, N.A., OLSEN, L.R., NOERHOLM, V. and ABILDGAARD, W., 2001. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. *Journal of Affective Disorders,* **6**6(2-3), pp. 159-164.

BECK, J.S., 1995. *Cognitive Therapy: Basics and Beyond.* London: Guilford Press.

BELL, C., DAY, D.S., JONES, P.P., CHRISTOU, D.D., PETITT, D.S., OSTERBERG, K., MELBY, C.L. and SEALS, D.R., 2004. High energy flux mediates the tonically augmented beta-adrenergic support of resting metabolic rate in habitually exercising older adults. *The Journal of Clinical Endocrinology and Metabolism,* **89**(7), pp. 3573-3578.

BENNETT, F.I., MCFARLANE-ANDERSON, N., WILKS, R., LUKE, A., COOPER, R.S. and FORRESTER, T.E., 1997. Leptin concentration in women is influenced by regional distribution of adipose tissue. *The American Journal of Clinical Nutrition,* **66**(6), pp. 1340-1344.

BERNSTEIN, R.S., THORNTON, J.C., YANG, M.U., WANG, J., REDMOND, A.M., PIERSON, R.N. JR, PI-SUNYER, F.X., VAN ITALLIE, T.B. ,1983. Prediction of the resting metabolic rate in obese patients. *American Journal of Clinical Nutrition,* **37**(4), pp. 595-602.

BILOUS, R., 2008. Microvascular disease: what does the UKPDS tell us about diabetic nephropathy? *Diabetic Medicine,* **25** (Suppl 2), pp. 25-29.

BISSCHOP, P.H., PEREIRA ARIAS, A.M., ACKERMANS, M.T., ENDERT, E., PIJL, H., KUIPERS, F., MEIJER, A.J., SAUERWEIN, H.P. and ROMIJN, J.A., 2000. The effects of carbohydrate variation in isocaloric diets on glycogenolysis and gluconeogenesis in healthy men. *The Journal of Clinical Endocrinology and Metabolism,* **85**(5), pp. 1963-1967.

BITZ, C., TOUBRO, S., LARSEN, T.M., HARDER, H., RENNIE, K.L., JEBB, S.A. and ASTRUP, A., 2004. Increased 24-h energy expenditure in type 2 diabetes. *Diabetes Care,* **27**(10), pp. 2416-2421.

BLAKE, G.J. and RIDKER, P.M., 2002. Inflammatory bio-markers and cardiovascular risk prediction. *Journal of Internal Medicine,* **252**(4), pp. 283-294.

BLAKEMORE, A.I. and FROGUEL, P., 2008. Is obesity our genetic legacy? *The Journal of Clinical Endocrinology and Metabolism,* **93**(Suppl 1), pp. S51-56.

BLOCH, A.S., 2005. Low carbohydrate diets, pro: time to rethink our current strategies. *Nutrition in Clinical Practice,* **20**(1), pp. 3-12.

BODEN, G., SARGRAD, K., HOMKO, C., MOZZOLI, M. and STEIN, T.P., 2005. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Annals of Internal Medicine,* **142**(6), pp. 403-411.

BOGARDUS, C., TASKINEN, M.R., ZAWADZKI, J., LILLIOJA, S., MOTT, D. and HOWARD, B.V., 1986. Increased resting metabolic rates in obese subjects with non-insulin-dependent diabetes mellitus and the effect of sulfonylurea therapy. *Diabetes,* **35**(1), pp. 1-5.

BOISSAN, M., BEUREL, E., WENDUM, D., REY, C., LECLUSE, Y., HOUSSET, C., LACOMBE, M.L. and DESBOIS-MOUTHON, C., 2005. Overexpression of insulin receptor substrate-2 in human and murine hepatocellular carcinoma. *The American Journal of Pathology,* **16**7(3), pp. 869-877.

BOLING, C.L., WESTMAN, E.C. and YANCY, W.S.,JR, 2009. Carbohydrate-restricted diets for obesity and related diseases: an update. *Current Atherosclerosis Reports,* **11**(6), pp. 462-469.

BRADLEY, C., 2006. *The Diabetes Treatment Satisfaction Questionnaire (DTSQ) Status and Change Versions. User Guidelines. rev 6.6.06.*

BRADLEY, C., 2005. *The Audit of Diabetes-Dependent Quality of Life (ADDQoL). User Guidelines. Rev 24.1.05A.*

BRADLEY, C., 1994. The Diabetes Treatment Satisfaction Questionnaire: DTSQ. In: C. BRADLEY, ed, *Handbook of Psychology and Diabetes: a guide to psychological measurement in diabetes research and practice.* Chur, Switzerland: Harwood Academic Publishers.

BRADLEY, C., TODD, C., GORTON, T., SYMONDS, E., MARTIN, A. and PLOWRIGHT, R., 1999. The development of an individualized questionnaire measure of perceived impact of diabetes on quality of life: the ADDQoL. *Quality of Life Research,* **8**(1-2), pp. 79-91.

BRAVATA, D.M., SANDERS, L., HUANG, J., KRUMHOLZ, H.M., OLKIN, I., GARDNER, C.D. and BRAVATA, D.M., 2003. Efficacy and safety of low-carbohydrate diets: a systematic review. *Journal of the American Medical Association,* **289**(14), pp. 1837-1850.

BREHM, B.J., SEELEY, R.J., DANIELS, S.R. and D'ALESSIO, D.A., 2003. A fized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *The Journal of Clinical Endocrinology and Metabolism,* **88**(4), pp. 1617-1623.

BREHM, B.J., SPANG, S.E., LATTIN, B.L., SEELEY, R.J., DANIELS, S.R. and D'ALESSIO, D.A., 2005. The role of energy expenditure in the differential weight loss in obese women on low-fat and low-carbohydrate diets. *The Journal of Clinical Endocrinology and Metabolism,* **90**(3), pp. 1475-1482.

BRINKWORTH, G.D., BUCKLEY, J.D., NOAKES, M. and CLIFTON, P.M., 2010. Renal function following long-term weight loss in individuals with abdominal obesity on a very-low-carbohydrate diet vs high-carbohydrate diet. *Journal of the American Dietetic Association,* **110**(4), pp. 633-638.

BRINKWORTH, G.D., NOAKES, M., BUCKLEY, J.D., KEOGH, J.B. and CLIFTON, P.M., 2009. Long-term effects of a very-low-carbohydrate weight loss diet compared with an isocaloric low-fat diet after 12 mo. *The American Journal of Clinical Nutrition,* **90**(1), pp. 23-32.

BRINKWORTH, G.D., NOAKES, M., KEOGH, J.B., LUSCOMBE, N.D., WITTERT, G.A. and CLIFTON, P.M., 2004b. Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. *International Journal of Obesity and Related Metabolic Disorders,* **28**(5), pp. 661-670.

BRINKWORTH, G.D., NOAKES, M., PARKER, B., FOSTER, P. and CLIFTON, P.M., 2004a. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with type 2 diabetes: one-year follow-up of a randomised trial. *Diabetologia,* **47**(10), pp. 1677-1686.

BRITISH NUTRITION FOUNDATION TASK FORCE, 2000. *Obesity. The Report of the British Nutrition Foundation Task Force.* Oxford: Blackwell Science.

BROMLEY, C., GIVEN, L., ORMSTON, R., CORBETT, J., DOBBIE, F., DOIG, M., D'SOUZA, J., GRAY, A.L., MACGREGOR, A., MARRYAT, L., MAW, T., MILLER, M., MINDELL, J., ROTH, M. and SHARP, C., 2010. *The Scottish Health Survey. Volume 1: Main Report.* Edinburgh, UK: The Scottish Government.

BROOM, J., FRASER, M.H., MCKENZIE, K., MILLER, J.D. and FLECK, A., 1986. The protein metabolic response to short-term starvation in man. *Clinical Nutrition,* **5**(1), pp. 63-65.

BRUNEROVA, L., SMEJKALOVA, V., POTOCKOVA, J. and ANDEL, M., 2007. A comparison of the influence of a high-fat diet enriched in monounsaturated fatty acids and conventional diet on weight loss and metabolic parameters in obese non-diabetic and Type 2 diabetic patients. *Diabetic Medicine,* **24**(5), pp. 533-540.

BRYANT, C.X. and GREEN, D.J., eds, 2008. *Lifestyle and Weight Management Coach Manual.* 2nd edn. American Council on Exercise.

BUTLAND, B., JEBB, S., KOPELMAN, P., MCPHERSON, K., THOMAS, S., MARDELL, J. and PARRY, V., 2007. *Foresight. Tackling Obesities: Future Choices - Project Report. 2nd Edition.* Government Office for Science.

CHAMBERS, M.J. and DOCKTOR, B.J., 1993. Fatigue scale lacks adequate validation. *Psychiatry Research,* **46**(2), pp. 207-210.

CHAN, J.M., RIMM, E.B., COLDITZ, G.A., STAMPFER, M.J. and WILLETT, W.C., 1994. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care,* **17**(9), pp. 961-969.

CLIFTON, P.M., KEOGH, J.B. and NOAKES, M., 2008. Long-term effects of a high-protein weight-loss diet. *The American Journal of Clinical Nutrition,* **87**(1), pp. 23-29.

COCKCROFT, D.W. and GAULT, M.H., 1976. Prediction of creatinine clearance from serum creatinine. *Nephron,* **16**(1), pp. 31-41.

COLBERG, S.R., SIGAL, R.J., FERNHALL, B., REGENSTEINER, J.G., BLISSMER, J.G., RUBIN, R.R., CHASAN-TABER, L., ALBRIGHT, A.L., BRAUN, B., AMERICAN COLLEGE OF SPORTS MEDICINE, AMERICAL DIABETES ASSOCIATION, 2010. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care,* **33**(12), pp. 2692-2696.

COLDITZ, G.A., WILLETT, W.C., ROTNITZKY, A. and MANSON, J.E., 1995. Weight Gain as a Risk Factor for Clinical Diabetes Mellitus in Women. *Annals of Internal Medicine,* **122**(7), pp. 481-486.

CONNOR, H., ANNAN, F., BUNN, E., FROST, G., MCGOUGH, N., SARWAR, T., THOMAS, B. and NUTRITION SUBCOMMITTEE OF THE DIABETES CARE ADVISORY COMMITTEE OF DIABETES UK, 2003. The implementation of nutritional advice for people with diabetes. *Diabetic Medicine,* **20**(10), pp. 786-807.

COOPER, Z., FAIRBURN, C.G., 2001. A new cognitive behavioural approach to the treatment of obesity. *Behaviour Research and Therapy,* **39**(5), pp. 499-511.

COOPER, Z., DOLL, H.A., HAWKER, D.M., BYRNE, S., BONNER, G., EELEY, E., O'CONNOR, M.E., FAIRBURN, C.G., 2010. Testing a new cognitive behavioural treatment for obesity: A randomized controlled trial with three-year follow-up. *Behaviour Research and Therapy,* **48**(8), pp. 706-713.

CORNIER, M.A., DONAHOO, W.T., PEREIRA, R., GUREVICH, I., WESTERGREN, R., ENERBACK, S., ECKEL, P.J., GOALSTONE, M.L., HILL, J.O., ECKEL, R.H. and DRAZNIN, B.,

2005. Insulin sensitivity determines the effectiveness of dietary macronutrient composition on weight loss in obese women. *Obesity Research,* **13**(4), pp. 703-709.

CRAIG, R., HIRANI, V., ARESU, M., CHAUDHURY, M., DIMENT, E., FULLER, E., GORDON-DSEAGU, V., GUNNING, N., MINDELL, J., NICHOLSON, S., OGUNBADEJO, T., ROBINSON, C., RODERICK, P., ROTH, M., SHELTON, N., TABASSUM, F. and WARDLE, H., 2010. *Health Survey for England 2009. Volume 1: Health and Lifestyles.* Leeds, UK: The NHS Information Centre for Health and Social Care.

DAFNE STUDY GROUP, 2002. Trainine in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: the dose adjustment for normal eating (DAFNE) randomised controlled trial. *British Medical Journal,* **325**, pp. 746-749.

D'AGOSTINO RB, S., VASAN, R.S., PENCINA, M.J., WOLF, P.A., COBAIN, M., MASSARO, J.M. and KANNEL, W.B., 2008. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation,* **117**(6), pp. 743-753.

DALY, M.E., PAISEY, R., PAISEY, R., MILLWARD, B.A., ECCLES, C., WILLIAMS, K., HAMMERSLEY, S., MACLEOD, K.M. and GALE, T.J., 2006. Short-term effects of severe dietary carbohydrate-restriction advice in Type 2 diabetes--a randomized controlled trial. *Diabetic Medicine,* **23**(1), pp. 15-20.

DANAEI, G., FINUCANE, M.M., LIN, J.K., SINGH, G.M., PACIOREK, C.J., COWAN, M.J., FARZADFAR, F., STEVENS, G.A., LIM, S.S., RILEY, L.M., EZZATI, M. and ON BEHALF OF THE GLOBAL BURDEN OF METABOLIC RISK FACTORS OF CHRONIC DISEASES COLLABORATING GROUP (BLOOD PRESSURE), 2011. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet,* **377**(9765), pp. 568-577.

DANSINGER, M.L., GLEASON, J.A., GRIFFITH, J.L., SELKER, H.P. and SCHAEFER, E.J., 2005. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *Journal of the American Medical Association,* **293**(1), pp. 43-53.

DASHTI, H.M., MATHEW, T.C., KHADADA, M., AL-MOUSAWI, M., TALIB, H., ASFAR, S.K., BEHBAHANI, A.I. and AL-ZAID, N.S., 2007. Beneficial effects of ketogenic diet in obese diabetic subjects. *Molecular and Cellular Biochemistry,* **302**(1-2), pp. 249-256.

DAVIDSON, M.H., HAUPTMAN, J., DiGIROLAMO, M., FOREYT, J.P., HALSTED, C.H., HEBER, D., HEIMBURGER, D.C., LUCAS, C.P., ROBBINS, D.C., CHUNG, J., HEYMSFIELD, S.B., 1999. Weight control abd risk factor reduction in obese subjects treated for 2 years with orlistat: a randomized controller trial. *The Journal of the American Medical Association,* **281**(3), 2350-242.

DAVIS, N.J., TOMUTA, N., SCHECHTER, C., ISASI, C.R., SEGAL-ISAACSON, C.J., STEIN, D., ZONSZEIN, J. and WYLIE-ROSETT, J., 2009. Comparative study of the effects of a 1-year dietary intervention of a low-carbohydrate diet versus a low-fat diet on weight and glycemic control in type 2 diabetes. *Diabetes Care,* **32**(7), pp. 1147-1152.

DE KONING, L., FUNG, T.T., LIAO, X., CHIUVE, S., RIMM, E.B., WILLETT, W.C., SPIEGELMAN, D. and HU, F.B., 2011. Low-carbohydrate diet scores and risk of type 2 diabetes in men. *The American Journal of Clinical Nutrition,* **93**(4), pp. 844-850.

DE LORENZO, A., TAGLIABUE, A., ANDREOLI, A., TESTOLIN, G., COMELLI, M., DEURENBERG, P., 2001. Measured and predicted resting metabolic rate in Italian males and females, aged 18-59 y. *European Journal of Clinical Nutrition,* 55(3), pp. 208-214.

DE LUIS, D.A., ALLER, R. and IZAOLA, O., 2006. Resting energy expenditure and insulin resitance in obese patients, differences in women and men. *European Review for Medical and Pharmacological Sciences,* **10**(6), pp. 285-289.

DEFRONZO, R., TOBIN, J. and ANDRES, R., 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *AJP - Endocrinology and Metabolism,* **237**(3), pp. E214-223.

DEPARTMENT OF HEALTH, 1991. *Diatary Reference Values for Food Energy and Nutrients for the United Kingdom. 41 Report of the Panel on dietary Reference Values of the Committee on Medical Aspects of Food Policy.* Norwich: The Stationery Office.

DHINDSA, P., SCOTT, A.R., DONNELLY, R., 2003. Metabolic and cardiovascular effects of very-low-calorie diet therapy in obese patients with Type 2 diabetes in secondary failure: outcomes after 1 year. *Diabetic Medicine,* **20**(4), pp. 319-324.

DIABETES UK, 2011. Position Statement: Low-carbohydrate diets for people with Type 2 diabetes. March 2011. Available: www.diabetes.org.uk, Printed on 12/04/2011.

DOUMAS, B.T., WATSON, W.A., BIGGS, H.G., 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta,* **31**(1), pp. 87-96.

DUE, A., TOUBRO, S., SKOV, A.R. and ASTRUP, A., 2004. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. *International Journal of Obesity and Related Metabolic Disorders,* **28**(10), pp. 1283-1290.

DURHAM, T.A., TURNER, J.R., 2008. *Introduction to Statistics in Pharmaceutical Trials.* London, UK: Pharmaceutical Press.

DUSSERRE, E., MOULIN, P., VIDAL, H., 2000. Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. *Biochimica Et Biophysica Acta,* **1500**(1), pp. 88-96.

DUVAL, K., MARCEAU, P., PERUSSE, L. and LACASSE, Y., 2006. An overview of obesity-specific quality of life questionnaires. *Obesity Reviews,* **7**(4), pp. 347-360.

DYSON, P.A., BEATTY, S. and MATTHEWS, D.R., 2007. A low-carbohydrate diet is more effective in reducing body weight than healthy eating in both diabetic and non-diabetic subjects. *Diabetic Medicine,* **24**(12), pp. 1430-1435.

ECKEL, R.H., 2008. Clinical practice. Nonsurgical management of obesity in adults. *The New England Journal of Medicine,* **358**(18), pp. 1941-1950.

