# PHYSIOLOGICAL ASPECTS OF

# WEIGHT LOSS IN OBESITY

Kishor Kantilal Patel

MB BCh MRCP (UK)

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## Abstract

Obesity continues to be a major cause of morbidity and mortality and worldwide prevalence rates continue to rise. The cornerstone for treating obesity remains diet and lifestyle, with the ultimate goal being normalising those parameters that are associated with ill health, for example hyperinsulinaemia and insulin resistance. Because obesity predominantly develops due to a mismatch between energy intake and utilisation, this thesis looked at the effects of dietary interventions upon Resting Energy Expenditure (REE) and substrate oxidation. In addition, the impact of popular dietary interventions upon body composition and insulin resistance was examined.

When phenotypic characteristics were investigated before and after weight loss by using hypocaloric diets, which differed in fat and carbohydrate content, Fat-Free Mass (FFM) and Fat Mass (FM), were strong predictors of REE before and after the intervention and weight loss rather than the specific dietary intervention, significantly predicted post intervention REE.

Fasting fat oxidation was found to be lower in obese subjects and they had a lower postprandial response to a high fat challenge. This implied that a diet high in fat is more likely to promote a positive energy balance an ultimate weight gain.

The final study compared 4 popular dietary interventions. Each was equally effective at achieving clinically significant weight loss and improvements in insulin sensitivity. Although none was significantly more superior, there was a trend supporting three of the diets (Atkins', Weight Watchers and Rosemary Conley) above the other (Slim-Fast) and it was the pattern of weight loss, i.e.

mainly loss of FM, which proved beneficial with regards to improving insulin sensitivity.

In summary, this thesis confirms that REE is mainly predicted by FFM and FM and that there is diminished fat oxidation on obese subjects. What this thesis also adds to previous research that it if a specific diet can improve the pattern of weight loss, this can be clinically beneficial.

## Acknowledgements

During the preparation and submission of this thesis has I travelled a long and winding path. I would like to take this opportunity to thank Professor Ian Macdonald for introducing me to clinical research and providing guidance, support and inspiration during the past 7 years. His persistent encouragement enabled me to achieve my goal in preparing and submitting this thesis.

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# Chapter 1

# Introduction

## 1.1 Background

Obesity has become a major problem over the past two decades, having an impact upon the nation's health and economy, both directly and indirectly. The recent figures for England would indicate that whilst obesity prevalence in 1980 was 6% for men and 8% for women, by 2002 this had risen to 23% for men and 25% for women (Department 2004). For the British Isles as a whole, 25% of men and 20% of women were obese (BMI>30) with a further 42% men and 32% women classed as overweight (BMI 25-30) (Swan 2004). Over the past decade the largest increase has been seen in the younger age group (figure 1.1)

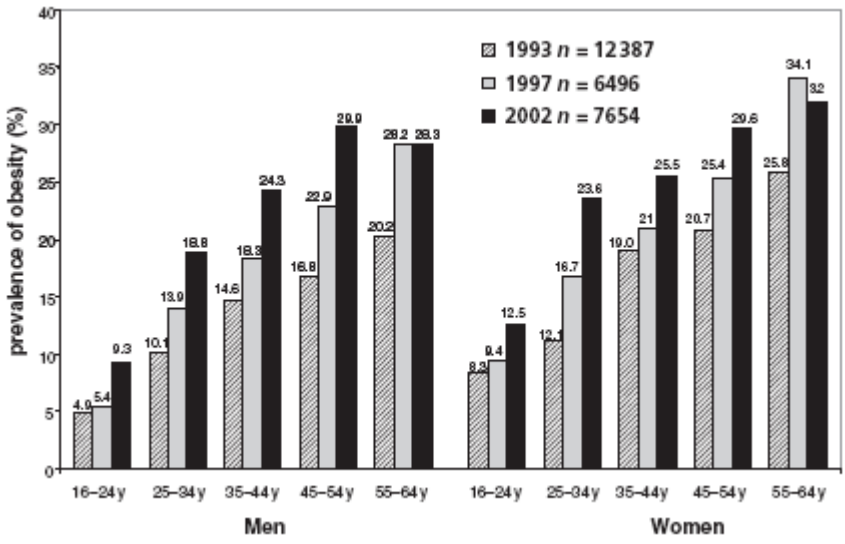


**Figure 1.1**: Secular trends in the prevalence of obesity (% with BMI > 30)
(Department of Health 2004)

Nonetheless, the older people become the more likely they are of becoming obese. There is also an association of obesity with low education attainment, low income and manual occupations (Rennie and Jebb 2005).

Similar increases in obesity have been seen in the United States (Caterson and Gill 2002) and elsewhere in the western world and more recently in developing countries.

It is estimated that obesity cost the NHS £ 0.5 billion directly with indirect costs to the economy reaching up to £7 billion (Health Select Committee 2004). The burden of obesity is therefore immense and one which is likely to increase, especially with the availability of processed, high-energy foods and a move towards a more sedentary lifestyle.

## 1.2 Definition

Obesity can be defined as state in which the amount of body fat within adipose tissue reaches a level which impacts upon health. Body fatness can be difficult to measure so, as a surrogate marker, Body Mass Index (BMI) is the commonly accepted measure of obesity. BMI is expressed as the ratio of weight to height (Wt (kg)/$Ht^2$ (m)). Obesity is defined as a BMI of 30 or more. The current WHO classifications for bodyweight are shown below (Table 1.1)

| Classification | BMI | Obesity associated health risks |
|---|---|---|
| **Normal range** | 18.5 to 24.9 | Average |
| **Overweight / Pre-obese** | 25.0 to 29.9 | Mildly Increased |
| **Obese Class 1** | 30.0 to 34.9 | Moderately Increased |
| **Obese Class 2** | 35.0 to 39.9 | Severely Increased |
| **Obese Class 3** | >40.0 | Very Severely Increased |

**Table 1.1**: WHO classification of overweight and obesity (in International Textbook of obesity (Bjorntorp 2001))

Another method of assessing body fatness is by measuring waist circumference. Some authorities advocate the use of waist circumference rather than BMI as a better indicator of central obesity due to its association with increased mortality (Table 1.2). Whichever model is adopted, it is clear that there is a major impact upon health with increasing obesity (Table 1.3).

| | **Risk of metabolic complications** | **Waist Circumference (cm)** | |
|---|---|---|---|
| | | **Men** | **Women** |
| **Zone 1** | Increased | 94 | 80 |
| **Zone 2** | Substantially increased | 102 | 88 |

**Table 1.2**: Waist circumference and its association with metabolic complications[Lean, Han et al. 1995]

| **Greatly increased (relative risk >3)** | **Moderately increased (relative risk 2-3)** | **Slightly increased (relative risk 1 –2)** |
|---|---|---|
| Type 2 Diabetes | Coronary Heart Disease | Certain cancers |
| Gallbladder Disease | Hypertension | Reproductive hormone abnormalities |
| Dyslipidaemia | Osteoarthritis (knees) | Polycystic Ovary Syndrome |
| Breathlessness | Hyperuricaemia & Gout | Low back pain |
| Sleep Apnoea | | Increased anaesthetic risk |
| | | Foetal defects in mothers with obesity |

**Table 1.3**: Relative risk of health problems associated with obesity [World Health Organisation 1997]

## 1.3 Medical consequences of obesity

The concept of adipose tissue being more than just a storage site has been well documented (Prins 2002). People who are obese often suffer from numerous health problems. The pathophysiological consequences of obesity occur as a result of adipose tissue hypertrophy or hyperplasia. This can be as a result of the physical burden and distribution of carrying excess adipose tissue or the metabolic and endocrine consequences relating to adipose tissue function (Figure 1.2).

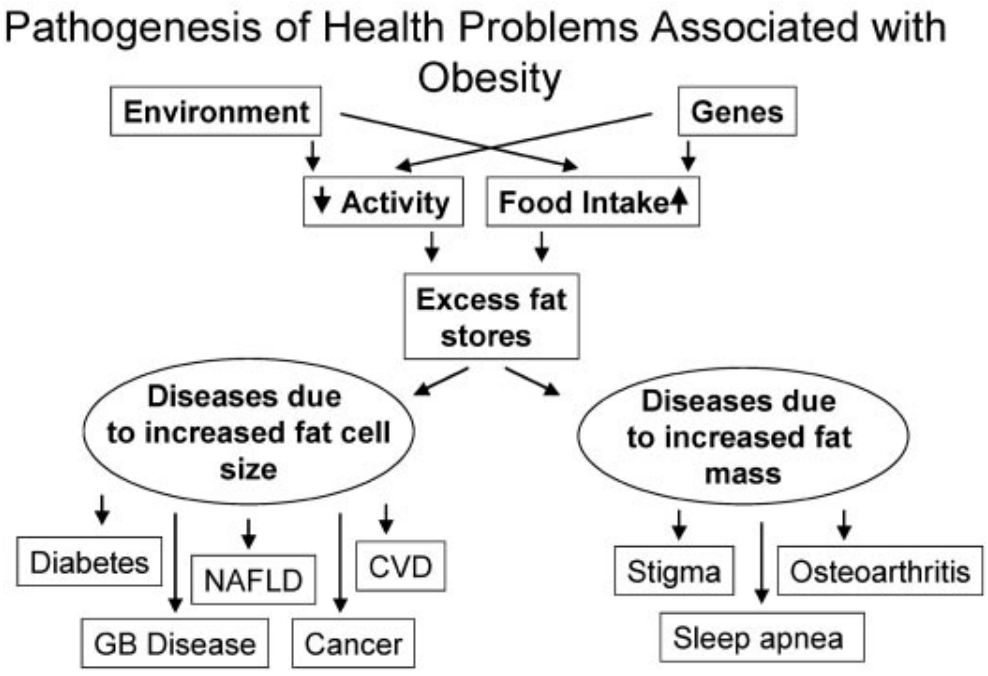


**Figure 1.2: The pathology of obesity produces the myriad of health related problems. CVD, Cadiovascular Disease; GB, Gallbladder; NAFLD, Non-Alcoholic Fatty Liver Disease**[(Bray 2004)]

**Insulin Resistance and Type 2 Diabetes**

It is well documented that the risk of developing Type 2 Diabetes increases with increasing BMI. Gaining >10% in weight is associated with an increased risk of diabetes (Colditz, Willett et al. 1995; Wannamethee and Shaper 1999).Thompson developed a model of lifetime health and economic consequences of obesity (Thompson, Edelsberg et al. 1999). From this he estimated that the risk of developing diabetes was increased 50-60% in individuals who were mildly obese, 2-fold in those classed as moderately obese and 3-4 fold in the severely obese. Not only is the level of obesity important but also the duration that an individual has been either overweight or obese (Wannamethee and Shaper 1999; Wilson, D'Agostino et al. 2002). Encouragingly, weight-loss achieved medically or surgically, has been shown to improve diabetic control (UKPDS 1998; Torgerson and Sjostrom 2001; Diabetes Prevention Program Research Group 2002) or even prevent diabetes from occurring (Tuomilehto, Lindstrom et al. 2001; Diabetes Prevention Program Research Group 2002; Torgerson, Hauptman et al. 2004).

**Dyslipidaemia**

Obesity is associated with both hyperinsulinaemia and insulin resistance. Both of these factors stimulate hepatic triglyceride formation, which ultimately results in the characteristic, atherogenic pattern of dyslipidaemia seen in obesity (raised triglyceride, smaller and denser LDL particles, raised apolipoprotein B and lower HDL concentrations) (Lewis, Carpentier et al. 2002; Lawrence and Kopelman 2004) .When looking at overweight and obese individuals within the Framingham cohort, the risk for developing

hypercholesterolaemia was 10% for men and 9% for women (Wilson, D'Agostino et al. 2002).

**Hypertension**

Although the exact mechanism by which obesity increases the risk of hypertension is not entirely certain, it is thought to be contributed to by a combination of increased sodium retention and an increase in sympathetic activity, resulting in an increase in intravascular volume and peripheral vascular resistance which is unable to respond appropriately to this increased volume load (Formiguera and Cantón 2004).

Whilst the physiological link with obesity may not be clear, epidemiological evidence is however, much greater. Data from NHANES 3 (Collins and Winkleby 2002) and other studies (Colditz, Willett et al. 1995; Thompson, Edelsberg et al. 1999) shows that there is a positive and linear relationship between BMI and hypertension. In the Swedish Obesity Study (Torgerson and Sjostrom 2001), 44-51% of individuals were hypertensive at baseline. After adjusting for smoking, Diabetes and hypercholesterolaemia, the prevalence of new hypertension in the Framingham cohort was 26% for men and 28% for women who were either overweight or obese (Wilson, D'Agostino et al. 2002). Field et al observed odds ratios of between 1.7 and 3.0 for developing hypertension in men and women who were either overweight or obese as compared to their leaner peers (Field, Coakley et al. 2001).

## Cardiovascular Disease

The incidence of cardiovascular disease has been shown to increase in a variety of populations with increasing BMI (Manson, Willett et al. 1995) as well as with increasing abdominal obesity (Lakka, Lakka et al. 2002). After accounting for other known risk factors, there was still a two-fold increase in the risk of coronary heart disease in obese individuals in the Framingham population (Hubert, Feinleib et al. 1983; Wilson, D'Agostino et al. 2002). This risk also seems to increase the more obese an individual is (Field, Coakley et al. 2001). The risk of coronary mortality has been estimated to increase by 1-1.5% for every 1kg increase in weight (Jousilahti, Tuomilehto et al. 1996). This increase in heart disease has been seen across gender, race and socio-economic group(Paeratakul, Lovejoy et al. 2002). It is clear that there is a close interrelationship between increasing BMI, waist circumference and dyslipidaemia, which increases an individual's risk of developing coronary artery disease(Dalton, Cameron et al. 2003; Park, Choi et al. 2009). It is not however, only atherosclerosis in the obese which leads to cardiovascular death. Changes to the heart muscle itself, independent of hypertension can lead to sudden death (Kortelainen and Sarkioja 1997).

## Cerebrovascular Disease

Unlike the established link between obesity and cardiovascular disease, the association between obesity and cerebrovascular disease is less clear. Earlier studies in Japanese-American men, found no association between BMI and risk of stroke (Curb and Marcus 1991). More recently, however, data has

provided more evidence to support the theory that obesity and cerebrovascular disease are linked.

Field et al (Field, Coakley et al. 2001) found that the risk of stroke, both fatal and non-fatal, increases with increasing BMI and that this risk was more pronounced in men than in women. As part of the Women's Health Study (Kurth, Gaziano et al. 2005), Kurth et al prospectively assessed the risk of stroke with increasing BMI and discovered an increase in the risk of total and ischaemic but not haemorrhagic stroke. The same group had previously demonstrated similar results in men for both ischaemic and haemorrhagic stroke (Kurth, Gaziano et al. 2002). Hu et al showed that not only BMI but also waist circumference or waist-to-hip ratio were associated with a greater risk of stroke (Hu, Tuomilehto et al. 2007). Results from the Northern Manhattan Stroke Study (Suk, Sacco et al. 2003) demonstrated that abdominal obesity was not only an independent risk factor for ischaemic stroke but that it was also a stronger risk factor than BMI.

**Obstructive Sleep Apnoea**

Obstructive Sleep Apnoea (OSA), is characterised by repetitive cessation of airflow during sleep, secondary to the collapse of the upper airway at the level of the pharynx (Kyzer and Charuzi 1998; Bjorntorp 2001), with the deposition of fat in the upper airways being implicated in the aetiology (Lowe, Fleetham et al. 1995). OSA occurs in 4–9% of middle-aged men and 1-2% of middle-aged women. Obesity has been shown to be the most significant risk factor. Millman et al reported that between 60 and 70% of patients with OSA were obese (Millman, Redline et al. 1991), whilst Frey reported figures as high as 90%(Frey and Pilcher 2003). The incidence is some 12 – 30 fold higher in the

morbidly obese than in the general population (Peiser, Lavie et al. 1984). OSA has been associated with an increased risk of fatal and non-fatal cardiovascular events (Rossner, Lagerstrand et al. 1991; Marin, Carrizo et al. 2005).

**Gallbladder Disease**

One of the main hepatobiliary disorders associated with obesity is cholelithiasis (gallstones), with women being particularly susceptible (Everhart 1993). More specifically, abdominal or central obesity is a strong predictor of gallstones in both men and women (Ruhl and Everhart 2001; Tsai, Leitzmann et al. 2004).

Cholesterol stones account for approximately 80% of all gallstones which are surgically removed from patients (Diehl, Schwesinger et al. 1994; Hyogo, Tazuma et al. 2002). This high incidence of cholesterol stones seems to be related to the higher excretion of cholesterol in bile which is seen in obese subjects, who tend to have higher plasma cholesterol and triglyceride levels (Formiguera and Cantón 2004).

**Polycystic Ovary Syndrome**

Polycystic Ovary Syndrome (PCOS) is characterised by hyperandrogenism (acne, hirsutism), menstrual irregularities (oligomenorrhoea / amenorrhoea) and infertility. Approximately 50% of women with PCOS are obese (Gambineri, Pelusi et al. 2002). A study in Spanish women found a PCOS prevalence of 28% in overweight and obese women as compared to prevalence of 6% in normal weight women (Alvarez-Blasco, Botella-Carretero et al. 2006).

Insulin resistance, a common feature of obesity, results in hyperinsulinaemia. Hyperinsulinaemia in turn favours increased synthesis and secretion of ovarian androgens, which is felt to be the primary defect in PCOS (Dunaif 1997; Nelson, Qin et al. 2001).

**Cancer**

Evidence for the relationship between obesity and the development of cancer has been steadily growing. Mechanisms behind this association may be related to changes in hormones and growth factors, which occur with obesity. In women, decreased sex-hormone binding globulin (SHBG) and an increase in aromatisation of ovarian androgens to oestrogens within adipose tissue may predispose to an increased risk of breast and endometrial carcinomas. Central obesity is linked to a high level of insulin-like growth factor-1 (IGF-1). This hormone has been shown to be capable of inhibiting cell apoptosis and thereby stimulating cell proliferation and potentially dysplasia.

Adami et al, reported that as BMI increased, so did the relative risk of death from cancer (Adami and Trichopoulos 2003). For people with a BMI of 30-35, relative risk of death was 1.3 and those with BMI > 40 had a relative risk of cancer related death which was 1.5 times greater than normal weight individuals. Calle et al prospectively studied over 900000 US men and women over 16 years (Calle, Rodriguez et al. 2003). They discovered that those who had a BMI >40 had cancer-related death rates that were 52% and 62% higher for men and women respectively, than their normal weight counterparts. When individual cancers were examined, BMI was positively associated with carcinoma of the oesophagus, colon and rectum, liver, gallbladder, pancreas and kidney in both men and women. In addition, obese men had a higher

incidence of stomach and prostate cancer and obese women, higher rates of carcinoma of the breast, cervix, uterus and ovary.

Results from the European Prospective Investigation into Cancer and Nutrition (EPIC) study have also demonstrated similar results. An increase in breast cancer rates was seen in obese, postmenopausal women (Lahmann, Lissner et al. 2004). General obesity was related to an increased risk of renal cell carcinoma in women, although in men, it was related more to higher waist-to-hip ratios (WHR) (Pischon, Lahmann et al. 2006). The same group also demonstrated that abdominal obesity, as measured by waist circumference or WHR, was strongly associated with an increased risk of developing colonic carcinoma in both men and women (Pischon, Lahmann et al. 2006).

## 1.4 Mechanisms of Development of Obesity

Obesity is a highly multifactorial condition in which social, behavioural and environmental influences might amplify some of the underlying metabolic and physiological processes underlying what has been termed the ‘thrifty phenotype’ (Prentice 2001). Figure 1.3 attempts to illustrate how these factors may interact to influence weight gain.

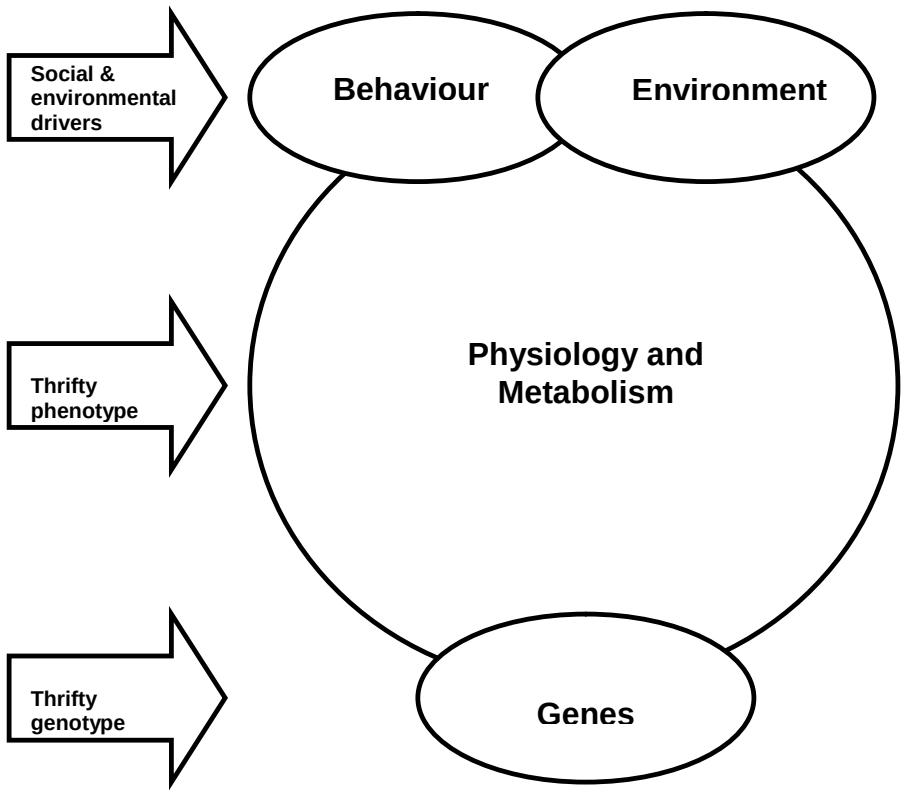


**Figure 1.3**: **Interaction of physiological, environmental and genetic factors which underlie the development of obesity**.

### 1.4.1 Genetics

The concept of the “thrifty genotype” was first coined by Neel in the early 1960s. The notion was that a gene or a set of genes would assist in laying down energy stores more efficiently during times of feasting and they would help conserve energy in times of famine (Neel 1962). Whilst beneficial to our ancestors, for many in modern day society, this acts as an obstacle to treating obesity (Lev-Ran 1999).

It has been estimated that genetic factors can contribute between 25 and 40% towards the pathophysiology of obesity (Ravussin and Bouchard 2000).

Certain genetic disorders, which have severe obesity as part of their clinical spectrum, have been known for some time. One example is Prader-Willi Syndrome, which results due to a deletion or non-functioning paternal gene on the long arm of chromosome 15. Another example is Bardet-Biedl Syndrome. This condition is more complex in that there are at least 14 gene abnormalities which have been associated which the phenotypic expression (Waters and Beales 2003).

More recently, monogenic mutations have been identified as causing certain rarer forms of obesity. O’Rahilly and colleagues have reported upon loss in the function of the leptin receptor in the hypothalamus and the absence of functional leptin in the circulation, leading to an absence in feedback from adipose tissue stores (Barsh, Farooqi et al. 2000). Characteristic features seen with leptin deficiency are severe early onset obesity, hyperphagia and abnormalities in T-Cell function, which lead to recurrent infections. Mutations in the Melanocortin-4 Receptor (MC4R) have been reported in several different populations. Some mutations have been implicated in accounting for

between 1 and 6% of severe forms of obesity (Marti, Corbalan et al. 2003; Hinney, Bettecken et al. 2006; Kublaoui and Zinn 2006; Lubrano-Berthelier, Dubern et al. 2006), whilst Young et al found in their meta-analysis of 3 population-based cohort studies that another polymorphism in the MC4R gene (V1031) had a protective effect against obesity (Young, Wareham et al. 2007). Deficiency in the gene product of Proopiomelanocortin (POMC) has been associated with early onset obesity and hyperphagia (Farooqi and O'Rahilly 2008).

Researchers have targeted specific candidate genes based on their perceived function in metabolic processes.

The β adrenoreceptor genes (ADRB2 / ADRB3 / ADRB1) have been studied due to the role played by the sympathetic nervous system in energy expenditure regulation and adaptive thermogenesis. One single nucleotide polymorphism (SNP) in the ADRB2 gene, where glutamine is substituted by glutamic acid at codon 27, has been associated with an increase in BMI, subcutaneous fat and raised triglyceride levels in men whereas the same SNP was associated with an increase in BMI, fat mass and waist-to-hip ratio in women (Corbalan, Marti et al. 2002; Corbalan, Marti et al. 2002; Macho-Azcarate, Calabuig et al. 2002; Macho-Azcarate, Marti et al. 2002). Park et al found an SNP in the ADRB3 gene which resulted in an increase in visceral fat in variety of populations (Park, Kim et al. 2005). The ADRB1 gene has also been a candidate gene for obesity due to its role in catecholamine-induced energy homeostasis in both adults and children (Linne, Dahlman et al. 2005; Li, Chen et al. 2006). Other genes which are under investigation are the uncoupling proteins (particularly UCP 2 and 3) due to the relationship between

SNPs in these genes and exercise efficiency, resting energy expenditure and energy metabolism (Marti, Corbalan et al. 2004; Alonso, Marti et al. 2005). Genes involved with adipogenesis have also been investigated as potential candidate genes with an SNP of peroxisome proliferator-activated receptor γ (PPARγ) found to be positively associated with obesity (Marti, Corbalan et al. 2002).

In the majority of individuals, susceptibility to obesity is felt to be a polygenic trait (Barsh, Farooqi et al. 2000; Boutin and Froguel 2001). One of the major difficulties in investigating these polygenic traits is that the individual variations in phenotype accounted for by any one gene may be very minor. Only through large-scale population studies might it therefore be possible to identify these small effects (Lango and Weedon 2008).

Recent advances in Genome-Wide Association studies have revealed novel and unexpected genetic factors with strong associations with diabetes (Korner, Kiess et al. 2008). Currently 10 million SNPs have been identified in the human genome (Sachidanandam, Weissman et al. 2001). The development of DNA “chips” has allowed several hundred of these SNPs to be assayed simultaneously. Recent genome-wide associations have linked obesity to variations in the fat mass and obesity-associated (FTO) gene (Frayling, Timpson et al. 2007). As well as its association with BMI (Frayling, Timpson et al. 2007), FTO has also been associated with body weight (Scuteri, Sanna et al. 2007), leptin (Andreasen, Stender-Petersen et al. 2008), subcutaneous fat (Frayling, Timpson et al. 2007), fat mass (Frayling, Timpson et al. 2007; Andreasen, Stender-Petersen et al. 2008) and waist circumference (Andreasen, Stender-Petersen et al. 2008). No association has been found with lean mass (Frayling, Timpson et al. 2007). The clinical

significance of FTO is that for each additional risk allele, BMI increases by approximately 0.1 to 0.13 $kgm^{-2}$ and weight by 1.3 to 2.1 kg (Frayling, Timpson et al. 2007; Scuteri, Sanna et al. 2007; Andreasen, Stender-Petersen et al. 2008). Cecil et al (Cecil, Tavendale et al. 2008) found that FTO was linked more to food intake and food choice rather than regulation of energy expenditure. More recently, Fischer et al (Fischer, Koch et al. 2009) have reported that in FTO deficient mice, fat mass is diminished as a consequence of increased energy expenditure and systemic sympathetic activity.

Although there is now more evidence regarding human genetics, the human gene pool has not changed dramatically over the past 20 years. During the same time, however, the obesity pandemic has increased in prevalence by almost 30%, suggesting that factors other than genetics must be having a significant influence (Filozof and Gonzalez 2000).

### 1.4.2 Environment

The dramatic change in lifestyle over recent decades has been a major contributor to obesity, with the inactivity associated with television watching being one of the major factors (Prentice and Jebb 1995). There has been growing evidence supporting a link between social deprivation and obesity. Ellaway et al discovered a link between the areas of poorer residence and increasing BMI(Ellaway, Anderson et al. 1997). This finding of higher BMI being associated with less favourable social circumstances has also been reported by Smith et al (Smith, Hart et al. 1998), although this was only demonstrated in women. Residential areas, which discouraged physical

activity due to their design, were also found to be strongly associated with obesity(Black and Macinko 2008).

