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**Relationships between exercise,
energy balance, appetite and dietary
restraint in overweight and obese
women.**

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Submitted in fulfilment of the requirements for the
Degree of Doctor of Philosophy

Human Nutrition

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Abstract

Background: Exercise may acutely and chronically up-regulate appetite and energy intake in overweight and obese women preventing body mass reduction in the long term. Overweight and obese women may be most prone to compensatory responses to exercise but the possible mechanism for this is unclear. Appetite regulating hormones have been investigated as a possible mechanism but to date the evidence is somewhat mixed.

Identifying compensatory energy intake responses in overweight and obese women is complicated by the high prevalence of dietary under-reporting in this group. The laboratory-based buffet meal method has frequently been used in research studies that have assessed food intake in these women, but this method has only undergone preliminary validation.

Dietary restraint may also affect individual appetite responses to exercise; it has been theorised that restraint may be a behavioural adaptation to diminished energy requirements, and differences in physical activity levels could also contribute. Evidence thus far has produced mixed results, possibly because two distinct sub-groups of restrained eaters exist, those with flexible and rigid control of restraint. It is not known if there are differences in energy requirements between these two sub-groups.

Participants and Methods:

Participants in all studies were sedentary, healthy, pre-menopausal, overweight and obese, adult women.

Study 1: Fourteen women completed four trials; two exercise and two control, following the same protocol as study 1. Energy intake at three buffet meals and subjective appetite ratings were measured, and the reproducibility of these values under control and exercise conditions was tested using intraclass correlation coefficient (r_i).

Study 2: Twenty-nine women completed two trials in a randomised, counterbalanced order; exercise and control. Each trial lasted 24 hours spanning 2 days; the afternoon of day 1 and morning of day 2. An exercise session to expend 1.65 MJ was completed on day 1 of exercise trials, and three buffet

meals were served during each trial to measure energy intake. Appetite was assessed using a visual analogue scale and blood samples were taken to determine acylated ghrelin (n=15) and peptide YY (n=10) concentrations. A repeated measures ANOVA was used to investigate the effects of trial and time on appetite hormones, EI and appetite.

Study 3: Fifteen women participated in a sixteen week exercise intervention to expend 8360 kJ week⁻¹. Participants exercised unsupervised in the University gym, and compliance was measured via heart rate monitoring. Sub-maximal fitness and body composition assessments were carried out at baseline, and after 8 and 16 weeks of exercise. Energy expenditure, energy intake, appetite, and acylated ghrelin (n=14) and peptide YY concentrations (n=11) were measured at baseline and after 8 weeks of exercise. Paired t tests were used to assess differences in time-averaged AUC for appetite, total and relative EI, metabolic rate, and exercise responses between trials. Repeated measures ANOVA was used to assess changes over time in body composition, appetite ratings, EI, acylated ghrelin, peptide YY, and cardiovascular fitness levels.

Study 4: Forty-one sedentary women in a one week observational study. Participants were classed as restrained or unrestrained using the three factor eating questionnaire, and the former group were further classified as having flexible or rigid control of restraint. All participants completed a food frequency questionnaire, sub-maximal fitness test, body composition assessment and two fasted metabolic rate measurements. Average daily energy expenditure was calculated from a seven day physical activity diary combined with continuous heart rate data. Differences between restrained and unrestrained eaters, and restrained eaters with flexible and rigid control, were assessed using a paired t-test.

Results

Study 1: The r_i for energy intake in control trials was significant but had large associated confidence intervals (r_i 0.50 (95% CI 0.03, 0.80) $p=0.0003$). The r_i was for energy intake in exercise trials was (r_i 0.04 (95% CI -0.53, 0.55; $p=0.45$) and for the difference between control and exercise trials was (r_i -0.05 (95% CI -0.54, 0.48; $p=0.57$) this was not significant. The r_i values for satiety, fullness and

desire to eat were significant in both control and exercise trials ($p < 0.05$), but the associated confidence intervals were large.

Study 2: There was no effect of exercise on subjectively rated appetite, acylated ghrelin, or peptide YY concentrations (all $p > 0.05$). Total energy intakes were not significantly different between trials (exercise: 10.9 ± 0.5 MJ, control: 10.8 ± 0.5 MJ; mean \pm SEM).

Study 3: Total exercise energy expenditure during the intervention was 80.8 ± 7.7 MJ, which resulted in a significant reduction in total body mass (-1.9 ± 0.9 kg), fat mass (-1.7 ± 0.7 kg) and BMI (-0.7 ± 0.4 kg m⁻²). However individual changes in body and fat mass ranged from $+2.8$ to -9.9 kg, and $+1.78$ to -6.55 kg respectively. There were no significant differences in appetite, energy intake, or expenditure after 8 weeks of exercise ($p > 0.05$).

Study 4: There were no differences in metabolic rate, daily energy expenditure or physical activity patterns between restrained and unrestrained eaters ($p > 0.05$), or between restrained eaters with flexible and rigid control of restraint ($p > 0.05$).

Conclusions:

Study 1: The laboratory-based buffet meal method of measuring energy intake does not provide reliable, reproducible values in overweight and obese, pre-menopausal women either under control or exercise conditions.

Study 2: A walking-based exercise session which induces a moderate energy deficit of 1.65 MJ does not appear to affect subsequent twenty four hour energy intake, subjectively rated appetite, or plasma acylated ghrelin and peptide YY concentrations during the subsequent twenty four hours.

Study 3: This study concluded that 16 weeks aerobic exercise in overweight and obese women produces a small, but significant, reduction in body and fat mass (-1.9 ± 0.9 kg); however the extent of these changes varies greatly between individuals ($+2.8$ to -9.9 kg). No evidence of compensatory changes in energy intake or expenditure, subjective appetite ratings, or circulating levels of acylated ghrelin and peptide YY was apparent after 8 weeks of exercise.

Study 4: This study concluded that there is no evidence of a difference in body composition or energy requirements between overweight and obese female

restrained and unrestrained eaters, or between sub-groups of restrained eaters. Dietary restraint does not appear to be an adaptation to diminished energy requirements.

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“It must be remembered that the purpose of education is not to fill the minds of students with facts... it is to teach them to think, if that is possible, and always to think for themselves.”

-Robert Hutchins

“I hear, and I forget.

I see, and I remember.

I do, and I understand.”

-Chinese Proverb

"Happiness is always a by-product. It is probably a matter of temperament, and for anything I know it may be glandular. But it is not something that can be demanded from life, and if you are not happy you had better stop worrying about it and see what treasures you can pluck from your own brand of unhappiness."

-Robertson Davies

Publications arising from this thesis

Short Communication:

G.L. Brown, M.E.J. Lean and C.R. Hankey (2011) Reproducibility of 24-h post-exercise changes in energy intake in overweight and obese women using current methodology. *Brit J Nutr*, doi: 10.1017/S0007114511005575.

Abstracts:

G.L. Brown, M.E.J. Lean and C.R. Hankey (2011) Post-exercise changes in energy intake using current methodology are not reproducible in overweight and obese women. *Proc Nutr Soc*, **70**, E1 doi: 10.1017/S002966511100033.

G.L. Brown, C.R. Hankey, E Manthou, J.M.R. Gill, D Malkova and M.E.J Lean (2010) Effect of a single exercise session on appetite, energy intake and appetite regulating hormones in overweight and obese women. British Association of Sports and Exercise Medicine annual conference, November 25th, London, UK.

Author's Declaration

I declare that the work contained in this thesis is original, and I am the author of this thesis. The planning and design of the studies reported within this thesis were completed by the author, in collaboration with Dr C Hankey, Professor M Lean and Dr D Malkova. Recruitment of study participants and delivery of study interventions were carried out by the author. Biochemical measurements were conducted by Mrs Frances Cousins, with assistance from the author in the Department of Biochemistry, Royal Hospital for Sick Children, Yorkhill, Glasgow. Data entry and analysis were carried out by the author with guidance from Dr David Young, Senior Lecturer, University of Strathclyde Department of Mathematics and Statistics. Dual X-Ray Absorptiometry measurements were carried out by Dr Sheila Khanna, Post-Doctoral Scientist, Department of Child Health, University of Glasgow.

List of Abbreviations

BMI	Body Mass Index
BM	Body Mass
BW	Body Weight
FM	Fat Mass
FFM	Fat Free Mass
LM	Lean Mass
RQ	Respiratory Quotient
$\dot{V}O_{2max}$	Maximum Oxygen Uptake
$\dot{V}O_{2peak}$	Peak Oxygen Uptake
EE	Energy Expenditure
ExEE	Exercise Energy Expenditure
EI	Energy intake
REI	Relative Energy Intake
CHO	Carbohydrates
PRO	Proteins
DTE	Desire to Eat
PFC	Prospective Food Consumption
DLW	Doubly Labelled Water
NEAT	Non-Exercise Activity Thermogenesis
RMR	Resting Metabolic Rate
BMR	Basal Metabolic Rate
ADMAR	Average Daily Metabolic Rate
SMR	Sleeping Metabolic Rate
$\dot{V}O_2$	Oxygen uptake
$\dot{V}CO_2$	Carbon dioxide production
HR	Heart Rate

HR _{max}	Maximum Heart Rate
HRR	Heart Rate Reserve
RPE	Rate of Perceived Exertion
AEE	Active Energy Expenditure
IAEE	Inactive Energy Expenditure
SEE	Sleeping Energy Expenditure
TEE	Total Energy Expenditure
MET	Metabolic Equivalent
TEF	Thermic Effect of Feeding
CVD	Cardiovascular Disease
CHD	Coronary Heart Disease
ACSM	American College of Sports Medicine
WHO	World Health Organisation
RIA	Radio-Immunoassay
PYY	Peptide YY
ANOVA	Analysis of Variance
AUC	Area-Under-the-Curve

CHAPTER 1: Literature review

1.1 Introduction

1.1.1 Definition, prevalence, and health effects of obesity and overweight

1.1.1.1 Definition

Obesity is a multi-factorial condition characterised by the presence of excess body fat. The terms overweight and obese describe the severity of the condition and, in adults, body mass index (BMI) is the generally accepted method of defining these terms (Grant *et al*, 2007). BMI is a means of expressing body mass independently of height, and is defined as $BMI = \text{body mass (kg)}/\text{height (m}^2\text{)}$. This formula was first devised by Adolphe Quetelet in 1832 and was originally known as the Quetelet index (Eknoyan, 2008), before being termed BMI in 1972 by Ancel Keys (Keys *et al*, 1972). International classification of overweight and obesity according to BMI is shown in table 1.1:

Table 1.1 The International Classification of adult underweight, overweight and obesity according to BMI.Source: World Health Organisation Global Database on Body Mass index (2006).

Classification	BMI(kg/m ²)	
	Principal cut-off points	Additional cut-off points
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	18.50 - 22.99
		23.00 - 24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	≥30.00	≥30.00
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

According to BMI classification, those with a BMI between 25 and 29.9 kg m⁻² are termed overweight and above 30 kg m⁻² are termed obese. There are different levels of obesity, as shown in table 1.1, and BMI > 40 kg m⁻² is often termed as “morbidly obese”.

Whilst BMI provides a quick and simple measure of adiposity which is particularly useful for defining the prevalence of obesity on a population level, there are limitations regarding its use in the individual. The accuracy of BMI for diagnosing moderate obesity has been questioned due to its inability to discriminate between lean mass and fat mass (Romero-Corral *et al*, 2006 & 2008); this can lead to misclassification of an individuals’ adiposity and associated health risks. To minimise this limitation other measures of body fatness are often used alongside BMI. One such measure is waist circumference which acts as a measure of abdominal adiposity and is strongly associated with BMI classification (Lean *et al*, 1995). Based on established cut-off values (>94cm for men and >80cm for women), waist circumference can reliably identify the presence of one or more cardiovascular risk factors in the individual (Han *et al*, 1995). Waist circumference is often expressed relative to hip circumference as the waist-hip ratio (WHR); value >0.95 in males and >0.85 in females are associated with increased health risks (Grant *et al*, 2007).

Waist circumference is a particularly useful measure since abdominal adiposity has been strongly associated with the presence of metabolic syndrome; a condition characterised by abdominal obesity, impaired glucose tolerance/insulin resistance, a pro-inflammatory state, hypertension, and atherogenic dislipidaemia (elevated triglycerides and reduced HDL cholesterol). Metabolic syndrome is associated with increased risk of developing diabetes and/or cardiovascular disease (Carr *et al*, 2004; Després & Lemieux’ 2006); the Framingham Heart Study found that amongst individuals with metabolic syndrome the relative risks of developing diabetes and cardiovascular disease were 3.97 and 3.01 respectively (Meigs *et al*, 2006). Correspondingly, 5-10cm reductions in waist circumference have been associated with improvements in these characteristics of metabolic syndrome; improved blood lipid profile, reductions in total and LDL cholesterol and decreased diastolic blood pressure

(Han *et al*, 1997). Definitions of metabolic syndrome vary between organisations; the World Health Organisation, the International Diabetes Federation, American Society of Clinical Endocrinologist, the National Cholesterol Education Program and the European Group for the Study of Insulin resistance all provide definitions which vary slightly in their clinical cut-off values (Grundy *et al*, 2004).

There is often a need to measure body composition, particularly in obesity management research. There many methods of measuring percentage body fat and all have some degree of associated inherent error due to their indirect nature (table 1.2; Lee & Gallacher, 2008). These methods can provide an informative and alternative method of defining overweight and obesity, although they are generally more expensive and difficult to carry out than waist circumference and BMI measurements, so are not widely utilised in a healthcare setting.

Table 1.2 The advantages and disadvantages of available non-invasive methods for measuring body composition in humans (Adapted from Lee & Gallacher, 2008).

Method	Primary measurements	Advantages	Disadvantages
Bio-electrical impedance (BIA)	TBW, extracellular and intracellular fluid spaces	Inexpensive, portable, simple, safe quick	Population specific, poor accuracy
Dual X-Ray Absorptiometry (DEXA)	Total and regional body fat and lean mass, bone mineral content and density	Easy to use, low radiation exposure, accurate for limb lean and fat mass	Bias: body size, sex and fatness, expensive equipment and specialised technician required to operate
Dilution techniques (doubly labelled water)	TBW and extracellular fluid	Acceptable for use in all age groups, easy to administer	Inaccurate in non-healthy individuals, expensive equipment and labour for analyses
Air Displacement Plethysmography	Total body volume, density and body fat	Relatively high accuracy, quick	Reduced accuracy in non-healthy individuals, expensive equipment
Magnetic Resonance Spectroscopy (MRS/MRI)	Total and regional adipose tissue (visceral, subcutaneous and intermuscular), skeletal muscle, organs (liver, heart, kidney, pancreas and spleen), lipid content in liver and muscle	High accuracy and reproducibility for whole-body and regional adipose tissue and skeletal muscle	Expensive
Underwater weighing (UWW)	Total body volume and density	High accuracy - "gold standard"	Non-portable, costly, labour intensive, time consuming, only suitable for highly mobile individuals, measurement can be affected by residual lung volume

Waist circumference	Abdominal mass	Inexpensive, portable, simple, safe quick, correlates highly with BMI and health risks	Not accurate in those with large lean mass content, does not distinguish regional fat mass, accuracy is reduced in those with BMI > 35 and high gynoid fat mass
Skinfold thicknesses	Subcutaneous fat	Inexpensive, portable, simple, safe quick, accurate when carried out correctly	Can be painful/uncomfortable, operators need to be trained to ensure accuracy, factors other than subcutaneous fat can affect skin thickness, does not measure visceral fat

Body fat percentage varies depending on factors such as age and sex. However there are no established body fat percentage cut-off values defining overweight and obesity, as there are for BMI. General guidelines indicate >25% body fat in men and >32% body fat in women represent obesity (table 1.3); these values have not been validated for clinical classification thus are usually described alongside BMI values.

Table 1.3 General body fat percentage categories (Source: American Council on Exercise).

Description	Women	Men
Essential Fat	10-13%	1-3%
Athletes	14-20%	6-13%
Fitness	21-24%	14-17%
Average	25-31%	18-24%
Obese	32%+	25%+

1.1.1.2 Prevalence of overweight and obesity

Obesity has become a worldwide epidemic (James *et al*, 2001 & 2004), with prevalence rising greatly over the past 30 years (Caballero, 2007). Estimates of the global prevalence of overweight and obesity vary, but all figures are worryingly high; the World Health Organisation (WHO) estimated in 2003 that 1.7

billion adults worldwide were overweight (Deitel, 2003), whilst other publications estimated in 2005 that there were 1.3 billion, or 23.2%, overweight or obese adults worldwide; it has been estimated that if current obesity trends continue at the same rate it is estimated that 3.3 billion adults, or 57.8 % of the world population, will be obese by 2030 (Kelly *et al*, 2008). Though once considered a problem only of wealthy, developed countries obesity is now a significant problem in developing and lower income countries too (Popkin & Doak, 1998; Prentice, 2006).

Obesity is a particular public health problem in the UK. In Scotland the prevalence of overweight and obesity increased from 55.6% to 66.3% in adult males and from 47.2% to 58.4% in adult women between 1995 and 2009. Of those percentages, 26.8% of men and 26.4% of women were classed as obese in 2009; this is a stark rise in comparison to 1995 values when rates of obesity in Scottish adult men and women were recorded as 15.9% and 17.3% respectively. The mean BMI in adult males has risen from 26.0 to 27.4 kg m⁻² and in adult females from 25.7 to 27.2 kg m⁻² during the same 14 year period (Scottish Health Survey 2009). Obesity is clearly an increasingly large problem in Scotland and the statistics for England are no better; in 2009 nearly a quarter of all adults in England were classed as obese; 22% of men and 24% of women. In total 66% of men and 57% of women were either overweight or obese in 2008, whilst only 33% of men and 41% of women had a BMI within the healthy range. Mean BMI was 27.0 kg m⁻² in both sexes (Statistics on obesity, physical activity and diet: England, 2010).

In the USA the situation is similarly grim; prevalence of overweight and obesity combined was 68% overall in the US in 2007-08. As in the UK a greater proportion of men were overweight compared to women; 72.3% versus 64.1%. Of these individuals 33.8% overall were obese with a greater proportion of women than men in this category; 35.5% versus 32.2%. Encouragingly, the rate of increase in the prevalence of overweight and obesity in the US does seem to be slowing, particularly in women (Flegal *et al*, 2010).

1.1.1.3 Health effects of overweight and obesity

The increasing prevalence of overweight and obesity is particularly worrying due to the serious co-morbidities associated with the presence of excess body fat. Hypertension and hyperlipidaemia are more common in overweight individuals than lean, and these conditions are causative factors in the development of serious cardiovascular disease (CVD) such as coronary heart disease (CHD) and congestive heart failure (Van Itallie, 1985). Other conditions associated with overweight and obesity are impaired glucose tolerance and type 2 diabetes mellitus, osteoarthritis, cerebral infarction, and increased risk of some cancers (Van Itallie, 1979; Mokdad *et al*, 2003). Obese individuals have a 1.5-3.5 fold higher risk of developing breast, endometrial, colon, kidney, and oesophageal cancer compared to those with a healthy BMI, and it is estimated that between 15 and 45% of the occurrences of these cancers in Europe may be attributable to overweight and obesity (Pischon *et al*, 2008).

The increasing prevalence of diabetes is particularly worrying due to the many serious complications of the disease; CVD, retinopathy, nephropathy, and neuropathy are all common and serious side effects of diabetes and can lead to sudden death, blindness, kidney failure and amputations respectively (Nathan, 2003). It is estimated that the prevalence of diabetes in the UK will rise by 20% between 2000 and 2036, and accordingly prevalence of diabetes related complications is expected to rise by 20-30% in the same period (Bagust *et al*, 2002).

Obesity has far reaching economic consequences in many countries as it places a huge burden on health services; it was estimated in 2000 that obesity expenditure in the USA was equal to 1.2% of the gross domestic product (GDP) (Yach *et al*, 2006). Estimated costs of obesity in European countries relative to GDP are presented in figure 1.1. In 2007, obesity is estimated to have cost the NHS in England and Scotland £4.2 billion and £312 million respectively, in absolute terms. These estimates do not include indirect costs of obesity such as loss of productivity due to obesity related health problems; in England these

indirect costs have been estimated as being anywhere between £2.6 - £15.8 billion, and total societal costs of obesity in Scotland in 2007/08 have been estimated at between £600 million and £1.4 billion (Morgan & Dent, 2010). The impact of the obesity epidemic is huge and has a profound effect on society as well as the individual.