EGGER, G. and DIXON, J., 2009. Obesity and chronic disease: always offender or often just accomplice? *The British Journal of Nutrition,* **102**(8), pp. 1238-1242.

EKELUND, U., BESSON, H., LUAN, J., MAY, A.M., SHARP, S.J., BRAGE, S., TRAVIER, N., AGUDO, A., SLIMANI, N., RINALDI, S., JENAB, M., NORAT, T., MOUW, T., ROHRMANN, S., KAAKS, R., BERGMANN, M.M., BOEING, H., CLAVEL-CHAPELON, F., BOUTRON-RUAULT, M.C., OVERVAD, K., JAKOBSEN, M.U., JOHNSEN, N.F., HALKJAER, J., GONZALEZ, C.A., RODRIGUEZ, L., SANCHEZ, M.J., ARRIOLA, L., BARRICARTE, A., NAVARRO, C., KEY, T.J., SPENCER, E.A., ORFANOS, P., NASKA, A., TRICHOPOULOU, A., MANJER, J., LUND, E., PALLI, D., PALA, V., VINEIS, P., MATTIELLO, A., TUMINO, R., BUENO-DE-MESQUITA, H.B., VAN DEN BERG, S.W., ODYSSEOS, A.D., RIBOLI, E., WAREHAM, N.J. and PEETERS, P.H., 2011. Physical activity and gain in abdominal adiposity and body weight: prospective cohort study in 288,498 men and women. *The American Journal of Clinical Nutrition,* **93**(4), pp. 826-835.

EKNOYAN, G. and NAGY, J., 2005. A history of diabetes mellitus or how a disease of the kidneys evolved into a kidney disease. *Advances in Chronic Kidney Disease,* **12**(2), pp. 223-229.

EL KHOURY, T.D., OBEID, O., AZAR, S.T. and HWALLA, N., 2006. Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Annals of Nutrition & Metabolism,* **50**(3), pp. 260-269.

ELHAYANY, A., LUSTMAN, A., ABEL, R., ATTAL-SINGER, J. and VINKER, S., 2010. A low carbohydrate Mediterranean diet improves cardiovascular risk factors and diabetes control among overweight patients with type 2 diabetes mellitus: a 1-year prospective randomized intervention study. *Diabetes, Obesity & Metabolism,* **12**(3), pp. 204-209.

FARNSWORTH, E., LUSCOMBE, N.D., NOAKES, M., WITTERT, G., ARGYIOU, E. and CLIFTON, P.M., 2003. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *The American Journal of Clinical Nutrition,* **78**(1), pp. 31-39.

FAROOQI, I.S. and O'RAHILLY, S., 2007. Genetic factors in human obesity. *Obesity Reviews,* **8** (Suppl 1), pp. 37-40.

FARZADFAR, F., FINUCANE, M.M., DANAEI, G., PELIZZARI, P.M., COWAN, M.J., PACIOREK, C.J., SINGH, G.M., LIN, J.K., STEVENS, G.A., RILEY, L.M., EZZATI, M. and ON BEHALF OF THE GLOBAL BURDEN OF METABOLIC RISK FACTORS OF CHRONIC DISEASES COLLABORATING GROUP (CHOLESTEROL), 2011. National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3.0 million participants. *Lancet,* **377**(9765), pp. 578-586.

FAWCETT, K.A. and BARROSO, I., 2010. The genetics of obesity: FTO leads the way. *Trends in Genetics,* **26**(6), pp. 266-274.

FERRARO, R., LILLIOJA, S., FONTVIEILLE, A.M., RISING, R., BOGARDUS, C. and RAVUSSIN, E., 1992. Lower sedentary metabolic rate in women compared with men. *The Journal of Clinical Investigation,* **90**(3), pp. 780-784.

FIELD, A., 2009. *Discovering Statistics Using SPSS.* 3rd Edition edn. London: SAGE.

FINUCANE, M.M., STEVENS, G.A., COWAN, M.J., DANAEI, G., LIN, J.K., PACIOREK, C.J., SINGH, G.M., GUTIERREZ, H.R., LU, Y., BAHALIM, A.N., FARZADFAR, F., RILEY, L.M., EZZATI, M. and ON BEHALF OF THE GLOBAL BURDEN OF METABOLIC RISK FACTORS OF CHRONIC DISEASES COLLABORATING GROUP (BODY MASS INDEX), 2011. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet*, **377**(9765), pp. 557-567.

FIORETTO, P., CARAMORI, M.L. and MAUER, M., 2008. The kidney in diabetes: dynamic pathways of injury and repair. The Camillo Golgi Lecture 2007. *Diabetologia*, **51**(8), pp. 1347-1355.

FIORETTO, P., SUTHERLAND, D.E., NAJAFIAN, B., MAUER, B., 2006. Remodelling of renal interstitial and tubular lesions in pancreas transplant recipients. *Kidney International*, **69**(5), pp. 907-912.

FLEGAL, K.M., CARROLL, M.D., OGDEN, C.L. and CURTIN, L.R., 2010. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA : the journal of the American Medical Association*, **303**(3), pp. 235-241.

FOOD AGRICULTURAL ORGANIZATION, 2010. FAOSTAT Food Balance Sheets. Available on: http://faostat.fao.org Last updated 02 June 2010. Printed on 29/03/2011.

FOOD AND NUTRITION BOARD, 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes.* Washington, D.C.: The National Academies Press.

FORLANI, G., DI BONITO, P., MANNUCCI, E., CAPALDO, B., GENOVESE, S., ORRASCH, M., SCALDAFERRI, L., DI BARTOLO, P., MELANDRI, P., DEI CAS, A., ZAVARONI, I. and

FOSTER, G.D., WYATT, H.R., HILL, J.O., MCGUCKIN, B.G., BRILL, C., MOHAMMED, B.S., SZAPARY, P.O., RADER, D.J., EDMAN, J.S. and KLEIN, S., 2003. A randomized trial of a low-carbohydrate diet for obesity. *The New England Journal of Medicine*, **348**(21), pp. 2082-2090.

FOSTER, G.D., WYATT, H.R., HILL, J.O., MAKRIS, A.P., ROSENBAUM, D.L., BRILL, C., STEIN, R.I., MOHAMMED, B.S., MILLER, B., RADER, D.J., ZEMEL, B., WADDEN, T.A., TENHAVE, T., NEWCOMB, C.W. and KLEIN, S., 2010. Weight and Metabolic Outcomes After 2 Years on a Low-Carbohydrate Versus Low-Fat Diet. *Annals of Internal Medicine*, **153**, pp. 147-157.

FRAYN, K.N., 2003. *Metabolic Regulation. A Human Perspective.* 2nd edition edn. Oxford: Blackwell Publishing.

FREY-HEWITT, B., VRANIZAN, K.M., DREON, D.M. and WOOD, P.D., 1990. The effect of weight loss by dieting or exercise on resting metabolic rate in overweight men. *International Journal of Obesity*, **14**(4), pp. 327-334.

FRIEDEWALD, W.T., LEVY, R.I. and FREDRICKSON, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6), pp. 499-502.

FYFE, C.L., STEWART, J., MURISON, S.D., JACKSON, D.M., RANCE, K., SPEAKMAN, J.R., HORGAN, G.W. and JOHNSTONE, A.M., 2010. Evaluating energy intake measurement in free-living subjects: when to record and for how long? *Public Health Nutrition,* **13**(2), pp. 172-180.

GAGLIARDI, L. and WITTERT, G., 2007. Management of obesity in patients with type 2 diabetes mellitus. *Current Diabetes Reviews,* **3**(2), pp. 95-101.

GANNON, M.C. and NUTTALL, F.Q., 2006. The metabolic response to a high-protein, low-carbohydrate diet in men with type 2 diabetes mellitus. *Metabolism.,* **55**(2), pp. 243.

GANNON, M.C. and NUTTALL, F.Q., 2004. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes,* **53**(9), pp. 2375-2382.

GANNON, M.C., NUTTALL, F.Q., SAEED, A., JORDAN, K. and HOOVER, H., 2003. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. *The American Journal of Clinical Nutrition,* **78**(4), pp. 734-741.

GARDNER, C.D., KIAZAND, A., ALHASSAN, S., KIM, S., STAFFORD, R.S., BALISE, R.R., KRAEMER, H.C. and KING, A.C., 2007. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *Journal of the American Medical Association,* **297**(9), pp. 969-977.

GIBNEY, M.J., VORSTER, H.H., KOK, F.J, eds, 2002. Introduction to Human Nutrition. Oxford, UK: Blackwell Publishing.

GOLDEN, S.H., SELVIN, E. and CUNNINGHAM, K.E., 2007. Glycaemic status and cardiovascular disease in type 2 diabetes mellitus: re-visiting glycated haemoglobin targets for cardiovascular disease prevention. *Diabetes, Obesity & Metabolism,* **9**(6), pp. 792-798.

GOUGEON, R., LAMARCHE, M., YALE, J.F. and VENUTA, T., 2002. The prediction of resting energy expenditure in type 2 diabetes mellitus is improved by factoring for glycemia. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* **26**(12), pp. 1547-1552.

GOUGEON, R., MORAIS, J.A., CHEVALIER, S., PEREIRA, S., LAMARCHE, M. and MARLISS, E.B., 2008. Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes. *Diabetes Care,* **31**(1), pp. 128-133.

GOUGEON, R., STYHLER, K., MORAIS, J.A., JONES, P.J. and MARLISS, E.B., 2000. Effects of oral hypoglycemic agents and diet on protein metabolism in type 2 diabetes. *Diabetes Care,* **23**(1), pp. 1-8.

GOUGH, S.C., KRAGH, N., PLOUG, U.J., HAMMER, M., 2009. Impact of obesity and type 2 diabetes on health-related quality of life in the general population in England. *Diabates, Metabolic Syndrome, and Obesity: Targets and Therapy*, **2**, 179-184.

GREEN, A.J., BAZATA, D.D., FOX, K.M., GRANDY, S., SHIELD STUDY GROUP, 2007. Health-related behaviours of people with diabetes and those with cardiometabolic risk factors: results from SHIELD. *International Journal of Clinical Practice,* **61**(11), pp. 1791-1797.

GROSS, J.L., DE AZEVEDO, M.J., SILVEIRO, S.P., CANANI, L.H., CARAMORI, M.L. and ZELMANOVITZ, T., 2005. Diabetic Nephropathy: Diagnosis, Prevention, and Treatment. *Diabetes Care,* **28**(1), pp. 164-176.

GRUNDY, S.M., CLEEMAN, J.I., DANIELS, S.R., DONATO, K.A., ECKEL, R.H., FRANKLIN, B.A., GORDON, D.J., KRAUSS, R.M., SAVAGE, P.J., SMITH, S.C.,JR, SPERTUS, J.A. and COSTA, F., 2005. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation,* **112**(17), pp. 2735-2752.

GUERCI, B., BENICHOU, M., FLORIOT, M., BOHME, P., FOUGNOT, S., FRANCK, P. and DROUIN, P., 2003. Accuracy of an electrochemical sensor for measuring capillary blood ketones by fingerstick samples during metabolic deterioration after continuous subcutaneous insulin infusion interruption in type 1 diabetic patients. *Diabetes Care,* **26**(4), pp. 1137-1141.

GUERRE-MILLO, M., 2008. Adiponectin: An update. *Diabetes & Metabolism,* **34**(1), pp. 12-18.

HAIMOTO, H., SASAKABE, T., WAKAI, K. and UMEGAKI, H., 2009. *Effects of a low-carbohydrate diet on glycemic control in outpatients with severe type 2 diabetes.* England: .
HALES, C.N. and BARKER, D.J., 2001. The thrifty phenotype hypothesis. *British Medical Bulletin,* **60**, pp. 5-20.

HALES, C.N., BARKER, D.J., CLARK, P.M., COX, L.J., FALL, C., OSMOND, C. and WINTER, P.D., 1991. Fetal and infant growth and impaired glucose tolerance at age 64. *British Medical Journal,* **303**(6809), pp. 1019-1022.

HAN, S.H., QUON, M.J., KIM, J.A. and KOH, K.K., 2007. Adiponectin and cardiovascular disease: response to therapeutic interventions. *Journal of the American College of Cardiology,* **49**(5), pp. 531-538.

HARDER, H., DINESEN, B. and ASTRUP, A., 2004. The effect of a rapid weight loss on lipid profile and glycemic control in obese type 2 diabetic patients. *International Journal of Obesity and Related Metabolic Disorders,* **28**(1), pp. 180-182.

HARRIS, J.A., BENEDICT, F.G., 1919. *A biometric study of basal metabolism in man.* Washington, DC: Carnegie Institute of Washington.

HASLAM, D., 2007. Obesity: a medical history. *Obesity Reviews,* **8** (Suppl 1), pp. 31-36.

HAY, W.H., 1934. Health via Food. London: George G. Harrap & Co. Ltd.

HAYS, N.P., STARLING, R.D., LIU, X., SULLIVAN, D.H., TRAPPE, T.A., FLUCKEY, J.D. and EVANS, W.J., 2004. Effects of an ad libitum low-fat, high-carbohydrate diet on body weight, body composition, and fat distribution in older men and women: a randomized controlled trial. *Archives of Internal Medicine,* **164**(2), pp. 210-217.

HEILBRONN, L.K. and CAMPBELL, L.V., 2008. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Current Pharmaceutical Design,* **14**(12), pp. 1225-1230.

HEILBRONN, L.K., NOAKES, M. and CLIFTON, P.M., 1999. Effect of energy restriction, weight loss, and diet composition on plasma lipids and glucose in patients with type 2 diabetes. *Diabetes Care,* **22**(6), pp. 889-895.

HELEN E. TURNER, JOHN A.H. WASS, ed, 2002. *Oxford Handbook of Endocrinology and Diabetes.* GSK edition. Oxford: Oxford University Press.

HENRY, C.J.K., 2005. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutrition,* **8**(7A), pp. 1133-1152.

HENSRUD, D.D., 2001. Dietary treatment and long-term weight loss and maintenance in type 2 diabetes. *Obesity Research*, **9** (Suppl 4), pp. 348S-353S.
HESSION, M., ROLLAND, C., KULKARNI, U., WISE, A. and BROOM, J., 2009. Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obesity Reviews,* **10**(1), pp. 36-50.

HICKMAN, I.J., RUSSELL, A.J., PRINS, J.B. and MACDONALD, G.A., 2008. Should patients with type 2 diabetes and raised liver enzymes be referred for further evaluation of liver disease? *Diabetes Research and Clinical Practice,* **80**(1), pp. e10-e12.

HILL, J.O., 2006. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocrine Reviews,* **27**(7), pp. 750-761.

HINNEY, A., VOGEL, C.I.G. and HEBEBRAND, J., 2010. From monogenic to polygenic obesity: recent advances. *European Child and Adolescent Psychiatry,* **19**, pp. 297-310.

HIVERT, M.F., JABLONSKI, K.A., PERREAULT, L., SAXENA, R., MCATEER, J.B., FRANKS, P.W., HAMMAN, R.F., KAHN, S.E., HAFFNER, S., THE DIAGRAM CONSORTIUM, MEIGS, J.B., ALTSHULER, D., KNOWLER, W.C., FLOREZ, J.C. and FOR THE DIABETES PREVENTION PROGRAM RESEARCH GROUP, 2011. An Updated Genetic Score Based on 34 Confirmed Type 2 Diabetes Loci Is Associated With Diabetes Incidence and Regression to Normoglycemia in the Diabetes Prevention Program. *Diabetes,* **60**(4), pp. 1340-1348.

HM GOVERNMENT, DEPARTMENT OF HEALTH, 2010. *White Paper Healthy Lives, Healthy People: Our strategy for public health in England.* UK: Crown Copyright.

HOLM, S., 2007. Obesity interventions and ethics. *Obesity Reviews,* **8**(Suppl 1), pp. 207-210.

HOWARD, A.N., 1981. The historical development, efficacy and safety of very-low-calorie diets. *International Journal of Obesity*, **5**(3), pp. 195-208.

HOWORKA, K., PUMPRLA, J., SCHLUSCHE, C., WAGNER-NOSISKA, D., SCHABMANN, A. and BRADLEY, C., 2000. Dealing with ceiling baseline treatment satisfaction level in patients with diabetes under flexible, functional insulin treatment: assessment of improvements in treatment satisfaction with a new insulin analogue. *Quality of Life Research,* **9**(8), pp. 915-930.

HUANG, K.C., KORMAS, N., STEINBECK, K., LOUGHNAN, G. and CATERSON, I.D., 2004. Resting metabolic rate in severely obese diabetic and nondiabetic subjects. *Obesity Research,* **12**(5), pp. 840-845.

INTERNATIONAL DIABETES FEDERATION, 2011a-last update, IDF Diabetes Atlas. Morbidity and Mortality. [Homepage of IDF], [Online]. Available: http://www.diabetesatlas.org/content/diabetes-mortality [02/19, 2011].

INTERNATIONAL DIABETES FEDERATION, 2011b-last update, Prevalence estimates of diabetes mellitus (DM), 2010 [Homepage of International Diabetes Federation], [Online]. Available: http://www.diabetesatlas.org/content/prevalence-estimates-diabetes-mellitus-dm-2010 [02/19, 2011].
INTERNATIONAL DIABETES FEDERATION, 2011c-last update, Prevalence estimates of diabetes mellitus (DM), 2030 [Homepage of International Diabetes Federation], [Online]. Available: http://www.diabetesatlas.org/content/prevalence-estimates-diabetes-mellitus-dm-2030 [02/24, 2011].

INTERNATIONAL DIABETES FEDERATION CONSENSUS GROUP, 2006. *The IDF consensus worldwide definition of the metabolic syndrome.* Brussels, Belgium: International Diabetes Federation.

