The effect of gender and rimonabant on energy expenditure, gene expression and body fat distribution in obesity

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Rimonabant, a cannabinoid antagonist, had emerged as a promising drug in the fight against obesity. In animals, rimonabant had been shown to affect both appetite and energy expenditure. Further investigation was required to determine the direct metabolic effect of rimonabant in humans.

Fourteen obese postmenopausal women with a mean body mass index (BMI) of 32.5kg/m\(^2\) (SEM ±0.44) were randomised to receive either 20mg rimonabant daily alongside an isocaloric diet (drug group) or a hypocaloric diet designed to result in matching weight loss to that in the drug group (diet group). The aim was to determine if, when energy intake was maintained at pre treatment levels, rimonabant induced weight loss through an effect on energy expenditure and to also determine the direct effects of rimonabant on body fat distribution and gene expression. As weight loss itself may alter metabolism, a control group undertaking a dietary intervention matched for the weight loss in the rimonabant group were investigated.

In this trial it was demonstrated, for the first time in humans, that treatment with rimonabant, whilst daily energy intake was maintained at pre-treatment levels, still induced weight loss (2.6±0.5kg). In a matched group, who followed a dietary intervention to achieve the same weight loss (3.1±1.1kg), there was a decrease in resting energy expenditure (REE) (p=0.05) which was not found in the rimonabant group, suggesting that rimonabant may influence energy expenditure. A decrease in total body fat (p=0.046) and subcutaneous abdominal fat (p=0.028) was found in the diet group only. No significant differences in gene expression were found in muscle, however in adipose tissue there was a difference in expression of PPAR (Peroxisome Proliferator-activated Receptor) delta between the two groups (p=0.006) with PPAR delta decreasing post treatment in the rimonabant treated group.
Abstract

The trial produced interesting results with regards to the effect of rimonabant on REE and reinforced that the endocannabinoid system is a good potential target in the treatment of obesity. As antagonism of central receptors has been found to cause depression, however, an alternative medication to rimonabant that only produces the beneficial peripheral effects on energy expenditure is required.

Previous research has demonstrated that not all obese individuals display the expected metabolic risk factors and there is a distinction between the metabolically ‘healthy’ and ‘unhealthy’. Factors that may influence metabolic risk, such as body fat distribution and variations in lipid metabolism, require further research in order that future treatments may be targeted effectively. Therefore a follow on study to the main rimonabant intervention was undertaken to gain a comprehensive understanding of the gender differences in energy expenditure, body fat distribution and lipid metabolism in 12 obese males (mean BMI 31.40 ± 0.34 kg/m²) and 12 obese postmenopausal females (mean BMI 32.39 ± 0.53 kg/m²). Results showed that absolute REE was significantly higher in males than females (p<0.01), however, once expressed per kg fat free mass (FFM), females had the significantly higher REE (p=0.01). Males had significantly higher levels of internal fat (p=0.001) and visceral fat (p=0.003) and significantly lower levels of total body fat (p=0.002) and subcutaneous abdominal fat (p=0.004) than females. In adipose tissue, a significantly greater expression of Lipoprotein Lipase (LPL) was identified in females than males (p=0.01), but there were no further significant differences in gene expression in adipose or muscle tissue between the genders.

As there are gender differences in metabolism in obesity, there may be gender specific responses to any newly developed anti-obesity medications and these differences need to be evaluated.
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DECLARATION OF ORIGINALITY

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.

The author was responsible for the following elements of each trial:

Pilot study
Writing and submitting the ethics application, production of study paperwork, study administration, recruitment of subjects and subject retention, screening of potential subjects, dietary intervention, analysis of diet diaries, anthropometric measurements, measurement of resting energy expenditure, analysis of resting energy expenditure recordings, measurement of activity energy expenditure, and analysis of activity energy expenditure recordings.

Rimonabant study
Alterations to study protocols, production of study paperwork, study administration, recruitment of subjects and subject retention, screening of potential subjects, dietary intervention, analysis of diet diaries, administration of the Beck Depression
Inventory II, anthropometric measurements, measurement of resting energy expenditure, analysis of resting energy expenditure recordings, analysis of fat and carbohydrate oxidation rates, measurement of activity energy expenditure, analysis of activity energy expenditure recordings, measurements of 24 hour urine collection and analysis of urinary nitrogen excretion data, analysis of MRI data provided by Hammersmith Hospital, assisting clinicians with muscle and fat biopsies, laboratory processing of biopsy samples and rtPCR measurement of gene expression, analysis of rtPCR results.

Gender differences study

Jointly writing the study protocol and submitting the ethics application, production of study paperwork, study administration, recruitment of subjects and subject retention, screening of potential subjects, analysis of diet diaries, anthropometric measurements, measurement of resting energy expenditure, analysis of resting energy expenditure recordings, analysis of fat and carbohydrate oxidation rates, measurement of activity energy expenditure, analysis of activity energy expenditure recordings, measurements of 24 hour urine collection and analysis of urinary nitrogen excretion data, analysis of MRI data provided by Hammersmith Hospital, assisting clinicians with muscle and fat biopsies, laboratory processing of biopsy samples and rtPCR measurement of gene expression, analysis of rtPCR results.
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<td>1,2-diacylglycerol</td>
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<td>2-AG</td>
<td>2-arachidonoylglycerol</td>
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<td>ACOX1</td>
<td>Acyl CoA Oxidase 1</td>
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<tr>
<td>AEE</td>
<td>Activity Energy Expenditure</td>
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<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
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<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
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<tr>
<td>AMARES</td>
<td>Advanced Method for Accurate, Robust and Efficient Spectral Fitting</td>
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<td>AT</td>
<td>Adipose Tissue</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BMR</td>
<td>Basal Metabolic Rate</td>
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<tr>
<td>CB₁</td>
<td>Cannabinoid Receptor Type 1</td>
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<tr>
<td>CB₂</td>
<td>Cannabinoid Receptor Type 2</td>
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<tr>
<td>CD36</td>
<td>Cluster of Differentiation 36</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>CIU</td>
<td>Clinical Investigation Unit</td>
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<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
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<td>CPT1</td>
<td>Carnitine Palmitoyltransferase 1</td>
</tr>
<tr>
<td>CPT2</td>
<td>Carnitine Palmitoyltransferase 2</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised Tomography</td>
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<td>CV</td>
<td>Coefficient of Variation</td>
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<td>DGL</td>
<td>Diacylglycerol Lipase</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>DIT</td>
<td>Dietary Induced Thermogenesis</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>EDTA</td>
<td>Ethylene Diamine Tetraacetic Acid</td>
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<td>EE</td>
<td>Energy Expenditure</td>
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<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
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<td>FAAH</td>
<td>Fatty Acid Amide Hydrolase</td>
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<td>FFA</td>
<td>Free Fatty Acid</td>
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<td>FFM</td>
<td>Fat Free Mass</td>
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<td>GAPDH</td>
<td>Glyceraldehyde-3-Phosphate-Dehydrogenase</td>
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<td>GEM</td>
<td>Gas Exchange Monitor</td>
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<td>GLP-1</td>
<td>Glucagon-Like Peptide-1</td>
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<td>GPO-PAP</td>
<td>Glycerophosphate Oxidase-Peroxidase-4-Aminophenazone</td>
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<td>HbA1c</td>
<td>Haemoglobin A1C</td>
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<td>High-density Lipoprotein</td>
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<td>HOMA-IR</td>
<td>Homeostasis Assessment Model of Insulin Resistance</td>
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<td>Hs-CRP</td>
<td>High-sensitivity CRP</td>
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<tr>
<td>HSL</td>
<td>Hormone Sensitive Lipase</td>
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<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
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<td>IHCL</td>
<td>Intrahepatocellular Lipid</td>
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<tr>
<td>IMCL</td>
<td>Intramyocellular Lipid</td>
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<tr>
<td>IOTF</td>
<td>International Obesity Task Force</td>
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<tr>
<td>ISE</td>
<td>Ion Selective Electrode</td>
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<td>LDL</td>
<td>Low-density Lipoprotein</td>
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<td>LPL</td>
<td>Lipoprotein Lipase</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>MAGL</td>
<td>Monoacylglycerol Lipase</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>MRUI</td>
<td>Magnetic Resonance User Interface</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated Fatty Acid</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NAPE</td>
<td>N-arachidonoyl-phosphatidylethanolamine</td>
</tr>
<tr>
<td>NAT</td>
<td>N-acyltransferase</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>NEAT</td>
<td>Non-Exercise Activity Thermogenesis</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PAL</td>
<td>Physical Activity Level</td>
</tr>
<tr>
<td>PAV</td>
<td>Percentage Atheroma Volume</td>
</tr>
<tr>
<td>PLD</td>
<td>Phospholipase D</td>
</tr>
<tr>
<td>PPARα</td>
<td>Peroxisome Proliferator-activated Receptor Alpha</td>
</tr>
<tr>
<td>PPARδ</td>
<td>Peroxisome Proliferator-activated Receptor Delta</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome Proliferator-activated Receptor Gamma</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated Fatty Acid</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular Nucleus</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Daily Amount</td>
</tr>
<tr>
<td>REE</td>
<td>Resting Energy Expenditure</td>
</tr>
<tr>
<td>RIO</td>
<td>Rimonabant In Obesity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RQ</td>
<td>Respiratory Quotient</td>
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<tr>
<td>rtPCR</td>
<td>real-time Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated Fatty Acid</td>
</tr>
<tr>
<td>SIMCL</td>
<td>Soleus Intramyocellular Lipid</td>
</tr>
<tr>
<td>TAV</td>
<td>Total Atheroma Volume</td>
</tr>
<tr>
<td>TEE</td>
<td>Total Energy Expenditure</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>TIMCL</td>
<td>Tibialis Intramyocellular Lipid</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour Necrosis Factor Alpha</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid Stimulating Hormone</td>
</tr>
<tr>
<td>Type II DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>UCP1</td>
<td>Uncoupling Protein 1</td>
</tr>
<tr>
<td>UCP2</td>
<td>Uncoupling Protein 2</td>
</tr>
<tr>
<td>UCP3</td>
<td>Uncoupling Protein 3</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scales</td>
</tr>
<tr>
<td>VCO₂</td>
<td>Carbon dioxide production in ml/min</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen consumption in ml/min</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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Chapter 1

Introduction
1.1 Levels of obesity

In recent decades levels of obesity have increased to pandemic proportions. The World Health Organisation (WHO) estimated that in 2005 approximately 1.6 billion adults were overweight (BMI > 25 kg/m²) and over 400 million obese (BMI > 30 kg/m²) worldwide and projected that by 2015, 2.3 billion adults would be overweight and 700 million obese (WHO, 2006). In England in 2004, two thirds of the population were estimated to be overweight or obese (House of Commons, 2004). This rise in obesity comes with a huge cost, not only in financial terms, but with regards to increases in mortality and morbidity. In socio-economic terms, obesity and its associated co-morbidities are sizeable and were estimated to cost approximately £3.5 billion in England in 2002 and account for up to 8% of overall health budgets in Europe (International Obesity Task Force (IOTF), 2002).

1.2 The consequences of obesity

Obesity is well documented to be linked to health problems such as cardiovascular disease, type II diabetes mellitus, and some cancers and respiratory diseases (Richard and Boisvert, 2006) and there are a number of metabolic disorders associated with obesity including hypertension, dyslipidaemia, elevated serum triglycerides, increased glucose levels and insulin resistance (National Cholesterol Education Program (NCEP), 2002). These metabolic disorders arise from excessive fat accumulation in muscle, adipose tissue and major organs involved in energy regulation resulting in inflammation and impaired tissue integrity (Maynadier et al., 2009).
1.3 Defining obesity and metabolic risk

Obesity has historically been defined using body mass index (BMI) a measure of weight in kg in relation to height in m$^2$ (WHO, 1997). In epidemiological studies increases in BMI have been shown to be related to an increase in incidence of conditions such as coronary heart disease and diabetes (National Institutes of Health, 1998). The relationship between levels of obesity and coronary heart disease risk is not straightforward however. Some obese patients, with high levels of body fat, may not display the expected metabolic risk factors, whereas their moderately obese counterparts may be found to have a number of metabolic complications (Despres et al., 2001). Work from the 1940s first suggested that the complications associated with obese individuals were more closely associated with the location of excess fat in the body rather than the level of excess weight itself (Despres et al., 2001). Studies have shown that high levels of abdominal fat are a major risk factor for coronary heart disease and that abdominal girth is actually a better predictor of heart disease than BMI or weight alone (Dagenais et al., 2005). Many studies have used simple anthropometric measures such as waist circumference or waist hip ratio to estimate abdominal adipose tissue, but more accurate methods are available such as magnetic resonance imaging (MRI) scanning. Of the anthropometric measurements utilised, waist circumference rather than waist hip ratio has been shown to be a more accurate predictor of visceral adipose tissue stores. A study following changes in waist hip ratio in women over a twenty year period identified that, although the participants gained weight and accumulated visceral adipose tissue, their simultaneous increases in both waist and hip ratios resulted in a misleading stable waist-hip ratio (Despres, 2001). Using these anthropometric measures it has been shown that, in addition to the increase in obesity rates as defined by BMI, there has been an increase in obesity
as defined by a high risk waist circumference (a circumference of >102cm in men and >88cm in women) (Ford et al., 2003). In 2000 36.9% of men and 55.1% of women in the USA were found to have a high risk waist circumference (Ford et al., 2003) and the incidence of abdominal obesity in the USA was found to have tripled over the past forty years (Okosun et al., 2004).

1.4 Energy balance

Energy balance refers to a state in which energy intake in the form of food and drinks matches energy expended by the body. Obesity occurs when there is a long term positive energy balance and energy intake exceeds energy expenditure (Prentice and Jebb, 1995). In a state of positive energy balance the body has surplus energy in relation to requirements and increases its energy stores.

1.4.1 Energy intake

Changes in lifestyle over recent decades have made it increasingly easy for individuals to have an energy intake exceeding their energy requirements. Energy dense foods are more readily available and in western countries there is a greater reliance on highly calorific convenience foods (House of Commons, 2004). In addition to the availability of energy dense foods, there are a number of elements that combine to influence energy intake. Environmental factors such as food availability, external factors such as stress and internal factors such as the effect of the central nervous system and hormones will all impact on energy intake (Gibney, 2002).
1.4.2 Energy storage

The energy consumed in the diet that is not utilised for fuel can be stored in the body in the form of fat, glycogen or protein. Fat is the major storage form accounting for between 10-35% body weight, whereas body protein contributes between 10 and 15% and glycogen only 0.6% to total weight in lean individuals. As described in section 1.2 excess energy intake may lead to excessive fat accumulation in muscle, adipose tissue and major organs negatively influencing energy regulation.

1.4.2.1 Gender differences in energy storage

Gender differences exist in both protein and fat storage. Body protein is generally higher in males than females due to a larger muscle mass (Gibney et al., 2002). Levels of body fat also differ and in adults of a healthy weight levels are 10-25% in males and 15-35% in females but these figures can be as high as 60-70% of body weight in obese individuals (Gibney et al., 2002). Gender differences are also found in fat distribution in obesity. In obese males excess fat frequently accumulates in the subcutaneous abdominal area and may be described as android or ‘apple shaped’ obesity. In premenopausal obese women subcutaneous fat accumulation more commonly occurs in the lower abdominal wall and gluteofemoral region and is described as gynoid or pear shaped’ obesity (Arner, 1997). Postmenopausal obese women, however, tend to accumulate more abdominal and visceral fat (Lovejoy and Sainsbury, 2009).

1.4.3 Energy expenditure

Total energy expenditure is comprised of REE, dietary induced thermogenesis (DIT) and non resting energy expenditure. REE accounts for approximately 60% of total
energy expenditure and refers to the energy required for metabolic and cellular processes. Dietary induced thermogenesis accounts for roughly 10% of total energy expenditure and this describes the energy expended in the digestion, transport and storage of macronutrients. The remaining 30% of total energy expenditure is accounted for through non resting energy expenditure (Leibel et al., 1995). Non resting energy expenditure, also described as activity thermogenesis, is comprised of physical activity, exercise and non-exercise thermogenesis (NEAT) and this is the most variable element of total energy expenditure between individuals (Levine, 2005). For individuals exercising regularly exercise related energy expenditure has been estimated at approximately 10% of total energy expenditure but for those undertaking no sporting exercise the level of exercise related energy expenditure is zero (Levine, 2005). NEAT refers to the energy expenditure of physical activities of daily living which includes fidgeting and maintaining posture when sitting or standing (Levine, 2005). As with exercise, levels of NEAT can be highly variable between individuals.

Levels of activity energy expenditure have fallen in recent decades contributing to the pandemic of obesity. In England in 2004, only a quarter of women and a third of men achieved the Department of Health’s target of 30 minutes of physical activity 5 times a week. Car usage has doubled in the past 30 years, whilst levels of walking and cycling have fallen (House of Commons, 2004). With the invention of numerous labour saving devices, and increased leisure time spent watching television and playing computer games, total energy expenditure has been reduced.
1.4.4 Measurement of energy expenditure

Energy expenditure may be measured using direct, indirect calorimetry or non-calorimetric techniques. Direct calorimetry measures heat production by the body and there are several different types of direct calorimeter including isothermal, heat sink and convection systems (Levine, 2005). As direct calorimeters are very expensive to build and run, their use is limited. Indirect calorimetry measures the heat released through oxidative processes in the body through respiratory gas analysis (Jéquier et al., 1987). There are a variety of approaches to the measurement of energy expenditure using indirect calorimetry including total collection systems, open-circuit indirect calorimetry systems, closed-circuit systems and respiratory chambers (Levine, 2005). Non-calorimetric methods estimate energy expenditure by extrapolation from physiological measurements. A key non-calorimetric method of estimating total energy expenditure is measurement of doubly labeled water. In this method an individual ingests a dose of heavy water isotopically labeled with both deuterium and oxygen-18. The differing rates of loss of these isotopes from the body are utilised to estimate the rate of carbon dioxide production and, from this, energy expenditure can be calculated (Gibney, 2002).

1.4.5 Gender differences in energy expenditure

Men tend to be taller, have a higher body weight and a greater proportion of fat free mass than women, and as levels of fat free mass are a key determinant of energy expenditure, men are reported, in absolute terms, to have greater energy expenditure than women. It is unclear, however, if these gender differences in energy expenditure are independent of body composition. Several studies have failed to find a gender difference in energy expenditure once adjustments have been made for body
composition (Lovejoy and Sainsbury, 2009). Other studies have concluded, however, that after adjusting for differences in fat free mass, women do have lower energy expenditure than men (Morio et al., 1997; Ferraro et al., 1992).

Gender differences in response to activity energy expenditure have also been explored. Studies by Paul et al. (2004) and Westerterp and Goran (1997) reported that in men higher levels of physical activity were associated with a reduction in percentage of body fat but that this relationship was not seen in women. These studies included a range of both healthy weight and over weight participants. Responses to an exercise training program also differed with women demonstrating a smaller reduction in body fat and weight loss than men. These differing responses may reflect gender differences in the metabolic response to exercise combined with an increase in energy intake detected in women but not in men post exercise (Lovejoy and Sainsbury, 2009).

1.5 Genes involved in lipid metabolism

In recent years a number of genes involved in lipid metabolism have been identified and research undertaken to try to clarify their role in the development of obesity and its associated complications such as insulin resistance and dyslipidaemia. Adiponectin is an adipocyte-derived hormone that has received much attention as a potential therapeutic target in the treatment of obesity related disease. It has a role in the regulation of lipid and glucose metabolism, body weight and homeostasis (Maynadier et al., 2009). In 2008, Yamauchi and Kadowaki demonstrated that in obese mice adiponectin decreases insulin resistance by reducing triglyceride content in both muscle and the liver. This effect was found to result from increased gene expression of molecules involved in fatty-acid transport, combustion and energy
dissipation such as Cluster of Differentiation 36 (CD36), acyl CoA oxidase (ACOX1) and Uncoupling protein 2 (UCP2) in muscle. Levels of expression of adiponectin and its receptors, adiponectin receptor 1 and adiponectin receptor 2, are decreased in obese individuals (Yamauchi and Kadowaki, 2008).

Much research is also being undertaken into peroxisome proliferator-activated receptors (PPARs). These are a family of nuclear receptors comprised of different isoforms (PPARα, PPARδ and PPARγ). PPARα and PPARγ have both been found to have roles in the regulation of lipid metabolism. PPARα is expressed in skeletal muscle, the heart and liver and controls the catabolism of fatty acids and PPARγ is expressed in adipose tissue where it has a role in inducing fat cell differentiation and improving insulin sensitivity (Pagano et al., 2008). PPARδ is found in both skeletal muscle and adipose tissue, increasing fat oxidation in muscle and lipolysis in the adipose tissue (de Lange et al., 2008).

There are many further genes being studied in addition to those described above and it is hoped that establishing a greater understanding of their role in energy metabolism may lead to improved treatments for both obesity and its associated metabolic disorders.

1.6 Gender differences in lipid metabolism

Women are at a lower risk of cardiovascular disease than men until they are postmenopausal when the risk becomes similar. High plasma triglycerides are independent and better predictors of cardiovascular disease than cholesterol in women (Meagher, 2004) suggesting that treatment of hypertriglyceridaemia may be more important in women than men. This finding may be related to differences in lipid metabolism between genders.
In young lean subjects it has been shown that very low density lipoprotein (VLDL) triglyceride production by the liver is higher in women than men, but since clearance is also higher, this results in lower plasma triglyceride in women than men (Magkos et al., 2007). There have been no studies of fatty acid kinetics and triglyceride metabolism in older obese men and postmenopausal women. A further interesting finding is that fatty acid production from adipose tissue has been shown to be greater in young lean women than men even when they are matched for adiposity (Mittendorfer et al., 2001). Fatty acid production rate correlates with resting energy expenditure and a study by Nielsen et al. (2003) found that young women (mean age 30 years) produced 40% more fatty acids in relation to energy expenditure than men. As free fatty acid (FFA) concentrations are not different in men and women, fatty acid clearance from the circulation must also be higher in women than men.

Further research into gender differences in lipid metabolism should aid in the targeting of novel anti-obesity treatments.

1.7 Treatment for obesity

Much research is being undertaken to try and prevent the development, and to aid in the treatment of obesity and its associated metabolic risk factors. Currently the three treatment options for obesity are: lifestyle modification, bariatric surgery and pharmacotherapy.
1.7.1 Lifestyle modification

Lifestyle modification is recommended for long term weight loss. A combination of sedentary lifestyle and a wealth of high energy foods contribute to the development of obesity and these issues may be addressed through dietary interventions and increasing physical activity. In reality, however, behavioural change is difficult to achieve and even harder to maintain in the longer term. A study comparing a range of popular dietary interventions in the USA showed that weight loss was modest with a mean reduction in weight of 3-4kg after one year. Additionally adherence to the diets was poor with drop out rates between 42 and 60% (Dasinger et al., 2005).

1.7.2 Bariatric surgery

Bariatric surgery refers to surgery on part of the digestive system to aid weight loss in obese individuals. Examples of bariatric surgery include gastric banding and gastric bypass. In order to be eligible for bariatric surgery strict criteria have to be met. Patients must have a BMI of >40kg/m² or 35kg/m² - 40kg/m² with a disease that may improve if weight were lost. Additionally, all other non-surgical options must have been trialled and proven not to have been of benefit over a six month period (National Institute for Health and Clinical Excellence (NICE), 2006). Weight losses with bariatric surgery tend to be greater than with other obesity treatments and a review of the clinical effectiveness of obesity surgery found that surgical intervention resulted in a significantly greater weight loss of 23-37kg more weight after two years than with non surgical treatment (Clegg et al., 2003). However, bariatric surgery is only undertaken in 1% of obese adults annually (Goodpaster et al., 2010).
1.7.3 Pharmacotherapy

Currently only one anti-obesity medication, Orlistat (Xenical) a lipase inhibitor, is available on prescription in Europe. The action of orlistat is to reduce dietary fat absorption by approximately 30% thereby promoting weight loss. A randomized, double blind, placebo-controlled study in which participants received 120mg orlistat three times daily plus a hypocaloric diet for one year reported a weight loss of 8.76 ± 0.37kg in the treatment group versus 5.81 ± 0.67kg in the placebo group (Davidson et al., 1999). Unfortunately orlistat is associated with a number of well documented adverse side effects including oily stools, oily spotting and passing flatus with discharge (Davidson et al., 1999).

Until recently Sibutramine (Reductil), a serotonin-adrenaline reuptake-inhibitor was available to prescribe to aid in the treatment of obesity, but in January 2010 the European Medicines Authority withdrew its licence after the cardiovascular risks of the drug were found to outweigh the benefits.

Other medications that have attracted interest recently due to their beneficial impact on weight reduction are the glucagon-like peptide-1 (GLP-1) analogues such as exenatide and liraglutide. These drugs prolong the half life of incretins resulting in delayed gastric emptying and induced satiety (Kennedy and Khoo, 2005). Liraglutide was initially developed for the treatment of Type II Diabetes but has been shown to induce dose dependent weight loss. In a clinical trial where liraglutide treatment for 20 weeks was compared to a placebo and to orlistat in obese individuals without diabetes, the mean weight loss with liraglutide was between 4.8-7.2kg (at doses between 1.2-3.0mg per day) in comparison to 4.1kg with orlistat and 2.8kg with the placebo (Astrup et al., 2009). It should be noted, however, that all participants were additionally instructed to follow a hypocaloric diet (500kcal/day
deficit) and to maintain or increase physical activity. Currently the National Institute for Health and Clinical Excellence has recommended that liraglutide at a dose of 1.2mg daily (in combination with metformin and/or sulfonylureas) only be used in the treatment of people with type II diabetes with a BMI \( \geq 35\text{kgm}^2 \) and poor blood glucose control (NICE, 2010).

One further group of medications which have been under development and which have been emerging as a promising treatment in the fight against obesity are drugs targeting the endocannabinoid system. This novel field of research is examined in section 1.8.

1.7.4 Gender differences in response to obesity treatments

Greater weight loss has been reported in males than females following hypocaloric diets but it has been suggested that this is due to a higher initial starting weight or greater compliance with the dietary restriction rather than an inherent gender difference (Lovejoy and Sainsbury, 2009). Studies found that men lost more abdominal, visceral fat during weight loss than women (Wirth and Steinmetz, 1998; Janssen and Ross 1999), and that postmenopausal women lost less visceral fat than premenopausal women (Park and Lee, 2003). A study investigating gender differences following gastric bypass surgery found that for participants with matched BMI, men lost a greater amount of weight post surgery than women (Tymitz et al., 2007). There are, however, limited studies examining gender differences in response to anti-obesity medications (Lovejoy and Sainsbury, 2009).
1.8 Discovery of the endocannabinoid system

Cannabis had been observed for many years to increase appetite but the active constituent of cannabis Δ9-tetrahydrocannabinol (THC) was not identified until 1964 (Gaoni and Mechoulam, 1964). The potential importance of THC as an orexigenic agent was highlighted when it was observed that through smoking marijuana, patients with acquired immune deficiency syndrome (AIDS) experienced an enhanced appetite and less weight loss (Mechoulam et al., 2006). A synthetic form of THC, dronabinol, was subsequently created and this has been used to treat the nausea and sickness associated with chemotherapy in addition to the anorexia related to the human immunodeficiency virus infection (Gelfand and Cannon, 2006). Following identification of THC, years of research finally led to the characterisation and cloning of a receptor activated by THC and this was named the cannabinoid receptor type 1 or CB₁ (Howlett et al., 2002). Further research was then undertaken to identify any further cannabinoid receptors and potential endogenous ligands for these receptors. The endocannabinoid system is comprised of these cannabinoid receptors, the endogenous ligands (endocannabinoids) and the proteins required for their synthesis and degradation (Pagotto et al., 2006).