### 1.4.3 Behaviour

Eating is influenced by multiple cognitive, economic and environmental variables (Schwartz 2004). One of the characteristic hallmarks for the development of obesity is hyperphagia, with either an increase in meal size or meal duration(Farooqi, Keogh et al. 2003). One of the difficulties faced by researchers when they look into eating habits and dietary intake is that obese subjects tend to under report food intake by as much as 30% (Lichtman, Pisarska et al. 1992). It can, however, be difficult to differentiate between the psychological control of eating behaviour and physiological processes which control appetite and feeding.

### 1.4.4 Central Nervous System control of Energy Intake

The regulation of feeding behaviour can be divided into short-term and long-term controls of food intake. When assessing the control of food intake, there are several issues, which need consideration (Jequier 2002):

1. Suppression of hunger, inducing the end of a meal – Satiation
2. The period of absence of hunger between meals – Satiety
3. Long-lasting control of food intake, related to the size of the adipose tissue mass

Satiation and satiety occur in response to both locally and centrally derived neuroendocrine responses. The distension of the gastrointestinal tract by food causes the release of gut-derived hormones such as Cholecystokinin (CCK).

CCK, released from the duodenum, causes signals to the hypothalamus, via the vagus nerve, which triggers a feeling of satiation and early satiety(Moran 2000).

The macronutrient composition of a meal also has an important role to play in satiety. Protein and carbohydrate have a greater capacity to induce satiation and satiety than fat (Prentice 1998; Jequier 2002). Therefore, meals that are higher in fat, which tend to also be more palatable, not only result in an increase in energy intake but also have a weaker signal for meal termination, resulting in increased energy consumption.

While these signals may regulate individual meal size, there are long-term signals, which reflect energy stores. Adipose tissue derived leptin reflects the body's fat stores and acts as an adiposity signal to the brain, which ultimately influences feeding patterns(Schwartz, Woods et al. 2000).

Several other peptides are involved in the regulation of feeding and fall into two categories: the anabolic (orexigenic) hormones, which increase food intake and decrease energy expenditure and the catabolic (anorexigenic) hormones, which have the opposite effect. Table 1.4 shows these peptides, which are predominantly hypothalamic neurotransmitters, and the impact that they have upon energy balance.

| NEUROPEPTIDES | ADIPOSITY SIGNAL | | EFFECT ON ENERGY BALANCE | |
|---|---|---|---|---|
| | **Fasting** | **Refeeding** | **Food intake** | **Energy Expenditure** |
| **Neuropeptide Y** | Increase | Decrease | Increase | Decrease |
| **Melanin-Concentrating Hormone** | | | | |
| **Agouti-Related Protein** | | | | |
| **Corticotrophin-Releasing Hormone** | Decrease | Increase | Decrease | Increase |
| **Cocaine/Amphetamine Regulating Transcript** | | | | |
| **Proopiomelanocortin** | | | | |

**Table 1.4: The interaction of adiposity signals upon hypothalamic neuropeptides and their effect on energy balance (adapted from (McMinn, Baskin et al. 2000))**

Several key areas within the brain have been shown to be associated with energy balance. Lesions within the paraventicular nucleus of the hypothalamus cause hyperphagia, decreased energy expenditure and pronounced weight gain. Lesions within the lateral hypothalamic area result in hypophagia and increased energy expenditure (reviewed in (McMinn, Baskin et al. 2000)).

One point to note however, is that this basic (homeostatic) control of eating can be over-ridden by hedonic or non-homeostatic factors operating via the limbic system and cortical dopaminergic reward pathways. Thus people can overeat even though they do not need to satisfy any homeostatic need (Bryant, King et al. 2008; Zheng and Berthoud 2008).

### 1.4.5 Energy Expenditure

Total Energy Expenditure (TEE) can be broken down into three major components:

- Basal Metabolic Rate (BMR)
- Thermic Effect of Feeding (TEF)
- Energy cost of Physical Activity (PA).

**Basal Metabolic Rate**

BMR is the largest component of TEE, accounting for up to 80% of TEE (Goran 2000). It is a measure of the energy consumed by an individual whilst lying at physical and mental rest in a thermoneutral environment, at least 12 hours after a meal. Often, one or more of the above conditions cannot be met to allow acute measurements. Under these circumstances, an alternative is to measure the Resting Energy Expenditure (REE), also termed Resting Metabolic Rate (RMR). REE refers to the sum of metabolic process maintaining normal body function and regulatory processes during rest. Numerous factors have been shown to influence REE.

Fat Free Mass (FFM) is the largest determinant of REE. Johnstone et al used linear regression models to determine the contribution of FFM to the between subject variance of REE (Johnstone, Murison et al. 2005). They reported that 63% of the variance in REE between subjects could be explained by differences in FFM. Previously, Nelson et al had reported a value close to 73% (Nelson, Weinsier et al. 1992). It must be noted however, that Nelson used a combination of published regression equations to estimate REE whereas Johnstone measured REE using indirect calorimetry, which may

account for the differences seen. Dehmark-Warhnefield et al, who investigated the weight gain which is often associated with chemotherapy for breast carcinoma, have demonstrated the importance of maintaining FFM to minimise changes in REE (Demark-Wahnefried, Peterson et al. 2001). They showed that rather than being related to over consumption of energy, weight gain was associated with a reduction in lean mass (FFM), a phenomenon they termed ‘sarcopenic obesity’.

As well as FFM, Fat Mass (FM) has also been proven to be of significance in determining REE. Armellini et al showed that after adjusting for FFM, body fat and body fat distribution (as measured by waist-to-hip ratios) was positively correlated with REE (Armellini, Robbi et al. 1992). Other studies have confirmed this finding (Bernstein, Thornton et al. 1983) and quantified this contribution to be in the region of 6% (Johnstone, Murison et al. 2005). As well as the absolute fat mass, the manner in which fat is distributed is also important to REE. When Busetto et al examined the distribution of body fat in obese subjects awaiting bariatric surgery, they discovered that REE was positively related to visceral fat both before and after surgery (Busetto, Perini et al. 1995). Tataranni et al also made similar observations in obese males (Tataranni, Larson et al. 1994). It was felt that the higher REE seen in men with upper body obesity was related to the greater turnover of lipids seen in the visceral fat depot in these individuals. One fact that must be remembered however is that the metabolic rate of adipose tissue is only approximately 1% of that of other more metabolically demanding organs such as the heart, brain, liver and kidneys (Nelson, Weinsier et al. 1992). And as such, FM only becomes relevant to estimates of REE when the absolute FM is very large, as in obesity (Karhunen, Franssila-Kallunki et al. 1997).

It is not only body composition that influences REE. For example in prepubescent and adolescent children, gender has a small but significant contribution to REE independent of body composition (Molnàr and Schutz 1997). This is not seen in adults and any gender effect disappears once REE is adjusted for FFM and FM (Johnstone, Murison et al. 2005).

Piers et al (Piers, Soares et al. 1998) examined REE in relation to both the quantitative and qualitative changes in lean tissue that are seen with aging. They discovered that the decline in REE seen with aging is partly due to an absolute reduction in FFM but also to a reduction in the metabolic activity within the lean tissue. Hunter et al confirmed this finding and extended it by including both FFM and FM partitioning in the analysis of the relationship between age and REE (Hunter, Weinsier et al. 2001).

The secretion of Growth Hormone is considerably reduced in obesity and with weight loss secretion can improve (Scacchi, Pincelli et al. 1999). Also, when growth hormone deficient patients are treated with recombinant human growth hormone, there is an increase in FFM and metabolic parameters, which leads to an increase in REE (Snel, Doerga et al. 1995) . Armellini et al investigated whether growth hormone indices could explain any of the variance in REE (Armellini, Zamboni et al. 2000). They concluded that at physiological levels, no relationship could be demonstrated between growth hormone and REE after the adjustment for body composition. It must be remembered however, that growth hormone will have an effect upon body composition.

Because serum leptin is a reflection of the body's fat stores (Schwartz, Woods et al. 2000), there was interest into whether leptin could influence REE. Sinha et al (Sinha, Opentanova et al. 1996) demonstrated that leptin circulated in two forms: one free leptin monomer (FL) and another bound to plasma

proteins (BL). FL tended to be the major fraction of total leptin in obese subjects whereas BL was the dominant fraction in lean subjects. Magni et al investigated the relationship of these two forms of leptin (FL/BL) with body composition and REE (Paolo Magni 2005). They found that BL correlated better with REE whereas FL increased with increasing fat mass, suggesting differing biological activities of the two forms of leptin. In their review of leptin and energy expenditure, Hukshorn et al (Hukshorn and Saris 2004) found that the predominant function of leptin was to control energy balance through its effects upon appetite, with little effect seen on energy expenditure.

Raised fasting insulin levels and subsequent insulin resistance, have been shown to be associated with lower rates of weight gain in diverse populations, such as non-diabetic Pima Indians (Swinburn, Nyomba et al. 1991) and patients with liver cirrhosis (Petrides, Stanley et al. 1998). The presence of insulin resistance has been associated with an increase in REE and liver transplantation, although improving insulin sensitivity is associated with a corresponding decline in REE (Perseghin, Mazzaferro et al. 2002). In healthy individuals, Sympathetic Nervous System (SNS) activity is directly related to plasma insulin levels (Scherrer, Randin et al. 1994). Because obesity is associated with a hyperinsulinaemic state, the resultant increase in SNS activity in turn, leads to an increase in energy expenditure by way of diet-induced thermogenesis, increased lipolysis and gluconeogenesis, and non-shivering thermogenesis (Landsberg 1986).

**Thermic Effect of Feeding (TEF)**

The consumption of food produces an increase in energy expenditure usually over a period of several hours. This is due to the energy required for

processes such as digestion, absorption and transportation of ingested micro and macronutrients. TEF typically accounts for approximately 10% of TEE (Van Zant 1992). There is growing evidence to support the notion that both diet composition and macronutrient oxidation are important in the development of obesity.

Studies by both Tremblay (Tremblay, Plourde et al. 1989) and Larson(Larson, Tataranni et al. 1995) have implicated a high intake of dietary fat in promoting subsequent weight gain. Heitman et al found this association only in women that were overweight and had at least one parent that was overweight (Heitmann, Lissner et al. 1995). This suggested that there must also be a familial element to weight gain, which had an additional impact to dietary fat. This concept of a familial influence was further supported by work from Saltzman et al (Saltzman, Dallal et al. 1997). They examined dietary energy intake in identical twins and discovered no major influence from differing proportions of dietary fat. Energy intake was however, similar within twin pairs for whichever diet being followed.

The measurement of Respiratory Quotient (RQ) allows an estimation to be made of the of the fuel mixture being used by the body. It is a ratio of the oxygen being consumed and the carbon dioxide being produced. Table 1.5 shows the RQ values for various macronutrients and the associated energy released.

| Nutrient | $O_2$ Consumed (l/g) | $CO_2$ Produced (l/g) | RQ | Energy Released (kJ/g) |
|---|---|---|---|---|
| **Glucose** | 0.746 | 0.746 | 1.00 | 15.44 |
| **Fat** | 1.975 | 1.382 | 0.70 | 39.12 |
| **Protein** | 0.962 | 0.770 | 0.80 | 18.52 |
| **Alcohol** | 1.434 | 0.957 | 0.667 | 29.75 |

**Table 1.5**: **Values for oxidation of major nutrients (from (Escott-Stump 2008))**

When fuel oxidation is predominantly fat, RQ is very close to 0.7 and when predominantly carbohydrate, RQ approaches 1.0. As can be seen from the table, the most efficient method of achieving negative energy balance with weight loss would be by increasing fat oxidation, represented by a low RQ.

In Pima Indians, in whom there is a high prevalence of obesity, those individuals who had a high 24 hour RQ (>90$^{th}$ percentile) were two and a half times as likely to gain more than 5kg in weight, during a follow up period of 6 years, than those with the lowest 24 hour RQ (<10$^{th}$ percentile) (Zurlo, Lillioja et al. 1990). When Toubro et al prospectively studied a Danish cohort they found that age, gender, prior energy balance, fasting insulin and fasting free fatty acids could explain a substantial proportion of the variance in 24 hour RQ. In addition, there was also a strong familial resemblance in measured macronutrient oxidation patterns (Toubro, Sorensen et al. 1998). In non-obese women, a high RQ was found to predispose to weight gain after a period of 6 years, especially in those women with the highest baseline RQ (>90$^{th}$ percentile) (Marra, Scalfi et al. 2004). The finding had previously been

reported in non-obese men (Seidell, Muller et al. 1992). Once obese individuals have lost sufficient weight to be classed as “formerly obese”, RQ values are higher than for “never obese” control subjects (Bessard, Schutz et al. 1983; Filozof, Murua et al. 2000), indicating a physiological adaptation which would contribute towards regaining weight.

Although the TEF would appear to be reduced in obesity, there have been inconsistencies in the literature. Ganatra (Granata and Brandon 2002) discovered in his review of 50 studies examining TEF that there were numerous methodological flaws in measuring and calculating TEF, making interpretation and comparison of studies difficult. For example, when Swaminathan et al looked at post-prandial macronutrient thermogenesis, only fat-induced thermogenesis was reduced in obesity, with no difference seen in carbohydrate or protein-induced thermogenesis (Swaminathan, King et al. 1985). In contrast, Golay et al did find a reduction in thermogenesis induced by carbohydrate (Golay 1993).

**Physical Activity**

The final element contributing to TEE is Physical Activity (PA), accounting for between 30 and 40% in certain individuals.

The importance of PA was seen in Pima Indians. Those with low levels of energy expenditure relating to PA had higher rates of obesity (Rising, Harper et al. 1994). As well as voluntary PA associated with walking and running, there is an involuntary component termed Spontaneous Physical Activity (SPA), Non-Activity Energy Thermogenesis (NEAT) or “fidgeting”. Zurlo et al found that SPA correlated inversely to the rate of subsequent weight gain and

this was also a familial trait (Zurlo, Ferraro et al. 1992). It has also be found that as humans overeat, activation of NEAT dissipates excess energy to preserve leanness but that failure to do so may result in weight gain (Levine, Eberhardt et al. 1999).

Although PA can be measured directly for each subject using either direct or indirect calorimetry, this process can be very laborious and by the very nature of the procedures, will place a degree of constraint on physical activity. An alternative approach is to use estimations of energy cost derived from tables. Estimations can be expressed as either Metabolic Equivalents (METS) or Physical Activity Ratios (PAR). MET represents the ratio of energy expended (kJ) divided by REE (kJ), either measured or estimated by body size. PAR represents the ratio of energy expended (kJ) divided by BMR. Tables 1.6 and 1.7 illustrate values for MET and PAR for common activities.

| MET | Activities | Weight (Kilograms) | | | |
|---|---|---|---|---|---|
| | | 40 | 50 | 60 | 70 |
| 1 | Sitting on toilet | 132 | 165 | 198 | 231 |
| 1.5 | Bathing-sitting<br>Typing | 198 | 248 | 297 | 347 |
| 2.0 | Standing and getting ready for bed<br>Dressing and undressing<br>Grooming<br>Eating and | 264 | 330 | 396 | 462 |
| 2.3 | Ironing | 304 | 380 | 456 | 531 |
| 2.5 | Serving food, setting table (implied walking or standing) | 330 | 413 | 495 | 578 |
| 3.0 | Walking downstairs<br>Carrying small talking child | 396 | 495 | 594 | 693 |
| 3.5 | Home activities- Vacuum<br>Walking and carrying infant | 462 | 578 | 693 | 809 |
| 3.8 | Scrubbing floor | 502 | 627 | 753 | 878 |
| 4.0 | Care for elder/ Disabled adult | 528 | 660 | 792 | 924 |
| 4.5 | Jogging on a mini tramp<br>Washing and waxing car | 594 | 743 | 891 | 1040 |
| 5.0 | Gardening-digging/spading | 660 | 825 | 990 | 1155 |
| 6.0 | Cycling(leisure/slow/light)<br>Moving furniture | 792 | 990 | 1188 | 1386 |
| 7.0 | Jogging<br>Swimming-back stroke | 924 | 1155 | 1386 | 1617 |
| 7.5 | Carrying groceries upstairs | 990 | 1238 | 1485 | 1730 |
| 8.0 | Cycling(moderate effort) | 1056 | 1320 | 1584 | 1848 |
| 11.0 | Swimming-breast stroke/butterfly stroke | 1452 | 1815 | 2178 | 2541 |

**Table 1.6: MET and corresponding calories (kcal/hr) for a variety of activities at different body weights (adapted from Ainsworth, Haskell et al. 2000)**

| | |
|---|---|
| **PAR 1.2 (1.0-1.4)**<br>Lying at rest:<br>Sitting at rest:<br>Standing at rest: | <br>reading.<br>watching TV, reading, writing, calculating, playing cards, listening to the radio, eating. |
| **PAR 1.6 (1.5-1.8)**<br>Sitting:<br>Standing: | <br>sewing, knitting, playing piano, driving.<br>preparing vegetables, washing dishes, ironing, general office & laboratory work. |
| **PAR 2.1 (1.9-2.4)**<br>Standing: | mixed household chores (dusting & cleaning), washing small clothes, cooking activities, hairdressing, playing snooker, bowling. |
| **PAR 2.8 (2.5-3.3)**<br>Standing:<br>Walking:<br>Industrial: | <br>dressing & undressing, showering, 'hoovering', making beds.<br>3-4 km/h, playing cricket<br>tailoring, shoe making, electrical, machine tool, painting & decorating. |
| **PAR 3.7 (3.4-4.4)**<br>Standing:<br>Walking:<br>Industrial: | <br>mopping floor, gardening, cleaning windows, table tennis, sailing.<br>4-6 km/h, golf.<br>motor vehicle repairs, carpentry, chemical, joinery, brick laying. |
| **PAR 4.8 (4.5-5.9)**<br>Standing:<br>Walking:<br>Exercise:<br>Occupational: | <br>polishing furniture, chopping wood, heavy gardening, volley ball.<br>6-7 km/h<br>dancing, moderate swimming, gentle cycling, slow jogging.<br>Labouring, hoeing, road construction, digging & shovelling, felling trees. |
| **PAR 6.9 (6.0-7.9)**<br>Walking:<br>Exercise:<br>Sports: | <br>uphill with load or cross-country, climbing stairs.<br>average jogging, cycling.<br>Football, more energetic swimming, tennis, skiing. |

**Table 1.7**: PAR values for common physical activities (Department of Health 1991)

## 1.5 Treatment of Obesity

Although net energy deficit will ultimately result in weight loss, how this is achieved has and continues to be a major challenge. Factors that have been a source of much debate include:

- Lifestyle and behaviour modification
- The influence of dietary composition
- Pharmacotherapy
- Surgical intervention.

### Lifestyle and Behaviour

Weight loss programmes, which tend to focus upon reduction of energy intake in combination with an increase physical activity, have been proven to be successful. One of the main issues, however, is that their effectiveness tends to be short lived and provide limited long-term benefit (Garner and Wooley 1991; Wilson 1994; Jeffery, Drewnowski et al. 2000).

The concept of psychological therapy is based upon the theory that the problem being addressed, for example obesity, is maintained by certain dysfunctional thoughts and beliefs (Duffy and Spence 1993; Cooper, Fairburn et al. 2003). Cognitive Behaviour Therapy (CBT) uses a combination of behavioural techniques, to help modify precipitants and consequences, along with cognitive techniques, which allow an individual to identify, evaluate and restructure these dysfunctional cognitions and beliefs. It is thought that this helps to promote generalisation and transfer of new skills and behaviours, which have been learnt, into everyday life as time goes by (Cooper, Fairburn et al. 2003; Butler, Chapman et al. 2006). Much of the work into CBT in

obesity has in the past focussed on those individuals with an underlying eating disorder (Wilson 1999). More recently, Werrij et al (Werrij, Jansen et al. 2009) have studied CBT in non-eating disorder obese subjects. They compared CBT to physical exercise when used in addition to diet. Whilst both groups demonstrated an initial weight loss of approximately 4%, during long-term (12 months) follow up, the CBT group were able to maintain this weight loss. This enduring effect of CBT to prevent relapse following weight loss has also been reported by others (Hollon 2003; Beck 2005).

It has also been demonstrated that adding CBT to either low fat or low carbohydrate diets achieved superior weight loss to diet alone (9% vs 4%) over a 6 month period (Rodriguez-Hernandez, Morales-Amaya et al. 2009). When Ashton et al used CBT in patients awaiting bariatric surgery, as little as 4 sessions proved helpful in decreasing the number of episodes of binge eating (Ashton, Drerup et al. 2009).

The setting for delivering CBT is also of interest. If effective group therapy were possible, this could have an enormous impact upon resources. Cresci and colleagues demonstrated that with non-eating disorder obese subjects, when CBT was delivered weekly over 10 weeks, in either a group or individual setting, although initial results were more favourable in terms of weight loss in those undergoing group therapy, after 36 months of follow up, results were similarly effective for both cohorts (Cresci, Tesi et al. 2007).

**Pharmacotherapy**

There have been a number of pharmaceutical agents used, with varying success, to treat obesity (Dexfenfluramine, Sibutramine, Rimonobant and Orlistat). Unfortunately, due to safety issues and serious side effects, only one of these (Orlistat) is currently licensed for use within the United Kingdom.

Orlistat is a pancreatic lipase inhibitor and as such stops approximately 30% of dietary fat from being absorbed from the gut (Williamson 1999). When used in combination with a low fat, hypocaloric diet, in comparison to placebo, not only was there significantly greater weight loss after 12 months of therapy with orlistat (~3kg) (Davidson, Hauptman et al. 1999; Karhunen, Franssila-Kallunki et al. 2000) but use of orlistat for 2 years resulted in less weight regain (35% vs. 63%) (Davidson, Hauptman et al. 1999; Karhunen, Franssila-Kallunki et al. 2000). In addition to significant weight loss, Orlistat has also been shown to reduce the incidence of Type 2 Diabetes in those obese individuals that have impaired glucose tolerance (Torgerson, Hauptman et al. 2004). Since 2007, Orlistat has been available at a lower dose (60mg) as a non-prescription, over-the-counter medication (Alli® - GlaxoSmithKline). At this lower dose, average weight loss of approximately 5% has been reported (Anderson 2007). One of the difficulties, however, in assessing whether over-the-counter medication will prove beneficial is that there will be no way of knowing who has purchased the medication, whether treatment has proved successful or for how long it was taken for.

Over the past few years, a new therapeutic modality has been developed which utilises the “incretin effect” (Bloomgarden 2004). The incretin effect is the rise in insulin levels that are seen after an oral carbohydrate (glucose) load. It is mediated by one of two gut derived hormones: Glucagon-Like Peptide -1 (GLP-1) and Glucose-dependent Insulinotropic Peptide (GIP). Following a meal containing carbohydrate, GLP-1 is released from the small intestine. It results in an increase in pancreatic insulin secretion from the beta cells as well as reducing glucagon levels from pancreatic alpha cells. The net

effect is to lower plasma glucose levels. In addition, GLP-1 causes a delay in gastric emptying and as a result acts as a satiety signal to the brain (Holz and Chepurny 2003; Nielsen, Young et al. 2004). In Type 2 diabetes there is a diminished response by the gut to release these hormones. Recently, drugs, which enhance the incretin effect, have been developed for use in patients that have both Type 2 Diabetes and obesity. Currently, there are two GLP-1 analogues licensed for use (Exenatide and Liraglutide). Long term (82 weeks) use of Exenatide has resulted in a fall in HbA1c of around 1% with associated weight loss of 4 to 6kg (Ratner, Maggs et al. 2006; Riddle, Henry et al. 2006). Because it is a newer drug, studies with Liraglutide have been of relatively shorter duration. Early results have been encouraging and over a 26 week period, HbA1c has decreased by 1% with weight decreasing by 2 to 4 kg (Buse, Rosenstock et al. 2009; Zinman, Gerich et al. 2009). Astrup et al have also studied the use of several doses of Liraglutide in obese, non-diabetic subjects, in addition to a 500 kcal hypocaloric diet (Astrup, Rossner et al. 2009). Following 20 weeks of therapy, compared to placebo, greater weight loss was seen in the each of the Liraglutide groups (2 – 3.5kg).

**Bariatric Surgery**

The use of surgical procedures has become an increasingly more popular method used to assist the treatment of obesity. In the United Kingdom, the most commonly performed procedures are:

- Laparoscopic Gastric Banding
- Sleeve Gastrectomy
- Roux-en Y Gastrectomy
- Biliopancreatetic Diversions

These procedures fall into two basic categories. The first are restrictive procedures, which reduce the volume of the stomach, thereby restricting the amount of food that can be consumed at any one time. The second type of procedure is a malabsorptive procedure. Here, part of the digestive tract is bypassed and therefore reducing the opportunity for macro and micronutrients to be absorbed.

**Laparoscopic Gastric Banding**

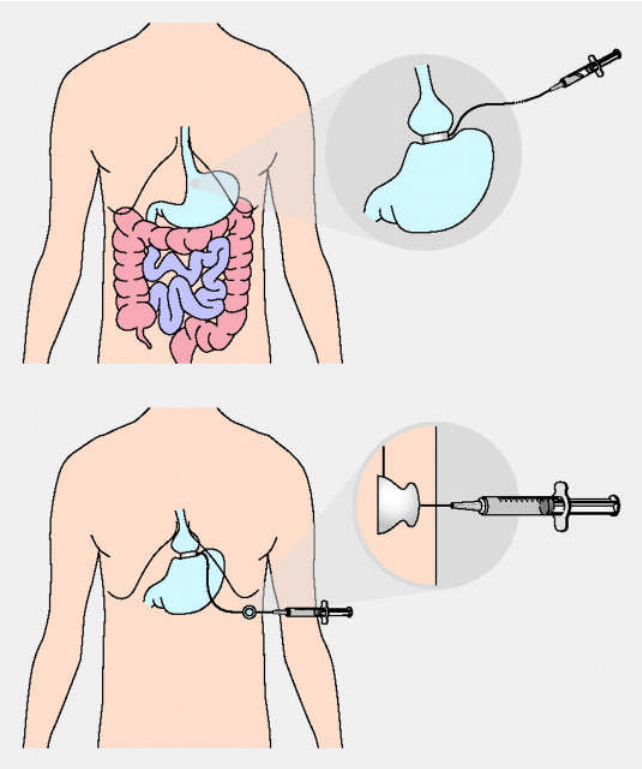

**Figure 1.4 Laparoscopic Gastric Banding** (Lett and Heath 2009)

This procedure involves placing an inflatable cuff around the upper part of the stomach. The band is connected to a port, which is placed under the skin and allows saline to be either injected or withdrawn which results in the band being inflated or deflated respectively (figure 1.4).

By restricting the volume of the stomach, the aim is to provide a feeling of satiety to encourage the consumption of smaller meals.

### Sleeve Gastrectomy

This procedure involves resecting the entire fundus and most of the body of the stomach, leaving behind a narrow tube of stomach tissue (figure 1.5).

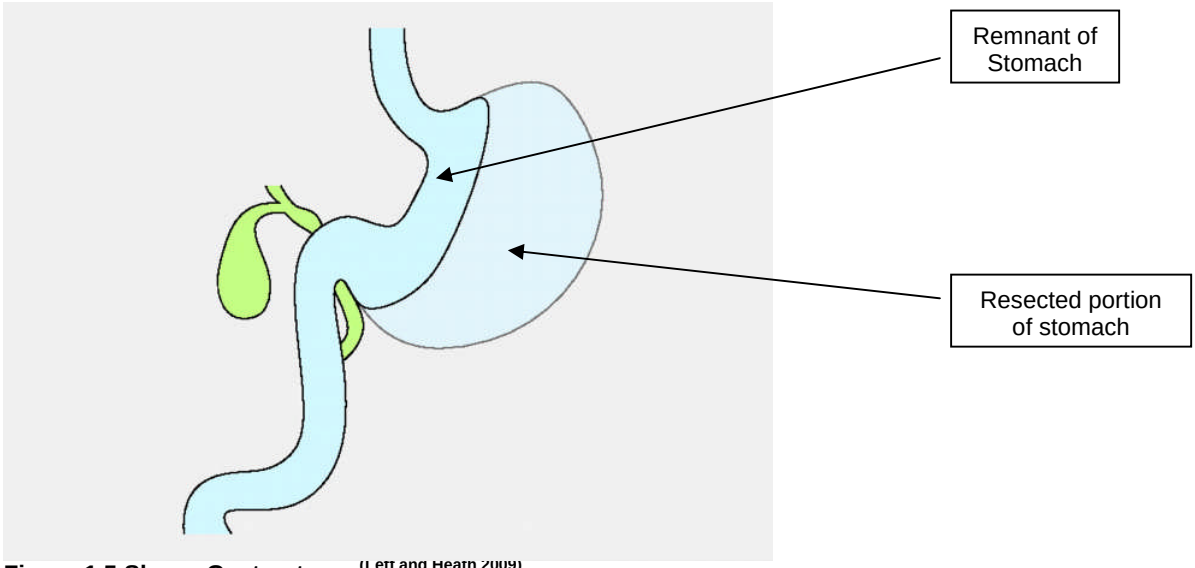


**Figure 1.5 Sleeve Gastrectomy** (Leff and Heath 2009)

### Roux-en-Y Gastric Bypass

This procedure creates a small pouch from the upper part of the stomach, which is then attached to the jejunem. Food therefore bypasses the majority of the stomach and upper small intestine (figure 1.6).