JAFFE, M., 1886. Uber den niederschlag, welchen pikrinsaure in normalen hrn erzeugt und uber eine neue reaction des kreatinins. *Zeitschrift für Physiologische Chemie*, **10**, pp. 391-400.

JAMES, W.P., 2008a. The epidemiology of obesity: the size of the problem. *Journal of Internal Medicine,* **263**(4), pp. 336-352.

JAMES, W.P.T., 2008b. The fundamental drivers of the obesity epidemic. *Obesity Reviews,* **9**(Suppl. 1), pp. 6-13.

JEBB, S.A., 2007. Dietary determinants of obesity. *Obesity Reviews,* **8**(Suppl 1), pp. 93-97.

JENNINGS, A.M., LEWIS, K.S., MURDOCH, S., TALBOT, J.F., BRADLEY, C. and WARD, J.D., 1991. Randomized trial comparing continuous subcutaneous insulin infusion and conventional insulin therapy in type II diabetic patients poorly controlled with sulfonylureas. *Diabetes Care,* **14**(8), pp. 738-744.

JOHNS, M.W., 1992. Reliability and factor analysis of the Epworth Sleepiness Scale. *Sleep,* **15**(4), pp. 376-381.

JOHNS, M.W., 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep,* **14**(6), pp. 540-545.

JOHNSTONE, A.M., RANCE, K.A., MURISON, S.D., DUNCAN, J.S., SPEAKMAN J.R., 2006. Additional anthropometric measures may improve the predictability of basal metabolic rate in adult subjects. *European Journal of Clinical Nutrition*, **60**(12), pp. 1437-1444.

JULIOUS, S., TAN, S.B., MACHIN, D., 2010. *An Introduction to Statistics in Early Phase Trials.* Chichester, UK: Wiley.

JUMEAN, M., OKORODUDU, D., ROMERO-CORRAL , A., SOMERS, V. and LOPEZ-JIMENEZ, F., 2008. Diagnostic Performance of Body Mass Index to Diagnose Obesity as Defined by Body Fatness: A Meta-Analysis. *Obesity,* **16**(Suppl 1), p. S328.

KANNEL, W.B., D'AGOSTINO, R.B., WILSON, P.W., BELANGER, A.J. and GAGNON, D.R., 1990. Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. *American Heart Journal,* **120**(3), pp. 672-676.

KARLSSON, C., LINDELL, K., OTTOSON, M., SJOSTROM, L., CARLSSON, B., CARLSSON, L.M., 1998. Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. *Journal of clinical Endocrinology and Metabolism*, **83**(11), pp. 3925-3929.

KEOGH, J.B., BRINKWORTH, G.D. and CLIFTON, P.M., 2007. Effects of weight loss on a low-carbohydrate diet on flow-mediated dilatation, adhesion molecules and adiponectin. *The British Journal of Nutrition,* **98**(4), pp. 852-859.

KEOGH, J.B., BRINKWORTH, G.D., NOAKES, M., BELOBRAJDIC, D.P., BUCKLEY, J.D. and CLIFTON, P.M., 2008. Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. *The American Journal of Clinical Nutrition,* **87**(3), pp. 567-576.

KEOGH, J.B., LUSCOMBE-MARSH, N.D., NOAKES, M., WITTERT, G.A. and CLIFTON, P.M., 2007. Long-term weight maintenance and cardiovascular risk factors are not different following weight loss on carbohydrate-restricted diets high in either monounsaturated fat or protein in obese hyperinsulinaemic men and women. *The British Journal of Nutrition,* **97**(2), pp. 405-410.

KEYS, A., BROZEK, J., HENSCHEL, A., MICKELSEN, O. and TAYLOR, H.L., 1950. *The Biology of Human Starvation. Volume I.* Minneapolis: The University of Minnesota Press.

KINZIG, K.P., HARGRAVE, S.L., HYUN, J. and MORAN, T.H., 2007. Energy balance and hypothalamic effects of a high-protein/low-carbohydrate diet. *Physiology & Behavior,* **92**(3), pp. 454-460.

KINZIG, K.P., SCOTT, K.A., HYUN, J., BI, S. and MORAN, T.H., 2005. Altered hypothalamic signaling and responses to food deprivation in rats fed a low-carbohydrate diet. *Obesity Research,* **13**(10), pp. 1672-1682.

KIP, K.E., MARROQUIN, O.C., KELLEY, D.E., JOHNSON, B.D., KELSEY, S.F., SHAW, L.J., ROGERS, W.J. and REIS, S.E., 2004. Clinical importance of obesity versus the metabolic syndrome in cardiovascular risk in women: a report from the Women's Ischemia Syndrome Evaluation (WISE) study. *Circulation,* **109**(6), pp. 706-713.

KIRK, J.K., GRAVES, D.E., CRAVEN, T.E., LIPKIN, E.W., AUSTIN, M. and MARGOLIS, K.L., 2008. Restricted-carbohydrate diets in patients with type 2 diabetes: a meta-analysis. *Journal of the American Dietetic Association,* **108**(1), pp. 91-100.

KOTRONEN, A., JUURINEN, L., HAKKARAINEN, A., WESTERBACKA, J., CORNER, A., BERGHOLM, R. and YKI-JARVINEN, H., 2008. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care,* **31**(1), pp. 165-169.

KORTH, O., BOSY-WESTPHAL, A., ZSCHOCHE, P., GLÜER, C.C., HELLER, M., MÜLLER, M.J., 2007. Influence of methods used in body composition analysis on the prediction of resting energy expenditure. **61**(5), pp. 582-589.

KRIEGER, J.W., SITREN, H.S., DANIELS, M.J. and LANGKAMP-HENKEN, B., 2006. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. *The American Journal of Clinical Nutrition,* **83**(2), pp. 260-274.

KURUKULASURIYA, L.R., STAS, S., LASTRA, G., MANRIQUE, C. and SOWERS, J.R., 2008. Hypertension in obesity. *Endocrinology and Metabolism Clinics of North America,* **37**(3), pp. 647-62, ix.

LACHAPELLE, D.L. and FINLAYSON, M.A., 1998. An evaluation of subjective and objective measures of fatigue in patients with brain injury and healthy controls. *Brain Injury,* **12**(8), pp. 649-659.

LAMARCHE, B., TCHERNOF, A., MOORJANI, S., CANTIN, B., DAGENAIS, G.R., LUPIEN, P.J. and DESPRES, J.P., 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation,* **95**(1), pp. 69-75.

LARSEN, R.N., MANN, N.J., MACLEAN, E. and SHAW, J.E., 2011. The effect of high-protein, low-carbohydrate diets in the treatment of type 2 diabetes: a 12 month randomised controlled trial. *Diabetologia,* **54**(4), pp. 731-740.

LAYMAN, D.K., EVANS, E., BAUM, J.I., SEYLER, J., ERICKSON, D.J. and BOILEAU, R.A., 2005. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *The Journal of Nutrition,* **135**(8), pp. 1903-1910.

LAZZER S., AGOSTI F., SILVESTRI P., DERUMEAUX-BUREL H., SARTORIO A., 2007. Prediction of resting energy expenditure in severely obese Italian women. *Journal of Endocrinological Investigation,* **30**(1), pp. 20-27.

LAZZER, S., AGOSTI F., RESNIK, M., MARAZZI, N., MORNATI, D., SARTORIO, A., 2007. Prediction of resting energy expenditure in severely obese Italian males. *Journal of Endocrinological Investigation,* **30**(9), pp. 754-761.

LEE, K.A., HICKS, G. and NINO-MURCIA, G., 1991. Validity and reliability of a scale to assess fatigue. *Psychiatry Research,* **36**(3), pp. 291-298.

LEFEBVRE, A.M., LAVILLE, M., VEGA, N., RIOU, J.P., VAN GAAL, L., AUWERX, J. and VIDAL, H., 1998. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes,* **47**(1), pp. 98-103.

LEITE, N.C., SALLES, G.F., ARAUJO, A.L., VILLELA-NOGUEIRA, C.A. and CARDOSO, C.R., 2008. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver International,* **29**(1), 113-119.

LEROITH, D., NOVOSYADLYY, R., GALLAGHER, E.J., LANN, D., VIJAYAKUMAR, A. and YAKAR, S., 2008. Obesity and type 2 diabetes are associated with an increased risk of developing cancer and a worse prognosis; epidemiological and mechanistic evidence. *Experimental and Clinical Endocrinology & Diabetes,* **116** (Suppl 1), pp. S4-6.

LEVEY, A.S., BOSCH, J.P., LEWIS, J.B., GREENE, T., ROGERS, N. and ROTH, D., 1999. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine,* **130**(6), pp. 461-470.

LEVEY, A.S., STEVENS, L.A., SCHMID, C.H., ZHANG, Y.L., CASTRO, A.F.,3RD, FELDMAN, H.I., KUSEK, J.W., EGGERS, P., VAN LENTE, F., GREENE, T., CORESH, J. and CKD-EPI

(CHRONIC KIDNEY DISEASE EPIDEMIOLOGY COLLABORATION), 2009. A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine,* **150**(9), pp. 604-612.

LIU-SEIFERT, H., ZHANG, S., D'SOUZA, D. and SKLJAREVSKI, V., 2010. A closer look at the baseline-observation-carried-forward (BOCF). *Patient Preference and Adherence,* 4, pp. 11-16.

LIU, L., IKEDA, K., SULLIVAN, D.H., LING, W., YAMORI, Y., 2002. Epidemiological evidence of the association between dietary protein intake and blood pressure: a meta-analysis of published data. *Hypertension Research,* **25**(5), pp. 689-695.

LIVINGSTON, E.H., KOHLSTADT, I., 2005. Simplified resting metabolic rate-predicting formulas for normal-sized and obese individuals. *Obesity Research,* **13**(7), pp. 1255-1262.

LLOYD, A., SAWYER, W., HOPKINSON, P., 2001. Impact of long-term complications on quality of life in patients with type 2 diabetes not using insulin. *Value in Health,* **4**(5), pp. 392-400.

LOOS, R.J. and BOUCHARD, C., 2003. Obesity-is it a genetic disorder? *Journal of Internal Medicine,* **254**(5), pp. 401-425.

LUSCOMBE, N.D., CLIFTON, P.M., NOAKES, M., FARNSWORTH, E. and WITTERT, G., 2003. Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects. *International Journal of Obesity and Related Metabolic Disorders,* **27**(5), pp. 582-590.

LUSCOMBE, N.D., CLIFTON, P.M., NOAKES, M., PARKER, B. and WITTERT, G., 2002. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. *Diabetes Care,* **25**(4), pp. 652-657.

LUSCOMBE-MARSH, N.D., NOAKES, M., WITTERT, G.A., KEOGH, J.B., FOSTER, P. and CLIFTON, P.M., 2005. Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. *The American Journal of Clinical Nutrition,* **81**(4), pp. 762-772.

MAIA-FERNANDES, T., RONCON-ALBUQUERQUE, R.,JR and LEITE-MOREIRA, A.F., 2008. Cardiovascular actions of adiponectin: pathophysiologic implications. *Portuguese Journal of Cardiology,* **27**(11), pp. 1431-1449.

MA, Y., OLENDZKI, B.C., HAFNER, A.R., CHIRIBOGA, D.E., CULVER, A.L., ANDERSEN, V.A., MERRIAM, P.A., PAGOTO, S.L., 2006. Low-carbohydate and high-fat intake among adult patients with poorly controlled type 2 diabetes mellitus. *Nutrition,* **22**(11-12), pp. 1129-1136.

MANNUCCI, E., PETRONI, M.L., VILLANOVA, N., ROTELLA, C.M., APOLONE, G., MARCHESINI, G. and QUOVADIS STUDY GROUP, 2010. Clinical and psychological correlates of health-related quality of life in obese patients. *Health and Quality of Life Outcomes,* **23**(8), p. 90, doi: 10.1186/1477-7525-8-90.

MANNUCCI, E., RICCA, V., BARCIULLI, E., DI BERNARDO, M., TRAVAGLINI, R., CABRAS, P.L. and ROTELLA, C.M., 1999. Quality of life and overweight: the obesity related well-being (Orwell 97) questionnaire. *Addictive Behaviors,* **24**(3), pp. 345-357.

MANTZOROS, C.S. (Ed), 2006. *Obesity and Diabetes*. Totowa, New Jersey: Humana Press.

MARCHESINI, G., 2008. Prevalence of elevated liver enzymes in Type 2 diabetes mellitus and its association with the metabolic syndrome. *Journal of Endocrinological Investigation,* **31**(2), pp. 146-152.

MARKOVIC, T.P., JENKINS, A.B., CAMPBELL, L.V., FURLER, S.M., KRAEGEN, E.W. and CHISHOLM, D.J., 1998. The determinants of glycemic responses to diet restriction and weight loss in obesity and NIDDM. *Diabetes Care,* **21**(5), pp. 687-694.

MARTINEZ-HERNANDEZ, A., ENRIQUEZ, L., MORENO-MORENO, M.J. and MARTI, A., 2007. Genetics of obesity. *Public Health Nutrition,* **10**(10A), pp. 1138-1144.

MASSI-BENEDETTI, M. and FEDERICI, M.O., 1999. Cardiovascular risk factors in type 2 diabetes: the role of hyperglycaemia. *Experimental and Clinical Endocrinology & Diabetes,* **107** (Suppl 4), pp. S120-S123.

MATTHEWS, D.R., HOSKER, J.P., RUDENSKI, A.S., NAYLOR, B.A., TREACHER, D.F. and TURNER, R.C., 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* **28**(7), pp. 412-419.

MAZZONI, R., MANNUCCI, E., RIZZELLO, S.M., RICCA, V. and ROTELLA, C.M., 1999. Failure of acupuncture in the treatment of obesity: a pilot study. *Eating and Weight Disorders,* **4**(4), pp. 198-202.

MCARDLE, W.D., KATCH, F.I. and KATCH, V.L., 2007. *Exercise Physiology, Energy, Nutrition and Human Performance.* Baltimore: Lippincott, Williams & Wilkins.

MCAULEY, K.A., HOPKINS, C.M., SMITH, K.J., MCLAY, R.T., WILLIAMS, S.M., TAYLOR, R.W. and MANN, J.I., 2005. Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. *Diabetologia,* **48**(1), pp. 8-16.

MCAULEY, K.A., SMITH, K.J., TAYLOR, R.W., MCLAY, R.T., WILLIAMS, S.M. and MANN, J.I., 2006. Long-term effects of popular dietary approaches on weight loss and features of insulin resistance. *International Journal of Obesity,* **30**(2), pp. 342-349.

MCLAUGHLIN, T., ABBASI, F., LAMENDOLA, C., LIANG, L., REAVEN, G., SCHAAF, P. and REAVEN, P., 2002. Differentiation between obesity and insulin resistance in the association with C-reactive protein. *Circulation,* **106**(23), pp. 2908-2912.

MCLAUGHLIN, T., CARTER, S., LAMENDOLA, C., ABBASI, F., YEE, G., SCHAAF, P., BASINA, M. and REAVEN, G., 2006. Effects of moderate variations in macronutrient composition on weight loss and reduction in cardiovascular disease risk in obese, insulin-resistant adults. *The American Journal of Clinical Nutrition,* **84**(4), pp. 813-821.

MCLENNAN W., P.A., 1998. *National Nutrition Survey. Nutrient Intakes and Physical Measurements. Australia 1995.* 4805.0. Australia: Australian Bureau of Statistics.

MCPHERSON, R., 2007. Genetic contributors to obesity. *The Canadian Journal of Cardiology,* **23 (**Suppl A), pp. 23A-27A.

MECKLING, K.A., O'SULLIVAN, C. and SAARI, D., 2004. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *The Journal of Clinical Endocrinology and Metabolism,* **89**(6), pp. 2717-2723.

MIFFLIN, M.D., ST JEOR, S.T., HILL, L.A., SCOTT, B.J., DAUGHERTY, S.A., KOH, Y.O., 1990. A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition,* **51**(2), pp. 241-247.

MIYASHITA, Y., KOIDE, N., OHTSUKA, M., OZAKI, H., ITOH, Y., OYAMA, T., UETAKE, T., ARIGA, K. and SHIRAI, K., 2004. Beneficial effect of low carbohydrate in low calorie diets on visceral fat reduction in type 2 diabetic patients with obesity. *Diabetes Research and Clinical Practice,* **65**(3), pp. 235-241.

MOBBS, C.V., MASTAITIS, J., YEN, K., SCHWARTZ, J., MOHAN, V., POPLAWSKI, M. and ISODA, F., 2007. Low-carbohydrate diets cause obesity, low-carbohydrate diets reverse obesity: a metabolic mechanism resolving the paradox. *Appetite,* **48**(2), pp. 135-138.

MONNIER, L., COLETTE, C., PERCHERON, C., BONIFACE, H., 2000. Very-low-calorie-diets: is there a place for them in the management of the obese diabetic? *Diabetes & Metabolism,* **26** (Suppl 3), pp. 46-51.

MONTAGUE, C.T., PRINS, J.B., SANDERS, L., DIGBY, J.E. and O'RAHILLY, S., 1997. Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes,* **46**(3), pp. 342-347.

MORAN, L.J., LUSCOMBE-MARSH, N.D., NOAKES, M., WITTERT, G.A., KEOGH, J.B. and CLIFTON, P.M., 2005. The satiating effect of dietary protein is unrelated to postprandial ghrelin secretion. *The Journal of Clinical Endocrinology and Metabolism,* **90**(9), pp. 5205-5211.

MORGAN, L., GRIFFIN, B., MILLWARD, D., DELOOY, A., FOX, K., BAIC, S., BONHAM, M., WALLACE, J., MACDONALD, I., TAYLOR, M. and TRUBY, H., 2008. Comparison of the effects of four commercially available weight-loss programmes on lipid-based cardiovascular risk factors. *Public Health Nutrition,* **12**(6), pp. 799-807.