1.9 Cannabinoid receptors

At this time two cannabinoid receptors have been identified; the CB₁ receptor cloned in 1990 and the CB₂ receptor cloned in 1993 (Munro et al., 1993). Both receptors are members of the class A (rhodopsin-like) seven transmembrane spanning domain G protein-coupled receptor family and their activation inhibits adenylate cyclase activity leading to a reduction in intracellular cyclic adenosine monophosphate production (Pertwee, 1997).
The two receptor subtypes have unique pharmacological profiles and are expressed in different tissues (Kyrou et al., 2006). CB₁ receptors are widely distributed throughout the body and have been found in the hypothalamus, nucleus accumbens, gastrointestinal tract, adipose tissue, liver and muscle (Duffy and Rader, 2007). CB₂ receptors are located throughout the immune system including the thymus, tonsils, spleen and mast cells (Cannon, 2005) and on blood cells (Pagotto et al., 2006). CB₂ receptors have also been identified in the central nervous system, for example on microglial cells (Pertwee, 2006).

1.10 Endogenous cannabinoids

In 1992, the first endogenous cannabinoid (endocannabinoid), arachidonoylethanolamide (anandamide) was discovered (Devane et al, 1992). At first, it was believed that this was a specific receptor agonist for the CB₁ receptor but it has since been shown to be a CB₂ receptor agonist too (Sharkey, 2006). Three years later a further endocannabinoid, 2-arachidonoylglycerol (2-AG) was identified with efficacy at both CB receptors (Mechoulam et al, 1995). These two endocannabinoids are metabolised via separate pathways as shown in Figures 1.1 and 1.2.
Figure 1.1 Anandamide synthesis and metabolism.

(A) Anandamide is synthesised de novo inside the cellular membranes. N-arachidonoyl-phosphatidylethanolamine (NAPE) is produced from phosphatidylethanolamine under the action of the enzyme N-acyltransferase (NAT). NAPE is subsequently hydrolysed to anandamide by a NAPE-selective phospholipase D (PLD).

(B) Intracellularly, anandamide is hydrolysed to arachidonic acid and ethanolamine under the catalytic action of fatty acid amide hydrolase (FAAH). From Kyrou et al., 2006 (text adapted).
Other potential endocannabinoids have been identified including the amino-acid amides, virodhamine and noladin ether (Porter et al., 2002, Hanus et al., 2001). These endogenous cannabinoids have a number of similarities. Firstly, they are amides, esters or ethers of arachidonic acid, and are derived from membrane phospholipids (Kyrou et al., 2006). They are synthesised ‘de novo’ and upon demand and are not stored but immediately released from the cell (Di Marzo and Deutsch, 1998). They are subsequently rapidly inactivated by enzymes found in most cells (Woods, 2007) and removed by cellular uptake (Matias et al., 2006). The
endocannabinoids are found in the brain and peripheral tissues with a similar distribution pattern to CB₁ receptors (Kyrou et al., 2006).

In the brain endocannabinoids act as retrograde signallers as they are released from postsynaptic neurons and travel backwards across the synapse modulating neurotransmitter release from presynaptic terminals through the activation of CB₁ receptors (Figure 1.3) (Alger, 2002; Freund et al., 2003).

**Figure 1.3. Endocannabinoids acting as retrograde messengers in the brain.**

(a) Excitation of the neuron causes its depolarization and an influx of calcium ions. This stimulates the synthesis of endocannabinoids such as 2-AG and anandamide which are released from the neuron.

(b) Endocannabinoids diffuse away and bind to CB₁ cannabinoid receptors on the presynaptic terminals of neurons that form synapses with the stimulated neuron. This reduces inhibitory neurotransmitters being released.

(c) Endocannabinoids are taken up into neuronal and glial cells via a transporter and are then broken down by fatty acid amide hydrolase.

From MacDonald and Vaughan, 2001.

Matias et al (2006) have suggested the likely existence of an endocannabinoid membrane transporter that regulates the intracellular and extracellular distribution of endocannabinoids. Although, at this time, such a transporter has not been characterised there is evidence indicating that endocannabinoid transport across the cell membrane is an active process not one of simple passive diffusion.
In a number of animal studies, endocannabinoids were found to increase food intake and promote weight gain through the activation of CB₁ receptors (Engeli and Jordan, 2006). This discovery led to further research to generate specific antagonists for cannabinoid receptors. A number of compounds with CB₁ receptor antagonistic properties have been characterised including SR141716 (now known as Rimonabant), SR14778, AM251, AM281, LY320135 and SLV319 (Pagotto et al., 2006).

1.11 Central effects of endocannabinoids

Studies in animals suggested that the brain endocannabinoid system is involved in the regulation of food intake and that this occurs through two mechanisms. Firstly, the endocannabinoid system reinforces the motivation to find and consume palatable foods, possibly through an effect on mesolimbic pathways connected with reward mechanisms. Studies utilising the CB₁ antagonist rimonabant discovered administration greatly reduced the consumption of palatable food (e.g. sweet food) in animals fed ad libitum and reduced the intake of normal food but not water in food deprived animals (Simiand et al., 1998; Colombo et al., 1998; Rowland et al., 2001). Secondly, it is activated in the hypothalamus after fasting and regulates other anorectic and orexigenic mediators of appetite (Di Marzo et al. 2005).

The importance of the role of the endocannabinoid system in the hypothalamus for the control of food intake has been shown in a number of animal studies and levels of endocannabinoids in the brain have been shown to alter during fasting and feeding (Bellocchio et al., 2006). A study by Kirkham et al. (2002) measured levels of anandamide and 2-AG in feeding-associated areas of rats brains during feeding and fasting. Their results detected an increase in anandamide and 2-AG levels in the
limbic forebrain, a region of the brain involved with the motivational aspects of eating during fasting. They also detected an increase in 2-AG in the hypothalamus during fasting although not to the same extent as that in the limbic forebrain. The level of 2-AG in the hypothalamus subsequently decreased during feeding. Further evidence for a hypothalamic site of action was provided by the finding that food intake was increased in rats following an injection of anandamide into the dorsomedial hypothalamic nucleus (Osei-Hyiaman et al., 2006).

Changes in endocannabinoid levels in the hypothalamus have also been shown to inversely correlate with levels of leptin in the blood. Leptin is the key signal through which the hypothalamus controls food intake, reducing food intake through the upregulation of anorexigenic neuropeptides and the down regulation of orexigenic factors such as neuropeptide Y. Di Marzo et al. (2001) investigated the relationship between leptin and endocannabinoid levels in rats and knockout mice. Di Marzo et al. (2001) found that acute leptin treatment of both normal rats and ob/ob mice reduced hypothalamic levels of anandamide and 2-arachidonoylglycerol and that defective leptin signalling was associated with elevated levels of endocannabinoids in the hypothalamus.

Animal studies also indicated that grehlin may be involved in the regulation of endocannabinoid levels. Ghrelin is a 28-amino-acid peptide, which is synthesised in the stomach and the brain during food deprivation and signals to the hypothalamus to increase food intake (Rigamonti et al., 2006). A study by Tucci et al. (2004) suggests that endocannabinoids are actually involved in ghrelin’s actions on appetite. They demonstrated that blocking the CB₁ receptors in the paraventricular nucleus (PVN) of the hypothalamus with rimonabant prevented the orexigenic action of an intra-PVN injection of ghrelin. It has been shown in rats that the hypothalamic
concentrations of anandamide and 2-arachidonoylglycerol alter during the different stages of food ingestion; levels are highest during fasting, when leptin levels are lowest and ghrelin levels are highest and lowest during feeding when leptin is highest and ghrelin lowest (Kirkham et al., 2002).

1.12 Peripheral effects of endocannabinoids

A study of marihuana smokers by Greenberg et al. (1976) provided the first indication that cannabinoids might affect energy homeostasis through mechanisms other than central effects on food intake. Under ward conditions, energy intake and body weight were monitored in marihuana users before, during and after 21 days of marihuana smoking. It was reported that marihuana initially caused an increase in food intake and weight gain. After the first few days, however, caloric intake was reduced yet the weight gain continued for the duration of the study indicating an independent effect on peripheral metabolism.

Effects on peripheral metabolism have also been reported in studies using the endocannabinoid receptor rimonabant. Colombo et al. (1998) administered rimonabant to non-obese Wistar rats and found that tolerance to the anorectic effect of the drug developed within five days, yet the reduced body weight seen in these treated rats was maintained for the entire 14 days of the treatment period. Bellochio et al. (2006) noted that similar observations were reported in a number of animal studies where the CB1 receptor was either removed or pharmacologically antagonised. All of the studies described a transient reduction in food intake but a persistent reduction in body weight following blockage of the cannabinoid receptor. CB1 receptor knockout mice were shown to resist developing diet induced obesity and insulin resistance whilst being fed a high fat diet (Ravinet Trillou et al., 2004).
These observations clearly suggested that weight reduction was being influenced by a factor independent of food intake, energy expenditure.

Further research was undertaken and results suggested that a number of peripheral sites were involved in endocannabinoid activity including the liver, white adipose tissue, brown adipose tissue and skeletal muscle (Bellocchio et al., 2006). A study by Osei-Hyiaman et al. (2005) showed that the liver is a site of action for endocannabinoids. They demonstrated that activation of CB₁ in mice increased de novo fatty acid synthesis. This occurred through an increased expression of sterol regulatory element binding protein-1C, a lipogenic transcription factor, and its target enzymes fatty acid synthase and acetyl coenzyme-A carboxylase-1. A high fat diet fed to the mice for three weeks led to an increase in hepatic anandamide and an increase in the basal rate of hepatic fatty acid synthesis. The increase in anandamide was not due to an increase in synthesis but rather to a decrease in its degradation by fatty acid amide hydrolase. Subsequent administration of the CB₁ antagonist, rimonabant, reduced both basal levels of fatty acid synthesis and development of hepatic steatosis caused by the high fat diet (Osei-Hyiaman et al., 2005).

Like the liver, adipose tissue is another important peripheral site of action for endocannabinoids. Studies have shown that adipocytes express the CB₁ receptor and that CB₁ expression is increased in mature adipocytes in comparison to preadipocytes suggesting that the receptors are important for the function of these mature cells (Engeli et al., 2005). A study by Jbilo et al. (2005) provided evidence as to the mechanisms of action of the endocannabinoid system on adipose tissue. They treated dietary induced obese mice with rimonabant and were able to reverse the phenotype of obese adipocytes. The weights of the lumbar white and brown fat adipose tissue were both reduced by more than 50% following treatment and they found that this
was not due to adipocyte apoptosis but rather a depletion of the adipocyte’s fat content. In addition they discovered that major alterations in gene expression levels induced by a high fat diet were mostly reversed in rimonabant treated obese mice. Analysis showed that the reduction of adipose mass by the drug was due to enhanced lipolysis through the induction of the enzymes of beta-oxidation and the tricarboxylic acid cycle; increased energy expenditure and regulation of glucose homeostasis (Jbilo et al., 2005).

A further important finding with regards to adipose tissue was that activation of CB1 receptors in adipocytes regulates the hormone adiponectin (Bensaid et al., 2003). Adiponectin is the most abundantly secreted adipokine and it has antiatherogenic and anti diabetic properties (Pagotto et al., 2006). In men, high levels of the adipokine are associated with a decreased risk of myocardial infarction (Pi-Sunyer, 2006) and increases in adiponectin have been shown to improve insulin sensitivity (Pagotto et al., 2006). Reduced adiponectin levels are reported in obese individuals and in those with type II diabetes, coronary artery disease, hypertension and polycystic ovary syndrome (Pi-Sunyer, 2006). Bensaid et al. (2002) found that the CB1 antagonist, rimonabant, stimulated adiponectin messenger ribonucleic acid (mRNA) expression in adipose tissue and decreased hyperinsulinaemia in obese (fa/fa) rats.

To further examine the relationship between adipose tissue and dysregulation of the endocannabinoid system, Bluher et al. (2006) studied the association between visceral adipose tissue accumulation and circulating endocannabinoid levels. They reported that levels of 2-AG were increased in obese compared to lean participants and that a significant correlation was found between circulating 2-AG and fat mass. They also identified a high expression of CB1 receptors in visceral adipose tissue and suggested that these could be a primary target for blockade with a CB1 antagonist.
Brown adipose tissue has recently been studied as another potential site of endocannabinoid activity. It has been suggested that blocking the endocannabinoid system may increase thermogenesis, as one study showed that mice with dietary induced obesity treated with the CB₁ antagonist AM251 increased levels of both uncoupling protein-1 (UCP1) and uncoupling protein-3 (UCP3) mRNA in brown adipose tissue (Pagotto et al., 2006).

Skeletal muscle is another tissue to express the CB₁ receptor although this expression is low in comparison to the brain (Engeli and Jordan, 2006). A study by Liu et al. (2005) investigated the effect of treatment with rimonabant for seven days on oxygen consumption, and thus thermogenesis, in obese (Lepob/Lepob) mice. They demonstrated that rimonabant had a direct effect on energy expenditure, significantly increasing basal oxygen consumption compared with vehicle treated animals and significantly increasing glucose uptake in isolated soleus muscle preparations. They concluded that rimonabant may directly impact energy expenditure at the skeletal muscle level and suggested that the antiobesity effect of rimonabant was due to a combination of hypophagia and activation of thermogenesis (Liu et al., 2005). The increase reported in glucose uptake in the soleus muscle during treatment with rimonabant may contribute to the improvement in glycaemic profile observed in other studies (Pagotto et al., 2006).

An overview of the metabolic effects of antagonism of CB₁ receptors is shown in Figure 1.4.
Chapter 1 — Introduction

1.13 Hyperactivity of the endocannabinoid system

Recent studies demonstrate a close relationship between obesity and overactivation of the endocannabinoid system (Bellocchio et al., 2006). This has been shown through an increase in endocannabinoid production or an increase in cannabinoid receptor expression in obese animals and humans. Bellocchio et al. (2006) have summarised the five possible sites of endocannabinoid activation as 1) the hypothalamus as increased levels of endocannabinoids were identified in the hypothalamus of obese ob/ob mice; 2) the liver as an increase in anandamide content and numbers of CB1 receptors following a high fat diet was observed in rodents; 3) skeletal muscle as an increase in CB1 receptor expression has been seen following a high fat diet; 4) pancreatic beta cells as elevated levels of anandamide and 2-
arachidonoylglycerol were seen under conditions mimicking hyperglycaemia and 5) white adipose tissue as higher levels of endocannabinoids have been observed in visceral tissue from obese individuals compared to lean participants.

The mechanism behind the overactivation of the endocannabinoid system seen in obesity has still not been identified but may be related to nutrition and genetics. A genetic mutation has been identified in obese participants in the enzyme that degrades anandamide, fatty acid amide hydrolase. An investigation of 2,667 black, white and Asian subjects found that the defective fatty acid amide hydrolase gene was significantly associated with obesity in both the black and white subjects (Sipe et al., 2005). Individuals with this mutation have approximately 50% enzymatic activity resulting in a potential impact on the clearance of endocannabinoids.

Woods (2007) proposed that a combination of factors may result in hyperactivity of the endocannabinoid system in obesity. He suggests that consumption of a high fat diet is related to elevated levels of omega-6 polyunsaturated fatty acids. As these fatty acids are the precursors for endocannabinoids then elevated levels may lead to overproduction of endocannabinoids. The overabundance of endocannabinoids will lead to obesity which may in turn contribute to leptin resistance. Dysfunction of other endocrine systems may then occur and finally a number of individuals may have a missense polymorphism in the fatty acid amide hydrolase gene resulting in defective clearance of endocannabinoids.

1.14 Rimonabant

Due to the emerging importance of the endocannabinoid system in food intake and energy balance regulation it was hypothesised that a cannabinoid antagonist would be of therapeutic benefit in treating obesity (Pagotto et al., 2006). Rimonabant or
SR141716 was developed by Sanofi-Synthelabo (now Sanofi-Aventis) in 1994 and its structure is shown in Figure 1.5. It was the first cannabinoid receptor antagonist to be characterised and binds to both CB₁ and CB₂ receptors with a 1000-fold higher affinity for the CB₁ than the CB₂ receptor (Tonstad, 2006).

![Figure 1.5. The chemical structure of rimonabant](image)

Rimonabant is an active lipophilic compound and is best taken with food (Wierzbicki, 2006). Its half-life varies according to BMI and for individuals with a BMI of 18-28 kg/m² the half life of rimonabant is 6-9 days and is 16 days for those with a BMI > 30 kg/m² (Wierzbicki, 2006). Rimonabant is metabolised by the liver, excreted in bile and eliminated in faeces (Padwal and Majumdar, 2007).

Rimonabant had been seen as a promising drug for the treatment of obesity as it had shown no significant interactions with other drugs commonly prescribed to patients for conditions associated with obesity such as hypertension and type II diabetes (Tonstad, 2006).

The results of rimonabant studies in animals had produced some positive results as described above. Blockade of the cannabinoid receptors had been shown to reduce food intake, influence peripheral metabolism and modify the appeal of palatable foods (Ravinet Trillou et al., 2004). Due to these findings it was hoped that equally positive results would be observed in humans and four large scale phase III clinical
trials were undertaken: RIO (Rimonabant In Obesity) - Europe, RIO-Lipids, RIO-North America and RIO-Diabetes (Costa, 2007).

1.15 Initial clinical trials in humans

The four clinical trials were very similar in design with the major differences being the duration of the trial and the population group studied. The RIO-Europe (Van Gaal et al., 2005) and RIO-North America (Pi-Sunyer et al., 2006) trials recruited obese or overweight adults with treated or untreated hypertension, dyslipidaemia or both. The RIO-Europe trial enrolled 1047 participants and was undertaken for a year in comparison to the 3045 subjects enrolled on to the RIO-North America trial, which ran for two years. Following these studies in obese and overweight people, the RIO-Lipids study (Despres et al., 2005) investigated 1036 overweight or obese adults with untreated dyslipidaemia to determine the effects of rimonabant in those with a higher risk of cardiovascular disease. Finally the RIO-Diabetes trial (Scheen et al., 2006) was undertaken to establish the efficacy and safety of rimonabant in overweight and obese patients with type II diabetes that was poorly controlled with metformin or sulphonylureas.

These were all double-blind placebo controlled studies in which participants were randomised to receive either a placebo, 5mg rimonabant or 20mg rimonabant daily for one year in addition to a hypocaloric diet (600kcal/day deficit). For the second year of the RIO-North America study the rimonabant groups were then rerandomised to receive either the placebo or to continue receiving the same rimonabant dose while the placebo group continued to receive the placebo.

Primary outcome measures for the different trials included weight loss and waist circumference with secondary measures including changes in haemoglobin AlC.
(HbA1c), fasting glucose, fasting insulin, lipid levels, C-reactive protein, leptin concentrations, waist circumference, blood pressure and prevalence of metabolic syndrome.

Fifty one to sixty six percent of all study participants completed the one year follow up and drop-out rates were similar in the placebo and treated groups. In all studies, the patients treated with 20mg of rimonabant daily had a significantly greater reduction in weight than the placebo groups. For the groups receiving 20mg rimonabant the mean weight loss was 5.3kg-8.6kg and between 49-67% of patients treated with the 20mg of rimonabant daily achieved ≥ 5% weight loss. This weight loss mainly occurred in the first nine months of the study after which weight stabilised. In the RIO-North America study (Pi-Sunyer et al., 2006), when the rimonabant groups were re-randomised in the second year, the participants that were switched from the 20mg rimonabant group to the placebo regained the majority of weight lost whereas those continuing to receive 20mg rimonabant maintained their weight loss.

In addition to weight loss, a significantly greater decrease was seen in waist circumference in the 20mg rimonabant groups compared to the placebo groups. The mean decrease in waist circumference in subjects treated with 20mg of rimonabant was 5.2-6.1cm.

With regards to the markers of cardiovascular disease that were measured, significantly greater improvements were seen in high-density lipoprotein (HDL) cholesterol, triglycerides and insulin resistance in the subjects receiving 20mg rimonabant compared to the placebo groups. HDL-cholesterol increased by 7.2-16.2% and triglycerides concentrations fell by 6.8-13.2% in the groups receiving 20mg rimonabant. Investigators used weight loss as a covariate to try and establish
what proportion of the observed improvements in HDL-cholesterol and triglycerides following treatment with 20mg rimonabant were independent of weight loss. In the RIO-Europe trial (Van Gaal et al., 2005) approximately 40% of the increase in HDL-cholesterol and 55% decrease in triglycerides were found to be independent of weight loss but in the RIO-Diabetes study (Scheen et al., 2006) when the positive effects on triglycerides in the 20mg rimonabant group were adjusted for weight loss, the results were no longer significant. There were no significant reductions in low-density lipoprotein (LDL) concentration reported but the RIO-Lipids study determined that the proportion of large LDL particles was greater in those receiving 20mg of rimonabant. When compared to the placebo group, there was a difference of 1.1Å in the peak LDL particle size and a 4.6% lower proportion of small LDL particles in the 20mg rimonabant group (Despres et al., 2005).

Improvements in insulin sensitivity were seen in the 20mg rimonabant groups compared to the placebo groups with a significant decrease in both fasting plasma insulin levels and areas under the curve for insulin and glucose during an oral glucose tolerance test. In the RIO-Diabetes study HbA1c levels were lower with both doses of rimonabant than with the placebo but only the reduction with the 20mg of rimonabant was significantly different. HbA1c was reduced by 0.6% in the 20mg rimonabant group compared to a rise of +1% in the placebo group. Further analysis was undertaken which demonstrated that the effect of the 20mg of rimonabant on HbA1c was approximately twice that attributable to weight loss alone (Scheen et al., 2006).

With regards to the additional secondary outcome measures which were considered in the RIO-Lipids study, improvements were seen in adiponectin, leptin and C-reactive protein levels. Mean adiponectin levels increased by 2.7μg/ml in the 20mg
rimonabant treated group compared with 0.8μg/ml in the placebo group. As weight loss is correlated with adiponectin levels, further covariate analysis was undertaken which determined that 57% of the increase in adiponectin observed in those treated with 20mg of rimonabant could not be attributed to weight loss. There was a positive correlation between the changes in adiponectin levels and the changes in HDL-cholesterol. The plasma leptin levels decreased significantly in those receiving the 20mg of rimonabant, as did the plasma levels of C-reactive protein (Despres et al., 2005).

In addition to these results, the RIO-Diabetes study reported that subjects receiving 20mg of rimonabant per day reported significantly lower appetite, less desire for sweets and less desire for high fat foods than the placebo group. A greater improvement in physical functioning as determined by a health survey questionnaire was also reported for the group treated with 20mg of rimonabant at one year.

A number of adverse events were reported during the trials and the frequency of these events was slightly higher in the groups treated with 20mg rimonabant than the placebo group. Commonly reported adverse events in the rimonabant treated groups were nausea, diarrhoea, vomiting, fatigue, dizziness and anxiety. These events were considered to be mild and transient in nature, occurring mainly in the first few months of the study. Depressed mood disorders were also seen more frequently in the participants treated with 20mg of rimonabant than in other groups, but the discontinuation rate due to this effect were reported to be very similar between the 20mg rimonabant groups and the participants receiving the placebo.

The results of these four trials together indicated that a 20mg dose of rimonabant could bring about significant reductions in weight loss and waist circumference measurements in overweight and obese individuals. Few significant findings were
reported in the studies with a 5mg daily dose of rimonabant. In addition to the reduction in cardiovascular risk brought about by the weight loss and decreased abdominal girth measurement with 20mg rimonabant, the drug appeared to be having a direct effect on metabolic risk factors independent of the weight loss. From the studies, positive changes have been reported in HDL-cholesterol levels, insulin sensitivity, adiponectin levels, leptin levels and plasma levels of C-reactive protein. To try and establish whether these improvements in cardiovascular risk factors would translate to a true reduction in cardiovascular events and the effects of rimonabant in other population groups a number of further human clinical trials were then undertaken.

1.16 Further Human Clinical Trials

STRADIVARIUS (Strategy to Reduce Atherosclerosis Development InVolving Administration of Rimonabant – the Intravascular Ultrasound study) (Nissen et al., 2008) investigated the effect of rimonabant on cardiovascular risk. Eight hundred and thirty nine abdominally obese patients with coronary artery disease were recruited and randomised to receive either a placebo or 20mg rimonabant for 18 months. An intravascular ultrasound was performed pre and post the treatment and the primary endpoint was the change in percentage atheroma volume (PAV) with a secondary endpoint of change in normalised total atheroma volume (TAV). As seen in the RIO trials, rimonabant had a beneficial effect on weight, waist circumference, HDL cholesterol levels, triacylglycerol levels and HbA1C. Rimonabant did slow the rate of progression in PAV but it was not significantly different to the placebo group (PAV increased by 0.25% in the rimonabat group versus 0.57% in the placebo
group, $P=0.22$). In the secondary endpoint, TAV, however, a significant decrease was seen in the rimonabant group.

The ADAGIO-Lipids study (An International Study of Rimonabant in Dyslipidemia with AtheroGenic Risk in Abdominally Obese Patients) examined the effects of rimonabant on cardiometabolic risk factors in 803 patients with severe abdominal obesity (Despres et al., 2009). Participants were randomised to receive either 20mg rimonabant daily or a placebo and were prescribed a diet which reduced their daily energy intake by 600 kcal. The results confirmed the previously reported positive effects of rimonabant on HDL-cholesterol and triglyceride levels but additionally a CT (computerised tomography) scan showed that rimonabant reduced visceral adipose tissue (-10.1% versus placebo, $P=<0.0005$) to a larger extent than subcutaneous fat (-5.1% versus placebo, $P=<0.005$) and significantly decreased fat accumulation in the liver.