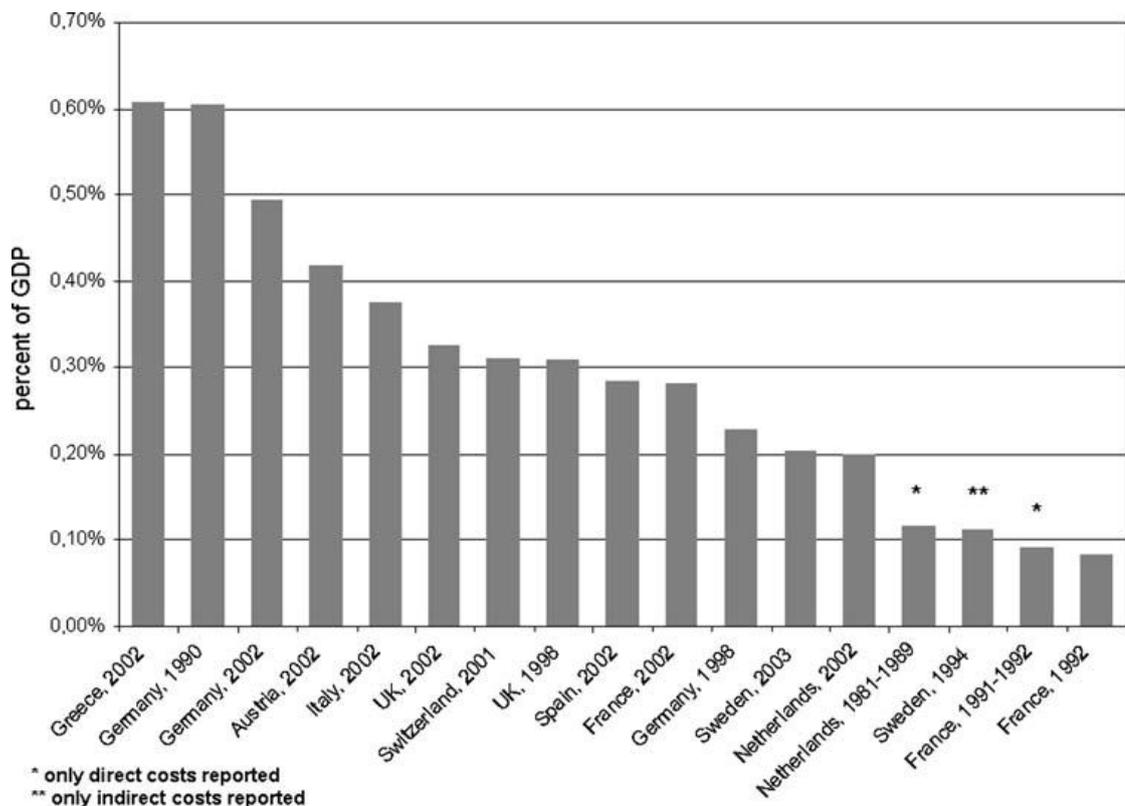


Figure 1.1 Country-specific costs attributable to obesity as a percentage of national gross domestic product (GDP). Source: Müller-Riemenschneider *et al* (2008).

1.1.2 Definitions of exercise and physical activity

Obesity can be prevented and treated by an increase in physical activity/exercise levels (At least five a week: Chief Medical Officers report, 2004). Regular PA participation, resulting in greater energy expenditure (EE), has been shown to lower risk of mortality (Leitzmann *et al*, 2007) from many of the chronic diseases associated with obesity (Warburton *et al*, 2006). The terms physical activity (PA) and exercise have specific definitions; PA encompasses

exercise and is defined as “any bodily movement produced by skeletal muscles which results in energy expenditure”, and can be further categorised into subcategories such as leisure time PA (activities carried out in an individuals free time) and occupational PA (physical activity in the workplace). Exercise is defined as a type of PA which is “planned, structured, and repetitive and has as a final or an intermediate objective; the improvement or maintenance of physical fitness” (Caspersen *et al*, 1985; At least five a week: Chief Medical Officers report, 2004).

Intensity of PA or exercise is often described using the terms light, moderate and vigorous intensity; these terms describes the difficulty and metabolic cost of an activity and this cost will be higher for a given effort in overweight and obese compared to lean (At least five a week: Chief Medical Officers report, 2004). Moderate and vigorous intensity activities have been strongly associated with reduced mortality risk (Lee & Paffenbarger, 2000), thus from a public health perspective it has become very important to accurately define these terms. Intensity is expressed relative to fitness level and this can be expressed in several ways; relative to maximal oxygen consumption ($\dot{V}O_{2max}$), rate of EE, or to individual rating of perceived exertion (RPE). Rate of EE is often measured in MET's (metabolic equivalent), which describes the rate of EE of an activity relative to resting metabolic rate i.e. an individual performing an activity of intensity 2 MET's is utilising energy at twice the rate compared to when they are at rest (At least five a week: Chief Medical Officers report, 2004). Detailed definitions of intensity of exercise relative to fitness are shown in table 1.4. Broadly speaking, light intensity activities are generally those performed at <3 MET's or ~30-45% $\dot{V}O_{2max}$, moderate are 3-6 MET's or ~45-65% $\dot{V}O_{2max}$, and vigorous >6 MET's or ~ >65% $\dot{V}O_{2max}$ (Kesaniemi *et al*, 2001; Haskell *et al*, 2007).

Table 1.4 Classification of physical activity intensity relative to cardiovascular fitness levels (Source: Kesaniemi *et al*, 2001).

ENDURANCE-TYPE ACTIVITY				
Intensity (METs and % $\dot{V}O_{2max}$) in healthy adults differing in $\dot{V}O_{2max}$				
INTENSITY	$\dot{V}O_{2max} = 12$ METs	$\dot{V}O_{2max} = 10$ METs	$\dot{V}O_{2max} = 8$ METs	$\dot{V}O_{2max} = 5$ METs

	METs	$\dot{V}O_{2max}$	METs	$\dot{V}O_{2max}$	METs	$\dot{V}O_{2max}$	METs	$\dot{V}O_{2max}$
Very Light	<3.2	<27	<2.8	<28	<2.4	<30	<1.8	<36
Light	3.2-5.3	27-44	2.8-4.5	28-45	2.4-3.7	30-47	1.8-2.5	36-51
Moderate	5.4-7.5	45-62	4.6-6.3	46-63	3.8-5.1	48-64	2.6-3.3	52-67
Hard	7.6-10.2	63-85	6.4-8.6	64-86	5.2-6.9	65-86	3.4-4.3	68-87
Very Hard	≥ 10.3	≥ 86	≥ 8.7	≥ 87	≥ 7.0	≥ 87	≥ 4.4	≥ 88
Maximal	12	100	10	100	8	100	5	100

1.1.2.1 Definitions of Physical Fitness

Aerobic fitness levels largely dictate individual capacity and tolerance for exercise. Oxygen is necessary for the muscle cells to sustain essential, aerobic, energy-producing metabolic processes during exercise, and the rate at which oxygen can be supplied to the muscle cells processes is a major limiting factor of exercise capacity. Endurance exercise training induces adaptations such an increase in cardiac output, and increasing efficiency of metabolic processes in the muscle cell, which enhance the oxygen supply and energy production rate in the muscle cell during exercise (Jones & Carter, 2000). As a result $\dot{V}O_{2max}$ values, which represent the rate of oxygen consumption in muscle cells, are generally considered the best indicator of cardiovascular endurance and aerobic fitness. $\dot{V}O_{2max}$ values are usually expressed relative to body mass, and age and gender must also be taken into account when interpreting fitness levels. Normative data for $\dot{V}O_{2max}$ is presented relative to age and gender (tables 1.5 & 1.6).

Table 1.5 Normative $\dot{V}O_{2max}$ data for females, expressed relative to age and body mass (values expressed as $ml.kg.min^{-1}$)

Age	Very Poor	Poor	Fair	Good	Excellent	Superior
13-19	<25.0	25.0 - 30.9	31.0 - 34.9	35.0 - 38.9	39.0 - 41.9	>41.9

20-29	<23.6	23.6 - 28.9	29.0 - 32.9	33.0 - 36.9	37.0 - 41.0	>41.0
30-39	<22.8	22.8 - 26.9	27.0 - 31.4	31.5 - 35.6	35.7 - 40.0	>40.0
40-49	<21.0	21.0 - 24.4	24.5 - 28.9	29.0 - 32.8	32.9 - 36.9	>36.9
50-59	<20.2	20.2 - 22.7	22.8 - 26.9	27.0 - 31.4	31.5 - 35.7	>35.7
60+	<17.5	17.5 - 20.1	20.2 - 24.4	24.5 - 30.2	30.3 - 31.4	>31.4

Table 1.6 Normative $\dot{V}O_{2\max}$ data for males, expressed relative to age and body mass (values expressed as ml.kg.min⁻¹)

Age	Very Poor	Poor	Fair	Good	Excellent	Superior
13-19	<35.0	35.0 - 38.3	38.4 - 45.1	45.2 - 50.9	51.0 - 55.9	>55.9
20-29	<33.0	33.0 - 36.4	36.5 - 42.4	42.5 - 46.4	46.5 - 52.4	>52.4
30-39	<31.5	31.5 - 35.4	35.5 - 40.9	41.0 - 44.9	45.0 - 49.4	>49.4
40-49	<30.2	30.2 - 33.5	33.6 - 38.9	39.0 - 43.7	43.8 - 48.0	>48.0
50-59	<26.1	26.1 - 30.9	31.0 - 35.7	35.8 - 40.9	41.0 - 45.3	>45.3
60+	<20.5	20.5 - 26.0	26.1 - 32.2	32.3 - 36.4	36.5 - 44.2	>44.2

1.1.2.2 Current physical activity/exercise recommendations for the prevention and management of obesity and overweight

Current population PA recommendations issued in the US and UK are aimed at prevention of chronic diseases such as heart disease and diabetes. For adults, 30 minutes of moderate activity five times per week, or 20 minutes vigorous intensity three times per week is recommended. These targets can be completed either in a single session or through several short bouts, each of at least 10 minutes duration, over the course of a day. For children the current recommendation is 60 minutes moderate PA every day (At least five a week: Chief Medical Officer's report, 2004; Haskell *et al*, 2007). Exercise targets for achieving a body mass reduction, or preventing body mass gain, are higher than those aimed at inducing health benefits. Current UK recommendations state that 45-60 minutes of moderate intensity activity every day may be needed to prevent body mass gain (At least five a week: Chief Medical Officers report, 2004). Current evidence based guidelines from the American College of Sports Medicine suggest that 150-250 minutes moderate intensity exercise per week can prevent body mass gain and hence lower risk of diseases related to excess body fat; this amount of activity may also be useful for inducing moderate body fat

reduction when employed in conjunction with energy restriction. If attempting to induce significant body mass reduction solely through exercise, or for long term maintenance of such losses, recommendations are much higher; it is thought at least 250 minutes (or ≥ 8368 kJ ExEE per week) of moderate exercise per week are required to achieve these goals (Jakicic *et al*, 2001; Donnelly *et al*, 2009). In fact it has been suggested that an even higher weekly ExEE of 10460 kJ may be most effective for promoting a significant body mass reduction in the long term (Jeffery *et al*, 2003). Thus these guidelines dictate that individuals seeking to reduce body mass should aim to complete significant amounts of PA, equivalent to an EE of at least 8368 kJ per week; this is the minimal recommendation for body mass reduction and participation in higher amounts of PA will further increase ExEE and improve outcomes.

1.2 Efficacy of Exercise as a Strategy to Reduce Body Mass

There has been increasing scientific interest in the effectiveness of methods of reducing body and/or fat mass in the last two decades. The majority of studies in this area focus on the effectiveness of various dietary interventions in conjunction with different forms of exercise and/or physical activity. Exercise alone has proven to be a relatively unsuccessful method of achieving a significant body mass reduction compared to other methods. A review of interventions found that regular exercise participation was associated with maintenance of intentional body mass loss, but exercise on its own produced the smallest body mass changes compared to dietary restriction interventions (Wing *et al*, 1999). This finding has been echoed in several meta-analyses (Garrow & Summerbell, 1995; Miller *et al*, 1997; Franz *et al*, 2007) which have all reported that exercise as the sole means of inducing body mass reduction is relatively unsuccessful compared to dietary interventions and pharmaceutical methods (figure 1.2).

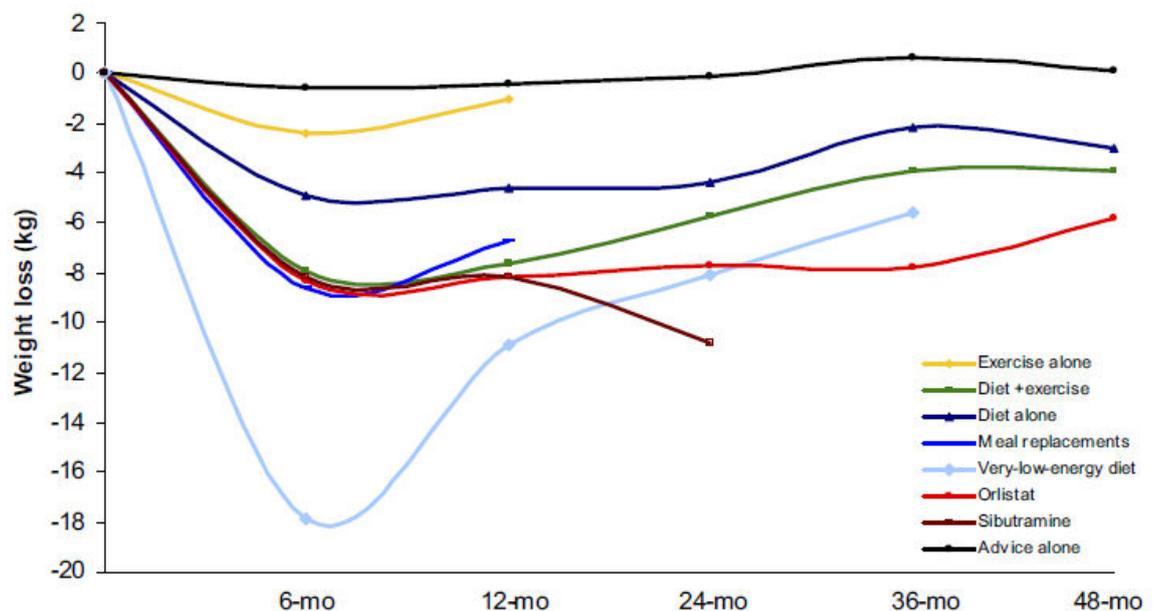


Figure 1.2 Average body mass reductions of participants completing a minimum one year intervention. Based on 80 studies, n = 18,199 completers (Franz *et al*, 2007).

Study Selection

The primary outcome of interest in this review was body composition changes as a result of medium and long term exercise interventions. Inclusion criteria for studies were:

- Published between 1970 and 2011.
- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Exercise and physical activity interventions resulting in increased energy expenditure (studies including a dietary intervention were included providing all groups were subject to the same intervention allowing exercise induced effects to be elucidated). Additional criteria for some sub-sections of this review were:
 - Section 1.2.1 - studies including at least two exercise intervention groups completing exercise of differing intensities (summarised in table 1.7).
 - Section 1.2.2 - studies including at least two exercise intervention groups completing different durations of exercise (summarised in table 1.7).
 - Section 1.2.3 - studies including at least two exercise intervention groups completing interventions comprised of either continuous or intermittent exercise bouts (summarised in table 1.8).
- Must include body mass and/or body fat measurements.
- Interventions >1 week in duration*.
- Body mass and/or fat mass changes as a primary outcome of interest.

*Please note there are no strict definitions of the duration of intervention classed as medium or long term in the literature, therefore for the purpose of this discussion interventions ranging from 1 week to 4 months in duration are referred to as “medium term”, whilst any exceeding 4 months in length are referred to as “long term”.

1.2.1 Effects of Exercise Intensity

Achievement of body mass reduction is dependent on inducing a state of chronic negative energy balance. Exercise is one of the means by which such an energy deficit can be induced. Higher intensity exercise produces greater energy deficit in a shorter time than lower intensity, and may acutely increase post-exercise lipid oxidation rate (Yoshioka *et al*, 2001). Increasing intensity of exercise may thus be expected to produce the greatest body mass reductions. Indeed two large cross sectional studies, largely conducted with lean men and women, have reported that those who engaged in the highest amounts of vigorous activity had the lowest subcutaneous adiposity of the sample. This trend remained even after correcting for differences in EE (Tremblay *et al*, 1990; Yoshioka *et al*, 2001).

Experimental evidence does not always illustrate a direct relationship between exercise intensity and body mass reduction. With identical ExEE, vigorous and moderate intensity exercise interventions often produce near identical reductions in fat mass in obese women over the medium term, whether in conjunction with energy restriction or as a sole means of inducing negative energy balance (Gregadian *et al*, 1995; Nicklas *et al*, 2009). Indeed, total body mass may appear significantly smaller in vigorous activity interventions due to increases in lean mass (Gregadian *et al*, 1995). High intensity exercise also confers significant cardiovascular fitness benefits that are not observed in response to lower intensity exercise (Nicklas *et al*, 2009).

Lower intensity exercise interventions may favour greater compliance in obese, sedentary individuals; the study of Nicklas *et al* (2009) illustrated that moderate intensity exercise was better tolerated than vigorous, with group attrition rates of 11.1% and 26.7% respectively. Although lower intensity activity may not confer the same fitness benefits as that of a higher intensity, compliance is an important issue when working with the sedentary, overweight and obese to promote a more active lifestyle, particularly for health benefits. As such it is

important to balance the benefits of an exercise programme against the likely compliance and long term participation in at-risk individuals. Additionally, low intensity exercise may also have beneficial metabolic effects in the obese that promoted fat loss. Obese adolescents combining low intensity exercise with energy restriction experienced more than double the fat losses than those completing high intensity exercise due to an increased fat oxidation rate. EI was restricted in both groups, but macronutrient intake did not differ therefore this effect was not attributable to dietary influences (Lazzer *et al*, 2011). Participants were resident in a specialised nursing facility during the intervention and all meals were supplied hence these results are likely to be highly accurate. Unfortunately this study was only 3 weeks in duration so it is unclear if this effect would persist; long term interventions with adult women do not observe similar changes in substrate oxidation (Kanaley *et al*, 2001; Potteiger *et al*, 2008), so it is possible this effect is short lived or specific to this age group.

Despite obvious benefits of low intensity exercise in the overweight and obese, there are undeniable benefits associated with vigorous activity participation. It seems that the greatest body fat reductions are seen at higher intensities, and this may confer greater health benefits to clinical populations. Obese women diagnosed with metabolic syndrome have been observed to experience significant fat losses when engaging in physical activity, and these losses were greatest in individuals completing high intensity exercise (Irving *et al*, 2008). Although these changes were modest, they may still be sufficient to induce significant health benefits in this clinical population. Vigorous exercise may also be effective for reducing abdominal adiposity, a known risk factor for metabolic syndrome, when total percentage body fat remains unchanged. This benefit has been observed in a small sample of overweight but otherwise healthy elderly adults participating in a 12 week exercise intervention (Coker *et al*, 2009), and may result in a lower risk of chronic diseases such as diabetes.

Low participant numbers, and lack of long term data somewhat limits this field of literature, often resulting from high attrition rates common to exercise intervention studies (table 1.7). Different methods of classifying exercise

intensity are also utilised between studies; intensity is often expressed relative to lactate threshold, % $\dot{V}O_{2max}$, or HR_{max} thus there may be variation in the intensity of activity between studies. Although some interventions reviewed here combine exercise with energy restriction, identical energy intake (EI) restriction between groups means that the effects of exercise can still be elucidated. However, it can be difficult to be assured of compliance to dietary interventions which may affect results. Many interventions are able to monitor compliance to exercise interventions closely by supervising all sessions, but without accurate dietary information it is not possible to accurately assess energy balance.

The evidence regarding vigorous intensity exercise and fat loss is mixed, and although such activity may not confer the greatest body fat reduction, associated health benefits include a reduction in abdominal adiposity, preservation of lean mass, an increase in cardiovascular fitness and elevated fat oxidation rate are compelling reasons to recommend participation in vigorous exercise. However, compliance to these interventions can be poor, particularly in sedentary, obese individuals. Difficulty of vigorous exercise may result in completion of a lower volume of exercise, and experimental studies manipulating intensity but not ExEE illustrate that vigorous exercise may not produce greater body mass reductions for this reason. Interventions should be designed to balance these issues in order to achieve maximum participant compliance and health benefits in sedentary, obese populations.

1.2.2 Effect of Exercise Duration

As well as intensity, exercise duration, or volume, should be considered. In the previously discussed studies, intensity is varied whilst ExEE is held constant between groups. Thus durations of exercise in these studies differed and it is possible that this may influenced observed body composition changes. Indeed a dose-response relationship between exercise volume and body fat reduction has been observed and well-documented in healthy individuals (Ross & Janssen, 2001; Ohkawara *et al*, 2007) in the medium term. Therefore it is important to

examine if increasing exercise duration is advantageous in terms of body composition changes.

Many intervention studies have illustrated the dose-response relationship in overweight men and women (table 1.7), even with moderate physical activity interventions. Behavioural intervention may increase the success of such interventions, and may also lead to adoption of other healthy behaviours which increase fat loss, such as restriction of dietary fat intake (Jakicic *et al*, 2011). However, regain of body mass is often an issue in the long term. Behavioural physical activity interventions have been observed to induce large reductions in body mass in the overweight, but follow up measures often show that losses are not maintained in the long term. Body mass regain may be lowest in those completing the highest levels of PA (Jeffery *et al*, 2003). However, the self reported nature of PA levels in some behavioural lifestyle interventions may lead to inaccuracies in activity data, and it is possible that concurrent changes or misreporting in dietary intake may contribute. Large sample size and long duration of these interventions presents a significant study design advantage that strengthens these findings however.