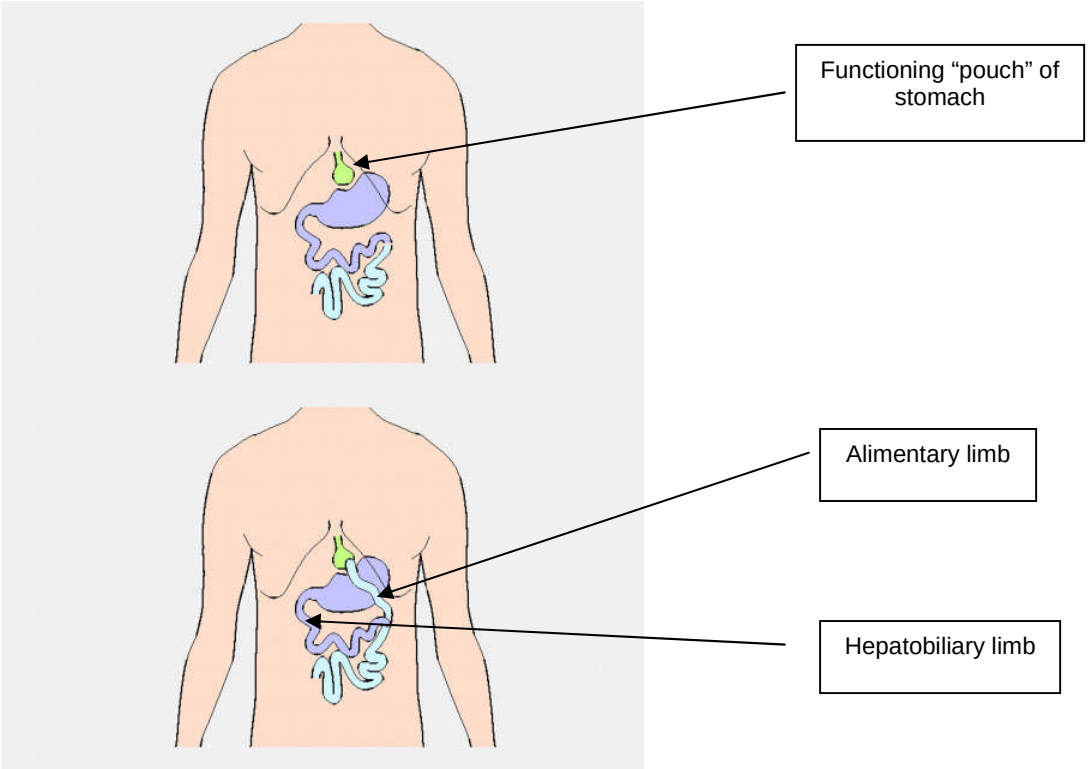


**Figure 1.6 Roux-en-Y gastrectomy** (Leff and Heath 2009)

**Biliopancreatetic Diversion**

The aim of these procedures is to divert food away from all but a small section of the small bowel, thereby creating only a small area from which nutrients can be absorbed.

The diagram on the left shows the gastrointestinal tract after a sleeve gastrectomy. This is followed by diversion surgery whereby the small intestine is divided at the pylorus (A) and distal ileum (B/C). The distal ileum (usually around 250cm in length) is then connected to the pylorus to act as the alimentary limb. The biliopancreatic limb connects the biliary tree to the distal ileum (see figure 1.7). This creates a shortened digestive tract, producing malabsorption.

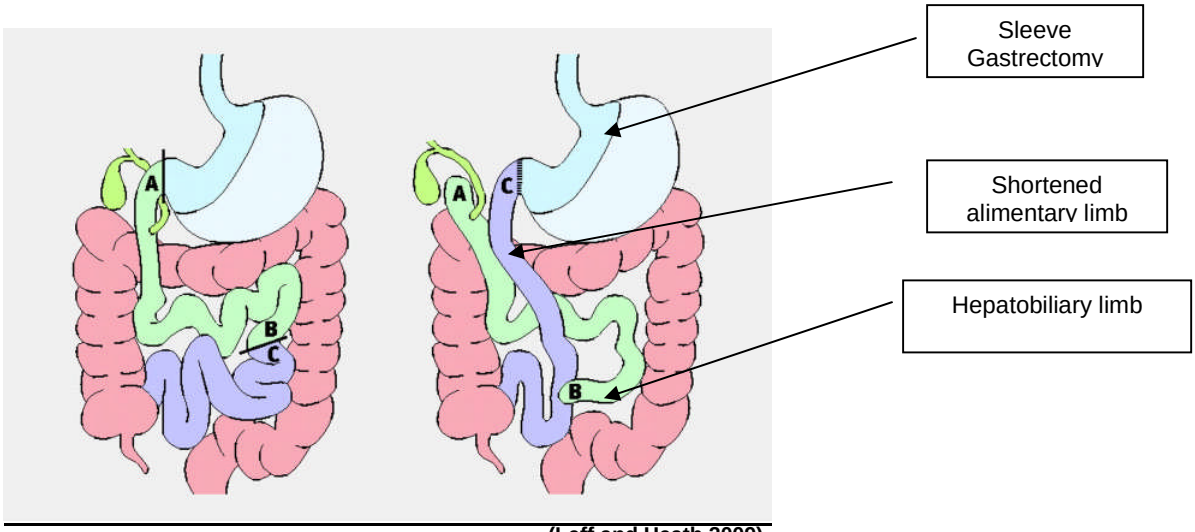


**Figure 1.7 Biliopancreatic Diversion**(Leff and Heath 2009)

Studies that have compared bariatric surgery to conventional therapy for losing weight have consistently found surgical intervention to be more superior at both achieving and maintaining weight loss. In the Swedish Obesity Study, bariatric surgery was compared to conventional, non-surgical therapy (Sjostrom, Narbro et al. 2007). Over the course of the 15 years of the study,

whilst the conventional group had weight that changed ± 2% from baseline, the surgical group achieved significantly more. During the first 2 years after surgery, weight loss of 32% (bypass surgery) and 20% (banding) was achieved. Although some weight was then regained, this did plateau after 8 to 10 years to 25% and 14% respectively.

Although Laparascopic Sleeve Gastrectomy (LSG) is a relatively newer procedure, early results suggest that weight loss similar to Roux-en-Y Gastric Bypass (RYGB) can be achieved. Lakdawala et al reported that Excess Weight Loss (EWL), the percentage of excess weight above ideal body weight which is lost, was in the region of 76% for LSG and 62% for RYGB 12 months after surgery (Lakdawala, Bhasker et al. 2009). Others have demonstrated slightly less dramatic, but still significant results with EWL of 49-51% (Mognol, Chosidow et al. 2006; Fuks, Verhaeghe et al. 2009).

The greatest weight loss is achieved using biliopancreatic diversion surgery, EWL in the region of 70%, 5 years after surgery, have been reported (Perez, Baltasar et al. 2005).

The long term benefits of weight loss using surgery versus conventional therapy are that there is a decrease in the incidence of hypertension (3% vs 10% respectively) (Sjostrom, Lindroos et al. 2004) and remission rates for type 2 diabetes of 78% when EWL was greater than 55% (Buchwald, Estok et al. 2009). Even before weight loss however, with either restrictive or malabsorptive surgery, improvements in glucose metabolism are seen almost immediately after surgery (Wickremesekera, Miller et al. 2005; Briatore, Salani et al. 2008). There is growing evidence that the incretin hormones, especially GLP-1, have a role in this early phenomenon as levels rise soon after bypass surgery (Bose, Olivan et al. 2009; Laferrere 2009).

It must be remembered that bariatric surgery is not without its problems. Whilst obese patients pose a significant anaesthetic risk, specific to the surgical procedures are erosion of the band into the stomach wall, or dilatation of the gastric pouch. With bypass surgery, issues relate to malabsorption of minerals, fat-soluble vitamins and anaemia as a result of vitamin B12 deficiency due to absence of intrinsic factor. In addition, some have reported that those patients with a pre-operative tendency towards binge eating seemed to do less well following surgery than those who did not (Green, Dymek-Valentine et al. 2004) although others have suggested this was not necessarily the case (Bocchieri-Ricciardi, Chen et al. 2006). For this reason, in the UK, it is recommended that a multidisciplinary approach is adopted to ensure patients are both physical and mentally prepared for the rigors that with associated bariatric surgery (NICE_CG43 2006).

## 1.6 Dietary aspects of weight loss

Whether or not the composition of the diet influences weight loss and weight maintenance continues to be a subject of ongoing debate.

Traditionally, when attempts at weight loss have been made, national guidelines have tended to recommend diets which are low in saturated fat, high in complex carbohydrates and energy deficient (National Heart Lung and Blood Institute 1998).

Diets, which are high in saturated fatty acids have been associated with impairment of insulin secretion and insulin sensitivity, which ultimately leads to the development of type 2 diabetes.

Lovejoy et al found that both total and saturated fat intake were strongly associated with insulin resistance, in subjects who had glucose tolerance which ranged from normal to diabetic (Lovejoy, Champagne et al. 2001). Similarly, when insulin sensitivity was assessed in the Insulin Resistance Atherosclerosis Study (Mayer-Davis, Monaco et al. 1997), higher fat intake was associated with lower insulin sensitivity, although this was seen in obese subjects rather than non-obese. High dietary fat translated into an increased risk of developing type 2 diabetes in Japanese-American men (Tsunehara, Leonetti et al. 1990), women in the Women's Health Study (Colditz, Manson et al. 1992) and in the Iowa women's Health Study (Meyer, Kushi et al. 2001). By reducing the intake of dietary fat, Tuomilehto et al demonstrated that the incidence of type 2 diabetes could be reduced (Tuomilehto, Lindstrom et al. 2001).

Based on the association between dietary fat and adverse health outcomes, dietary intervention studies have investigated the effects of reducing fat intake.

Hays et al discovered that in comparison to a control group of individuals, consuming a diet consisting of 45% fat, those in the intervention group, who consumed an ad libitum diet comprising of only 18% fat, demonstrated a greater loss in weight and body fat (Hays, Starling et al. 2004). Although this was a relatively short intervention period (14 weeks), similar results have been reported by longer studies (Mueller-Cunningham, Quintana et al. 2003).

Results from studies comparing diets low in fat to those high in Monounsaturated Fatty Acids (MUFA) have been inconclusive. One study suggested that a low fat diet achieved greater weight loss than one high in MUFA (Gerhard, Ahmann et al. 2004) although another failed to demonstrate any difference, both achieving a similar degree in weight and fat mass loss with no deleterious effect upon glycaemic control or lipid profile (Clifton, Noakes et al. 2004). The conflicting results may in part be related to a difference in study design; the former being a very small (n=11) crossover study in diabetic women, whereas the latter included 62 non-diabetic women.

One concern that has previously been raised was that diets, which were relatively high in carbohydrate and low in fat, might prove to have a detrimental effect upon glycaemic control (increased glucose and insulin levels) and lipid profile (increased triglycerides) in both diabetic and non-diabetic subjects (Coulston, Hollenbeck et al. 1987; Garg, Bantle et al. 1994; Abbasi, McLaughlin et al. 2000). Gerhard et al have provided contrary evidence more recently (Gerhard, Ahmann et al. 2004).

Attempts at adjusting the macronutrient content of diets have not only focussed upon fat. Low carbohydrate diets have become increasingly popular and formed the basis recent commercial diet programmes, for example: The Atkins' Diet (Atkins 1999) and The South Beach Diet (Agatston 2003). Clinical trials have confirmed the beneficial effects of low carbohydrate diets. In a 6 month study comparing a ketogenic, low carbohydrate diet with a conventional low fat diet, Yancy et al reported greater weight loss and a superior improvement in the atherogenic lipid profile (lower triglyceride, higher HDL-cholesterol) in the group following the low carbohydrate diet (Yancy, Olsen et al. 2004). In a longer study by Stern et al, a low carbohydrate diet (<30g/day) achieved similar weight loss to a conventional low fat diet over 12 months (Stern, Iqbal et al. 2004). There was also a favourable outcome, albeit marginal, with respect to dyslipidaemia and glycaemic control. The long-term beneficial effects of ketogenic, low carbohydrate diets, with regards to body weight and lipid profile has also been demonstrated in obese subjects with (Dashti, Mathew et al. 2007) or without (Dashti, Al-Zaid et al. 2006) diabetes. Regardless of whether the low carbohydrate diet was high in fat or protein, Luscombe-Marsh et al demonstrated similar levels of weight and body fat loss and associated improvements in insulin resistance (Luscombe-Marsh, Noakes et al. 2005). Low carbohydrate diets have also demonstrated greater loss of fat mass and in particular trunk fat mass, than low fat diets (Volek, Sharman et al. 2004).McAuley et al compared low carbohydrate diets, which differed in fat and protein content, to high carbohydrate diets. After 6 months, low carbohydrate diets that varied in fat and protein content achieved similar and more superior reductions in body weight, body fat and waist circumference than a high carbohydrate diet. What was also noted was that by 12 months,

there was a rapid regression towards baseline in the high fat group, whereas the high protein group were able to sustain the reductions achieved in the first 6 months (McAuley, Smith et al. 2006). As well as showing improvements in physiological parameters, low carbohydrate diets have been shown to improve psychological aspects in obese subjects, such as reduction in the feeling of hunger and an improvement in elements relating to negative affect and cognitive functioning (McClernon, Yancy et al. 2007). Concerns have been raised about the safety of low carbohydrate diets, particularly regarding increased cardiovascular risk associated with high fat diets and deleterious effects upon lipid profile. Results from the Nurses Health Study (Halton, Willett et al. 2006), which examined validated food frequency questionnaires over a period of 20 years, did not support this concern and in fact, when vegetable sources for fat and protein were chosen, a reduction in the risk of coronary heart disease was seen.

Other forms of dietary intervention have also proven to be successful, not only in reducing weight, but also improving physiological and metabolic parameters. Das et al investigated the effect of energy-restricted diets which had either a high or low glycaemic index (Das, Gilhooly et al. 2007). Both dietary regimens demonstrated significant, but comparable, reductions in body weight and fat, lipids and insulin levels over a 12 month period. This tended to indicate that it was the energy restriction rather than the glycaemic load which was relevant, which supported results from Raatz et al (Raatz, Torkelson et al. 2005).

The use of meal replacements has also been shown to be an effective and safe weight management strategy, producing significant and sustained weight loss as well as improvement in weight-related risk factors (reviewed in (Heymsfield, van Mierlo et al. 2003). Meal replacements have also been shown to be as effective at losing weight as structured weight loss diets (Noakes, Foster et al. 2004).

## Aims of the thesis

This thesis will aim to investigate the relationship of phenotypic characteristics with:

1. Resting Energy Expenditure and changes that occur with weight loss using a dietary intervention that differs in fat content.
2. Fat oxidation in obese subjects and a reference group of lean individuals before and after a high fat liquid test meal
3. Different dietary intervention programmes, in relation to body composition and insulin resistance.

# Chapter 2

# General Methods

## 2.1 Body Composition

Body composition can be assessed by several different procedures with varying degrees of accuracy. The “Gold Standard” or “Criterion” method is that which provides the closest and most accurate representation of actual body composition. It is against this method, which all others are then compared. For body composition, it is generally accepted to be the 4 Compartment (4C) Model. This model incorporates measurements of Total Body Water (TBW), Body Density (BD) and Total Body Bone Mineral (TBBM) to estimate the fourth component: Percentage Body Fat and thus Fat Mass (FM) and Fat Free Mass (FFM) can be estimated if one knows the accurate body weight.

**Total Body Water (TBW)**

This can be calculated using labelled water ($^2H_2O$). An oral dose of deuterium labelled $H_2O$ ($^2H_2O$) is given. Samples of saliva are collected before and for several hours after the dosing. As $^2H_2O$ reaches equilibrium within body fluids, measurements are made of the concentration of $^2H_2O$ appearing in the saliva. TBW can then be calculated by the “net” $^2H_2O$ concentration (Lukaski and Johnson 1985).

**Body Density (DB)**

Body Density can reliably be measured using Underwater Weighing (Katch, Michael et al. 1967; Heymsfield and Wang 1993). This method is based upon the “Archimedes principle”. Body fat has a lower density than water and thus can increase the body’s buoyancy. In contrast, FFM has a greater density and

will therefore result in a tendency for the body to sink in water. It must also be remembered that the lung's residual volume will need to be determined, as this too will add to the buoyancy in water. Ideally lung volume should be measured, for example using the nitrogen washout technique (Wilmore 1969) but can be estimated my multiplying Vital Capacity by 0.24 (males) and 0.28 (females). Subjects are weighed on land and then are submerged completely within the underwater chamber and weight under water, having exhaled as much as possible. This is repeated up to ten times with the average of the final three measurements being taken as the underwater weight. Body Density is then calculated using the following equation:

$$\text{Body Density} = \frac{\text{Dry Weight}}{[\text{Dry Weight} - \text{Wet Weight}/\ \text{Density of Water}] - \text{RV} - 0.1}$$

(Weight in kg, RV = residual volume (L) and 0.1 represents volume (L) of gas within the gastrointestinal tract).

Using the following equation (Siri 1961) : Percentage Fat = [(495 / Body Density) – 450 x 100].

**Total Body Bone Mineral (M)**

Dual Energy X-ray Absorptiometry (DEXA) is able to measure total body mineral content. Two x-ray beams of differing (low) energy are aimed at the bones. The absorption of energy from soft tissue is extracted and bone mineral density can then be calculated by the absorption of each energy beam by bone.

The 4C method uses the equation:

**Percentage Body Fat = (2.747/DB – 0.714W + 1.146M-2.0503) x 100**, where DB is in kg/L, W is TBW (kg) as a fraction of weight (kg) and M is bone mineral content (kg) as a fraction of weight (kg) (Sopher, Thornton et al. 2004).

While this 4C method provides the ideal way of assessing body composition it is not practical for all research settings. In such situations, indirect methods, which estimate body composition, are used, and two such methods were used within the experimental chapters presented in this thesis:

- Bioelectrical Impedance Analysis (BIA)
- Dual Energy X-Ray Absorptiometry

**Bioelectrical Impedance Analysis**

One of the basic principles upon which BIA is based is that the resistance, R, of a length of homogenous conductive material of uniform cross sectional area is proportional to its length (L) and inversely proportional to its cross sectional area (A). When used in the context of measuring composition of the human body, BIA makes a number of assumptions:

1. The human body is viewed as a cylinder (Suzuki, Rounds et al. 1996).
2. Fluid contained within the body has a relatively constant resistance (Suzuki, Rounds et al. 1996).
3. FFM contains a relatively constant proportion of water compared to solids (Lukaski and Johnson 1985).

Whilst the human body is not a cylinder, a relationship can be established between lean body mass and height$^2$/R.

Electrodes applied to the limbs allow a means of both providing and detecting electrical current. When measured at a frequency of 50kHz, the current passes through both the intracellular and extracellular fluid but at varying proportions from tissue to tissue (Kyle, Bosaeus et al. 2004).
FFM is everything that is not fat (visceral protein, bone mineral, intracellular and extracellular water). By using equations derived from multiple regression analysis, parameters such as weight, height age and gender can be used to provide an estimate for FFM, and FM.

The benefits of using BIA are that it is non-invasive, relatively cheap, portable and requires minimal training in its use. This makes it a very attractive tool to use in population based, epidemiological and clinical research.
It must, however, be remembered that there are certain limitations. The estimations of body composition are prone to variations in certain circumstances.
In experiments involving patients with end-stage renal disease on either haemodialysis or peritoneal dialysis, although BIA was felt to be accurate at predicting lean body weight when subjects were normally hydrated, in situations when they were either dehydrated or suffering from fluid overload, BIA performed poorly at accurately measuring lean body mass (de Fijter, de Fijter et al. 1993; Rallison, Kushner et al. 1993). When used in malnourished patients with inflammatory bowel disease, BIA was found to overestimate FFM by almost 6% in comparison to the reference method of doubly labelled water (Royall, Greenberg et al. 1994). Even in healthy individuals, performance of BIA can be affected by ambient temperature, with both cooler and hot

environments causing variations in measurements of resistance and FFM (Caton, Mole et al. 1988; Gudivaka, Schoeller et al. 1996). Changes in skin blood flow are thought to be the likely underlying aetiology of this phenomenon (Buono, Burke et al. 2004).

For comparative studies and intraindividual measurements, the positioning of the limbs must be the same, for example, supine, as variations in BIA recordings have been reported when moving from a standing to a lying position within the same individual (Roos, Westendorp et al. 1992; Berg, Tedner et al. 1993).

It is vital that the "electrical circuit" for measuring BIA does not become compromised by the crossing of limbs or the touching of the extremities together or with the trunk, otherwise spurious results with errors of 18% and 43% may be seen for crossed limbs and contact with central body parts respectively (Kushner, Gudivaka et al. 1996).

These variations mean that it is vital that standardised conditions are observed during the estimation process.

During the experimental procedures in chapters 3 and 4, fat mass and fat-free mass was assessed by multifrequency bio-impedance (Bodystat®; QuadScan 4000, Isle of Man, British Isles) (www.bodystat.com). Before measurements were performed, subjects rested in the supine position for 5 minutes, the room was kept at a constant temperature of $25^0$C and individual data on gender, age, weight, and height were entered into the device. It was checked that no parts of the body were touching each other. Two electrodes were placed on the dorsum of the right hand and two electrodes were placed on the dorsum of the right foot. Following manufacturer's instructions, the source leads were

connected to electrodes placed at sites corresponding to the head of the second and third metacarpal bone, and the head of the second metatarsal bone, respectively. The measuring leads were connected to electrodes placed between the styloid processes of ulna and radius, and between the medial and lateral maleolus. Although the QuadScan 4000 device records body impedance at four frequencies (5khz, 50khz, 100khz, 200khz), only the 50 kHz impedance is used for calculations using the manufacturer's software.

Whilst it must be remembered that this technique is an estimation based upon calculations, clinical studies have compared the performance of BIA with other more direct measures of body composition.

Utter et al compared BIA with underwater weighing (Utter, Nieman et al. 1999) and found that BIA was accurate at assessing FFM with a non-significant standard error of 3.7kg. This was performed in a diverse population of individuals in whom BMI varied between 21 and 34. Close agreement has also be shown when compared to DEXA (Pateyjohns, Brinkworth et al. 2006; Thomson, Brinkworth et al. 2007) in both the basal state and following weight loss. This has also been reported with comparison to whole body MRI (Bosy-Westphal, Later et al. 2008).

It has been questioned how well BIA performs in the morbidly obese (Coppini, Waitzberg et al. 2005). Deurenberg suggested that once BMI is greater than 35, there may be an underestimation of FM related to alterations in extracellular water seen in these individuals (Deurenberg 1996). This was in contrast to work by another group over a wide range of BMI, including morbidly obese, who found that in the group as a whole, good agreement with

DEXA was seen although it was commented that for any given individual, there were wide limits of agreement (Pateyjohns, Brinkworth et al. 2006).
It would therefore seem that within controlled clinical conditions, BIA can be a valid method for estimating body composition but because of factors both internal to and external of the subject, it may have limited use in everyday clinical practice (Dehghan and Merchant 2008).

**Dual-Energy X-Ray Absorptiometry**

The use of DEXA for determining body composition has become increasingly popular. It is based on the 3-compartment model of body composition (Withers, LaForgia et al. 1998). Two x-ray energy sources in combination with a detector allow the measurement of differential attenuation of high and low energy protons to measure the relative amounts of bone mineral-free lean tissue, fat mass and bone mineral within each pixel of the scan area (LaForgia, Dollman et al. 2009).
Advantages of DEXA are that it is a non-invasive, quick, low radiation exposure procedure. It is however, quite expensive. There are, however, still some limitations to the technique that for the time being prevents it from becoming a criterion method for body composition measurement.
In lean individuals for example, DEXA was shown to underestimate percentage body fat when compared to the reference method of 4C modelling (van der Ploeg, Withers et al. 2003). This has also been seen in a paediatric population (Sopher, Thornton et al. 2004). This same study also found an overestimation of body fat in children who were overweight (Sopher, Thornton et al. 2004). This overestimation in obese individuals has been confirmed more recently in adults (LaForgia, Dollman et al. 2009). It is felt that the bias

seen in DEXA is associated with tissue thickness which then affects the attenuation of the signal by low energy x-rays. When tissue thickness is low (lean individuals), there is a higher attenuation of this signal and a lower attenuation when tissue thickness is larger (Goodsitt 1992; van der Ploeg, Withers et al. 2003).

Modern DEXA hardware and software must strive to overcome these obstacles to improve the accuracy of a very useful technique.

When having the scan done, subjects must lay still in the supine position for approximately twelve minutes for the computer software to produce an image of the tissues (Figure 2.1). The results may be viewed as whole body estimates of body fat, muscle, and bone mineral as well as regional body estimates.

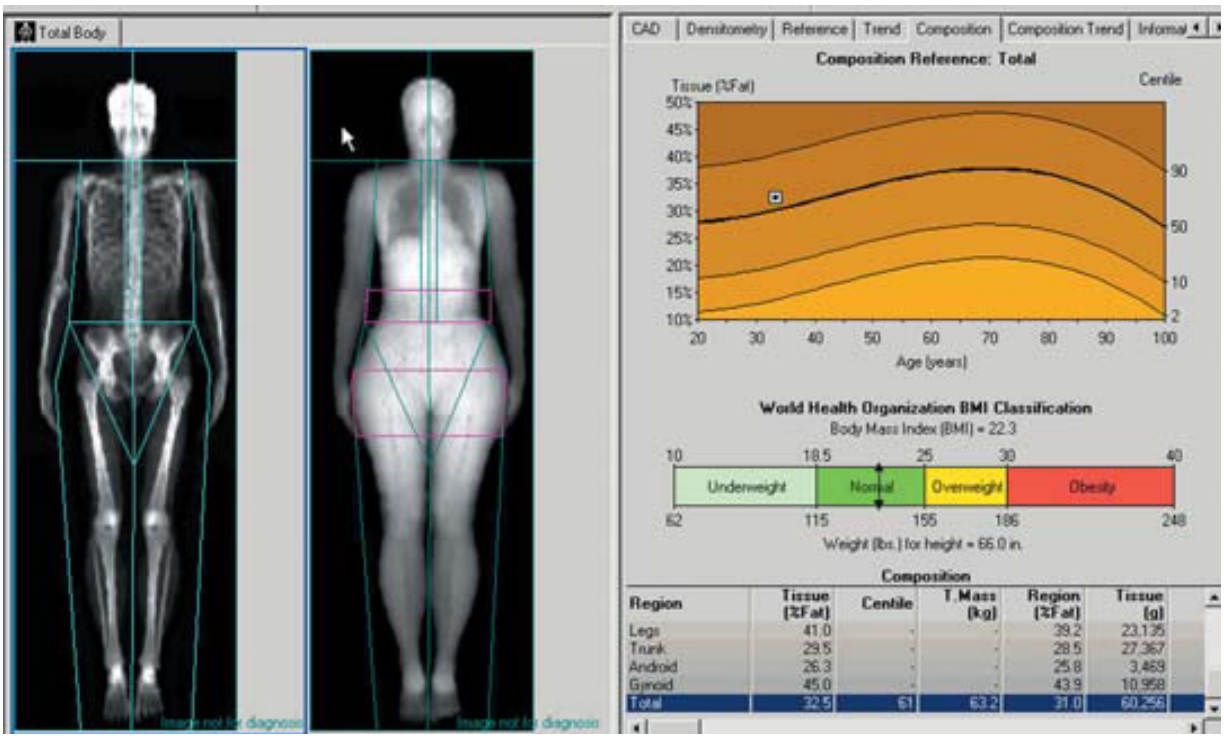


**Figure 2.1: Sample DEXA report. A digital image displaying bone and soft tissue and numerical measurements of total and regional fat and lean tissue**

Within chapter 5, among the 5 different centres, 2 different types of DEXA machine were used: General Electric Lunar Prodigy™ densitometer (GE Healthcare Inc., Waukesha, WI) or Hologic™ densitometer (Hologic Inc, Bedford MA).