A summary of findings from key rimonabant papers may be seen in Table 1.1.
### Table 1.1 Summary of findings from key rimonabant trials.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Primary end points†</th>
<th>Secondary end points†</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIO-Europe</td>
<td>1507 (309 males, 1198 females) BMI 27-40kg/m²</td>
<td>Rimonabant 5mg, 20mg or placebo daily for 1 yr</td>
<td>4.7 kg weight loss*, 4cm waist decrease</td>
<td>HOMA-IR ↓, HDL-C ↑</td>
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<tr>
<td>(Van Gaal et al., 2005)</td>
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<tr>
<td>RIO-Lipids</td>
<td>1033 (407 males, 626 females) BMI 27-40kg/m²</td>
<td>Rimonabant 5mg, 20mg or placebo daily for 1 yr</td>
<td>5.4kg weight loss*, 5.7cm waist decrease</td>
<td>HDL-C ↑, LDL particle size ↑, Adiponectin ↑</td>
</tr>
<tr>
<td>(Despres et al., 2005)</td>
<td></td>
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<tr>
<td>RIO-Diabetes</td>
<td>1045 (513 males, 532 females) Type II DM (treated with oral agents) BMI 27-40kg/m²</td>
<td>Rimonabant 5mg, 20mg or placebo daily for 1 yr</td>
<td>3.9 kg* weight loss, 3.3 cm weight decrease HbA1c ↓</td>
<td>TG↓, HDL-C ↑</td>
</tr>
<tr>
<td>(Scheen et al., 2006)</td>
<td></td>
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<tr>
<td>RIO-North America</td>
<td>3040 (588 males, 2452 females) BMI 27-40kg/m²</td>
<td>Rimonabant 5mg, 20mg or placebo daily for 1 yr</td>
<td>4.7kg* weight loss, 5.4 cm waist decrease</td>
<td>HOMA-IR ↓, HDL-C ↑, Weight regained once drug stopped</td>
</tr>
<tr>
<td>(Pi-Sunyer et al., 2006)</td>
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<tr>
<td>STRADIVARIUS</td>
<td>839 (545 males, 294 females) &gt;88cm in females, &gt;102cm in males</td>
<td>Rimonabant 20mg daily vs. placebo for 18-20 mths.</td>
<td>No significant difference in rate of progression in PAV</td>
<td>TAV↓, Weight ↓, Waist circ. ↓</td>
</tr>
<tr>
<td>(Nissen et al., 2008)</td>
<td></td>
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<tr>
<td>ADAGIO</td>
<td>799 (371 males, 428 females) &gt;88cm in females, &gt;102cm in males</td>
<td>Rimonabant 20mg daily vs. placebo for 1 yr</td>
<td>TG↓, HDL-C ↑</td>
<td>Abdominal AT ↓, Visceral AT ↓, Weight ↓, Waist circ. ↓</td>
</tr>
<tr>
<td>(Despres et al., 2009)</td>
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</table>

Mean placebo subtracted one year weight loss; ADAGIO, An International Study of Rimonabant in Dyslipidemia with Atherogenic Risk in Abdominally Obese Patients; AT, Adipose Tissue; Diabetes Mellitus; HbA1C, haemoglobin A1c; HDL-C, High Density Lipoprotein Cholesterol; HOMA-IR, Homeostasis Assessment Model of Insulin Resistance; PAV, percentage atheroma volume; STRADIVARIUS, Strategy to Reduce Atherosclerosis Development Involving Administration of Rimonabant – the Intravascular Ultrasound study; TAV, total atheroma volume; TG, Triglycerides; Type II DM, type 2 diabetes mellitus.

† Results are for rimonabant 20mg/day versus placebo (p<0.05).
1.17 Rationale for the current studies

1.17.1 Rimonabant Study

As described in section 1.11, animal studies had demonstrated that the effect of rimonabant on appetite was transient, yet weight loss persisted, suggesting a major impact on energy expenditure. Further animal studies had provided evidence that the effect on energy expenditure was due to an increase in fatty acid oxidation. As this previous research was undertaken in animals, further investigation was required to determine whether rimonabant increased energy expenditure in humans. Previous research had also not determined to what extent the metabolic effects seen from rimonabant treatment were due to a direct effect of the drug rather than a consequence of weight loss.

The RIO studies described above had attempted to determine the proportion of the metabolic effects that arose as a direct effect of the drug and that which had occurred secondary to the reduction in body weight through covariate analysis but did not measure this directly. Additionally in these studies visceral fat, liver fat and muscle fat were not assessed but the weight loss reported would be expected to have resulted in significant effects on fat distribution as shown in other weight loss studies of obesity (Markovic et al., 1998; Tiikkainen et al., 2003). The ADAGIO trial examining visceral adipose tissue as a secondary outcome measure was published three years after this study commenced.

Weight loss due to dietary intervention had been reported to reduce energy expenditure (Ravussin et al., 1985; De Boer et al., 1986), fasting rate of lipolysis (Nicklas et al., 1997) and whole body fat oxidation (Nicklas et al., 1997; Franssila-Kallunki et al., 1992). This was the opposite of the expected effect of rimonabant treatment. Weight loss due to dietary intervention had been shown to reduce both
visceral fat and liver fat (Goodpaster et al., 1999; Tiikkainen et al., 2003) resulting in health benefits such as improvements in insulin sensitivity. Few studies had been undertaken examining the effects of weight loss due to energy restriction on levels of intramyocellular lipid (IMCL).

In order to elucidate further the direct effects of rimonabant in obese individuals a trial examining the direct effect of the drug on energy expenditure, body fat distribution and gene expression of regulators of fatty acid metabolism in adipose tissue and muscle was planned. Since the aim of the study was to measure the effects of drug treatment on energy expenditure and not energy intake in individuals treated with rimonabant, energy intake was to be maintained during the study at a level matched to energy expenditure determined in a run-in period. To understand how much of the effects on body composition and metabolism were actually a direct effect, rather than a consequence of weight loss, a control group was to be studied with weight loss due to dietary intervention matched for the weight loss in the rimonabant treated group.

This was a collaborative study involving another PhD student (who examined the effects of rimonabant on fatty acid and triglyceride metabolism), colleagues at the Postgraduate Medical School, the Royal Surrey County Hospital and the MRC Clinical Sciences Centre, at Imperial College School of Medicine, Hammersmith Hospital.

Hypothesis for rimonabant study

When energy intake was maintained at pre treatment levels in the rimonabant group weight loss would be induced due to an increase in energy expenditure. The fuel for the increased energy expenditure would be provided by increased fat oxidation and
an increase in gene expression of regulators of fat oxidation would be seen. Accompanying the weight loss, a reduction in total body fat, visceral fat, and liver and muscle fat would be found in the rimonabant group. In a control group following a dietary intervention, with matching weight loss to that in the rimonabant group, there would be a decrease in energy expenditure and fat oxidation and a reduction in visceral fat, liver fat and muscle fat similar to that of the rimonabant group.

Prior to commencement of the Rimonabant study a Pilot Study was to be undertaken to test the dietary methodology proposed for both groups in the trial.

1.17.2 Pilot Study

In the rimonabant study design a diet of commercially available modular energy controlled foods (Slim.Fast, Unilever) was to be used for the seventeen week duration of the trial to allow for energy and macronutrient control of the diet in both the diet and the control group. A pilot study was undertaken in order to establish whether the Slim.Fast products were acceptable to participants and whether they could be consumed at the level that was to be required for the duration of the rimonabant study. Additionally it was important to assess whether a tightly controlled level of weight loss was achievable if the energy provided by Slim.Fast products prescribed was lower than energy requirements and these products were the sole source of nutrition.
1.17.3 Gender differences study

There was, and still is, a huge clinical need for the development of an effective treatment which can produce a sustainable loss in body weight in obesity. As described in section 1.3-1.6, there was some evidence for gender differences in body fat distribution and lipid metabolism and, as obesity is inextricably linked with both, a better understanding of gender differences in these areas could aid in the targeting of different anti-obesity treatments. Although previous research had investigated gender differences in individual elements of energy expenditure and body fat distribution, no one study had comprehensively researched resting energy expenditure, activity energy expenditure, body fat distribution and gene expression of key regulators of lipid and glucose metabolism in the same participants. It was proposed that this approach would provide a fuller understanding of the differences in metabolism in obese men and women.

A study was undertaken in obese men and postmenopausal women, matched for age and BMI, to compare differences in energy expenditure, body fat distribution and gene expression of regulators of fatty acid metabolism in adipose tissue and muscle.

Hypothesis for gender differences study

REE would be lower in women than men. Total body fat would be higher in females than males and men would have greater levels of visceral fat. There would be a greater expression of genes involved in both the clearance of triglycerides and the regulation of lipolysis in adipose tissue in women. In muscle there would be a greater expression of genes involved in fatty acid transport into muscle in females than males.
1.18 Summary of studies

As outlined above, three studies were undertaken:

1. A pilot study to establish the feasibility and acceptability of meeting energy requirements in target study participants through the use of a commercially available modular energy controlled diet (Slim.Fast, Unilever).

2. A study to determine if a) when energy intake was maintained at pre treatment levels, rimonabant still induced weight loss through an effect on energy expenditure and b) the direct effects of rimonabant on body fat distribution and gene expression.

3. A study to determine how gender influences energy expenditure, body fat distribution and gene expression in obesity.
Chapter 2

General Methods
This chapter outlines the general methods used in the studies. Individual comprehensive protocols are provided for each study in the relevant chapter along with any further detail or alterations to the methods described below.

2.1 Participant recruitment

Participants were recruited for the studies through recruitment emails sent to staff at the University of Surrey and posters displayed throughout the University campus, local GP surgeries and nearby hospitals. In addition recruitment letters were sent to previous participants of studies and adverts and articles appeared in local newspapers.

2.2 Screening of participants

Prior to the screening visit participants were requested to refrain from drinking alcohol and to fast for a twelve hour period. All participants had been provided with a participant information sheet and the study design was fully explained before written consent was obtained.

During the screening visit a number of measurements were undertaken including weight, height, percentage body fat, blood pressure and waist and hip circumferences. A physical examination was performed by a doctor and a medical history and any current medications recorded. Blood was taken to measure haemoglobin, white blood cell count, platelet count, sodium, potassium, creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), thyroid stimulating hormone (TSH), triglyceride, total cholesterol, blood glucose and insulin (more details are provided in section 2.2.4 below). A self certified medical
questionnaire (Appendix 1) was completed to assess whether participants met the inclusion and exclusion criteria. A Dutch Eating Behaviour Questionnaire (Appendix 2) was completed for the pilot and rimonabant studies to establish eating behaviours and potential participants for the rimonabant study were additionally administered with a Beck Depression Inventory-II questionnaire (Appendix 3) in order to assess current levels of depression. (Further details on all questionnaires provided below).

If a participant fulfilled all of the inclusion criteria and none of the exclusion criteria they were accepted into the study. A letter was then sent to the participant’s GP to explain the trial and notify them of their patient’s involvement.

2.2.1 Self-certified medical questionnaire

Exclusion criteria were assessed through the completion of the self-certified medical questionnaire (Appendix 1). Participants were excluded from the studies if they reported a history of diabetes, cardiovascular disease, endocrine disease, hepatic and renal disorders, neurological or psychological illness, eating disorders, drug or alcohol abuse, depression or if they had undergone a previous surgical procedure for weight loss. Exclusion criteria also included the prescribed use of any medications known to alter body weight or appetite and β-blockers, fibrates and metformin. For studies involving an MRI scan it was confirmed that participants did not have any metal implants within the body and that they were not claustrophobic.

2.2.2. Dutch Eating Behaviour Questionnaire

As the measurement of food intake was a key element of the pilot and rimonabant studies, restrained eaters were to be excluded and therefore potential participants’
eating behaviour was established using the Dutch Eating Behaviour Questionnaire (Appendix 2). Participants were excluded from the study if they had a restrained eating score of >4.

The Dutch Eating Behaviour Questionnaire was developed by Van Strein et al. in 1986 with the aim of understanding eating behaviours in obese individuals. The questionnaire is constructed of 33 questions. The 33 questions are divided into three different behavioural patterns: emotional eating (13 questions), restrained eating (10 questions) and external eating (10 questions). The responses that may be given are Never, Seldom, Sometimes, Often and Very Often and for 12 of the questions there is a Not Relevant option.

The answers are scored as follows: Never (1), Seldom (2), Sometimes (3), Often (4), Very Often (5) and Not relevant (0) with the exception of question 26 which is scored in reverse. Scores are then totalled for each of the three separate categories i.e. emotional, external and restrained eating. The final score for the category is determined by dividing the total score calculated by the number of questions for that category. Each time a participant chooses the Not Relevant option then the number of questions used as a division factor is reduced by one for that category.

\[
\text{e.g. Emotional eating score} = \frac{\text{Total score from emotional eating questions}}{\text{Number of emotional eating questions}}
\]

\[
\text{e.g. Emotional eating score} = \frac{37}{13} = 2.8
\]

See Appendix 2 for an annotated copy of the questionnaire.
2.2.3 Beck Depression Inventory II

The Beck Depression Inventory II is a questionnaire consisting of 21 items for the self reporting of levels of depression in adults and adolescents over the age of 13 (Beck et al. 1996). It measures the severity of depression and is a self administered questionnaire taking between 5-10 minutes to complete (Appendix 3).


Each item has four alternative responses provided. These responses are descriptive statements of symptoms and attitudes and are organized according to the severity of their content. They are rated on a four point scale from 0-3 with 0 being the score for the null option through to 3 for the statement with the greatest severity of content.

   e.g. Loss of Interest:
      0   I have not lost interest in other people or activities
      1   I am less interested in other people or things than before
      2   I have lost most of my interest in other people or things
      3   It’s hard to get interested in anything

Participants were instructed to read each statement and to choose the one statement which best represented how they had been feeling over the past two weeks. The total score was calculated by summing the ratings for the 21 items. Levels of depression were then categorized as:
If the total score for a participant was 14 or above then they were excluded from participating in the rimonabant study. The Beck Depression Inventory II was additionally used on a fortnightly basis to monitor levels of depression in participants undertaking the rimonabant study.

2.2.4 Screening blood samples

A 14ml blood sample was taken at the screening visit by a doctor. Venous blood was collected in one 2ml tube containing sodium fluoride/potassium oxalate for the analysis of glucose, three 4ml tubes containing serum separation clot activator for the biochemical profile, thyroid function test and insulin analysis, and one 4ml tube containing EDTA (ethylene diamine tetraacetic acid) to measure full blood count. The vacuettes were sent within 30 minutes to the Biochemistry Laboratory at the Royal Surrey County Hospital for analysis. The methods used to analyse the samples are shown in Table 2.1.
Table 2.1 Methods used for analysis of screening blood samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Analyser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Indirect ISE</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>Indirect ISE</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>Alkaline Picrate Jaffe/O'Leary</td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
<td>Biuret</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>Bromocresol Green</td>
<td>Siemens Advia 1650</td>
</tr>
<tr>
<td>ALT</td>
<td>IFCC Conditions</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>IFCC (AMP buffer)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol oxidase</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Enzymic (GPO/PAP)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Hexokinase</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>ELISA</td>
<td>Mercodia Iso-insulin ELISA kit</td>
</tr>
<tr>
<td>TSH</td>
<td>Chemiluminescence</td>
<td>Siemens Advia Centaur</td>
</tr>
</tbody>
</table>

ISE, ion selective electrodes; IFCC, International Federation of Clinical Chemistry; GPO-PAP, glycerophosphate oxidase-peroxidase-4-aminophenazone; ELISA, Enzyme-linked immunosorbent assay.

2.2.5 Screening diet diaries

Following the screening appointment each participant potentially participating in the rimonabant or gender differences study was asked to complete a four day diet diary. For potential participants of the gender differences study this was simply to ensure that individuals were consuming a diet of a composition similar to the national average. For the rimonabant study participants this was in order to ensure that they were competent to comprehensively complete a diary for the duration of the study and to check for under-reporting. As under-reporting of dietary intake is a problem frequently encountered when requesting free living participants to record their food consumption and has the potential to affect the validity of the study results, it was essential to identify this. Participants classified as under-reporters, as defined by Goldberg et al (1991), were excluded from taking part in the rimonabant study. The reported energy intake from the participants four day diet diary was examined to
identify under-reporting. Cut off limits as defined by Goldberg et al. (1991) were utilised to test whether the energy intake recorded by each participant was actually a plausible measure of the food consumed over the four day period. The basal metabolic rate (BMR) was estimated using the Schofield equation (1985) for each participant and the BMR to energy intake ratio calculated. Participants with an energy to BMR ratio of less than 1.06 were categorised as under-reporters.

2.3 Anthropometric measurements

2.3.1 Height
Height was only measured at the screening visit. Participants were asked to stand on a stadiometer with their heels together and their back straight and gentle traction was applied to the mastoid processes to ensure that the participant was stretched upwards as fully as possible. Height was measured to the nearest 0.5 centimetre.

2.3.2 Weight
The same set of calibrated electronic scales was used for the entire duration of each study. For the pilot study Seca 864 scales (Birmingham, United Kingdom) were utilised and Tanita BC-418 MA Scales (Tokyo, Japan) for the rimonabant trial and gender differences study. For the pilot study participants were asked to remove any heavy clothing and shoes before their weight was recorded. In the rimonabant trial and gender differences study participants were asked to remove shoes and all clothing with the exception of underwear and wear a provided dressing gown of known weight (0.8kg). The participant’s weight was recorded in kg (to the nearest 0.1kg) and their BMI was calculated from the weight and height measurements using the following equation:

\[
\text{BMI} = \frac{\text{Weight in kg}}{\text{height in metres}^2}.
\]
2.3.3 Waist and hip circumference

The participant was asked to stand in an upright but relaxed position. The waist circumference was measured around the umbilicus and the hip circumference around the widest part of the participant’s body to the nearest 0.5cm. Each measurement was repeated three times and undertaken by the same investigator to minimise interoperator error.

2.3.4 Body fat

Body composition was measured using a Tanita BC-418 MA Scales (Tokyo, Japan). The Tanita uses bioimpedance to calculate percentage of body fat and water. Participants were asked to remove socks and empty their bladder prior to standing upon the machine.

2.4 Diet diaries

Four day screening diet diaries were completed for all studies as described above and seven day diet diaries for the pilot and rimonabant study. Participants were instructed to record all food and drinks consumed and photographs of varying portion sizes of common foods were provided to aid in portion size estimation. Participants were encouraged to provide as much information as possible such as brands of foods, types of food, quantities consumed and cooking methods. Each diet diary was examined with the participant prior to analysis to ensure that the information provided was as comprehensive and accurate as possible.

The dietary information recorded in the diaries was analysed using the nutritional analysis programme Windiets Professional 2005 (The Robert Gordon University, Aberdeen, Scotland). This programme contains the nutritional breakdown for an
extensive number of generic foods available in the United Kingdom. If specific brands of food were provided by the participant then the nutritional composition for the food was obtained and manually added to the analysis programme.

Mean four day or weekly intakes of energy and macronutrient composition of the diet were calculated for each food diary for every participant.

2.5 Visual Analogue Scales

Visual Analogue Scales (VAS) were only completed by participants undertaking the pilot study. VAS were developed as a measure of subjective feelings of hunger, fullness etc (Blundell and Rogers, 1980). The scales consist of a 10 cm line with words at each end describing extreme sensations.

\[
\begin{array}{c|c}
\text{Nothing at all} & \text{A lot} \\
\hline
\end{array}
\]

\text{How much do you think you can eat?}

Participants were asked to rate their own feelings by drawing a vertical mark through the line indicating how they felt at that exact moment in time. The position of the mark on the line was then measured and converted to a score. The minimum score on the scale is 0 with a maximum score of 10.

The participants were given the visual analogue scales as a booklet with each scale printed onto a separate page so that previous ratings could not be seen. Participants were asked to rate their feelings on four topics: hunger, appetite, satiety and thirst.

2.6 Resting energy expenditure measurement

On the day prior to the measurement of REE, participants were requested to refrain from heavy exercise and from consuming alcohol. They were provided with a
standardised low fat, low fibre meal the evening before the study and were instructed
to fast for 12 hours before the appointment. On arrival the participants were weighed
and then allowed to rest for 30 minutes in a quiet room. Their REE was then
measured using a Gas Exchange Monitor (GEM), which is ventilated hood open-
circuit indirect calorimetry (GEMNutrition Limited, Cheshire, UK). A 40 minute
measurement of REE was taken to ensure that a steady state measurement was
achieved. The GEM was calibrated each morning prior to the study using a zero gas
(high purity N₂) and a span gas (20% O₂, 1% CO₂, balance N₂) supplied by BOC
(Guildford, UK).

2.6.1 GEM operation
The GEM was designed for the measurement of energy expenditure and substrate
oxidation in spontaneously breathing patients. Its performance has been assessed
using reference gas injections and has been reported to produce a mean error of
0.3±2% in oxygen consumption (VO₂), 1.8±1% in carbon dioxide production
(VCO₂) and 14±1.5% in the respiratory quotient (RQ) (Nicholson et al. 1996).
The GEM is controlled by Windows style software on a standard computer and
allows for real time collection and analysis of data. To measure REE participants
were required to lie down and a clear plastic hemispherical hood was placed over
their head. This hood was attached to the calorimeter by a length of 22mm diameter
flexible tubing. Air was drawn through the hood by a pump and the rate of this air
flow was controlled using a pump controller. The mean level of CO₂ was provided
every 60 seconds as it was essential that the rate of air flow was sufficient to
maintain CO₂ levels in the hood of approximately 0.5%. If the CO₂ concentration
falls too low then errors in measurement may occur and if it rises above 1% both
respiratory effort and energy expenditure will be increased. Alterations to air flow were made as required.

Participants were requested to relax and attempt to breathe normally and mean values for VO\(_2\) and VCO\(_2\) were computed over each one minute during the 40 minute measurement period by the GEM. From these measurements the GEM calculated REE using the modified Weir formula (Nicholson et al., 1996):

\[
\text{REE (Kcal)} = 1.44 \times (3.9\text{VO}_2 + 1.1\text{VCO}_2)
\]

Where \(\text{REE} = \) Energy expenditure in kcal/24 hours
- \(\text{VO}_2 = \) \(\text{O}_2\) consumption in ml/min
- \(\text{VCO}_2 = \) \(\text{CO}_2\) production in ml/min

### 2.6.2 Resting energy expenditure measurement validation

Prior to the start of the studies a new CardiO\(_2\) Ultima (Medgraphics, Minnesota, USA) was purchased for the purpose of measuring REE. Extensive validation work was undertaken and showed that the CardiO\(_2\) Ultima was unable to provide a reliable measurement of REE.

Following the difficulties experienced with the CardiO\(_2\) Ultima it was decided that the GEM (GEMNutrition Limited, Cheshire, UK) would be utilised to measure REE. To ensure that the measurements of REE provided by the GEM indirect calorimeter were reproducible validation work was undertaken. Three individuals had their REE measured for forty minutes on 5-10 occasions. Participants undertook a variable number of readings, but each individual had REE measurements taken on separate days to establish whether REE varied from day to day, as well as repeated measurements on the same day to examine any changes in REE with time rested. Both male and female and normal weight and overweight individuals were studied to
ensure that the results would be representative of future study samples. These results are illustrated in Figures 2.1-2.3 below.

As can be seen from Figure 2.1, the readings were very similar with mean REE of 1374±17 kcal/day and a Coefficient of Variation (CV) of 4%.

**Figure 2.1 Repeated REE measurements for participant V03 using GEM indirect calorimeter.** Two forty minute measurements were taken each day for five days. Data expressed as means ± SEM.
Figure 2.2 Repeated REE measurements for participant V04 using GEM indirect calorimeter. Three forty minute measurements were taken each day for three days. Data expressed as means ± SEM.

As before, the REE measurements for participant V04 were shown to be repeatable with a mean REE of 1811±10 kcal/day and a CV of 1.7%.

Figure 2.3 Repeated REE measurements for participant V05 using GEM indirect calorimeter. One forty minute measurement was taken on day 1 and two forty minute measurements on day 2 and 3. Data expressed as means ± SEM.
As shown in Figure 2.3, REE measurements were similar for participant V05 with a mean reading of 1342±13 kcal/day and a CV of 2.2%.

All validation measurements undertaken indicated that the GEM indirect calorimeter produced reproducible recordings of REE and would be suitable for use in the rimonabant study.

2.7 Carbohydrate, fat and protein oxidation

Carbohydrate and fat oxidation were calculated from the measurements of oxygen consumption and carbon dioxide production taken using the GEM. Protein oxidation was also calculated through the measurement of urinary nitrogen excretion. The following equations (Frayn, 1983) were used to calculate substrate oxidation:

Carbohydrate (g/min) = \(4.55 \times V_{CO_2} - 3.21 \times V_{O_2} - 2.87n\)

Fat (g/min) = \(1.67 \times V_{O_2} - 1.67 \times V_{CO_2} - 1.92n\)

Protein (g/min) = 6.25n

Where \(V_{CO_2}\) = CO₂ production in litres per minute
\(V_{O_2}\) = O₂ consumption in litres per minute
\(n\) = Urinary nitrogen in grams per minute

To establish urinary nitrogen excretion, participants were requested to collect a 24 hour urine collection. The collection commenced in the morning. Upon waking, participants were requested to empty their bladder as usual but not to retain the specimen. Participants were instructed to record the time and the collection started from this point. For the next 24 hours participants urinated into a plastic jug and then transferred their urine into the 24 hour urine collection container. The importance of
retaining every sample was highlighted and it was explained that one missed sample would invalidate the results. The urine collection finished exactly 24 hours after it commenced and to ensure the collection was complete participants were asked to empty their bladder, even if they did not have the urge to urinate, five minutes prior to the end time.

Five grams of boric acid powder was added to the urine collection containers to prevent nitrogen loss through evaporation. The total volume of urine was measured and nitrogen concentration was measured by colleagues at the University of Newcastle using a LECO FP-428 (LECO Corporation, St. Joseph, MI, USA) nitrogen analyser.

2.8 Activity energy expenditure measurement

Activity energy expenditure (AEE) was measured in both the rimonabant trial and gender differences study with an Actiheart monitor.

2.8.1 The Actiheart Monitor

The Actiheart monitor combines measurements of acceleration with heart rate data to accurately estimate activity energy expenditure. The Actiheart computer software provides an estimate of daily total energy expenditure based on a combination of measured (or estimated) REE, activity energy expenditure and dietary induced thermogenesis (the software calculates this as 10% of total daily energy expenditure). Validation work undertaken by Brage et al. (2005) confirmed that the Actiheart is able to accurately record activity energy expenditure during walking and running. The reported CV was 0.5% for movement and 0.03% for heart rate. This study, however, did not assess validity during other activities or free living.
As NEAT is variable between individuals (Levine, 2005), and contributes to total energy expenditure, a small validation study was undertaken to establish if the actiheart monitor could identify activities such as fidgeting and standing. Three individuals were fitted with a monitor and AEE recorded for a five minute period for each of the following activities: finger tapping, foot tapping, leg shaking, pen tapping, typing and standing. No movement was allowed with the exception of the activity being tested. No AEE was recorded for any of the activities indicating that the Actiheart Monitor was unable to identify elements of NEAT.

2.8.2 Activity energy expenditure measurement

The Actiheart monitor was worn on two standard electrocardiogram (ECG) pads (Pulse Medical Limited, Woking, UK) placed on the upper chest of the participant. Prior to application of the pads the top layer of the skin was removed using Cardio-Prep Single use abrasive pads (Pulse Medical Limited, Woking, UK) to ensure that the R wave signal of the heart could be captured by the device. A five minute signal test was undertaken to ensure that the level of the R wave signal being picked up by the Actiheart was adequate. The monitors were then calibrated before use by each participant using an 8 minute linear ramped step test. The step test commenced at a speed of 15 steps per minute increasing to 33 steps per minute at the end of the 8 minute period. Once calibrated, the participants were asked to wear the monitor continuously for a five day period. To try and ensure measurements were an accurate representation of weekly activity participants were asked to wear the monitor during both week and weekend days. The Actiheart data was downloaded at the end of each five day period and AEE calculated using a branched chain equation model (Brage et al. 2005). The model allows the intensity and energy expenditure of
physical activity to be calculated from the activity data or heart rate data alone or from both.