The dose-response relationship has also been observed in clinical populations; overweight patients with dislipidaemia exhibited greatest body fat reduction in response to high volumes of activity, particularly when that activity was vigorous (Slentz *et al*, 2004). Comparison of two groups completing vigorous activity in different volumes illustrated that the dose-response relationship was attributable to exercise duration and not intensity, although increasing either would lead to greater ExEE and presumably greater body fat changes.

Vigorous activity may at times be counterproductive; there is evidence that high levels of exercise may be associated with an increase in EI or decrease in non exercise EE in overweight women. High volumes of exercise, inducing a similarly high exercise energy expenditure (ExEE), may in fact induce smaller body mass reduction than those completing smaller volume of exercise because of such compensatory changes (Church *et al*, 2009). Measuring changes in EE and EI is difficult due to reliance on accurate self-reporting in many interventions. As a

result it is not uncommon to observe smaller than expected body fat reductions in response to an intervention with no apparent explanation. However, a failure to detect compensatory responses does not necessarily mean that they did not occur. It is interesting that no apparent compensation has been observed in response to physical activity interventions, and this indicates that compensation may occur only at very high ExEE and that these responses may be subject to a threshold. The evidence surrounding the existence of compensatory responses will be addressed in more detail in section 1.3

It is encouraging that data regarding the dose-response relationship comes from long term studies, commonly of around 18 months duration, with hundreds of participants. Such study design strengths lend considerable weight to these findings. Attrition is often high in intervention studies, and achievement of such a large sample size is particularly impressive because of this. It seems clear that a dose-response relationship applies to exercise and body fat reduction in overweight and obese, although participants may need to be wary of compensatory behaviours at higher levels of exercise.

Table 1.7 Evidence regarding the effects of exercise intensity and duration on chronic exercise-induced body composition changes.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Church <i>et al</i> (2009)	464 obese, post-menopausal women	n=411	57.2 ± 6.4 (mean ± SD)	$\dot{V}O_{2peak}$ 15.6 ± 2.8 (mean ± SD)	31.7 ± 3.8 (mean ± SD)	6 months	Participants randomised to one of 4 groups: 1. Control - no exercise 2. Low volume - exercise to expend 16.7 kJ/kg/week (72 mins) 3. Moderate - exercise to expend 33.4 kJ/kg/week (136 mins) 4. High - exercise to expend 50.2 kJ/kg/week (194 mins)	Not reported	Control: -0.9 Low: -1.4 ± 0.5 Moderate: -2.1 ± 0.7 High: -1.5 ± 0.7 (mean ± SD)	% body fat: Control: 1.0 (-0.1, 2.1) Low: -0.7 (-1.6, 0.2) Moderate: 0.5 (-1.7, 0.6) High: -0.1 (-1.2, 1.0) (mean (95% CI))	11.4%
Coker <i>et al</i> (2009)	18 overweight, elderly men (n=9) and women (n=9)	n=18	High intensity: 73 ± 2 Moderate intensity: 70 ± 1 Control: 67 ± 3 (mean ± SEM)	High intensity: 1.4 ± 0.1 Moderate intensity: 1.3 ± 0.1 Control: 1.5 ± 0.1 (L/kg/min) (mean ± SEM)	High intensity: 30 ± 1 Moderate intensity: 28 ± 1 Control: 31 ± 1 (mean ± SEM)	12 weeks	Participants randomised to one of three groups: 1. High intensity - 75% $\dot{V}O_{2peak}$ (n=6) 2. Moderate intensity - 50% $\dot{V}O_{2peak}$ (n=6) 3. Control - no exercise (n=6) Exercise groups expended 4180 kJ/week.	High intensity: 30 ± 1 Moderate intensity: 28 ± 1 Control: 31 ± 1 (mean ± SEM)	Not reported	Not reported. Reduction in abdominal visceral fat of -39cm ² in high intensity group only.	Not reported
Grediagin <i>et al</i> (1995)	18 women	n=12	31 ± 6 (mean ± SD)	31.5 ± 3.8 (mean ± SD)	Low intensity: 26.2 ± 1.4 High intensity: 23.8 ± 2.3 (mean ± SD)	12 weeks	4 exercise sessions per week to expend 1254 kJ/session at either a high (80% $\dot{V}O_{2max}$; n=6) or low intensity (50% $\dot{V}O_{2max}$; n=6).	Not reported	Low intensity: -1.5 ± 1.2 High intensity -0.3 ± 1.2 (mean ± SD)	Both groups -2.3 ± 2.6 (mean ± SD)	33%
Irving <i>et al</i> (2008)	37 obese women with metabolic	n=27	51 ± 9 years (mean ± SD)	Control: 21.6 ± 4.1	Control : 32.7 ± 3.8	16 weeks	Participants randomised to one of three groups: 1. Control - no	Control : 32.4 ± 3.8	Change not reported.	Change not reported.	24%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
	syndrome			LIET: 21.0 ± 3.5 HIET: 21.7 ± 4.1 (mean ± SD)	LIET: 34.7 ± 7.5 HIET: 34.7 ± 6.8 (mean ± SD)		exercise (n=7) 2. LIET - 5 exercise sessions/week below LT (n=11) 3. HIE - 3 exercise sessions/week above LT (n=9) Progressive intervention till a maximum ExEE of 1672 kJ/session reached in week 5.	LIET: 33.9 ± 6.5 HIET: 33.4 ± 5.6 (mean ± SD)	<u>Baseline</u> Con: 89.6 ± 11.2 LIET: 97.2 ± 22.0, HIET: 93.5 ± 18.3 <u>Post-intervention</u> Con: 88.7 ± 10.6 LIET: 95.1 ± 19.3 HIET: 90.0 ± 15.6 (mean ± SD)	<u>Baseline</u> Con: 40.4 ± 6.2 LIET: 43.1 ± 11.5 HIET: 41.0 ± 70.2 <u>Post-intervention</u> Con: 40.1 ± 6.3 LIET: 41.8 ± 11.2 HIET: 38.2 ± 10.7 (mean ± SD)	
Jakicic <i>et al</i> (2011)	269 overweight adults	n=196	Control: 44.7 ± 7.9 Moderate PA: 43.5 ± 8.8 High PA: 45.0 ± 8.4 (mean ± SD)	Not reported	Control: 27.1 ± 1.7 Moderate PA: 27.2 ± 1.8 High PA: 27.0 ± 1.6 (mean ± SD)	18 months	Participants randomised to one of 3 groups: 1. Control - self help intervention only (booklet and weekly newsletter; n=69) 2. Moderate PA - 150 mins PA/day at 55-85% HR _{max} (n=55) 3. High PA - 300 mins PA/day at 55-85% HR _{max} (n=72)	Control: 26.9 ± 2.1 Moderate PA: 26.9 ± 2.0 High PA: 26.7 ± 2.4 (mean ± SD)	Controls: -0.7 ± 4.6 % Moderate PA: -0.9 ± 4.7 % High PA: -1.2 ± 5.6 % (mean ± SD)	% body fat reduced from 33.4 ± 4.2% to 32.9 ± 5.2% with no significant differences between groups.	27.1%
Jeffery <i>et al</i> (2003)	202 overweight men and women	n=168 (58% women)	42.2 ± 6.4 (mean ± SD)	Not reported	31.7 ± 2.6 (mean ± SD)	18 months	Participants randomised to one of 2 groups: 1. SBT - behavioural therapy encouraging participants to expend 4180 kJ/week in PA 2. HPA - personal coaching and incentive to	Not reported	SBT: -4.1 ± 8.3 HPA: -6.7 ± 8.1 (mean ± SD)	Not reported	16.8%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ¹ /min ¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							increase EE by 10,450 kJ/week				
Lazzer <i>et al</i> (2011)	24 obese adolescent boys	n=20	Low intensity: 16.3 ± 1.1 High Intensity: 16.1 ± 1.0 (mean ± SD)	Low intensity: 0.0052 ± 0.005 High intensity: 0.0048 ± 0.004 (L/kg FFM/min) (mean ± SD)	Low intensity: 36.5 ± 3.6 High intensity: 38.7 ± 3.7 (mean ± SD)	3 weeks	Participants randomised to one of two groups, both of which restricted EI to 1.2xBMR, and completed exercise to expend 2.8 MJ/day on 5 days of the week: 1. Low intensity - 40% $\dot{V}O_{2max}$ 2. High intensity - 70% $\dot{V}O_{2max}$	Low intensity: 33.7 ± 3.3 High intensity: 36.7 ± 3.7 (mean ± SD)	Low intensity: -8.1 ± 1.6 High intensity: -5.9 ± 1.6 (mean ± SD)	Low intensity: -4.2 ± 1.9 High intensity: -2.3 ± 1.7 (mean ± SD)	16.7%
Nicklas <i>et al</i> (2009)	112 post-menopausal, overweight and obese women	n=95	CR: 58.4 ± 6.0 CR+M: 57.7 ± 5.5 CR+V: 59.0 ± 5.0 (mean ± SD)	CR: 20.6 ± 2.9 CR+M: 21.5 ± 3.2 CR+V: 20.3 ± 3.7 (mean ± SD)	CR: 33.9 ± 4.0 CR+M: 33.7 ± 3.5 CR+V: 32.9 ± 3.7 (mean ± SD)	20 weeks	Three intervention groups, all of which reduced EI by 1672 kJ/day. 1. CR - energy restriction only (n=29). 2. CR+M - EI restriction and moderate intensity exercise (45-50% HRR; n=36). 3. CR+V - EI restriction and moderate intensity exercise (70-75% HRR; n=30). Exercise groups expended 2926 kJ/week.	Not reported	CR: -11.8 ± 4.1 CR+M: -12.2 ± 4.5 CR+V: 12.3 ± 4.9 (mean ± SD)	CR: -7.4 ± 2.8 CR+M: -8.2 ± 3.2 CR+V: -8.5 ± 3.8 (mean ± SD)	17.2% in the on exercising group, 11.1% in the moderate intensity group, 26.7% in the vigorous intensity group
Stentz <i>et al</i> (2004)	182 overweight and obese men and women	n=120 (n=55 females, n=65 males)	52.8 ± 6.4 (mean ± SD)	Not reported	29.7 ± 3.2 (mean ± SD)	8 months	Participants randomised to one of 4 groups: 1. High amount/vigorous intensity (HA/VI) - caloric equivalent to jogging 20 miles at 65-80% $\dot{V}O_{2peak}$,	Not reported	CON: +1.0 ± 2.1 LA/MI: -0.9 ± 1.8 LA/VI: -0.6 ± 2.0	CON: 0.4 ± 3.0 LA/MI: -2.0 ± 2.6 LA/VI: -2.5 ± 3.4	34%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ¹ /min ¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							2. low amount/vigorous intensity exercise (LA/VI) - caloric equivalent to jogging 12 miles at 65-80% $\dot{V}O_{2peak}$, 3. low amount/moderate intensity exercise (LA/MI) - caloric equivalent to jogging 12 miles at 40-55% $\dot{V}O_{2peak}$ 4. Control - No exercise		HA/VI: -2.9 ± 2.8 (mean ± SD)	HA/VI: -4.8 ± 3.0 (mean ± SD)	

1.2.3 Efficacy of Continuous and Intermittent Exercise

Current public health guidelines in the UK recommend participation in 30 minutes moderate activity a day in order to accrue health benefits.

Recommendations state that these 30 minutes may be accumulated in multiple smaller bouts of no less than 10 minutes duration. Additionally, compliance to exercise interventions may be improved when exercise is prescribed in shorter bouts. As a result the relative effectiveness of continuous and intermittent exercise interventions has been examined in the literature (table 1.8).

Body mass reduction seems not to vary between continuous and intermittent exercise, when duration of exercise is kept equal (Schmidt *et al*, 2001). In the long term continuous exercise may be most effective for maintenance of body mass losses. Similar body fat mass reduction has been observed after 9 months in obese women completing either continuous or intermittent exercise, but after 18 months only the continuous exercisers had progressively continued to reduce body fat whilst intermittent exercisers regained to baseline values (Jakicic *et al*, 1999; Donnelly *et al*, 2000). Exercise volume in this intervention was low and reductions in body mass unlikely to have clinical significance, but maintenance of a lower body mass is nonetheless encouraging. Optimum fat loss occurred after about 6 months, and provision of exercise equipment, rather than duration and frequency of exercise bouts, may have been a key factor leading to greater compliance and fat loss maintenance over the course of this intervention (Jakicic *et al*, 1999).

There may be an element of individual variation related to the efficacy of intermittent exercise as a body mass reduction method. Whilst some obese women participating in a 32 week intermittent, brisk walking intervention have successfully reduced body mass, others have actually experienced an increase (Snyder *et al*, 1997). No changes in EI or differences in adherence were apparent to explain these differences, but it is possible that these self reported measures were not accurate. More recent interventions have also documented great

individual variability in response to a long term, continuous exercise intervention (King *et al*, 2008). It is possible that compensatory responses play a role, and that susceptibility to these responses varies on an individual basis for some unknown reason.

Higher compliance to moderate, intermittent exercise may also result in achievement of greater ExEE and greater body mass reduction. As a result attrition rates may be relatively low for such long term exercise interventions (Jakicic *et al*, 1995). Provision of exercise equipment at home may also be an aid to increase compliance, as Jakicic and colleagues supplied participants with treadmills at home to exercise and experienced a relatively low attrition rate for an intervention of this nature (7.4%). This may be because Intermittent, home-based interventions are more convenient and achievable for sedentary, overweight individuals, who may feel self conscious exercising in company. Body mass regain in the long term has been observed to be minimised with short bout exercise programmes that can be completed at home, and this could also be attributable to greater long term compliance (Jakicic *et al*, 1999). The dose response relationship also appears to apply to intermittent exercise interventions, and as such total volume of exercise seems an important factor contributing to body mass reduction, regardless of duration of individual sessions.

It seems that there may be a dose-response relationship in the medium term between exercise duration and body mass reduction for most individuals, regardless of whether exercise is continuous or intermittent. This is encouraging as compliance and adherence is often greater in such interventions. However, there may be individual variation in the efficacy of intermittent exercise between individuals for as yet unidentified reasons. Evidence suggests that regardless of frequency and duration of exercise bouts, improving compliance and adherence to exercise in the long term are critical to achieving and maintaining exercise induced body fat reduction.

Table 1.8 Evidence regarding the effects of continuous and intermittent exercise on chronic body composition changes in overweight and obese men and women.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ¹ /min ¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Donnelly <i>et al</i> (2000)	22 obese females	n=22	Con: 54 ± 9 Int: 49 ± 8 (mean ± SD)	Not reported	Con: 30.1 ± 2.5 Int: 32.3 ± 5.1 (mean ± SD)	9 months	Three 30 minute exercise sessions/week, completed in either in a single bout (Con; n=11), or in two 15 min sessions (Int; n=11).	Con: 29.6 ± 2.3 Int: 31.8 ± 5.1 (mean ± SD)	Change not reported. Baseline Con: 34.0 ± 3.7 INT: 36.7 ± 7.0 9 months Con: 32.7 ± 4.0 Int: 35.0 ± 6.7 (mean ± SD)	Change not reported.	Not reported
Jakicic <i>et al</i> (1995)	56 overweight women	n=52	LB: 40.9 ± 7.3 SB: 40.4 ± 5.9 (mean ± SD)	Not reported	LB: 33.8 ± 4.7 SB: 34.1 ± 3.5 (mean ± SD)	20 weeks	Participants exercised for 40 minutes on 5 days of the week, either in one uninterrupted bout (LB) or in 4x10 minute sessions (SB).	LB: 31.3 ± 5.0 SB: 30.9 ± 3.8 (mean ± SD)	LB: -6.4 SB: -8.9 (range not reported) (mean ± SD)	Not reported	7.4%
Jakicic <i>et al</i> (1999)	148 overweight women	n=115	37.1 ± 5.4 (mean ± SD)	Not reported	32.4 ± 3.8 (mean ± SD)	18 months	Participants randomised to one of three groups: SB: 4x10 min bouts 5 days/week SQEQ: as above with treadmill provided LB: 1x40 min bout 5 days/week	SB: 31.9 ± 4.6 SBEQ: 29.5 ± 5.1 LB: 30.8 ± 5.1 (mean ± SD)	SB: -7.5 ± 5.4 SBEQ: -9.3 ± 5.6 LB: -8.2 ± 5.5 (mean ± SD)	Change not reported.	22%
Schmidt <i>et al</i> (2001)	48 overweight, sedentary women	n=38	Control: 20.8 ± 1.6 1x30: 20.7 ± 2.5 2x15: 18.3 ± 0.5 3x10: 19 ± 0.9 (mean ± SD)	Control: 1.83 ± 0.1 1x30: 1.72 ± 0.04 2x15: 1.75 ± 0.04 3x10: 1.96 ± 0.1 (L/min)	Control: 31.4 ± 2.5 1x30: 31.2 ± 3.8 2x15: 30.4 ± 3.3 3x10: 32.6 ± 3.9 (mean ± SD)	12 weeks	Participants randomised to one of 4 groups. All groups restricted EI to 80% of resting EE and three groups also exercised for 30 mins on 5 days/week at 75% HRR: 1. Control - No exercise 2. 1x30 - exercise in one bout 3. 2x15 - two 15 min	Only change in BMI reported: Control: 0.0 ± 0.9 1x30 minutes group: -1.1 ± 0.6 2x15 minutes group: -1.1 ±	Control: 0.06 ± 0.2 1x30 minutes group: -2.7 ± 1.1 2x15 minutes group: -3.0 ± 1.3 3x10 minutes	Not reported	20.8%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ ($ml/kg^{-1}/min^{-1}$)	Mean baseline BMI (kg/m^2)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m^2)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
				(mean \pm SD)			4. exercise bouts 3x10 - three 10 min exercise bouts	0.4 3x10 minutes group: -1.6 \pm 0.9 (mean \pm SD)	group: -4.4 \pm 2.3 (mean \pm SD)		
Snyder <i>et al</i> (1997)	15 obese females	n=13	43 \pm 11 (mean \pm SD)	24.0 \pm 4.6 (mean \pm SD)	32.5 \pm 8.0 (mean \pm SD)	32 weeks	Three ten minute bouts of risk walking exercise on 5 days/week	32.4 \pm 7.8 (mean \pm SD)	-0.1 \pm 1.9 (mean \pm SD)	+0.5 \pm 1.9 (mean \pm SD)	13.3%

1.2.4 Effect of Length of Intervention

Medium and long term interventions are most informative as they provide valuable information about the long term efficacy of exercise as a method of controlling or reducing body mass. Many medium term interventions produce a statistically significant, but often not clinically meaningful, body mass reduction, and a dose-response relationship between exercise and body mass change is well documented (Ross & Janssen, 2001; Ohkawara *et al*, 2007). However, over a period of years the dose-response relationship between exercise volume and body mass reduction does not appear to exist as strongly (Miller *et al*, 1997). Body mass regain over the long term is an issue common to most diet and exercise interventions, possibly because compliance diminishes over time. Data from meta-analyses have highlighted the relatively short duration of exercise interventions in the published literature as a general limitation in this field; most exercise interventions with overweight and obese range from 13-21 weeks duration, and tend to focus heavily on middle-aged, moderately obese participants (Miller *et al*, 1997). Indeed figure 1.2, taken from the meta-analysis of Franz *et al* (2007), illustrates the relatively short duration of available exercise intervention compared to other methods. Exercise and dietary interventions are typically very labour intensive with high attrition rates, often in excess of 20%, and it is probable that these factors are the major contributors to the relative lack of long term data in this field. As a result of these factors the efficacy of both medium and long term interventions has been considered.

1.2.4.1 Medium Term Interventions

Many high volume, medium term exercise interventions with overweight and obese males have produced relatively large body mass changes (table 1.9). Large volumes of aerobic exercise, completed for 3-4 months, have produced apparently large reductions in body mass (Leon *et al*, 1979; Bouchard *et al*, 1990). However, comparison of predicted (based on known ExEE) to actual changes revealed actual losses to be only 50% of predicted in some cases,

despite self reported EI data indicating a reduction in EI occurring during the intervention (Leon *et al*, 1979). Well controlled interventions have met with greater success; overweight men resident in an experimental facility for 100 days completing a rigorously controlled intervention with direct monitoring of EI achieved almost 100% of predicted losses, based on ExEE (Bouchard *et al*, 1990). Sample size was small at 5 participants, most probably because the nature of the study made a larger sample size unfeasible. However, the robust design and methodology of this study strengthen these findings considerably. Discrepancies in results between these studies are most likely attributable to differences in the study design; the former being free-living and the latter residential, with strict control over participants' dietary intake. It is interesting that attrition rate for the free-living study was high at 40%, whilst 100% of participants completed the residential intervention. Motivations may have varied between participants since participants in the free-living study of Leon *et al* offered a one hundred dollar bonus to completers.