## 2.2 Measurement of Insulin Resistance

The reference, criterion, method for assessing insulin secretion and resistance has been describe by DeFronzo et al with the hyperglycaemic clamp providing a measure of insulin secretion and the euglycaemic clamp quantifying insulin resistance (DeFronzo, Tobin et al. 1979).

One of the main disadvantages of this method however, is that it is time consuming, requiring frequent blood sampling over a 3 hour period. This therefore makes it an unattractive method for use in large, population-based research.

Matthews et al developed a computer model based upon varying degrees of beta cell deficiency and insulin resistance (Matthews, Hosker et al. 1985). Using fasting values for glucose and insulin, the model allows a quantitative assessment / estimate of how both beta cell dysfunction and insulin resistance contribute to fasting hyperglycaemia. The model has demonstrated good correlation with the euglycaemic clamp ($r = 0.88$, $p<0.0001$) and the hyperglycaemic clamp ($r = 0.61$, $p=0.01$). There were issues relating to the actual precision of the model with a coefficient of variation of 31% for insulin resistance and 32% for beta cell function. This early model can be closely represented by the following equation:

$$\text{HOMA-IR} = \frac{\text{Glucose (mmol/L)} \times \text{Insulin(mU/L)}}{22.5}$$

This model has subsequently been modified by Levy et at (HOMA2) (Levy, Matthews et al. 1998) and is available for use free of charge for academic use (http://www.dtu.ox.ac.uk/homacalculator).

The HOMA model has performed well across a spectrum of glucose tolerances from normal to type 2 diabetes (Hermans, Levy et al. 1999) as well as in other insulin resistant states (Lansang, Williams et al. 2001). There have however, been some reports when HOMA has not performed as well as expected in certain groups of individuals, for example in lean type 2 diabetics (Kang, Yun et al. 2005) and older individuals with type 2 diabetes (Ferrara and Goldberg 2001).

One suggestion as to why HOMA may not perform as well on occasions may be related to the fact that it is based upon fasting insulin and glucose measurements, and in the basal state the majority of glucose uptake occurs in an insulin-independent manner (Ferrara and Goldberg 2001). The model does not incorporate any measures of insulin sensitive tissues, such as muscle, in the postprandial state but rather relies upon extrapolation of basal results. This is not the case for the euglycaemic clamp, which measures across physiological, levels. This is not supported by other research by Bonara et al (Bonora, Targher et al. 2000).

Overall, it would appear that for large population-based studies, using HOMA modelling provides an easy, and relatively cheap means by which to assess insulin resistance. One caveat to this though is the difficulties in standardising measurements of serum insulin across laboratories, which limits its use in everyday clinical practice.

## 2.3 Energy expenditure and substrate utilisation

Resting metabolic rate and postprandial energy expenditure were measured using a ventilated hood system.

Calorimetry is based on the principle that as substrates are oxidised to produce energy, oxygen is consumed and carbon dioxide is produced in proportion to the heat generated. By measuring oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$), energy expenditure can be calculated. The most widely used equation for measuring EE is that developed by Weir(Weir 1949):

**EE (kJ) = 16.489*$VO_2$ (l) + 4.628*$VCO_2$ (l) – 9.079*N (g).**

For the measurements in this thesis, it was assumed that protein accounted for 15% of total energy expenditure, the equation then became:

**EE (kJ) = 16.318*$VO_2$ (l) + 4.602*$VCO^2$ (l)**

Subjects arrived at the research centre having fasted for a minimum of 12 hours and a preceding 3-day dietary run in period, in which they had to keep to their habitual diet, and avoid excessive physical activity and alcohol consumption. The room was kept thermoneutral at 25 $^0$C. Subjects had EE measured for 30 minutes in the fasting state only (chapter 3) or for 30 minutes before and 3 hours after a high fat test meal (chapter 4). Each 30-minute period of measurement comprised of 5 minutes to calibrate the calorimeter with $CO_2$ and $O_2$ and 20 minutes to collect subject data.

To ensure valid results were being obtained from the calorimeter, a series of alcohol burns were performed, with measurement of oxygen consumed and

estimation of Respiratory Quotient (RQ). For alcohol, a RQ value of 0.667 would be expected for 100% recovery of oxidised gases. Also, to check for intra-individual variability, repeated calorimeter measurements were performed on a separate group of volunteers on 2 separate occasions. The results for these data are shown in table 2.1 and 2.2. Table 2.1 shows the intra-individual variability was small with a coefficient of variation (CV) of 3.66% for energy expenditure and of 2.8% for measured RQ. These data would indicate that repeated measures in the same subject are valid. The accuracy of the system is reflected in Table 2.2 with mean RQ for alcohol of 0.668 and CV of 1.27%. The recovery of the oxygen consumption for alcohol combustion was 99.5% with a CV of 2.02%. These data indicate that the system was operating correctly in view of RQ for alcohol being very near the expected value and the recovery of oxygen consumption being almost 100%, both with low CVs.

| | ENERGY EXPENDITURE (KJ/DAY) | | | RQ | | |
|---|---|---|---|---|---|---|
| Subject | Mean | SD | CV (%) | Mean | SD | CV (%) |
| 1 | 1581 | 66.5 | 4.2 | 0.873 | 0.004 | 0.5 |
| 2 | 1653 | 36.1 | 2.2 | 0.892 | 0.037 | 4.2 |
| 3 | 1652 | 9.9 | 0.6 | 0.884 | 0.045 | 5.0 |
| 4 | 1944 | 94.8 | 4.9 | 0.831 | 0.007 | 0.9 |
| 5 | 1064 | 21.2 | 2.0 | 0.921 | 0.028 | 3.1 |
| 6 | 1347 | 130.8 | 9.7 | 0.760 | 0.030 | 4.0 |
| 7 | 1318 | 13.4 | 1.0 | 0.861 | 0.014 | 1.6 |
| 8 | 1161 | 80.6 | 6.9 | 0.905 | 0.006 | 0.7 |
| 9 | 2207 | 68.6 | 3.1 | 0.860 | 0.065 | 7.5 |
| 10 | 1450 | 28.3 | 2.0 | 0.769 | 0.004 | 0.5 |
| | Mean | | **3.66** | Mean | | **2.8** |
| | 95% confidence intervals | | **-2.04 to 9.36** | 95% confidence intervals | | **-1.93 to 7.54** |

**Table 2.1: Intra-individual reproducibility data for indirect calorimetry**

| MEASUREMENT | BURN DURATION (MINS) | ALCOHOL COMBUSTED (G) | RQ | RECOVERY (%) |
|---|---|---|---|---|
| 1 | 34 | 6.035 | 0.668 | 98.6 |
| 2 | 31 | 4.464 | 0.673 | 104.1 |
| 3 | 30 | 5.615 | 0.659 | 99.8 |
| 4 | 25 | 5.922 | 0.684 | 99.3 |
| 5 | 25 | 5.346 | 0.679 | 96.6 |
| 6 | 25 | 5.346 | 0.665 | 97.4 |
| 7 | 30 | 4.261 | 0.671 | 100.5 |
| 8 | 30 | 9.010 | 0.661 | 100.2 |
| 9 | 30 | 6.657 | 0.661 | 99.6 |
| 10 | 30 | 6.467 | 0.661 | 98.9 |
| | | Mean | **0.668** | **99.5** |
| | | SD | **0.008** | **2.01** |
| | | CV (%) | **1.27** | **2.02** |

**Table 2.2: Alcohol burn recovery data**

## 3.4 Statistics

All data are expressed as means with either standard deviation (SD) or 95% confidence intervals.

When comparing means between two observations or groups (e.g. low fat vs. moderate fat), independent Student t-test was used. For comparing differences in mean values at baseline and following intervention, paired Student t-test was used. When comparison of means across more than two groups was required, Analysis of Variance (ANOVA) was applied. If variables did not comply to conditions of normality, even after transformation, where applicable, Mann-Whitney –U test was used for non-parametric data. During regression analysis, dependent variables were checked for normality and where necessary, natural log or $Log_{10}$ transformation was performed. Independent variables were checked for collinearity and if they were closely associated ($r>0.80$), these variables were not entered simultaneously into the regression models.

A p-value of $<0.05$ was accepted as being statistically significant

During regression analysis, dependent variables were checked for normality and where necessary, natural log or $Log_{10}$ transformation was performed. Independent variables were checked for collinearity and if they were too closely associated ($r>0.80$), these variables were not entered simultaneously into the regression models.

A p-value of $<0.05$ was accepted as being statistically significant.

## 3.5 Background to study data

The data present in this thesis are derived from two separate multi-centre trials:

1. Nutrient –Gene Interactions in Human Obesity – Implications for Dietary Guideline (NUGENOB).
2. BBC Diet Trials.

I started work as a Clinical Research Fellow in April 2001. I was employed primarily for the NUGENOB project. This was a multi- centre study involving 8 centres across Europe: Copenhagen, Maastricht, Stockholm, Nottingham, Prague, Paris, Pamplona and Toulouse.

The main aims of the study were to:

- Identify and characterise novel nutrient-sensitive candidate genes for obesity.
- Assess differential gene expression in adipose tissue in relation to the acute intake of a high fat meal as well as long-term intake of a hypocaloric diet with either a high or a low fat content.
- Assess effects of functional variants of the candidate genes on physiological responses in obese subjects to a high-fat test meal: appetite, energy expenditure, partitioning, and circulating obesity related hormones and metabolites.
- Identify on this basis predictors of changes in body weight and composition during dietary intervention, including changes in fat intake.

Each centre was also invited to perform additional sub-projects based upon data collected from the study. This could be done independently or in collaboration with other member centres.

The study design, protocols and ethical approval had already been agreed prior to my appointment. My specific role was to recruit the Nottingham cohort of subjects (100 obese and 15 lean). I was then to perform the clinical investigations, which involved anthropometric measurements, estimation of body composition, obtaining samples of subcutaneous fat from obese subjects, measuring energy expenditure and obtaining blood samples before and after a high-fat test meal. This was performed with the assistance of Sarah Jones and Liz Simpson who helped with measurements of energy expenditure and preparation of blood samples for storage and subsequent shipment to a central institution in Maastrict for analysis.

Once obese subjects were randomised to a low fat or moderate fat diet, the study dietitians, Sue Bridgwater and Moira Taylor, saw them at weekly intervals for 10 weeks. During this time, if necessary, I provided medical input for the subjects to ensure that they came to no harm as a consequence of the study.

At the end of the intervention periods, subjects had repeat blood tests and measurement of resting energy expenditure.

A number of publications have thus far been derived from the multi-centre work, four of which I am a co-author. My contribution to the development of these papers was to review draft manuscripts and offer opinions and suggestions were necessary.

- Impaired Fat-Induced Thermogenesis in Obese Subjects: The NUGENOB Study (Blaak, Hul et al. 2007).
- Fat Oxidation before and after a high fat load in the obese insulin resistant state (Blaak, Hul et al. 2006).
- Total adiponectin and adiponectin multimeric complexes in relation to weight loss-induced improvements in insulin sensitivity in obese women: the NUGENOB study (Polak, Kovacova et al. 2008).
- Several obesity and nutrient-related gene polymorphisms but not FTO and UCP variants modulate post-absorptive resting energy expenditure and fat-induced thermogenesis in obese individuals: the NUGENOB study (Goossens, Petersen et al. 2009).

In chapter 3, data has been analysed from several centres whereas data for chapter 4 is only from subjects recruited in Nottingham.

In addition to the work for NUGENOB, I was also had minor involvement in the recruitment of subjects for the BBC Diet Trials project. My involvement was restricted to the screening of potential subjects and ensuring that they were medically fit to enter into the study. Although not involved in the two publications derived from this study (Truby, Baic et al. 2006; Morgan, Griffin et al. 2008), I have subsequently analysed the data on body composition and insulin resistance. This data is presented in chapter 5.

Having completed my research in February 2003, I returned to full time clinical medicine to take up a post as Specialist Registrar in Diabetes and Endocrinology. I have successfully obtained a Certificate of Completion of Training in January 2008 and I am currently working full time as a Consultant Physician.

# CHAPTER 3

## The variation in energy expenditure associated with weight loss is explained more by weight and body composition and not by the type of dietary intervention adopted.

## 3.1 Introduction

The global epidemic of obesity shows no signs of relenting with prevalence figures in the USA approaching 33% in 2004 (Ogden, Carroll et al. 2006) and figures for Europe reaching 30% (International 2005)

The development of obesity requires a chronic mismatch between energy intake and energy expenditure (Rosenbaum, Leibel et al. 1997), for example an over consumption of 100kcal/day above energy requirements, can lead to a weight gain of 5Kg in a year.

The determinants of energy expenditure are physical activity, diet-induced thermogenesis and resting energy expenditure (REE) with the latter contributing the largest component of between 68% and 80% (Goran 2000). Studies have shown that numerous factors have an influence upon REE. By far the largest contribution to REE is from Fat-free mass (FFM) (Nielsen, Hensrud et al. 2000) (Karhunen, Franssila-Kallunki et al. 1997) (Johnstone, Murison et al. 2005). Fat mass (FM) also seems to have a small yet significant contribution although this only becomes apparent when FM is large, such as in obesity (Karhunen, Franssila-Kallunki et al. 1997). Not only does the absolute amount of fat have an influence upon REE but also the fat distribution. Waist-hip ratio or waist circumference does seem to contribute to REE although this phenomenon is only seen in Caucasian subjects (Karhunen, Franssila-Kallunki et al. 1997) (Okura, Koda et al. 2003) (Weinsier, Hunter et al. 2001). As we age there is a gradual decline in REE which in part can be explained by the decrease in FFM (Nelson, Weinsier et al. 1992), however, even when this is taken into account, there still seems to

be an independent effect of ageing upon REE (Hunter, Weinsier et al. 2001). It has also been shown that there is a positive association between fasting insulin levels and REE(Karhunen, Franssila-Kallunki et al. 1997; Fan, Anderson et al. 2006). Other hormones such as leptin (Hukshorn and Saris 2004) and growth hormone (Armellini, Zamboni et al. 2000) do not seem to make a significant contribution to REE. A reduction in weight is accompanied by a decrease in REE, but it is not clear whether this is entirely due to a decrease in FFM or whether there is a contribution from energy conserving mechanisms, which are activated in states of negative energy balance.

The purpose of the study was first to use phenotypic information obtained from obese subjects studied in the Nutrient-Gene Interactions in Human Obesity-Implications for Dietary Guidelines (NUGENOB) multicentre project, to predict baseline REE and then to determine what effect of a 10-week, low energy diet (either high in fat or carbohydrate) would have upon the ability of these parameters remaining valid predictors of REE after weight loss.
The hypothesis to be tested was that post-weight loss REE is influenced by the nutrient composition of the diet being followed.

## 3.2 Methods

### Subjects

Although the NUGENOB project was conducted in eight different centres in seven European countries, only six centres (Stockholm, Nottingham, Prague, Paris, Toulouse, Pamplona) performed measurement of REE after the dietary intervention period and therefore participated in this NUGENOB subproject. 439 obese subjects, with a body mass index ($kg/m^2$) of 30 or more, aged between 20 and 50, were recruited through the media, from obesity clinic waiting lists, ongoing population studies, by self-referral or referral from a general physician or other clinical units and local obesity organisations. Subjects were excluded if their weight had changed more than 3kg within the 3 months prior to the study, or if they had hypertension, diabetes or hyperlipidaemia treated with drugs, untreated thyroid disease, obesity which had had been treated either surgically or with drugs, pregnancy, were participating in other trials simultaneously or were suffering from drug or alcohol misuse. Recruitment of subjects was undertaken from May 2001 until September 2002.

The Ethical Committee at each of the participating centres approved the study protocol. Volunteers were informed about the nature of the study, and written consent was obtained prior to study participation.

**Procedures**

All subjects underwent a 1-day clinical investigation protocol before and after the dietary intervention program. Subjects arrived at the research centre at 8.00 a.m. after a 12 hours overnight fast and a preceding 3-day dietary run in period, in which they had to keep to their habitual diet and avoid excessive physical activity and alcohol consumption. After the subjects voided their bladder, they underwent anthropometric and body composition assessments, the details of which have been described previously (Petersen, Taylor et al. 2005). Following this they underwent measurement of Resting Energy Expenditure (REE) and fasting blood samples were taken to determine metabolites and hormones.

**Resting Energy Expendituire**

The experimental room was kept at 25 $^{o}$C. Energy expenditure was measured using an open circuit ventilated hood system for 30 minutes. The equipment used varied between centres, however, all equipment and procedures were standardised for the different centres using a standardised validation protocol involving both ethanol recovery and reproducibility measurements in human subjects (see general methods sections). For the all centres combined, the mean and SD in RQ were 0.668 ± 0.006. Likewise the within-subject coefficient of variation was 2.73 ± 1.10% and 2.89 ± 1.19% for RQ and EE respectively.

**Blood sampling**

At least 30 minutes before the start of the resting measurement, a Teflon catheter was inserted in an antecubital forearm vein for blood sampling. Blood was drawn in the fasting state for measurement of glucose, free fatty acids, insulin, cortisol, triglycerides, IGF-1 and leptin.

**Blood analyses**

All blood analyses were performed at one of the centres, or at a subcontracted commercial laboratory (for cortisol and IGF-1). Plasma glucose concentrations (ABX diagnostics, Montpellier, France), and triglycerides (Sigma, St Louis, USA; ABX diagnostics, Montpellier, France) were measured on a COBAS MIRA automated spectro-photometric analyser (Roche diagnostica, Basel, Switzerland). Free fatty acids (NEFA C kit; Wako Chemicals, Neuss Germany) were measured on a COBAS FARAH centrifugal spectro-photometer (Roche Diagnostica, Basel, Switzerland). Standard samples with known concentrations were included in each run for quality control. Plasma insulin and serum leptin concentrations were measured with a double antibody radio-immunoassay (Insulin RIA 100; Kabi-Pharmacia, Uppsala, Sweden; Human leptin RIA kit, Linco research, Inc, St.Charles, Missouri, USA). Cortisol and IGF-1 were both determined with ELISA assays (IGF-1: Diagnostics Systems Laboratories, Inc, Texas, USA, cortisol: ADVIA Centaur, Bayer Health Care LLC).

**Dietary intervention**

Prior to commencing the dietary intervention, subjects were asked to complete a weighed, 3-day food diary, on two weekdays and one weekend day. Having completed the diary, each centre then analysed these records, using databases routinely used in clinical practice, to calculate energy intake and macronutrient composition for each subject. This process was repeated during the final week of the 10-week intervention period.

The target macronutrient composition of the two diets was: **low-fat diet**: 20-25% of total energy from fat, 15% from protein and 60-65% from carbohydrate; **moderate-fat diet**: 40-45% of total energy from fat, 15% from protein and 40-45% from carbohydrate. Both diets were designed to provide 600 kcal/day (2500 kJ) less than the individually estimated energy requirement, based on their initial resting metabolic rate multiplied by 1.3. Subjects were given verbal and written instructions on how to achieve these targets. Instructions were also given on how to minimize differences between the two diets in other components such as sources and type of fat, amount and type of fibre, type of carbohydrate, fruit and vegetables, and meal frequency. Subjects were requested to abstain from alcohol consumption. Dietary advice reflected local customs and all food items were purchased by the subjects themselves. The dietary instructions were reinforced weekly.

**Calculations**

EE was calculated according to the equation of Weir (Weir 1949). The homeostasis model assessment of insulin resistance ($HOMA_{IR}$) was calculated from fasting insulin and glucose levels according to the equation of

Matthews et al(Matthews, Hosker et al. 1985). Fat oxidation was calculated according to the equations of Frayn (Frayn 1983)

**Statistical analysis**

Data are expressed as means ± SD, or mean and 95% confidence intervals. Statistical analysis was performed with SPSS 11.5 for Windows. Where variables were not normally distributed, natural log transformation was performed to satisfy conditions of normality.

Initially, REE, RQ and Fat Oxidation was analysed before and at the end of the dietary intervention period with comparisons made between the two diets using analysis of covariance. Adjustments for phenotype were made in a step-wise manner, when appropriate, if the variable was significantly associated with REE, RQ or Fat Oxidation. For analysis of change (Δ) of REE, RQ and Fat oxidation, values were also adjusted for the corresponding baseline values.

For the predictors of baseline REE before and after dietary intervention, EE was studied as the dependent variable in linear regression models. Firstly, research centre, age, sex and gender were included (Model 1). Secondly, weight, % body fat and waist-hip ratio were included (Model 2). Thirdly, energy intake and HOMA-IR were included (Model 3). Finally, free fatty acids, leptin, cortisol and IGF-1 were included (Model 4). The effect of the independent variables is expressed as the β coefficients.

In the analysis looking at the impact of the dietary intervention, research centre, age, gender, weight, % body fat, waist-hip ratio, energy intake, $HOMA_{IR}$ and baseline REE were included (Model 1). Weight loss and diet

were then included (Model 2). To avoid multicolliniarity within the regression models, any independent variables whose correlation was > 0.8 were not simultaneously included within the model.

Post-hoc power calculations were performed. For an alpha level of 0.05, having used 11 predictors within the regression model that obtained an $R^2$ of 0.70 and a sample size of 439, a power of 0.999 was achieved.

## 3.3 Results

Table 3.1 shows the baseline characteristics of the subjects according to which dietary intervention they were randomised to. Overall, there were more women than men but there were equal proportions in both groups (78.2% vs.78.5%). Statistically, there was no significant difference between the groups based upon their baseline characteristics. We also looked at those subjects who had been recruited for the original NUGENOB study but were excluded from the current subgroup analysis. Only leptin was found to be significantly different, being 15% higher in the excluded group (p = 0.04).

| | **Low Fat** | **Moderate Fat** | **Sig.** |
|---|---|---|---|
| **Number subjects** | 225 | 214 | |
| **Male** | 21.8 % | 21.5 % | |
| **Age** | 36.2 (± 8.0) | 36.4 (± 8.0) | ns |
| **Mean weight (Kg)** | 101.6 (± 17.8) | 100.8 (± 16.2) | ns |
| **BMI** | 36.3 (± 5.0) | 36.0 (± 5.0) | ns |
| **Mean waist (cm)** | 106.5(± 13.5) | 105.7 (± 12.7) | ns |
| **Mean hip** | 120.2 (± 10.3) | 119.6 (± 10.5) | ns |
| **Waist hip ratio** | 0.89 (± 0.09) | 0.89 (± 0.09) | ns |
| **FFM (Kg)** | 59.0 (± 11.6) | 58.7 (± 11.1) | ns |
| **FM (Kg)** | 42.7(± 12.4) | 42.2 (± 11.5) | ns |
| **Fat%** | 41.7 (± 7.6) | 41.6 (± 7.3) | ns |
| **RQ** | 0.81 (± .05) | 0.81 (± .05) | ns |
| **Resting Energy Expenditure (kJ/min)** | 5.46 (± 0.94) | 5.48 (± 0.92) | ns |
| **Energy intake (MJ/day)** | 8.916 (± 2.792) | 9.033 (± 2.875) | ns |
| **Leptin (ng/ml)** | 30.5 (± 13.9) | 30.5 (± 15.0) | ns |
| **Cortisol (nM)** | 207.8 (± 112.5) | 202.9 (± 116.0) | ns |
| **Insulin-like Growth Factor-1 (ng/ml)** | 22.3 (± 8.5) | 23.8 (± 9.6) | ns |
| **HOMA-IR** | 2.4 (± 1.9) | 2.7 (± 2.1) | ns |

**Table 3.1: Baseline anthropometric and biochemical characteristics of subjects, according to dietary intervention**
Results represent means (± sd). Independent T-Tests was used to compare between group means
ns= not statistically significant

**Dietary Compliance**

Figure 3.1 shows the dietary fat contents at baseline and at the end of the intervention periods, based upon analysis of the food diaries recorded before and during the final week of the intervention period. At baseline, both groups consumed similar proportions of fat (37.2% vs. 37.5%, p=0.67). During the final week, the low fat group reported a mean fat intake of 24.9% whilst the moderate fat group reported a mean fat intake of 40.7%. Both groups showed significant changes from baseline (p<0.001 for both groups), indicating overall compliance with the level of macronutrient restriction that was requested of them.

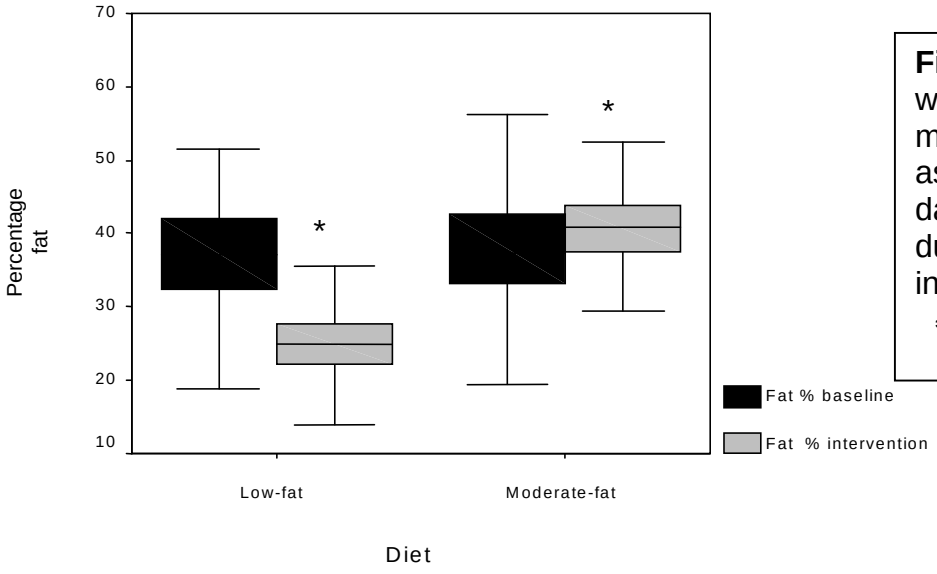


**Figure 3.1**: Box and whisker plot representing mean percentage fat intake as assessed by weighed 3-day food diaries before and during final week of dietary intervention

* P<0.001

**Weight Loss**

The subjects on a low fat diet had an unadjusted mean weight loss of 6.57kg (sd ± 3.46) and those on a moderate fat diet lost an unadjusted mean of 6.34kg (sd ± 3.38), with no significant difference observed between the two diets (p=0.476).