2.9 Body fat distribution

Measurements of body fat, intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL) were undertaken by colleagues at the MRC (Medical Research Council) Clinical Sciences Centre, Hammersmith Hospital. Following a ten hour fast participants were asked to attend the unit where three procedures were undertaken: a 15 minute magnetic resonance imaging (MRI) scan of the total body fat, a 15 minute magnetic resonance spectroscopy (MRS) examination of the liver and a 15 minute MRS examination of the calf.

Whole body MR imaging of body fat content, IHCL and IMCL levels were acquired on an Intera 1.5T Achieva multinuclear system (Philips Medical Systems, Best, Holland) as previously reported (Thomas et al. 2005).

2.9.1 Total body fat measurement

Total and regional adipose tissue volumes were measured after imaging data was analysed using the SliceOmatic image analysis program (Tomovision, Montreal, Quebec, Canada). Participants were scanned from their fingertips to their toes by acquiring 10 mm thick transverse images with 30mm gaps between slices in their arms and legs and 10 mm gaps between slices in their abdomen. All images were acquired as single slices at the isocentre of the magnet to avoid image distortion.
2.9.2 Intrahepatocellular lipid and intramyocellular lipid measurement

Both intrahepatocellular lipid and intramyocellular lipid were measured by $^1$H-magnetic resonance spectroscopy. Intrahepatocellular lipid spectra were acquired from the right lobe of the liver and intramyocellular lipid spectra from the tibialis muscle group in the left lower leg. All spectra were analysed in the time domain using the AMARES (advanced method for accurate, robust and efficient spectral fitting) algorithm included in the MRUI (Magnetic Resonance User Interface) software package (Naressi et al. 2001). IMCL was expressed as a ratio to the muscle creatine signal. IHCL was expressed as a ratio to liver water content.

2.10 Muscle and adipose tissue biopsies

Muscle and adipose tissue biopsies were taken in order that key regulators of lipid metabolism could be measured by real-time polymerase chain reaction (rtPCR). In the adipose tissue samples CD36, adiponectin, adiponectin receptor 1, adiponectin receptor 2, UCP2, lipoprotein lipase, hormone sensitive lipase, PPAR gamma and PPAR delta were measured and in muscle CD36, adiponectin receptor 1, adiponectin receptor 2, UCP2, UCP3 (Uncoupling protein 3), lipoprotein lipase, CPT1 (Carnitine palmitoyltransferase 1), CPT2 (Carnitine palmitoyltransferase 2), ACOX1, PPAR delta and PPAR alpha. A summary of the key functions of these genes is shown in Table 2.2.
Table 2.2 Key functions of genes studied.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue in which gene expression measured</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>Muscle and adipose</td>
<td>Binds long-chain fatty acids and facilitates their transport into cells, influencing muscle lipid utilization, adipose energy storage, and gut fat absorption</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Adipose</td>
<td>Has insulin-sensitizing effects in tissues involved in glucose and lipid metabolism</td>
</tr>
<tr>
<td>UCP2</td>
<td>Muscle and adipose</td>
<td>Involved in the oxidation of fatty acids and glucose.</td>
</tr>
<tr>
<td>UCP3</td>
<td>Muscle</td>
<td>Involved in mediating energy expenditure and in fatty acid metabolism</td>
</tr>
<tr>
<td>Lipoprotein Lipase</td>
<td>Muscle and adipose</td>
<td>Hydrolysis of triglycerides from chylomicrons and VLDLs</td>
</tr>
<tr>
<td>Hormone sensitive lipase</td>
<td>Adipose</td>
<td>Degradation of triglycerides to diacylglycerol and free fatty acids</td>
</tr>
<tr>
<td>CPT1</td>
<td>Muscle</td>
<td>Mediates transport of long chain fatty acids</td>
</tr>
<tr>
<td>CPT2</td>
<td>Muscle</td>
<td>Mediates transport of long chain fatty acids</td>
</tr>
<tr>
<td>PPARα</td>
<td>Muscle</td>
<td>Controls catabolism of fatty acids</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Adipose</td>
<td>Induces fat cell differentiation</td>
</tr>
<tr>
<td>PPARδ</td>
<td>Muscle and adipose</td>
<td>Increases fat oxidation in muscle and lipolysis in adipose tissue</td>
</tr>
<tr>
<td>ACOX1</td>
<td>Muscle</td>
<td>Involved in fatty acid oxidation</td>
</tr>
</tbody>
</table>

CD36, Cluster of Differentiation 36; UCP2, Uncoupling protein 2; UCP3, Uncoupling protein 3; CPT1, Carnitine palmitoyltransferase 1; CPT2, Carnitine palmitoyltransferase 2; PPARα, Peroxisome proliferator-activated receptor alpha; PPARγ, Peroxisome proliferator-activated receptor gamma; PPARδ, Peroxisome proliferator-activated receptor delta, ACOX1, Acyl CoA Oxidase 1.

2.10.1 Biopsy procedure

Whilst participants were fasted, two separate biopsies were taken under sterile conditions. An adipose tissue sample from the lower back and a muscle tissue sample from the vastus lateralis muscle. Both sites were cleaned with betadine solution and local anaesthetic (2% lidocaine hydrochloride) injected. Approximately
100mg of adipose tissue was taken using a standard biopsy pack (Synergy Healthcare, Derby, UK) and an Bergström muscle biopsy needle (Hillside Medical, Newark, Nottinghamshire) was utilized to obtain 100mg of muscle tissue. A stitch was placed in the incision on the lower back and steri strips applied to the thigh incision and both sites were covered with a sterile dressing. The adipose and muscle tissue samples were cleaned of any visible blood and were snap frozen in liquid nitrogen and then stored at -80°C before batch analysis of gene expression was undertaken.

2.11 Adipose and muscle gene expression

2.11.1 Tissue processing

The frozen muscle and adipose tissue biopsies were hand-homogenized using a liquid nitrogen cooled pestle and mortar. Once samples had been ground to a very fine powder they were transferred to a sterile cooled tube and weighed to determine the amount of tissue acquired.

2.11.2 RNA extraction

The SV Total RNA Isolation System (Promega, USA) was used to isolate the RNA from both the muscle and adipose tissue. Levels of RNA was analysed using the Nanodrop spectrophotometer (Thermo Scientific, Delaware, USA). 1.2 μl of each sample were loaded on the Nanodrop and only samples with an mRNA level of 10mg/μl or greater were reverse transcribed. Any samples found to have an mRNA level of between 5 and 10mg/μl were concentrated to double the amount of mRNA available.
2.11.3 Reverse transcription and cDNA Synthesis

First strand complementary DNA (cDNA) was synthesized using the Reverse Transcription System (Promega USA). For the reverse transcription 10μl of total RNA extracted from the fat and muscle tissue was reverse transcribed using both Oligo(dT)$_{15}$ and random primers.

2.11.4 Primers for rtPCR

PCR amplification of the first-strand cDNA was undertaken using the primers (Eurogentec, Seraing, Belgium) shown in Table 2.3.

Table 2.3 Primers used for rtPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer Sequence</th>
<th>Reverse Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>tgcaaaaagctgctgacggtca</td>
<td>gcaaaaaaacatcaccacacca</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>aagggagacatcgggtaaaaccgga</td>
<td>aagcgaatgggcatgtttggga</td>
</tr>
<tr>
<td>Adiponectin receptor 1</td>
<td>tgcacatcgtgcgcgagt</td>
<td>agcgcataaggccagctcca</td>
</tr>
<tr>
<td>Adiponectin receptor 2</td>
<td>tgcgctggccattgcagccatt</td>
<td>agaggttgccatcagcatcga</td>
</tr>
<tr>
<td>UCP2</td>
<td>cctgcagcggccaaatgacgtt</td>
<td>tcgggcaatggtcttgtgagc</td>
</tr>
<tr>
<td>UC P3</td>
<td>tcagtcacacactccccctgca</td>
<td>acgttccaggcctccaacagca</td>
</tr>
<tr>
<td>Lipoprotein Lipase</td>
<td>aaaaactgtggtgcccctgt</td>
<td>actcctgcaatgcacagcaca</td>
</tr>
<tr>
<td>Hormone sensitive lipase</td>
<td>tgcagggcagttttgcccgt</td>
<td>aaggatactgcggcgcggt</td>
</tr>
<tr>
<td>CPT1B</td>
<td>ttccaaaaacagttgccagcge</td>
<td>tgcagccagcatgcaaaaa</td>
</tr>
<tr>
<td>CPT2</td>
<td>agccactgccccgattcagc</td>
<td>tgggccattgctgcttttgg</td>
</tr>
<tr>
<td>PPARα</td>
<td>acggcagcggcttcacagctt</td>
<td>agctgctgcgacactgtcata</td>
</tr>
<tr>
<td>PPARγ</td>
<td>acggccaggctttgtgatgt</td>
<td>agctgcagctgtttgcgcaca</td>
</tr>
<tr>
<td>PPARδ</td>
<td>actggtggctctttgttg</td>
<td>tgcgggcttccttttgg</td>
</tr>
<tr>
<td>ACOX1</td>
<td>taaggccgctacagttgcac</td>
<td>agggcatgaaagtcaagcagt</td>
</tr>
<tr>
<td>GAPDH</td>
<td>atggggaaggatgatgtcga</td>
<td>gagatgatgacctttggc</td>
</tr>
</tbody>
</table>

CD36, Cluster of Differentiation 36; UCP2, Uncoupling protein 2; UCP3, Uncoupling protein 3; CPT1, Carnitine palmitoyltransferase 1; CPT2, Carnitine palmitoyltransferase 2; GAPDH, Glyceraldehyde-3-phosphate-dehydrogenase PPARα, Peroxisome proliferator-activated receptor alpha; PPARγ, Peroxisome proliferator-activated receptor gamma; PPARδ Peroxisome proliferator-activated receptor delta, ACOX1, Acyl CoA Oxidase 1.
2.11.5 PCR Amplification

PCR amplification of the first-strand cDNA was undertaken using a Stratagene Mx3005P™ System as per the manufacturer’s instructions. The rtPCR was performed in a final volume of 50μl containing 2μl of cDNA, 25μl of GoTaq® qPCR Master Mix, 21μl of nuclease-free water and 1μl each of the specific forward and reverse primers. The temperature profile used for the PCR amplification was: a denaturing step at 95°C for 10 minutes and 40 cycles consisting of 95°C for 30 seconds, 62°C for 1 minute and 72°C for 30 seconds. All samples were measured in triplicate and the mean relative mRNA expression levels results normalized against expression of the housekeeping gene GAPDH.

2.12 Statistical Analysis

All statistical analysis was carried out using SPSS Version 17.0 (SPSS Inc., Chicago, USA). The results are presented as means ± SEM unless otherwise stated. Statistical significance was assumed at P<0.05. All variables were checked for normality using the Kolmogorov-Smirnov test. Within group changes were analysed by paired T-test and Wilcoxon Signed Ranks Test as appropriate and differences between groups by Independent Sample T-tests or Mann-Whitney U Test. If outliers were identified using SPSS, then the data was reanalysed removing any outliers and this is clearly indicated in the relevant results section.
Chapter 3

Pilot study to establish the feasibility and acceptability of meeting energy requirements in target participants using a modular energy controlled diet.
3.1 Introduction

3.1.1 Background

A study was planned to examine the effect of rimonabant on energy expenditure, gene expression and body fat distribution. The rimonabant study aimed to examine the direct effects of the drug i.e. independent of weight loss, and therefore a group of participants taking the rimonabant were to be compared to a group of participants subject to a dietary intervention. The weight loss in the dietary intervention group was to match that found in the rimonabant group. Based on previous studies, it was estimated that rimonabant treatment would lead to a 0.5kg weight loss per week. As the aim of the rimonabant study was to measure the effects of treatment on energy expenditure and not energy intake in the rimonabant group, it was essential for energy intake to be maintained during the study. The energy intake was to be maintained at a level matching the participant’s energy expenditure determined in a run-in period. In the dietary intervention group a very specific level of weight loss would be required.

The original rimonabant project was designed prior to commencement of this PhD and it had been proposed that a diet of commercially available modular energy controlled foods (Slim.Fast, Unilever) be used for the seventeen week duration of the trial to allow for energy and macronutrient control of the diet in both the rimonabant and dietary treated groups. Due to the Author’s concerns regarding this dietary methodology, a pilot study was undertaken in order to ascertain the acceptability and feasibility of adopting such an approach.
3.1.2 The Modular Diet

Slim.Fast products had been selected for the study as they allowed for energy and macronutrient control of the diet and were available on the market for the public to purchase. The products were to be used for both the treatment group and the diet group in the rimonabant study. The treatment group was to be given an energy prescription to meet their expenditure and was, therefore, to be prescribed an appropriate number of Slim.Fast products to consume daily to meet their energy requirements. In the control group weight loss was required and therefore the energy prescription was to be lower than energy requirements. This weight loss would have to match that achieved in the rimonabant group so the rate of weight loss would need to be able to be tightly controlled.

Slim.Fast food products had previously been shown to be effective when used as a weight loss tool (Morgan et al., 2008) but are marketed as a low fat meal replacement not as a sole source of nutrition. The advice provided with Slim.Fast products is that one or two meals a day should be replaced with either a Slim.Fast shake, smoothie, soup or meal bar. Previous research had shown that when used as a twice daily meal replacement, Slim.Fast products resulted in a mean weight loss of 3.7 ± 3.5kg over a two month period (Truby et al., 2006). In order to meet the participant’s daily energy requirements (even the reduced requirements in the diet group) they would need to consume a much greater number of products each day than is recommended by the manufacturer. Although vitamin/mineral fortified liquid meals have been used as a sole nutrient source before, this has been in cases where the diet provided has been very low in calories and rapid weight loss has been urgently required (Heymsfield et al. 2003). Studies have not been undertaken in which Slim.Fast products replace all meals and snacks.
3.1.3 Study rationale

A pilot study was required in order to establish whether the Slim.Fast products were acceptable to participants and whether they could be consumed at the level that was to be required for the 17 week duration of the rimonabant study. Additionally it was important to assess whether a tightly controlled level of weight loss was achievable if the energy provided by Slim.Fast products prescribed was lower than energy requirements and these products were the sole source of nutrition.

3.2 Aim

To establish whether a diet of Slim.Fast products was feasible and acceptable to the target study group.

3.3 Objectives

- To investigate whether the Slim.Fast products could be consumed by the participants at the level that would be required for the 17 week duration of the study.
- To assess whether a 0.5kg weight loss per week was achievable, if the energy provided by Slim.Fast products prescribed was 500 kcal lower per day than energy requirements.

3.4 Methodology

3.4.1 Participants

Four overweight female participants, closely matched to the target study group, were recruited for the pilot study through a recruitment email sent to all staff at the
University of Surrey and posters displayed throughout the University campus. The participants mean age was $46 \pm 4.8$ years and their mean BMI was $31.5 \pm 2.1 \text{ kg/m}^2$.

The inclusion and exclusion criteria for the study can be seen in Table 3.1. With the exception of restrained eating, the exclusion criteria were assessed through the completion of a self-certified medical questionnaire (Appendix 1). As the measurement of food intake was a key objective of the study, restrained eaters were identified using the Dutch Eating Behaviour Questionnaire (see chapter two, section 2.2.2).

Table 3.1 Inclusion and exclusion criteria.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-65 years</td>
<td>History of diabetes or endocrine disease</td>
</tr>
<tr>
<td>BMI of 28 -35</td>
<td>History of cardiovascular disease</td>
</tr>
<tr>
<td>Weight stable for a 3 month period</td>
<td>History of hepatic and renal disorders</td>
</tr>
<tr>
<td></td>
<td>History of neurological or psychological illness</td>
</tr>
<tr>
<td></td>
<td>History of depression</td>
</tr>
<tr>
<td></td>
<td>Previous surgical procedure for weight loss</td>
</tr>
<tr>
<td></td>
<td>Taking medication that may affect weight or appetite</td>
</tr>
<tr>
<td></td>
<td>Food allergies or intolerances</td>
</tr>
<tr>
<td></td>
<td>Restrained eaters</td>
</tr>
</tbody>
</table>

All participants were provided with a participant information sheet and the study design was explained before written consent was obtained. Ethical approval for the study protocol was obtained from the University of Surrey Ethics Committee (EC/2007/14/SBMS). Participants were not paid for their participation.

3.4.2 Study Design

The pilot study was undertaken over a five week period. In the first week REE and activity energy expenditure were measured. In weeks two and three weight
maintenance was the aim. Participants were given an energy prescription to match their estimated total energy expenditure and prescribed an appropriate number of Slim.Fast products to consume daily to meet their energy requirements. In weeks four and five weight loss was desired and therefore participants were prescribed a reduced number of Slim.Fast products providing an energy intake lower than energy requirements.

3.4.3 Nutritional information for Slim.Fast products

A range of products was prescribed including shakes, smoothies, soups and snack bars. Each Slim.Fast product had a precise balance of fat, protein and carbohydrate with essential vitamins and minerals and the diets prescribed to the participants comprised of 55% carbohydrate, 21% fat and 24% protein. A full breakdown of the nutritional composition of the products utilised in the study is shown in Appendix 4.

3.4.4 Protocol

All participants were asked to attend the Clinical Investigation Unit (CIU) at the University of Surrey on four occasions (Figure 3.1). Details of the four visits are provided below.

![Figure 3.1 Outline of study.](image-url)
3.4.4.1 Visit 1: Energy expenditure calculation

Resting energy expenditure measurement

The participants were weighed and their height was taken (chapter two, section 2.6). Their REE was measured using ventilated hood open-circuit indirect calorimetry (GEM, GEMNutrition Limited). See chapter two, section 2.6 for further details.

Activity energy expenditure measurement

In order to then estimate the amount of energy expended in everyday activities each participant was fitted with an Actiheart monitor (chapter two, section 2.8). Participants were asked to wear the monitor for a five day period. An estimation of dietary induced thermogenesis (10% of total energy expenditure) was combined with the results from the indirect calorimetry and the Actiheart monitor to estimate total energy expenditure.

3.4.4.2 Visit 2: Dietary intervention – Weight maintenance

On the second visit each participant was weighed. The total energy expenditure calculated for each participant was then used to provide a personal dietary prescription.

Dietary prescription

An example dietary prescription may be seen in Appendix 5. The aim was to provide a daily energy intake for each participant equal to their energy requirement. The products were supplied from the Slim.Fast range and had been donated by Unilever. A range of products was prescribed including shakes, smoothies, soups and snack bars. Participants were asked to consume a variety of the different
products so that acceptability of different foods could be assessed. In order to enhance compliance, each participant was provided with a list of all Slim.Fast products available (Appendix 6) and asked to indicate if any were unacceptable to them. Participants were asked to follow this eating plan for a period of two weeks and to refrain from drinking alcohol for the duration of the pilot study.

Assessment of compliance and side effects

In order to aid assessment of suitability of Slim.Fast products for use in a longer study, participants were asked to complete a 24-hour diet diary on every day of the two week period. They were asked to write down all of the foods and drinks that they consumed during each day. They were asked to note which Slim.Fast products they had eaten, and when, and to note occasions on which foods or drinks were only partially eaten/drunk or omitted and reasons for this. Space was available in the diary for participants to provide comments on any adverse side effects that they experienced.

Assessment of satiety

On the final day of week two of the study participants were asked to complete a Visual Analogue Scale six times daily. These were utilised as an additional means of ascertaining levels of satiety, appetite and thirst as these may potentially impact on compliance. They were undertaken at this time point as participants would have had several days to adjust to their new dietary regimen. The minimum score on the scale was 0 with a maximum score of 10. A mean visual analogue score for all participants was calculated for four questions at six different time points during the day. The four questions asked were:
How hungry do you feel?
How much do you think you can eat?
How full do you feel?
How thirsty do you feel?

Participants were asked to rate their feelings on these topics pre and post breakfast, lunch and dinner as described in chapter two, section 2.5.

### 3.4.4.3 Visit 3: Dietary intervention – Weight loss

Following the two week period on the energy controlled diet, participants were invited to the CIU for the third visit. All participants were weighed to assess whether they had remained weight stable following the eating plan. Twenty four hour diet diaries and Visual Analogue Scales were collected. Participants were then provided with their second personal dietary prescription. In this stage of the study weight loss was desired and therefore participants were prescribed a reduced number of Slim.Fast products to provide an energy intake lower than their energy requirements. The aim was for a weight loss of approximately 0.5 kg per week and, therefore, the dietary prescription was reduced by about 500 kcal per day. As before, this energy controlled diet was to be followed for two weeks and participants were asked to keep a 24 hour diet diary daily.

### 3.4.4.4 Visit 4

On completion of the two week dietary intervention, participants attended the CIU for a fourth visit. On this final visit participants were weighed. Completed diet diaries were requested and participants were asked a series of questions to try and obtain as much qualitative feedback as possible regarding the pilot study.
Participants were asked questions regarding compliance to the diet, adverse side effects and ability to tolerate the products (see Appendix 7 for End of Pilot Questionnaire).

3.5 Statistical Analysis

This was a feasibility study and one of the main aims was to understand participant compliance. The data obtained was mainly qualitative. For the element of the pilot study that assessed potential weight loss, simple comparison statistics were used.

3.6 Results

3.6.1 Total energy expenditure

Table 3.2 shows the baseline REE measurements, activity energy expenditure measurements and estimates of dietary induced thermogenesis and total energy expenditure (TEE) for the four participants.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>REE (kcal/day)</th>
<th>AEE (kcal/day)</th>
<th>DIT (kcal/day)</th>
<th>TEE (kcal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>49</td>
<td>91.6</td>
<td>1116 ± 5</td>
<td>712 ± 178</td>
<td>203</td>
<td>2031</td>
</tr>
<tr>
<td>P02</td>
<td>54</td>
<td>95.0</td>
<td>1001 ± 6</td>
<td>290 *</td>
<td>143</td>
<td>1434</td>
</tr>
<tr>
<td>P03</td>
<td>32</td>
<td>76.0</td>
<td>1053 ± 8</td>
<td>295 ± 18</td>
<td>149</td>
<td>1497</td>
</tr>
<tr>
<td>P04</td>
<td>49</td>
<td>89.6</td>
<td>1310 ± 8</td>
<td>680 ± 61</td>
<td>221</td>
<td>2211</td>
</tr>
</tbody>
</table>

Results were calculated from a combination of resting energy expenditure (REE) readings, estimated dietary induced thermogenesis (DIT) and activity energy expenditure (AEE) measured by the Actiheart Monitor.

(* No SEM available due to technical problems with Actiheart monitor resulting in one days recording only).

As can be seen in Table 3.2, the AEE for participants P02 and P03 was very low with a mean reading of 290-295kcal/day. These low levels of physical activity were
confirmed with the participants. The AEE recordings for P01 and P04 were higher resulting in higher estimates of total energy expenditure. These estimates of total energy expenditure were utilised to provide the personal dietary prescriptions for the participants.

3.6.2 Dietary analysis

Participants kept a record of all Slim.Fast products consumed and the mean daily intake of energy was calculated. A comparison of the prescribed energy intake and the mean daily intake of energy reported during both the two week weight maintenance intervention and the two week weight loss intervention is shown in Figures 3.2 and 3.3.

![Figure 3.2](image)

**Figure 3.2** Mean daily intake of energy for four participants during the maintenance intervention in comparison to prescribed daily energy intake. Data expressed as means ± SEM.
As can be seen in Figure 3.2, all participants reported a mean daily energy intake lower than that prescribed in the weight maintenance intervention. During the weight loss intervention the daily prescribed energy intake was reduced by 500 kcal yet, as shown in Figure 3.3, participants P02 and P03 continued to report a mean daily energy intake lower than that prescribed. One participant had to withdraw from the trial during the weight loss intervention stage due to diagnosis of an unexpected health problem (unrelated to the study).

**3.6.3 Analysis of weight change**

All participants were weighed at the beginning and the end of the two week weight maintenance intervention period. The weight measurements are shown in Figure 3.4.
Figure 3.4 Weight measurements for four participants at the beginning and the end of a two week weight maintenance intervention period.

As shown in Figure 3.4 all participants had a reduction in weight over the two week period with a mean weight loss of 1.6 ± 0.4kg.

Participants were subsequently weighed before and after the two week weight loss intervention and these results are shown in Figure 3.5.

Figure 3.5 Weight measurements for three participants at the beginning and the end of a two week weight loss intervention period.
As shown in Figure 3.5, all participants lost weight during the weight loss intervention period with a mean loss of 2.3 ± 0.1kg.

### 3.6.4 Visual Analogue Scores

The mean VAS scores for participants for the four separate questions *How hungry do you feel?* *How much do you think you can eat?* *How full do you feel?* and *How thirsty do you feel?* were calculated and these are shown in Figures 3.6-3.9 below.

![Figure 3.6](image1.png) **Figure 3.6** Mean VAS scores ± SEM for all participants pre and post meal for the question ‘*How hungry do you feel?*’.

![Figure 3.7](image2.png) **Figure 3.7** Mean VAS scores ± SEM for all participants pre and post meal for the question ‘*How much do you think you can eat?*’.
The VAS scores indicated that participants were moderately full all day with the highest satiety scores being recorded following the evening meal. Similar hunger scores were recorded prior to the three meals of the day with a subsequent reduction in hunger following eating. Participants reported moderate thirst throughout the day.
3.6.5 Questionnaire results

All participants reported that they would feel unable to consume a diet of Slim.Fast products for a 17 week period. One participant suggested that they might have been able to comply with the diet for 17 weeks if more savoury products had been provided.

Changes in bowel habits reported included more frequent passing of stools and mild diarrhoea, although one participant reported no changes. Side effects experienced whilst following the diet included flatulence, occasional bloating and nausea.

3.7 Discussion

During the initial weight maintenance two weeks of the trial every participant lost weight, which was probably due to reduced energy intake, since activity energy expenditure was unchanged. All participants reported a mean daily energy intake lower than that prescribed in the weight maintenance period and this would account for the weight loss recorded. Participants reported that it was difficult to consume the number of Slim.Fast products that they were prescribed for each day and therefore could not meet their energy requirements. Although underreporting is a well documented phenomenon when using diet diaries (Goldberg et al., 1991) this does not appear to have been an issue in this study as reported levels of intake corresponded with weight lost.

The estimated energy requirements for participants P02 and P03 were low if compared to REE calculated using the Schofield equation (1985) and this may have been due to measurement error. These two participants also had very low recordings of AEE but reported that they incorporated very little activity into their usual day e.g. they travelled to work by car, had sedentary jobs and relaxed on a sofa every
evening. Despite the apparently low energy requirements for P02 and P03, and thus a lower energy prescription, neither participant was able to consume all of the products prescribed. It should be taken into consideration that the participants of the pilot study were overweight and even though the study objectives were explained to them may have felt motivated or wished to lose weight. Even with low measurements of TEE in two individuals, all participants failed to meet their estimated total energy expenditure requirements in the weight maintenance phase utilising the Slim.Fast products and no participant was able to consume all of the products prescribed.

In the second two weeks where the aim was for a weight loss of approximately 0.5 kg per week the average weight loss was over four times greater. This weight loss greatly exceeded the required loss of 1kg over the two week period. The daily prescribed energy intake had been reduced by 500 kcal, therefore requiring fewer products to be consumed each day, however participants P02 and P03 continued to report a mean daily energy intake lower than prescribed. In comparison to the mean daily energy intake over the first two weeks a further mean daily reduction of approximately 300kcal was reported during this two week period and this would account for the greater weight loss. It should be noted that previous research has identified that the fastest rate of weight loss in individuals undertaking a meal replacement weight loss intervention is during the initial two months (Truby et al., 2006).