MORRATO, E.H., HILL, J.O., WYATT, H.R., GHUSHCHYAN, V., SULLIVAN, P.W., 2007. Physical activity in U.S. adults with diabetes and at risk of developing diabetes. *Diabetes Care,* **30**, pp. 203-209.

MORRISON, K., 2005. Low carbohydrate diets for diabetes control. *The British Journal of General Practice,* **55**(520), p. 884.

MORSE, S.A., BRAVO, P.E., MORSE, M.C. and REISIN, E., 2005. The heart in obesity-hypertension. *Expert Review of Cardiovascular Therapy,* **3**(4), pp. 647-658.
MORTON, G.J., CUMMINGS, D.E., BASKIN, D.G., BARSH, G.S., SCHWARTZ, M.W., 2006. Central nervous system control of food intake and body weight. *Nature,* **443**(7109), pp. 289-295.

MUTLU, E.A., KESHAVARZIAN, A. and MUTLU, G.M., 2006. Hyperalbuminemia and elevated transaminases associated with high-protein diet. *Scandinavian Journal of Gastroenterology,* **41**(6), pp. 759-760.

MUZIO, F., MONDAZZI, L., HARRIS, W.S., SOMMARIVA, D. and BRANCHI, A., 2007. Effects of moderate variations in the macronutrient content of the diet on cardiovascular disease risk factors in obese patients with the metabolic syndrome. *The American Journal of Clinical Nutrition,* **86**(4), pp. 946-951.

MÜLLER, M.J., BOSY-WESTPHAL, A., KLAUS, S., KREYMANN, G., LÜHRMANN, P.M., NEUHÄUSER-BERTHOLD, M., NOACK, R., PIRKE, K.M., PLATTE, P., SELBERG, O., STEINIGER, J., 2004. World Health Organization equations have shortcomings for predicting resting energy expenditure in persons from a modern, affluent population: generation of a new reference standard from a retrospective analysis of a German database of resting energy expenditure. *American Journal of Clinical Nutrition*, 80(5), pp. 1379-1390.

NAIR, K.S., HALLIDAY, D. and GARROW, J.S., 1984. Increased energy expenditure in poorly controlled Type 1 (insulin-dependent) diabetic patients. *Diabetologia,* **27**(1), pp. 13-16.

NARAYAN, K.M., GREGG, E.W., FAGOT-CAMPAGNA, A., ENGELGAU, M.M. and VINICOR, F., 2000. Diabetes-a common, growing, serious, costly, and potentially preventable public health problem. *Diabetes Research and Clinical Practice,* **50** (Suppl 2), pp. S77-S84.

NATIONAL CHOLESTEROL PROGRAM (NCEP) EXPERT PANEL ON DETECTION, EVALUATION, AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS (ADULT TREATMENT PANEL III), 2002. Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation,* **106**(25), pp. 3143-3421.

NATIONAL HEALTH SERVICE, 2006. *General Practice Physical Activity Questionnaire.* [Online]. Available: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_063812 [03/02 2011]

NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE, 2008. *Type 2 diabetes. The management of type 2 diabetes.* NICE clinical guideline 66. London: National Institute for Health and Clinical Excellence.

NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE, 2006. *Obesity. Guidance on the prevention, identification, assessment, and management of overweight and obesity in adults and children.* NICE clinical guideline 43. London: National Institute for Health and Clinical Excellence.

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES, 2008. Very low-calorie diets. Factsheet No 03-3894. Available at http://www.win.niddk.nih.gov.

NEEL, J.V., 1962. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *American Journal of Human Genetics,* **14**, pp. 353-362.

NIELSEN, J.V. and JOENSSON, E., 2006. Low-carbohydrate diet in type 2 diabetes. Stable improvement of bodyweight and glycemic control during 22 months follow-up. *Nutrition & Metabolism,* **3**, pp. 22.

NOAKES, M., KEOGH, J.B., FOSTER, P.R. and CLIFTON, P.M., 2005. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on

weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *The American Journal of Clinical Nutrition,* **81**(6), pp. 1298-1306.

NORDMANN, A.J., NORDMANN, A., BRIEL, M., KELLER, U., YANCY, W.S.,JR, BREHM, B.J. and BUCHER, H.C., 2006. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Archives of Internal Medicine,* **166**(3), pp. 285-293.

NUTTALL, F.Q. and GANNON, M.C., 2004. Metabolic response of people with type 2 diabetes to a high protein diet. *Nutrition & Metabolism,* **1**(1), pp. 6, doi: 10.1186/1743-7075-1-6.

NUTTALL, F.Q., SCHWEIM, K., HOOVER, H. and GANNON, M.C., 2008. Effect of the LoBAG30 diet on blood glucose control in people with type 2 diabetes. *The British Journal of Nutrition,* **99**(3), pp. 511-519.

O'CONNELL, D. and VELICER, W.F., 1988. A decisional balance measure and the stages of change model for weight loss. *The International Journal of the Addictions,* **23**(7), pp. 729-750.

OLSEN, L.R., JENSEN, D.V., NOERHOLM, V., MARTINY, K. and BECH, P., 2003. The internal and external validity of the Major Depression Inventory in measuring severity of depressive states. *Psychological Medicine,* **33**(2), pp. 351-356.

OWEN, O.E., HOLUP, J.L., D'ALESSIO, D.A., CRAIG, E.S., POLANSKY, M., SMALLEY, K.J., KAVLE, E.C., BUSHMAN, M.C., OWEN, L.R., MOZZOLI, M.A., 1987. A reappraisal of the caloric requirements of men. *American Journal of Clinical Nutrition,* **46**(6), pp. 875-885.

OWEN, O.E., KAVLE, E., OWEN, R.S., POLANSKY, M., CAPRIO, S., MOZZOLI, M.A., KENDRICK, Z.V., BUSHMAN, M.C., BODEN, G., 1986. A reappraisal of caloric requirements in healthy women. *American Journal of Clinical Nutrition,* **44**(1), pp. 1-19.

PACKARD, C.J., 2003. Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochemical Society Transactions,* **31**(Pt 5), pp. 1066-1069.

PARK, K.G., PARK, K.S., KIM, M.J., KIM, H.S., SUH, Y.S., AHN, J.D., PARK, K.K., CHANG, Y.C. and LEE, I.K., 2004. Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Research and Clinical Practice,* **63**(2), pp. 135-142.

PARKER, B., NOAKES, M., LUSCOMBE, N. and CLIFTON, P., 2002. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care,* **25**(3), pp. 425-430.

PHELAN, S., WYATT, H., NASSERY, S., DIBELLO, J., FAVA, J.L., HILL, J.O. and WING, R.R., 2007. Three-year weight change in successful weight losers who lost weight on a low-carbohydrate diet. *Obesity,* **15**(10), pp. 2470-2477.

PI-SUNYER, F.X., 2005. Weight loss in type 2 diabetic patients. *Diabetes Care,* **28**(6), pp. 1526-1527.

POCOCK, G. and RICHARDS, C.D., 2006. *Human Physiology. The basis of medicine.* Third Edition. Oxford University Press.

POLGAR, S. and THOMAS, S.A., 2008. *Introduction to Research in the Health Sciences.* Fifth Edition. Philadelphia, USA: Churchill Livingstone Elsevier.

POUWER, F., GEELHOED-DUIJVESTIJN, P.H., TACK, C.J., BAZELMANS, E., BEEKMAN, A.J., HEINE, R.J., SNOEK, F.J., 2010. Prevalence of comorbid depression is high in out-patients with Type 1 or Type 2 diabetes mellitus. Results from three out-patient clinics in the Netherlands. *Diabetic Medicine*, **27**(2), pp. 217-224.

PRENTICE, A.M., HENNIG, B.J. and FULFORD, A.J., 2008. Evolutionary origins of the obesity epidemic: natural selection of thrifty genes or genetic drift following predation release? *International Journal of Obesity (2005)*, **32**(11), pp. 1607-1610.

RAHMOUNI, K., CORREIA, M.L., HAYNES, W.G. and MARK, A.L., 2005. Obesity-associated hypertension: new insights into mechanisms. *Hypertension*, **45**(1), pp. 9-14.

RANA, J.S., NIEUWDORP, M., JUKEMA, J.W. and KASTELEIN, J.J., 2007. Cardiovascular metabolic syndrome - an interplay of, obesity, inflammation, diabetes and coronary heart disease. *Diabetes, Obesity & Metabolism*, **9**(3), pp. 218-232.

RANKINEN, T., ZUBERI, A., CHAGNON, Y.C., WEISNAGEL, S.J., ARGYROPOULOS, G., WALTS, B., PERUSSE, L. and BOUCHARD, C., 2006. The human obesity gene map: the 2005 update. *Obesity*, **14**(4), pp. 529-644.

RAVAL, A., DHANARAJ, E., BHANSALI, A., GROVER, S., TIWARI, P., 2010. Prevalence & determinants of depression in type 2 diabetes patients in a tertiary care centre. *The Indian Journal of Medical Research*, **132**, pp. 195-200.

RIGBY, N. and JAMES, P., 2003. The obesity campaign view of diabetes prevention. *Diabetes Voice*, **48**, pp. 20-23.

ROBERTSON, A.M., BROOM, J., MCROBBIE, L.J. and MACLENNAN, G.S., 2002. Low carbohydratediets in treatment resistant overweight patienets with Type 2 diabetes. *Diabetic Medicine*, **19**(Suppl. 2), pp. 24.

ROLLAND, C., HESSION, M., MURRAY, S., WISE, A., BROOM, I., 2009. Randomized clinical trial of standard dietary treatment versus a low-carbohydrate/high-protein diet or the LighterLife Programme in the management of obesity. *Journal of Diabetes*, **1**(3), pp. 207-217.

ROSS, R., 1999. Atherosclerosis - An Inflamattory Disease. *New England Journal of Medicine*, **340**(2), pp. 115-126.

ROZA, A.M., SHIZGAL, H.M., 1984. The Harris Benedict equation reevaluated: resting energy requirements and the body cell mass. *American Journal of Clinical Nutrition*, **40**(1), pp. 168-182.

RUSSELL, W.R., GRATZ, S.W., DUNCAN, S.W., HOLTROP, G., INCE, J., SCOBBIE, L., DUNCAN, G., JOHNSTONE, A.M., LOBLEY, G.E., WALLACE, R.J., DUTHIE, G.G., FLINT, H., 2011. High-protein, reduced-carbohydrate weight-loss diets promote metabolic profiles likely to be detrimental to colonic health. *American Journal of Clinical Nutrition*, First published online on March 9, doi: 10.3945/ajcn.110.002188.

RYAN, M.C., ABBASI, F., LAMENDOLA, C., CARTER, S. and MCLAUGHLIN, T.L., 2007. Serum alanine aminotransferase levels decrease further with carbohydrate than fat restriction in insulin-resistant adults. *Diabetes Care,* **30**(5), pp. 1075-1080.

SACKS, F.M., BRAY, G.A., CAREY, V.J., SMITH, S.R., RYAN, D.H., ANTON, S.D., MCMANUS, K., CHAMPAGNE, C.M., BISHOP, L.M., LARANJO, N., LEBOFF, M.S., ROOD, J.C., DE JONGE, L., GREENWAY, F.L., LORIA, C.M., OBARZANEK, E. and WILLIAMSON, D.A., 2009. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *The New England Journal of Medicine,* **360**(9), pp. 859-873.

SAMAHA, F.F., IQBAL, N., SESHADRI, P., CHICANO, K.L., DAILY, D.A., MCGRORY, J., WILLIAMS, T., WILLIAMS, M., GRACELY, E.J. and STERN, L., 2003. A low-carbohydrate as compared with a low-fat diet in severe obesity. *The New England Journal of Medicine,* **348**(21), pp. 2074-2081.

SANDBAEK, A., GRIFFIN, S.J., RUTTEN, G., DAVIES, M., STOLK, R., KHUNTI, K., BORCH-JOHNSEN, K., WAREHAM, N.J. and LAURITZEN, T., 2008. Stepwise screening for diabetes identifies people with high but modifiable coronary heart disease risk. The ADDITION study. *Diabetologia,* **51**(7), pp. 1127-1134.

SARGRAD, K.R., HOMKO, C., MOZZOLI, M. and BODEN, G., 2005. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin A1c, and blood pressure in patients with type 2 diabetes mellitus. *Journal of the American Dietetic Association,* **105**(4), pp. 573-580.

SARZANI, R., SALVI, F., DESSI-FULGHERI, P. and RAPPELLI, A., 2008. Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: an integrated view in humans. *Journal of Hypertension,* **26**(5), pp. 831-843.

SAUNDERS, C.L., CHIODINI, B.D., SHAM, P., LEWIS, C.M., ABKEVICH, V., ADEYEMO, A.A., DE ANDRADE, M., ARYA, R., BERENSON, G.S., BLANGERO, J., BOEHNKE, M., BORECKI, I.B., CHAGNON, Y.C., CHEN, W., COMUZZIE, A.G., DENG, H.W., DUGGIRALA, R., FEITOSA, M.F., FROGUEL, P., HANSON, R.L., HEBEBRAND, J., HUEZO-DIAS, P., KISSEBAH, A.H., LI, W., LUKE, A., MARTIN, L.J., NASH, M., OHMAN, M., PALMER, L.J., PELTONEN, L., PEROLA, M., PRICE, R.A., REDLINE, S., SRINIVASAN, S.R., STERN, M.P., STONE, S., STRINGHAM, H., TURNER, S., WIJMENGA, C. and COLLIER, D.A., 2007. Meta-analysis of genome-wide linkage studies in BMI and obesity. *Obesity,* **15**(9), pp. 2263-2275.

SCHOFIELD, W.N., 1985. Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition. Clinical Nutrition,* **39** (Suppl 1), pp. 5-41.

SCOTT, R., BEST, J., FORDER, P., TASKINEN, M.R., SIMES, J., BARTER, P., KEECH, A. and FIELD STUDY INVESTIGATORS, 2005. Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study: baseline characteristics and short-term effects of fenofibrate. *Cardiovascular Diabetology,* **4**, p. 13, doi: 10.1186/1475-2840-4-13

SCOTTISH INTERCOLLEGIATE GUIDELINES NETWORK, 2010. *Management of Diabetes. A national clinical guideline. Report 116.* Edinburgh: Scottish Intercollegiate Guideline Network.

SENECHAL, M., ARGUIN, H., BOUCHARD, D.R., CARPENTIER, A.C., ARDILOUZE, J.L., DIONNE, I.J. and BROCHU, M., 2010. Interindividual variations in resting metabolic rate during

weight loss in obese postmenopausal women a pilot study. *Metabolism: Clinical and Experimental,* **59**(4), pp. 478-485.

SESHADRI, P., IQBAL, N., STERN, L., WILLIAMS, M., CHICANO, K.L., DAILY, D.A., MCGRORY, J., GRACELY, E.J., RADER, D.J. and SAMAHA, F.F., 2004. A randomized study comparing the effects of a low-carbohydrate diet and a conventional diet on lipoprotein subfractions and C-reactive protein levels in patients with severe obesity. *The American Journal of Medicine,* **117**(6), pp. 398-405.

SHAI, I., SCHWARZFUCHS, D., HENKIN, Y., SHAHAR, D.R., WITKOW, S., GREENBERG, I., GOLAN, R., FRASER, D., BOLOTIN, A., VARDI, H., TANGI-ROZENTAL, O., ZUK-RAMOT, R., SARUSI, B., BRICKNER, D., SCHWARTZ, Z., SHEINER, E., MARKO, R., KATORZA, E., THIERY, J., FIEDLER, G.M., BLUHER, M., STUMVOLL, M., STAMPFER, M.J. and DIETARY INTERVENTION RANDOMIZED CONTROLLED TRIAL (DIRECT) GROUP, 2008. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *The New England Journal of Medicine,* **359**(3), pp. 229-241.

SHARMAN, M.J., GOMEZ, A.L., KRAEMER, W.J. and VOLEK, J.S., 2004. Very low-carbohydrate and low-fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *The Journal of Nutrition,* **134**(4), pp. 880-885.

SHINE, B., DE BEER, F.C. and PEPYS, M.B., 1981. Solid phase radioimmunoassays for human C-reactive protein. *Clinica Chimica Acta,* **117**(1), pp. 13-23.

SIMS, E.A., DANFORTH, E.,JR, HORTON, E.S., BRAY, G.A., GLENNON, J.A. and SALANS, L.B., 1973. Endocrine and metabolic effects of experimental obesity in man. *Recent Progress in Hormone Research,* **29**, pp. 457-496.

SKOV, A.R., TOUBRO, S., BULOW, J., KRABBE, K., PARVING, H.H. and ASTRUP, A., 1999a. Changes in renal function during weight loss induced by high vs low-protein low-fat diets in overweight subjects. *International Journal of Obesity and Related Metabolic Disorders,* **23**(11), pp. 1170-1177.

SKOV, A.R., TOUBRO, S., RONN, B., HOLM, L. and ASTRUP, A., 1999b. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *International Journal of Obesity and Related Metabolic Disorders,* **23**(5), pp. 528-536.

SPEAKMAN, J.R., 2008a. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. *International Journal of Obesity,* **32**(11), pp. 1611-1617.

SPEAKMAN, J.R., RANCE, K.A., JOHNSTONE, K.M., 2008b. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. *Obesity,* **16**(8), pp. 1961-1965.

SPEAKMAN, J.R., 2007. A nonadaptive scenario explaining the genetic predisposition to obesity: the "predation release" hypothesis. *Cell Metabolism,* **6**(1), pp. 5-12.