**Resting Energy Expenditure**

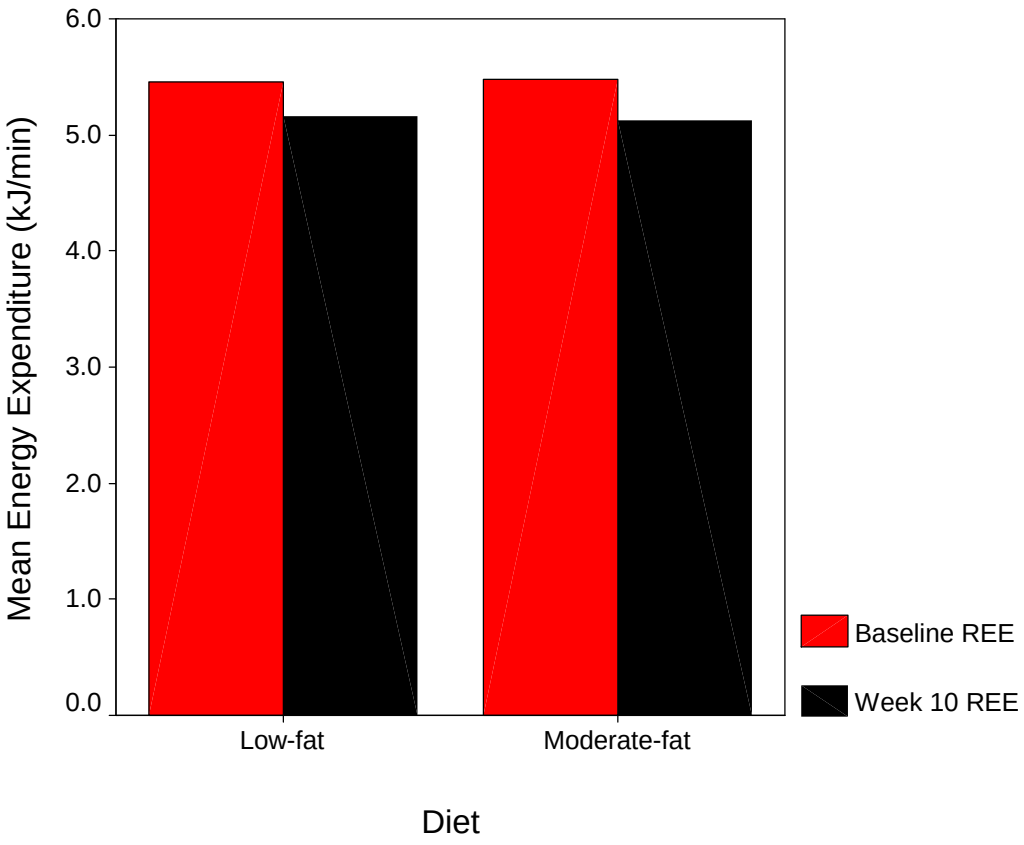


Figure 3.2. Unadjusted REE before and after dietary intervention

| | Diet | | |
|---|---|---|---|
| | Low Fat | Moderate Fat | P value |
| Baseline REE (kJ/min) | 5.37 | 5.41 | |
| Baseline REE $_{\%\ Fat}$ | 5.38 (5.26 to 5.49) | 5.41 (5.30 to 5.53) | 0.663 |
| Baseline REE $_{\%\ Fat + Weight}$ | 5.36 (5.29 to 5.42) | 5.43 (5.36 to 5.50) | 0.161 |
| Baseline RQ | 0.810 | 0.811 | |
| Baseline RQ $_{Weight}$ | 0.810 (0.804 to 0.816) | 0.811 (0.804 to 0.816) | 0.904 |
| Baseline RQ $_{Weight + Insulin}$ | 0.811 (0.805 to 0.817) | 0.810 (0.803 to 0.816) | 0.740 |
| Baseline Fat Oxidation (mg/min) | 67.8 | 67.7 | |
| Baseline Fat Oxidation $_{Weight}$ | 67.9 (64.0 to 71.0) | 67.6 (65.0to 72.0) | 0.8.66 |
| Baseline Fat Oxidation $_{Weight + \%\ Fat}$ | 67.0 (64.0 to 71.0) | 68.0 (64.0 to 72.0) | 0.823 |
| Baseline Fat Oxidation $_{Weight + \%\ Fat + Insulin}$ | 67.0 (64.0 to 71.0) | 69.0 (65.0 to 72.0) | 0.592 |
| Week 10 REE (kJ/min) | 5.09 | 5.06 | |
| Week 10 REE [a] $_{\%\ Fat}$ | 5.10 (5.04 – 5.17) | 5.05 (4.98 – 5.11) | 0.251 |
| Week 10 REE [a] $_{\%\ Fat + Weight}$ | 5.09 (5.03 – 5.15) | 5.06 (5.00 – 5.12) | 0.443 |
| Week 10 RQ | 0.813 | 0.806 | |
| Week 10 RQ [a] $_{\%\ Fat}$ | 0.813 (0.807 to 0.820) | 0.805 (0.799 to 0.812) | 0.094 |
| Week 10 RQ [a] $_{\%\ Fat + Insulin}$ | 0.813 (0.807 to 0.820) | 0.805 (0.799 to 0.812) | 0.082 |
| Week 10 Fat Oxidation (mg/min) | 65.9 | 67.9 | |
| Week 10 Fat Oxidation [a] $_{Weight}$ | 66.0 (63.0 to 69.0) | 68.0 (65.0 to 71.0) | 0.268 |
| Week 10 Fat Oxidation [a] $_{Weight + Insulin}$ | 65.0 (62.0 to 69.0) | 68.0 (65.0 to 71.0) | 0.242 |
| Δ REE (kJ/min) | -0.29 | -0.35 | |
| Δ REE [a] $_{\%\ Fat + Weight}$ | -0.30 (-0.36 to -0.24) | -0.34 (-0.40 - -0.27) | 0.415 |
| Δ RQ | 0.0034 | -0.0052 | |
| Δ RQ [a] $_{\Delta\%\ Fat}$ | 0.003 (-0.003 to 0.010) | -0.005 (-0.012 to 0.001) | 0.054 |
| Δ Fat Oxidation (mg/min) | -2.1 | 0.6 | |
| Δ Fat Oxidation [a] $_{Gender}$ | -2.0 (-5.0 to 1.0) | 0.0 (-3.0 to 4.0) | 0.342 |
| Δ Fat Oxidation [a] $_{Gender + \Delta Insulin}$ | -2.0 (-5.0 to 2.0) | 0.01 (-3.0 to 3.0) | 0.476 |

**Table 3.2: REE and Fat Oxidation before and after dietary intervention**
Data are means with 95% confidence intervals
[a] = Week 10 means also adjusted for baseline value
Means are adjusted for %Fat, Weight, Gender or Insulin when indicated below the variable

Table 3.2 summaries the unadjusted and adjusted means for resting energy expenditure (REE), respiratory quotient (RQ) and fat oxidation, according to dietary intervention, before and after weight loss. The unadjusted REE is represented graphically in figure 3.2.

Both groups had similar anthropometric and biochemical matching at baseline (table 3.1) and no significant difference for REE, RQ and fat oxidation at baseline. This would suggest that metabolically, there was good matching at baseline. Regarding the change in REE (∆REE) after the dietary intervention, there was no significant difference between the diets even after adjusting for weight and % body fat and baseline REE (-0.30 vs. -0.34 kJ/min, p=0.415). The mean change in RQ (∆RQ) over the intervention period was +0.003 and -0.005 for the low fat and moderate fat diets respectively. This trend for a change in substrate utilisation towards carbohydrates on the low fat diet and towards fat on the moderate fat diet did not reach statistical significance (p=0.054).

When the change (∆) in fat oxidation was adjusted for baseline fat oxidation, gender and change in insulin levels, there was no significant difference between the groups (p=0.476), although numerically the subjects on low fat diets had decreased their fat oxidation by 2.0mg/min, whilst the moderate fat group showed no change in fat oxidation.

| | *Model 1* | | | *Model 2* | | | *Model 3* | | | *Model 4* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value |
| Intercept | 1.723 | 1.655 to 1.792 | < 0.001 | 0.989 | 0.846 to 1.131 | < 0.001 | 1.072 | 0.928 to 1.216 | < 0.001 | 1.004 | 0.836 to 1.172 | <0.001 |
| Dependent Variable | | | | | | | | | | | | |
| Age | -0.002 | -0.004 to -0.001 | 0.009 | -0.001 | -0.002 to 0.0 | 0.107 | -0.001 | -0.002 to 0.001 | 0.310 | -0.001 | -0.002 to 0.001 | 0.329 |
| Gender Female | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gender Male | 0.228 | 0.196 to 0.259 | <0.001 | 0.042 | -0.019 to 0.104 | 0.177 | 0.044 | -0.015 to 0.103 | 0.146 | 0.029 | -0.031 to 0.088 | 0.342 |
| Weight (kg) | | | | 0.007 | 0.006 to 0.008 | <0.001 | 0.006 | 0.006 to 0.007 | <0.001 | 0.007 | 0.006 to 0.008 | <0.001 |
| % Body Fat | | | | -0.004 | -0.007 to –0.001 | 0.009 | -0.004 | -0.007 to –0.001 | 0.005 | -0.004 | -0.008 to –0.001 | 0.005 |
| Waist to Hip Ratio | | | | 0.164 | 0.014 to 0.315 | 0.032 | 0.044 | -0.107 to 0.194 | 0.570 | 0.068 | -0.083 to 0.218 | 0.379 |
| Energy Intake (kcal/day) | | | | | | | $1.550^{-05}$ | $1.087^{-06}$ to $2.991^{-05}$ | 0.035 | $1.789^{-05}$ | $3.618^{-06}$ to $3.217^{05}$ | 0.014 |
| HOMA-IR | | | | | | | 0.013 | 0.008 to 0.018 | <0.001 | 0.013 | 0.008 to 0.018 | <0.001 |
| Free Fatty Acid | | | | | | | | | | $8.813^{-05}$ | $1.950^{-05}$ to $1.567^{-04}$ | 0.012 |
| Leptin | | | | | | | | | | $-4.360^{-}_{04}$ | $-1.348^{-03}_{04}$ to $4.759^{-}$ | 0.348 |
| Cortisol | | | | | | | | | | $1.010^{-04}$ | $2.112^{-05}$ to $1.808^{-04}$ | 0.013 |
| IGF-1 | | | | | | | | | | $2.103^{-04}$ | $-1.291^{-03}$ to $8.790^{-04}$ | 0.702 |
| **Adjusted $R^2$** | | 0.323 | | | 0.668 | | | 0.694 | | | 0.703 | |

**Table 3.3: Predictor of baseline Resting Energy Expenditure in a linear regression model**
β= ln (REE**)**
Models also include research centre as well as the indicated variables.

Table 3.3 demonstrates how baseline REE is affected by various independent variables in a linear regression model.

In model 1 it can be seen that men have a significantly higher REE compared to women (25.6% $p<0.001$) and with increasing age, there is reduction in REE, with a fall of 0.2% every year ($p=0.009$).

Once body composition is taken into consideration (model 2), the effects of age and gender are no longer significant. Body weight is a strong predictor, with REE increasing by 0.7% for every kg increase in weight ($p<0.001$). The composition of weight also seems to be important as REE reduces by 0.3% for every % increase in body fat% ($p=0.009$). The distribution of body fat centrally, as reflected by an increase in waist to hip ratio (WHR), resulted in an increase in REE of 1.65% for every 10% increase in WHR ($p=0.032$).

Baseline energy intake was included in Model 3. This demonstrated that for every 100kcal increase there was a 0.16% increase in REE ($p=0.035$). With increasing insulin resistance (HOMA-IR), there was an increase in REE, with REE increasing by 1.31% for every unit increase in HOMA-IR ($p<0.001$). Body weight and body fat % continued to have a significant impact upon REE but WHR was no longer a significant determinant of REE.

The final model (4) introduced baseline fasting metabolites and hormones. Positive effects upon REE were seen with free fatty acids ($8.813^{-03}$ % increase, $p =0.012$) and cortisol (0.01% increase, $p=0.013$). Serum leptin and IGF-1 did not have a significant impact upon REE ($p= 0.348$ and 0.702 respectively).

Overall, the final model was able to predict over 70% of the variation in REE with major contributions from weight, body fat %, energy intake, HOMA-IR, free fatty acids and cortisol levels.

| | *Model 1* | | | *Model 2* | | | *Model 3* | | | *Model 4* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value | β | 95% Confidence Interval | p-value |
| Intercept | 1.642 | 1.570 to 1.713 | <0.001 | 0.828 | 0.692 to 0.963 | <0.001 | 0.853 | 0.713 to 0.993 | <0.001 | 0.854 | 0.684 to 1.024 | <0.001 |
| Dependent Variable | | | | | | | | | | | | |
| Age | -0.002 | -0.004 to 0.0 | 0.030 | -0.001 | -0.002 to 0.0 | 0.143 | -0.001 | -0.002 to 0.001 | 0.277 | -0.001 | -0.002 to 0.001 | 0.307 |
| Gender Female | 0 | | | 0 | | | 0 | | | 0 | | |
| Gender Male | 0.236 | 0.202 to 0.270 | <0.001 | 0.021 | -0.035 to 0.078 | 0.456 | 0.012 | -0.043 to 0.066 | 0.680 | 0.010 | -0.045 to 0.066 | 0.718 |
| Weight (kg) $_{week\ 10}$ | | | | 0.008 | 0.007 to 0.009 | <0.001 | 0.007 | 0.006 to 0.008 | <0.001 | 0.007 | 0.006 to 0.008 | <0.001 |
| % Body Fat $_{week\ 10}$ | | | | -0.005 | -0.008 to –0.002 | <0.001 | -0.005 | -0.007 to –0.002 | 0.001 | -0.005 | -0.008 to –0.002 | 0.002 |
| Waist to Hip Ratio $_{week\ 10}$ | | | | 0.269 | 0.120 to 0.418 | <0.001 | 0.164 | 0.014 to 0.314 | 0.033 | 0.176 | 0.023 to 0.329 | 0.024 |
| Energy Intake (kcal/day) $_{week\ 10}$ | | | | | | | $8.848^{-05}$ | $4.879^{-05}$ to $1.282^{-04}$ | <0.001 | $8.843^{-05}$ | $4.855^{-05}$ to $1.283^{-04}$ | <0.001 |
| HOMA-IR $_{week\ 10}$ | | | | | | | 0.005 | 0.001 to 0.010 | 0.023 | 0.005 | 0.001 to 0.010 | 0.027 |
| Free Fatty Acid $_{week\ 10}$ | | | | | | | | | | $-2.010^{-05}$ | $-8.620^{-05}$ to $4.609^{-05}$ | 0.551 |
| Leptin $_{week\ 10}$ | | | | | | | | | | $1.133^{-04}$ | $-9.736^{-04}$ to $1.200^{-03}$ | 0.838 |
| Cortisol $_{week\ 10}$ | | | | | | | | | | $3.607^{-05}$ | $-4.426^{-05}$ to $1.164^{-04}$ | 0.378 |
| IGF-1 $_{week\ 10}$ | | | | | | | | | | $-3.024^{-04}$ | $-1.346^{-03}$ to $7.414^{-04}$ | 0.569 |
| **Adjusted $R^2$** | 0.320 | | | 0.699 | | | 0.718 | | | 0.717 | | |

**Table 3.4: Predictor of post-dietary intervention Resting Energy Expenditure in a linear regression model**
β= ln (REE**)**
Models also include research centre as well as the indicated variables.

To test whether the original determinants of REE remained valid predictors after weight loss, the linear regression model firstly was repeated using results of variables obtained at the end of the 10-week dietary intervention period (table 3.4), with no adjustment for baseline values.

A similar pattern was seen with men still having a REE that was 26.6% higher than women ($p<0.001$) and increasing age having a negative effect (-0.2% per year, $p=0.030$). Once again, the introduction of body composition negated the effects of gender and age. Weight, body fat % and WHR had a significant impact upon REE (0.8% per kg, -0.5% per kg, and 2.73% per 10% increase in WHR respectively, all $p<0.001$).

Although energy intake was reduced during the intervention period, it continued to influence REE (0.89 % increase in REE per 100kcal/day, $p<0.001$), and HOMA-IR at week 10 also had a significant influence (0.5% increase in REE per unit of HOMA-IR, $p=0.023$).

The contribution of fasting metabolites and hormones were not significant at week 10.

Despite the fact that fewer of the original predictor variables remained significant, the overall model was able to predict nearly 72% of the variation in REE.

| | *Model 1* | | | *Model 2* | | |
|---|---|---|---|---|---|---|
| | **β** | **95% Confidence Interval** | **p-value** | **β** | **95% Confidence Interval** | **p-value** |
| **Intercept** | 0.802 | 0.660 to 0.944 | <0.001 | 0.842 | 0.706 to 9.77 | <0.001 |
| **Dependent Variable** | | | | | | |
| **Age** | -0.001 | -0.002 to 0.0 | 0.176 | -0.001 | -0.002 to 0.001 | 0.311 |
| **Gender** **Female** | 0 | | | 0 | | |
| **Male** | -0.001 | -0.059 to 0.056 | 0.968 | 0.007 | -0.047 to 0.061 | 0.807 |
| **Weight (kg)** | 0.004 | 0.002 to 0.005 | <0.001 | 0.004 | 0.003 to 0.005 | <0.001 |
| **% Body Fat** | -0.003 | -0.006 to 0.0 | 0.048 | -0.003 | -0.006 to 0.0 | 0.021 |
| **Waist to Hip Ratio** | 0.039 | -0.107 to 0.184 | 0.833 | 0.072 | -0.066 to 0.209 | 0.305 |
| **Energy Intake (kcal/day)** | $2.167^{-06}$ | $-1.183^{-05}$ to $1.617^{-05}$ | 0.761 | $8.744^{-06}$ | $-4.567^{-06}$ to $2.205^{-05}$ | 0.197 |
| **HOMA-IR** | 0.004 | -0.001 to 0.009 | 0.103 | 0.002 | -0.003 to 0.007 | 0.397 |
| **Baseline REE (kJ/min)** | 0.097 | 0.080 to 0.114 | <0.001 | 0.087 | 0.071 to 0.104 | <0.001 |
| **Diet** | | | | -0.002 | -0.018 to 0.014 | 0.781 |
| **Weight loss (kg)** | | | | -0.009 | -0.012 to –0.007 | <0.001 |
| **Adjusted $R^2$** | | 0.745 | | | 0.774 | |

**Table 3.5**: **Linear regression model for predictors of post intervention Resting Energy Expenditure including the impact of weight loss and diet**
β= ln (REE**)**
Models also include research centre as well as the indicated variables

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Although the model in table 3.4 is still able to predict a large proportion of REE, it is important to appreciate that both the absolute and relative change in REE following weight loss must take into account the baseline REE. With this in mind, the regression model in table 3.5 was created, also including variables that are strongly related to baseline REE.

Within model 1, the only significant contributors to post-intervention REE are weight (0.4% increase in REE per kg, $p<0.001$), body fat % (0.3% decrease in REE per kg, $p=0.048$) and baseline REE (0.97% increase in REE per 10% increase in baseline REE, $p<0.001$).

When weight loss is added to the model (model 2), there is a strong negative impact, with REE decreasing 0.9% per kg weight loss, $p<0.001$. The type of diet has no significant effect upon REE ($p=0.781$).

Even though there are fewer significant predictors for post-intervention REE, the model is able to predict over 77% of the variability in REE.

## 3.4 Discussion

This study set out to investigate whether factors known to have an effect upon baseline REE, remained valid predictors after weight loss. What was discovered was that the baseline weight, body fat percentage and starting REE were the only baseline variables which had a significant contribution to post-weight loss REE. The amount of weight that was lost also significantly affected REE.

**Baseline REE**

Previous studies have shown that REE decreases with increasing age (Johnstone, Murison et al. 2005). Some have shown that the decline which is seen is related mainly to the reduction in FFM (Piers, Soares et al. 1998; Bosy-Westphal, Eichhorn et al. 2003), whereas others have shown that even after adjusting for body composition (FFM and FM) there is still an independent effect of age (Hunter, Weinsier et al. 2001; Van Pelt, Dinneno et al. 2001). The present study showed that age was only a significant determinant of the variance in REE in the simplest regression model, REE decreased by 0.2% per year ($p=0.009$). Once body composition was accounted for, age was no longer relevant. The differing results between this study and that of Hunter et al (Hunter, Weinsier et al. 2001) could be explained by the quite marked difference in populations studied. Our subjects were clinically obese, with a mean BMI of 36.0 whereas the subjects in the study by Hunter et al were of normal weight, mean BMI 23.7. Also, the age range in the present study was smaller (20 - 50) than in the study by Hunter et al.

As in other studies (Johnstone, Murison et al. 2005) we found that any difference in REE that was observed between genders disappeared once adjustments were made for body composition.

The dominant factors influencing REE were body weight and body percentage fat. This is consistent with other studies (Karhunen, Franssila-Kallunki et al. 1997; Nielsen, Hensrud et al. 2000; Johnstone, Murison et al. 2005), although these studies tended to report FFM and FM. We chose to look at weight and percentage fat mass as there is some suggestion that especially in obese women, weight explains more of the variation in REE than FFM alone (Dore, Hesp et al. 1982; Mifflin, St Jeor et al. 1990; Karhunen, Franssila-Kallunki et al. 1997).

As the daily energy intake increased, so did REE. For every extra 100kcals consumed, REE increased by 0.16%. This is in keeping with the finding that with increased energy intake there is an increase in the thermic effect of food (D'Alessio, Kavle et al. 1988).

Higher fasting insulin levels, as reflected in a raised HOMA-IR, are associated with a rise in REE. This is consistent with studies in both diabetic (Weyer, Snitker et al. 1999) and non-diabetic (Fan, Anderson et al. 2006) subjects. This is likely to be explained in part by the observation that insulin stimulates sympathetic nervous system (SNS) activity, which in turn increases REE (Vollenweider, Tappy et al. 1993), and also possibly that in the insulin resistant state there may be a failure to suppress the energy expenditure associated with hepatic gluconeogenesis. Although HOMA-IR is a highly significant predictor of REE, the absolute value by which HOMA-IR changes is usually only a fraction of a whole unit. The 95% confidence intervals reveal

that for every unit increase in HOMA-IR, REE increased between 0.8% and 1.8%. In reality however, the contribution, which HOMA-IR makes to REE, would be much less.

The final model introduced hormones and metabolites associated with REE. No significant contribution was made by leptin. This is in keeping with previous studies which suggested that the influence leptin has upon energy balance in humans is through its effect upon appetite rather than regulation of energy expenditure (Rosenbaum, Nicolson et al. 1997; Hukshorn and Saris 2004). While studies looking at administering Growth Hormone (GH) and Insulin-like growth factor (IGF-1) have shown that weight loss can be achieved without compromising FFM (Thompson, Butterfield et al. 1998), our study and that of Armellini et al (Armellini, Zamboni et al. 2000) suggest that at least in the basal state, GH/IGF-1 had no significant contribution towards REE.

An increase in REE was observed with increasing levels of fasting free fatty acids. This is consistent with other studies (Schiffelers, Saris et al. 2001), which showed that both thermogenesis and fat oxidation can be increased by intravenous infusion of intralipid. The present study also showed that cortisol levels were positive associated with REE. The role of glucocorticoids in affecting REE has been reported by Chong et al (Chong, Jung et al. 1992) who demonstrated that intravenous administration of corticotrophin releasing factor (CRF), increased energy expenditure in both lean and obese individuals.

**Post intervention REE**

It has been shown that weight loss or weight gain results in a decrease or increase in total energy expenditure respectively, so that normal weight can be restored (Leibel, Rosenbaum et al. 1995). The success of Very Low Calorie Diets (VLCDs) in achieving and maintaining significant weight loss, in the face of marked restriction in metabolic rate (Kreitzman, Coxon et al. 1992) suggests that there must be some cognitive adaptation to overcome the natural tendency to regain weight.

When the determinants of post weight loss REE were investigated, the original regression model (table 3.2) was used, substituting values obtained at week 10 for those at baseline. Not surprisingly, the model yielded similar results for age and gender. The contribution that weight made towards the variance in REE remained unchanged. When the proportion of body fat increased, REE was reduced by a greater amount (0.5% for every % increase in body fat). Heshka et al (Heshka, Yang et al. 1990) demonstrated that following conservative weight loss, there was a reduction in REE and even though body fat (i.e. FM) was not a significant contributor to the variance in REE at baseline, following weight loss, FM became a significant determinant of REE. This was mainly due to the greater loss of FM than FFM. The implications being that the loss of FM associated with weight loss will reduce REE unless compensated for by an increase in metabolically more active FFM.

Following weight loss, waist-hip ratio was now a significant contributor to the variance in REE even after taking into account body composition. Notably,

insulin sensitivity now contributed much less to REE. With weight loss comes improved insulin sensitivity (Webber, Donaldson et al. 1994; Raatz, Torkelson et al. 2005), which corresponds to a reduction in central obesity and a reduction in fasting insulin levels. This is likely to explain why WHR now plays a greater role in predicting REE as the contribution of waist (i.e. central obesity) is much less relative to the contribution of hip measurement (i.e. FFM).

Although daily energy intake continued to provide a significant contribution towards REE, those fasting metabolites and hormones which did originally help determine REE, now no longer added to the regression model. Rennaries et al (Ranneries, Bulow et al. 1998) found that in formerly obese women, despite an intact ability to mobilise fat at rest and during exercise, there was a subnormal fat oxidation rate despite higher levels of fatty acids. This may well explain why in the present study FFA no longer had a positive effect upon REE as it is likely that the physiological response would be to reduce fat oxidation and thus attempt to regain the weight which was lost. Although cortisol had a positive influence upon baseline REE, following the intervention period, this significant contribution to REE disappeared. Despite the fact that cortisol has an important part to play in fat metabolism, there does not appear to be any correlation between weight loss and serum cortisol (Christeff, Melchior et al. 1999).

Although this model was able to predict almost 72% of the variance in REE following weight loss, it is still important to take into account those variables that had a strong influence upon baseline REE. The final regression model did just this and the only variables, which now significantly predicted post

intervention REE were, baseline weight and percentage fat and baseline REE. The introduction of the dietary intervention made no difference, however, the amount of weight lost, not surprisingly, did. Leibel e al (Leibel, Rosenbaum et al. 1995) reported that an increase or decrease of 10% in body weight resulted in either a 16% increase or 15% decrease in total energy expenditure respectively. In this study, REE decreased by 0.9% for every kg weight loss. Assuming that TEE approximates to ~ 150% of REE, we can calculate for the group as a whole that for a 10% reduction in weight, TEE will reduce by 13.5%, a figure not dissimilar to that reported by Leibel.

One of the limitations of this study is that although baseline REE was performed at a stable weight, the post intervention REE was calculated during or just after the final week of the intervention during a period of dynamic weight change. This may influence some of the results and future work may wish to address this by allowing a period of weight stabilisation before repeating metabolic profiling.

Despite this, the model offers a simple basis to predict changes in REE and thus energy requirement to help obese subjects not only to lose weight but also succeed in the much harder process of maintaining weight loss.

# Chapter 4

# Variations in energy expenditure and fat oxidation before and after a high fat test meal

## 4.1 Introduction

The first law of thermodynamics states “The increase in the internal energy of a system is equal to the amount of energy added by heating the system, minus the amount lost as a result of the work done by the system on its surroundings”. This concept is equally applicable to the human body, with energy balance and weight maintenance dependent upon equilibrium between the intake and utilisation of dietary macronutrients (carbohydrate, protein and fat).

The body’s stores of carbohydrate, in the form of glycogen, are relatively small. For an average 70kg male, only 0.2kg of energy is stored as carbohydrate (Rosenbaum, Leibel et al. 1997). As a result, there may only be sufficient carbohydrate to allow oxidation for 1 to 2 days. This means that carbohydrate consumption needs to be rapidly and efficiently adjusted, a process mainly mediated by the actions of insulin (Blaak and Saris 1996). Fat stores, on the other hand, are considerably larger (Rosenbaum, Leibel et al. 1997). As a result, it is not essential for fat oxidation to be able to adjust as rapidly to differences in fat intake (Flatt, Ravussin et al. 1985). If an individual therefore consumes meals, which are high in dietary fat, they are more likely to gain weight due to a net, positive, energy balance. This is supported by studies that have shown fat oxidation to be much slower at adapting to an increase in dietary fat than other macronutrients (Schutz, Flatt et al. 1989; Schrauwen, van Marken Lichtenbelt et al. 1997). During overfeeding studies, subjects were able to adapt better to carbohydrate overfeeding than to fat

overfeeding (Horton, Drougas et al. 1995; Jebb, Prentice et al. 1996). This meant that more of the excess energy was stored during fat overfeeding than when overfed with carbohydrate. Expansion of the body's fat stores continues until a new equilibrium is reached between fat mass and fat oxidation rates (Astrup, Buemann et al. 1994; Kunz, Schorr et al. 2002). However, some studies have suggested that fat oxidation does not correlate with fat mass but instead more with gender and fat free mass (Nagy, Goran et al. 1996).

It has also been demonstrated in obese subjects that there is a diminished ability to utilise fat as a fuel (Thomas, Peters et al. 1992), a feature also seen in post-obese women (Buemann, Toubro et al. 1998; Faraj, Jones et al. 2001) and those individuals at increased risk of developing obesity (Giacco, Clemente et al. 2003). This might well explain why having lost weight, obese subjects have a tendency to regain weight.