Long term resistance exercise may induce similar body fat reductions to aerobic exercise, despite the typically lower ExEE of this form of exercise (Broeder *et al*, 1992a). With no measurable differences in metabolic rate this may be attributable to unobserved changes in dietary intake occurring during the intervention. Lean mass preservation or gain may be another key benefit of both resistance and aerobic exercise (Broeder *et al* 1992a; Ross *et al*, 2004). Evidence shows that aerobic exercise may not always serve to preserve lean mass; overweight and obese men completing high volumes of aerobic exercise (ExEE of 2926 kJ/day) have been observed to experience a loss of lean mass (Ross *et al*, 2000). This evidence indicates that resistance exercise interventions may prove similarly beneficial to aerobic exercise for some, and may be of value for reducing body fat in overweight and obese. It is possible that overweight and obese may also find resistance exercise programmes easier to adhere to as it may be perceived as less strenuous exercise for individuals with low cardiovascular fitness.

Some medium term studies have observed little or no change in body fat mass in groups of overweight and obese women. At times these small losses are

expected due to low volume of exercise and low ExEE, though self reported EI data indicate a small reduction in EI which is not reflected in body composition changes (Barwell *et al*, 2009). It is often difficult to definitively prove the reasons for such modest body fat changes in response to medium term exercise due to misleading EI data such as this. Additionally, other interventions with low ExEE have found that changes in non exercise activity may also contribute to failure to reduce body mass (Manthou *et al*, 2010). Despite completing a low volume of exercise, some women seem to alter their lifestyle activity in a compensatory manner, preventing body mass reduction. It is worth noting that body fat mass in the latter study was assessed by bio-impedance, and this method may not have been sensitive enough to accurately detect the small magnitude of changes in fat mass which was expected in this study. These studies also focused exclusively on women. Interventions including overweight men and women have observed fat losses close to predicted values during a 3 month intervention (King *et al*, 2008; Martins *et al*, 2010a); it may be that exercise is more effective for overweight men. Discrepancies between studies may be because mean values mask a large degree of individual variability in body fat mass change in some cases (King *et al*, 2008). Explanatory individual changes in EI add to the growing evidence that compensatory mechanisms may play a large role in exercise interventions, and the role of such mechanisms will be discussed in full in chapter 1.3.

There are notable limitations in this field of evidence; firstly, many medium term intervention studies with overweight and obese participants have relatively small sample sizes. Additionally, studies often do not accurately quantify ExEE making it difficult to gauge the relative success of the intervention. Individual variability and gender differences may contribute, which can make it difficult to compare participants. Finally there is considerable variability in intervention duration, exercise protocol, and methodology and it is at times difficult to draw meaningful direct comparisons between studies. However, the evidence that does exist indicates that medium term interventions lasting several months do appear to have a modest role in reducing fat mass, and may serve to preserve lean mass. Emerging evidence indicates that responses to such interventions are highly individual, and this may contribute in large part to the mixed results reported in the medium term literature.

1.2.4.2 Long Term Interventions

Many long term exercise interventions, often observing participants for a year or more, observe rather disappointing body composition changes (table 1.10). Often ExEE is not quantified making it difficult to gauge the true success of such interventions. Morbidly obese men and women have been observed to reduce fat mass by only 3kg over a 6 month period (Bjorntorp *et al*, 1973). This seems a somewhat disappointing change but no measurements of ExEE were made hence it is not possible to define expected body fat changes. Exercise intensity was by necessity low for these participants but greater losses may have been expected in these participants. Indeed a body mass reduction equivalent to only 40% of expected values was observed in fifteen overweight men completing a similar exercise protocol over 6 months (Turner *et al*, 2010). In comparison overweight women participating in a moderate, aerobic exercise protocol have been observed to lose as little as 0.5kg body mass over 3 months, and a 1.4kg loss after 12 months. Unfortunately studies only reporting body mass changes do not inform us as to the degree of change in adiposity, and it may be that exercise-induced increases in lean mass present misleading results (Foster-Schubert *et al*, 2005). However, these findings are echoed with obese women completing a similar intervention to that of Bjorntorp *et al* (1973), with a higher volume of exercise. A non-significant reduction in body fat mass was observed after 3 months of training, and this small loss was regained after 6 months of training. Participants in this study had varying degrees of obesity, and it was found that those with the lowest number of fat cells experienced greatest body fat reductions, whilst those with higher numbers gained body fat. Extent of loss appeared to be related to degree of adiposity, and the physiological constant of fat cell numbers (Spalding *et al*, 2008). This finding may also explain, in part, the modest reductions observed in other interventions.

Significant fat mass reduction and additional health benefits have been observed in overweight men with dislipidaemia participating in longer term interventions. Two studies observing overweight men for one year did observe a significant reduction in body fat mass (approximately 4kg), though arguably losses were still modest considering the intervention duration (Fortmann *et al*, 1988; Wood *et al*,

1988). ExEE was not quantified but the exercise protocols were moderate therefore larger changes may not be expected. Additionally, both studies observed significant health benefits accompanying these body composition changes in the form of a reduction in blood pressure and improved lipid profile, though it should be noted that similar health benefits were observed in a group restricting EI in the study of Wood and colleagues who achieved greater reductions in fat mass. Associated health benefits are therefore dependent on changes in adiposity, and are not exercise induced *per se*.

There is evidence that exercise interventions including the teaching of behavioural strategies may be most useful for sustaining small body fat losses in the long term in overweight and obese men and women. Weekly motivation classes teaching “correct” exercise technique have produced sustainable moderate body mass loss; losses were maintained a year after the intervention. ExEE was not quantified so expected losses cannot be predicted. Participants were followed up a year after the intervention and it was found that body mass was still significantly lower than baseline (Skender *et al*, 1996). Other evidence has shown that small, non-clinically significant, body fat reductions may be achieved after a 6 month exercise intervention, and maintained or even improved upon over a period of 18 months with a behaviourally focused maintenance intervention (Dunn *et al*, 1997; 1999). It is interesting that the same authors observed a similar six month body fat loss (approximately -2%) induced by behavioural intervention teaching strategies to alter behaviour and increase PA, with no actual activity intervention. Greater losses may in fact be achieved and maintained during an 18 month maintenance phase with this approach (Dunn *et al*, 1997; 1999). Although these behavioural interventions did not achieve a clinically significant body fat reduction, and therefore are unlikely to have gained specific health benefits, it may be beneficial to use such behavioural strategies to achieve and maintain small body fat losses, rather than eliciting large, unsustainable reductions through demanding exercise protocols.

Studies which control and accurately quantify exercise induced energy deficit have witnessed greater success over a period of 1 year. Significant fat mass reduction, with no loss of fat free mass, was seen in middle aged, lean and

overweight men and women who participated in the exercise arm of another intervention study. The exercise intervention was designed to induce a 16% energy deficit in the first three months and a 20% deficit for the subsequent nine months. Participants in this study were mostly overweight, but did include some lean individuals therefore it is difficult to assess the effects on the overweight exclusively. However, participants in an energy restriction arm of the study achieved similar fat mass losses, along with a reduction in lean mass, therefore exercise was a successful intervention in comparison to energy restrictions in this case (Weiss and Holloszy, 2007). It is not clear why this intervention induced greater losses than some other long term studies, but it may be that the controlled energy deficit may have resulted in the relative success of this particular intervention. Individual quantification of ExEE and associated energy deficit thus seems important for a successful exercise intervention.

Significant, but rather modest, body and fat mass reduction is possible in the long term through exercise, with the additional benefit of lean mass preservation and improvement of health related variables in some cases. Unfortunately and it is hard to truly measure success of many interventions where losses cannot be predicted. In those studies that do quantify ExEE success is quite varied, 100% achievement of predicted losses is rare. Moderate intensity exercise may still be useful for stabilisation of body mass in long term and improving health, but there are many considerations to be taken into account when designing a successful exercise programme. Most studies closely supervise exercise sessions and it is not clear what the effect of removing this level of supervision would be on compliance. Attrition rates experienced in some studies are in excess of 20%, and sometimes as much as 50%, Very obese individuals also need to be accommodated as they are physically limited in the type and intensity of exercise. These types of studies can be demanding and labour intensive for researchers because of such issues.

Table 1.9 Evidence regarding the effects of medium term exercise interventions on body composition changes in overweight and obese men and women.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Barwell <i>et al</i> (2009)	55 overweight women	n=55	34.7 ± 6.4 (mean ± SD)	31.3 ± 5.1 (mean ± SD)	27.5 ± 4.7 (mean ± SD)	7 weeks	Progressive, aerobic exercise intervention. Participants exercised for 30 minutes three times a week at baseline at 65-80% HRmax, and progressed to a maximum of five 60 minutes sessions in weeks 6 and 7.	Not reported. Change: -0.2 ± 0.7 (mean ± SD)	Not reported	-1.0 ± 1.5 (mean ± SD)	Not reported
Bouchard <i>et al</i> (1990)	5 overweight men	n=5	25 ± 3 (mean ± SD)	44.9 ± 7.6 (mean ± SD)	27.5 ± 2.9 (mean ± SD)	100 days	Participants were resident in an experimental facility and completed two exercise sessions at 55% $\dot{V}O_{2max}$ on six days of the week to expend 4.2 MJ day ⁻¹ , resulting in a total ExEE of 352.8 MJ during the intervention.	25.0 (range not reported)	9 (range not reported)	-10.4 ± 7.5 (mean ± SD)	Not reported
Broeder <i>et al</i> (1992a)	64 overweight men	n=47	Not reported Range 18-35 years.	48.8 ± 1.1 (mean ± SEM)	Con: 25.3 ± 1.0 Endurance: 25.1 ± 1.1 Resistance: 25.5 ± 1.1 (mean ± SEM)	12 weeks	Participants randomised to one of three groups. Control: No exercise (n=19) Endurance: Aerobic exercise 4 days/week, increasing intensity to a maximum of 70-85% $\dot{V}O_{2max}$ for 50 minutes per session in week 8 (n=15) Resistance: 1 hour resistance training 4 days/week (n=13)	Con: 25.3 ± 1.0 Endurance: 24.8 ± 1.0 Resistance: 25.4 ± 1.0 (mean ± SEM)	Change not reported Baseline Control: 79.6 ± 3.1 Endurance: 79.0 ± 208 Resistance: 81.6 ± 3.9 Post-intervention Control: 79.9 ± 3.1 Endurance: 77.9 ± 3.4 Resistance: 81.6 ± 3.8	Control: 0% Endurance: -9.6% Resistance: -11.8%	26.6%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
									(mean \pm SEM)		
Leon <i>et al</i> (1979)	10 obese men	n=6	25 (range not reported)	Not reported	38.2 \pm 1.5 (mean \pm SEM)	16 weeks	Six 90 minute sessions/week of treadmill walking.	36.4 \pm 1.3 (mean \pm SEM)	Not reported. Baseline: 99.1 \pm 6.8 Post-intervention: 93.4 \pm 6.4 (mean \pm SEM)	Not reported. Baseline: 23.3 \pm 4.6 Post-intervention: 17.4 \pm 4.2 (mean \pm SEM)	40%
King <i>et al</i> (2008)	35 overweight men and women	n=35, 10 men and 25 women	39.6 \pm 11.0 (mean \pm SD)	28.4 \pm 5.8 (mean \pm SD)	31.8 \pm 4.1 (mean \pm SD)	12 weeks	Aerobic exercise sessions to expend 2090 kJ/session at 70% HR _{max} on 5 days/week.	Not reported	-3.7 \pm 3.6 (mean \pm SD)	-3.7 \pm 2.6 (mean \pm SD)	Not reported
Manthou <i>et al</i> (2010)	34 overweight women	n=34	31.7 \pm 8.1 (mean \pm SD)	2.1 \pm 0.4 L/min (mean \pm SD)	29.3 \pm 4.4 (mean \pm SD)	8 weeks	150 minutes of supervised cycling exercise per week at 90-95% of their lactate threshold. ExEE of the intervention was 30.2 MJ	Not reported. Change: -0.05 \pm 0.11 (mean \pm SEM)	-0.15 \pm 0.28 (mean \pm SEM)	-0.04 \pm 0.24 (mean \pm SEM)	Not reported
Martins <i>et al</i> (2010a)	22 overweight and obese men and women	n=15, 8 men and 7 women.	36.9 \pm 8.3 (mean \pm SD)	32.9 \pm 6.6 (mean \pm SD)	31.3 \pm 2.3 (mean \pm SD)	12 weeks	Aerobic exercise session 5 times a week at 75% of HR _{max} .	30.1 \pm 2.3 (mean \pm SD)	Change not reported Baseline: 96.1 \pm 12.0 Week 12: 92.6 \pm 11.7 (mean \pm SD)	Change not reported Baseline: 34.1 \pm 4.9 Week 12: 31.2 \pm 5.0 (mean \pm SD)	31.8%
Ross <i>et al</i> (2000)	18 overweight and obese men	n=16	45.0 \pm 7.5 (mean \pm SD)	3.8 \pm 0.8 L/min (mean \pm SD)	32.3 \pm 1.9 (mean \pm SD)	12 weeks	Daily treadmill exercise conducted at 70% $\dot{V}O_{2peak}$ to expend 2090 kJ/day	Not reported	-7.6 \pm 0.6 (mean \pm SD)	-6.1 \pm 1.5 (mean \pm SD)	11.1%
Ross <i>et al</i> (2004)	23 obese women	n=17	43.2 \pm 5.1 (mean \pm SD)	2.1 \pm 0.5 L/min (mean \pm SD)	32.8 \pm 3.9 (mean \pm SD)	14 weeks	Daily treadmill exercise conducted at 70% $\dot{V}O_{2peak}$ to expend 2090 kJ/day	30.4 \pm 3.7 (mean \pm SD)	-6.1 \pm 1.2 (mean \pm SD)	-6.7 \pm 1.9 (mean \pm SD)	26.1%

Table 1.10 Evidence regarding the effects of long term exercise interventions on body composition changes in overweight and obese men and women.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Bjorntorp <i>et al</i> (1973)	11 morbidly obese men and women	n=8, 5 women and 3 men	Not reported, age of women ranged from 21-37 years and men from 30-47 years	2.5 ± 0.5 L/min (mean ± SD)	Not reported, mean body mass 112 ± 20 kg (mean ± SD)	6 months	35 minute circuit training classes 3 times a week.	Not reported, mean body mass 113 ± 9 kg (mean ± SD)	+1 ± 5 (mean ± SD)	-3 ± 5 (mean ± SD)	27.3%
Dunn <i>et al</i> (1999)	114 overweight men and women	n=90	46.2 ± 6.5 (mean ± SD)	26.5 ± 6.2 (mean ± SD)	28.0 ± 3.8 (mean ± SD)	6 months	Aerobic exercise on 5 days/week, 20-60 minute sessions at an intensity of 50-85% of maximal aerobic power.	Not reported	-1.3 (-1.9, -0.6) (mean (95% CI))	-1.7% (-2.1, -1.2) (mean (95% CI))	19.1%
Foster-Schubert <i>et al</i> (2005)	91 overweight women	n=87	60.7 ± 6.7 (mean ± SD)	20.0 ± 3.5 (mean ± SD)	30.4 ± 4.1 (mean ± SD)	12 months	Moderate intensity aerobic exercise, 45 minutes sessions at 60-75% HRmax on five days/week	Not reported	-1.4 ± 0.4 (mean ± SD)	Not reported	2.9%
Krotkiewski <i>et al</i> (1979)	38 obese women	n=27	37.0 ± 7.5 (mean ± SD)	Not reported	Not reported, mean body mass 78.8 ± 9.8 kg (mean ± SD)	6 months	Three aerobic 55 min exercise classes per week (consisting of 15 minutes jogging/dancing warm up, 25 minutes intermittent high intensity exercise on cycle ergometer, and 15 minute cool down)	Not reported	Not reported Body mass: Baseline 78.8 ± 9.8 3 months 78.8 ± 10.1 6 months 80.0 ± 12.1 (mean ± SD)	Not reported Body fat: Baseline 34.1 ± 5.5 3 months 33.2 ± 5.7 6 months 34.1 ± 9.8 (mean ± SD)	29%
Skender <i>et al</i> (1996)	43 overweight men and women	n=30, 16 men and 14 women	Not reported, age range 25-45 years	25.3 ± 7.8 (mean ± SD)	Not reported. Baseline body mass 93.7 ± 21.1 kg (mean ± SD)	12 months	Behavioural and exercise intervention including weekly exercise motivation and technique classes 45 minute vigorous walking exercise sessions 3-5 times/ week.	Not reported	-2.9 ± 7.4 (mean ± SD)	Not reported. Body fat Baseline: 30.5 ± 0.5 % 12 months: 24.3 ± 5.9 % (mean ± SD)	32.3%
Turner <i>et al</i> (2010)	27 overweight,	n=15	Ex: 55 ± 5	34.5 ± 4.2	Ex: 28.5 ± 2.9	6 months	Progressive, aerobic exercise intervention,	27.8 ± 2.5	-1.8 ± 2.2	Not reported	16.7%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ¹ /min ¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
	men		(mean ± SD)	(mean ± SD)	(mean ± SD)		increasing from three 30 min sessions at 50% $\dot{V}O_{2max}$ per week to four 60 min sessions at 70% $\dot{V}O_{2max}$	(mean ± SD)	(mean ± SD)		

1.2.5 Gender Differences

Some intervention studies including both men and women report their results by gender to elucidate possible gender-based differences. The findings of such studies have indicated that exercise is relatively less effective for reducing body mass in women compared to men (table 1.11). It has been suggested that only women with greater initial body fat levels than men experience comparable, significant, exercise-induced body fat losses. Comparisons between men and women most similar in body composition revealed exercise is significantly more effective in men, over a 3 month period (Andersson *et al*, 1991). Indeed lean men and women completing identical training regimes over 40 weeks in preparation for a half marathon showed differing body composition changes. Similar to the previous study, some women were comparable to men in terms of body fat content, whilst others had higher levels of body fat; women with a similar body composition to the male participants did not experience the same magnitude of exercise induced body mass reduction. The authors found that these women tended to increase EI and this explained the relatively smaller loss of fat mass (Westerberp *et al*, 1992).

Evidence indicates that exercise may be most effective for prevention of body mass gain in women. Overweight men experienced a significant 5kg body mass change as a result of a 16 month exercise intervention, in stark contrast to women who experienced no changes. Female control participants gained 3 kg over the same period so it seems exercise had prevented body mass gain in the exercising females. The authors found that inherent sex-based body composition differences resulted in men expending relatively more energy during exercise (Donnelly *et al*, 2003a). However, the reported power calculation stated that sample size was sufficient to observe a 4-7kg difference in body mass with 80% power so it is possible that changes in women were not detected due to insufficient statistical power. Other evidence indicates that exercise may be effective for body mass reduction in women only in combination with EI restriction. Analysis of covariance results of a large, 2 year, behavioural intervention revealed that restricting fat intake was more effective than exercising for reducing body mass in both men and women; exercise seemed to

be effective only in the males. In women, high volumes of exercise resulted in a -0.2 kg/m^2 change in BMI, and a reduction in fat intake resulted in -0.8 kg/m^2 change in BMI. Larger changes were induced by a combination of these methods, equivalent to a -1.8 kg/m^2 change in BMI. Exercise was slightly more effective in men (-0.5 kg/m^2), but there was no additive effect of a combination of exercise and dietary fat restriction (Dunn *et al*, 2006). Though power calculations were not reported for these studies, a considerable strength of these studies is that sample sizes numbered in their hundreds.

It should be noted that not all studies have observed sex-based differences in the efficacy of exercise as a body fat reduction method. The Heritage family study found that both men and women achieved negligible reduction in fat mass over a 20 week period. The authors predicted a fat mass reduction of 1.3 kg based on mean whole group exercise intensity, duration and $\dot{V}O_2$ measured during exercise sessions, but actual losses determined by hydrostatic weighing were smaller than predicted in men and women. Observed changes were statistically significant for both sexes but, as with total body mass losses, could not be considered clinically significant (Wilmore *et al*, 1999). It is not immediately clear why this intervention was so unsuccessful, as exercise sessions were rigorously controlled. The large variability in adiposity of participants may have contributed; the greater homogeneity of participants in other intervention may explain why they have observed greater losses. However, this intervention had a much larger sample size than most interventions, with hundreds of participants. Unfortunately sex based differences were not analysed and EI and EE were not monitored, which makes it difficult to understand the findings in either men and women in this study. Dietary restraint was not measured in this study and the authors speculated that dietary restraint levels may have varied due to the presence of restrained eaters in this participant group; it was postulated that these individuals may have experienced a loss of restraint as a result of exercise participation and increased their EI. The authors were unable to conclusively explain their modest findings with the available data, but some form of compensatory response seems likely (Wilmore *et al*, 1999). Additionally, a meta-analysis compared the effect of exercise in combination with EI restriction in both genders and found no significant difference in body fat mass reductions induced by either method. Lean mass losses were 50% lower in

men and women in the combination group, showing once again that exercise is crucial for preservation of muscle tissue during a body mass reduction programme (Ballor & Poehlman, 1994).