SPEAKMAN, J.R., 2006. Thrifty genes for obesity and the metabolic syndrome--time to call off the search? *Diabetes & Vascular Disease Research,* **3**(1), pp. 7-11.

ST. JEOR, S.T., HOWARD, B.V., PREWITT, T.E., BOVEE, V., BAZZARRE, T. and ECKEL, R.H., 2001. Dietary Protein and Weight Reduction. A Statement for Healthcare Professionals From the

Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Circulation.*, **104**, pp. 1869-1874.

ST.JEOR, S.T., 2000. High Protein Diets. Online article available on http://www.medscape.com, Posted on 11/09/2000, Printed on 16/02/2011.

STEFANOVIC, A., KOTUR-STEVULJEVIC, J., SPASIC, S., BOGAVAC-STANOJEVIC, N. and BUJISIC, N., 2008. The influence of obesity on the oxidative stress status and the concentration of leptin in type 2 diabetes mellitus patients. *Diabetes Research and Clinical Practice,* **79**(1), pp. 156-163.

STERN, L., IQBAL, N., SESHADRI, P., CHICANO, K.L., DAILY, D.A., MCGRORY, J., WILLIAMS, M., GRACELY, E.J. and SAMAHA, F.F., 2004. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Annals of Internal Medicine,* **140**(10), pp. 778-785.

ST-PIERRE, A.C., CANTIN, B., DAGENAIS, G.R., MAURIEGE, P., BERNARD, P.M., DESPRES, J.P. and LAMARCHE, B., 2005. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. *Arteriosclerosis, Thrombosis, and Vascular Biology,* **25**(3), pp. 553-559.

STUBBS, P.J., ALAGHBAND-ZADEH, J., LAYCOCK, J.F., COLLINSON, P.O., CARTER, G.D. and NOBLE, M.I., 1999. Significance of an index of insulin resistance on admission in non-diabetic patients with acute coronary syndromes. *Heart,* **82**(4), pp. 443-447.

STUNKARD, A.J., HARRIS, J.R., PEDERSEN, N.L. and MCCLEARN, G.E., 1990. The body-mass index of twins who have been reared apart. *The New England Journal of Medicine,* **322**(21), pp. 1483-1487.

SWINBURN, B. and EGGER, G., 2004. The runaway weight gain train: too many accelerators, not enough brakes. *British Medical Journal,* **329**(7468), pp. 736-739.

SWINBURN, B., EGGER, G. and RAZA, F., 1999. Dissecting obesogenic environments: the development and application of a framework for identifying and prioritizing environmental interventions for obesity. *Preventive Medicine,* **29**(6 Pt 1), pp. 563-570.

TAKESHITA, H., SHIMADA, Y., KOBAYASHI, Y., NISHIOKA, H., EHARA, S., KATAOKA, T. and YOSHIYAMA, M., 2008. Impact of body mass index and Framingham risk score on coronary artery plaque. *Osaka City Medical Journal,* **54**(1), pp. 31-39.

TATTERSALL, R., 2009. *Diabetes: the Biography*. Oxford: Oxford University Press.

THE NHS INFORMATION CENTRE, 2009. *Health Survey for England 2008. Volume 1: Physical Activity and Fitness.*

THE SCOTTISH GOVERNMENT, 2010. *Preventing Overweight and Obesity in Scotland. A Route Map Towards Healthy Weight.* Edinburgh: The Scottish Government.

THE SCOTTISH GOVERNMENT, 2009. *The Scottish Health Survey 2008.* Edinburgh: The Scottish Government.

TOKUDA, K., TANIMOTO, K., 1993. New method of measuring serum bilirubin using vanadic acid. *Japanese Journal of Clinical Chemistry,* **22**(2), pp.116-122.

TONG, P.C., KONG, A.P., SO, W.Y., YANG, X., HO, C.S., MA, R.C., OZAKI, R., CHOW, C.C., LAM, C.W., CHAN, J.C. and COCKRAM, C.S., 2007. The usefulness of the International Diabetes Federation and the National Cholesterol Education Program's Adult Treatment Panel III definitions of the metabolic syndrome in predicting coronary heart disease in subjects with type 2 diabetes. *Diabetes Care,* **30**(5), pp. 1206-1211.

TRAYHURN, P., 2007. Adipocyte biology. *Obesity Reviews,* **8** (Suppl) 1, pp. 41-44.

TRAYHURN, P., 2005. Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiologica Scandinavica,* **184**(4), pp. 285-293.

TREASURE, T., MACRAE, K.D., 1998. Minimisation: the platinum standard for trials? Randomisation doesn't guarantee similarity of group; minimisation does. *British Medical Journal*, **317**(7155), pp. 362-363.

TSAI, A.G., WADDEN, T.A., 2006. The evolution of very-low-calorie diets: an update and meta-analysis. *Obesity*, **14**(8), pp. 1283-1293.

TURNER H., W.J., 2002. *Oxford Handbook of Endocrinology and Diabetes.* Oxford University Press.

UK PROSPECTIVE DIABETES STUDY GROUP, 1991. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. *Diabetologia*, **34**, pp. 877-890.

ULIJASZEK, S.J., 2008. Seven models of population obesity. *Angiology,* **59**(2 Suppl), pp. 34S-38S.

ULIJASZEK, S.J., 2007. Obesity: a disorder of convenience. *Obesity Reviews,* **8** (Suppl 1), pp. 183-187.

UNGER, R.H., 2008. Reinventing type 2 diabetes: pathogenesis, treatment, and prevention. *Journal of the American Medical Association,* **299**(10), pp. 1185-1187.

UNITED NATIONS, 2010. *The Millennium Development Goals Report 2010.* New York: United Nations.

VARLEY, H., 1969. *Practical Clinical Biochemistry.* 4th edn. New Delhi: Arnold-Heinemann Publishers Pvt Ltd.

VIBERTI, G., LACHIN, J., HOLMAN, R., ZINMAN, B., HAFFNER, S., KRAVITZ, B., HEISE, M.A., JONES, N.P., O'NEILL, M.C., FREED, M.I., KAHN, S.E., HERMAN, W.H. and FOR THE ADOPT STUDY GROUP, 2006. A Diabetes Outcome Progression Trial (ADOPT): baseline characteristics of Type 2 diabetic patients in North America and Europe. *Diabetic Medicine,* **23**(12), pp. 1289-1294.

VIMALESWARAN, K.S. and LOOS, R.J., 2010. Progress in the genetics of common obesity and type 2 diabetes. *Expert Reviews in Molecular Medicine,* **12**, pp. e7, doi: 10.1017/S1462399410001389

VISOCKIENE, Z., KALIBATIENE, D., 2006. Resting energy expenditure in type 2 diabetes subjects: is obesity important? *Acta Medica Lituanica*, **13**(4), pp. 258-264.

VOLEK, J.S. and FEINMAN, R.D., 2005a. Carbohydrate restriction improves the features of Metabolic Syndrome. Metabolic Syndrome may be defined by the response to carbohydrate restriction. *Nutrition & Metabolism*, **2**, pp. 31.

VOLEK, J.S., FERNANDEZ, M.L., FEINMAN, R.D. and PHINNEY, S.D., 2008. Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Progress in Lipid Research*, **47**(5), pp. 307-318.

VOLEK, J.S., SHARMAN, M.J. and FORSYTHE, C.E., 2005b. Modification of lipoproteins by very low-carbohydrate diets. *The Journal of Nutrition*, **135**(6), pp. 1339-1342.

VOLEK, J.S., SHARMAN, M.J., GOMEZ, A.L., DIPASQUALE, C., ROTI, M., PUMERANTZ, A. and KRAEMER, W.J., 2004. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *Journal of the American College of Nutrition*, **23**(2), pp. 177-184.

WADDEN, T.A., STERNBERG, J.A., LETIZIA, K.A., STUNKARD, A.J. and FOSTER, G.D., 1989. Treatment of obesity by very low calorie diet, behavior therapy, and their combination: a five-year perspective. *International Journal of Obesity*, **13** (Suppl 2), pp. 39-46.

WALLACE, T.M., LEVY, J.C. and MATTHEWS, D.R., 2004. Use and abuse of HOMA modeling. *Diabetes Care*, **27**(6), pp. 1487-1495.

WANG, R.T., KORETZ, R.L. and YEE, H.F.,JR, 2003. Is weight reduction an effective therapy for nonalcoholic fatty liver? A systematic review. *The American Journal of Medicine*, **115**(7), pp. 554-559.

WARDLE, J. and BONIFACE, D., 2008. Changes in the distributions of body mass index and waist circumference in English adults, 1993/1994 to 2002/2003. *International Journal of Obesity (2005)*, **32**(3), pp. 527-532.

WAUTERS, M., MERTENS, I., CONSIDINE, R., DE LEEUW, I. and VAN GAAL, L., 1998. Are leptin levels dependent on body fat distribution in obese men and women? *Eating and Weight Disorders*, **3**(3), pp. 124-130.

WEEKS, I., STURGESS, M., SIDDLE, K., JONES, M.K., WOODHEAD, J.S., 1984. A high sensitivity immunochemiluminometric assay for human thyrotropin. Clinical Endocrinology, 20(4), pp. 489-495.

WEIJS, P.J.M., 2008. Validity of predictive equations for resting energy expenditure in US and Dutch overweight and obese class I and II adults aged 18-65. *American Journal of Clinical Nutrition*, **88**(4), 959-970.

WELLE, S., NAIR, K.S. and LOCKWOOD, D., 1988. Effect of a sulfonylurea and insulin on energy expenditure in type II diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism*, **66**(3), pp. 593-597.

WESTERTERP-PLANTENGA, M.S., 2007. How are normal, high- or low-protein diets defined? *The British Journal of Nutrition*, **97**(2), pp. 217-218.

WESTMAN, E.C., YANCY, W.S., JR and HUMPHREYS, M., 2006. Dietary treatment of diabetes mellitus in the pre-insulin era (1914-1922). *Perspectives in Biology and Medicine,* **49**(1), pp. 77-83.

WESTMAN, E.C., FEINMAN, R.D., MAVROPOULOS, J.C., VERNON, M.C., VOLEK, J.S., WORTMAN, J.A., YANCY, W.S. and PHINNEY, S.D., 2007. Low-carbohydrate nutrition and metabolism. *The American Journal of Clinical Nutrition,* **86**(2), pp. 276-284.

WESTMAN, E.C., YANCY, W.S.,JR, MAVROPOULOS, J.C., MARQUART, M. and MCDUFFIE, J.R., 2008. The effect of a low-carbohydrate, ketogenic diet versus a low-glycemic index diet on glycemic control in type 2 diabetes mellitus. *Nutrition & Metabolism,* **5**, pp. 36.

WESTMAN, E.C., YANCY, W.S.,JR, OLSEN, M.K., DUDLEY, T. and GUYTON, J.R., 2006. Effect of a low-carbohydrate, ketogenic diet program compared to a low-fat diet on fasting lipoprotein subclasses. *International Journal of Cardiology,* **110**(2), pp. 212-216.

WESTMAN, E.C., YANCY, W.S., EDMAN, J.S., TOMLIN, K.F. and PERKINS, C.E., 2002. Effect of 6-month adherence to a very low carbohydrate diet program. *The American Journal of Medicine,* **113**(1), pp. 30-36.

WEYER, C., SNITKER, S., RISING, R., BOGARDUS, C., RAVUSSIN, E., 1999. Determinants of energy expenditure and fuel utilization in man: effects of body composition, age, sex, ethnicity, and glucose tolerance in 916 subjects. *International Journal of Obesity*, **23**, pp. 715-722.

WILD, S., ROGLIC, G., GREEN, A., SICREE, R. and KING, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care,* **27**(5), pp. 1047-1053.

WILLEIT, J. and KIECHL, S., 2000. Biology of arterial atheroma. *Cerebrovascular Diseases,* **10** (Suppl 5), pp. 1-8.

WING, R.R., 1995. Use of very-low-calorie diets in the treatment of obese persons with non-insulin-dependent diabetes mellitus. *Journal of the American Dietetic Association,* **95**(5), pp. 569-572.

WING, R.R., BLAIR, E.H., BONONI, P., MARCUS, M.D., WATANABE, R. and BERGMAN, R.N., 1994. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care,* **17**(1), pp. 30-36.

WING, R.R., MARCUS, M.D., SALATA, R., EPSTEIN, L.H., MIASKIEWICZ, S., BLAIR, E.H., 1991. Effects of a very-low-calorie diet on a long-term glycaemic control in obese type 2 diabetic subjects. *Archives of Internal Medicine,* **151**(7), pp. 1334-1340.

WITTHAUS, E., STEWART, J. and BRADLEY, C., 2001. Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with Type 1 diabetes. *Diabetic Medicine,* **18**(8), pp. 619-625.

WORLD HEALTH ORGANIZATION, 2011a-last update, Diabetes. Fact sheet No 312 [Homepage of World Health Organization], [Online]. Available: http://www.who.int/mediacentre/factsheets/fs312/en/ [12/21, 2011].

WORLD HEALTH ORGANIZATION, 2011b-last update, Obesity and Overweight. Fact sheet No 311. [Homepage of World Health Organization], [Online]. Available: http://www.who.int/mediacentre/factsheets/fs311/en/index.html [12/21, 2011].

WORLD HEALTH ORGANIZATION, 2003. *Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation.* WHO Technical Report Series 916. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION, 2000. *Obesity: preventing and managing the global epidemic. Report of a WHO Consultation.* WHO Technical Report Series 894. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION, 1999. *Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation.* Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION and INTERNATIONAL DIABETES FEDERATION, 2006. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia. Report of a WHO/IDF consultation.* Geneva, Switzerland: World Health Organization.

WORLD HEALTH ORGANIZATION, FOOD AND AGRICULTURE ORGANIZATION / UNITED NATIONS UNIVERSITY, 1985. *Energy and Protein Requirements. World Health Organization Technical Report.* Geneva, Switzerland: World Health Organization.

WORTH, J. and SORAN, H., 2007. Is there a role for low carbohydrate diets in the management of type 2 diabetes? *QJM : Monthly Journal of the Association of Physicians,* **100**(10), pp. 659-663.

WOZNIAK, S.E., GEE, L.L., WACHTEL, M.S. and FREZZA, E.E., 2008. Adipose Tissue: The New Endocrine Organ? A Review Article. *Digestive Diseases and Sciences,* **54**(9), pp. 1847-1856.

YANCY, W.S., JR, FOY, M., CHALECKI, A.M., VERNON, M.C. and WESTMAN, E.C., 2005. A low-carbohydrate, ketogenic diet to treat type 2 diabetes. *Nutrition & Metabolism,* 2, p. 34, doi: 10.1186/1743-7075-2-34.

YANCY, W.S.,JR, OLSEN, M.K., GUYTON, J.R., BAKST, R.P. and WESTMAN, E.C., 2004. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Annals of Internal Medicine,* **140**(10), pp. 769-777.

YANG, W., KELLY, T. and HE, J., 2007. Genetic epidemiology of obesity. *Epidemiologic Reviews,* **29**, pp. 49-61.

ZALESIN, K.C., FRANKLIN, B.A., MILLER, W.M., PETERSON, E.D. and MCCULLOUGH, P.A., 2008. Impact of obesity on cardiovascular disease. *Endocrinology and Metabolism Clinics of North America,* **37**(3), pp. 663-684.

ZHANG, H., PARK, Y., WU, J., CHEN, X., LEE, S., YANG, J., DELLSPERGER, K.C. and ZHANG, C., 2009. Role of TNF-alpha in vascular dysfunction. *Clinical Science,* **116**(3), pp. 219-230.

9 Appendices

9.1 Appendix 1 – Protein Sparing Modified Fast booklet

PROTEIN SPARING MODIFIED FAST

INTRODUCTION

This diet is high in protein and low in carbohydrate. We are using this combination of nutrients to treat your medical problem - obesity. If this diet is going to work, you have to follow it very carefully.

The Protein Sparing Modified Fast makes your body use its fat stores for energy. Your body can only do this well, and for the long term, if there is less than 40g of carbohydrate (CHO), per day, in your diet.

Your doctor has prescribed this for you. It is unsafe to give it to other people.

During the first 7-10 days on this diet, you will lose weight very quickly. You will probably pass more urine. You may feel dizzy or light headed on standing up.

After this time your brain and body will have learned to use fat for energy provided you have followed this diet strictly. You will no longer feel dizzy and may find yourself much less hungry. Your weight loss will be slower, but you will be burning fat without losing muscle. The weight loss will continue while you stick to the diet. If you have a lapse, you will get a rapid weight gain and feel hungry.

VITAMIN & MINERAL SUPPLEMENTS

You will be prescribed vitamin and mineral supplements, please take them as directed to keep you healthy.

DAILY ALLOWANCES

QUANTITIES 12-16oz/300g-400g of:

LEAN MEAT - eg. beef, pork, lamb, game, chicken, turkey etc.

OR

OTHER **COOKED MEATS**-eg. corned beef, smoked sausage (Check labels of other processed meats for carbohydrate content)

OR

FISH - eg. haddock, plaice, salmon, tuna, mackerel herring etc.

OR

EGGS - 1 egg – 2oz

OR

TOFU or **QUORN** (for vegetarians only)

AND

2oz (50g) **CHEESE**

CARBOHYDRATE SOURCES

Many foods contain carbohydrate as starch or sugar. These include processed foods, any food that comes in a jar, tin or bottle. Please check the labels beforehand.
Milk, milk products, bread, rice, pasta, fruit and vegetables all contain carbohydrate and have to be restricted.
Foods containing sugar, e.g. biscuits, sweets, cakes, chocolate, etc. have to be avoided.