All participants reported that they would feel unable to consume a diet of Slim.Fast products for a 17 week period. This supports findings by Dasinger et al. (2005), when examining a range of popular diets, that the more extreme the eating plan the poorer the adherence. Reasons cited for not being able to follow a diet using
Slim.Fast foods for 17 weeks included that it was hard to eat out socially whilst consuming this diet and it was difficult to adhere to when cooking for a family. The Slim.Fast products were additionally reported as being either too sweet or tasteless. All participants reported introducing additional foods during the four week period of the trial. The reasons provided for this were the desire to eat ‘normal’ foods at social occasions and a need to eat ‘proper’ foods after a few days. These findings are in agreement with previous research in which lifestyle barriers to successful dieting had been reported as family commitments, social life and work (Herriot et al., 2008). It is unsurprising that participants wished to eat ‘normal’ foods as Slim.Fast products are designed to replace one or two meals a day and are not used as a sole source of nutrition. In a previous study, 14.5% of participants following the Slim.Fast diet withdrew from the trial with the reason provided that they could not tolerate the diet (Truby et al., 2006). Further detail was not provided as to whether this was due to practical reasons, taste or adverse side effects.

In the end of study questionnaire, three participants reported that the Slim.Fast products were satiating, particularly the shakes and that usual feelings of hunger disappeared. One participant, however, felt very hungry and that the foods ‘gave no satisfaction’. This finding corresponds with disadvantages of ‘feeling hungry’ and finding the products ‘boring’ cited by a group of participants undertaking a study using the Slim.Fast meal replacements twice a day (Herriot et al., 2008). Differing feelings of satiety may have been related to the type of product consumed, as previous work by Tieken et al. (2007) has shown that solid meal replacement products are more satiating than liquid meal replacement products. All participants reported increased thirst which was reflected in the VAS scores. Although there is anecdotal evidence that meal replacement products may increase thirst levels, few
studies have investigated this potential side effect. A study examining the hunger changes in obese participants following ingestion of a meal replacement bar did also measure thirst, and found it to be below baseline levels for three hours following consumption (Rothacker and Watemberg, 2004). However the participants were instructed to take their meal replacement bar with a glass of water which may have aided in the reduction of thirst sensation. Additionally, participants in this trial were taking large quantities of replacement products so direct comparisons are invalid. It is possible that, as participants in the study were having several shakes each day, they reduced their intake of additional or alternative fluids such as water. Few additional drinks were recorded on the diet diaries.

The side effects reported during the study of flatulence, bloating and nausea were cited as a further reason for not being able to comply with the diet for a seventeen week period. It is possible that these effects may have been due to the high fibre content of the diet as the prescribed diets had 35-40g fibre per day. This is likely to be higher than the participants’ usual fibre intake as a recent investigation reported average fibre intake in the UK adult population to be 21.2g per day (Cust et al., 2009). Higher intakes of fibre can be tolerated by most individuals but fibre intake needs to be gradually increased over time, however this was not the situation with this study in which fibre intake was likely to have increased abruptly. The major side effect of a high fibre diet is increased flatulence but this does decrease as individuals adapt to a high fibre diet (Anderson et al., 1994).

Slim.Fast products are designed as a meal replacement and have not been used in previous studies as a sole source of nutrition. The major issues regarding the products as a sole nutrient source are palatability and the safety of providing participants with large doses of micronutrients. As the Slim.Fast products were
designed as meal replacements each shake/smoothie/meal bar provides between 20-59% of the recommended daily amount (RDA) for each micronutrient. Therefore if two products are consumed per day as advised by Slim.Fast, individuals may meet their RDA for each micronutrient. In the pilot study, however, participants were consuming up to eight products each day and the RDAs for each micronutrient were being greatly exceeded. Water-soluble vitamins are excreted from the body but there is the potential for the fat-soluble vitamins to be stored. As the rimonabant study was only to be of 17 weeks duration this would have been unlikely to cause health problems for the participants but it was an additional consideration.

From the results of the pilot study it was established that the Slim.Fast products were not acceptable to participants and that they were not able to be consumed at the level that was to be required for the 17 week duration of the rimonabant study. It was found that weight loss was achievable if the energy provided by Slim.Fast products prescribed was lower than energy requirements, as would be required in the diet group of the rimonabant study, but this weight loss may have been greater than necessary and could not be tightly controlled. On the basis of these findings it was decided that utilising foods normally consumed by participants would be more effective at meeting energy requirements in the rimonabant clinical trial. This approach was considered appropriate for the group receiving rimonabant, in which energy intake would be required to meet energy requirements, and would also allow for prescription of a reduced energy intake in the diet group. Macronutrient composition of the diet could still be monitored through diet diary analysis and compliance to the diet was likely to be greater than was seen with Slim.Fast products.
Chapter 4

The effect of rimonabant on energy expenditure, gene expression and body fat distribution
4.1 Introduction/Background

In light of the ever increasing worldwide problem of obesity, new approaches for the prevention and treatment of obesity need to be identified. Weight loss through lifestyle modification is challenging and largely unsuccessful. There is thus a huge clinical need for the development of an effective treatment which can produce a sustainable loss in body weight.

The endocannabinoid system is involved in the control of food intake and energy balance regulation (Pagotto, 2006) and has emerged as a promising target in the fight against obesity. The system is overactivated in obesity, which is demonstrated through an increase in endocannabinoid production, or an increase in cannabinoid receptor expression in obese animals and humans (Bellocchio et al. 2006).

Rimonabant, a CB₁ antagonist, was shown to reduce food intake in obese Zucker rats (Bensaïd et al., 2003) and in diet induced obese mice. The effect on food intake was only transient, however, while a decrease in body weight was prolonged also suggesting energy expenditure was affected. A study investigating the effect of rimonabant treatment in obese (Lep^{ob}/Lep^{ob}) mice showed that basal oxygen consumption significantly increased compared to control animals and that there was a significant increase in glucose uptake in isolated soleus muscle preparations (Liu et al. 2005). The cause of the increase in energy expenditure was unclear but in the diet induced obese mouse, rimonabant increased gene expression in adipose tissue of key enzymes in fatty acid oxidation, suggesting an increase in fatty acid oxidation was potentially the mechanism (Jbilo et al., 2005).

The four large clinical RIO trials, discussed in chapter one, examined the effects of rimonabant in obese humans. The results of these four trials together indicated that a daily 20mg dose of rimonabant for one year, in addition to a hypocaloric diet
(600kcal/day deficit), resulted in weight loss, reductions in waist circumference and positive changes in HDL-cholesterol, adiponectin and leptin levels and insulin sensitivity.

As discussed in chapter one, further investigation was still required as to the metabolic effects of rimonabant. The rationale and hypothesis for the study are described in chapter one, section 1.17.

4.2 Aims

Study aim:
The aim of this study was to determine if, when energy intake was maintained at pre treatment levels, rimonabant still induced weight loss through an effect on energy expenditure and the direct effects of rimonabant on body fat distribution and gene expression. As weight loss itself may result in alterations in metabolism, the study investigated changes in energy expenditure, body fat distribution and gene expression in a control group undertaking a dietary intervention matched for the weight loss in the rimonabant group.

Specific aims: To investigate in obese participants the following:

1. If, when food intake is maintained at pre treatment levels, rimonabant reduces body weight.
2. If a reduction in body weight was induced in the rimonabant group, whether this was due to an increase in resting energy expenditure.
3. The effects of rimonabant treatment on fat and carbohydrate oxidation rate.
4. The effect of rimonabant on whole body fat distribution, intrahepatocellular lipid (IHCL) and intramyocellular (IMCL) lipid.
5. The effect of rimonabant on adipose tissue and muscle mRNA levels of key regulators of fatty acid metabolism.

6. In a control group following a dietary intervention, with weight loss matched to that achieved by the drug group, changes in REE, fat and carbohydrate oxidation rate, body fat distribution and gene expression in comparison to the rimonabant group.

4.3 Methodology

4.3.1 Study power

The power calculation was undertaken by a medical statistician. The primary endpoint of the study was an increase in total energy expenditure. It had been assumed for the purposes of the power calculation that there would be no change in total energy expenditure in the diet group, as it was difficult to predict how much this would decrease. Over 12 weeks, rimonabant treatment of 20mg per day resulted in a weight loss of approximately 5.0kg in the clinical trials whereas the placebo group lost approximately 2.5kg. If it was assumed that the extra 2.5kg weight loss was due to an increased energy expenditure, this was approximately 178 kcals/day. Based on an SD of REE measured by indirect calorimetry of 12% (Nielsen et al., 2003) it was calculated that this study required 15 participants in each group. The probability was 80% that the study would detect this increase in energy expenditure in the rimonabant group after 12 weeks treatment compared to the dietary intervention group at a two-sided 5% level of significance.
4.3.2 Participants

Fourteen obese postmenopausal females aged 50-65 years were recruited for the study from the local Guildford population using the recruitment strategies outlined in Chapter 2. To be eligible for the study participants required a BMI of 30-35kg/m² and must have been weight stable for at least a three month period. Participants were excluded from the study if they fulfilled any of the exclusion criteria detailed in Chapter 2 (section 2.2.1). Over two hundred potential participants were excluded during an initial screening telephone call and the three primary reasons for exclusion were a past history of depression, not meeting the BMI criteria and the use of medications prohibited for the trial.

All participants were provided with a participant information sheet and the study design was fully explained before written consent was obtained. This study was registered with ClinicalTrials.gov (NCT00584389) and was approved by the UK Medicines and Healthcare Products Regulatory Agency (Eudract 2006-006424-18), the University of Surrey Ethics Committee and the East Kent Research Ethics Committee (07/Q1803/1).

4.3.3 Study design

In the original study design 32 participants were to be randomised into two groups for the study:

Group 1: 16 participants. Rimonabant dose 20mg/day for 13 weeks with energy intake matched for energy expenditure determined in the run-in period.

Group 2: 16 participants. Dietary intervention for 13 weeks, with the aim of achieving the same weight loss as group 1.
Participants were to be studied in blocks of four i.e. four participants randomised to group 1 were to be studied in the first block, followed by four participants in group 2, then this was to be repeated. This allowed for the monitoring of weight loss in the drug treated group which then set the intervention for the dietary intervention group.

Half way through the trial, in November 2008, the licence for Rimonabant was suspended by the European Medicines Agency. Rimonabant was subsequently withdrawn from the market and the study was prematurely terminated. At this time, 14 participants had completed the study. Group 1 (n=7) received rimonabant 20mg/day for 13 weeks with energy intake matched to their energy requirements and group 2 (n=7) followed a dietary intervention to achieve the same weight loss as group 1.

As planned, four participants in group 1 were studied first in order to monitor weight loss and thus set the intervention for group 2. Eight weeks after group 1 began the study four participants from group 2 started the dietary intervention. This was then repeated with a block of three participants in each group.

The study was of 17 weeks duration and comprised of a run-in period of four weeks, a treatment period of twelve weeks and post treatment tests of one week (treatment continued during the post treatment tests) (Figure 4.1 and 4.2).
4.3.4 Protocol

Screening

Visit 1: Details of the screening visit are in Chapter 2 (section 2.2). Due to the potential side effects of rimonabant, participants were asked to complete a Beck Depression Inventory-II questionnaire in order to assess current levels of depression.
A Dutch Eating Behaviour Questionnaire was also completed to establish eating behaviour. Following the screening visit, participants were requested to complete a four day diet diary in order that under reporters could be excluded from the trial. If the participant fulfilled all of the inclusion criteria and none of the exclusion criteria they were accepted into the study. A letter was then sent to the participant’s GP to explain the trial and notify them of their patient’s involvement.

Once 8 participants were accepted into the study they were randomised to the rimonabant treatment or the dietary intervention. Participants were randomised through the selection of a sealed envelope containing either the rimonabant or dietary treatment option by an independent individual not involved in the trial. Due to the considerable amount of time required for Visits 5 and 7 only one participant was able to commence on the study each week. For the participants allocated to the dietary intervention group there was a delay of eight weeks before they started this part of the study. This was in order to establish the mean amount of weight loss being achieved by the rimonabant group.

![Figure 4.2 Study design.](image)
**Run-in period**

**Visit 2:** The purpose of this visit was to establish the participants' energy expenditure. Participants were weighed. REE was measured as described in Chapter 2 (section 2.6).

In order to then estimate the amount of energy expended in everyday activities and exercise each participant was fitted with an Actiheart monitor (Chapter 2/section 2.8).

**Visit 3:** At this visit the total energy expenditure calculated for each participant was used to provide a personal dietary prescription. The aim was for a participant's energy intake to match the estimated total energy expenditure determined during the first week of the run-in period. In both groups the macronutrient composition of the diet was required to be equal and this was continually monitored through the analysis of diet diaries. It was decided to aim for a diet with a macronutrient composition representative of the Guildford population (approximately 46% carbohydrate, 37% fat and 17% protein) as previously reported in a study by Wilkinson *et al.* (2005). Participants that usually consumed moderate or high quantities of alcohol were asked to reduce their intake during the study. At this visit participants in the dietary intervention group undertook a comprehensive dietary interview in order to establish regular eating habits. This interview was utilised to form the basis of their personalised diet plan, which was to be followed for the 12 week treatment period.

**Visit 4:** This was undertaken two weeks after visit 3 to check compliance with the dietary prescription and to ensure that the diet was meeting the participant’s energy requirements. If the participant was weight stable (less than 1 kg variation), they
proceeded to visit 5, otherwise the diet was adjusted as appropriate and the visit was repeated a fortnight later.

**Visit 5:** This visit was undertaken during the last week of the run-in period. Participants were weighed and, as before, REE was measured by indirect calorimetry. To establish urinary nitrogen excretion, participants were requested to collect a 24 hour urine collection throughout the day before the visit as described in Chapter 2 (section 2.7).

**Visit 6:** Measurements of body fat, intrahepatocellular lipid and intramyocellular lipid were taken at the MRC Clinical Sciences Centre, Hammersmith Hospital. Any participants with metal implants or claustrophobia were excluded from this section of the study (Chapter 2. Section 2.9).

**Visit 7:** This visit was scheduled 5-7 days after visit 5. Participants were weighed and a needle biopsy of the vastus lateralis muscle in the thigh and subcutaneous adipose tissue was taken under local anaesthesia by a physician trained in this procedure. The biopsies were snap frozen in liquid nitrogen and stored at -80°C in preparation for batch analysis. These biopsies were utilised to measure mRNA levels of key regulators of fatty acid metabolism (Chapter 2. Section 2.11).

**Dietary intervention/Rimonabant treatment**

At the end of the study day each participant was advised on their dietary intake for the next two weeks. Participants in the rimonabant group had a consultation with a doctor who prescribed the drug and provided advice on possible side effects.
Each participant commenced with either rimonabant treatment or dietary intervention on the following day. If a participant had been randomised to the drug treatment group they were asked to maintain their dietary intake at the same level as during the run-in period (i.e. their energy intake should match their measured energy requirements) and if they had been randomised to the dietary intervention group they were provided with a very detailed diet plan to reduce their dietary intake. The energy prescription provided to participants in the diet group was based on the estimate of the energy deficit required to create equal weight loss to that seen in the rimonabant treated group. i.e. the participant’s total daily energy expenditure as determined during the run-in period minus the appropriate number of kcal daily to achieve equal weight loss to the drug group. The diet plan utilised foods usually consumed by the participant for convenience and to aid with compliance. Participants were asked to complete a seven day diet diary for the full 17 week duration of the study.

**Treatment Period**

**Visit 8-12:** These visits took place on a fortnightly basis. The participants were weighed and dietary compliance discussed. Participants were advised as to any changes that were required to the diet and self reported levels of physical activity were recorded. For participants in the rimonabant treated group medication compliance was assessed by tablet counting and levels of depression monitored through the completion of the Beck Depression Inventory-II. Any adverse events or potential side effects of the medication were also recorded and any necessary action taken. Between these visits each participant received a fortnightly telephone call to review dietary and medication compliance and to record any self reported changes in physical activity levels.
On visit 12 participants were fitted with an Actiheart monitor to remeasure energy expenditure for a five day period during week 12.

Post treatment tests

Visit 13: This took place after the 12 week intervention period and was identical to visit 5.

Visit 14: This visit was undertaken after the 12 week intervention period and was identical to visit 6.

Visit 15: This took place 5-7 days after visit 13 and was identical to visit 7.

Participants in the rimonabant group continued with their treatment, and in the dietary control group the hypocaloric diet, until the post treatment tests were completed.

4.4 Results

4.4.1 Screening data

All participants were screened at Visit 1 and a summary of their characteristics may be seen in Table 4.1. There was no significant difference in any of these characteristics between the two groups.
Table 4.1 Screening characteristics. Data expressed as mean ± SEM

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rimonabant Group (n=7)</th>
<th>Diet Group (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.14 ± 1.87</td>
<td>57.43 ± 1.86</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.10 ± 2.20</td>
<td>82.96 ± 2.86</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.50 ± 1.22</td>
<td>159.57 ± 2.60</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32.47 ± 0.72</td>
<td>32.54 ± 0.58</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>97.71 ± 2.99</td>
<td>96.57 ± 2.54</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>116.00 ± 1.69</td>
<td>114.64 ± 3.18</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.84 ± 0.03</td>
<td>0.84 ± 0.02</td>
</tr>
<tr>
<td>Fat mass (%)*</td>
<td>45.46 ± 0.77</td>
<td>43.66 ± 1.11</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.53 ± 0.08</td>
<td>5.29 ± 0.15</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.47 ± 0.44</td>
<td>5.64 ± 0.38</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.87 ± 0.43</td>
<td>1.12 ± 0.14</td>
</tr>
</tbody>
</table>

* Fat mass as measured by impedance.

4.4.2 Dietary analysis

Diet diary analysis was undertaken on a weekly basis to ensure that each participant was consuming a diet that provided the correct amount of energy to meet their requirements.

Mean daily energy intake was maintained in the rimonabant group throughout the run-in period and treatment period as shown in Figure 4.3. In the rimonabant group the mean daily intake of kcal per day was 1991±99 in the run-in period versus 1963±73 kcal/day during the treatment period (p= 0.630).

In the diet group there was a difference in daily energy intake between the run-in period and during the treatment as per the study design. There was a reduction in mean daily energy intake between the run-in and treatment period of 341±55 kcal/day (p<0.001).
The change in energy intake between the run-in period and treatment period was significantly different (p=0.002) between the two groups (Table 4.2).

Figure 4.3 Mean daily energy intake during the four week run-in period and the treatment period in the rimonabant and diet group. Data are expressed as mean daily energy intake in kcal/day ±SEM.

Figure 4.4 shows that the mean energy intake was very steady in both groups during the run-in period as per the study design. There was a reduction in energy intake in the diet group during the treatment period as prescribed and the mean energy intake was very consistent every week in the rimonabant group.
Figure 4.4 Mean energy intake during the four week run-in period and the treatment period in the rimonabant and diet group. Data are expressed as mean energy intake in kcal/day ±SEM.

In the dietary intervention group the reduction in mean energy intake was due to a decrease in fat intake, mainly saturated and monounsaturated fatty acids and carbohydrate intake (Table 4.2).

Table 4.2 Mean daily macronutrient intake during the run-in and the intervention period for the rimonabant and diet group.

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group</th>
<th>Diet Group</th>
<th>( \Delta ) rimonabant versus ( \Delta ) diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>Run-In</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal/day)</td>
<td>1991±99</td>
<td>1905±36</td>
<td>1564±56* P=0.002</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>87±5</td>
<td>82±3</td>
<td>61±4* P=0.001</td>
</tr>
<tr>
<td>SFA (g/day)</td>
<td>31±2</td>
<td>28±2</td>
<td>21±1* P=0.046</td>
</tr>
<tr>
<td>PUFA (g/day)</td>
<td>14±1</td>
<td>15±1</td>
<td>12±1* NS</td>
</tr>
<tr>
<td>MUFA (g/day)</td>
<td>28±3</td>
<td>24±1</td>
<td>19±1* NS</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>215±21</td>
<td>215±10</td>
<td>185±10* P=0.03</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>78±2</td>
<td>78±4</td>
<td>73±2 NS</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids. *significantly different from run-in period, p<0.05. Data expressed as mean ± SEM.
The mean percentage contribution of each macronutrient to total energy intake during both the run-in period and intervention was also calculated for both groups and this is shown in Table 4.3. There were no significant differences between the run-in period and intervention period in macronutrient composition of the diet in the rimonabant group. In the diet group, however, there was a significant decrease in the proportion of energy provided by fat \((p=0.02)\) and a significant increase in percentage of energy provided by protein \((p<0.01)\) between the run-in and intervention periods. When comparing between groups there was no significant difference in the percentage contribution of fat or protein to total energy intake during the run-in period but the mean percentage contribution of carbohydrate was higher \((p=0.02)\) and mean percentage of alcohol lower \((p<0.01)\) in the diet group than the rimonabant group during the run-in period. During the intervention period the mean percentage contribution of fat \((p<0.01)\) and alcohol \((p<0.01)\) to energy intake was significantly lower in the diet group and the mean percentage contribution of protein \((p<0.01)\) and carbohydrate \((p=0.04)\) to energy intake lower in the rimonabant group.
Table 4.3 Mean percentage contribution of macronutrients to total energy intake during the run-in and the intervention period for the rimonabant and diet group.

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group</th>
<th>Diet Group</th>
<th>p Rimonabant Group versus Diet Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>Run-In</td>
<td>Intervention</td>
<td>Run-In</td>
<td>Intervention</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>38.65 ± 1.39</td>
<td>38.22 ± 1.51</td>
<td>34.39 ± 1.44</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>40.89 ± 2.56</td>
<td>43.23 ± 1.66</td>
<td>45.20 ± 2.04</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>16.23 ± 0.72</td>
<td>16.68 ± 1.01</td>
<td>19.25 ± 1.01</td>
</tr>
<tr>
<td>Alcohol (% of energy)</td>
<td>4.22 ± 1.36</td>
<td>3.76 ± 0.88</td>
<td>1.82 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*p* significantly different from run-in period, *p*<0.05

### 4.4.3 Body weight

As shown in Figure 4.5 participants in the rimonabant group were weight stable during the run-in period of the study as per the study protocol and then, despite mean weekly energy intake being maintained during the intervention period, there was a reduction in body weight.

![Figure 4.5](image-url)
During the intervention period mean energy intake was reduced in the dietary group and a corresponding weight reduction was observed (Figure 4.6).

Mean weight loss was very similar in both the rimonabant and diet group as per the study design. In the rimonabant group, despite energy intake maintenance during the study, the mean weight loss was 2.6±0.5kg (p=0.003). In the dietary intervention group, where energy intake was reduced during the treatment period, a weight loss of 3.1±1.1kg (p=0.027) was achieved (Figure 4.7). There was no significant difference between groups.
4.4.4 Resting energy expenditure

REE was measured at visit 2 and visit 5. The measurement taken at visit 2 was a practice reading to familiarise the participants with the procedure and the REE measurement taken at visit 5 was used as the baseline REE for all participants in the trial. There was no significant difference between the mean REE measurements taken at visit 2 and visit 5 (Figure 4.8).

Figure 4.7 Mean body weight pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM (n=7 in each group).
Figure 4.8 Comparison of mean REE measured at visit 2 and visit 5 for all participants. Data expressed as mean ± SEM.

Pre and post REE results were analysed for both groups. REE decreased in the dietary intervention group (p=0.054) as expected with weight loss, but was maintained in the rimonabant group despite a reduction in weight (Figure 4.9). There was no significant difference between groups. Once adjusted to REE/kg fat free mass, the significant difference in the diet group was lost.
Chapter 4 – Rimonabant Study

Figure 4.9 Mean REE pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM (n=7 in each group).

To ensure that the REE readings recorded were realistic for participant’s body mass the measured REE was compared to predicted REE using the Schofield equations (Schofield et al. 1985) and the results were very similar (Table 4.4).

Table 4.4 Mean measured REE versus mean predicted REE for both the rimonabant and diet group. All figures mean ± SEM (n=7 in each group).

<table>
<thead>
<tr>
<th></th>
<th>Mean Weight Pre (kg)</th>
<th>Mean Predicted REE (kcal/day)</th>
<th>Mean Measured REE Pre (kcal/day)</th>
<th>Mean Measured REE as % of predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rimonabant group</td>
<td>89.0 ± 2.2</td>
<td>1539 ± 19.2</td>
<td>1460 ± 51.9</td>
<td>94.7 ± 2.5</td>
</tr>
<tr>
<td>Diet group</td>
<td>84.2 ± 2.9</td>
<td>1487 ± 28.7</td>
<td>1453 ± 70.2</td>
<td>97.7 ± 4.3</td>
</tr>
</tbody>
</table>

As may be seen in Table 4.4 the mean measured REE for the rimonabant group was 94.7% of that predicted and in the diet group the mean measured REE was 97.7% of that predicted.
### 4.4.5 Carbohydrate and fat oxidation

Carbohydrate and fat oxidation rates did not change significantly in either group pre and post treatment (Table 4.5). There was no significant difference between groups.

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group (n=7)</th>
<th>Diet Group (n=7)</th>
<th>( p ) * rimonabant vs. ( \Delta ) diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate oxidation (g/min)</td>
<td>Pre: 0.11 ±0.01</td>
<td>Post: 0.11 ±0.02</td>
<td>0.12 ±0.01</td>
</tr>
<tr>
<td>Fat oxidation (g/min)</td>
<td>Pre: 0.04 ±0.01</td>
<td>Post: 0.04 ±0.01</td>
<td>0.03 ±0.01</td>
</tr>
<tr>
<td>Respiratory Quotient</td>
<td>Pre: 0.85 ±0.01</td>
<td>Post: 0.86 ±0.03</td>
<td>0.86 ±0.01</td>
</tr>
</tbody>
</table>

### 4.4.6 Activity energy expenditure

AEE was analysed for a five day period for each participant pre and post treatment. Data was not available for two participants in the rimonabant group. One participant was found to have a malfunctioning monitor after a five day recording period and declined a repeat measurement, and for the second participant it was not possible to obtain a strong heart beat signal. Different monitors were trialed, but the participant had large adipose tissue deposits interrupting the R wave signal, thus preventing a recording.

The Actiheart software provided data on both heart rate changes as well as movement and, as described in Chapter 2 (section 2.8.2). An example of one participants AEE recording for one day can be seen in Figure 4.10.

A summary of REE, AEE, DIT and TEE for the five day period could also be downloaded for each participant as shown in Figure 4.11. The bar graph in the
summary shows the average distribution of AEE over 24 hours during the five day recording period.

![Activity, Heart Rate and Energy Expenditure](image)

**Figure 4.10 AEE recording for one participant using the Actiheart monitor.** The red line represents the heart rate data and the black blocks movement detected by the accelerometer. The blue line indicates a time when the monitor is not detecting an adequate R wave of the heart beat. On this occasion the blue line coincided with the time that the participant had removed the monitor whilst showering.
Figure 4.11 AEE daily energy expenditure summary for one participant using the Actiheart monitor for a five day period. The figures in bold represent days upon which the monitor was recording for a full 24 hour period whereas the figures in grey represent recordings of a part day only. REE, Resting Energy Expenditure; AEE, Activity Energy Expenditure; DIT, Dietary Induced Thermogenesis (an estimated value of 10% of TEE); TEE, Total Energy Expenditure; PAL, Physical Activity Level (TEE/REE).