A pronounced gender based difference in the efficacy of exercise as a body mass reduction method, possibly due in large part to the inherent greater lean and total body mass of men resulting in greater EE than women. As a result exercise induced body mass reduction requires more effort from women in order to expend the same amount of energy, which could have great implications for exercise compliance rates in women. Additionally, some evidence indicates that women may be particularly prone to increasing EI in response to regular exercise participation, a tendency not seen regularly seen in men. Though these results are mostly obtained from self-reported EI, this method seems to have been sufficient to detect changes in this context, possibly because achieving a change in body mass was not a major goal of participants in this study. Problematically, changes in EI are very difficult to measure accurately, particularly in overweight and obese participants. It does seem that exercise is most effective for body fat loss in men, and prevention of body mass gain in women. As exercise induced changes in women are often modest, it seems that a combination of EI restriction and exercise may be necessary to achieve significant fat losses. As a result, it is important to review evidence of body mass changes as a result of such combination interventions in order to fully understand the efficacy of exercise as a body fat reduction method, in comparison with other available methods of achieving such a loss.

Table 1.11 Evidence regarding gender differences in extent of body composition change in response to chronic exercise participation in lean and overweight/obese.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Andersson <i>et al</i> (1991)	22 slightly overweight men and women	n=22; 9 men and 22 women	Men: 37 ± 2.4 Women: 36 ± 1.4 (mean ± SEM)	Men: 42.0 ± 2.8 Women: 34.6 ± 3.1 (mean ± SEM)	Men: 25.7 ± 0.4 Women: 24.6 ± 0.3 (mean ± SEM)	3 months	Three supervised one-hour exercise sessions per week. Sessions consisted of intermittent short bouts of high intensity exercise, with longer periods of more moderate jogging activity in between.	Not reported. <u>Δ BMI</u> Men: -0.6 ± 0.2 Women: -0.2 ± 0.7 (mean ± SEM)	Men: -2.0 ± 0.6 Women: -0.7 ± 1.9 (mean ± SEM)	Men: -2.9 ± 1.4 kg Women: -2.6 ± 0.8 (mean ± SEM)	Not reported
Donnelly <i>et al</i> (2003)	87 overweight and obese men and women	n=41; 16 men and 25 women	Men: 22 ± 4 Women: 24 ± 5 (mean ± SD)	Men: 39.2 ± 5.2 Women: 32.8 ± 4.2 (mean ± SD)	Men: 29.7 ± 2.9 Women: 28.7 ± 3.2 (mean ± SD)	16 months	Progressive aerobic exercise intervention, beginning with 20 minutes sessions performed at 60% of HRR and progressing to 45 minutes at 75% HRR after 6 months. Sessions were of a duration sufficient to expend 1672 kJ	Men: 28.1 ± 2.5 Women: 28.8 ± 3.5 (mean ± SD)	Men: -5.2 ± 4.7 Women: + 0.6 ± 3.8 (mean ± SD)	Men: -4.9 ± 4.4 Women: 0.3 ± 2.7 (mean ± SD)	52.9%
Dunn <i>et al</i> (2006)	Overweight and obese men and women	n=962; 674 women and 288 men	Men: 56.0 ± 11.4 Women: 51.2 ± 11.6 (mean ± SD)	Not measured.	Men: 32.9 ± 4.4 Women: 33.9 ± 6.1 (mean ± SD)	2 years	Behavioural intervention delivered via mail or phone that taught behavioural strategies to increase EE and decrease EI, specifically through reducing dietary fat intake. Ten interactive, sequential lessons were delivered via phone or mail. Each lesson included instructional material describing a rationale for a specific behaviour change strategy, specific	Men: 32.5 ± 4.5 Women: 33.5 ± 6.0 (mean ± SD)	Not reported. <u>Δ BMI (kg/m₂)</u> Men: -0.4 ± 2.2 Women: -0.5 ± 2.6 (mean ± SD) N.B: ANCOVA analysis separated effects of diet and exercise. Restricting fat intake was more effective than exercising for reducing body	Not measured.	Not reported

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ¹ /min ¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							behaviour change goals related to that strategy, and homework to be completed before beginning the next lesson (e.g., keeping records of eating and exercise). Topics covered in the lessons included nutrition, physical activity, behaviour management techniques such as behavioural assessment, goal setting, stimulus control, social support, and self-motivation/ Weekly feedback was given by a health professional after completion of each lesson.		mass in both men and women; exercise seemed to be effective only in the males.		
Westerterp <i>et al</i> (1992)	32 active, lean and slightly overweight men and women	n=23; 12 men and 11 women	Not reported, range 28-41 years	Not reported	Not reported, range 19.4 - 26.4	44 weeks	Aerobic exercise intervention with the aim of training to complete a half marathon. Participants trained four sessions/week, increasing running time to 10-30 min, 20-60 min and 30-90 minutes per training session after 8, 20 and 40 weeks respectively.	Not reported	Men: -1.0 ± 4.5 Women: -0.9 ± 7.0 (median ± range)	Men: -4.2 ± 7.0 Women: -2.8 ± 5.9 (median ± range)	28.1%
Wilmore <i>et al</i> (1999)	Sedentary, overweight parents and children	n=557, 258 men and 299 women.	Men: 35.2 ± 14.2 Women: 33.1 ±	Not reported	Men: 26.0 ± 4.3 Women: 24.9 ± 4.8	20 weeks	Progressive, aerobic exercise session consisting of three sessions/week	Not reported	Men: -0.4 ± 0.1 Women: -0.1 ± 0.1	Men: -0.9 ± 0.1 Women: -0.5	Not reported

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
	(over 17 years old)		13.1 (mean ± SD)		(mean ± SD)		beginning with 30 minutes cycling exercise at 55% $\dot{V}O_{2max}$ and progressing to a maximum of 50 minutes cycling at 75% $\dot{V}O_{2max}$.		(mean ± SD)	± 0.1 (mean ± SD)	

1.2.6 Effect of Exercise Combined with Dietary Restriction

Meta-analyses consistently find exercise to be most successful for body mass reduction in combination with EI restriction (Wood *et al*, 1998; Skender *et al*, 1996; Pritchard *et al*, 1997; Weiss & Holloszy, 2007). Figure 1.2 shows that exercise is one of the least successful body mass reduction methods available. Likewise, the meta-analysis of Miller *et al* (1997) reported the same conclusion; mean body fat losses resulting from the interventions reviewed were -7.8 ± 0.7 kg, -3.3 ± 0.5 kg, or -9.0 ± 1.0 kg for EI restriction, exercise, and combination interventions respectively. A combination of energy restriction and exercise seems more effective than either method alone. The evidence from randomised controlled trials supports the findings of such meta-analyses (table 1.12). Analysis of data from overweight men completing a 16 week intervention reported a significant main effect only of EI restriction on body and fat mass. Body and fat mass reductions attributable to dietary changes were large, but analysis showed there was very little additional benefit of including vigorous exercise in terms of body composition changes (Cox *et al*, 2003). These findings echo those reported for the male participants of the behavioural intervention of Dunn *et al* (2006).

It is often suggested that exercise participation may preserve lean mass during a period of negative energy balance, but experimental results are conflicting. Some have observed lower lean mass losses in overweight women completing either resistance or aerobic exercise alongside EI restriction; skeletal muscle losses of 1kg were observed in individuals restricting EI, and the inclusion of either form of exercise halved this loss (Janssen *et al*, 2002). Others have found no protective effect in obese women of either resistance, aerobic, or a combination of both modes of exercise during a period of EI restriction (Wadden *et al*, 1997). The latter study was of much longer duration than the former, and this may explain the discrepancy; it is possible that exercise only protects lean mass in the shorter term.

It seems that exercise may need to exceed a specific intensity before conferring additional benefits during an EI restriction programme. Low intensity exercise has not been shown to enhance body fat mass losses in obese men and women. No significant differences in total body, lean or fat mass losses between groups restricting EI with and without exercise participation were observed in one medium term intervention (van Aggel-Leijssen *et al*, 2001). Exercise in this studies was conducted at an intensity of 40% $\dot{V}O_{2max}$ exercise; higher intensity exercise may induce a greater energy deficit hence it is perhaps logical that exercise may need to be moderate to vigorous to add benefit to an energy restriction programme. These results were mirrored in elderly, overweight and obese adults completing aerobic and resistance exercise programme over 18 months. The combination of EI restriction and exercise proved slightly, but not significantly, more successful than diet alone in these participants (Messier *et al*, 2004). These participants were older and suffered from arthritis and as a result the exercise programme was not strenuous. However, there were additional benefits as only participants who exercised reported an improvement in mobility and pain levels. Such an improvement in quality of life provides a compelling reason to include exercise in a body mass reduction programme in some clinical populations.

Inclusion of exercise in a body mass reduction program may be most beneficial in the long term to prevent regain of body mass. Evidence from a one year intervention has shown that a combination of exercise and energy restriction combined may be most effective for maintaining losses. In this study, overweight men and women who only restricted EI had regained all body mass lost after a year. Those who exercised in combination with EI restriction also experienced some regain but remained maintained greater losses at follow than either the exercise or energy restriction only groups (Skender *et al*, 1996). Benefits of adding exercise to dietary restriction may emerge only in the maintenance phase, and serve to minimise the problem of body mass regain that commonly occurs in the long term.

There are limitations in this field of literature which affect the interpretation of results. As noted by Miller *et al* (1997) the majority of studies focused on a rather narrow study population. Thirteen years later, lack of diversity in study populations in this field is still an issue. As the prevalence of obesity in all age groups rises (Low *et al*, 2009), it is important to have a range of studies on younger and older age groups, with varying degrees of overweight/obesity. Attrition rates in exercise intervention studies are typically high, and are often time-consuming and laborious to complete. As a result these interventions range greatly in sample size; the most intensive interventions often have less than ten participants, whilst other studies have sample sizes numbering in their hundreds. In the smaller scale studies where power calculations are not reported, lack of statistical power may mean that true effects of exercise are not observed, and conclusions may be misleading. High attrition rates often contribute to low sample sizes, and minimising attrition and achieving a satisfactory sample size presents a significant challenge for researchers undertaking such work.

Although the evidence is not entirely straightforward, the inclusion of exercise in an EI restriction programme seem to be effective for reducing body mass, and women may experience the greatest benefits from this combination. Dietary restriction may contribute more to body mass reduction than exercise, but the inclusion of exercise may also preserve muscle tissue and minimise significant body mass regain in the long term.

Table 1.12 Evidence regarding the effects of dietary energy restriction combined with chronic exercise participation on body composition changes in overweight and obese men and women.

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2\max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
Cox <i>et al</i> (2003)	59 overweight men	n=51	42.0 ± 5.0 (mean ± SD)	26.9 ± 3.5 (mean ± SD)	31.1 ± 3.9 (mean ± SD)	16 weeks	Subjects were randomised to one of four groups: 1. Low EI/light exercise (n=14) 2. Normal EI/light exercise (n=17) 3. Low EI/vigorous exercise (n=15) 4. Normal EI/vigorous exercise (n=13) Participants randomised to low EI were restricted to 6279 kJ/day. Light exercise groups completed flexibility exercise and cycling exercise against zero resistance. Vigorous exercise groups completed cycling at 60-70% maximum workload. Both exercise groups completed three 30 minute sessions/week.	Not reported	ANOVA analysis revealed that body mass change attributable to energy restriction was 10.1 (-12.2, -8.0), and to exercise was -1.1 (-3.2, 1.0). (Mean (95% CI))	ANOVA analysis revealed that fat mass change attributable to energy restriction was -7.7 (-9.6, -5.9), and to exercise was -1.4 (-3.3, 0.5). (Mean (95% CI))	15%
Janssen <i>et al</i> (2002)	38 obese women	n=38	DO: 40.1 ± 6.7 DA: 37.5 ± 6.0 DR: 34.8 ± 5.8 (mean ± SD)	Not reported	DO: 33.7 ± 4.1 DA: 36.0 ± 7.1 DR: 31.6 ± 4.3 (mean ± SD)	16 weeks	Participants were randomised to one of three groups: 1. dietary restriction only (DO, n=13) 2. dietary restriction and aerobic exercise (DA, n=11) 3. dietary restriction and resistance exercise (DR, n=14)	Not reported Δ BMI DO: -4.0 ± 1.4 DA: -4.2 ± 1.2 DR: -3.9 ± 1.0	DO: -10.0 ± 3.9 DA: -11.1 ± 4.4 DR: -10.0 ± 3.0 (mean ± SD)	DO: -7.8 ± 3.1 DA: -9.9 ± 4.6 DR: -8.6 ± 2.4 (mean ± SD)	Not reported

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²) (mean ± SD)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							All participants were asked to reduce their EI by 4180 kJ day ⁻¹ and limit fat intake to <30% of total intake. The aerobic exercise program consisted of 5 sessions per week; at baseline each session was 15 minutes in duration and this was gradually increased to 60 minute sessions. Resistance exercise was carried out 3 times a week for approximately 30 minutes				
Messier <i>et al</i> (2004)	316 overweight and obese men and women	n=252	C: 69 ± 0.1 D: 68 ± 0.7 E: 69 ± 0.8 DE: 69 ± 0.8 (mean ± SEM)	Not reported	C: 34.2 ± 0.6 D: 34.5 ± 0.6 E: 34.2 ± 0.6 DE: 34.0 ± 0.7 (mean ± SEM)	18 months	Participants were randomised to one of four groups: 1. control (C) 2. energy restriction only (D) 3. exercise only (E) 4. energy restriction and exercise (DE) Exercisers completed three 30 minute sessions/week, which included aerobic (at 50-75% HRR), and resistance exercise. Participants in the energy restriction groups attended weekly classes which taught techniques to help them reduce their EI in order to reduce body mass by	Not reported	C: -1.1 (-3.0, 5.2) D: -4.6 (-0.4, -8.8) E: -3.5 (-0.8, 7.7), DE: 5.2 (-0.9, -9.6) (mean (95% CI))	Not reported	20.3%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							5%.				
Skender <i>et al</i> (1996)	127 overweight men and women	n=86, 43 men and 43 women	Not reported, age range 25-45 years	25.3 ± 7.8 (mean ± SD)	Not reported. <u>Baseline body mass</u> D: 98.5 ± 25.9 E: 93.7 ± 21.1 DE: 100.1 ± 27.4 (kg) (mean ± SD)	12 months	Participants were randomised to one of three groups. 1. Diet only (D; n=29) 2. Exercise only (E; n=30) 3. Diet and exercise (DE; n=27) The dietary intervention was designed to induce 1kg loss body mass per week. The exercise intervention included weekly motivational classes teaching exercise technique, and completion of three to five 45 minute vigorous walking exercise sessions/week.	Not reported	D: -6.8 ± 7.8 E: -2.9 ± 7.4 DE: -8.9 ± 11.5 (mean ± SD)	Not reported. <u>Baseline % body fat</u> D: 31.9 ± 4.9 E: 30.5 ± 5.0 DE: 32.0 ± 6.1 <u>Post-intervention % body fat</u> D: 24.5 ± 6.7 E: 24.3 ± 5.9 DE: 24.9 ± 7.2 (mean ± SD)	32.3%
van Aggel-Leijssen <i>et al</i> , 2001	40 obese men	n=37	D: 38.6 ± 6.5 DE: 39.3 ± 7.7 (mean ± SD)	D: 44.7 ± 5.8 DE: 43.3 ± 4.5 (ml/kg FFM/min) (mean ± SD)	D: 32.0 ± 2.1 DE: 32.6 ± 2.5 (mean ± SD)	10 weeks	All participants restricted EI, and were also randomised to sedentary (n=20) or low intensity exercise (n=17). For the first 6 weeks participants were provided with a formula diet which provided 2.1 MJ day ⁻¹ , reduced to 1.4 MJ day ⁻¹ energy obtained from the formula and 3.5 MJ from food in week 7, and to 0.7 MJ day ⁻¹ of energy from formula and 4.9 MJ	D: 27.5 ± 1.8 DE: 27.8 ± 2.5 (mean ± SD)	D: -14.8 ± 5.3 DE: 15.2 ± 6.3 (mean ± SD)	Not reported. <u>Baseline body fat%</u> D: 34.6 ± 4.9 DE: 33.5 ± 4.2 <u>Post-intervention body fat %</u> D: 25.9 ± 6.1 DE: 25.0 ± 5.0 (mean ± SD)	7.5%

Authors	Participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Δ body mass (kg)	Δ body fat (kg)	Attrition rate
							<p>day⁻¹ from food in week 9.</p> <p>Exercisers participated in four 1 hour aerobic exercise sessions/week at 40% $\dot{V}O_{2max}$ (cycling, walking or aqua jogging).</p>				
Wadden <i>et al</i> (1997)	128 obese women	n=99	<p>D: 41.0 ± 8.8</p> <p>DA: 40.8 ± 7.9</p> <p>DS: 40.0 ± 9.1</p> <p>DAS: 42.8 ± 8.3</p> <p>(mean ± SD)</p>	Not measured	<p>D: 36.4 ± 5.5</p> <p>DA: 37.3 ± 5.1</p> <p>DS: 36.5 ± 6.0</p> <p>DAS: 35.3 ± 4.4</p> <p>(mean ± SD)</p>	48 weeks	<p>Participants in this study were assigned to one of 4 groups for a 48 week intervention: 1. energy restriction only (D; n=29)</p> <p>2. energy restriction plus aerobic exercise (DA; n=31)</p> <p>3. energy restriction plus strength training (DS; n=31)</p> <p>4. energy restriction plus aerobic and strength training (DAS; n=29).</p> <p>All participants consumed 3766-3870 kJ/day from weeks 2-17, 5230 kJ/day from weeks 18 to 22, and 6270 kJ/day thereafter. For the first 28 weeks exercisers completed three moderate intensity 40 minute exercise sessions /week, and thereafter this was reduced to two sessions/week</p>	Not reported	<p>D: -14.4 ± 6.2</p> <p>DA: -13.7 ± 8.7</p> <p>DS: -17.2 ± 9.4</p> <p>DAS: -15.2 ± 9.1</p> <p>(mean ± SD)</p>	<p>Not reported.</p> <p><u>% body mass loss from fat</u></p> <p>D: 81.7 ± 28.3</p> <p>DA: 79.6 ± 19.3</p> <p>DS: 83.5 ± 18.3</p> <p>DAS: 91.2 ± 39.2</p> <p>(mean ± SD)</p>	6.3%

1.3 Exercise induced compensatory responses

The relatively modest efficacy of exercise as a body mass reduction method is thought to be attributable to compensatory responses. Some studies have reported that exercise induces an up-regulation of EI and/or down-regulate non-exercise physical activity resulting in negation of ExEE (King *et al*, 2008; Manthou *et al*, 2010). Potential mechanisms driving these changes, such as exercise induced alterations in appetite regulating hormones, have been hypothesised but have not yet been definitively proven. Metabolic compensatory responses may also contribute; metabolic rate often decreases in response to significant body mass reduction and acts to oppose further losses. There is evidence to suggest that the magnitude of compensatory responses may vary between individuals differing in sex, age, adiposity and possibly cardiovascular fitness level, creating a complex field of literature. Inherent problems associated with accurate observation of EI and EE (Acheson *et al*, 1980; Lara *et al*, 2004) makes detection of compensatory responses difficult and may explain why many studies find no evidence of compensation. The existing evidence regarding these compensatory responses and their possible mechanisms will be reviewed in this chapter.

1.3.1 Types of compensatory responses and potential mechanisms

Compensatory mechanisms may be due to innate physiological and behavioural differences between individuals. A brief overview of the factors that may contribute to exercise induced compensatory responses presented in figure 1.3.