Your daily intake of carbohydrate (CHO) must be restricted to a maximum of 40g. This must be taken on a daily basis. You may not have 30g one day and 50g the next day. Having more than your daily allowance can mean that you will feel hungry and not lose weight.

GETTING STARTED

To begin, keep your choice of carbohydrate foods simple.
A typical days carbohydrate allowance will be:

		CARBOHYDRATE
½ pt (200ml) MILK; semi-skimmed or skimmed		10g
or	150ml low calorie yoghurt	
1 (25g) SLICE BREAD, small loaf, medium slice		10g
or	50g (2 oz) potato (egg size)	
or	12g (½ oz) 1TBLS rice (raw weight)	
or	12g (½ oz) pasta (raw weight)	
or	25g (1 oz) oats (raw weight)	
1 PIECE OF FRUIT- see list for weight		10g
VEGETABLES - see list for quantities		10g
	DAILY TOTAL	**40g**

When you become more familiar with carbohydrate sources you can then be more flexible with your choice of foods. You must always remain within the 40g carbohydrate daily limit.

A little fat may be added to food, e.g. salad dressing, cream on fruit, etc. Fat reduced products may have more carbohydrate than the full fat versions so always check the labels.

For the first week or so it may be helpful to stock the fridge with cold meats, cooked chicken pieces, hard boiled eggs, which can be eaten if feeling hungry.

DAILY FLUIDS

It is essential to drink at least 2 litres (4 pints) of fluids to keep your kidneys working properly.

Water, mineral water, still or sparkling
Tea or coffee (using milk from allowance)
Oxo, Marmite, Bovril, Vegemite
Diet drinks - limit to 1 litre/day

FRUIT

The following quantities of fruit will give **10g** of carbohydrate.

100g/4oz	150g/6oz	200g/8oz	400g/1lb
Apple	Apricots	Melon	Gooseberries
Cherries	Blackcurrants	Cherry tomatoes	Grapefruit
Kiwi fruit	Brambles	Red currents	
Nectarine	Raspberries	Cranberries	
Orange	Strawberries		
Peach			
Pineapple			
Plums			
Pear			
Satsumas			
Tangerines			

UNLIMITED
Olives
Rhubarb (stewed with artificial sweetener)
Sugar free jelly

* AVOID GRAPES & BANANAS

VEGETABLES

The following quantities of vegetables will provide **5g** of carbohydrate.

300g/12oz	200g/8oz	100g/4oz
Asparagus	Aubergine	Bearsprouts
Broccoli (raw)	Baby corn	Beetroot (pickled)
Cauliflower (boiled)	Cabbage (boiled)	Brussel sprouts
Courgette	Cauliflower (raw)	Cabbage (raw)
Cucumber	Chicory	Carrots (cooked)
Fennel	Gherkins (pickled)	Green beans
Lettuce	Green Pepper	Mangetout
Spinach (raw)	Leeks	Onions (boiled)
Spring greens	Marrow	Tomato (raw)
	Okra	Tomato (canned)
	Spring onions	Turnip/Swede (raw)
	Swede/turnip (boiled)	

UNLIMITED
Broccoli (boiled)
Celery
Kale
Mushrooms
Mustard/cress
Spinach (boiled)
Watercress

Boiled Beetroot, Peas, Sweetcorn, Raw Carrots, Raw Onions, Mixed Vegetables, Red/Yellow peppers and parsnip are high in carbohydrate and **should be avoided.**

MENU IDEAS

Remember to keep to a total of 40g of carbohydrate and drink **at least 2 litres/4 pints of fluid each day.**

SEE RECIPE BOOKLET

Breakfasts (suggestions)

Bacon, egg and mushrooms	no CHO
Porridge, 4oz	10g CHO
Fruit and 1 slice of toast	20g CHO
Scrambled eggs	no CHO
Cheese on toast	10g CHO
Grapefruit, 8oz	5g CHO
Cottage cheese, 2oz; Melon, 8oz	10g CHO

Light Meals (suggestions)

Cold meat, 1 slice of bread, unlimited veg	10g CHO
Tuna and scrambled eggs	no CHO
Chicken and broccoli soup,1 slice of bread	14g CHO
Meat roll-ups with spicy cottage cheese	2g CHO
Mackerel pate with celery sticks	1g CHO
Chicken and mushroom soup	3g CHO
Bacon open sandwich, 1 slice of bread	10g CHO

Main Meals (suggestions)

Omelette Pizza	6g CHO
Stir fried pork with vegetables	5g CHO
Bacon and vegetable chilli	10g CHO
Fish and mushroom crust	3g CHO
Turkey and ham kebabs	3g CHO
Gammon steak and vegetables	5g CHO
Steak, vegetables with melted cheese	5g CHO

COOKING HINTS

SOUPS

Make soups that contain meat and vegetables from 'Unlimited List'. Clear soup can be made with stock cubes. Broth mix, lentils and split peas cannot be used as they contain too much carbohydrate. You can add potatoes but you must take the amount of carbohydrate into consideration. If you have a freezer, make a pot of soup and freeze in individual portions.

HERBS AND SPICES

Using different herbs and spices will add variety and flavour to your meals e.g. garlic, parsley, basil, chives. Be adventurous!

LEMON JUICE

A few drops of lemon juice in water, soda water or mineral water is refreshing.

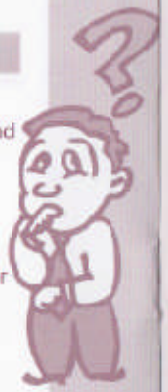
SALAD DRESSING

Home made Vinaigrette dressing can be added to salads. Always check labels of bottled dressings to check for carbohydrate.

VINAIGRETTE

1 tbsp. Olive oil
1 tbsp. Vinegar
1 tbsp. Water
Salt, pepper, herbs and spices to taste. Mix in a jug and pour over salad. Add a drop of artificial sweetener if too sour.

QUESTIONS



How quickly will I lose weight?
You may lose several kilos quickly at first. Much of this is fluid and you will notice that you pass more urine. After the first weight loss aim for 3kgs (½ stone) every 3 weeks. You may have times when weight loss is faster or slower.

Are there any side effects?
For the first 7-10 days, you may have headaches, palpitations, or feel light-headed. If you have palpitations for the first time or if palpitations worsen see your doctor.

Will I be constipated?
You may have fewer bowel motions than before. It is quite normal to have only three bowel movements in a week. You are not constipated if you are not straining.
If you think you are constipated increase your fluid intake to at least 4 pints a day and try to walk or swim daily.
Do not take laxatives unless they are recommended or prescribed by your doctor.

What about manufactured foods?
If you are taking manufactured foods, check the label, any carbohydrate present must be added in with your daily allowance.

Gravy?
Flour or cornflour can be used. 1 tablespoon of either will provide 10g of carbohydrate.

Alcohol?
It is recommended that you do not exceed the limit of 21 units/week for men, 14 units/week for women, and that you have 1 or 2 alcohol-free days per week. A unit is 25ml (2 TBLS) spirits, or 125ml wine. Dry red or white wine or spirits with diet mixer are allowed.

Beer, cider, liqueurs, alcopops and sweet wines should not be taken.

10g CHO Exchanges

BREAKFAST CEREAL	Measure	
All Bran/ Bran Flakes	5 Tablespoons	(20g)
Cornflakes	5 Tablespoons	(10g)
Weetabix	1 Biscuit	(15g)
Porridge	7 Tablespoons	(120g)
Shredded Wheat	⅔ Biscuit	(10g)
Rice Krispies	6 Tablespoons	(10g)
Muesli (unsweetened)	2 Tablespoons	(15g)

BEANS		
Baked	5 tablespoons	(75g)
Dried	2 tablespoons	(20g)

NUTS	
Almonds	240g
Brazils	240g
Chestnuts	30g
Peanuts(shelled)	120g
Walnuts	210g

CRISPBREADS	No.
Crackerbread	2
Ryvita (not multigrain or current crunch)	2
Dutch Crispbread	1
Cream Crackers	2
Carrs Table Water	4
Choice Grain	2
Tuc Biscuits	3
Cheddars	5
Oatcakes (will depend on size-check label)	

Please note - 1oz is taken as 25g

Label Reading on PSMF

If you are using any food from a packet, tin, bottle or jar, check the label for carbohydrate (CHO) content.
To work out the carbohydrate content, calculate:

EITHER (1) The carbohydrate content per serving.

OR (2) The carbohydrate content per weight of portion size.

Label Example

Nutrient	Per 110g serving	Per 100g
Energy	106 kcal 443 kJ	96 kcal 402 kJ
Protein	1.9g	1.7g
Carbohydrate	**9.1g**	**8.3g**
Of which sugars	6.1g	5.5g
Fat	6.7g	6.1g
Of which saturates	4.8g	4.4g
Fibre	0.8g	0.7g
Sodium	0.3g	0.3g

Pack contains 4 servings of 110g

Carbohydrate content per serving

If the label only shows the ingredients per 100g then weigh your usual portion and work out the carbohydrate content.

e.g. If a portion is 30g and the carbohydrate per 100g is 10g then:

$$\frac{10\text{g CHO} \times 30\text{g}}{100\text{g}} = 3\text{g carbohydrate per portion}$$

Checklist

- Check carbohydrate content
- Check carbohydrate content per serving
- Check number of servings per pack
- Check portion size
- Check if water has to be added to food and whether label applies to the food with the water added.

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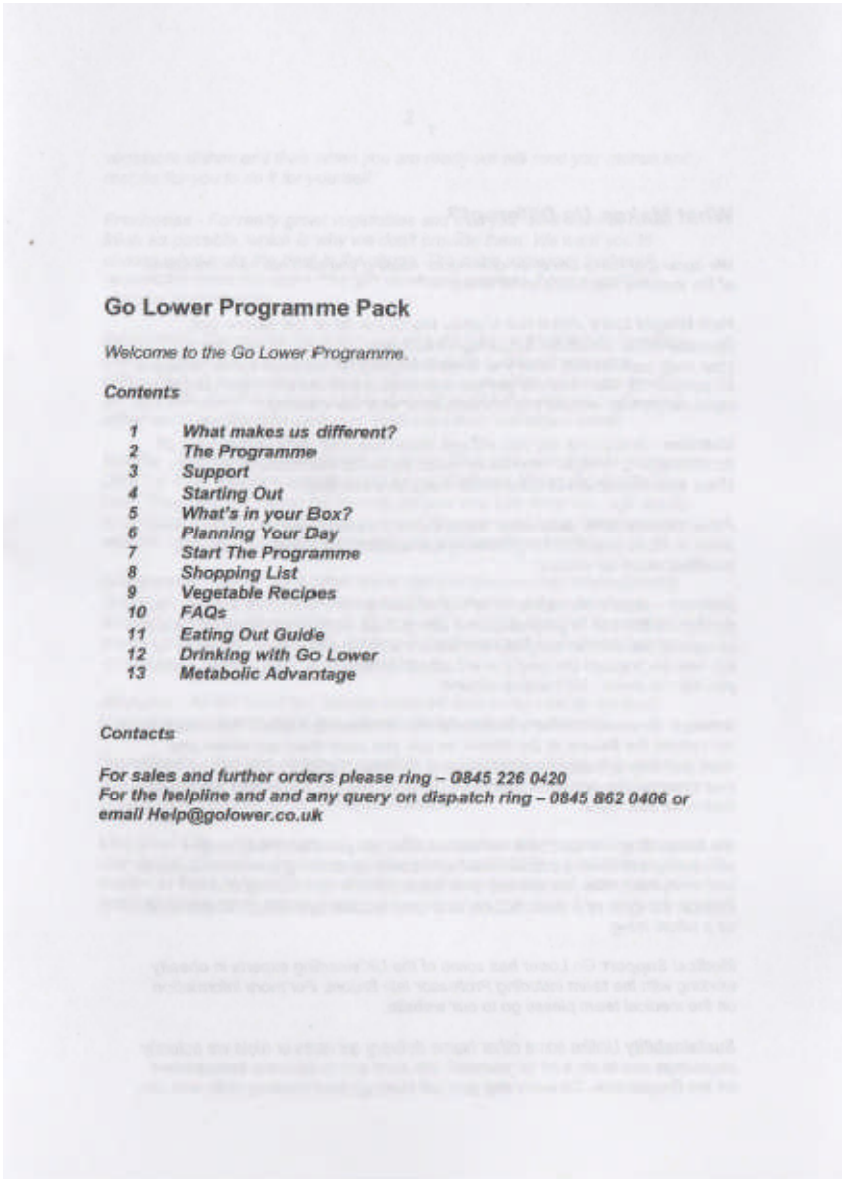
CARBOHYDRATE VALUES OF COMMON FOODS

		CHO
15g	Cheese spread	1g
20g	Processed cheese	1g
30g	Packet of crisps	15g
25g	Peanuts	2g
130g	½ Avocado	2g
22g	2 poppadoms	9g
150g	Low calorie/natural yoghurt	10g

Notes

10

9.2 Appendix 2 – Go Lower booklet



Go Lower Programme Pack

Welcome to the Go Lower Programme.

Contents

Contacts

For sales and further orders please ring – 0845 226 0420
For the helpline and and any query on dispatch ring – 0845 862 0406 or email Help@golower.co.uk

What Makes Us Different?

We know that there are other options for helping you slim but here are some of the features that make us different: -

Fast Weight Loss *-this is not another low GI,low fat or low calorie diet. Because of the Metabolic Advantage (read more on page 18) we can help your body burn fat fast while you keep eating highly nutritious foods. Most low GI and low GL diets doe not get you into ketosis and therefore can not get you rapid weight loss without lots of exercise or very few calories.*

Nutrition *- In any one day you will get more than your daily requirement of fibre, omega 3, omega 6 and all the other essential vitamins and minerals. Many nutritionists like Dr John Briffa really love this diet.*

Price *– Unlike other most other home delivery services we can get the price down to £8.80 day (including delivery and support) so it is affordable and excellent value for money.*

Delivery *– orders are delivered by our chosen courier company but you you don't need to be in to get your box. If you give us clear instructions on where to leave it, the courier company will leave it without a signature. Because of the way we prepare the food it won't affect the quality or safety of the meals if you are not there. No hanging around.*

Storage *-Because we use a traditional French cooking method, the meals do not require the freezer or the fridge- so you can store them anywhere you want and they will stay fresh without any artificial ingredients. In fact we know that some customers leave everything at work and just take home the meals they want that night.*

No Measuring *- At Go Lower we want to liberate you from measuring calculating and driving yourself mad with counting carbs, grams of fats, points and everything else. We will help you begin a life free of looking at food through the eyes of a manufacturer and food technologist. Begin to see food as a whole thing.*

Medical Support *Go Lower has some of the UK'sleading experts in obesity working with the team including Professor Iain Broom. For more information on the medical team please go to our website.*

Sustainability *Unlike some other home delivery services or diets we actually encourage you to do a bit for yourself. We want you to became independent on the Programme. So every day you will keep up your cooking skills with the*

vegetable dishes and then when you are ready we will send you menus and recipes for you to do it for yourself.

Freshness *- For really great vegetables and fruit you need them to be as fresh as possible, which is why we don't provide them. We want you to choose what looks the best in the shops. The more you move fruit and vegetables about the more they get damaged and lose those essential vitamins and minerals.*

Education *- Our goal is for you to not only do it for yourself but to be able to understand why it works and the science behind it. We will send you information at the touch of a button to keep you as informed as you want to be. Just ask about any aspect of food nutrition diet or wellbeing and we will either send you the information or seek it out from our expert panel.*

Quality *- Our meals are prepared for us by chefs who cook food for some the UK's top food suppliers including M&S and Waitrose. We work with the very best. They take our clever fat burning recipes and turn them into high quality ready meals, in kitchens that are some of the very best in the UK complying with the very highest standards of hygiene and health and safety.*

Information *- Unlike most other home delivery services and other catering outlets we comply with retail standards of information. All our foods are labelled providing information on ingredients, calories, levels of fat, carbs and protein. Nothing is hidden. We don't need to do this but we believe that you should know exactly what you are eating.*

Allergies *- All our foods are allergy declared and we can make up food boxes for people who have nut, wheat, gluten and other allergies.*

Ingredients *– We use wherever possible local produce and we only use sustainable nuts and palm oil in our bars and cereal. The meat is all sourced in local farm assured farms in Northumberland.*

Live your Life *- We want you to live a life away from the Programme so not only do we give you recipes we will also give you the Dining and Drinking Out Guides in this booklet. But you can always phone us up to ask about specific restaurant or holiday destination..*

The Go Lower Programme

The Go Lower programme is split into three easy stages.
There are three stages to the Programme

- Inch/ Weight Loss
- Maintenance and Education
- Support and Resource.

Inch / Weight Loss

This first part of the Programme is to get you losing weight and inches as quickly as possible without compromising your wellbeing or life. To ensure that you get the very best support we provide you with consultations and counselling to keep you motivated and on track.

This diet does discriminate between fat and muscle and fat and is designed to get your body to burn fat and not muscle. Indeed we hope you will actually improve your muscle tone and therefore you may have dramatic inch loss even if your weight stabilises because fat weighs less than muscle.

The support we provide is on line and by telephone. You can ring or email us whenever you want and discuss your progress and your needs. Whilst it is not a 24 hour service there is normally a fast response to most questions unless they are medical in which case we normally require extra time to speak with one of our specialists. We want to hear from you but we won't hassle you.

Maintenance and Education

When you have achieved your goal, or at any time after 4 weeks of the inch/Weight Loss phase, you can move on to the Maintenance Programme.

At this stage you will ready to do it for yourself and so we have designed a unique plan, which provides support, food and education to ensure that you don't go back to the bad old days of yo yo dieting.

Support and Resource

We will be here at any time ready to answer any questions you have on food, diet, cooking, labelling or any other related topic.