AEE was measured in participants pre and post treatment. In the rimonabant group mean AEE was unchanged at 367±78 kcal/day pre treatment to 361±56 kcal/day post treatment (p=0.93) (Figure 4.12). AEE decreased from 533±99 kcal/day to 476±82 kcal/day in the dietary intervention group, but this difference was not significant (p=0.10).
4.4.7 Waist and hip circumference changes

Although energy intake was maintained in the rimonabant group there was a mean waist circumference decrease of 3.7±1.4cm (p=0.036). In the diet group following a hypocaloric diet a reduction of 5.7±1.7cm (p=0.016) in waist circumference was achieved (Figure 4.13). There was no significant difference between the two groups.
Figure 4.13 Mean waist circumference pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM (n=7 in both groups).

Changes in hip circumference and waist hip ratio are shown in Table 4.6. Despite weight loss there was no significant reduction in hip circumference or waist hip ratio pre and post treatment in the rimonabant group. A significant reduction in hip circumference was seen in the diet group post treatment, but this did not translate to a significant change in waist hip ratio. There was no significant difference in either hip circumference or waist hip ratio between groups.

Table 4.6 Changes in hip circumference and waist hip ratio pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM (n=14).

<table>
<thead>
<tr>
<th>Anthropometric Measure</th>
<th>Rimonabant Group (n=7)</th>
<th>Diet Group (n=7)</th>
<th>Δ rimonabant vs Δ diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>115.87 ± 2.14</td>
<td>114.38 ± 1.61</td>
<td>112.45 ± 2.64</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.94 ± 0.03</td>
<td>0.92 ± 0.02</td>
<td>0.96 ± 0.04</td>
</tr>
</tbody>
</table>

*significantly different from Pre measurement, p< 0.05
4.4.8 Body fat distribution

Two participants in the rimonabant group and one participant in the control group were unable to have scans due to metal implants.

Participants initially undertook an MRI scan of body fat in which they were scanned from their fingertips to their toes as described in Chapter 2 (section 2.9). An example of the images taken may be seen in Figure 4.14.

![Muscle and Fat](image)

**Figure 4.14 Example single slice MRI images of body fat in the upper thigh and pelvic area for one participant.** Participants were scanned from their fingertips to their toes by acquiring 10 mm thick transverse images with 30mm gaps between slices in their arms and legs and 10 mm gaps between slices in their abdomen. The white areas of fat can clearly be distinguished in comparison to the dark muscle.

MRI results showed a significant decrease in total body fat \( (p=0.046) \), subcutaneous fat \( (p=0.028) \), subcutaneous abdominal fat \( (p=0.028) \) and subcutaneous peripheral fat...
(p=0.028) in the diet group pre and post treatment, however there were no significant differences in the rimonabant group or between treatments (Table 4.7).

Table 4.7 MRI measurements of body fat pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM (Rimonabant group, n=5. Diet group, n=6).

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group (n=5)</th>
<th>Diet Group (n=6)</th>
<th>( p ) rimonabant vs ( \Delta ) diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Total body fat (litres)</td>
<td>46.91 ± 1.82</td>
<td>46.84 ± 1.82</td>
<td>44.17 ± 2.41</td>
</tr>
<tr>
<td>Subcutaneous fat (litres)</td>
<td>38.13 ± 1.62</td>
<td>37.72 ± 1.30</td>
<td>36.82 ± 2.50</td>
</tr>
<tr>
<td>Internal fat (litres)</td>
<td>8.78 ± 1.17</td>
<td>9.12 ± 1.40</td>
<td>7.35 ± 0.70</td>
</tr>
<tr>
<td>Subcutaneous abdominal fat (litres)</td>
<td>11.53 ± 0.69</td>
<td>11.04 ± 0.76</td>
<td>10.93 ± 1.21</td>
</tr>
<tr>
<td>Subcutaneous peripheral fat (litres)</td>
<td>26.61 ± 1.18</td>
<td>26.32 ± 0.58</td>
<td>25.89 ± 1.48</td>
</tr>
<tr>
<td>Visceral fat (litres)</td>
<td>5.45 ± 0.94</td>
<td>5.47 ± 1.05</td>
<td>4.01 ± 0.36</td>
</tr>
<tr>
<td>Non visceral fat (litres)</td>
<td>3.33 ± 0.33</td>
<td>3.65 ± 0.40</td>
<td>3.34 ± 0.37</td>
</tr>
</tbody>
</table>

*significantly different from Pre measurement, p<0.05.

As mean total body fat appeared unchanged in the rimonabant group despite weight loss the individual measurements were examined to identify if participants had increases, decreases or maintenance of body fat levels (Figure 4.15).

Figure 4.15 Individual measurements of total body fat (litres) for all participants pre and post treatment. Blue lines = rimonabant group, pink lines = diet group.
Figure 4.15 shows that although three of the participants treated with rimonabant lost body fat, participants RIM10R and RIM11R actually had an increase in total body fat despite weight loss. In the diet group a reduction in total body fat was seen in all participants with the exception of RIM12D in which total body fat was stable (35.98 litres pre versus 36.37 litres post).

The relationship between fat free mass measured by MRI and the Tanita bioimpedance method was investigated using Pearson product-moment correlation coefficient (Figure 4.16). There was a strong positive correlation between the two variables, $r = 0.918$, $n = 11$, $p < 0.001$.

![Figure 4.16 Correlation between MRI and Tanita measurements of fat free mass in kg post intervention (n=11).](image-url)
4.4.9 Intrahepatocellular lipid and intramyocellular lipid

Both intrahepatocellular lipid and intramyocellular lipid were measured by $^1$H-magnetic resonance spectroscopy as described in Chapter 2 (section 2.9). An example of an intrahepatocellular lipid spectra for one participant pre and post treatment may be seen in Figure 4.17.

![Figure 4.17 Example proton magnetic resonance spectra for one participant pre and post treatment. Resonances from water and IHCL are shown. IHCL, intrahepatocellular lipid.](image)

Levels of IHCL were expressed as a ratio to liver water content and the percentage change in IHCL pre and post treatment calculated. No significant differences were found in IHCL pre and post treatment in either group or between groups (Figure 4.18). The results for participant RIM09R were confirmed with the MRC Clinical Sciences Centre who undertook the MRS scan. This participant is an outlier and despite weight loss was found to have an increase in IHCL. The results were
reanalysed excluding this participant but this did not alter the findings and no significant differences in IHCL were found pre and post treatment in either group or between groups.

![Box plot](image)

**Figure 4.18** Box plots of the \% difference in intrahepatocellular fat pre and post treatment for the rimonabant and diet group. Data expressed as median and interquartile range (Rimonabant group, n=5. Diet group, n=6).

Levels of intramyocellular lipid were also calculated. Despite weight loss the rimonabant group showed an increase in both soleus and tibialis intramyocellular lipid (SIMCL and TIMCL) following treatment (Table 4.8). These increases were not statistically significant. The diet group also had an increase in TIMCL, but a decrease in SIMCL was found following treatment. There was a borderline
significant difference in SIMCL between the diet and the rimonabant group ($p = 0.05$).

Table 4.8 MRS measurements of SIMCL and TIMCL pre and post treatment for the rimonabant and diet group. Data expressed as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group (n=5)</th>
<th>Diet Group (n=6)</th>
<th>Rimonabant Group (n=5)</th>
<th>Diet Group (n=6)</th>
<th>$\Delta$ rimonabant vs $\Delta$ diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Mean % change</td>
</tr>
<tr>
<td>SIMCL</td>
<td>10.42 ± 2.14</td>
<td>11.85 ± 2.63</td>
<td>11.09 ± 2.21</td>
<td>9.59 ± 2.13</td>
<td>13.7 ± 2.21</td>
</tr>
<tr>
<td>TIMCL</td>
<td>6.19 ± 0.99</td>
<td>6.84 ± 1.21</td>
<td>8.13 ± 0.88</td>
<td>10.98 ± 2.01</td>
<td>25.26 ± 2.92</td>
</tr>
</tbody>
</table>

SIMCL, soleus intramyocellular lipid; TIMCL, tibialis intramyocellular lipid.

4.4.10 Adipose and muscle gene expression

In adipose tissue, a significant difference was found in gene expression of PPAR delta between the rimonabant and diet group pre and post treatment ($p = 0.006$). There were no further significant differences in gene expression in adipose tissue found pre-post treatment in either the diet or the rimonabant group or between groups (Figure 4.19).

No significant differences in gene expression in muscle tissue were found pre-post treatment in either the diet or the rimonabant group or between groups (Figure 4.20).
Figure 4.19 Post:pre gene expression in adipose tissue normalized to the housekeeping gene GAPDH. Data expressed as ratios. (Rimonabant group, n=4. Diet group, n=5). CD36, Cluster of Differentiation 36; A, Adiponectin; AR1, adiponectin receptor 1; UCP2, Uncoupling protein 2; LPL, lipoprotein lipase; HSL, Hormone sensitive lipase; PG, Peroxisome proliferator-activated gamma; PD, Peroxisome proliferator-activated delta.

Figure 4.20 Post:pre gene expression in muscle normalized to the housekeeping gene GAPDH. Data expressed as ratios. (Rimonabant group, n=2. Diet group, n=4). CD36, Cluster of Differentiation 36; AR1, adiponectin receptor 1; UCP2, Uncoupling protein 2; UCP3, Uncoupling protein 3; LPL, lipoprotein lipase; PD, Peroxisome proliferator-activated delta; PA, Peroxisome proliferator-activated alpha; ACOX1, acyl CoA oxidase; CPT1, Carnitine palmitoyltransferase 1; CPT2, Carnitine palmitoyltransferase 2.
### 4.4.11 Beck depression scores

In the rimonabant treated group participants levels of depression were monitored fortnightly through the completion of the Beck Depression Inventory II. The mean depression scores at each fortnightly visit are shown in Table 4.9.

**Table 4.9 Mean Beck Depression Inventory II scores for the rimonabant group.**
Data are expressed as mean ± SEM (n=7). The maximum score on the inventory is 63.

<table>
<thead>
<tr>
<th>Visit</th>
<th>Mean Beck Depression Inventory II Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>6.86 ± 0.46</td>
</tr>
<tr>
<td>Visit 8</td>
<td>1.86 ± 0.46</td>
</tr>
<tr>
<td>Visit 9</td>
<td>3.43 ± 1.34</td>
</tr>
<tr>
<td>Visit 10</td>
<td>3.29 ± 2.16</td>
</tr>
<tr>
<td>Visit 11</td>
<td>3.00 ± 1.38</td>
</tr>
<tr>
<td>Visit 12</td>
<td>3.43 ± 1.02</td>
</tr>
</tbody>
</table>

A score of 0-13 on the Beck Depression Inventory II is categorised as minimal depression and the mean score at each visit belonged to this category. The highest mean depression score recorded was actually at the screening visit with the lowest mean score two weeks into the drug treatment period.

The statements of symptoms and attitudes that scored most highly are shown in Figure 4.21.
The statements with the highest scores at the screening visit were tiredness or fatigue and loss of energy. During the treatment period an increase in sadness and agitation scores were seen in comparison to the screening visit. Changes in appetite were also evident for the duration of the treatment period. Only one participant undertaking the study reported fleeting suicidal thoughts and this was at visit 11 near the end of the treatment period.

4.5 Discussion

The aim of this clinical trial was to determine if, when energy intake was maintained at pre-treatment levels, rimonabant still induced weight loss through an effect on energy expenditure and the direct effects of rimonabant on body fat distribution and gene expression.

In this trial it was demonstrated, for the first time in humans, that treatment with rimonabant for a 13 week period, whilst daily energy intake was maintained at pre-
treatment levels, did induce weight loss. Additionally it was shown that, despite weight loss following rimonabant treatment, there was no change in REE and, since REE would be expected to decrease, this could have contributed to the weight loss.

Weight loss

Over the course of the 13 week trial both the rimonabant and diet group lost similar amounts of weight as per the study design. The weight loss in the rimonabant group was less than the average loss of 4kg–5.5kg (Van Gaal et al., 2005; Despres et al., 2005; Pi-Sunyer et al., 2006; Scheen et al., 2006) over the first 12 week period in the RIO human trials. It is reasonable that the weight loss seen in this study was less as in the study design for the RIO trials all participants were advised to adhere to a hypocaloric diet (600 kcal/day deficit) and instructed to increase their level of physical activity throughout the study, which should have increased levels of weight loss in comparison to drug treatment alone. Levels of adherence to the diet and increased activity were not reported in the RIO papers, however, and a 600 kcal/day deficit alone over a 12 month period should have resulted in greater annual weight loss than 5.3-8.6kg suggesting non compliance from participants.

In order to induce a weight loss of 1kg a deficit of 7000kcal of energy is required. The weight loss in the diet group can be explained by their daily reduction in energy intake during the treatment period. The rimonabant group, however, maintained energy intake thereby indicating that a change in energy expenditure was responsible for the weight reduction in this group.
Energy expenditure

Total energy expenditure is comprised of REE, non-resting energy expenditure (including AEE) and DIT. REE contributes 60%, non-resting energy expenditure and AEE 30% and dietary induced thermogenesis approximately 10% of TEE. A study in obese participants by Leibel et al. (1995) found that a weight reduction of 10% was associated with a decrease in TEE of 8kcal/day/kg fat free mass. It was shown that approximately half the decrease in TEE was due to a decrease in REE and half to a decrease in non-resting energy expenditure. In this study this would correspond to the weight loss in the diet group being associated with a decrease in TEE of 120kcal/day. The mean decrease in REE in the diet group was actually 67kcal/day which is consistent with these previous findings and in line with the expected reduction of REE in the hypothesis. The remaining decrease in energy expenditure is likely to be accounted for by reductions in non-resting energy expenditure. AEE, an element of non resting energy expenditure was measured for a five day period at the end of the treatment period and did decrease by 57kcal/day in the diet group, although this decrease was not statistically significant.

In the rimonabant group the maintenance in REE can partially account for the weight loss but cannot fully explain it. As there was no change in AEE in the rimonabant group pre and post treatment this also cannot explain the weight reduction. It must be taken into consideration, however, that AEE was only measured for two five day periods and not for the duration of the intervention period. In addition, participants were instructed not to alter exercise patterns over the course of the trial and questioned regarding their activity levels at every visit to try and minimise variability.
To induce the weight reduction recorded in the rimonabant group an increase in TEE of approximately 200 kcal/day was required and therefore an increase in REE was expected as stated in the hypothesis. The initial study power calculation determined that 30 participants would be required in total to detect differences in REE, yet at the point at which the trial was prematurely terminated only 14 participants had been studied. It is possible that due to the reduced number of participants indirect calorimetry may not have been sufficiently sensitive to detect any small changes in REE.

There are some disadvantages associated with measuring REE using indirect calorimetry. Firstly REE was only measured at two time points during the study and over a recording period of 40 minutes. It is questionable whether this provides an accurate picture of REE over a thirteen week period although there was no significant difference in the REE measurements at Visit 2 and Visit 5. An alternative method of measuring energy expenditure, however, such as doubly labeled water may have yielded more accurate results. Secondly there are a number of assumptions utilized when using indirect calorimetry to measure REE. For example, an assumption is made that the body pools that can affect the respiratory exchange measurements, such as the urea pool and the bicarbonate pool, remain stable (Jéquier et al., 1987). Finally, some participants found laying under the Perspex hood for 40 minutes slightly distressing and it is possible that the associated hyperventilation contributed to an increased RQ. The reported RQ for pure fat oxidation is 0.707 and for carbohydrate oxidation 1.0 (Gibney et al., 2002). The mean RQ varied between 0.85±0.01 and 0.88±0.01 and as the SEMs were small suggested a systematic overestimation in RQ. Interestingly this may be explained by the reported mean error in RQ of 14±1.5%.
when the GEM performance was assessed using reference gases (Nicholson et al., 1996).

Doubly labeled water is the gold standard method for calculating total energy expenditure. In this method an individual ingests a dose of heavy water isotopically labeled with deuterium and oxygen-18. The differing rates of loss of these isotopes from the body are utilised to estimate the rate of carbon dioxide and energy expenditure (Gibney, 2002). The major advantages of this method in comparison to indirect calorimetry are that it is non-invasive and energy expenditure can be measured over an extended period. The REE measurements in this trial were conducted over a forty minute recording period whereas using doubly labeled water energy expenditure may be measured for up to 7-14 days. Additionally the doubly labeled water method allows for individuals to have energy expenditure measured under free-living conditions. There are some disadvantages to the method. Not only is there a requirement for expensive equipment to analyse the samples but the $^{18}$O isotope costs approximately £400 for a 70kg adult (Gibney, 2002) so for a trial of this nature, when energy expenditure was to be measured twice in participants with higher body weight, the expense would have been great. Additionally no information may be obtained regarding the different components of activity thermogenesis (Levine, 2005).

Several problems were also encountered in the measurement of AEE. As previously described in the methods chapter, the Actiheart Monitor was unable to detect various fidgeting elements of NEAT such as leg shaking, finger tapping, foot tapping etc. The Actiheart monitor can only detect movement in the vertical plane, hence the
requirement for the branched chain equation model (Brage et al. 2005) allowing the intensity and energy expenditure of physical activity to be calculated from the activity data or heart rate data alone or from both. As the monitor was unable to detect some components of NEAT, a potential contributor to TEE had been omitted. NEAT has been found to vary immensely between individuals, and for the many individuals not participating in regular purposeful physical activity, is the major contributor to activity thermogenesis (Levine and Kotz, 2005).

One methodological problem encountered whilst measuring AEE was an inability to obtain a strong R wave signal in several participants. As all participants were female and obese, several had abundant breast tissue. Research had been published with recommendations to place the ECG pads on the breast, rather than under (Rautaharju et al., 1998), which was followed, but signal problems were still encountered. This may have led to a slight underestimation in AEE as the estimations of AEE would have been based more heavily on the accelerometer data and, as discussed previously, the monitor could only detect movement in the vertical plane.

*Fat and carbohydrate oxidation and gene expression*

Fat and carbohydrate oxidation measured by indirect calorimetry were not significantly different in either group pre and post treatment. This was contrary to the hypothesis that a reduction in fat oxidation would be seen in the dietary intervention group, as weight loss due to dietary intervention had previously been reported to reduce whole body fat oxidation (Nicklas et al., 1997; Franssila-Kallunki et al., 1992). The anticipated increase in oxidation rates in the rimonabant group, as had previously been reported in animal studies, were also not discovered. The
oxidation rates did correspond well to measurements of REE but it is possible that this method was too insensitive to detect any changes due to the reduced numbers of participants in this study. Interestingly, Dr Sarac (collaborating PhD student on the trial) used stable isotope techniques to measure fatty acid oxidation and identified an increase in fatty acid oxidation rate in the rimonabant group compared to the diet group, in which fatty acid oxidation rate decreased. The mean increase in fatty acid oxidation rate in the rimonabant group would have yielded 220 kcals per day, which could fully account for the weight loss with rimonabant. The decrease identified in fatty acid oxidation in the diet group could also explain the reduction found in REE in this group.

In the hypothesis it was proposed that an increase in gene expression of regulators of fat oxidation would be seen in the rimonabant group yet no significant differences were found pre and post treatment. A significant difference was identified, however, between the rimonabant and diet group in expression of PPAR delta in adipose tissue. PPAR delta actually decreased post treatment in the rimonabant treated group and, as the role of PPAR delta involves increasing fat oxidation in muscle and lipolysis in the adipose tissue (de Lange et al., 2008), this was the opposite effect of that predicted in the hypothesis. It was interesting that no change was found in the rimonabant group in adiponectin or the adiponectin receptors gene expression, as in the RIO-Lipids study (Despres et al., 2005) adiponectin levels were increased in the rimonabant treated group compared to the placebo group and it was hypothesized that this may be the mechanism by which rimonabant causes weight loss. In this study (data from thesis of Dr Ivana Sarac) there was no significant change in plasma adiponectin found in the rimonabant group despite an increase in fatty acid oxidation,
suggesting the mechanism of weight loss is not mediated by adiponectin. It is possible that the lack of significant findings with regards to gene expression in this study is directly related to the small participant number. As biopsies were optional, a number of participants did not undertake them resulting in underpowering.

*Waist circumference*

As was found in previous human trials using rimonabant (Van Gaal *et al.*, 2005; Despres *et al.*, 2005; Pi-Sunyer *et al.*, 2006; Scheen *et al.*, 2006) weight loss was accompanied by a decrease in waist circumference. In this study waist circumference decreases were found both in the rimonabant and diet group. All participants had a high risk waist circumference measurement pre treatment, with one participant in the diet group reducing her waist circumference measurement to the lower risk category post treatment. No significant changes were seen in waist-hip ratio pre and post treatment in either group, but as previously mentioned measurements of waist-hip ratio have limited use due to the simultaneous fat reduction from both areas during weight loss.

*Body fat distribution*

As waist circumference decreased it was surprising that a corresponding significant reduction in subcutaneous abdominal fat measured by MRI was not found in the rimonabant group, although a significant decrease was identified pre and post treatment in the diet group. This is contradictory to the findings of the ADAGIO trial (Despres *et al.*, 2009) which reported a significant reduction in subcutaneous abdominal fat accompanying a significant reduction in waist circumference in a rimonabant treated group when compared to a placebo group. In the ADAGIO trial,
however, participant numbers were far greater and 20mg of rimonabant was given daily for a one year period resulting in a greater mean weight loss of 5.8kg. It is possible that if early termination of this trial had not resulted in underpowering that a significant result might have been achieved.

From the MRI scans it was shown that fat free mass declined in two of the rimonabant treated participants, yet interestingly this decrease was not accompanied by a reduction in REE in either of these participants. This finding was in line with the hypothesis that rimonabant would increase (or in this case maintain) REE. It has been shown in previous studies that the loss of lean body mass is correlated with a reduction in energy expenditure (Ravussin et al., 1982) so a decrease might have been expected had these participants lost weight through dietary intervention. As proposed in the hypothesis a reduction in energy expenditure was observed in the diet group, however reductions in total body fat were recorded in all but one of the participants, as opposed to reductions in fat free mass.

MRI results showed a significant decrease in total body fat, subcutaneous fat, subcutaneous abdominal fat, and subcutaneous peripheral fat in the diet group as expected, however the hypothesised differences in body fat distribution in the rimonabant group were not seen. This finding was surprising, as the weight loss reported in both groups would be expected to have resulted in significant effects on fat distribution as shown in other weight loss studies of obesity (Markovic et al., 1998; Tiikkainen et al., 2003). A further unexpected result was the non significant change in visceral fat in both groups. This finding contradicts the significant reduction in visceral fat reported in the rimonabant treated group compared to the
placebo group in the ADAGIO trial (Despres et al., 2009). However, due to the differences between this trial and the ADAGIO trial as outlined above, it is hard to draw direct comparisons. The non-significant finding in the diet group also contradicts previous research, in which significant reductions in visceral fat were identified following weight loss interventions (Goodpaster et al., 1999; Goodpaster et al., 2010). Once again, the underpowering of this trial and the larger weight loss and longer duration of the Goodpaster et al. studies must be taken into consideration.

**IHCL**

IHCL had been predicted to decrease in both the rimonabant and diet group. Surprisingly, no significant differences were found in IHCL pre and post treatment in either group or between groups. This is contrary to previous research, in which both weight loss due to dietary intervention (Tiikkainen et al., 2003) and with rimonabant treatment (Despres et al., 2009) had been shown to reduce liver fat. In these studies, however, greater weight loss was achieved and in the ADAGIO trial the rimonabant was taken for one year as opposed to 12-13 weeks.

**IMCL**

Previous studies have presented contradictory findings with regards to changes in IMCL following weight loss. IMCL has been found to be increased in obesity and there is a strong correlation between levels of IMCL and insulin resistance (Simoneau et al., 1999). As expected the levels of SIMCL and TIMCL in all participants were greater than that reported in healthy individuals in a previous study. Li et al. (2008) reported SIMCL to be 2.6±1.5 (IMCL/water %) and TIMCL to be 0.6±0.2 (IMCL/water %) in insulin sensitive healthy weight participants (mean BMI
24.2±3.2) compared to SIMCL measurements of 9-11 (IMCL/water %) and TIMCL of 6-10 (IMCL/water %) in this trial. Two studies of obese participants with weight losses of 14kg and 15kg respectively both reported significant reductions in IMCL (Greco et al., 2002; Goodpaster et al., 1999) whereas a small study of obese participants with an 11kg weight loss found no effect on IMCL (Malenfant et al., 2001). The results from this study also do not provide a consistent message. Despite weight loss the rimonabant group showed no significant change in either soleus or tibialis IMCL following treatment. The diet group were also reported to have an increase in TIMCL post weight loss, but a decrease in SIMCL resulting in a significant difference in SIMCL between the diet and the rimonabant group (p = 0.05).

Rimonabant side effects

The Rimonabant was well tolerated and any side effects noted were mild. In the RIO trials (Van Gaal (2005), Despres (2005), Pi-Sunyer (2006), Scheen (2006)) commonly reported adverse events in the rimonabant treated groups were nausea, diarrhoea, vomiting, fatigue, dizziness, anxiety and depressed mood disorder. During this study no participants reported any physical side effects such as nausea, vomiting or diarrhoea. In the RIO trials the Hospital Anxiety and Depression Scale was utilised to assess psychological well being, but in this trial a similar tool, The Beck Depression Inventory II, was used. In the RIO trials psychiatric disorders (depression, anxiety, agitation and sleep disorders) were reported in 2.1-7% of participants and were the most common reason given for prematurely withdrawing from the trial. In this study a lowering of mood was noted by one participant in the last week of the treatment period, but this may also have been influenced by personal
problems reported by the individual at that time. Another participant reported fleeting suicidal thoughts although interestingly not accompanied by feelings of sadness or crying. Overall in this trial, although increased sadness and crying was noted by three participants, the mood lowering effects of the rimonabant appeared minimal and no participants withdrew from the trial. It was during the CRESCENDO trial (Topol et al., 2010), in which the effect of rimonabant on cardiovascular disease was being investigated, that it was discovered that serious psychiatric disorders were more common in patients prescribed rimonabant than previously reported in the clinical trials. Four patients in the rimonabant group and one in the placebo group in the trial had committed suicide resulting in immediate termination of the trial and the decision to suspend the licence for rimonabant.

In this study the most frequently scored categories on the Beck Depression Inventory II during the treatment period were not lowering in mood, but changes in sleep pattern and changes in appetite. These changes in appetite were reductions in hunger and these changes were expected from previous studies in animals (Bellochio et al., 2006). However, several participants reported loss of appetite for the duration of the treatment period, which is contradictory to the finding of only a transient reduction in appetite seen in rats (Colombo et al., 1998). Participants were requested to continue with their usual food intake despite experiencing a reduced appetite and the findings from the analysed diet diaries confirm their compliance.