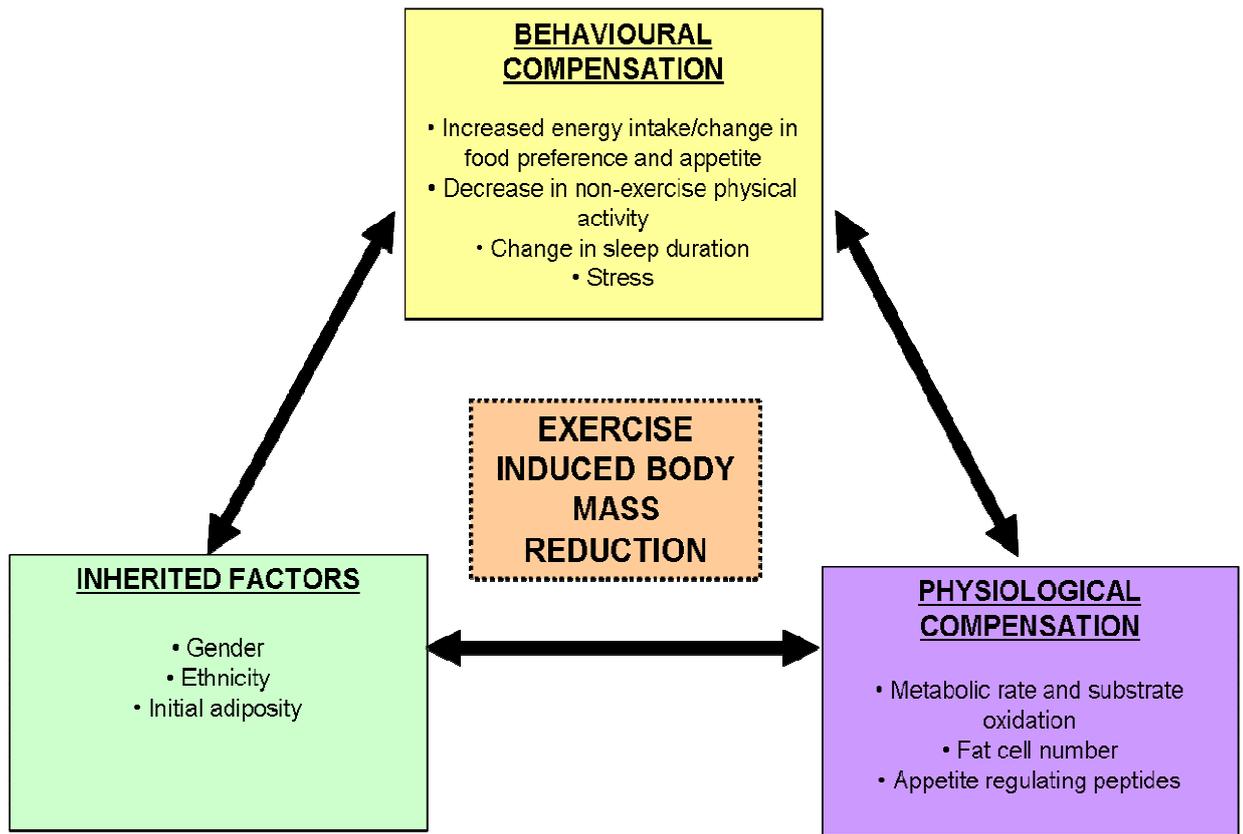


Figure 1.3 Behavioural, genetic and physiological factors that may influence individual exercise induced body mass reduction. (Diagram adapted from Boutcher and Dunn, 2009).

1.3.1.1 Behavioural

Changes in perceived appetite and desire for food have been identified in overweight and obese individuals who do not achieve predicted body mass reduction during a supervised 12 week exercise intervention. Emerging evidence indicates that even a single session of exercise may increase liking, wanting, and perceived reward value of food in some individuals (Finlayson *et al*, 2009 & 2011). This may be accompanied by an increased drive to eat and a greater satiety response to food ingestion (King *et al*, 2009). It has not yet been proven that these changes in appetite result in altered food consumption either; further investigation is required to quantify the contribution such a change may make to compensatory EI changes. Exact mechanisms driving these changes in appetite are yet to be identified; dopamine deficiency may be involved since this

neurotransmitter modulates the reward value of food (Wang *et al*, 2001). These changes could also be the result of behavioural influences such as dietary restraint (Coletta *et al*, 2009). Some individuals seem to increase EI post-exercise out of a desire to “reward” themselves for exercising (King *et al*, 2007). Obese individuals are particularly prone to inaccurately estimating ExEE and EI (Lichtman *et al*, 1992); overestimating ExEE and thus the size of “reward” that has been earned may lead to overconsumption and a state of positive energy balance despite exercise participation.

Behavioural mechanisms may also drive compensatory reductions in non-exercise physical activity. Increased feelings of fatigue from participation in vigorous activity may result in increased sedentary behaviour outside of exercise sessions (Manthou *et al*, 2010). Sleep duration and ghrelin concentrations have been shown to be negatively correlated (Spiegel *et al*, 2004); an alteration in sleeping time, and thus total EE, could feasibly occur in response to increased exercise participation and influence appetite. Psychological stress could also play a role as it stimulated cortisol production, which in turn inhibits fat oxidation (McMurray and Hackney, 2005), which may result in lower ExEE and body mass losses.

1.3.1.2 Physiological

Appetite regulating hormones, such as ghrelin and peptide YY, have been hypothesised to be involved in the exercise induced up-regulation of EI, and have been the subject of much research interest. Both of these hormones are secreted in the gut and have specific forms which are able to cross the blood-brain barrier and exert their influence via the hypothalamic circuits (figure 1.5). Ghrelin is involved in initiating feelings of hunger (Lim *et al*, 2010), and is the only known orexigenic peptide at present. Peptide YY is one of many hormones which regulate satiety responses to food ingestion (le Roux and Bloom, 2005). These hormones and their potential contribution to compensatory mechanisms in the short and long term will be explored in depth in section 1.4.

Initial adiposity also plays a role as those with the highest BMI at baseline consistently experience the greatest body mass reductions (Hainer *et al*, 2005). Fat cell numbers in adults are fixed and may partially dictate the magnitude of exercise-induced body fat mass reduction in the individual since it is only possible to reduce the size, but not the number, of these cells (Spalding *et al*, 2008). Size of the cells in the individual may be associated with exercise induced body fat reduction in males; those with the largest fat cells experienced the greatest body mass reduction during 20 weeks of exercise training (Despres *et al*, 1984). This effect was not seen in female participants indicating potential sex based differences in adipocyte adaptation to exercise training. Fat oxidation capacity also seems to play a role in females; changes in resting fat oxidation rate have been positively correlated with the magnitude of body mass reduction in response to exercise in overweight women (Barwell *et al*, 2009).

Reducing body mass often results in a reduction in resting metabolic rate, and hence total EE may decline and oppose further perturbations in energy balance (Elliot *et al*, 1989; Leibel *et al*, 1995; Doucet *et al*, 2003). Individuals who experience frequent fluctuations in body mass may be most prone to these metabolic adaptations. Such behaviour is associated with a relative decline in muscle mass and increase in fat mass, resulting in a significant decline in metabolic rate in some cases (Manore *et al*, 1991; Froidevaux *et al*, 1993). Maintaining high levels of physical activity may minimise this compensatory decline (Gilliat-Wimberly *et al*, 2001).

1.3.1.3 Inherited (genetic) factors

Some factors which play a role in individual susceptibility to compensatory behaviours may be determined by genetics; these factors are not modifiable and will not be examined in any detail in this thesis except to note that they can affect the outcome of exercise participation. Gender has a profound influence on exercise induced body mass reduction due to inherent body composition and metabolic differences, enabling males to achieve higher ExEE for any given effort compared to women (Donnelly *et al*, 2003a). There are also inherent

differences in body composition, metabolic factors such as insulin resistance (Wulan *et al*, 2010), and levels of appetite regulatory peptides such as ghrelin (Kasa-Vubu *et al*, 2007) between different ethnic groups which may affect the extent of exercise induced body composition change.

There may be other physiological factors contributing to compensatory responses to exercise training but behavioural changes in EI and EE are of most interest in this thesis since these variables are modifiable and thus theoretically preventable. Current evidence regarding the existence and magnitude of these changes are reviewed in this chapter.

1.3.1.4 Is energy intake matched to energy expenditure?

Preload studies have investigated the sensitivity of EI regulation; these studies involve the manipulation of the energy content of a preload, often in the form of a drink or milkshake, and subsequent monitoring of subsequent EI at a normal meal. Many such studies have found that lean habitual exercisers are able to regulate EI more sensitively than non exercisers in this case (Goldberg *et al*, 1998; Long *et al*, 2002; Martins *et al*, 2007a; Whybrow *et al*, 2008). This has led to speculation that regular exercise participation may increase sensitivity of energy balance regulation. Experimental evidence has supported this hypothesis; both cross-sectional and intervention studies have observed that EI regulation in response to a high and low energy preloads is more sensitive in lean individuals who exercise regularly compared to their sedentary counterparts (Lluch *et al*, 2000; Martins *et al*, 2007a). However, it is difficult to determine if this effect is wholly attributable to exercise participation, and findings may not be applicable to overweight and obese. Thus exercise may improve EI regulation, a change which could theoretically decrease likelihood of compensatory behaviours.

Conversely, it has been put forward that chronic inactivity diminishes the ability to self regulate energy balance, leading to a state of positive energy balance resulting in a transition to overweight or obesity over time. Evidence has shown

that a seven day regime of inactivity within a whole-body calorimeter does not result in a matching down-regulation of EI (Stubbs *et al*, 2004a), but in the longer term EI restriction gradually induces adaptations in EE level. A rather unique study observed eight healthy, lean and overweight men and women who resided in a biosphere for 2 years. Participants consumed a low energy diet for the first 6 months and after exiting the biosphere it was observed that their spontaneous PA levels were lower than control participants. Interestingly, this depression in activity levels persisted six months after their exit from the biosphere, despite regaining body mass to baseline levels (Weyer *et al*, 2000). Regulatory systems seem sensitive to EI restriction, but not to forced inactivity. Though this may seem contradictory initially, when we consider from an evolutionary perspective this inability to down-regulate EI to match EE makes perfect sense. Evolutionary and genetic research has shown that human-beings evolved as “hunter-gatherers” who endured periods of both feast and famine. To ensure survival in such an environment consuming a high EI whenever food was plentiful, regardless of EE, would have been a pivotal survival strategy (Chakravarthy and Booth, 2004). Human-beings have thus evolved to be fuel-efficient but this has become a hindrance in the modern “obesogenic” environment with constant, abundant fuel supply, and little requirement to be routinely active (Egger and Swinburn, 1997).

1.3.2 Evidence regarding compensatory changes in appetite and energy intake

Study Selection

1.3.2.1 Acute effect of exercise on energy intake in overweight and obese

The primary outcome of interest in this section of the review was acute post-exercise EI. Inclusion criteria for studies in this section (summarised in table 1.13) were:

- Published between 1999 and 2011.

- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Study design including both an aerobic exercise session and a control trial.
- Measurement of post-exercise EI.

There is a large field of acute studies observing EI responses to a single exercise session, these studies are typically less than 24 hours in duration, and many are conducted over just a few hours. The majority of this evidence focuses on lean individuals; evidence from overweight and obese is sparse in comparison. As a result the acute effect of exercise on EI in overweight and obese women is still somewhat unclear. Existing evidence suggests that acute post-exercise EI responses vary depending on adiposity; differing post-exercise responses have been observed between lean and obese women (Kissileff *et al*, 1990).

Overweight women have also been observed to consume greater EI than lean women under both sedentary and exercise conditions (George and Morgenstein, 2003). Both of these studies directly observed EI in the laboratory, a method commonly used in acute studies, but not without its limitations. One of the challenges associated with accurate assessment of EI is the adverse effect of atypical settings on eating behaviour (Herman and Polivy, 2005), and as a result it is often difficult to determine if EI observed in the laboratory in acute studies such as these is typical of normal eating behaviour. Differing palatability of test foods may also have an effect on EI measurements; Kissileff and colleagues served a liquefied meal whilst George and Morgenstein allowed participants to self-select cafeteria food. Additionally, neither study appears to have measured dietary restraint; this may be an important factor in acute studies since it has been demonstrated that post-exercise EI responses may vary depending on individual level of dietary restraint. Overweight restrained eaters, who reported that they were also currently dieting, had significantly lower 12 hour EI on sedentary days compared to those with exercise. This effect was not seen in non-dieting women or those with low restraint (Visona and George, 2002). Other cognitive influences, such as mood, may be affected by very brief exercise participation, and in turn contribute to differing individual EI responses in overweight and obese. A three minute step test was sufficient to result in increased snack food consumption in overweight, sedentary men who

experienced increased negative mood on the profile of mood states questionnaire after the step test. It was found that this effect was specific to the most overweight participants (Schneider *et al*, 2009). Post exercise mood deterioration may be more likely to occur in overweight, sedentary participants due to their poor fitness levels and greater perceived exertion (Ekkekakis and Lind, 2006). Thus cognitive and emotional factors may have a significant influence on post-exercise EI responses, and may contribute to individual differences observed in overweight and obese.

Acute exercise studies involving only overweight and obese men and women have largely found no evidence of EI compensation. Buffet meal EI measured in the laboratory one hour after a moderate walking exercise session (approximately 40 minutes duration) did not differ from control conditions in one small study of overweight and obese women, illustrating that short term negative energy balance can be induced by relatively small doses of exercise (Unick *et al*, 2010). Findings from overweight and obese men have echoed this; a single session of cycling exercise was sufficient to induce short term negative energy balance, and overweight and obese achieved greater negative energy balance than lean men participating in the same protocol, in part due to their larger body size resulting in greater ExEE (Ueda *et al*, 2009a). However, both studies had small sample sizes (<20 participants) and no reported power calculations, thus these non-significant findings could be a result of a lack of statistical power. Additionally, these studies observed EI at only one meal served shortly after exercise; it is not clear if acute EI compensatory responses would become evident in overweight and obese over a longer observation period. There is a lack of evidence observing these responses over multiple meals in this participant group, and as a result it is difficult to ascertain if negative energy balance achieved is compensated for after experimental observation. Participants may be influenced by perceived social pressure induced by the knowledge that researchers conducting these studies can observe EI (Herman and Polivy, 2005), and may be more likely to limit consumption during observation and increase EI by consuming food later. It is possible that longer observation periods may increase the likelihood of observing normal eating behaviour. Although this is in part purely speculative, and would be impossible to prove experimentally, it is important to consider and minimise all possible

adverse influences on eating behaviour in order to achieve the most accurate results.

1.3.2.2 Chronic effect of exercise on energy intake in overweight and obese

The primary outcome of interest in this section of the review was the effect of chronic exercise participation on EI. Inclusion criteria for studies in this section (summarised in table 1.14) were:

- Published between 1980 and 2011.
- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Supervised aerobic exercise intervention with known exercise energy expenditure, of >12 weeks duration.
- Measurement of ad-libitum EI at baseline and during intervention as a primary outcome.
- Including measurement of exercise-induced body composition changes.

Exercise may acutely induce short-term negative energy balance, but longer term evidence is required to assess the prevalence of compensatory responses in the long term, and the potential impact on exercise-induced body composition changes. Findings from acute studies cannot be used to make assumptions about exercise as a method of maintaining or reducing body mass due to their short term nature, although it is tempting to do so, and many authors have attempted to apply short term findings in this context.

It has been suggested that energy balance regulation seems to be more sensitive in lean individuals than overweight or obese (King *et al*, 1997a) therefore it may be expected that overweight and obese would be less likely to compensate for ExEE. This has largely been shown not to be the case in longer term interventions with overweight and obese; many longer interventions have failed to observe the existence of EI compensatory responses in EI despite body composition indicating otherwise. This is likely due to inaccuracy of EI measures masking EI changes,

rather than providing robust evidence that compensatory changes do not occur. No significant changes in EI have been observed in overweight men and women completing a 16 month exercise intervention, despite the fact that the latter group did not significantly reduce body mass or fat during the intervention (Donnelly *et al*, 2003a,b). EI was directly observed in a cafeteria for two weeks periods at three monthly intervals in this study, but this seems not to have provided an adequate measure of EI. The abnormal setting and knowledge that EI was observed may have affected these participants EI. Body composition changes may provide better indication of the presence of compensatory responses because of these limitations. The body composition results indicate that some form of compensatory response must have occurred, either in EI or none exercise PA. A lower ExEE in the female participants may also have contributed to these disappointing body mass changes, but this difference cannot completely explain these body composition results.

The previously mentioned study of King *et al* (2008) did find robust evidence of EI compensatory responses in overweight men and women by analysing data on an individual basis. As the majority of studies analyse and report only mean changes, it is possible that many studies may simply have failed to observe compensatory responses. Overweight and obese men and women in this study exercised for twelve weeks, and did achieve a significant mean reduction in body fat, but there was also huge individual variability in the extent of these changes. Only ~50% of participants achieved or exceeded predicted body fat loss, whilst the remaining 50% fell short, in some cases gaining body fat mass. Analysing these participants as separate groups revealed a clear, explanatory, divergent EI response; the latter increased daily whilst the former decreased EI. Further evidence of these individual responses gathered from a greater number of overweight and obese men and women completing the same study protocol were published in 2009. It was found that exercise induced an increase in fasting and average daily hunger, as well as an increase in satiety responses to food ingestion. The authors argued that not all individuals experience both of these changes to the same degree; individuals who do not experience increased satiety would be predisposed to increase EI in response to exercise (King *et al*, 2009). These authors also assessed acute EI responses to exercise before and after the twelve week intervention; some individuals reported an increase in hedonic

response to food after a single exercise session at baseline and after twelve weeks of exercise participation. Those individuals failing to reduce body mass also seems to have a greater innate preference for high-fat, sweet foods, which is unaffected by exercise participation (Finlayson *et al*, 2011). EI and food choice was not measured in this study however, so it is unclear if these changes translate to effects on subsequent EI. These results present compelling evidence of highly individual responses to exercise which contribute to susceptibility to compensatory mechanisms.

1.3.2.3 Gender differences

The literature reviewed thus far has shown that susceptibility to compensatory mechanisms may vary between genders. Evidence from exercise intervention studies (table 1.15) often observes smaller body composition changes in women compared to men, and this may be in part because women seem most likely to compensate for ExEE. One possible explanation for this observation is that compensatory behaviours act as a means of conserving energy stores and thus defending reproductive function in women, since low energy availability had been observed to affect reproductive hormone levels in women (Loucks *et al*, 1998; Loucks and Thuma, 2003). Evidence regarding these differences is limited, and the majority of existing studies in this area are conducted with lean individuals; it is not clear if gender differences exist between overweight and obese men and women, and due to the lack of data from overweight and obese findings obtained from lean men and women will be included and discussed in this section.

The previously mentioned study of Westerterp *et al* (1992) observed that women training for a half marathon had a tendency to increase EI over the long term, whilst the men decreased EI in the latter half of the observation period. Changes in EI were not statistically significant, but small perturbations in EI over the long term can have a significant effect on body composition. A pair of small, medium term studies conducted with lean men and women also found that women were more likely than men to compensate for ExEE. A seven day exercise protocol

induced negative energy balance in men but a partial EI compensatory response was seen in women completing the same protocol (Stubbs *et al*, 2002a,b). Although sample sizes were small, participants lean and EI assessed by self-reported intake, these results are supported by findings of another, similar study (Whybrow *et al*, 2008).

However, not all studies have found evidence of a gender based differences in lean men and women. A small study of lean participants completing a 5 day exercise protocol found that it was actually men who increased EI, whilst remaining unchanged in the women (Staten *et al*, 1991). The discrepancy between results of this study and similar protocols may be attributable to dietary influences; Stubbs *et al* used self-reported EI measures, whereas this study supplied participants with a liquid meal replacement formula, and restricted the food items participants could choose to consume from. The observed decrease in EI may be due to poor palatability of the diet affecting intake.

Evidence regarding gender differences in compensatory responses is mixed and it is not clear if women are more susceptible to these changes; body composition changes suggest this may be the case but other factors may contribute. Further research is needed to determine if overweight and obese men and women differ in their response to exercise as at present no definitive evidence exists with this population group.

1.3.2.4 Rate and extent of compensatory changes in energy intake

There is evidence that compensatory changes are gradual and partial, although this evidence is obtained entirely from relatively small samples of lean individuals. As these results provide some interesting information that could potentially apply to obese, or present a potential avenue for future research with overweight and obese, some discussion of these findings seems relevant.

Stubbs *et al* (2002b) provided evidence that compensation for ExEE in the short term was partial in lean women (33%). Further evidence suggested that these responses were also gradual in nature. Regression analyses of data from lean men and women completing a seven day exercise protocol revealed that small, gradual compensatory changes in both EE and EI occurred, acting to restore neutral energy balance in men and women. Changes in EI were equivalent to 0.2 MJ day^{-1} and EE 0.35 MJ day^{-1} (Stubbs *et al*, 2004). These findings were corroborated by the findings of another study which reported that both male and female lean participants compensated for ExEE by approximately 30% during a 16 day exercise protocol (Whybrow *et al*, 2008), a figure strikingly similar to that previously reported in Stubbs *et al* (2002b; 2004b). There was also considerable individual variability in the extent of compensation in these participants, a finding which agrees with those of King *et al*, 2008. The small and gradual nature of compensatory changes observed here may also go some way to explaining why so many studies do not detect these responses; studies of shorter and duration may not have the statistical power to detect changes which are gradual and therefore of rather small magnitude in the short term. Additionally, it is not clear from this evidence if full compensation would eventually occur in the long term. It should be noted that both of these studies are of short duration and small sample size, which may limit the strength of findings considerably.

There are mixed findings in the literature regarding the presence of exercise-induced compensatory EI responses, and a problem in this field is that such responses may occur and escape detection. It has been illustrated that there are three key factors which may contribute to this problem:

- Methods of assessing EI are lacking in accuracy, and many studies rely on self-reported measures which may give misleading values.
- Compensatory responses to exercise may be highly individual, and studies analysing only group mean values may fail to observe EI changes occurring in some participants.
- Compensatory changes may be partial and/or gradual, and therefore changes may be very small and thus even more difficult to detect.