Support

We know how important it is to be able to speak with someone to answer your questions or provide moral support as you begin to change your life. We are here for you when you want. Just phone this number to speak with one of our team .

Consultation

When you have been on the programme for 1 week you can arrange for a one to one consultation to discuss your diet worries and goals. Just Ring the help line or email help@golower.co.uk to book your consultation with one of the councillors .

Counselling

If you just want to have a general chat or have general enquires about the programme then ring our help line or email help@golower.co.uk and we have a team of people happy to answer any of your questions.

Starting Out:-

- **RELAX.** Stress is a key factor in weight gain and so it is really important that you begin to relax and stop seeing this as an ordeal. This is fun and easy and all you do is eat. Your body will do the work for you. We call this **Metabolic Advantage.**
- **TAKE OUT THE FOOD** Separate dinners from lunches and breakfasts. These may be stored in a cupboard or anywhere you want. There is no need to refrigerate or freeze. Read more about what is in the box on the next page.
- **BUY THE FRESH GREENS AND FRUIT.** Go to the shopping list on page 7 and keep the fresh produce in the fridge
- **GET STARTED!** Often getting started is the hardest step; we sometimes look for reasons to put it off. Remember that every journey starts with one small step. It really is easy.

What is in the Box?

Please check the contents of the box against the packing sheet enclosed and

If there is anything missing please ring our help line or email
Help@golower.co.uk

Many thanks for your patience.

Planning Your Day

Breakfast

We provide you with seven portions of our unique granola. This can be eaten on its own but we do recommend that you add as much milk (full fat or otherwise) or full fat Greek strained yogurt or single cream as you want. From experience we have found that using PRO BIOTIC yoghurt is really effective for anyone suffering from sensitive stomachs who are not used to high levels of fibre. We also strongly recommend that you also add a handful of fresh raspberries or strawberries or blueberries or blackberries. You will find the extras on the shopping list on page 9.

Lunch

If you feel that you need something extra with the soup or the mushroom stroganoff then please feel free to add any amount of full fat cheese. The cheese can be hard or soft or blue or otherwise. There is no need to measure the amount of cheese you would like.

Dinner

There are seven main meals. Please add one portion of the vegetables listed on the shopping list and you may cook them any way you like but we have provided some recipes on page 10. If you want more ideas then please email us at help@golower.co.uk or ring our help line.

There is no requirement to choose any meal for any day. You choose how and when you want to eat them.

Bars

The nut bars are provided as a daily snack but they can also be used as a breakfast replacement when you are on the go. The crème bars and the brazil nuts can make a tasty treat for every alternate day.

If you feel hungry at any time please call our help line or email **Help@golower.co.uk** and we will sort your problem out in a flash.

Drinking

You should drink at least 8 large glasses of water each day. This does not include the coffees and teas that you may also have. Add as much milk (of any kind) to your tea and coffee. We recommend decaffeinated tea and coffee as these will help your inch/weight loss. If you want to add calorie free sweeteners or flavourings to your water please do so. You can have calorie-free or sugar free soft drinks. You may drink fruit and herb teas.

Start the Programme

Decide what you want to eat for the day and set to one side.

Choose the vegetables you wish to eat with your main course and review the recipes on page 10.

Remember do not get the kitchen scales out at any time. If you are in doubt about how much to eat please just ring our help line or email help@golower.co.uk

Ketosis

To get the fast results we need you to slip gently into ketosis.

Ketosis is the point at which your body is burning its own body fat for energy. The diet encourages your body to do this because we deprive it of easy energy in the form of sugar and starch (ie grain;pulses;sugar;rice)

When your body switches its energy supply from starch and sugar to body fat it releases ketones.

Here are some of the side effects of being in ketosis which only last for a short time:-
Lack of hunger
Sudden unexpected headache
Sickness
Extreme tiredness
Bad breath
Dramatic inch loss / weight loss

Normally these symptoms pass and your body carries on as normal

If after 4/5 days you have not slipped into ketosis we need you to tell us so that we can make some minor changes to the Programme to ensure that YOU do go into ketosis.

We are all different so don't worry if we have to make some small changes.

Shopping List

Please feel free to buy any of the following vegetables to accompany the meals form the list below but we suggest you only have one portion per meal

Summer 08

Food	Amount
Courgette	Two
Green Beans	A handful
Pak choi	Two
Spinach	One Bag
Mushrooms -field	Large handful /10
Leeks	Two trimmed
Celery	One plant
Cauliflower	One Handful /one large florets
Rocket	One large handful
Aubergine	One small
Celeric	One quarter / half
Kale	One large bunch
Sweet heart cabbage	Half
Asparagus	handful
Milk (soya or otherwise)or yoghurt or cream (always natural and no sugar)	Half a pint a day
Berries	One handful a day

At any time you can choose to have salad instead of vegetables. Choose either half a Cos or Romaine lettuce or a quarter of an iceberg lettuce with a little chopped up cucumber and green pepper. We recommend either mayonnaise with the salad or an oil dressing which is 10 parts oil to 1 part vinegar with a good shake of salt and some garlic (if you like it). Another wonderful salad option is an avocado with the dressing suggested above.

Do Not Eat potatoes, carrots, swede, turnip, onion, sweet potato, rice pasta, bread , any fruit other than a handful of berries with your granola.

Recipes

Cauliflower Fritters
Separate the florets and boil in salted water for 10 mins. Drain.
Whip up an egg and dip the drained florets into the whipped egg and lightly fry the dipped florets. Sprinkled with grated cheese and serve.

Chopped Cauliflower.
Cook the cauliflower in boiling salted water and once soft drain and put to one side. Chop finely and add to some chopped ham and a table spoon of sour cream and a sprinkling of cheese.

Srambled Spinach
Take a large bag of spinach and cook it until soft in the microwave or in a pan with water. You can use frozen spinach but you need to defrost it first. Drain the defrosted or cooked spinach.
Beat up an egg and add the drained spinach and now cook as you would scrambled eggs. Add a squeeze of lemon and some grated cheese of your choice.

Cucumber Salad
Peel and slice one cucumber. Sprinkle with salt and put to one side for an hour. Wash well and drain.
Make up a dressing (see page 9) . Mix well with the drained cucumber and chill in the fridge for 30 mins. Serve with chopped parsley.

Go Lower Quiche Lorraine
Whisk one egg and add a tablespoon of cream. Add salt and peper and some freshly grated nutmeg (optional) Add a handful of chopped ham or chopped cooked bacon and a handful of grated gruyere or swiss cheese. Bake in a medium oven in a buttered dish for 15 mins.

Lemon Chicory
Cut the chicory length ways and then put the chicory in a pan of boiling water and cook for 10 mins and drain. Then add to a pan the juice of one lemon and a clove of garlic and then when hot add the drained chicory and cover the pan for moments just long enough to allow the flavours to go into the chicory.

Spinach Terrine
Take one portion of frozen spinach defrosted and drained or cooked and drained , Pre heat the oven to 220c or gas mark 7.chop the drained spinach and beat up one eggs and add salt and pepper. Add two tablespoons of double cream. Then add the drained chopped spinach. Mix well.
Pour into a buttered baking dish and place in the oven 20mins . Serve warm or wait until cold and serve as cold slices.

Green Beans with sour cream

Boil green beans for approx 5 mins. Drain and add 3 table spoons of sour cream with a sprinkling of nutmeg and caraway seeds. Add extra butter for taste if required.

Cauliflower and cashew nut stir fry.

Par boil the cauliflower and break up into florets. Heat up a wok with a splash of oil. Add a handful of salted cashews and fry. Add 3 spring onions chopped. Add the partly cooked cauliflower and continue to stir fry. Add a sprinkling of ginger or a tablespoon of freshly grated root ginger (if you like ginger) and now add a splash of soy sauce to taste. Serve

Celeriac puree

Peel and chop a quarter of a celeriac and boil until soft. Then either mash or put through a food processor. When sufficiently smooth add a mixture of butter and cream with salt and pepper to taste. A few pine nuts can really bring it alive.

FAQs

There are numerous questions that people ask us but here are some of the regular enquiries: -

Side Affects- are there any and if so what are they?

When the body stops getting readily accessible energy in the form of starch or sugar it needs to find energy from its own fat resources. This is known as ketosis. Once the body has made the switch it will operate as well as if the energy was coming from starch or sugar. However some people can experience the following side effects before the switch is fully made:-

Headaches. These normally don't last more than 12-24 hours and a paracetamol usually sorts it out.

Tiredness. Again this can last a few days but as soon as the body finds its fat resources it will start to feel energised. If you do a lot of exercise you will notice a difference in your performance in the gym but clinical studies over many years have consistently shown that after a matter of days the body will perform just as well. Please contact us further if you want more information.

Hunger. Because the body is looking for quick energy (as you are not providing it with sugar and starch) it will send a message to your brain that it is hungry. We are always happy to provide extra bars but often this symptom will pass. As soon as it taps into the fat its energy needs are satisfied and so it stops telling you it 's hungry.

Sick. Some people, fortunately not many feel really sick for a short time. This tends to pass after a few hours and we recommend that you just drink water and rest until you feel better.

Grumpiness. Some might say that this is just an excuse for bad behaviour but some people do experience a short period of grumpiness before the switch over happens. This will pass.

Bad Breath. Some people do get bad breath and so we recommend a good mouthwash or some sugar free gum.

Wind. We have ensured that you have a large amount of fibre in this diet for several reasons but this can cause some people to create a lot of wind. Your body will get used to it so don't panic. If you do find that this does not pass after a few days please ring us and we will be able to sort the problem out.

Constipation. Funnily enough the very opposite can affect others. If you find yourself constipated ...don't suffer in silence and either ring us or if you are keen to solve the problem for yourself simply buy some psylium husk from any good health food shop and use as directed on the packet.

Bloated tummies. This can be one of the short term side affects of changing your eating pattern from low fibre to high fibre. At first your body struggles with the amount of fibre in this diet (due to lack of practice) but after a while this will improve. Drink extra water to help the fibre work it's way through your lower gut and take some PRO BIOTIC yoghurt.

Why do we recommend that you drink so much water?

There are two main reasons why you should drink lots of water. The first is that it helps with ketosis.

Secondly we often confuse in our mind the message of thirst with hunger. Often we eat when our body is telling us to drink. We want to make sure that you drink first.

Extra water can really help with the side affects listed above.

Can I have extra snacks?

Yes you can but we do recommend that you stick with cheese or other protein snacks such as a pepperami but remember the salt levels can be high so go easy. Try olives as a snack or some macadamia nuts.

Do remember that if you feel hungry please ring or email us.

Can I use gum?

Yes as long as it is sugar free and does not contain any poyols

Eating Out Guide

We really want you to enjoy your life while you are getting slim and to learn the basic rules of smart eating so that you will never have to "diet " again. These rules will apply after you finish Go Lower as well as during the programme.

You will see that this note is really long and for those of you who can't be bothered to read everything I will leave you with this thought. When choosing anything outside the Go Lower range of food you need ask your self only one simple question. What would I eat if I were living in the UK 10,000 years ago?

So imagine yourself just for a moment walking through the wilderness and think like a hunter-gatherer. If it is animal or fish or a product of an animal or a fish you can always eat it. If it grows above the ground and you can pick and eat it in its raw form you can eat it. If the food has grown underground or requires cooking to be eaten or has been processed – DO NOT EAT IT. This does not mean you should only eat raw food but don't eat something that must be cooked to be edible.

For those of you that want more info read on.

There are three key things to remember

First

There are only three basic food groups – I will talk about fibre later but fibre is a subsection of Carbohydrates.

Protein	Meat, Chicken, Fish, Quorn, Nuts, Eggs
Fats	Butter ,Cream, Oils, Cheese
Carbohydrates	All Fruit, All Vegetables, Bread Pasta Rice Noodles any Grains and Beans or Pulses such as Lentils or Chick Peas Sugar Honey

Second

You can eat anything you want from the first two boxes. So you can eat chicken, meat, fish, eggs and you can have these with any sauce as long as it is not based on tomatoes sugar or honey. Pick creamy or wine or butter sauces. Once you have achieved your target weight /size you can have tomato based sauces but remember tomatoes are high in sugar.

Third

You need to eat certain carbohydrates but not all carbohydrates are good for you. **The Good the Bad and the Ugly!**

Food Groups	Type of Foods	Characteristics
Green leafy Vegetables **THE GOOD**	Cabbage, Lettuce, spinach, broccoli, green beans, fine beans. These are all vegetables that can be picked and eaten without cooking in their whole form. This does not include peas, which you remove from their natural jackets. Peanuts	These foods are rich in essential vitamins and minerals and are very high in fibre…these are great and we want you to eat these every day.
Starch **THE BAD**	Bread, pasta, rice, potatoes, carrots, root vegetables, cereals	These are not full of essential vitamins and minerals unless the large food companies add them or they change when cooked.
Sugars **THE UGLY**	Sugar fructose	Whilst these taste nice they have no nutritional properties at all and just provide instant energy which if unused causes a large insulin release. Honey does have some nutritional properties but it will still trigger an insulin response and therefore work against your weight loss.
Pulses **BAD NOW but GOOD LATER**	Beans, lentils, chickpeas,	These foods do contain some great minerals and vitamins and also provide some protein and fibre but they are still high in carbs and will not help you if you want to lose weight. When you have reached your chosen weight or size you can eat these foods in moderation.
Fruit **CAN BE GOOD BAD and UGLY**	Tomatoes citrus fruits bananas apples pears etc	Full of vitamins and minerals but also sugar. You need to focus on only those fruits that are high in fibre but also low in sugar. Therefore eat Berries and if that is too boring you may have the occasional apple or pear. BUT never ever drink fruit juice or smoothies.
Nuts and Seeds **ALWAYS GOOD**	All types of nuts other than peanuts	Full of essential vitamins and minerals , essential fats and protein

So as an example you could choose these meals in the following restaurants

Chinese	Shredded duck soup or crab soup; king prawn or chicken with a ginger sauce with green stir-fried vegetables. You can have shredded duck with bean sprout or Crispy Aromatic Duck with out the pancakes. BUT No sweet and sour and no noodles and no rice and no sticky honey sauces. No dumplings and no won ton. No prawn crackers.
Indian	Any meat curry but no rice Naan, chapatti or poppadoms. Any green vegetable dish such as sag paneer so long as it does not contain any chickpeas or Dahl or potatoes. No samosas or pakoras or bhajis.
French	This is really easy. Just don't have the potatoes the rice or pasta or bread. Most French food is high in protein and very low in starchy carbs. Have lots of rich French foods and add salad or spinach or green French beans. Not all beans are the same so if they aren't the type that is green don't buy them.
Greek	Easy Peasy. There is lots of meat and fish and cheese in the Greek menu. Just don't eat the bread or the rice or the pasta or the potatoes. Greek salad is cool as are the aubergine dishes
Spanish	This is really the same as France but Tapas is cool because most of them are protein based. Don't eat the tortilla as that has loads of potatoes in it.
Mexican	This is difficult because the Mexican menu is dependent on cheap starch. Stick with the burgers or the meat dishes but just leave to the side any of the bread or wrap or fajita or rice.
Thai	This is a lot like the Chinese option and just avoid any thing with rice noodle or other starchy foods.
Italian	Avoid pasta, rice, potatoes, and pizza. Go for the prawns cold meats veal and chicken dishes. Eat the main meat course only or have the bressaola to start or a green salad with avocado or mozzarella or seafood. Try to avoid tomatoes but that can be hard in an Italian restaurant.
Lebanese / Turkish	These are a little like the Greek restaurants but they are bigger on the Mezzes. Eat meat based meals or starters such as the kebabs or the meatballs. Eat lots of salad but stay off the couscous and tabbouli . Don't be tempted to eat the carrots when the humus comes and just dip with celery and spring onion.

Drinking with Go Lower

This is easier than the food choices.

The basic rule is that any drink that contains sugar is a no no. So you can forget all fruit juices (pure or otherwise) all smoothies and all fruit cordials. If you want to drink sugar free fruit juices then please do so but we do not advocate the use of artificial sweeteners and if you would like further information on this please email rhelp@golower.co.uk for further information.

You can drink red wine in moderation – which is equal to no more than a small glass a night. If you drink more it will affect the progress of the diet.

Do not drink white wine, rose wine, or cider or beer of any type. The difference is that white wine, cider , rose, and beer are much higher in sugar.

With regard to spirits you may drink vodka or whisky and whilst these are low carb if you drink too much it will still affect the diet. With the whisky and vodka you may have sugar free or calorie free mixers or soda.

Our best advice is to drink water during the day and feel free to have a small glass of red wine in the evening if it suits your lifestyle.

Finally with regard to tea and coffee we do recommend that you cut back as much as possible because caffeine can trigger insulin release therefore you could choose decaf coffee or tea or herbal or green teas for the duration of the first phase of the Programme.

Metabolic Advantage

Go Lower gets great results with real food by doing lots of things at once.

Here are the unique combined features of Go Lower: -

Fat Burning – by removing easily accessible energy in the form of starch and sugar we force your body to use its own fat supplies as energy. This is known as ketosis and you can read more about ketosis at www.golower.co.uk

Feeling Full – even though we reduce your calorific intake to 1500 calories a day (or thereabouts) you will feel full because we ensure that there is sufficient natural protein in your diet to keep the hunger at bay. Protein (such as meat or fish or eggs) contains essential amino acids, which contain peptides that tell your brain that you are full. How clever is that?

Build and protect muscle – The weakness with low fat diets is that they don't discriminate between the loss of weight from fat or muscle. On the Go Lower Programme you are eating lots of protein, which means that you have a diet rich in essential amino acids, which help build muscle, and therefore your weight loss is focused on excess fat. It is for this reason that often you can lose inches but not pounds because fat weighs less than muscle. Also by retaining muscle your metabolic rate is likely to stay higher. To read more about this go to FAQ on our website.