Dietary data

One major consideration whilst undertaking the study was the potential for participants to misreport their dietary intake. During the run-in period, and the treatment period for the diet group, there was the concern of under reporting with the
converse problem of over reporting a potential issue for the rimonabant group. It has been demonstrated that there is a high level of under reporting in studies where dietary intake is recorded. Goldberg et al. (1991) have shown that measurements of seven day weighed food intake are lower than energy expenditure measured simultaneously using doubly labeled water and Prentice et al. (1986) identified a self recorded energy intake of only 67% of isotopically measured energy expenditure in obese individuals. As the doubly labeled water was too expensive to be used in this study, a number of methods were utilised to try and ensure dietary intake was accurately provided. Initially Goldberg's cut off values were utilised to ensure dietary information provided by participants was feasible. Dietary intake was then recorded daily throughout the run-in period, whilst weight was simultaneously monitored to ensure participants were remaining weight stable. Estimated energy intake was also compared to energy expenditure and finally reported levels of protein excretion were compared to measured urinary nitrogen excretion. The reported levels of dietary protein were found to correspond well to the nitrogen excretion (data not shown) but it must be acknowledged that the urine samples were only taken over two 24 hour periods.

General limitations

In addition to the methodological limitations outlined above, one of the major limitations of this study was that it was underpowered due to the suspension of rimonabant's licence and early termination of the trial. Recruitment for the study was slow as, although there was much interest regarding the trial, many individuals had to be excluded as they did not meet the BMI inclusion criteria or had a previous history of depression. Less than 10% of individuals responding to recruitment
advertisements and letters were actually eligible to participate in the study. Additionally during the months of recruiting for the study there was some negative publicity in the press surrounding the possible side effects of rimonabant resulting in several individuals withdrawing their interest in the trial.

Conclusions and future work

In conclusion this trial did demonstrate, for the first time, that, when energy intake was maintained at pre treatment levels, rimonabant still induced weight loss and that this reduction in weight could be partly explained by maintenance of REE. Although significant increases in oxidation rates and gene expression of key regulators of lipid metabolism and decreases in total body fat, IHCL and IMCL were not identified it is likely that this is due, in part, to the early termination and subsequent underpowering of the trial.

Rimonabant did show good potential as an anti-obesity medication but due to its negative side effects is unlikely to be utilised in the future. Further research is required to create other medications that antagonise the endocannabinoid system but that selectively target the peripheral and not the central receptors.

Due to the limitations discussed above regarding the measurement of activity and resting energy expenditure, were a similar trial to be repeated in the future, it would be advisable to utilise the doubly labeled water method of measuring TEE in addition to the described REE and AEE methodologies. This would provide more comprehensive information on the different elements of energy expenditure.
Chapter 5

The effect of gender on energy expenditure, gene expression and body fat distribution
5.1 Background

Much research has been conducted in an attempt to better understand the mechanisms by which obesity is related to chronic diseases. It has been shown that gender is an important factor. In the European Union, in 2008, it was estimated that a greater number of males than females were overweight (exclusive of obesity), >82 million versus 61 million respectively, however a greater number of females were obese than males, 37 million in comparison to 31 million (International Association for the Study of Obesity (IASO), 2008)

A variety of studies have examined gender differences in REE and body fat distribution as described in chapter one. There have also been studies which suggest that metabolism in obesity is different between genders. In a study by Nielsen et al. (2003) women were reported to release approximately 40% more free fatty acids from fat tissue than men relative to REE. However, a major limitation of these studies is that the number of participants was small or that the gender groups were not matched for the degree of obesity.

Although previous research had investigated gender differences in individual elements of energy expenditure and body fat distribution, no one study had comprehensively researched resting energy expenditure, activity energy expenditure, body fat distribution and gene expression of key regulators of lipid and glucose metabolism in the same participants. It was proposed that this approach would provide a fuller understanding of the differences in metabolism in obese men and women.

This study was a continuation of the work undertaken studying the effect of rimonabant in obese postmenopausal woman. At the time that the rimonabant study
was prematurely terminated, fourteen women had been studied, but only ten had completed all of the tests. The aim of this study was to recruit a further six females and sixteen BMI and age matched males to participate in a cross sectional study to determine gender differences in obesity. Permission was obtained from the ten women completing all the baseline tests in the rimonabant study to utilise their data in this study. The rationale and hypothesis for the study are described in chapter one, section 1.17.

5.2 Overall aim

The overall aim of this study was to determine how gender influences energy expenditure, body fat distribution and gene expression in obesity.

This was a collaborative study involving another PhD student (who examined the effects of gender on insulin sensitivity, fatty acid and triglyceride metabolism), colleagues at the Postgraduate Medical School, the Royal Surrey County Hospital and the MRC Clinical Sciences Centre, at Imperial College School of Medicine, Hammersmith Hospital.

5.3 Specific aims

To investigate in obese participants the effect of gender on the following:

1. Resting energy expenditure and activity energy expenditure.

2. Fat and carbohydrate oxidation rate.

3. Whole body fat distribution, intrahepatocellular lipid (IHCL) and intramyocellular (IMCL) lipid.

4. Adipose tissue and muscle mRNA levels of key regulators of fatty acid metabolism.
5.4 Methodology

5.4.1 Participants

Thirteen males and two postmenopausal females aged 50-70 years were recruited for the study from the local Guildford population using the recruitment strategies outlined in Chapter 2 and additionally through GP practices with the help of the Primary Care Research Network, and through a search of a study volunteer database of individuals who had previously participated in research and wished to volunteer for future studies at the Clinical Research Centre, University of Surrey. To be eligible for the study participants were required to have a BMI of 30-35kg/m$^2$ and must have been weight stable for at least a three month period. Participants were excluded from the study if they fulfilled any of the exclusion criteria detailed in chapter two (section 2.2.1).

All participants were provided with a participant information sheet and the study design was fully explained before written consent was obtained. Ethical approval for the study protocol was obtained from the University of Surrey Ethics Committee (EC/2009/09/FHMS).
5.4.2 Study design

The study was of 4 weeks duration and the study schedule is shown in figure 5.1

<table>
<thead>
<tr>
<th>Screening (Visit 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE and 5 day measurement of AEE by Actiheart (Visit 2)</td>
</tr>
<tr>
<td>Repeated REE, Acetate infusion and Clamp* (Visit 3)</td>
</tr>
<tr>
<td>Fat distribution (MRI/MRS) (Visit 4)</td>
</tr>
<tr>
<td>Adipose and muscle tissue biopsies taken for gene expression. TG/FFA metabolism* (Visit 5)</td>
</tr>
</tbody>
</table>

*These procedures were undertaken by another PhD student.

REE, resting energy expenditure; AEE, activity energy expenditure; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; TG, triglyceride; FFA, free fatty acid.

5.4.3 Protocol

Screening

Visit 1: Details of the screening visit are described in chapter two. If the participant fulfilled all of the inclusion criteria and none of the exclusion criteria they were accepted into the study. Following the screening visit, participants were requested to complete a four day diet diary.
**Visit 2:** The purpose of this visit was to establish the participants' energy expenditure. Participants were weighed. REE was measured as described in chapter two (section 2.6). This first REE measurement was undertaken to familiarise participants with the equipment.

In order to then estimate the amount of energy expended in everyday activities and exercise each participant was fitted with an Actiheart monitor (Chapter two/section 2.8).

To establish urinary nitrogen excretion, participants were requested to collect a 24 hour urine collection throughout the day before the visit. See chapter two/section 2.7.

**Visit 3:** This visit was undertaken at least a week after visit 2. As before, REE was measured by indirect calorimetry. This REE reading was used in the study analysis.

**Visit 4:** Measurements of body fat, intrahepatocellular lipid and intramyocellular lipid were taken at the MRC Clinical Sciences Centre, Hammersmith Hospital as described in chapter two/section 2.9.

**Visit 5:** This visit was scheduled 5-7 days after visit 3. Participants were weighed and a needle biopsy of the vastus lateralis muscle in the thigh and subcutaneous adipose tissue was taken under local anaesthesia by a physician trained in this procedure. The biopsies were snap frozen in liquid nitrogen and stored at -80 °C in preparation for batch analysis. These biopsies were utilised to measure mRNA levels of key regulators of fatty acid metabolism (Chapter two/section 2.11).
5.4.4 Study power

The power calculation was undertaken by a medical statistician. The primary outcome measure for the study was triglyceride synthesis rate (examined by Dr Sarac). Secondary outcome measures included gene expression of key regulators of glucose and fat metabolism, total visceral and peripheral fat content, energy expenditure, fatty acid production and oxidation rate.

For the primary outcome measure of the difference in triglyceride secretion rate between genders, the SD is 50% according to the published literature. Based on previous data a difference in triglyceride secretion rate of 70% was expected between genders. From this information it was calculated that 16 participants would be required in each gender group to detect this difference with 87% probability at a two-sided 1% level of significance.

5.5 Results

5.5.1 Participant characteristics

All participants were screened at Visit 1 and a summary of their characteristics are shown in Table 5.1.

Of the 15 participants initially recruited for the study one male did not complete the trial, due to a negative reaction during one of the study days, and has been excluded from analysis.
Table 5.1 Participant characteristics at screening. All figures are mean ± SEM

<table>
<thead>
<tr>
<th></th>
<th>Females (n=12)</th>
<th>Males (n=12)</th>
<th>p males versus females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.92 ± 1.67</td>
<td>58.08 ± 1.92</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.69 ± 2.16</td>
<td>104.28 ± 3.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.58 ± 2.01</td>
<td>180.38 ± 2.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32.39 ± 0.53</td>
<td>31.40 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>95.67 ± 2.04</td>
<td>110.38 ± 1.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>114.83 ± 2.18</td>
<td>112.29 ± 1.98</td>
<td>NS</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.84 ± 0.02</td>
<td>0.98 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (%)*</td>
<td>43.24 ± 0.92</td>
<td>30.28 ± 0.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.28 ± 0.09</td>
<td>5.69 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.91 ± 0.24</td>
<td>5.44 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.28 ± 0.10</td>
<td>1.76 ± 0.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Fat mass as measured by impedance.

There was no significant difference in age between the two genders. Body weight was 18% greater in males than females primarily due to a greater amount of lean body mass. BMI, however, was not significantly different between individuals. Both waist circumference and waist hip ratio were significantly greater in males than females. Mean hip circumference was larger in females than males but the difference was not statistically significant. A significantly higher level of fasting glucose in mmol/l was measured in males than females with no difference in levels of cholesterol or triglyceride.

5.5.2 Dietary analysis

Diet diary analysis was undertaken to compare the average intake of the males and females (Table 5.2). Dietary data was unavailable for one male participant.
Table 5.2 Comparison of mean daily macronutrient intake for males and females reported in the four day screening diet diary.

<table>
<thead>
<tr>
<th></th>
<th>Males (n=12)</th>
<th>Females (n=12)</th>
<th>p males versus females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/day)</td>
<td>2632 ± 158</td>
<td>2002 ± 62</td>
<td>p = 0.0006</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>97 ± 6</td>
<td>84 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>SFA (g/day)</td>
<td>35 ± 4</td>
<td>29 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA (g/day)</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA (g/day)</td>
<td>31 ± 3</td>
<td>26 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>275 ± 13</td>
<td>235 ± 12</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>105 ± 5</td>
<td>78 ± 4</td>
<td>p = 0.0004</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>44 ± 15</td>
<td>5 ± 1</td>
<td>p = 0.01</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids. Data expressed as mean ± SEM.

In absolute terms, males reported a higher mean daily intake of energy (p = 0.0006), carbohydrate (p = 0.03), protein (p=0.0004) and alcohol (p = 0.01) than females over a four day period. No significant difference was found in fat intake between the genders.

Due to the greater body weight of males versus females, the reported nutrient intake was analysed per kg of body weight (Table 5.3). Once expressed in relation to body weight, the significant differences in mean energy, carbohydrate and protein intake were lost. Alcohol intake still remained significantly higher in males (p = 0.02) and PUFA was higher in females (p = 0.05).
Table 5.3 Comparison of mean daily macronutrient intake per kg body weight for males and females reported in the four day screening diet diary.

<table>
<thead>
<tr>
<th></th>
<th>Males (n=11)</th>
<th>Females (n=12)</th>
<th>p males versus females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/day)</td>
<td>26.18 ± 1.72</td>
<td>23.45 ± 0.73</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.96 ± 0.06</td>
<td>0.99 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>0.34 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.00</td>
<td>p = 0.05</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>0.31 ± 0.03</td>
<td>0.30 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>2.73 ± 0.13</td>
<td>2.75 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.05 ± 0.06</td>
<td>0.92 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.45 ± 0.15</td>
<td>0.06 ± 0.02</td>
<td>p = 0.02</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids. Data expressed as mean ± SEM.

The mean percentage contribution that each macronutrient provided to total daily energy intake in males and females is shown in figures 5.2 and 5.3.

![Macronutrient composition of the diet in males](image)

Figure 5.2 Mean percentage contribution of macronutrients to total daily energy intake over a four day period in males (n=11).
Macronutrient composition of the diet in females

- Fat
- Carbohydrate
- Protein
- Alcohol

15.35% 37.18%
1.87%
45.60%

Figure 5.3 Mean percentage contribution of macronutrients to total daily energy intake over a four day period in females (n=12).

A significantly greater percentage of energy was provided by fat in females than males (p = 0.02) and a significantly greater percentage of total energy provided by alcohol in males (p = 0.009). No significant differences were found in macronutrient composition of the diet in terms of carbohydrate or protein between the two groups.

5.5.3 Resting energy expenditure

In absolute terms, REE was significantly higher in the male group than the female group (p <0.01) (Figure 5.4).
Figure 5.4 Mean REE in males and females. Data expressed as mean ± SEM (males n=12, females n=12).

Once REE measurements were expressed per kg fat free mass, significantly higher REE measurements were seen in the female group rather than the male. As shown in Figure 5.5 males had a mean REE/kg fat free mass of 26.38 ± 0.66 versus 29.44 ± 0.91 in the female group (p = 0.01).

Figure 5.5 Mean REE per kg lean body mass in males and females. Data expressed as mean ± SEM (males n=12, females n=12).
5.5.4 Carbohydrate and fat oxidation

In absolute terms, a significantly higher rate of carbohydrate oxidation was measured in males than females (p = 0.003). There was no difference between genders in fat oxidation or respiratory quotient (Table 5.4).

Table 5.4 Carbohydrate and fat oxidation rates in g/min for males and females.
Data expressed as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Males (n=12)</th>
<th>Females (n=12)</th>
<th>p males vs. females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate oxidation (g/min)</td>
<td>0.20 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.0003</td>
</tr>
<tr>
<td>Fat oxidation (g/min)</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.00</td>
<td>NS</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>0.88 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Once oxidation rates were adjusted per kg/fat free mass (FFM) no significant differences were found in either carbohydrate or fat oxidation rates (Table 5.5).

Table 5.5 Carbohydrate and fat oxidation rates in kcal/kg/FFM for males and females. Data expressed as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Males (n=12)</th>
<th>Females (n=12)</th>
<th>p males vs. females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate oxidation (kcal/kg/FFM)</td>
<td>15.71 ± 4.40</td>
<td>14.75 ± 1.05</td>
<td>NS</td>
</tr>
<tr>
<td>Fat oxidation (kcal/kg/FFM)</td>
<td>6.99 ± 1.06</td>
<td>8.52 ± 1.17</td>
<td>NS</td>
</tr>
</tbody>
</table>

5.5.5 Activity energy expenditure

AEE was measured in all participants for a five day period. AEE recordings were not available for three of the participants due to problems with malfunctioning monitors for two individuals and an adverse skin reaction to the ECG pads for the other. In
the male group absolute mean AEE was significantly higher at 849±103 kcal/day in comparison to 448±69 kcal/day (p = 0.005) in the female group (Figure 5.6). If, however, the AEE was expressed per kg of body weight, the significant difference between the two genders was lost (p = 0.06) as shown in Figure 5.7.

**Figure 5.6** Mean activity energy expenditure for males and females over a five day period. Data expressed as mean ± SEM (males n=11, females n=10).

**Figure 5.7** Mean activity energy expenditure adjusted for body weight for males and females over a five day period. Data expressed as mean ± SEM (males n=11, females n=10).
Further analysis was undertaken to determine if there was any variation in AEE between week days and weekend days for both genders (Figure 5.8). As shown there was no significant difference for either gender between week days and weekend days and no significant difference in mean AEE during week days and the weekend between the two groups (p = 0.11).

![Figure 5.8 Comparison of mean activity energy expenditure between week days and weekends for males and females. Data expressed as mean ± SEM (males n=11, females n=10).](image)

**5.5.6 Total energy expenditure**

Total energy expenditure was estimated for participants utilising the measured REE and AEE values in conjunction with an estimate of DIT (10% of TEE). The estimated DIT and TEE were calculated utilising Actiheart software. The estimated mean TEE was then compared to reported mean kcal intake in the four day diet diary (Table 5.6).
Table 5.6 Comparison of estimated TEE to mean daily kcal intake in males and females. Data are mean values.

<table>
<thead>
<tr>
<th></th>
<th>Measured REE</th>
<th>Measured AEE</th>
<th>Estimated DIT</th>
<th>Estimated TEE</th>
<th>Mean daily kcal intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01M</td>
<td>1699</td>
<td>497</td>
<td>243</td>
<td>2439</td>
<td>2416</td>
</tr>
<tr>
<td>02M</td>
<td>1711</td>
<td>840</td>
<td>283</td>
<td>2834</td>
<td>1969</td>
</tr>
<tr>
<td>04M</td>
<td>1942</td>
<td>704</td>
<td>293</td>
<td>2939</td>
<td>2697</td>
</tr>
<tr>
<td>05M</td>
<td>1753</td>
<td>1204</td>
<td>328</td>
<td>3285</td>
<td>2679</td>
</tr>
<tr>
<td>06M</td>
<td>1751</td>
<td>995</td>
<td>305</td>
<td>3051</td>
<td>3445</td>
</tr>
<tr>
<td>08M</td>
<td>2082</td>
<td>607</td>
<td>298</td>
<td>2987</td>
<td>2424</td>
</tr>
<tr>
<td>09M</td>
<td>1532</td>
<td>647</td>
<td>242</td>
<td>2421</td>
<td>2397</td>
</tr>
<tr>
<td>12M</td>
<td>1969</td>
<td>564</td>
<td>281</td>
<td>2814</td>
<td>2000</td>
</tr>
<tr>
<td>14M</td>
<td>2184</td>
<td>712</td>
<td>321</td>
<td>3217</td>
<td>3452</td>
</tr>
<tr>
<td>15M</td>
<td>2126</td>
<td>1671</td>
<td>421</td>
<td>4218</td>
<td>2455</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>1894</td>
<td>883</td>
<td>308</td>
<td>3085</td>
<td>2613</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>220</td>
<td>359</td>
<td>49</td>
<td>494</td>
<td>537</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>69</td>
<td>114</td>
<td>16</td>
<td>156</td>
<td>170</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Measured REE</th>
<th>Measured AEE</th>
<th>Estimated DIT</th>
<th>Estimated TEE</th>
<th>Mean daily kcal intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01F</td>
<td>1730</td>
<td>625</td>
<td>261</td>
<td>2616</td>
<td>2366</td>
</tr>
<tr>
<td>03F</td>
<td>1340</td>
<td>522</td>
<td>206</td>
<td>2068</td>
<td>2234</td>
</tr>
<tr>
<td>04F</td>
<td>1267</td>
<td>307</td>
<td>174</td>
<td>1748</td>
<td>2246</td>
</tr>
<tr>
<td>05F</td>
<td>1561</td>
<td>670</td>
<td>247</td>
<td>2478</td>
<td>1987</td>
</tr>
<tr>
<td>06F</td>
<td>1610</td>
<td>360</td>
<td>218</td>
<td>2188</td>
<td>1759</td>
</tr>
<tr>
<td>07F</td>
<td>1224</td>
<td>222</td>
<td>160</td>
<td>1606</td>
<td>1776</td>
</tr>
<tr>
<td>09F</td>
<td>1558</td>
<td>237</td>
<td>199</td>
<td>1994</td>
<td>2211</td>
</tr>
<tr>
<td>11F</td>
<td>1264</td>
<td>258</td>
<td>169</td>
<td>1691</td>
<td>1825</td>
</tr>
<tr>
<td>12F</td>
<td>1411</td>
<td>875</td>
<td>253</td>
<td>2539</td>
<td>1973</td>
</tr>
<tr>
<td>14F</td>
<td>1219</td>
<td>407</td>
<td>180</td>
<td>1806</td>
<td>1952</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>1418</td>
<td>448</td>
<td>207</td>
<td>2073</td>
<td>2033</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>184</td>
<td>218</td>
<td>37</td>
<td>371</td>
<td>217</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>58</td>
<td>69</td>
<td>12</td>
<td>117</td>
<td>69</td>
</tr>
</tbody>
</table>

REE, Resting Energy Expenditure; AEE, Activity Energy Expenditure; DIT, Dietary Induced Thermogenesis.

No significant differences were found between the estimated mean TEE and the reported mean daily energy intake in kcal for either males or females. There was a significant difference in estimated TEE between the two groups (p < 0.001). Due to
differences in body size between males and females the estimated TEE was calculated per kg of FFM (Figure 5.9). This adjustment resulted in the significant difference in TEE between the genders being lost ($p = 0.93$).

![Figure 5.9](image)

**Figure 5.9 Mean estimated TEE adjusted for kg of FFM for males and females.** Data expressed as mean ± SEM (males n=11, females n=10).

### 5.5.7 Body fat distribution

Females had a significantly higher level of total body fat ($p = 0.002$), subcutaneous fat ($p < 0.001$), subcutaneous abdominal fat ($p = 0.004$) and subcutaneous peripheral fat ($p = 0.001$) than males. Conversely, levels of internal fat ($p = 0.001$), visceral fat ($p = 0.003$) and non visceral fat ($p = 0.002$) were significantly higher in males than females (Table 5.7).
Table 5.7 MRI measurements of body fat for males and females. Data are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Male (n=12)</th>
<th>Female (n=12)</th>
<th>p males vs. females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body fat (litres)</td>
<td>38.50 ± 2.04</td>
<td>46.16 ± 1.48</td>
<td>0.002</td>
</tr>
<tr>
<td>Subcutaneous fat (litres)</td>
<td>26.87 ± 1.88</td>
<td>38.36 ± 1.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Internal fat (litres)</td>
<td>11.64 ± 0.59</td>
<td>7.80 ± 0.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Subcutaneous abdominal fat (litres)</td>
<td>7.86 ± 0.77</td>
<td>11.22 ± 0.67</td>
<td>0.004</td>
</tr>
<tr>
<td>Subcutaneous peripheral fat (litres)</td>
<td>19.01 ± 1.23</td>
<td>27.14 ± 0.97</td>
<td>0.001</td>
</tr>
<tr>
<td>Visceral fat (litres)</td>
<td>6.48 ± 0.38</td>
<td>4.33 ± 0.42</td>
<td>0.003</td>
</tr>
<tr>
<td>Non visceral fat (litres)</td>
<td>5.15 ± 0.31</td>
<td>3.48 ± 0.30</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Total body fat = total body adipose tissue content; Subcutaneous fat = all subcutaneous adipose tissue; Internal fat = all internal adipose tissue compartments i.e. all non-subcutaneous adipose tissue; Subcutaneous abdominal fat = subcutaneous adipose tissue in the abdominal area, defined as the distance from the slice containing the base of the lungs/top of the liver, to the slice containing the femoral heads; Subcutaneous peripheral fat = all the other subcutaneous adipose tissue, i.e. subcutaneous fat in peripheral and non-abdominal parts of the body; Visceral fat = internal adipose tissue in the abdominal area, defined as the distance from the slice containing the base of the lungs/top of the liver, to the slice containing the femoral heads; Non visceral fat = all the other internal adipose tissue in the body, i.e. internal adipose tissue in peripheral and non-abdominal parts of the body, (in muscle, chest, pelvis, head and neck etc).

As waist circumference is used as a surrogate indicator for subcutaneous abdominal fat, a Pearson product-moment correlation coefficient was used to assess the relationship between the two measurements in males (Figure 5.10) and females (Figure 5.11). In males there was a weak correlation between the two variables, r = 0.215, n = 12, p = 0.503. In females there was a stronger correlation between the two variables, r = 0.638, n = 12, p = 0.026.
Figure 5.10 Correlation between subcutaneous abdominal fat (litres) and waist circumference in males (n=12).
Figure 5.11 Correlation between subcutaneous abdominal fat (litres) and waist circumference in females (n=12).

5.5.8 Intrahepatocellular lipid and intramyocellular lipid measurement

Levels of intrahepatocellular lipid and intramyocellular lipid were not significantly different between the genders (Table 5.8 and Figure 5.12).
Table 5.8 MRS measurements of SIMCL, TIMCL and IHCL in males and females.

<table>
<thead>
<tr>
<th></th>
<th>Male (n=12)</th>
<th>Female (n=12)</th>
<th>p males vs. females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SIMCL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>17.40</td>
<td>12.43</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum</td>
<td>9.39</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>25.17</td>
<td>28.63</td>
<td></td>
</tr>
<tr>
<td><strong>TIMCL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7.85</td>
<td>6.73</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum</td>
<td>5.22</td>
<td>2.71</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>16.41</td>
<td>10.73</td>
<td></td>
</tr>
<tr>
<td><strong>IHCL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>14.37</td>
<td>2.17</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.95</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>85.16</td>
<td>65.04</td>
<td></td>
</tr>
</tbody>
</table>

SIMCL, soleus intramyocellular lipid; TIMCL, tibialis intramyocellular lipid; IHCL, Intrahepatocellular lipid.

![Box plots of levels of intrahepatocellular fat for males and females. Data expressed as median and interquartile range (Males, n=12, Females, n=12).](image)

Figure 5.12 Box plots of levels of intrahepatocellular fat for males and females. Data expressed as median and interquartile range (Males, n=12, Females, n=12).
As alcohol intake was significantly greater in males than females ($P = 0.01$), a Pearson product-moment correlation coefficient was used to assess the degree of association between alcohol intake and IHCL in males (Figure 5.13). An initial analysis indicated a very weak correlation between the two variables, $r = 0.159$, $n = 11$, $p = 0.641$. As shown in Figure 5.13 there are two outliers, participant numbers 06M and 14M. As the $p$ value obtained indicated a low level of confidence in the results, a further analysis was undertaken excluding the two outlying cases (Figure 5.14). This second analysis indicated a stronger correlation between the two variables, $r = 0.735$, $n = 9$, $p = 0.024$.

![Graph showing correlation between mean alcohol intake (g/day) and intrahepatocellular lipid (arbitrary units)](image)

**Figure 5.13** Correlation between mean alcohol intake (g/day) and intrahepatocellular lipid in males ($n=11$).
5.5.9 Adipose and muscle gene expression

In adipose tissue, a significantly greater expression of LPL was identified in females than males ($p = 0.01$). There were no further significant differences in gene expression in adipose tissue found between the genders (Figure 5.15).

No significant differences in gene expression in muscle tissue were found between males and females (Figure 5.16).
Figure 5.15 Levels of gene expression in adipose tissue normalized to the housekeeping gene GAPDH. (Female group, n=11, Male group, n=11). CD36, Cluster of Differentiation 36; A, Adiponectin; AR1, adiponectin receptor 1; UCP2, Uncoupling protein 2; LPL, lipoprotein lipase; HSL, Hormone sensitive lipase; PG, Peroxisome proliferator-activated gamma; PD, Peroxisome proliferator-activated delta.