There are clearly many factors to consider in this field, and there is a need for future studies to be more robust and try to minimise these problems. It may be that comparison of actual to expected body mass changes will provide a superior method of detecting compensatory responses since it is unlikely there will ever be an EI assessment method of sufficient accuracy to do so consistently.

Table 1.13 Evidence regarding the acute effects of exercise on energy intake in lean and overweight/obese men and women.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention(s)	Control trial?	Test meal	Results
George & Morgenstein (2003)	12 lean and 12 overweight women	Lean: 22 ± 1 Overweight: 28 ± 1 (mean ± SD)	35 ± 8 years (mean ± SD)	Not measured	1 hour treadmill walking session conducted at 60% HR _{max}	Yes	Buffet lunch chosen from the normal selection of lunch foods served in the university cafeteria.	Estimated ExEE: 628-837 kJ Exercise had no effect on EI in either group, though overweight women ate significantly more than lean women at rest and post-exercise (p=0.03). EI (kJ): Lean Control: 1845 ± 540 Exercise: 1502 ± 536 (mean ± SD) Obese 2393 ± 724 2410 ± 1100
Kissileff <i>et al.</i> , (1990)	9 lean and 9 obese women	Lean: 22.1 ± 1.8 Obese: 27.7 ± 0.9 (mean ± SD)	Lean: 22.7 ± 4.9 Obese: 24.3 ± 4.9 (mean ± SD)	Not measured	Two 40 minute cycling exercise sessions at conducted at high (90W) and low (30W) intensity	Yes	Liquefied test meal served 15 minutes after intervention period.	ExEE (kJ): Lean Low intensity: 476 ± 154 High intensity: 1033 ± 102 (mean ± SD) Obese 599 ± 139 992 ± 105 Intake of test meal was significantly lower (p<0.05) after high intensity cycling in the lean women but there were no differences in intake in the obese participants (p>0.05). Food intake (g): Lean Control: 708 ± 305 Low intensity exercise: 754 ± 251 High intensity exercise: 621 ± 327 (mean ± SD) Obese 633 ± 481 583 ± 252 531 ± 265
Schneider <i>et al.</i> (2009)	65 overweight men (33.8%) and women (66.2%)	33.5 ± 5.5 (mean ± SD)	34.4 ± 10.8 (mean ± SD)	Not measured	3 minute step test	Yes	Snack foods - cookies and crisps.	ExEE: not reported No difference in EI between trials (p>0.05). Those who reported increased negative mood after the step test consumed an average of 288 kJ more post-exercise than those who reported no changes or a decrease in negative mood (p<0.05).
Visona & George (2003)	36 overweight women	D-HR: 28 ± 3 ND-HR: 27 ± 2	D-HR: 31 ± 8 ND-HR: 23 ± 3	Not measured	60 minutes treadmill walking exercise at 60-70% HR _{max}	Yes	Buffet lunch chosen from the normal selection of lunch foods served in the university cafeteria and participants self-reported EI for the rest of the day.	ExEE: 916 kJ No effect of exercise was seen on whole group EI at a post-exercise lunch test meal, or EI over the whole day (p>0.05).

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention(s)	Control trial?	Test meal	Results																				
		ND-LR: 30 ± 3 (mean ± SD)	ND-LR: 25 ± 8 (mean ± SD)					12 hour EI was significantly lower on day of control trial compared to exercise trial day in dieting women with high restraint only (p<0.01). EI (kJ): <table border="1"> <thead> <tr> <th colspan="2">Lunch meal</th> <th colspan="2">12 hour</th> </tr> <tr> <th>Exercise</th> <th>Control</th> <th>Exercise</th> <th>Control</th> </tr> </thead> <tbody> <tr> <td>D-HR: 2540 ± 1071</td> <td>1849 ± 782</td> <td>6259 ± 1862</td> <td>4452 ± 2205</td> </tr> <tr> <td>D-HR: 2364 ± 1042</td> <td>2899 ± 1364</td> <td>5213 ± 937</td> <td>5950 ± 1590</td> </tr> <tr> <td>ND-LR: 3707 ± 1494</td> <td>2858 ± 1418</td> <td>6782 ± 1954</td> <td>6192 ± 2360</td> </tr> </tbody> </table> (mean ± SD)	Lunch meal		12 hour		Exercise	Control	Exercise	Control	D-HR: 2540 ± 1071	1849 ± 782	6259 ± 1862	4452 ± 2205	D-HR: 2364 ± 1042	2899 ± 1364	5213 ± 937	5950 ± 1590	ND-LR: 3707 ± 1494	2858 ± 1418	6782 ± 1954	6192 ± 2360
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Ueda <i>et al</i> (2009a)	7 lean and 7 obese males	Lean: 22.4 ± 2.4 Obese: 30.0 ± 3.1 (mean ± SD)	Lean: 22.4 ± 4.2 Obese: 22.9 ± 3.4 (mean ± SD)	Lean: 46.6 ± 3.9 Obese: 34.0 ± 6.3 (mean ± SD)	60 mins cycle ergometer exercise at 50% $\dot{V}O_{2max}$	Yes	Pasta based meal.	ExEE: Not reported EI and REI were lower in exercise than control trial in both groups (p<0.001). EI and REI during exercise trial were significantly lower in obese participants than lean participants (p<0.05). No effect of exercise on appetite (p>0.05). EI (kJ): <table border="1"> <thead> <tr> <th colspan="2">Lean</th> <th colspan="2">Obese</th> </tr> <tr> <th>Resting</th> <th>Exercise</th> <th>Resting</th> <th>Exercise</th> </tr> </thead> <tbody> <tr> <td>EI: 3503 ± 477</td> <td>2893 ± 447</td> <td>EI: 3946 ± 736</td> <td>2567 ± 364</td> </tr> <tr> <td>REI: 2642 ± 485</td> <td>819 ± 451</td> <td>REI: 2767 ± 640</td> <td>-389 ± 468</td> </tr> </tbody> </table> (mean ± SD)	Lean		Obese		Resting	Exercise	Resting	Exercise	EI: 3503 ± 477	2893 ± 447	EI: 3946 ± 736	2567 ± 364	REI: 2642 ± 485	819 ± 451	REI: 2767 ± 640	-389 ± 468				
Lean		Obese																										
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EI: 3503 ± 477	2893 ± 447	EI: 3946 ± 736	2567 ± 364																									
REI: 2642 ± 485	819 ± 451	REI: 2767 ± 640	-389 ± 468																									
Unick <i>et al</i> (2010)	19 overweight and obese women	32.5 ± 4.3 (mean ± SD)	28.5 ± 8.3 (mean ± SD)	Fitness 6.5 ± 3.3 (submax MET's) (mean ± SD)	Moderate intensity walking for 40mins at 70-75% age-predicted maximum.	Yes	Buffet lunch meal consisting of a selection of snack foods.	ExEE: 1471 ± 301 kJ (mean ± SD) No difference in total EI (p=0.92), REI was significantly lower in exercise trial (p<0.001). EI (MJ): <table border="1"> <thead> <tr> <th>Control</th> <th>Exercise</th> </tr> </thead> <tbody> <tr> <td>EI: 2.29 ± 1.2</td> <td>2.31 ± 1.0</td> </tr> <tr> <td>REI: 2.11 ± 1.2</td> <td>0.83 ± 1.1</td> </tr> </tbody> </table> (Mean ± SD)	Control	Exercise	EI: 2.29 ± 1.2	2.31 ± 1.0	REI: 2.11 ± 1.2	0.83 ± 1.1														
Control	Exercise																											
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REI: 2.11 ± 1.2	0.83 ± 1.1																											

Table 1.14 Evidence regarding the chronic effects of exercise on energy intake in overweight and obese men and women.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean VO _{2max} (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy intake assessment	Δ body fat mass (kg)	Energy Intake results	Attrition rate
Donnelly <i>et al</i> (2003a,b)	Overweight men (Control group n=15, exercise group n=16) and women (Control group n=18, exercise group n=25)	Men Control group: 29.0 ± 3.0 Exercise group: 29.7 ± 2.9 Women Control group: 29.3 ± 2.3 Exercise group: 28.7 ± 3.2 (mean ± SD)	Not reported, age range 17-35 years.	Men Control group: 39.5 ± 5.7 Exercise group: 39.2 ± 5.0 Women Control group: 32.4 ± 3.1 Exercise group: 32.8 ± 4.2 (mean ± SD)	16 months	Five 45 min sessions of moderate (60-75% HRR) treadmill walking exercise per week.	Ad-libitum EI was directly observed every three months for two weeks; during these periods participants consumed all meals in the university cafeteria and food intake was observed, any snacks consumed outside cafeteria were reported by participants.	Men Control group: -0.5 (SD not reported) Exercise group: -4.9 ± 4.4 Women Control group: 2.1 ± 4.8 Exercise group: -0.3 ± 2.7 (mean ± SD)	ExEE: 1674 kJ/session No differences in EI between groups group (p>0.05). EI (MJ): Men Baseline 16 months Control 14.7 ± 3.2 13.5 ± 3.2 Exercise 12.9 ± 2.4 13.2 ± 3.3 Women Baseline 16 months Control 9.9 ± 2.1 10.3 ± 2.5 Exercise 10.7 ± 2.1 10.1 ± 2.2 (mean ± SD)	52.9%
King <i>et al</i> (2008)	35 overweight and obese men (n=10) and women (n=25)	31.8 ± 4.1 (mean ± SD)	39.6 ± 11.0 (mean ± SD)	28.4 ± 5.8 (mean ± SD)	12 weeks	Aerobic exercise sessions to expend 2090 kJ/session at 70% HR _{max} on 5 days/week.	Ad-libitum buffet lunch and dinner test meals served in laboratory at baseline and week 12.	Whole group: -3.7 ± 2.6 Compensators: -5.3 ± 2.2 Non-compensators: -2.1 ± 2.3 (mean ± SD)	ExEE: 10.5 MJ/week There was no significant change in EI between weeks 0 and 12 for the whole group (p>0.05). Non-compensators decreased EI by -544 kJ/day and C increased EI by 1121 kJ/day. The difference in EI was significant between groups (p<0.05).	-
King <i>et al</i> (2009)	58 overweight and obese men (n=19) and women (n=39)	31.8 ± 4.5 (mean ± SD)	39.6 ± 9.8 (mean ± SD)	29.1 ± 5.7 (mean ± SD)	12 weeks	Aerobic exercise sessions to expend 2090 kJ/session at 70% HR _{max} on 5 days/week.	Ad-libitum buffet lunch and dinner test meals served in laboratory at baseline and week 12.	Whole group: -3.2 ± 2.2 kg Responders: -15.3 % Non-responders: -4.7 %	ExEE: 10.5 MJ/week Non-responders increased EI by 686 kJ/day, and responders decreased EI by 527 kJ/day, between baseline and week 12.	-

Table 1.15 Evidence regarding gender differences in the effect of medium and long term exercise interventions on energy intake responses in lean and overweight men and women.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy intake assessment	Δ body fat mass (kg)	Energy Intake results									
Donnelly <i>et al</i> (2003b)	Refer to table 1.14 for details																	
Staten <i>et al</i> (1991)	10 lean men and 10 lean women	Not reported Height: 168 ± 12 cm Body mass: 62.5 ± 8.3 kg (mean ± SD)	Not reported	42.1 ± 7.7 (mean ± SD)	5 days	1 hour treadmill exercise at 70% $\dot{V}O_{2max}$ every day	Participants consumed all meals at research centre and intake was weighed and recorded by staff.	Not reported	<p>ExEE (kJ/day): Men: 2801 ± 528 Women: 1846 ± 385</p> <p>EI was significantly greater in the exercise protocol, compared to control, for men only (p<0.05).</p> <p>EI (kJ/day):</p> <table> <tr> <td></td> <td>Exercise</td> <td>Control</td> </tr> <tr> <td>Men</td> <td>11242 ± 787</td> <td>10366 ± 691</td> </tr> <tr> <td>Women</td> <td>7662 ± 381</td> <td>7666 ± 431</td> </tr> </table> <p>(mean ± SEM)</p>		Exercise	Control	Men	11242 ± 787	10366 ± 691	Women	7662 ± 381	7666 ± 431
	Exercise	Control																
Men	11242 ± 787	10366 ± 691																
Women	7662 ± 381	7666 ± 431																
Stubbs <i>et al</i> (2002a)	6 lean men	23.3 ± 2.4 (mean ± SD)	31.0 ± 5.3 (mean ± SD)	42.7 ± 6.1 (mean ± SEM)	7 days, preceded by a two day diet standardisation period.	Participants completed 3 seven day protocols: 1. Nex - no exercise, 2. Mex - two 40 minute cycle ergometer exercise sessions/day 3. Hex - three 40 minute cycle ergometer exercise sessions/day	Weighed seven day food record using portable electronic tape recording automated scales.	<p>Body mass change (kg) Nex: -0.72 Mex: -0.58 Hex: -1.2 (range not reported)</p>	<p>ExEE (MJ/day): Mex: 1.6 Hex: 3.2</p> <p>No significant differences in EI between trials (p>0.05).</p> <p>Total daily EE (MJ/day) Nex: 11.7 Mex: 12.9 Hex: 16.8</p> <p>EI (MJ/day) Con: 11.6 Mex: 11.8 Hex: 11.8</p> <p>(ranges not reported)</p>									
Stubbs <i>et al</i> (2002b)	6 lean females	21.4 ± 1.0 (mean	23.0 ± 0.6 (mean ±	33.4 ± 2.5 (mean ± SEM)	7 days, preceded by a two day diet standardisation	Participants completed 3 seven day protocols:	Weighed seven day food record using portable	<p>Body mass change (kg) Nex: -0.4</p>	<p>ExEE (MJ/day) Mex: 1.9 Hex: 3.4</p>									

Authors	Participants	Mean BMI (kg/m ²) ±SEM)	Mean Age (years) SEM)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy intake assessment	Δ body fat mass (kg)	Energy Intake results
					period.	1. Nex - no exercise, 2. Mex - two 40 minute cycle ergometer exercise sessions/day 3. Hex - three 40 minute cycle ergometer exercise sessions/day	electronic tape recording automated scales.	Mex: -0.5 Hex: -0.8 (range not reported)	There was a significant differences in EI between trials (p=0.035). Total daily EE (MJ/day) Nex: 9.2 Mex: 11.0 Hex: 12.1 EI (MJ/day) Con: 8.9 Nex: 9.2 Mex: 10.0 (ranges not reported)
Westerterp <i>et al</i> (1992)	32 lean and slightly overweight men (n=12) and women (n=11)	Not reported, range 19.4 - 26.4	Not reported, range 28-41 years	Not reported	44 weeks	Aerobic exercise intervention with the aim of training to complete a half marathon. Participants trained four sessions/week, increasing running time to 10-30 min, 20-60 min and 30-90 minutes per training session after 8, 20 and 40 weeks respectively.	7 day weighed food record	Men: -3.8 Women: -2.0 (median)	ExEE: Not reported There was a tendency for men to increase EI and women to decrease, though this was not significant (p>0.05). EI (MJ): Baseline Week 40 Men: 11.8 ± 8.0 10.2 ± 7.1 Women: 9.1 ± 4.3 9.5 ± 6.3 (median ± range)

1.3.3 Compensatory reductions in habitual physical activity

Research into compensatory mechanisms has thus far been mainly focused on EI changes, but as this is only one part of overall energy balance it is necessary to consider the contribution of compensatory changes in non exercise EE. EE is easier to measure than EI due to the existence of physiological measurement techniques such as doubly labelled water, heart rate monitoring and calorimetry which do not rely on accurate participant self reporting.

The primary outcome of interest in this section of the review was the effect of chronic exercise participation on EI. Inclusion criteria for studies in this section (summarised in table 1.16) were:

- Published between 1980 and 2011.
- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Supervised aerobic exercise intervention with known exercise energy expenditure, of >8 weeks duration. Studies with a dietary energy restriction are included if energy restriction is constant for all participants for the duration of the study.
- Measurement of total and/or physical activity energy expenditure at baseline and during intervention as a primary outcome.
- Including measurement of exercise-induced body composition changes.

1.3.3.1 Evidence from overweight and obese

As reported by Stubbs *et al* (2004b), compensatory changes in EE may be gradual in lean individuals in the short term. Longer term evidence from obese has corroborated this finding. Obese women completing an eight week exercise intervention accompanied by EI restriction exhibited no change in PA EE levels during the protocol, indicating partial compensation occurring through a

reduction in non ExEE. This effect was not seen in the group who restricted EI without exercising (Kempen *et al*, 1995). A similar 8 week protocol observed the highly individual nature of compensatory EE changes in overweight and obese women. Women in this study failed to reduce body mass despite high compliance to the intervention, and there was a large degree of individual variability in fat mass change. A diverging response in non exercise EE was observed to explain this variability; women who successfully reduced body fat increased their daily EE out with exercise sessions, whilst those who did not achieve a body fat reduction reduced total EE in a compensatory manner (Manthou *et al*, 2010). As with the EI findings reported by King *et al* (2008), compensatory EE changes vary greatly between overweight and obese individuals.

Evidence suggests that middle-aged adults may be less susceptible to compensatory EE changes. Middle-aged, overweight men and women randomised to groups completing differing volumes and intensities of exercise for eight months all increased their total EE levels, with high amount/vigorous intensity group achieving the greatest increase as expected (Hollowell *et al*, 2009). Body mass changes were not reported so it is not possible to fully assess whether other compensatory responses may have occurred. A larger sample of overweight, middle-aged adults with moderate dislipidaemia also found no evidence of compensatory EE changes during an 8 month aerobic or resistance exercise intervention (Rangan *et al*, 2011). Total EE was unchanged for participants in the resistance exercise group, despite no apparent change in non ExEE, and this may be due to the low ExEE of resistance exercise rather than any compensatory response. These results were further corroborated by another study of middle-aged, overweight men completing a 6 month exercise intervention who also observed an increase in total EE with no observed reduction in non-exercise EE. Body mass losses were only approximately 40% of predicted losses, based on known ExEE, and the authors postulated that these men partially compensated via EI during this intervention (Turner *et al*, 2010). Similar to middle-aged women, compensatory changes in EE do not seem to occur in this group but as ever this does not rule out the presence of compensatory changes in EI.

1.3.3.2 Evidence from elderly individuals

Existing evidence consistently indicates that elderly adults are prone to EE compensatory responses. One eight week progressive exercise protocol with a small sample of lean and overweight elderly observed no significant change in TEE, indicating a reduction in PA out with exercise sessions (Goran and Poehlman, 1992). Likewise, a twelve week study of overweight elderly men and women completing an intervention composed of resistance and endurance exercise observed no difference in PA between exercise and control participants. Compensation seemed likely to be due to increased fatigue since non exercise activity levels were significantly lower on days exercise was completed compared to sedentary days (Meijer *et al*, 1999). Morio *et al* (1998) reached the same conclusion using a study protocol similar to that of Goran and Poehlman (1992). Overweight, elderly individuals completing a progressive 14 week exercise intervention gradually reduced their non exercise EE levels throughout the intervention. This supports earlier evidence suggesting that compensatory changes are gradual and partial (Stubbs *et al*, 2004; Whybrow *et al*, 2008), and provides evidence that this holds true for elderly, overweight men and women.

1.3.4 General limitations of the Literature

It is important to appreciate the general limitations of the literature in this area. As mentioned previously, the main limitation in this field is the difficulty of obtaining a reliable estimate of EI. All available methods have some serious limitations and therefore EI estimates can be questioned. Obese participants, particularly females, may be prone to underreporting dietary intakes in self recorded diaries (Macdiarmid and Blundell, 1998). This may be part of the reason that 65% of studies examining compensatory responses to exercise fail to detect them (Blundell and King, 1999). Many studies also fail to assess ExEE, making it difficult to use body mass changes to assess the likelihood of compensation in the absence of accurate EI measured since expected changes cannot be

predicted. Differences between protocols, exercise mode and intensity, and the characteristics of participants make it difficult to readily compare studies too. Unfortunately conclusions from a lean population cannot be readily generalised to obese and thus there is a lack of short term regulatory information from obese and a lack of long term information from lean. Small samples sizes with no reported power calculation are also common in this field. As a result of these limitations, some evidence may be inconclusive. Research seeking to detect EI compensatory responses is more inconclusive than that regarding EE responses, in large part because the methodology available to measure the latter is far more robust and objective. In many cases it seems EI compensation does not occur, but it is likely that responses are not detected with small sample sizes and available methodology.