In addition to impaired fat oxidation, there is debate as to whether the thermic effect of food is also impaired in obesity. Postprandial thermogenesis was reduced in obese subjects (Bessard, Schutz et al. 1983; Schutz, Bessard et al. 1984) and this reduction in postprandial thermogenesis persisted following weight loss (Bessard, Schutz et al. 1983). This finding was confirmed in a study by Nelson et al (Nelson, Weinsier et al. 1992), who postulated that the reduced thermic effect of food was a cause rather than a consequence of obesity. Results, however, have been inconsistent and have been the subject of several reviews (D'Alessio, Kavle et al. 1988; de Jonge and Bray 1997; Granata and Brandon 2002). These discordant results have been related to different methodological approaches between studies, not controlling for age,

degrees of obesity, insulin resistance, levels of physical activity and habitual diet.

The objective for this study was therefore to examine the determinants of fat oxidation before and after a high fat challenge as well as to study the determinants of postprandial energy expenditure in a group of obese subjects and a lean reference group.

The hypothesis being tested was that fat oxidation is diminished in both the fasting and postprandial state in obese subjects.

## 4.2 Methods

The subjects recruited for this study, formed the Nottingham cohort investigated within the Nutrient-Gene Interactions in Human Obesity – Implications for Dietary Guidelines multi-centre project (NUGENOB: www.nugenob.org).

Subjects (100 obese and 14 normal BMI) were recruited after responding to advertisements in the local media, by self-referral or following referral by a Primary or Secondary Care physician.

The basic inclusion criteria were body mass index $\geq$ 30 kg/m$^2$ for the obese subjects and between 18.5 and 25 for the lean reference group and age 20 - 50 years. Subjects were excluded at screening if they reported weight change greater than 3 kg within the 3 months prior to the study start. Hypertension, diabetes or hyperlipidaemia, treated by drugs, along with untreated thyroid disease were also criteria for exclusion. Subjects were also excluded if they had undergone surgical or drug-treated weight loss, were pregnant or had a history of alcohol or drug abuse. Additional exclusion criteria for the reference group were a past history of BMI greater than 25 and medication other than oral contraception.

The local Ethics Committee approved the study protocol and all participants provided informed, written consent.

**Study Design and Procedures**

All subjects participated in a clinical investigation protocol. Subjects arrived at the research centre at 8.00 a.m. following a 12 hour, overnight fast and a preceding 3-day dietary run in period, during which time they were required to keep to their habitual diet, avoid excessive physical activity and alcohol consumption. After the subjects voided their bladder, they underwent anthropometric (height, weight and waist and hip circumference) and body composition assessments. Thereafter, subjects remained on the bed for 3.5 hours, during which time thermogenesis and substrate utilization was measured. Pre- and postprandial blood samples were taken to determine metabolites and hormones

Prior to the study, subjects completed a detailed, 3-day weighed food diary, which was analysed using a standard database used in routine clinical practice. Additionally, habitual physical activity was estimated through the Baecke questionnaire using the sum of work, sport and leisure scores recorded in the questionnaire. This index of physical activity has previously been validated against average daily energy expenditure measured by the doubly labelled water method (Montoye, Saris et al. 1996; Philippaerts and Lefevre 1998).

Body weights were measured using calibrated scales. Waist and hip circumferences were measured with the participant wearing only non-restrictive underwear. Body height was measured with a calibrated stadiometer. The mean of three measurements was recorded for each variable. Fat mass and fat-free mass were assessed by multifrequency bio-

impedance (Bodystat®; QuadScan 4000, Isle of Man, British Isles), with subject lying in the supine position.

**Test Meal**

To study the effect of nutrient partitioning, the response to a saturated fat load was measured. The liquid test meal comprised of double cream, (with 40% fat/100 g) and consisted of 95 en% (% of energy content), saturated fat, 3 en% carbohydrate and 2 en% protein. Based on the predicted metabolic rate (WHO, technical report series 724, Geneva, 1985) the energy content was fixed at 50 % of predicted basal metabolic rate. Subjects were asked to drink the test meal within 10 minutes.

**Energy Expenditure Measurement**

The experimental room was kept thermoneutral at 25 $^{o}$C. Energy expenditure was measured using an open circuit ventilated hood system for 30 minutes, in the fasting state and at hourly intervals for 3 hours after the test drink. The equipment was standardised using a validation protocol involving both ethanol recovery and reproducibility measurements in human subjects (see general methods).

**Blood Sampling**

At least 30 minutes before the start of the resting measurement, a Teflon coated cannula was inserted in an antecubital forearm vein for blood sampling. Blood was drawn in the fasting state and every 60 minutes following the test meal. Concentrations of glucose, free fatty acids, insulin, cortisol,

triglycerides, and free glycerol were determined at pre- and postprandial time points. IGF-1 and leptin were only determined in baseline samples.

**Biochemical Analysis**

All blood analyses were performed at one of the centres within the NUGENOB consortium, or at a subcontracted commercial laboratory (for cortisol and IGF-1). Plasma glucose concentrations (ABX diagnostics, Montpellier, France), and triglycerides (Sigma, St Louis, USA; ABX diagnostics, Montpellier, France) were measured on a COBAS MIRA automated spectro-photometric analyser (Roche diagnostica, Basel, Switzerland). Free fatty acids (NEFA C kit; Wako Chemicals, Neuss Germany) were measured on a COBAS FARAH centrifugal spectro-photometer (Roche Diagnostica, Basel, Switzerland). Standard samples with known concentrations were included in each run for quality control. Plasma insulin and serum leptin concentrations were measured with a double antibody radio-immunoassay (Insulin RIA 100; Kabi-Pharmacia, Uppsala, Sweden; Human leptin RIA kit, Linco research, Inc, St.Charles, Missouri, USA). Cortisol and IGF-1 were both determined with ELISA assays (IGF-1: Diagnostics Systems Laboratories, Inc, Texas, USA, cortisol: ADVIA Centaur, Bayer Health Care LLC).

**Calculations**

EE was calculated according to the equation of Weir (Weir 1949). The homeostasis model assessment of insulin resistance ($HOMA_{IR}$) was calculated from fasting insulin and glucose levels according to the equation of Matthews et al(Matthews, Hosker et al. 1985). Fat oxidation was calculated

according the equations of Frayn (Frayn 1983), ignoring protein oxidation. For comparing postprandial responses, the area under the curve (AUC) was calculated according to the trapezium rule.

**Statistical Analysis**

Data are expressed as means and 95% confidence intervals or mean ± standard error of the mean. Statistical analysis was performed using SPSS version 11 for Windows. Variables were assessed for normal distribution and where necessary were log10 transformed to satisfy conditions of normality. Fasting and postprandial energy expenditure, fat oxidation and RQ were compared among predefined categories of BMI using analysis of covariance. Adjustments for phenotype were made in a step-wise manner, when appropriate, if the variable was significantly associated with REE, RQ or Fat Oxidation. In addition, for postprandial values, adjustments were also made for the fasting value of the variable and the energy content of the test meal. Associations between fat oxidation and energy expenditure were examined using Pearson's correlation coefficients. Fasting fat oxidation, postprandial fat oxidation and postprandial energy expenditure were studied as the dependent variables in a linear regression models. The physical, metabolic, hormonal and dietary characteristics from Table 4.1 were entered into the model in a stepwise manner. Variable entry was set at $p<0.005$ and removal at $p>0.10$. The level of statistical significance was set at $p<0.05$ for all tests.

To avoid multicolliniarity within the regression models, any independent variables whose correlation was > 0.8 were not simultaneously included within the model.

Post Hoc power calculations were performed for the data set used within this study. Using an alpha level of 0.05 and 4 predictors within the regression model (table 4.6) to achieve an $R^2$ of 0.364, a power of 0.999 was achieved.

## 4.3 Results

Table 4.1 indicates baseline characteristics according to whether subjects were lean or obese and also according to BMI category. There were more women than men (95 women and 19 men) in the group as a whole, and obese subjects were older (p=0.004).

With increasing BMI, there was an increase in body fat (both in absolute terms and as percentage of total body weight), FFM, waist circumference and WHR. Analysing weighed food diaries revealed that there was no significant difference in the proportion of energy consumed as fat between any of the groups. The level of physical activity, as assessed by the Baecke questionnaire, decreased with increasing BMI. Fasting glucose as well as insulin resistance ($HOMA_{IR}$) both increased significantly with increasing BMI. As BMI increased, fasting values for cortisol and IGF-1 decreased.

| | Lean **(BMI<25)** | Obese **(BMI>30)** | **p value** [a] | Obese BMI category | | | |
|---|---|---|---|---|---|---|---|
| | | | | **30 – 35** | **35 – 40** | **>40** | **p value** [b] |
| **Number** | 14 | 100 | | 46 | 25 | 29 | |
| **Male (%)** | 21.4 | 16.0 | | 15.2 | 16.0 | 17.2 | |
| **Age** | 31.0 (27.6 to 34.4) | 36.9 (35.4 to 38.5) | **0.004** | 37.3 (34.8 to 39.8) | 38.3 (35.6 to 41.0) | 35.2 (32.2 to 38.2) | **0.004** |
| **Body Fat (%)** | 23.2 (19.3 to 27.0) | 43.4 (42.0 to 44.9) | **<0.001** | 38.9 (37.2 to 40.5) | 44.0 (41.5 to 46.5) | 49.9 (47.9 to 51.9) | **<0.001** |
| **Fat Mass (kg)** | 14.8 (12.4 to 17.2) | 45.4 (42.5 to 48.3) | **<0.001** | 34.5 (33.0 to 36.1) | 45.4 (42.7 to 48.1) | 62.3 (57.4 to 67.3) | **<0.001** |
| **Fat Free Mass (kg)** | 49.5 (44.7 to 54.3) | 57.9 (55.8 to 60.0) | **<0.001** | 54.6 (51.9 to 57.4) | 58.3 (54.0 to 62.6) | 62.5 (58.2 to 66.8) | **<0.001** |
| **Waist (cm)** | 74.9 (71.3 to 78.4) | 109.7 (106.8 to 112.7) | **<0.001** | 99.3 (96.9 to 101.6) | 110.5 (107.1 to 114.0) | 126.1 (121.1 to 131.1) | **<0.001** |
| **Waist to Hip Ratio** | 0.75 (0.73 to 0.78) | 0.88 (0.86 to 0.89) | **<0.001** | 0.85 (0.83 to 0.88) | 0.89 (0.86 to 0.92) | 0.91 (0.88 to 0.94) | **<0.001** |
| **Dietary Fat intake (% of total EI)** | 31.1 (28.4 to 37.8) | 31.7 (29.7 to 33.6) | NS | 31.1 (28.5 to 33.6) | 29.4 (25.1 to 33.6) | 34.5 (30.6 to 38.5) | NS |
| **Habitual Physical Activity (AU)** | 8.49 (7.90 to 9.09) | 7.26 (6.99 to 7.52) | **0.001** | 7.29 (6.89 to 7.70) | 7.30 (6.61 to 7.99) | 7.15 (6.80 to 7.51) | **0.001** |
| **Glucose (mmol/l)** | 4.89 (4.79 to 4.99) | 5.34 (5.27 to 5.51) | **<0.001** | 5.25 (5.11 to 5.38) | 5.28 (5.10 to 5.47) | 5.54 (4.99 to 5.54) | **0.009** |
| **$HOMA_{IR}$** | 0.70 (0.48 to 0.92) | 2.56 (2.11 to 3.00) | **<0.001** | 1.86 (1.59 to 2.12) | 2.29 (1.79 to 2.79) | 3.90 (2.57 to 5.23) | **<0.001** |
| **Leptin (ng/ml)** | 8.93 (6.3 to 11.6) | 36.5 (33.2 to 39.9) | **<0.001** | 29.2 (25.5 to 32.9) | 35.8 (29.0 to 42.7) | 48.8 (42.6 to 55.0) | **<0.001** |
| **Free Fatty Acids (µmol/l)** | 387 (326 to 449) | 528 (497 to 560) | **0.001** | 516 (464 to 569) | 521 (458 to 585) | 553 (500 to 606) | **<0.001** |
| **Triglyceride (µmol/l)** | 596 (470 to 722) | 1122 (998 to 1247) | **<0.001** | 1035 (903 to 1168) | 1212 (844 to 1580) | 1183 (952 to 1415) | **<0.001** |
| **IGF-1 (ng/ml)** | 33.4 (26.3 to 40.4) | 22.0 (20.6 to 23.5) | **0.001** | 23.5 (21.4 to 25.6) | 22.9 (19.9 to 25.8) | 19.0 (16.1 to 21.9) | **0.004** |
| **Cortisol (mmol/l)** | 326 (228 to 425) | 203 (174 to 232) | **0.002** | 251 (197 to 305) | 159 (127 to 192) | 163 (122 to 193) | **0.001** |

**Table 4.1: Baseline Characteristics for subjects according to BMI**

EI, energy intake; AU, Arbitrary units; $HOMA_{IR,}$ homeostasis model assessment of insulin resistance

Means and 95% confidence intervals are shown.

[a] p value for difference between lean and obese groups (t-test)

[b] p value for ANOVA for difference between lean reference group and categories of obese BMI.

Figure 4.1 illustrates the unadjusted concentrations of metabolites and hormones before and after a high fat test meal according to BMI category. Fasting free fatty acid (FFA) concentrations were significantly lower in the lean subjects in comparison to obese subjects and concentrations increased with increasing BMI ($P<0.001$). FFA concentrations decreased over the first hour but then increased during the following 2 hours (Fig.4.1A), with postprandial values remaining significantly lower in the reference group than in any of the obese groups ($p<0.001$).

Triglyceride (TAG) concentrations increased following the test meal (Fig.4.1B), with higher concentrations in the obese groups than in the lean subjects ($p<0.001$ for AUC).

There was an initial decrease in postprandial glucose concentrations during the first hour, after which, concentrations increased between 1 and 3 hours. Postprandial glucose was significantly lower in the lean group in comparison to the obese group ($p=0.007$ for AUC) (Fig.4.1C).

Insulin levels increased in the first hour following the test meal and then tended to decrease. Values for postprandial insulin AUC (Fig.4.1D) were significantly different between the lean and obese subjects ($p<0.001$).

Figure 4.1 also illustrates how energy expenditure (EE) and fat oxidation varied following the test meal (Fig. 4.1E & 4.1F). There was a significant difference in fasting energy expenditure ($p<0.001$) and a gradual increase in postprandial energy expenditure ($p<0.01$) with increasing BMI.

Fat oxidation was lower in the lean group in the fasting state ($p=0.005$) than in the obese groups. Postprandial fat oxidation AUC was significantly different between the groups, with the lowest values in the lean group, intermediate

values in those with BMI 30 to 40 and highest recorded measurements in those with BMI>40 ($p<0.001$).

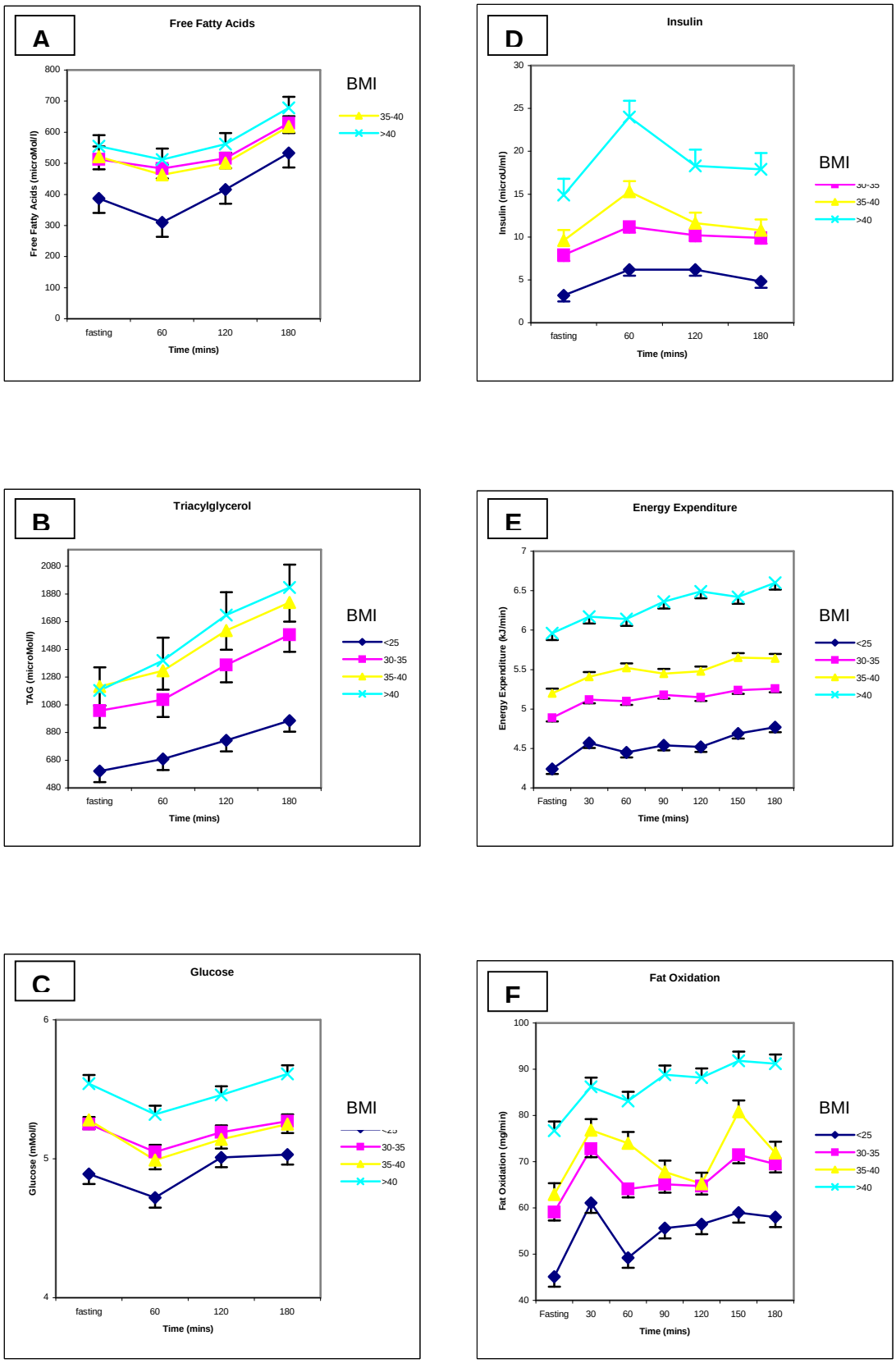



**Figure 4.1: Circulating fasting and postprandial concentrations for FFAs (A), TAGs (B), Glucose (C), Insulin (D) and EE (E) and Fat Oxidation (F) after a high fat liquid challenge according to BMI category**.
Data represent unadjusted means ± SEM.

Resting energy expenditure is seen to increase with increasing BMI (Table 4.2, Fig.4.1E) and remained significant after adjusting for FFM ($p<0.001$). Once adjustments for FM were made, similar values were seen across BMI categories. No difference was seen in fasting RQ, even after adjusting for FM. There was however, a tendency for lean subjects to have a lower RQ than the more obese subjects whose BMI was greater than 35 (Fig. 4.2A).

Fat Oxidation did increase with increasing BMI ($p=0.012$). When adjusted for FFM and FM, although no longer significant, there was a tendency for fat oxidation, to decrease with increasing BMI (Fig. 4.2B). This trend was however, not as evident when fat oxidation was expressed as percentage of EE (Fig.4.2C).

During the postprandial period, EE AUC was not significantly different between lean and obese subjects, although there was a trend towards a lower EE response in the more obese subjects (Fig. 4.3A).

As with fasting RQ and fat oxidation, there was a trend towards a higher postprandial RQ and a lower postprandial fat oxidation with increasing BMI (Fig. 4.3B & 4.3C).

| | Lean | Obese | p value[a] | Obese BMI categories | | | |
|---|---|---|---|---|---|---|---|
| | | | | 30 – 35 | 35 – 40 | >40 | p value[b] |
| **REE (kJ/min)** | 4.22 (3.82 to 4.61) | 5.27 (4.62 to 5.44) | <0.001 | 4.83 (4.42 to 5.05) | 5.24 (4.95 to 5.53) | 5.99 (5.72 to 6.26) | <0.001 |
| **$REE_{FFM}$ (kJ/min)** | 4.61 (4.34 to 4.90) | 5.22 (5.10 to 5.33) | 0.002 | 4.96 (4.81 to 5.12) | 5.16 (4.96 to 5.37) | 5.69 (5.49 to 5.88) | <0.001 |
| **$REE_{FFM + FM}$ (kJ/min)** | 5.14 (4.87 to 5.67) | 5.13 (5.03 to 5.23) | NS | 5.14 (4.97 to 5.30) | 5.07 (4.87 to 5.26) | 5.18 (4.88 to 5.48) | NS |
| **RQ** | 0.827 (0.802 to 0.853) | 0.816 (0.806 to 0.825) | NS | 0.814 (0.800 to 0.829) | 0.823 (0.804 to 0.842) | 0.813 (0.794 to 0.829) | NS |
| **$RQ_{FM}$** | 0.806 (0.766 to 0.846) | 0.817 (0.807 to 0.827) | NS | 0.809 (0.792 to 0.825) | 0.826 (0.806 to 0.846) | 0.828 (0.799 to 0.857) | NS |
| **Fat Oxidation (mg/min)** | 44.7 (29.8 to 59.5) | 63.1 (57.7 to 68.8) | 0.025 | 57.2 (49.0 to 65.4) | 61.9 (5.8 to 73.0) | 73.7 (63.3 to 84.0) | 0.012 |
| **Fat $Oxidation_{FFM}$ (mg/min)** | 52.1 (37.7 to 66.6) | 62.2 (56.9 to 67.4) | NS | 59.7 (51.8 to 67.5) | 60.4 (49.9 to 70.9) | 67.9 (57.7 to 78.0) | NS |
| **Fat $Oxidation_{FFM + FM}$ (mg/min)** | 80.6 (59.5 to 101.7) | 60.2 (54.9 to 65.4) | NS | 67.2 (58.6 to 75.8) | 56.4 (46.1 to 66.6) | 46.0 (30.3 to 61.6) | NS |
| **Fat Oxidation as %EE** | 39.0 (29.8 to 48.2) | 44.8 (41.4 to 48.2) | NS | 44.7 (39.6 to 49.8) | 44.0 (37.1 to 50.9) | 45.6 (39.3 to 52.0) | NS |
| **Fat $Oxidation_{FM}$ as %EE** | 48.4 (34.2 to 62.6) | 44.3 (40.8 to 47.9) | NS | 47.3 (41.4 to 53.1) | 42.7 (35.7 to 49.7) | 38.4 (27.9 to 48.9) | NS |

**Table 4.2: Fasting Resting Energy Expenditure, RQ and Fat Oxidation according to BMI category**

Data represent means and 95% confidence intervals.
Means are adjusted for FFM and FM when indicated below the variable.
[a] p value for difference between lean and obese groups
[b] p value for difference between lean group and different obese BMI categories

| | Lean | Obese | p value[a] | Obese BMI Categories | | | |
|---|---|---|---|---|---|---|---|
| | | | | 30 – 35 | 35 – 40 | >40 | p value[b] |
| Postprandial EE* (kJ/min x 180min) | 982 (950 to 1014) | 973 (963 to 984) | NS | 971 (955 to 987) | 975 (954 to 996) | 976 (952 to 1000) | NS |
| Postprandial $EE^*_{FFM + FM}$ (kJ/min x 180min) | 1001 (957 to 1044) | 973 (963 to 984) | NS | 976 (958 to 994) | 970 (948 to 991) | 972 (939 to 1003) | NS |
| Postprandial RQ* | 0.801 (0.787 to 0.816) | 0.803 (0.798 to 0.808) | NS | 0.802 (0.795 to 0.811) | 0.805 (0.796 to 0.815) | 0.800 (0.789 to 0.812) | NS |
| Postprandial $RQ^*_{FM}$ | 0.783 (0.763 to 0.804) | 0.804 (0.799 to 0.809) | NS | 0.798 (0.790 to 0.807) | 0.809 (0.799 to 0.819) | 0.815 (0.799 to 0.831) | NS |
| Postprandial Fat Oxidation* (g/min x 180 mins) | 12.8 (11.2 to 14.3) | 12.9 (12.4 to 13.4) | NS | 12.7 (11.9 to 13.5) | 12.9 (11.8 to 13.9) | 13.4 (12.2 to 14.5) | NS |
| Postprandial Fat $Oxidation^*_{FFM +FM}$ (g/min x 180 mins) | 15.1 (12.9 to 17.3) | 12.8 (12.3 to 13.3) | NS | 13.3 (12.4 to 14.2) | 12.5 (11.4 to 13.5) | 11.8 (10.2 to 13.4) | NS |
| Postprandial Fat Oxidation* as % of EE AUC | 49.5 (44.4 to 54.7) | 49.7 (48.0 to 51.5) | NS | 49.7 (47.0 to 52.3) | 49.4 (45.9 to 52.8) | 40.2 (46.4 to 54.0) | NS |
| Postprandial Fat $Oxidation^*_{FM}$ as % of EE AUC | 52.5 (45.2 to 59.9) | 49.6 (47.8 to 51.4) | NS | 50.5 (47.5 to 53.5) | 48.9 (45.4 to 52.) | 48.0 (42.5 to 53.5) | NS |

**Table 4.3: Postprandial Energy Expenditure, RQ and Fat Oxidation following a high fat load**

Values represent means and 95% confidence intervals

* Postprandial values adjusted for corresponding fasting values and energy content of test meal.