Figure 5.16 Levels of gene expression in muscle tissue normalized to the housekeeping gene GAPDH. (Female group, n=11, Male group, n=11). CD36, Cluster of Differentiation 36; AR1, adiponectin receptor 1; UCP2, Uncoupling protein 2; UCP3, Uncoupling protein 3; LPL, lipoprotein lipase; PD, Peroxisome proliferator-activated delta; PA, Peroxisome proliferator-activated alpha; ACOX1, acyl CoA oxidase; CPT1, Carnitine palmitoyltransferase 1; CPT2, Carnitine palmitoyltransferase 2.
5.6 Discussion

The overall aim of this study was to comprehensively research how gender influences energy expenditure, body fat distribution and gene expression in obesity. It was hoped that this novel approach of studying all of these separate elements of metabolism in the same participants would provide a greater understanding of some of the previously reported differences in metabolism in obese men and women.

Resting energy expenditure

Interestingly, previous research regarding gender differences in REE has been mixed. In the hypothesis it was proposed that males would have a higher REE than females. In absolute terms males were found to have a higher REE than the females, as would be expected, since REE is highly correlated with lean body mass (Ravussin et al., 1982). This finding is in line with previous studies (Tooze et al., 2007; Carpenter et al., 1998). When the REE measurements in this study were adjusted for lean body mass, however, the females were found to have a greater REE per kg of lean body mass than the males. This is contradictory to findings from previous studies (Morio et al., 1997; Ferraro et al., 1992) in which, once adjustments had been made for differences in fat free mass, women were found to have lower REE than men. There are several possible explanations for this. It has been shown that organ masses are the body compartments that predominantly contribute to REE and that organs including the brain, heart and liver have a higher resting metabolic rate than skeletal muscle. The brain, liver, kidneys and heart, account for only approximately 5–6% of body weight, but contribute to 80% of REE (Bosy-Westphal et al., 2009). In comparison to males, females have a greater proportion of their FFM as these high metabolic rate organs and tissues and this could provide a potential explanation for
the finding in this study that females had a greater REE per kg of lean body mass than the males. Another possible explanation is provided by the work of Hendersen et al. (2009) in which they demonstrated that muscle and whole body protein synthesis is positively associated with REE, and that women actually have higher muscle protein synthesis and higher whole-protein turnover than men.

Fat and carbohydrate oxidation

In this study a significantly higher rate of carbohydrate oxidation was measured in males than females in absolute terms but once adjusted per kg/FFM no significant differences were found in either carbohydrate or fat oxidation rates. This was unexpected as, due to the findings of Nielsen et al. (2003), a greater level of fat oxidation was anticipated in women. No significant difference was found in RQ between the two groups in this study and this corresponded with the findings in a study by Nielsen et al. (2003) which reported no significant difference in respiratory quotient measured by indirect calorimetry between obese males and females. It is possible that the indirect calorimetry method was too insensitive to detect any differences in oxidation rates in this study and, as in the rimonabant study, stable isotope investigations may have provided a more accurate view of differences in oxidation rates.

Gene expression

It was hypothesised that a greater expression of genes involved in lipolysis would be identified in females than males following previous research by Nielsen et al. (2003) in which women were reported to release approximately 40% more free fatty acids from fat tissue than men relative to REE. No significant differences were found in
gene expression in this study which could explain this higher fatty acid release. Interestingly, in this study, the one significant finding was a greater expression of LPL in adipose tissue in females than males. This actually corresponds with the findings of Arner et al. (1991) who previously described significantly higher LPL mRNA levels in women than men in abdominal and gluteal subcutaneous tissue. As LPL will be influencing lipid storage within fat cells, this increased LPL activity may contribute to the well documented gender differences in total body fat and body fat distribution.

It is possible that few significant differences were found in gene expression between genders as the data was variable and the SDs were high for most genes. Large differences would need to have been identified in gene expression to detect a significant difference between groups. There is little previous research in the literature to calculate the exact number of participants that would have been required to accurately achieve statistical significance but greater than the 11 participants in each group in this study.

*Body fat distribution*

Previous research by Martin and Jensen (1991) had concluded that upper-body subcutaneous fat was more lipolytically active than lower body fat in women and it was suggested that this upper body fat was the source of excess FFA release in upper-body obese women. As the female participants in this study were postmenopausal they had greater levels of upper body subcutaneous fat than the males, as reflected in the MRI results, and therefore would possibly have greater amounts of more lipolytically active fat tissue than men. It is possible that the
expected differences in gene expression were not identified due to fairly low participant numbers of 11 in each group.

It is well documented that there are gender differences in fat mass and body fat distribution. Females are reported to have higher levels of total body fat which was reflected in the results from this study. As described in chapter one, body fat distribution varies between the genders and changes post menopause in females. In this study post menopausal females were compared to males and it is well documented that post menopausal females tend to accumulate more abdominal and visceral fat due to a reduction in oestrogen levels. Levels of subcutaneous abdominal fat were found to be greater in the female group than the male, but as expected males were still found to have higher levels of abdominal visceral fat. These increased abdominal visceral fat depots put males at higher risk of obesity related chronic diseases (Lovejoy and Sainsbury, 2009).

**Dietary data**

Dietary analysis indicated that, in absolute terms, males reported a higher mean daily intake of energy and macronutrients than females over a four day period. This finding is consistent with results reported from a study by Paul et al. (2004), in which dietary recalls indicated that both energy and macronutrients were greater in men than women. Paul et al. found that the differences were no longer significant when macronutrient intake was expressed as a percentage of total daily energy intake. In this study, once the same analysis was undertaken, a significantly greater percentage of energy was provided by fat in females than males and a significantly greater percentage of total energy provided by alcohol in males. Although no difference was
found in IHCL between men and women, there was a moderately strong association between alcohol intake and IHCL in males.

*Activity energy expenditure*

Absolute mean AEE was significantly higher in males than females and this finding was unsurprising as males have a greater body weight, and therefore may expend more energy in daily activities than females with a lower body weight. Once AEE was expressed per kg of body weight, the significant difference between the two genders was lost. This finding is consistent with the results of a study by Ferraro et al. (1992) in which, once adjustments had been made for body composition, no significant difference was seen in physical activity levels. A previous study by Paul et al. (2004) had also reported no gender difference in physical activity level, however physical activity was calculated as TEE-REE and not measured by an activity monitor such as the Actiheart. Additionally it should be noted that the study participants were of a healthy weight and not obese. One clear limitation of the activity measurement in this study is that AEE measured over a five day recording period may not reflect habitual levels of physical activity. It has been suggested that study participants may knowingly, or otherwise, increase their levels of physical activity during times of AEE measurement (Westerterp and Goran, 1997).

*Total energy expenditure*

Estimated absolute levels of TEE were found to be higher in males than females and this was expected, as TEE is known to be highly correlated with FFM (Tooze et al., 2007). This finding fits with that of a previous study by Paul et al. (2004) in which TEE measured by the doubly labeled water method was found to be higher in healthy
weight men than women. Once TEE was adjusted per kg of FFM, however, the significant finding was lost. Interestingly, this finding contradicts that of Tooze et al. (2007) who reported that TEE was actually higher in females than males once adjusted per kg of FFM but, contrary to our results, they also reported a higher AEE in females which accounted for the greater TEE.

No significant differences were found between the estimated TEE and the reported mean daily energy intake in kcal for either males or females, yet if the individual participant data in Table 5.5 is examined there are substantial differences in reported intake to TEE in some participants. Subject 15M actually has an estimated TEE 1763 kcal higher than reported energy intake in kcal. There are several possible explanations for this. Firstly, the TEE is estimated and, therefore, may not be accurate. Secondly, both the AEE measurement and diet diary recordings were taken over a 4-5 day period only, which may not have been representative of habitual activity or eating patterns. Finally the participant may have under reported his dietary intake.

**Under reporting**

There is much research suggesting that under reporting is an issue when analysing dietary information provided by an obese study population. Results of a dietary survey by Gregory et al. (1990) indicated that if using a ratio of energy intake to BMR the reported energy intakes were unacceptably low in 30% of males and 40% of females. In a further study Macdiarmid et al. (1998) found that in obese individuals these levels of under reporting were found in 60% of males and 70% of females. Previous research has suggested that in obese individuals, fat may be
selectively under reported (Goris et al., 1999) and obese women have been shown to selectively under report sweet, high fat foods (Macdiarmid et al. 1998).

*Waist circumference*

Mean measurements of waist circumference were in the high risk category for both genders. Only one male and one female had a waist circumference that was not in the high risk category. Both males and females were also found to have high risk waist-hip ratios. Some large epidemiological studies have suggested that a high waist hip ratio is a better predictor of cardiovascular disease and all cause mortality than a large waist circumference alone (Canoy et al., 2007). However, as described in chapter one, measurements of waist hip ratio may be misleading. Findings from further studies have suggested that high levels of abdominal fat and therefore a greater abdominal girth are a major risk factor for coronary heart disease and therefore waist circumference is actually a better predictor of heart disease (Dagenais et al., 2005). For this reason it was interesting to find that there was only a weak correlation between waist circumference and subcutaneous abdominal fat in men but a stronger correlation in women.

*Conclusion*

In conclusion, although absolute mean daily REE was higher in males in females as predicted in the hypothesis, once expressed as REE/kg lean body mass the findings were reversed with significantly higher REE values in the female group. Females were found to have greater total body fat and subcutaneous fat and males, higher visceral fat as predicted. Although no significant difference was identified in gene expression in muscle, a greater expression of LPL in adipose tissue was measured in
females than males. This increased LPL activity may contribute to the well documented gender differences in total body fat and body fat distribution.

A further study involving a greater number of participants may have yielded more revealing results with regards to the gene expression data. Further research is still required regarding gender differences in obesity in these key genes involved in lipid and glucose metabolism. These genes may provide novel targets for future anti-obesity medications.
Chapter 6

General Discussion
General Discussion

Development of research skills

This project has provided invaluable experience in the development of independent research skills. Training was provided in various scientific techniques including the measurement of resting energy expenditure using indirect calorimetry, use of Actiheart activity monitors and laboratory techniques for the extraction of mRNA and rtPCR for measurement of gene expression. Considerable experience was also gained in the practical elements of research management, such as setting up and coordinating of a clinical trial and recruitment. Additionally, and most importantly, it has also provided a greater understanding of methods of critically analysing data and questioning research findings.

Anti-obesity Medications

The recent licence suspensions for rimonabant and sibutramine have resulted in only one option of anti-obesity medication, orlistat, being available for prescription. Over the past few years, much progress has been made treating high cholesterol and raised blood pressure with a selection of medications, but due to negative side effects of the weight loss medications and their subsequent withdrawal from the market, treatment for obesity still remains a problem. Further research is still required to find a pharmacological way to target obesity with its related metabolic complications.

An important consideration is that there are different types of obesity and perhaps these different types of obesity need to be treated in alternative ways. Although obesity is associated with a number of comorbidities, a sub group of obese individuals have been found not to have the associated problems and remain
metabolically ‘healthy’ (Blüher, 2010). It is important to identify the ‘unhealthy’ obese, who will potentially benefit the most from anti-obesity treatments from the ‘healthy’ obese. As demonstrated in both the rimonabant and gender differences study, fat distribution is not uniform between individuals. Men and post menopausal women have greater levels of abdominal obesity which is associated with insulin resistance, high triglyceride levels and low HDL cholesterol and thus greater cardiovascular risk. An anti-obesity medication which specifically targets high risk abdominal obesity is needed. Anti-obesity medications have historically been prescribed on the basis of BMI but, if drugs were developed that were more effective in a certain sub group of obese individuals than others, then prescription could be on the basis of, for example, waist circumference.

Limitations and future studies

It is disappointing that rimonabant was found to have more serious side effects than previously thought and that its licence was suspended half way through the trial as interesting results were emerging regarding the medications effects on energy expenditure. Had the medication not lost its licence it would have been important to have undertaken a study similar to the one described in this thesis using doubly labeled water which provides a robust measure of TEE over a two week period in a free living environment. This approach would compensate for some of the limitations of REE and AEE measurements.

Due to the many reasons outlined in this thesis, the endocannabinoid system is still a good potential target in the treatment of obesity. Due to the negative side effects of anxiety and depression seen with rimonabant, further research is required to find a
medication that only acts on peripheral and not central receptors. If a drug could be
developed that could be restricted to the peripheral receptors and could be prevented
from crossing the blood brain barrier, the mood lowering effects of rimonabant could
be eliminated (Di Marzo and Szallasi, 2008). However, it must be considered that
one of the mechanisms of action of rimonabant is through its central effects on
appetite and reward mechanisms and therefore a peripheral antagonist might be less
effective as an anti-obesity medication.

Future work also needs to establish the maximum length of time that an individual
can safely tolerate any newly developed anti-obesity medications. Once patients
stopped taking rimonabant in the RIO trials they regained their lost weight. It would
have been interesting to determine how quickly REE returned to baseline levels
during this period of weight regain.

As all of the weight loss medications developed at this time have only resulted in
modest weight reductions they should not to be used in isolation but as an adjunctive
therapy. Rimonabant’s ability to maintain or increase REE made it particularly
promising as an adjunct to lifestyle modification since, as shown in the diet group of
the current trial, obese people who lose weight through non pharmacological means
have metabolic alterations such as a reduced REE. Accompanying this lowered REE
tend to be feelings of hunger which promote increased food intake and make it
difficult to maintain a lower body weight. In order to maintain weight loss either
without, or post treatment with an anti-obesity medication, it is essential to
encourage obese individuals to maintain lean body mass, and thus increase both
resting and total energy expenditure, through increasing physical activity.
At this time the commercial weight loss industry is largely targeted towards women and much of the clinical research undertaken has been in females. There are limited studies examining gender differences in response to anti-obesity medications. The large human clinical trials using rimonabant were undertaken in both genders but there was no sub analysis as to the differing effects in males and females. As shown in this thesis, there are gender differences in metabolism in obesity, and therefore the potential for gender differences in response to anti-obesity medications which need to be evaluated. Given that there are also gender differences in response to commercial diet plans and physical activity programmes for weight loss, it is likely that gender-specific treatment approaches to weight loss would be advantageous.

**Conclusion**

This project has provided an interesting insight into the metabolic effects of endocannabinoid antagonists in humans, and undoubtedly supplies evidence to suggest that further research focusing on novel endocannabinoid antagonists is warranted. The novel gene expression work has also highlighted the impact that both medication and gender may have on genes involved in lipid and glucose metabolism and the potential importance of these genes as targets in the development of new anti-obesity medications.
References
References


HENDERSON, G.C., DHATARIYA, G., FORD, C., KLAUS, K.A., BASU, R.,
Higher muscle protein synthesis in women than men across the lifespan, and failure
of androgen administration to amend age-related decrements. The FASEB Journal,
23, 631-641.

(2008) A qualitative investigation of individuals' experiences and expectations before
and after completing a trial of commercial weight loss programmes. J Hum Nutr
Diet., 21 (1), 72-80.

HEYMSFIELD, S. B., VAN MIERLO, C. A. J., VAN DER KNAAP, H. C. M.,
strategy: meta and pooling analysis from six studies. International Journal of
Obesity, 27, 537-


HOWLETT, A. C., BARTH, F., BONNER, T. I., CABRAL, G., CASELLAS, G.,
DEVANE, W. A., FELDER, C. C., HERKENHAM, M., MACKIE, K., MARTIN, B.
Pharmacology XXVII. Classification of cannabinoid receptors. Pharmacol. Rev,

IASO (International Association for the Study of Obesity) (2008) Overweight and
Obesity in the EU27.

IOTF (International Obesity Task Force) and EASO (The European Association for


Appendices
APPENDIX 1

Self-Certificate Medical Questionnaire

Pilot study to establish the feasibility and acceptability of meeting energy requirements in target study participants through the use of a commercially available modular energy controlled diet (Slim.Fast, Unilever).

Please tick all/any of the following that apply:

☐ I have no prior/present history of Coronary Heart Disease, Angina or Stroke
☐ I have no prior/present history of Type 1 or Type 2 diabetes
☐ I have no prior/present history of liver disease
☐ I have no prior/present history of endocrine disorders
☐ I have no prior/present history of, nor am I currently being treated for, clinical depression and/or other psychological disorders
☐ I have no prior/present history of eating disorders, including anorexia or bulimia nervosa
☐ I have no prior/present history of drug or alcohol abuse within the last 2 years
☐ I have not had any surgical procedure to aid weight loss
☐ I have no known food allergies or intolerances
☐ I am not currently taking or have taken any regular medication prescribed by my GP in the last 6 months

Signed ___________________________ Date ___ / ___ / __________

Name: .................................................  DOB:
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Address:
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Contact Telephone Number:
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GP Name: .........................................................

GP Address:
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APPENDIX 2

Dutch Eating Behaviour Questionnaire (DEBQ)

Participant code _____________________ Date ___/___/___

Please answer the following questions as carefully and honestly as possible. Read each question and simply fill in the column which best applies to you.

<table>
<thead>
<tr>
<th>1. If you have put on weight, do you eat less than you usually do? *</th>
<th>Never</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
<th>Not relevant</th>
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<tbody>
<tr>
<td>2. Do you have a desire to eat when you are irritated? *</td>
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<td>3. If food tastes good to you, do you eat more than you usually do? *</td>
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<td>4. Do you try to eat less at meal times than you would like to eat? *</td>
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<td>5. Do you have a desire to eat when you have nothing to do? *</td>
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<td>6. Do you have a desire to eat when you are depressed or discouraged? *</td>
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<td>7. If food smells and looks good, do you eat more than you usually eat? *</td>
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<td>8. How often do you refuse food or drink offered because you are concerned about your weight? *</td>
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<td>9. Do you have a desire to eat when you are feeling lonely? *</td>
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<td>10. If you see or smell something delicious, do you have a desire to eat it? *</td>
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<td>11. Do you watch exactly what you eat? *</td>
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<td>12. Do you have a desire to eat when somebody lets you down? *</td>
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<td>13. If you have something delicious to eat, do you eat it straight away? *</td>
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<td>14. Do you deliberately eat foods that are slimming? *</td>
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<td>15. Do you have a desire to eat when you are cross? *</td>
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<td>16. Do you have a desire to eat when you are approaching something unpleasant to happen? *</td>
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<td>17. If you walk past the baker do you have a desire to buy something delicious? *</td>
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<td>18. When you have eaten too much, do you eat less than usual the following days? *</td>
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<td>19. Do you get a desire to eat when you are anxious, worried or tense? *</td>
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<td>20. If you walk past a snack bar or café, do you have a desire to buy something delicious? *</td>
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<td>21. Do you deliberately eat less in order not to become heavier? *</td>
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<td>22. Do you have a desire to eat when things are going against you, or things have gone wrong? *</td>
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<td>23. If you see others eating, do you have also the desire to eat? *</td>
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<td>24. How often do you try not to eat between meals because you are watching your weight? *</td>
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<td>25. Do you have a desire to eat when you are frightened? *</td>
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<td>26. Can you resist eating delicious food? *</td>
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<td>27. How often in the evening do you try not to eat because you are watching your weight? *</td>
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<td>28. Do you have a desire to eat when you are disappointed? *</td>
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<td>29. Do you eat more than usual when you see other eating? *</td>
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<td>30. Do you take your weight into account when you eat? *</td>
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<td>31. Do you have a desire to eat when you are emotionally upset? *</td>
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<td>32. When preparing a meal are you inclined to eat something? *</td>
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<td>33. Do you have a desire to eat when you are bored or restless? *</td>
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</tbody>
</table>
**DEBQ Categories:**

* Emotional eating – 13 items
* External eating – 10 items
* Restrained eating – 10 items
APPENDIX 3

Name: ___________________________________________ Marital Status: __________ Age: ________ Sex: ______

Occupation: _______________________________________ Education: _______________________________________

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
   0 I do not feel sad.
   1 I feel sad much of the time.
   2 I am sad all the time.
   3 I am so sad or unhappy that I can’t stand it.

2. Pessimism
   0 I am not discouraged about my future.
   1 I feel more discouraged about my future than I used to be.
   2 I do not expect things to work out for me.
   3 I feel my future is hopeless and will only get worse.

3. Past Failure
   0 I do not feel like a failure.
   1 I have failed more than I should have.
   2 As I look back, I see a lot of failures.
   3 I feel I am a total failure as a person.

4. Loss of Pleasure
   0 I get as much pleasure as I ever did from the things I enjoy.
   1 I don’t enjoy things as much as I used to.
   2 I get very little pleasure from the things I used to enjoy.
   3 I can’t get any pleasure from the things I used to enjoy.

5. Guilty Feelings
   0 I don’t feel particularly guilty.
   1 I feel guilty over many things I have done or should have done.
   2 I feel quite guilty most of the time.
   3 I feel guilty all of the time.

6. Punishment Feelings
   0 I don’t feel I am being punished.
   1 I feel I may be punished.
   2 I expect to be punished.
   3 I feel I am being punished.

7. Self-Dislike
   0 I don’t feel particularly about myself as ever.
   1 I have lost confidence in myself.
   2 I am disappointed in myself.
   3 I dislike myself.

8. Self-Criticalness
   0 I don’t criticize or blame myself more than usual.
   1 I am more critical of myself than I used to be.
   2 I criticize myself for all of my faults.
   3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
   0 I don’t have any thoughts of killing myself.
   1 I have thoughts of killing myself, but I would not carry them out.
   2 I would like to kill myself.
   3 I would kill myself if I had the chance.

10. Crying
    0 I don’t cry anymore than I used to.
    1 I cry more than I used to.
    2 I cry over every little thing.
    3 I feel like crying, but I can’t.
### Appendices

11. Agitation
- 0 I am no more restless or wound up than usual.
- 1 I feel more restless or wound up than usual.
- 2 I am so restless or agitated that it’s hard to stay still.
- 3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest
- 0 I have not lost interest in other people or activities.
- 1 I am less interested in other people or things than before.
- 2 I have lost most of my interest in other people or things.
- 3 It’s hard to get interested in anything.

13. Indecisiveness
- 0 I make decisions about as well as ever.
- 1 I find it more difficult to make decisions than usual.
- 2 I have much greater difficulty in making decisions than I used to.
- 3 I have trouble making any decisions.

14. Worthlessness
- 0 I do not feel I am worthless.
- 1 I don’t consider myself as worthwhile and useful as I used to.
- 2 I feel more worthless as compared to other people.
- 3 I feel utterly worthless.

15. Loss of Energy
- 0 I have as much energy as ever.
- 1 I have less energy than I used to have.
- 2 I don’t have enough energy to do very much.
- 3 I don’t have enough energy to do anything.

16. Changes in Sleeping Pattern
- 0 I have not experienced any change in my sleeping pattern.
- 1a I sleep somewhat more than usual.
- 1b I sleep somewhat less than usual.
- 2a I sleep a lot more than usual.
- 2b I sleep a lot less than usual.
- 3a I sleep most of the day.
- 3b I wake up 1–2 hours early and can’t get back to sleep.

17. Irritability
- 0 I am no more irritable than usual.
- 1 I am more irritable than usual.
- 2 I am much more irritable than usual.
- 3 I am irritable all the time.

18. Changes in Appetite
- 0 I have not experienced any change in my appetite.
- 1a My appetite is somewhat less than usual.
- 1b My appetite is somewhat greater than usual.
- 2a My appetite is much less than before.
- 2b My appetite is much greater than usual.
- 3a I have no appetite at all.
- 3b I crave food all the time.

19. Concentration Difficulty
- 0 I can concentrate as well as ever.
- 1 I can’t concentrate as well as usual.
- 2 It’s hard to keep my mind on anything for very long.
- 3 I find I can’t concentrate on anything.

20. Tiredness or Fatigue
- 0 I am no more tired or fatigued than usual.
- 1 I get more tired or fatigued more easily than usual.
- 2 I am too tired or fatigued to do a lot of the things I used to do.
- 3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex
- 0 I have not noticed any recent change in my interest in sex.
- 1 I am less interested in sex than I used to be.
- 2 I am much less interested in sex now.
- 3 I have lost interest in sex completely.

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**Total Score**

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195
## Macronutrient composition of Slim Fast products

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<th>Fruits of the Forest Meal Bar</th>
<th>Yoghurt Meshi Meal Bar</th>
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# Macronutrient composition of Slim.Fast products

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APPENDIX 5

DIETARY PRESCRIPTION

Participant number:

Week numbers: 1 and 2

Please find your daily dietary prescription below.
Please eat the products listed below on each day and record your dietary intake in the diet diary attached.
As you need to fit these foods into your day in a way that works for you, the table below has been provided so that you can tick each food product as you eat it and easily keep track of what you need to consume over the course of the day.

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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 x Milk Shake</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x Smoothie</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x Smoothie</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x Meal Bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x Meal Bar</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 x Soup</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 x Snack Bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x Snack (either Cheddar Bites or Pretzels)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
### APPENDIX 6

**SLIMFAST PRODUCTS AVAILABLE**

**Participant number:**

Products marked with an X are unacceptable to the participant.

<table>
<thead>
<tr>
<th>Product Name</th>
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</thead>
<tbody>
<tr>
<td>Banana Milk Shake</td>
</tr>
<tr>
<td>Chocolate Milk Shake</td>
</tr>
<tr>
<td>Strawberry Milk Shake</td>
</tr>
<tr>
<td>Vanilla Milk Shake</td>
</tr>
<tr>
<td>Café Classic Milk Shake</td>
</tr>
<tr>
<td>Banana Milk Shake Powder</td>
</tr>
<tr>
<td>Chocolate Milk Shake Powder</td>
</tr>
<tr>
<td>Vanilla Milk Shake Powder</td>
</tr>
<tr>
<td>Fruits of the Forest Meal Bar</td>
</tr>
<tr>
<td>Yoghurt Muesli Meal Bar</td>
</tr>
<tr>
<td>Mediterranean Bean and Tomato Soup</td>
</tr>
<tr>
<td>Hearty Chicken and Vegetable Pasta Soup</td>
</tr>
<tr>
<td>Cream of Chicken and Mushroom Soup</td>
</tr>
<tr>
<td>Fruits of the Forest Smoothie</td>
</tr>
<tr>
<td>Raspberry Smoothie</td>
</tr>
<tr>
<td>Peach and Mango Smoothie</td>
</tr>
<tr>
<td>Apricot and Cashew Snack Bar</td>
</tr>
<tr>
<td>Cranberry and Almond Snack Bar</td>
</tr>
<tr>
<td>Peanut Snack Bar</td>
</tr>
<tr>
<td>Caramel Snack Bar</td>
</tr>
<tr>
<td>Cheddar Bites</td>
</tr>
<tr>
<td>Sour Cream and Chive Pretzels</td>
</tr>
</tbody>
</table>
APPENDIX 7

END OF PILOT QUESTIONNAIRE

Pilot study to establish the feasibility and acceptability of meeting energy requirements in target study participants through the use of a commercially available modular energy controlled diet (Slim.Fast, Unilever).

Do you think that you could follow the diet for a seventeen week period? If not, for what reasons?

Did you eat any other foods whilst on the diet? If so, what was the reason for that?

Did you experience any changes in your bowel habits whilst following the diet?

Did you experience any other side effects whilst on the diet?

Any other comments?