High in fibre – we ensure that you get loads of fibre from the combination of nuts seeds and vegetables. We also use a pre biotic fibre in our bars, which means that not only do you feel fuller for longer you actually help your digestive system.

Low GI and Low GL- By ensuring that all our meals and snacks are low GI and low GL we can be sure that your body does not over produce insulin which is the hormone that causes our bodies to store fat. Excessive insulin production can cause diabetes and other health problems- to read more about Low GI and Low GL go to FAQ.

Calorie burn – By eating lots of protein you will actually burn more calories than the usual low fat diet without a trip to the gym. This is because your body has to work really hard to turn protein into energy.

And why does it last when other diets don't?

Real Food – Because you eat real natural food the diet as such doesn't end.

No Sugar – Because we are a very low sugar diet with no sweeteners your taste buds begin to change so that very sweet foods are no longer attractive.

Metabolic Rate – Because we are not very low calorie and we help you keep muscle tone, your metabolic rate does not drop so dramatically during the actual weight loss / inch loss period.

Education – Because we tell you how to cook everything for yourself you can do it all for you and your family and friends when you are ready to go alone.

Lifestyle- because the diet works with your life it doesn't need to stop.

Healthy- because you feel so great on the diet you won't want to change the way you eat when you reach your target weight/size.

Support – Because we hold your hand.

If you have more questions on the Go Lower Programme and why it works so successfully please ring or email us

9.3 Appendix 3 – Audit of Diabetes Dependent Quality of Life

ADDQoL

This questionnaire asks about your quality of life and the effects of your diabetes on your quality of life. Your quality of life is how good or bad you feel your life to be.

Please shade the circle which best indicates your response on each scale.

There are no right or wrong answers; we just want to know how you feel about your life now.

I) In general, my present quality of life is:

○ excellent	○ very good	○ good	○ neither good nor bad	○ bad	○ very bad	○ extremely bad

For the next statement please consider the effects of your diabetes, its management and any complications you may have.

II) If I did not have diabetes, my quality of life would be:

○ very much better	○ much better	○ a little better	○ the same	○ a little worse	○ much worse	○ very much worse

Please respond to the 18 more specific statements on the pages that follow.

For each statement, please consider the effects of your diabetes, its management and any complications you may have on the aspect of life described by the statement.

In each of the following boxes:

a) shade a circle to show how diabetes affects this aspect of your life;

b) shade a circle to show how important this aspect of your life is to your quality of life.

Some statements have a "not applicable" option. Please shade this "not applicable" circle if that aspect of life does not apply to you.

1a) If I did not have diabetes, my working life and work-related opportunities would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse	not applicable
○	○	○	○	○	○	○	○

1b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

2a) If I did not have diabetes, my family life would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse	not applicable
○	○	○	○	○	○	○	○

2b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

3a) If I did not have diabetes, my friendships and social life would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

3b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

4a) If I did not have diabetes, my sex life would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse	not applicable
○	○	○	○	○	○	○	○

4b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

5a) If I did not have diabetes, my physical appearance would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

5b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

6a) If I did not have diabetes, the things I could do physically would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
○	○	○	○	○	○	○

6b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

7a) If I did not have diabetes, my holidays or leisure activities would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

7b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

8a) If I did not have diabetes, ease of travelling (local or long distance) would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

8b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

9a) If I did not have diabetes, my confidence in my ability to do things would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
O	O	O	O	O	O	O

9b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

10a) If I did not have diabetes, my motivation to achieve things would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
O	O	O	O	O	O	O

10b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

11a) If I did not have diabetes, the way society at large reacts to me would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
O	O	O	O	O	O	O

11b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

12a) If I did not have diabetes, my worries about the future would be:

very much decreased	much decreased	a little decreased	the same	a little increased	much increased	very much increased
O	O	O	O	O	O	O

12b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

13a) If I did not have diabetes, my finances would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

13b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

14a) If I did not have diabetes, my need to depend on others for things I would like to do for myself would be:

very much decreased	much decreased	a little decreased	the same	a little increased	much increased	very much increased
○	○	○	○	○	○	○

14b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

15a) If I did not have diabetes, my living conditions would be:

very much better	much better	a little better	the same	a little worse	much worse	very much worse
○	○	○	○	○	○	○

15b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

16a) If I did not have diabetes, my freedom to eat as I wish would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
○	○	○	○	○	○	○

16b) This aspect of my life is:

very important	important	somewhat important	not at all important
○	○	○	○

17a) If I did not have diabetes, my enjoyment of food would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
O	O	O	O	O	O	O

17b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

18a) If I did not have diabetes, my freedom to drink as I wish (e.g. sweetened hot and cold drinks, fruit juice, alcohol) would be:

very much increased	much increased	a little increased	the same	a little decreased	much decreased	very much decreased
O	O	O	O	O	O	O

18b) This aspect of my life is:

very important	important	somewhat important	not at all important
O	O	O	O

If there are any other ways in which diabetes, its management and any complications affect your quality of life, please say what they are below:

9.4 Appendix 4 – ORWELL 97 Questionnaire

ORWELL 97 Questionnaire

Date: _____
PIN: _____

This questionnaire consists of 18 pairs of questions. After reading each question carefully, underline only one of the four answers which best describes your current situation.

1. R How important is it for you to exercise regularly?
   not at all *just a little* *not so much* *much*
   O Is your weight an obstacle for your physical activity?
   not at all *just a little* *not so much* *much*

2. R How important is it for you to have regular sexual activity?
   not at all *just a little* *not so much* *much*
   O Does your weight represent a physical obstacle for your sexual activity?
   not at all *just a little* *not so much* *much*

3. O Do you suffer from shortness of breath?
   not at all *just a little* *not so much* *much*
   R Does shortness of breath represent an obstacle for your daily activities?
   not at all *just a little* *not so much* *much*

4. O Do you feel sleepy?
   never *occasionally* *sometimes* *often*
   R Does sleepiness interfere with your daily activities?
   not at all *just a little* *not so much* *much*

5. O Do you suffer from excessive sweating?
   not at all *just a little* *not so much* *much*
   R Does sweating interfere with your daily activities?
   not at all *just a little* *not so much* *much*

6. O Mass media (TV, newspapers, etc.) often report that obesity is a major risk for health. Do you pay attention to this subject?
   not at all *just a little* *not so much* *much*
   R Does this information increase your preoccupation with your health?
   not at all *just a little* *not so much* *much*

7. R Is it important for you to live in a serene family environment?
   not at all *just a little* *not so much* *much*
   O Does being overweight prompt discussions in your family?
   not at all *just a little* *not so much* *much*

8. R Is it important for you to be successful in your job?
   not at all *just a little* *not so much* *much*
   O Does your weight represent an obstacle in your job?
   not at all *just a little* *not so much* *much*

9. R Is it important for you to spend your free time with friends?
not at all *just a little* *not so much* *much*
O Does your weight interfere with your social activities?
not at all *just a little* *not so much* *much*

10. O Do you feel uneasy in showing your body?
not at all *just a little* *not so much* *much*
R Does this uneasiness interfere with your social activities?
not at all *just a little* *not so much* *much*

11. O Is it important for you to be sexually attractive?
not at all *just a little* *not so much* *much*
R Does being overweight make you less sexually attractive?
not at all *just a little* *not so much* *much*

12. O *Do others ever tease you about your weight?*
never *occasionally* *sometimes* *often*
R If this happens, does it worsen your mood?
not at all *just a little* *not so much* *much*

13. R Do you feel excessively worried about unimportant matters?
not at all *just a little* *not so much* *much*
O Do you think that being overweight makes you more apprehensive?
not at all *just a little* *not so much* *much*

14. R Do you ever feel sad?
not at all *just a little* *not so much* *much*
O Do you ever feel sad because of being overweight?
not at all *just a little* *not so much* *much*

15. R Do you ever feel very nervous?
not at all *just a little* *not so much* *much*
O Does being overweight make you more nervous?
not at all *just a little* *not so much* *much*

16. R Do you have a negative opinion of yourself?
not at all *just a little* *not so much* *much*
O Does being overweight interfere with your opinion of yourself?
not at all *just a little* *not so much* *much*

17. R Do you ever experience a feeling of immediate danger with no apparent reason?
not at all *just a little* *not so much* *much*
O Do you feel more exposed to risks because of being overweight?
not at all *just a little* *not so much* *much*

18. O The world of fashion and entertainment pursues a model of lean persons. How far do you feel from this model?
not at all *just a little* *not so much* *much*
R Would it be important for you to reach this model of thinness?
not at all *just a little* *not so much* *much*

9.5 Appendix 5 – Diabetes Treatment Satisfaction Questionnaire s

Diabetes Treatment Satisfaction Questionnaire: DTSQs

The following questions are concerned with the treatment for your diabetes (including insulin, tablets and/or diet) and your experience over the past few weeks. Please answer each question by circling a number on each of the scales.

1. How satisfied are you with your current treatment?

very satisfied	6	5	4	3	2	1	0	very dissatisfied

2. How often have you felt that your blood sugars have been unacceptably high recently?

most of the time	6	5	4	3	2	1	0	none of the time

3. How often have you felt that your blood sugars have been unacceptably low recently?

most of the time	6	5	4	3	2	1	0	none of the time

4. How convenient have you been finding your treatment to be recently?

very convenient	6	5	4	3	2	1	0	very inconvenient

5. How flexible have you been finding your treatment to be recently?

very flexible	6	5	4	3	2	1	0	very inflexible

6. How satisfied are you with your understanding of your diabetes?

very satisfied	6	5	4	3	2	1	0	very dissatisfied

7. Would you recommend this form of treatment to someone else with your kind of diabetes?

Yes, I would definitely recommend the treatment	6	5	4	3	2	1	0	No, I would definitely not recommend the treatment

8. How satisfied would you be to continue with your present form of treatment?

very satisfied	6	5	4	3	2	1	0	very dissatisfied

Please make sure that you have circled one number on each of the scales.

9.6 Appendix 6 – General Practice Physical Activity Questionnaire

General Practice Physical Activity Questionnaire

Date……

Name……..

1. Please tell us the type and amount of physical activity involved in your work.

		Please mark one box only
a	I am not in employment (e.g. retired, retired for health reasons, unemployed, full-time carer etc.)	
b	I spend most of my time at work sitting (such as in an office)	
c	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, childminder, etc.)	
d	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
e	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. During the *last week*, how many hours did you spend on each of the following activities? *Please answer whether you are in employment or not*

Please mark one box only on each row

		None	Some but less than 1 hour	1 hour but less than 3 hours	3 hours or more
a	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
b	Cycling, including cycling to work and during leisure time				
c	Walking, including walking to work, shopping, for pleasure etc.				
d	Housework/Childcare				
e	Gardening/DIY				

3. How would you describe your usual walking pace? Please mark one box only.

Slow pace (i.e. less than 3 mph)		Steady average pace	
Brisk pace		Fast pace (i.e. over 4mph)	

9.7 Appendix 7 – Weight Decisional Balance

Weight: Decisional Balance

Client ID#_____
Date: _____/_____/_____
Assessment Point: _____

Each statement represents a thought that might occur to a person who is deciding whether or not to lose weight. Please indicate how IMPORTANT each of these statements might be to you if you were considering a decision to lose weight. There are FIVE possible responses to each of the items that reflect your answer to the question "How important would this be to you?" Please circle the number that best describes how important each statement would be to you if you were deciding whether or not to lose weight.

1=Not important at all
2=Slightly important
3=Moderately important
4=Very important
5=Extremely important

PLEASE READ EACH STATEMENT AND CIRCLE THE NUMBER ON THE RIGHT TO INDICATE HOW YOU RATE ITS LEVEL OF IMPORTANCE AS IT RELATES TO YOUR MAKING A DECISION ABOUT WHETHER TO LOSE WEIGHT.

How important is this to me?	Importance in making a decision about losing weight:				
	Not at all	Slightly	Moderately	Very	Extremely
1) The exercises needed for me to lose weight would be drudgery.	1	2	3	4	5
2) I would feel more optimistic if I lose weight.	1	2	3	4	5
3) I would be less productive.	1	2	3	4	5
4) I would feel sexier if I lose weight.	1	2	3	4	5
5) In order to lose weight I would be forced to eat less appetizing foods.	1	2	3	4	5
6) My self-respect would be greater if I lose weight.	1	2	3	4	5
7) My dieting could make meal planning more difficult for my family or housemates	1	2	3	4	5
8) My family would be proud of me If I lose weight.	1	2	3	4	5

How important is this to me?	Importance in making a decision about losing weight:				
	Not at all	Slightly	Moderately	Very	Extremely
9) I would not be able to eat some of my favorite foods if I were trying to lose weight.	1	2	3	4	5
10) I would be less self-conscious if I lost weight.	1	2	3	4	5
11) Dieting would take the pleasure out of meals.	1	2	3	4	5
12) Others would have more respect for me if I lose weight.	1	2	3	4	5
13) I would have to cut down on some of my favorite activities if I try to lose weight.	1	2	3	4	5
14) I could wear more attractive clothing if I lost weight	1	2	3	4	5
15) I would have to avoid some of my favorite places if I were trying to lose weight	1	2	3	4	5
16) My health would improve if I lost weight.	1	2	3	4	5
17) Trying to lose weight could end up being expensive when everything is taken into account.	1	2	3	4	5
18) I would feel more energetic if I lost weight.	1	2	3	4	5
19) I would have to cut down on my favorite snacks if I were dieting.	1	2	3	4	5
20) I would be able to accomplish more if I carried fewer pounds.	1	2	3	4	5

9.8 Appendix 8 – Major Depression Inventory

Psychiatric Research Unit
WHO Collaborating Centre in Mental Health

Major (ICD-10) Depression Inventory

The following questions ask about how you have been feeling over the last two weeks. Please put a tick in the box which is closest to how you have been feeling.

	How much of the time ...						
		All the time	Most of the time	Slightly more than half the time	Slightly less than half the time	Some of the time	At no time
1	Have you felt low in spirits or sad?	☐	☐	☐	☐	☐	☐
2	Have you lost interest in your daily activities?	☐	☐	☐	☐	☐	☐
3	Have you felt lacking in energy and strength?	☐	☐	☐	☐	☐	☐
4	Have you felt less self-confident?	☐	☐	☐	☐	☐	☐
5	Have you had a bad conscience or feelings of guilt?	☐	☐	☐	☐	☐	☐
6	Have you felt that life wasn't worth living?	☐	☐	☐	☐	☐	☐
7	Have you had difficulty in concentrating, e.g. when reading the newspaper or watching	☐	☐	☐	☐	☐	☐
8a	Have you felt very restless?	☐	☐	☐	☐	☐	☐
8b	Have you felt subdued or slowed down?	☐	☐	☐	☐	☐	☐
9	Have you had trouble sleeping at night?	☐	☐	☐	☐	☐	☐
10a	Have you suffered from reduced appetite?	☐	☐	☐	☐	☐	☐
10b	Have you suffered from increased appetite?	☐	☐	☐	☐	☐	☐

*Name:*_____ *Date:*_____

9.9 Appendix 9 – Epworth Sleepiness Scale

PIN: _____ Date: _____

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 your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:

- 0 = no chance of dozing
- 1 = slight chance of dozing
- 2 = moderate chance of dozing
- 3 = high chance of dozing

Situation	Chance Of Dozing
Sitting and reading	
Watching TV	
Sitting inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in traffic	

9.10 Appendix 10 – Fatigue Visual Analogue Scales

PIN: …….. Date: ……....
Time: ……

Lee Fatigue Scale

This questionnaire will check your level of energy at the moment. There are 18 items. Completing should take only about 1 minute.

Directions: Place an 'X' through these lines to indicate how you are feeling right now.

Please complete the following items:

Not at all tired	———————————————	Extremely tired
Not at all sleepy	———————————————	Extremely sleepy
Not at all drowsy	———————————————	Extremely drowsy
Not at all fatigued	———————————————	Extremely fatigued
Not at all worn out	———————————————	Extremely worn out
Not at all energetic	———————————————	Extremely energetic
Not at all active	———————————————	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 give one answer to each.

Thank you!

9.11 Appendix 11 – Poster for GP surgeries

THE ROBERT GORDON UNIVERSITY ABERDEEN

NHS Grampian

Do you have type 2 diabetes and want to lose weight?

Take part in a weight loss study!

We need people who:

- ✓ Have poorly controlled type 2 diabetes (HbA1c ≥ 7.5%)
- ✓ Are overweight with a BMI ≥ 30kg/m²
- ✓ Are 18 years of age or over
- ✓ Have no history of kidney, liver or cardiovascular disease

Benefits for you:

- ✓ Dietary advice and guidance for 12 months
- ✓ Possible weight loss and improvements in blood sugar levels

To find out more about the randomized clinical trial of low carbohydrate diets, please contact Miss Ania Gryka at 01224 553739 or by email: a.a.gryka@rgu.ac.uk

9.12 Appendix 12 – Investigating reasons for dropout

Date

Dear volunteer,

We would like to thank you for taking part in our weight loss study and visiting Westburn House. We truly appreciate your time and commitment. We would be grateful if you could help us once more and answer one question. It does not matter whether you have completed the study or not. Your reply is anonymous. Please return your answer in the enclosed envelope. Thank you very much.

Kind regards,

Prof Iain Broom
Dr Catherine Rolland
Miss Anna Gryka

Diet:

a) Go Lower (ready meals)

b) Protein Sparing Modified Fast (own cooking)

Completion of one-year diet programme:

a) Yes

b) No

Could you describe in a few sentences what made you complete the one-year diet programme? If you have not completed, could you describe the obstacles that made it difficult?

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