Means adjusted for FFM and FM when indicated below the variables

[a] p value for difference between lean and obese group means

[b] p value for difference between means for lean and obese BMI categories

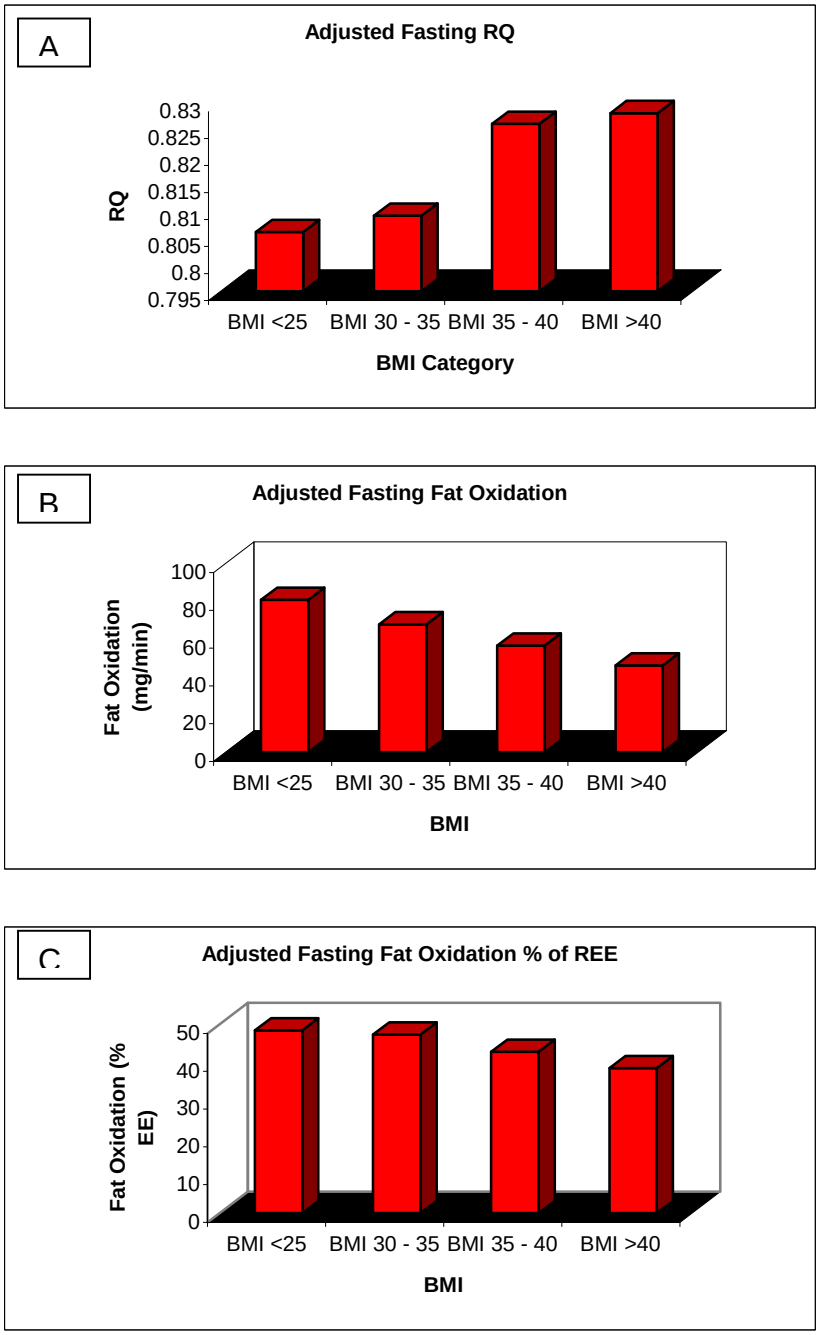
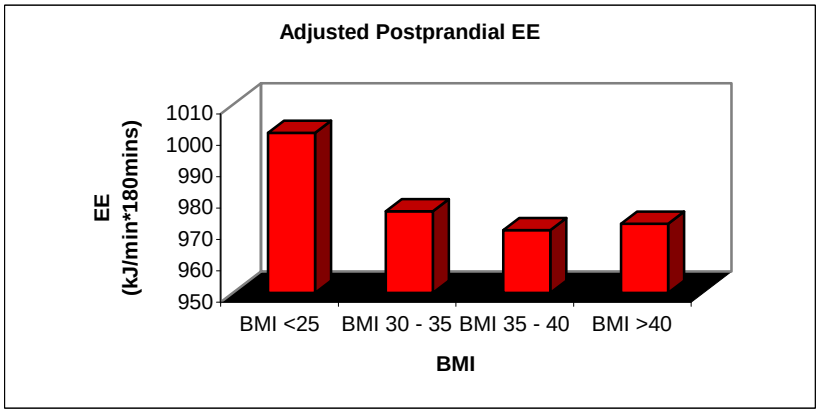
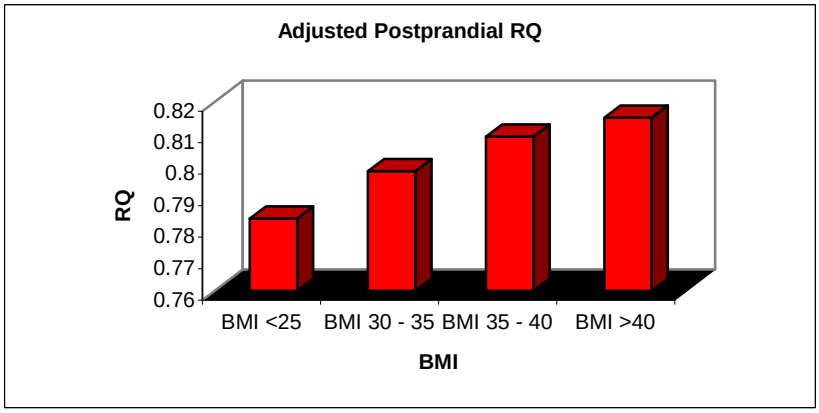



**Figure 4.2: Adjusted fasting RQ and fat oxidation demonstrating trend for increase in RQ and decrease in fat oxidation with increasing BMI**



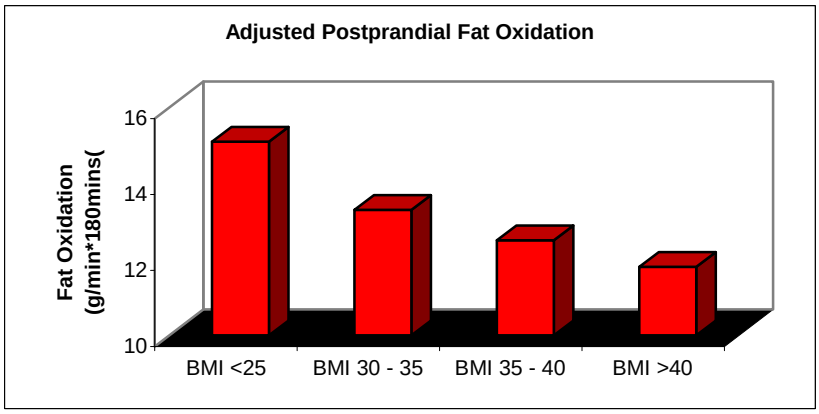




**Figure 4.3: Adjusted Postprandial EE, RQ and Fat Oxidation demonstrating trend for EE to decrease, RQ to increase and fat oxidation to decrease during the postprandial period**.

Table 4.4 summarises the relationships between either fasting or postprandial fat oxidation and physical, metabolic and dietary parameters. Whilst FFM correlated with fat oxidation when BMI was below 40, above this value, no significant correlation was seen. Instead, it was interesting to see that only FM became a significant correlate to fat oxidation when BMI was in excess of 40. In the post prandial state, these patterns persisted, with FFM correlating better to fat oxidation when BMI was below 40 and FM having a strong correlation with postprandial fat oxidation, only at the highest BMI category. As would be expected, postprandial fat oxidation is closely correlated to fasting fat oxidation.

A further, more detailed analysis of REE and postprandial EE is show in table 4.5. The strongest correlation for REE was with FFM (Table 4.5a). Only when BMI was very large (>40), did FM become significantly correlated to REE (Table 4.5a).

For postprandial EE, FFM continued to have a strong, positive correlation with postprandial EE across all levels of BMI. As with postprandial fat oxidation, postprandial EE correlated significantly with REE.

**Table 4.4a: Relationship between fasting fat oxidation and physical, metabolic and dietary parameters**

| Variable | Fasting Fat Oxidation (mg/min) | | | |
|---|---|---|---|---|
| | BMI Category | | | |
| | **<25** | **30 – 35** | **35 – 40** | **>40** |
| **Fat-Free Mass (kg)** | .589* | .374* | .497* | |
| **Fat Mass (kg)** | | | | .637** |
| **$HOMA_{IR}$** | | | | |
| **TAG (µmol/l)** | .626* | | | |
| **FFA (µmol/l)** | | | | .380* |
| **Leptin (ng/ml)** | .732* | | | |
| **Cortisol (nmol/l)** | .537* | | | |
| **IGF-1 (ng/ml)** | .645* | | | |

Values represent Pearson correlation coefficients. Parameters from table 4.1, which were not correlated to fat oxidation for any of the BMI categories, are not shown.
Blank spaces indicate no significant correlation.
* p< 0.05; * p< 0.01.

**Table 4.4b: Relationship between postprandial fat oxidation and physical, metabolic and dietary parameters**

| Variable | Postprandial Fat Oxidation $_{AUC}$ (mg/min*180 mins) | | | |
|---|---|---|---|---|
| | BMI Category | | | |
| | **<25** | **30 – 35** | **35 – 40** | **>40** |
| **Fat-Free Mass (kg)** | .710** | .683* | .440* | |
| **Fat Mass (kg)** | | | | .714** |
| **WHR** | | .334* | | |
| **$FFA_{AUC}$ (µmol/l * 180mins)** | | | .421* | |
| **Leptin (ng/ml)** | | -.380* | | |
| **Cortisol $_{AUC}$ (nmol/l*180mins)** | | | .569** | |
| **Fasting fat Oxidation (mg/min)** | .751** | .680** | .816** | .847** |

Values represent Pearson correlation coefficients. Parameters from table 1, which were not correlated to fat oxidation for any of the BMI categories, are not shown.
Blank spaces indicate no significant correlation. For postprandial hormones and metabolites, variables represent AUC for the postprandial period
* p< 0.05; * p< 0.01.

**Table 4.5a: Relationship between Resting Energy Expenditure and physical, metabolic and dietary parameters in lean and obese subjects**

| Variable | Resting Energy Expenditure (kJ/min) | | | |
|---|---|---|---|---|
| | BMI Category | | | |
| | <25 | 30 – 35 | 35 – 40 | >40 |
| **Fat-Free Mass (kg)** | .752** | .829** | .776** | .648** |
| **Fat Mass (kg)** | | | | .529** |
| **WHR** | | .384** | | .487** |
| **$HOMA_{IR}$** | | | | .700** |
| **TAG (µmol/l)** | | | | .408* |
| **Glucose (mmol/l)** | | .380** | | .599** |
| **Leptin (ng/ml)** | | -.466** | | |
| **IGF-1 (ng/ml)** | | | | -.460** |

Values represent Pearson correlation coefficients. Parameters from table 1, which were not correlated to resting energy expenditure for any of the BMI categories, are not shown.
Blank spaces indicate no significant correlation.
* p< 0.05; * p< 0.01.

**Table 4.5b: Relationship between Postprandial Energy Expenditure and physical, metabolic and dietary parameters in lean and obese subjects**

| Variable | Postprandial Energy Expenditure (kJ/min*180mins) | | | |
|---|---|---|---|---|
| | BMI Category | | | |
| | <25 | 30 – 35 | 35 – 40 | >40 |
| **Fat-Free Mass (kg)** | .928** | .875** | .756** | .589** |
| **Fat Mass (kg)** | | -.313* | | .651** |
| **WHR** | | .455** | | .439 |
| **$HOMA_{IR}$** | | | | .531** |
| **Glucose $_{AUC}$ (mmol/l*180mins)** | | .416** | | |
| **Leptin (ng/ml)** | | -.498** | | |
| **IGF-1 (ng/ml)** | | .333** | | -.480* |
| **REE (kJ/min)** | .911** | .922** | .881** | .940** |

Values represent Pearson correlation coefficients. Parameters from table 1, which were not correlated to resting energy expenditure for any of the BMI categories, are not shown.
For postprandial metabolites, variables represent AUC for the postprandial period
Blank spaces indicate no significant correlation.
* p< 0.05; * p< 0.01.

Table 4.6 shows the results from a stepwise multiple linear regression analysis for the determinants of fasting fat oxidation. The independent variables used in the models were taken from table 4.1. Results are represented for individual BMI categories as well as the group as a whole.

For lean subjects, fat oxidation was best determined by gender, fasting triglycerides and fasting leptin (adjusted $R^2$ 0.69). For the group with BMI 30 to 35, gender and WHR best determined fat oxidation (adjusted $R^2$ 0.252), whereas FFM and fasting cortisol levels best determined fat oxidation in those with a BMI of 35 to 40 (adjusted $R^2$ 0.385). In the group with the highest BMI, FM and habitual physical activity were the strongest determinants of fat oxidation (adjusted $R^2$ 0.519).

When the group was taken as a whole, over 36% of the variation in fat oxidation could be explained by FFM, FM, WHR and fasting FFA concentrations.

The stepwise regression models in Table 4.7 are the significant determinants of postprandial fat oxidation. Results are once again reported for individual BMI categories and the group as a whole. It was not surprising to discover that all models contained fasting fat oxidation as a significant determinant. In addition, for the lean subjects, FFM, FM and cortisol AUC were the significant determinants of postprandial fat oxidation (adjusted $R^2$ 0.951). When BMI was 30 to 35, FFM and FFA AUC, in addition to fasting fat oxidation explained just over 70% of the variance in postprandial fat oxidation. If BMI was 35 to 40, habitual physical activity became a significant determinant (adjusted $R^2$ 0.736). Similar variance in postprandial fat oxidation was explained by gender and fasting fat oxidation in the highest BMI group (adjusted $R^2$ 0.736). The group as a whole had nearly 64% of the variance in

postprandial fat oxidation explained by BMI and fasting fat oxidation (adjusted $R^2$ 0.639).

The final regression model (Table 4.8), examines the determinants of postprandial energy expenditure, across the different BMI categories.

Whilst REE had a strong influence upon all of the models, the influence of FFM is seen mainly in those subjects with relatively lower BMI: BMI <25 (adjusted $R^2$ 0.976), BMI 30 to 35 (adjusted $R^2$ 0.867). With high BMI (>40), WHR had a positive influence, with the model explaining 89%of the variance in postprandial EE.

Overall, REE, FFM and FFA AUC explained over 90% of the variance in postprandial EE.

| | Model | Variable selected | Regression Equation | Adjusted $R^2$ |
|---|---|---|---|---|
| **BMI <25** | 1 | Gender | 0.083- (0.044*female) | **0.300** |
| | 2 | TAG (µmol/l) | $0.119 – (0.037*female) – (7.06^{-05}*TAG)$ | **0.559** |
| | 3 | Leptin (ng/ml) | $0.112 – (0.018*female) – (6.01^{-05} TAG) – (0.003*Leptin)$ | **0.690** |
| **BMI 30 - 35** | 1 | Gender | 0.074 – (0.019*female) | **0.121** |
| | 2 | WHR | 0.199 –(0.036*female) – (0.131*WHR) | **0.252** |
| **BMI 35 - 40** | 1 | FFM (kg) | -0.022 + (0.01*FFM) | **0.203** |
| | 2 | Cortisol (nmol/l) | $-0.054 + (0.02*FFM) + (1.49^{-04}*Cortisol)$ | **0.385** |
| **BMI >40** | 1 | FM (kg) | -0.043 + (0.02*FM) | **0.422** |
| | 2 | Physical Activity (AU) | -0.176 + (0.02*FM) + (0.017*Physical Activity) | **0.519** |
| **All subjects** | 1 | FFM (kg) | -0.013 + (0.001*FFM) | **0.210** |
| | 2 | FM (kg) | -0.019 + (0.001*FFM) + (0.001*FM) | **0.273** |
| | 3 | WHR | 0.035 + (0.001*FFM) + (0.001*FM) – (0.096*WHR) | **0.308** |
| | 4 | FFA (µmol/l) | $4.96^{-05} +(0.002*FFM) + (4.83^{-04}*FM) –(0.1*WHR) + (4.96^{-05}*FFA)$ | **0.364** |

**Table 4.6: Stepwise linear regression analysis for determinants of fasting fat oxidation**
TAG, Triglyceride; WHR, Waist-to-Hip Ratio; FFM, Fat Free Mass; FM, Fat Mass, AU, Arbitrary units

| | Model | Variable selected | Regression Equation | Adjusted $R^2$ |
|---|---|---|---|---|
| **BMI <25** | 1 | Fasting Fat Oxidation (FO) (g/min) | 6.295 + (85.0*FO) | **0.513** |
| | 2 | FM | -3.687 + (110.9*FO) + (0.589*FM) | **0.672** |
| | 3 | FFM | -13.671 + (77.3*FO) + (0.685*FM) + (0.2*FFM) | **0.868** |
| | 4 | Cortisol (nmol/l) | -16.99 + (100*FO) + (0.68*FM) + (0.187*FFM) + ($6.09^{-05}$*Cortisol) | **0.951** |
| **BMI 30 - 35** | 1 | FFM | -0.367 + (0.225*FFM) | **0.449** |
| | 2 | Fasting Fat Oxidation (FO) (g/min | -1.789 + (0.165*FFM) + (81*FO) | **0.652** |
| | 3 | $FFA_{AUC}$ (µmol/l*180mins) | -6.421 + (0.198*FFM) + (67.6*FO) + ($3.86^{-05}$*FFA) | **0.706** |
| **BMI 35 - 40** | 1 | Fasting Fat Oxidation (FO) (g/min) | 5.904 + (110.5*FO) | **0.651** |
| | 2 | Physical Activity (AU) | 11.646 + (108.2*FO) – (0.76* Physical Activity) | **0.736** |
| **BMI >40** | 1 | Fasting Fat Oxidation (FO) (g/min) | 6.665 + (117.9*FO) | **0.661** |
| | 2 | Gender | 3.724 + (115.6*FO) + (3.73*Female) | **0.736** |
| **All subjects** | 1 | Fasting Fat Oxidation (FO) (g/min) | 5.681 + (115.2*FO) | **0.613** |
| | 2 | BMI | 2.557 + (106.8*FO) + (0.104*BMI) | **0.639** |

**Table 4.7: Stepwise linear regression analysis for determinants of postprandial fat oxidation (g/min*180mins)**
FFA, Free Fatty Acids; WHR, Waist-to-Hip Ratio; FFM, Fat Free Mass; FM, Fat Mass, AU, Arbitrary units

| | Model | Variable selected | Regression Equation | Adjusted $R^2$ |
|---|---|---|---|---|
| **BMI <25** | 1 | FFM (kg) | 195.63 + (12.5*FFM) | **0.873** |
| | 2 | REE (kJ/min) | -20.45 + (7.05*FFM) + (113.7*REE) | **0.915** |
| | 3 | $Cortisol_{AUC}$ (nmol/l*180mins) | -222.84 + (5.8*FFM) + (158*REE) + (0.002*$Cortisol_{AUC}$) | **0.954** |
| | 4 | $FFA_{AUC}$ (µmol/l*180mins) | -241.8 + (9.14*FFM) + (91.9*REE) + (0.002*$Cortisol_{AUC}$) + (0.002*$FFA_{AUC}$) | **0.976** |
| **BMI 30 - 35** | 1 | REE (kJ/min) | 60.78 + (117.4*REE) | **0.821** |
| | 2 | FFM (kg) | 132.95 + (109.9*REE) + (4.68*FFM) | **0.867** |
| **BMI 35 - 40** | 1 | REE (kJ/min) | 117.7 + (168.2*REE) | **0.759** |
| **BMI >40** | 1 | REE (kJ/min) | 176.57 + (159.7*REE) | **0.865** |
| | 2 | WHR | -108.72 + (142.5*REE) + (422.1*WHR) | **0.891** |
| **All Subjects** | 1 | REE (kJ/min) | 66.88 + (177.1*REE) | **0.882** |
| | 2 | FFM (kg) | 42.68 + (149.9*REE) + (2.89*FFM) | **0.899** |
| | 3 | $FFA_{AUC}$ (µmol/l*180mins) | -0.92 + (138.6*REE) + (3.65*FFM) + (0.001*$FFA_{AUC}$) | **0.905** |

**Table 4.8: Stepwise linear regression analysis for determinants of postprandial Energy Expenditure (kJ/min*180mins).**

FFA, Free Fatty Acids; WHR, Waist-to-Hip Ratio; FFM, Fat Free Mass; AU, Arbitrary units

## 4.4 Discussion

This study aimed to investigate the association between phenotypic and metabolic parameters and fat oxidation before and after a high fat challenge. It was also the aim to investigate whether any of these same parameters had an influence on postprandial energy expenditure. The main findings were that in the fasting state, fat oxidation tended to decrease with increasing BMI and was partially determined by fat free mass, fat mass, waist-to-hip ratio and fasting free fatty acid concentrations. Following the high fat test meal, fat oxidation continued to demonstrate a fall with increasing BMI and apart from fasting fat oxidation, the main determinant for postprandial fat oxidation was habitual physical activity value. Following the test meal, postprandial energy expenditure decreased with increasing BMI and was predicted mainly by resting energy expenditure, fat free mass and postprandial levels of free fatty acids.

**Fasting Fat Oxidation**

In the present study, fasting fat oxidation increased with increasing BMI category. This effect was due to the contribution of FFM and FM and once these variables were adjusted for, there was no difference in fasting fat oxidation across BMI categories.

There remains uncertainty as to the exact relationship between fat oxidation and body composition. Because a major determinant of REE is FFM (Ravussin, Lillioja et al. 1986; Nielsen, Hensrud et al. 2000), it would seem logical that FFM would be the major site for fat oxidation to occur and thus be a significant determinant of fasting fat oxidation. This has been confirmed in some studies (Nagy, Goran et al. 1996;

Toth, Sites et al. 1999) whereas others have found that fasting fat oxidation to be strongly correlated to FM (Schutz, Tremblay et al. 1992; Astrup, Buemann et al. 1994).

Using the slope of the regression of FFM on fasting fat oxidation, it can be estimated that if FFM were to increase by 10kg, fasting fat oxidation would increase by 29g/day. A similar increase in FM would result in fasting fat oxidation increasing by 7g/day. It must however be remembered that in the fasting state, fat oxidation provides a much greater proportion of energy expenditure than for the entire 24 hours (Buemann, Astrup et al. 1992). Therefore, extrapolating fasting fat oxidation to 24 hours is likely to lead to an overestimation.

Although questions have been raised about the covariation of FFM with FM, in the stepwise regression model, both were entered as independent variables and both remained significantly correlated to fat oxidation, although the association for FFM was greater than for that of FM.

Finding an association between fasting fat oxidation and FFA is consistent with work by Groop et al (Groop, Bonadonna et al. 1991). They determined that in the fasting state, FFA oxidation accounted for approximately 50% of basal lipid oxidation and that additionally, there was a positive correlation between circulating FFA and both FFA oxidation and total fat oxidation. From the regression analysis in the present study, FFA concentrations accounted for almost 9% of the variance in fasting fat oxidation.

As WHR was found to be negatively associated with fasting fat oxidation, it could be implied that central obesity and therefore insulin resistance, might influence fat oxidation in a negative manner, a finding reported in previous studies (Colberg, Simoneau et al. 1995; Kelley, Goodpaster et al. 1999)

**Postprandial Fat Oxidation**

During the postprandial period, there was a trend for fat oxidation to decrease with increasing BMI, although this was not statistically significant. The main determinant of postprandial fat oxidation was fasting fat oxidation, which is not surprising due to their very strong correlations across all categories of BMI (r = 0.751 to 0.847). 61% of the variation in postprandial fat oxidation could be explained purely by fasting fat oxidation. From the regression analysis, the only additional significant, albeit small, determinant of postprandial fat oxidation was habitual physical activity, explaining a further 2.5% in postprandial fat oxidation variance. The beta coefficient from the regression model indicated that increasing habitual physical activity has a negative association with postprandial fat oxidation. The relevance of this biologically is likely to be limited. This data conflicts with that of Aldred et al who found that following a high fat meal, increased levels of prior physical activity resulted in reduced postprandial insulin concentrations, which would encourage an increase in fat oxidation (Aldred, Hardman et al. 1995). The discordant results may in part be explained by differences in the methodology. In the present study, subjects were required to have stable body weight for the 3-month period prior to the study, whereas those subjects studied by Aldred underwent an exercise activity programmed for 3 months, before and after investigation, with associated weight loss.

Postprandial fat oxidation has been reported to be much higher in those individuals that have a habitual diet higher in dietary fat (Blundell, Cooling et al. 2002). In the current study, subjects tended to consume similar proportions of fat within their habitual diets, as reported by weighed food diaries. This might well explain why there

was no significant difference in postprandial fat oxidation seen between the different groups.

**Postprandial Energy Expenditure**

Although a lot of research has focussed on the thermic effect of food, the majority of studies have investigated thermogenesis following a carbohydrate load or a mixed meal (Segal, Albu et al. 1992; de Jonge and Bray 1997; Marques-Lopes, Forga et al. 2003). In the present study, the high fat load resulted in a very blunted response to postprandial energy expenditure in all subjects, although there was a tendency for lower values with increasing BMI. This finding is consistent with previous reports that a high fat load is unable to promote an increase in postprandial energy expenditure (Swaminathan, King et al. 1985). This is likely to explain why individuals who consistently consume a diet that is high in fat are more likely to gain weight over time.

Apart from REE, which was the dominant determinant of postprandial energy expenditure, a small but significant contribution was provided by FFM and $FFA_{AUC}$. The positive effect of FFAs upon postprandial energy expenditure has been reported by Schiffelers et al (Schiffelers, Saris et al. 2001). It has been suggested that FFA may stimulate mitochondrial uncoupling within the myocytes and, in doing so, generate energy (Schrauwen and Hesselink 2002). Whilst other studies have reported an association between postprandial thermogenesis and insulin resistance (Landsberg and Young 1993), this was not demonstrated in the present study, despite postprandial triglycerides being elevated.

What remains uncertain is whether the blunted fat-induced thermogenesis is a primary defect or an adaptation to the obese state.

In summary, fat oxidation tended to decrease with increasing BMI in the fasting and postprandial state, with an associated blunted postprandial response of thermogenesis from a high fat load. In combination, these factors would tend to encourage weight gain or regain in subjects consuming a diet that is habitually high in fat.

# Chapter 5

# The effect of four popular commercial weight loss programmes upon body composition and insulin sensitivity

## 5.1 Introduction

With the obesity epidemic showing no signs of relenting, the market for commercial dietary weight loss programmes is continuing to flourish. In 2004, the overall UK diet food and drink market had a value of £4.6billion and is estimated to increase to £5.3billion in 2009 (source Datamonitor).

Weight loss with energy restriction is accompanied by a variable reduction in lean and fat mass(Ross, Dagnone et al. 2000; Janssen, Fortier et al. 2002). Ideally, the aim of weight loss would be to reduce fat mass whilst maintaining lean mass. This is particularly important in older adults, in whom ageing itself is associated with loss in lean mass (Doherty 2001). Randomised controlled trials, in which macronutrient content has varied, have had varied success in achieving weight loss which is both clinically significant and metabolically beneficial. Bopp et al (Bopp, Houston et al. 2008) demonstrated that during negative energy balance, irrespective of whether subjects exercised or not, there was a linear relationship between protein intake and loss of lean mass. The greater the protein content of the diet, the smaller the proportion of weight that was lost as lean mass. Diets, which have a restricted carbohydrate content, for example the “Atkins Diet” (Atkins 1999.), have proven to be effective at achieving significant weight loss (Foster, Wyatt et al. 2003; Samaha, Iqbal et al. 2003). Brehm and colleagues discovered however, that although greater weight loss was achieved with a very low carbohydrate diet when compared to a low fat, calorie restricted diet, as well a significantly greater loss in fat mass, a

significantly greater reduction in lean mass was also seen (Brehm, Seeley et al. 2003).

As well as the change in body composition, macronutrient manipulation within diets can also have an impact upon hormones and metabolites. An improvement in insulin sensitivity has been seen with energy-restricted, low fat diets which varied in protein and carbohydrate content (Brinkworth, Noakes et al. 2004). The level of weight loss in this study was similar to that in the Diabetes Prevention Program (Rubin, Fujimoto et al. 2002) and the Finnish Diabetes Prevention Study (Tuomilehto, Lindstrom et al. 2001). Both studies demonstrated that the onset of type 2 diabetes could be delayed with weight loss and lifestyle alteration. When diets, which were either high in fat, protein or carbohydrate, were compared (McAuley, Hopkins et al. 2005), all had a significant reduction in fasting insulin and glucose levels although none was found to be more superior.

The BBC Diet Trials research project compared four commercial diet plans regularly followed in the United Kingdom: Atkins, Weight Watchers, Rosemary Conley and Slimfast. The results on weight loss (Truby, Baic et al. 2006) and cardiovascular risk markers (Morgan, Griffin et al. 2008) have already been reported.

In the present analysis, the aim was to see what impact these dietary interventions upon body composition and insulin sensitivity. The hypothesis being tested was that diet composition influenced body composition and insulin sensitivity after weight loss.

## 5.2 Methods

The study was an unblinded, randomised controlled parallel dietary intervention study. The dietary regimen represented some of the most common commercially available programmes in the United Kingdom. These were: 1) Dr Atkins' New Diet Revolution (Atkins 1999.) (a low carbohydrate diet); 2) Rosemary Conley's "Eat Yourself Slim" diet and fitness plan (an energy controlled, low fat healthy eating diet and weekly group exercise class); 3) Weight Watchers Pure Points Programme (an energy controlled, low fat, healthy eating diet) and 4) The Slim-Fast plan (a meal replacement programme). The control group were asked to delay entering into active weight loss and dieting for 6 months.

1. **Dr Atkins' New Diet Revolution.**

   This is a low carbohydrate diet. Subjects were provided with a copy of the book (Atkins 1999). There was an initial induction phase during which time carbohydrate intake was restricted to less than 20g per day. It is designed to promote lipolysis and ketosis. Subjects are maintained in this phase for a minimum of 2 weeks. Following on from this is the second phase – "on-going weight loss". During this phase the carbohydrate intake is gradually increased at approximately 5g per week until a level is reached when weight is not being lost. At this point carbohydrate intake is reduced by 5g, allowing weight loss to continue. Once a goal weight is achieved, a weight-maintenance phase is followed allowing sufficient carbohydrate to avoid weight gain.

2. **Rosemary Conley's "Eat Yourself Slim" Diet and Fitness Plan**.

   This diet plan involves selecting a variety of meals from menu suggestions outlined in the book. The meals are low fat, energy controlled. Breakfast, lunch and dinner along with a dessert are to be eaten. In addition, ¾ pint of skimmed milk daily, 3 pieces of fruit and 2 servings of vegetables and salad. In addition, subjects were to attend a weekly "Rosemary Conley exercise class". Weight Watchers Pre Points Programme.

3. **Weight Watchers Pure Points Plan.**

   This plan is based upon assigning a point value to everyday food. By knowing the number of calories and saturated fat content, using a "point calculator", a numeric value can be placed upon any meal or snack. Based upon the subject's weight and gender, a daily pot allowance is calculated. Subjects are then advised to plan their daily food plan to ensure the correct numbers of points are consumed. Subjects also attended a weekly group meeting where in addition to being weighed, general advice is provided on diet and lifestyle.

4. **The Slim-Fast Plan.**

   This plan is based upon meal replacements. For 2 meals a day, the normal meal is replaced by a liquid "shake" with a calorie content of approximately 220kCal. The main meal of the day was then to provide 500-600kCal per day and was chosen from a suggested menu. In addition, throughout the day, 3 snacks of 100kCal each were also recommended.

**Subject Recruitment and study design**

Five UK centres were involved in this study (Universities of Bristol, Nottingham, Surrey and Ulster (Coleraine), together with Queen Margaret University College Edinburgh).

Volunteers responded to advertisements in the media. Inclusion criteria were age 18 to 65 and a Body Mass Index (BMI) of between 27 and 40. Exclusion criteria were: coronary artery disease; diabetes (Type 1 or Type 2); renal, liver or respiratory failure; gout; obesity with a known cause (Cushing's disease, hypothyroidism); previous bariatric surgery; clinical depression; eating disorders; drug or alcohol misuse; lipid-lowering or antihypertensive medication; current usage of anti-obesity medication (Orlistat or Sibutramine); treatment for cancer; pregnancy or breast-feeding.

Patients provided full, informed written consent and ethical approval was obtained from the South-East Multi-Centre Research Ethics Committee.

Each centre aimed to recruit 60 subjects, allowing 12 subjects for each of the 4 interventions and 12 control subjects. It was calculated that 300 subjects would need to be recruited in total, to demonstrate a weight loss of 4kg (3kg body fat), with a significance level of 0.05 to achieve a power of 80%. All baseline tests were performed prior to randomisation. Of the 300 subjects recruited, 7 were excluded prior to randomisation due to either not being eligible for the study (4) or withdrawing consent (3).

**Biochemical analysis and Body Composition**

Plasma glucose and Triacylglycerol (TAG) were measured by standard automated spectrophotometric methods (reagent kits from Randox, County Antrim, UK). The inter-assay CV was <5% for these assays. Insulin was measured by immunochemiluminometric assay (Molecular Light Technologies, Cardiff, UK). The inter-assay CV was <10%. Insulin sensitivity was calculated using the updated homeostasis model assessment (HOMA2)(Levy, Matthews et al. 1998).

Body composition (lean and fat mass) was measured using whole body dual x-ray absorptiometry (DEXA). Three of the centres used a General Electric Lunar Prodigy™ densitometer (GE Healthcare Inc., Waukesha, WI) and the other two centres used Hologic™ densitometer (Hologic Inc, Bedford MA).

**Statistical Analysis**

The main analysis is based on an intention-to-treat basis with baseline values carried forward to replace missing values. Data are expressed as means ± sd or means ± 95% confidence intervals. Statistical analysis was performed using SPSS 11.5 for Windows. Before parametric testing, when variables were not normally distributed, natural log transformations were performed to satisfy conditions of normality.

Analysis of covariance showed that study centre did not affect weight loss and there was no difference in withdrawal rates between the 5 centres. Data for all participants was therefore analysed together.

ANOVA was used to examine differences between group means at baseline and 6 months. When a significant effect was indicated, post-hoc pair-wise testing was performed with Tukey's HSD (honestly significantly different) test.

Comparisons for changes in body composition and changes in insulin sensitivity were examined by analysis of covariance, using diet and gender as fixed (between subject) factors and baseline variables as covariates. A p value 0f <0.05 was considered as being statistically significant.

## 5.3 Results

Table 5.1 shows the baseline characteristics for the subjects according to the group into which they were randomised. Although overall, there were more women than men (73% vs. 27%), there were similar proportions in all 5 groups. There was no significant difference seen between the groups at baseline with respect to demographics, body composition or fasting biochemical markers. By the end of the study 83 subjects (28%) had withdrawn from the study. No difference in the diet, sex or centre was seen.

| | | Intervention Group | | | | |
|---|---|---|---|---|---|---|
| | | **Control** | **Atkins'** | **Rosemary Conley** | **Weight Watchers** | **Slimfast** |
| **Number subjects** | | 61(40) | 57(40) | 58(41) | 58(47) | 59(42) |
| **Gender** | **Male** | 15 | 15 | 16 | 16 | 17 |
| | **Female** | 46 | 42 | 42 | 42 | 42 |
| **Age** | | 41.2 (9.4) | 40.9 (9.8) | 40.3 (10.2) | 40.0 (10.9) | 38.9 (10.8) |
| **Height (m)** | | 1.66 (0.09) | 1.68 (0.10) | 1.68 (0.09) | 1.68 (0.09) | 1.67 (0.09) |
| **Fat Mass (%)** | | 39.2 (6.2) | 40.5 (6.1) | 39.4 (7.4) | 39.3 (6.6) | 40.6 (6.1) |
| **Lean Mass (%)** | | 57.7 (6.0) | 56.4 (5.9) | 57.5 (7.2) | 57.5 (6.5) | 56.4 (6.0) |
| **Glucose (mmol/L)** | | 5.48 (0.46) | 5.47 (0.48) | 5.57 (0.62) | 5.45 (0.52) | 5.54 (0.62) |
| **Insulin (pmol/L)** | | 63.7 (30.7) | 72.6 (40.0) | 77.9 (41.3) | 67.7 (40.3) | 80.5 (56.0) |
| **Triglyceride (mmol/L)** | | 1.56 (0.88) | 1.38 (0.69) | 1.40 (0.80) | 1.39 (0.70) | 1.50 (0.85) |

**Table 5.1**: **Baseline characteristic of subjects, according to dietary intervention group**
Results represent means (± sd). Independent T-Tests compared between group means.
Value in () for number of subjects represent number of subjects completing 6 months of the study

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### Change in weight

Figure 5.1 (and table 5.2) shows how the weight changed from baseline. No significant weight loss was seen in the control group. Each of the intervention groups lost a significant amount of weight, (mean (kg) ± sd), Atkins' 6.12 ± 6.39, Rosemary Conley 5.92 ± 5.41, Weight Watchers 6.50 ± 5.37 and Slim-Fast 4.60 ± 5.66, all p<0.001. Whilst each group loss significantly more weight than the control group (p < 0.001 for each group), there was no significant difference in weight loss between the intervention groups.

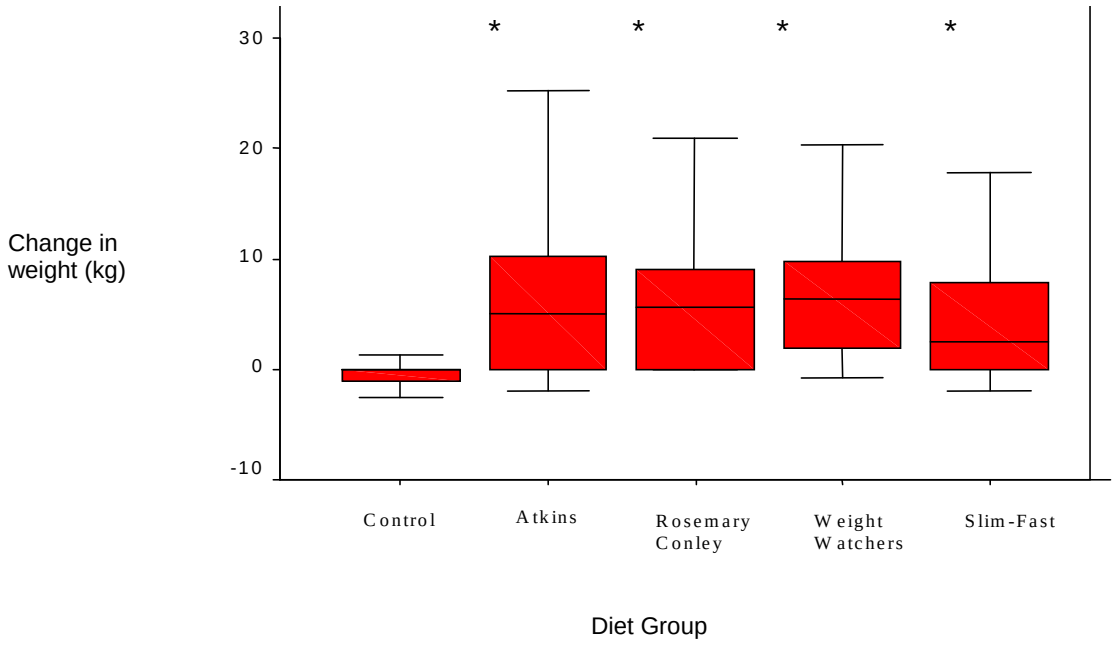


**Figure 5.1: Mean weight change (kg) after 6 months of dietary intervention**.
Error bars represent 95% confidence intervals.
* p < 0.001 for changes from baseline and for difference between control.

Table 5.2 also shows the mean values for BMI, waist circumference, body composition and insulin sensitivity.

All groups had a significantly lower BMI after 6 months in comparison to controls ($p<0.001$). There was also a significant difference in BMI between those subjects following Weight Watchers and Slim-fast (29.0 vs. 30.7, $p = 0.015$).

Waist circumference, after 6 months, differed significantly from controls for Atkins' ($p = 0.039$), Rosemary Conley ($p = 0.006$) and Weight Watchers ($p< 0.001$).

When body composition was assessed after 6 months, the mean fat mass for Rosemary Conley ($p = 0.043$) and Weight Watchers ($p = 0.007$) were the only significant decreases seen compared to the control group. There was no significant difference seen in lean mass between any of the groups after 6 months of intervention.

No change in insulin sensitivity, (HOMA-IR), was seen in any of the intervention groups after 6 months in comparison to the control groups ($p > 0.05$ for all groups). There was however, a significant improvement in HOMA-IR from baseline ($p<0.05$).

| | | Intervention Group | | | | |
|---|---|---|---|---|---|---|
| | | **Control** | **Atkins'** | **Rosemary Conley** | **Weight Watchers** | **Slim-Fast** |
| BMI | Baseline | 31.4 (2.8) | 31.9 (2.2) | 31.6 (2.6) | 31.3 (2.7) | 32.3 (3.0) |
| | 6 months | 31.6 (2.9) | **29.9 (2.6)[a]** | **29.5 (3.6)[a]** | **29.0 (3.0)[a]** | **30.7 (3.1)[a b]** |
| **Weight (kg)** | Baseline | 87.1 (12.9) | 90.3 (12.7) | 89.4 (12.9) | 88.8 (13.2) | 90.3 (14.2) |
| | 6 months | 87.7 (13.2) | **84.3 (10.4)[a]** | **83.5 (13.8)[a]** | **82.3 (13.3)[a]** | **85.7 (12.5)[a]** |
| **Waist Circumference (cm)** | Baseline | 101.3 (14.5) | 102.1 (10.6) | 100.9 (11.2) | 100.0 (10.2) | 101.3 (11.6) |
| | 6 months | 99.3 (10.3) | **94.0 (9.5)[a]** | **92.9 (10.5)[a]** | **91.1 (10.3)[a]** | 94.9 (9.9) |
| **Fat Mass (kg)** | Baseline | 33.5 (6.2) | 35.7 (5.8) | 34.5 (7.2) | 34.1 (6.5) | 36.0 (6.7) |
| | 6 months | 33.9 (6.4) | 31.1 (7.1) | **30.0 (9.0)[a]** | **29.3 (7.3)[a]** | 32.5 (6.8) |
| **Lean Mass (kg)** | Baseline | 49.8 (10.8) | 50.4 (10.6) | 50.8 (11.1) | 50.7 (11.2) | 50.6 (11.3) |
| | 6 months | 50.0 (10.5) | 49.6 (10.0) | 49.9 (10.4) | 49.3 (10.6) | 49.8 (10.7) |
| **HOMA-IR** | Baseline | 1.212 (0.699) | 1.372 (0.732) | 1.500 (0.801) | 1.285 (0.746) | 1.523 (1.048) |
| | 6 months | 1.277 (0.699) | **1.171 (0.745)[c]** | **1.234 (0.717)[c]** | **1.137 (0.752)[c]** | **1.456 (1.071)[c]** |

**Table 5.2**: **Unadjusted Mean (± sd) for baseline and 6 months anthropometric, body composition and metabolic hormone measurements**.

a = significant difference from control value (p = 0.01)
b = significant difference from Weight Watchers (p= 0.01)
c= significant difference from baseline value (p<0.05)

Table 5.3 shows how the change in body composition and insulin sensitivity varies according to diet groups. Means have been adjusted as indicated.

All 4 diet groups lost significantly more fat mass than the control group ($p < 0.001$). Although there was no significant difference between the diets, there was a suggestion that subjects on the Slim-Fast plan lost less fat mass than then those on the other 3 plans.

The only diet to have a differing amount of lean mass loss compared to controls was the Weight Watchers group ($p = 0.016$).

Even after adjusting means, insulin sensitivity did not change significantly in any of the groups relative to controls. Despite this, there did appear to be a very trivial decrease on HOMA-IR in the Slim-Fast group relative to the other 3 diet plans.

| | Intervention Group | | | | |
|---|---|---|---|---|---|
| | **Control** | **Atkins'** | **Rosemary Conley** | **Weight Watchers** | **Slim-Fast** |
| **Δ Fat Mass (kg)** | -0.32 | 4.57 | 4.50 | 4.89 | 3.46 |
| **Adjusted Δ Fat Mass** [a] | 1.04 (-0.02 to 2.10) | **4.35*** **(3.34 to 5.36)** | **4.43*** **(3.41 to 5.46)** | **4.18*** **(3.17 to 5.19)** | **3.59*** **(2.60 to 4.58)** |
| **Δ Lean Mass (kg)** | -0.22 | 0.968 | 0.919 | 1.488 | 0.745 |
| **Adjusted Δ Lean Mass** [b] | 0.35 (-0.07 to 0.772) | 0.80 (0.41 to 1.20) | 0.71 (0.31 to 1.10) | **1.22**** **(0.83 to 1.60)** | 0.71 (0.33 to 1.09) |
| **Δ HOMA-IR** | -0.082 | 0.233 | 0.267 | 0.147 | 0.082 |
| **Adjusted Δ HOMA-IR** [c] | 0.075 (-0.089 to 0.239) | 0.143 (-0.001 to 0.287) | 0.102 (-0.050 to 0.254) | 0.022 (-0.118 to 0.163) | 0.003 (-0.138 to 0.144) |

**Table 5.3: Adjusted means for change in fat mass, lean mass and HOMA-IR**

* $p < 0.001$; ** $p = 0.016$.

Means were adjusted for gender, baseline weight, height and waist in addition to those factors indicated below the variables:

a = adjusted for lean mass and delta lean mass

b = adjusted for delta fat mass

c = adjusted for delta lean mass, delta fat mass and baseline HOMA-IR

## 5.4 Discussion

The aim of this study was to investigate what effects four commercially available diet regimes would have upon body composition and insulin sensitivity. Each diet resulted in significant weight loss, the majority of weight being lost as fat mass with minimal changes in lean mass. When the diets were compared, no one diet was shown to better with regards to total weight loss. With respect to insulin sensitivity, three of the diets (Atkins', Weight Watchers and Rosemary Conley) were found to have improved insulin sensitivity following the 6 months of dieting. After adjusting for baseline variables, although no significant difference was seen, there was a trend that these three diets tended to be more effective at improving insulin sensitivity that the Slim-Fast plan controls.

### Body Composition

The present study demonstrated that, over a 6 month period, fat mass loss of between 3.6 and 4.4kg could be achieved with any one of four of the most popular commercially weight loss programmes currently followed in the United Kingdom. This represented a reduction of between 6.9% and 9.3% of baseline weight. This degree of weight loss was achieved in the Xendos study (Torgerson, Hauptman et al. 2004) using the anti-obesity agent Orlistat, which demonstrated that in doing so, the incidence of type 2 diabetes could be reduced. The Diabetes Prevention Programme (Rubin, Fujimoto et al. 2002) also showed that weight reduction of 7%, in combination with exercise, could achieve a relative risk reduction in the incidence of Type 2 Diabetes of 48%. Both of these studies were, however, looking at sustained weight loss

between 3 and of 4 years rather than the relatively short duration within the present study.

Similar to other studies (Dansinger, Gleason et al. 2005; Gardner, Kiazand et al. 2007), the present study compared several popular diet programmes to see which, if any, was better at achieving significant weight loss as well as looking from which components of body composition this was achieved. The present study, however, also used a control group for comparison.

All diets achieved a significant weight loss from baseline as well as in comparison to the control group. There was however, no significant difference demonstrated between the diets. These findings are similar to those of Dansinger et al (Dansinger, Gleason et al. 2005), who found that the weight loss achieved by Atkins', Weight Watchers, Zone (macronutrient balanced) and Ornish (low fat) diets correlated to the level of adherence to the diets rather than to the diet itself. In contrast, when Gardner et al (Gardner, Kiazand et al. 2007) compared Atkins', Zone, Ornish and LEARN (high carbohydrate) diets, they found that after 6 months, the Atkins' group lost significantly more weight than the other three. In all three studies, as in the present study, analysis was performed on an intention to treat basis. One explanation for the differences seen with weight loss could be explained by variations in the attrition rates seen within the studies. In the present study, 28% of subjects dropped out during the course of the study and in Dansinger et al (42%), both of which are markedly higher than in Gardner et al (20.3%).

As well as losing similar amounts of weight, each diet lost approximately 75% of this weight as fat. This degree of reduction in fat mass has also been seen

when meal replacements with or without exercise have been used in pre-menopausal women (Deibert, Konig et al. 2007). When Johnston et al studied ketogenic and non-ketogenic low carbohydrate diets (Johnston, Tjonn et al. 2006), although they found no significant difference in the proportion of weight that was lost as fat mass, there was tendency for more of the weight to be lost as fat with the non-ketogenic (76%) as compared to the ketogenic diet (54%). This study was, however, of short duration, 6 weeks, with a small number of participants in each group, 9 and 10 respectively.

In another study using diets which were either high in carbohydrate, fat or protein (McAuley, Smith et al. 2006), again the majority of weight was lost as fat, although there were some non-significant differences between the groups (70%, 65% and 57% respectively). When Meckling et al compared low fat and low carbohydrate diets(Meckling, O'Sullivan et al. 2004), both demonstrated significant reductions in fat mass, with the low fat diets losing more weight as fat (79%) compared to 59% in the low carbohydrate group, although this was not statistically significant.

With the majority of weight being lost as fat, Atkins', Rosemary Conley and Slim-Fast diets lost approximately 16% of weight as lean mass, which was not statistically significant. In contrast, there was almost 23% weight loss as lean mass in the group following Weight Watchers. Whilst some studies have suggested that maintaining dietary protein can minimise the amount of lean mass lost during dieting (Meckling, O'Sullivan et al. 2004; Bopp, Houston et al. 2008), others have not (Noakes, Keogh et al. 2005). When food diaries returned by subjects in the present study were analysed by Morgan et al (Morgan, Griffin et al. 2008), there did not appear to be any major difference in

the proportion of energy as protein (26% Atkins', 20% Rosemary Conley, 20% Weight Watchers and 20% Slim-Fast). It must be remembered however, that only a small proportion of subjects returned food diaries and therefore the lack of difference may be related to an effect of underreporting. Nevertheless, it would appear that the pattern of weight loss achieved in the present study is consistent with and on occasion better than published data, and represents an advantageous way of losing weight with preservation of metabolically active lean tissue.

**Insulin Sensitivity**

When compared to baseline, three of the diets (Atkins', Rosemary Conley and Weight Watchers) demonstrated an improvement in insulin sensitivity as measured by HOMA-IR. There was however, no significant difference between the groups or indeed, in comparison to the control group. It has previously been shown that insulin sensitivity is correlated to fat mass (Ouyang, Sung et al. 2004); the finding that those on the Slim-Fast diet did less well at improving HOMA-IR could therefore be explained by the smaller reduction in fat mass that was seen in this group than in the other three groups. That none of the diets demonstrated significant reductions in HOMA-IR are similar to findings in studies that have compared energy restricted diets with or without exercise (Markovic, Jenkins et al. 1998; Janssen, Fortier et al. 2002), or diets with different macronutrient composition (Dansinger, Gleason et al. 2005; McLaughlin, Carter et al. 2006; Gardner, Kiazand et al. 2007). It would appear that it is weight loss and specifically loss of fat mass, which is

important in improving insulin sensitivity rather than the specific type of dietary intervention followed.

One of the advantages of this study is that it allowed dietary intervention programmes to be compared to a control group of subjects who also expressed a desire to lose weight. The fact that the control group had minimal change in weight can provide reasonable assurance that observed changes are truly reflective of the intervention and not due to selection of highly motivated individuals.

Subjects also had minimal contact with research staff during the six months of the study; the only assistance received was that which was part of their weight loss programme (Weight Watchers, Rosemary Conley).

One of this study's limitations is that the attrition rate was relatively high. We therefore made assumptions about missing data, replacing missing data by baseline values. This may well have acted as a confounding factor in the interpretation of the results. Assuming that baseline variables remained constant for those subjects who did not complete the study has been shown to be a reasonable, if somewhat imprecise estimation (Ware 2003).

Nonetheless, this study demonstrates that any of the common weight loss programmes followed by individuals in the United Kingdom can help loss weight in a manner which facilitates the majority of weight loss as fat which has a metabolically beneficial effect in improving insulin sensitivity and thus potentially reducing the risk of developing type 2 diabetes.

# Chapter 6

# General Discussion

The aim of this thesis was to evaluate the relationship of phenotypic characteristics to: resting energy expenditure and changes with weight loss, fat oxidation in obesity and diet and body composition and insulin resistance in obesity.

In chapter 3, the dominant factors, which determined REE, were body weight and percentage body fat, with an additional contribution from $HOMA_{IR.}$ After the dietary intervention, body weight and percentage body fat remained significant predictors of post intervention REE. As one might expect, baseline REE had a major influence upon post intervention REE. The absolute amount of weight that was lost also influenced post intervention REE. From the regression analysis, 77% of the variance in post intervention REE can be represented by the equation:

**Ln (REE kJ/min) = 0.842 + (0.004 * weight (kg)) – (0.003 * % body fat) + (0.087 * baseline REE (kJ/min) – (0.009 * weight loss (kg)).**

Additionally, in chapter 4, postprandial energy expenditure was investigated following a high fat load. EE was found to decrease with increasing BMI and from regression analysis, by measuring REE, FFM and postprandial levels of FFA, 90% of postprandial fat oxidation could be predicted by using the following equation:

**Fat oxidation (kJ/min*180min) = -0.92 + (138.6 * REE (kJ/min) + (3.65 * FFM (kg)) + (0.001 * $FFA_{AUC}$ (µmol/l*180min).**

Chapter 4 examined how phenotypic and metabolic characteristics influenced fat oxidation in the fasting and postprandial state. FFM and FM were the main determinants of fasting fat oxidation, with additional contributions from WHR and FFA concentrations. The high fat test meal resulted in a blunted postprandial response in fat oxidation, with only habitual physical activity providing any significant, albeit small, influence to the fasting fat oxidation rate in the postprandial state. This suggested that obese subjects may be less well equipped to deal with high dietary fat, thereby encouraging weight gain in the long term due to a net positive fat energy balance.

The study in chapter 5 involved investigating 4 different dietary approaches for weight loss, commonly followed in the UK, to see whether differences could be detected with regards to patterns of weight loss and the influence of these diets upon insulin sensitivity. Each of the dietary programmes (Atkins', Weight Watchers, Rosemary Conley and Slim-Fast) was equally effective at achieving weight loss in a metabolically favourable manner, as the majority of weight which was lost was as FM. Although insulin sensitivity did improve in 3 of the 4 diets, the improvement was more a reflection of reductions in FM rather than the particular dietary intervention.

During the two intervention studies, significant weight loss was achieved over varying lengths of time (10 weeks to 6 months).

Weight loss is the most effective treatment for obesity-related physical and psychological co-morbidities. Modest weight loss can result in major

improvements in co-morbidity such as diabetes (Wing, Venditti et al. 1998) and dyslipidaemia (Poobalan, Aucott et al. 2004) even if BMI after weight loss remains in the obese range. It has also been shown that more favourable outcomes are seen in those individuals that were weight stable after weight loss in comparison to those who were naturally at a similar weight (Dixon, Anderson et al. 2004).

Weight loss has a potent ability at improving insulin sensitivity most specifically by the reduction in visceral adiposity (Goodpaster, Kelley et al. 1999). In the study described in chapter 5, weight loss achieved by dietary interventions resulted in an improvement in $HOMA_{IR}$, with the best results in those diets achieving the greatest reduction in fat mass. The finding that waist circumference, which is known to correlate to visceral obesity (Valsamakis, Chetty et al. 2004; Ness-Abramof and Apovian 2008), was significantly reduced in these diets, suggests that a significant amount of fat mass loss must have occurred from visceral areas.

In both diet studies, it was weight loss per se that proved to be the important factor whether looking at either energy expenditure or insulin sensitivity. Also, although epidemiological evidence would suggest that hyperinsulinaemia and insulin resistance is linked to the increased ingestion of saturated fats (Mayer, Newman et al. 1993; Parker, Weiss et al. 1993), neither the moderate fat diet (40%-45%) from chapter 3 or the Atkins' diet (high fat, low carbohydrate) in chapter 5, demonstrated any unfavourable outcomes with regard to weight loss or insulin sensitivity. Stern et al have reported a more favourable outcome with low carbohydrate, high fat diets in relation to blood lipids over 12

months (Stern, Iqbal et al. 2004), even when weight loss was similar in comparison to a conventional (low fat) weight loss diet.

Weight loss, with its associated reduction in FFM and FM, would be expected to result in a decreased REE (Doucet, St Pierre et al. 2000) and that this would be in proportion to the decreases in body substance (Doucet, St-Pierre et al. 2001). With weight loss of 10%, Leibel et al (Leibel, Rosenbaum et al. 1995) reported that REE decreased by 573kJ/d more than predicted from baseline characteristics. There seem therefore, mechanisms in place to improve energetic efficiency - 'adaptive thermogenesis', which impedes weight loss and favours a gradual regain in weight. Factors, which are thought to influence adaptive thermogenesis, include insulin (Schwartz, Figlewicz et al. 1992; Porte, Baskin et al. 2002), thyroid hormone (Toubro, Sorensen et al. 1996) and sympathetic nervous system activity (Rosenbaum, Hirsch et al. 2000). Also, fat depletion in itself has also been considered as a determining factor for adaptive thermogenesis (Dulloo and Jacquet 2001). Therefore, the effect of different dietary regimens upon adaptive thermogenesis, poses an attractive area for future research.

## Future work

One of the limitations of the study in chapter 3 was that it was of relatively short duration. It would therefore be interesting to investigate whether a longer period of dietary intervention, say 6 months, would provide similar findings with regards to post intervention REE. It would also allow the evaluation of the two diets as to whether, over a longer period of time, subjects were able to

adapt better to the higher fat content and therefore demonstrate better improvements in fat oxidation.

Further work, using the different diet plans in chapter 5, could be to specifically study a more insulin resistant population. Ideally, we would want to directly measure insulin sensitivity, as inclusion criteria, however, this may prove to be both time consuming and expensive. Because of the link between waist circumference and visceral fat (Valsamakis, Chetty et al. 2004; Ness-Abramof and Apovian 2008), and visceral fat being a marker of insulin resistance (Gastaldelli, Cusi et al. 2007; Hayashi, Boyko et al. 2008), measuring waist circumference  at screening could therefore be used as an inclusion criteria.

# Chapter 7

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