Table 1.16 Evidence regarding the effects of chronic exercise participation on habitual physical activity levels and energy expenditure in overweight and obese men and women.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate
Goran & Poehlman (1992)	11 overweight, elderly men (n=5) and women (n=6)	Not reported Body fat: 30.5 ± 8.9 % (mean ± SD)	66 ± 6 (mean ± SD)	2.0 ± 0.7 L/min (mean ± SD)	8 weeks	Progressive, aerobic exercise intervention. Participants began by completing three cycle exercise sessions/week at 60% $\dot{V}O_{2max}$ to expend 627 kJ at a time, and progressed to a maximum of three sessions per week completed at 85% $\dot{V}O_{2max}$, to expend 1254 kJ per session.	Doubly labelled water	-0.89 ± 0.71 (mean ± SD)	There was no significant change in TEE between weeks 0 and 8 (p>0.05). TEE (MJ/day): Baseline: 10.1 ± 2.0 Week 8: 10.4 ± 2.1 (mean ± SD) PAEE was reduced by 62% at week 8 compared to baseline (p<0.01). PAEE (MJ/day): Baseline: 2.4 ± 1.6 Week 8: 1.4 ± 1.9 (mean ± SD)	Not reported.
Hollowell <i>et al</i> (2009)	50 overweight men (52%) women (48%)	Con: 31 ± 3 LAVI: 30 ± 3 LAMI: 29 ± 3 HAVI: 30 ± 2 (mean ± SD)	Con: 51 ± 7 LAVI: 54 ± 6 LAMI: 57 ± 6 HAVI: 51 ± 6 (mean ± SD)	$\dot{V}O_{2peak}$: Con: 28 ± 7 LAVI: 29 ± 7 LAMI: 28 ± 7 HAVI: 29 ± 5 (mean ± SD)	8 months	Participants were randomised to one of four groups: 1. Non-exercising controls (n=8) 2. Low amount/vigorous intensity exercise (LAVI: ExEE 5023 kJ week ⁻¹ at 65-80% $\dot{V}O_{2peak}$; n=14) 3. Low amount/moderate intensity exercise (LAMI: ExEE 5023 kJ week ⁻¹ at 40-55% $\dot{V}O_{2peak}$; n=14)	Accelerometer and heart rate monitoring	Not reported	All exercise groups significantly increased PAEE (p<0.05). The intervention resulted in significantly greater PAEE in HAVI compared to control and LAVI groups (p<0.05). Change in total PAEE (kJ/hour): Con: 7.5 ± 58.6 LAVI: 49.4 ± 115.1 LAMI: 74.9 ± 101.3 HAVI: 137.3 ± 122.2 (mean ± SD) Exercise groups increased non exercise PAEE, though not significantly (p=0.08 for HA and p=0.24 for LA groups). Non exercise PAEE (kJ/hour): Con: -6.3 ± 47.3 LA: 26.0 ± 103.8	32.6%

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate									
						4. High amount/vigorous intensity (HAVI: ExEE 8372 kJ week ⁻¹ at 65-80% $\dot{V}O_{2peak}$; n=14)			HA: 62.0 ± 85.4										
Kempen <i>et al</i> (1995)	20 obese women	D: 31.7 ± 0.9 DE: 32.4 ± 1.3 (mean ± SEM)	D: 36.6 ± 2.3 DE: 39.3 ± 5.4 (mean ± SEM)	Approximately 44 ml/kg FFM/min (exact values not reported)	10 weeks	Participants were randomised to one of two groups. All participants restricted dietary intake to 3.5 MJ per day (D), consuming a mixture of low energy formula meal replacements and solid foods. 1. Dietary restriction only group (D; n=10) 2. Dietary restriction plus participation in three 90 min aerobics classes per week, 50-60% $\dot{V}O_{2max}$ (DE; n=10)	Indirect calorimetry and doubly labelled water	D: -5.5 ± 0.8 DE: -7.8 ± 0.8 (mean ± SEM)	DE were in significantly greater negative energy balance than D (p<0.05), but there was no difference in activity EE between groups at any point (p>0.05). Average daily energy balance (MJ): D: -3.2 ± 0.5 DE: -4.9 ± 0.6 Activity EE (MJ/day): <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>Week 8</th> </tr> </thead> <tbody> <tr> <td>D:</td> <td>4.0 ± 0.3</td> <td>3.9 ± 0.3</td> </tr> <tr> <td>DE:</td> <td>3.8 ± 0.4</td> <td>4.0 ± 0.5</td> </tr> </tbody> </table> (mean ± SEM)		Baseline	Week 8	D:	4.0 ± 0.3	3.9 ± 0.3	DE:	3.8 ± 0.4	4.0 ± 0.5	Not reported.
	Baseline	Week 8																	
D:	4.0 ± 0.3	3.9 ± 0.3																	
DE:	3.8 ± 0.4	4.0 ± 0.5																	
Manthou <i>et al</i> (2010)	34 overweight women	29.3 ± 4.4 (mean ± SD)	31.7 ± 8.1 (mean ± SD)	2.1 ± 0.4 L/min (mean ± SD)	8 weeks	150 minutes of supervised cycling exercise per week at 90-95% of their lactate threshold. ExEE of the intervention was 30.2 MJ	7 day PA diary combined with HR monitoring	Whole group: 0.04 ± 0.24 Responders: -1.75 ± 0.19 Non-	Daily TEE increased significantly for the whole group (p=0.01). The increase in daily TEE tended to be higher in responders than non-responders (p=0.08). Δ TEE (MJ/day) Whole group: +0.62 ± 0.30 Responders: +1.44 ± 0.49 Non-responders: +0.29 ± 0.36 (mean ± SEM)	Not reported									
								Non-	Increase in daily active EE as significantly higher in										

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate									
								responders: 0.62 ± 0.60 (mean ± SEM)	responders compared to non-responders (p=0.05). <u>Δ active EE (MJ/day)</u> Responders: +0.79 ± 0.50 Non-responders: -0.62 ± 0.39 (mean ± SEM)										
Meijer <i>et al</i> (1999)	22 overweight, elderly adults	Control: 25.1 ± 1.1 Exercise: 25.6 ± 3.1 (mean ± SD)	Control: 57.4 ± 2.6 Exercise: 58.9 ± 3.5 (mean ± SD)	Control: 1.99 ± 0.5 Exercise: 2.03 ± 0.5 (L/min; mean ± SD)	12 weeks	Participants in the exercise group (n=15) completed a 1 hour group aerobic session led by an instructor, and a 90 min individual session including aerobic and resistance exercises, every week. Control participants maintained normal lifestyle (n=7).	14 days tri-axial accelerometry monitoring	<u>Δ Body fat %:</u> Control: +1.6 ± 0.4 Exercise: +0.5 ± 0.4 (mean ± SEM)	Physical activity did not differ between baseline and week 12 in the exercise or control group (p>0.05). <u>Physical activity (counts/day)</u> <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>Week 12</th> </tr> </thead> <tbody> <tr> <td>Exercise group:</td> <td>25572 ± 5016</td> <td>23932 ± 5093</td> </tr> <tr> <td>Control group:</td> <td>23990 ± 5176</td> <td>24568 ± 6358</td> </tr> </tbody> </table> At week 12 the difference in physical activity between training and non-training days in the exercise group did not reach statistical significance. (p=0.06) <u>Physical activity (counts/day)</u> Training Day: 25234 ± 4690 Non-training day: 23575 ± 5543 (all values mean ± SD)		Baseline	Week 12	Exercise group:	25572 ± 5016	23932 ± 5093	Control group:	23990 ± 5176	24568 ± 6358	Not reported
	Baseline	Week 12																	
Exercise group:	25572 ± 5016	23932 ± 5093																	
Control group:	23990 ± 5176	24568 ± 6358																	
Morio <i>et al</i> (1998)	13 overweight, elderly adults (5 men, 8 women)	Not reported. Body fat %: 32.6 ± 7.9 (mean ± SD)	62.8 ± 2.3 (mean ± SD)	1720 ± 531 (ml/min; mean ± SD)	14 weeks	Participants completed three cycling exercise sessions/week. Sessions were 20 min in duration at an intensity of 50% $\dot{V}O_{2max}$ at baseline, and duration and intensity progressively increased until ExEE was 0.6 MJ/day.	7 day activity record and indirect calorimetry	Not reported Week 14 % body fat: 30.0 ± 9.4 (mean ± SD)	Daily TEE was unchanged throughout the intervention (p=0.34). <u>TEE (MJ/day):</u> Baseline: 10.3 ± 2.4 Week 7: 10.6 ± 2.4 Week 14: 10.3 ± 2.5 (mean ± SD)	Not reported									

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate															
Rangan <i>et al</i> (2011)	82 overweight and obese men (n=32) and women (n=50)	AT: 30.5 ± 3.1 AT/RT: 30.9 ± 3.2 RT: 30.3 ± 3.1 (Mean ± SD)	AT: 51.8 ± 9.2 AT/RT: 47.5 ± 11.0 RT: 50.6 ± 12.4 (mean ± SD)	AT: 27.3 ± 5.6 AT/RT: 26.9 ± 5.5 RT: 25.9 ± 6.3 (mean ± SD)	8 months	Participants were randomised to one of four groups: 1. AT: 59 kJ/kg body mass per week, energetically equivalent to approximately 12 miles/week, at 65%-80% $\dot{V}O_{2peak}$ 2. AT/RT: the combination of the full AT and the full RT programs 3. RT: 3 days/week, 3 sets per day on 8 different major muscle groups, 8-12 repetitions per set.	Tri-axial accelerometry	Not reported	There was non significant change in non-exercise PA in any group during the intervention (p>0.1). There was no change in total PA EE in the RT group (p=0.43), but PA EE was significantly higher in the AT and AT/RT groups as a result of the intervention (p<0.004). <u>Δ Total PAEE (kJ/hour)</u> AT: 38.4 ± 65.1 AT/RT: 55.8 ± 57.7 RT: 7.7 ± 51.2 <u>Δ Non exercise PA (kJ/hour)</u> AT: 1.6 ± 57.0 AT/RT: 19.1 ± 61.7 RT: -1.8 ± 44.4 (mean ± SD)	38.5%															
Turner <i>et al</i> (2010)	29 overweight men	Con: 27.6 ± 3.0 Ex: 28.1 ± 2.7 (mean ± SD)	Con: 53 ± 4 Ex: 55 ± 5 (mean ± SD)	Not reported	18 weeks	Participants were randomised to one of two groups: 1. Control - No exercise 2. Exercise - Progressive, aerobic exercise intervention consisting of four 1 hour sessions per week conducted at 70% $\dot{V}O_{2max}$	7 day heart rate and accelerometer recording	Δ Body mass (kg) Con: +0.2 ± 2.2 Ex: -1.8 ± 2.2 (mean ± SD)	Total PAEE was only significantly greater in the exercise group at week 18 of the intervention (p=0.004). Mean daily time engaged in PA (>3 METs) was significantly greater in exercise group throughout the intervention (p<0.04). <u>Mean daily PA time (minutes):</u> <table border="1"> <thead> <tr> <th></th> <th>Control</th> <th>Exercise</th> </tr> </thead> <tbody> <tr> <td>Baseline</td> <td>28 ± 14</td> <td>37 ± 23</td> </tr> <tr> <td>Week 2</td> <td>26 ± 20</td> <td>54 ± 33</td> </tr> <tr> <td>Week 9</td> <td>21 ± 16</td> <td>67 ± 45</td> </tr> <tr> <td>Week 18</td> <td>24 ± 26</td> <td>68 ± 42</td> </tr> </tbody> </table> (mean ± SD) Mean daily PAEE was not significantly different		Control	Exercise	Baseline	28 ± 14	37 ± 23	Week 2	26 ± 20	54 ± 33	Week 9	21 ± 16	67 ± 45	Week 18	24 ± 26	68 ± 42	16.7%
	Control	Exercise																							
Baseline	28 ± 14	37 ± 23																							
Week 2	26 ± 20	54 ± 33																							
Week 9	21 ± 16	67 ± 45																							
Week 18	24 ± 26	68 ± 42																							

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate
									between groups, or over time, when low intensity and moderate-vigorous activity intensity were analysed separately (p>0.05).	

1.3.5 Physiological compensatory mechanisms

Metabolic changes that have an impact on TEE may take place in response to body mass reduction and act as physiological compensatory mechanisms. TEE is made up of several factors; resting metabolic rate, activity EE, and the thermic effect of feeding. These components and their approximate relative contribution to total EE are shown in figure 1.4.

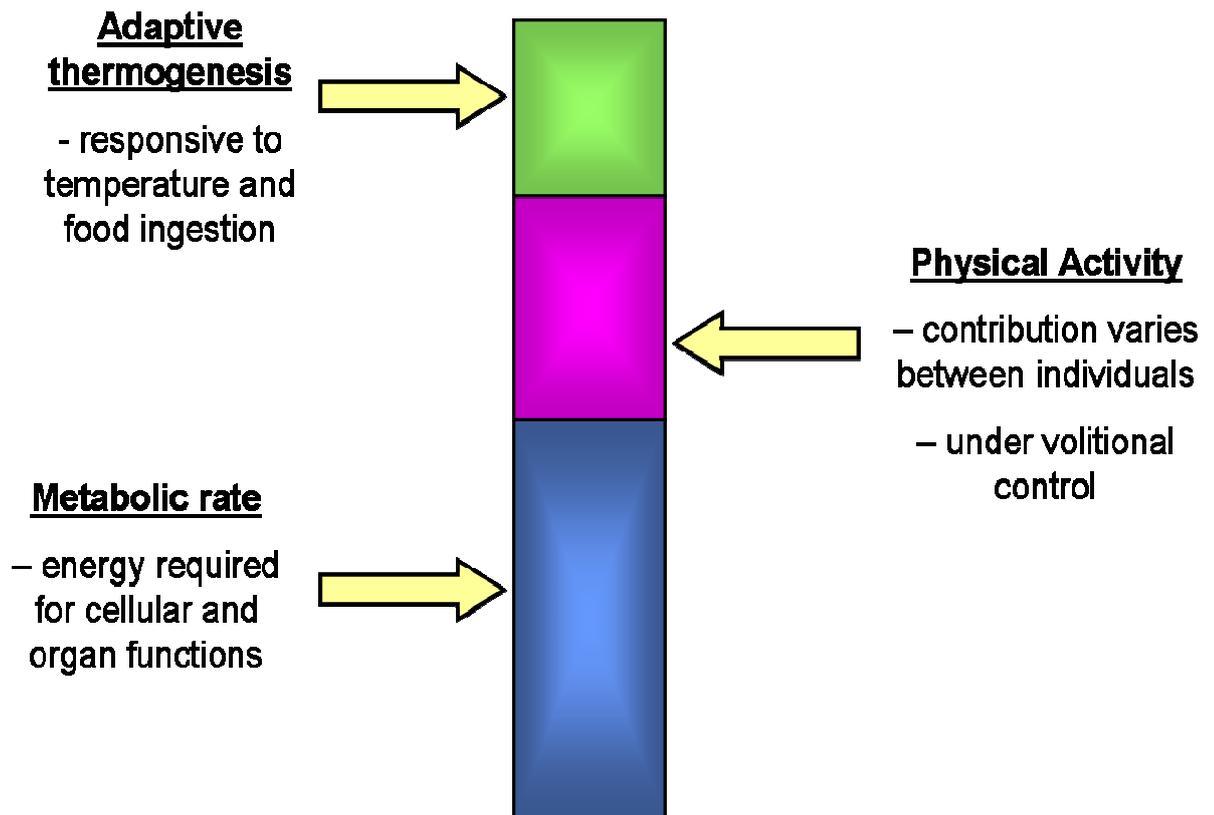


Figure 1.4 The components of total energy expenditure

Resting metabolic rate (RMR) is defined as the amount of energy expended while at rest in a neutrally temperate environment, in the post-absorptive state; this value represents the energy required to maintain cellular and organ function and constitutes 60-70% of total EE depending on the individuals age, sex and body composition, and prior exercise participation (Poehlman, 1989). The thermic effect of feeding (TEF) is defined as the cumulative increase in EE above resting levels due to the cost of processing food for storage and use and constitutes ~10% of daily TEE (Poehlman, 1989). Physical activity is the final factor contributing to total energy requirements; this will vary depending on individual activity level hence the relative contribution shown in the figure may vary.

Activity energy requirements include the excess post-exercise oxygen consumption (EPOC), which is the increased rate of oxygen uptake that occurs after strenuous exercise. This can be elevated for up to 24 hours after a vigorous exercise session and can make a further contribution to EE after exercise is finished (Wilmore, 1996). High activity levels may also be associated with higher lean mass content, which in turn may contribute to elevated RMR, though this is debated. Compensatory changes in TEF and RMR and their effect on body composition changes will be considered in this section.

1.3.5.1 Aerobic fitness and Resting Metabolic Rate

Highly active individuals will have greater activity EE, higher levels of aerobic fitness and therefore increased energy requirements. RMR may be slightly augmented in physically fit individuals compared to sedentary due to a larger lean mass, but it is not entirely clear if $\dot{V}O_{2max}$ *per se* is significantly associated with RMR. Broeder *et al* (1992b) studied this association in a cross-sectional study with 69 men and found that RMR relative to FFM was not different between trained and untrained individuals. Others have also reported that RMR and $\dot{V}O_{2max}$ are not related (Poehlman *et al*, 1990), whilst others have found a significant association independent of FFM (Davis *et al*, 1983; Tremblay *et al*, 1986; Poehlman *et al*, 1988, 1989). Differences in study populations; these studies have observed a mixture of lean and obese men of varying ages, and methodological issues such as timing of metabolic rate measurements may contribute to discrepancies between these results. Theoretically those with a higher metabolic rate would be less susceptible to metabolic compensatory adaptations, but it is not clear if being aerobically fit results in an increased metabolic rate. Fat free mass content may be more strongly related to RMR, with an augmentation of RMR per kg fat free mass being observed in overweight women during a twelve week exercise intervention. This change was not associated with cardiovascular fitness level indicating that RMR is not directly related to fitness in women (Shinkai *et al*, 1994). It seems activity level and body composition may be more strongly associated with RMR than cardiovascular fitness level itself.

1.3.5.2 Body mass changes and energy expenditure

The primary outcome of interest in this section of the review was the effect of body mass change on RMR and/or TEE. Inclusion criteria for studies in this section (summarised in table 1.17) were:

- Published between 1980 and 2011.
- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Including an intervention which induces a statistically significant body mass reduction; either through dietary energy restriction, exercise participation, or a combination of both.
- Measurement of the change in RMR and/or TEE in response to body mass change as a primary outcome.
- Including measurement of exercise-induced body composition changes.

Evidence shows that physiological compensatory changes in EE, induced by body mass reduction, may be greater in obese than lean individuals. Leibel *et al* (1995) demonstrated that obese have greater total EE than lean individuals, and compensatory changes in EE occur to oppose any body composition changes in both groups. Total EE was measured in obese and those who had never been obese, these participants were then under or over fed in order to reduce or increase body mass by 10%. As expected, a 10% reduction of body mass resulted in a significant reduction in EE per kg FFM. Absolute changes did not differ between obese and lean, but absolute EE values remained higher in the former group. For those who increased body mass by 10% the opposite was true, with an increase in EE per kg FFM⁻¹ day⁻¹ observed in all participants. Resting EE, non resting EE and TEF all contributed to these changes and there was also an association between FFM, fat mass, and these EE changes. Metabolic energy requirements seem very sensitive to body mass changes in lean and obese, and this regulation may in part prevent achievement of a healthy body mass.

In agreement with the findings of Leibel *et al* (1995), large body fat mass changes may result in a sustained depression of metabolic rate in the obese. Elliot *et al* (1989) studied 7 obese women who underwent a protein-sparing

modified fast (PSMF) with a very low energy content for 6-7 weeks, with subsequent gradual transition to a solid food diet with slightly higher energy content over a subsequent 9 week period. Body mass reduction resulting from this intervention was very large (-28.3 kg), and RMR was significantly depressed at the onset of the PMSF, before these body mass changes occurred. This depression persisted throughout the study, even after the PSMF had ended and participants maintained stable body mass. Admittedly, sample size was small and the authors themselves acknowledged that undetected compensatory mechanisms may have occurred since habitual activity levels were not monitored. However, similar works have echoed these findings. Another small sample of women undergoing a PSMF experienced smaller body mass changes than the latter study (-14 kg), but RMR was also significantly depressed in these women. As in the previous study, this depression persisted after the end of the intervention. Lipid oxidation rates were also reduced after body mass reduction and this reduction was found to be predictive of body mass regain (Froidevaux *et al*, 1993). Changes in RMR and lipid oxidation rate associated with body mass changes have been observed in other studies (Weyer *et al*, 2000), thus it seems that excess body fat levels may predispose a person to metabolic adaptations which oppose body mass reduction.

Exercise participation may actually increase RMR. Potteiger *et al* (2008) observed a significant increase in RMR in overweight and obese men and women, with no accompanying changes in substrate oxidation rates, during a 16 month exercise intervention. RMR changes remained significant when expressed relative to lean mass, and these changes did not seem to be body mass dependent since lean mass was unchanged. Increases were also similar in men and women despite the latter group failing to reduce body mass (Potteiger *et al*, 2008). This study included an exercise intervention in line with ACSM recommendations for body mass reduction (Donnelly *et al*, 2009), and found that exercise participation may stimulate resting energy requirements. Further research with overweight men and women found that 6 months of exercise participation maintained total daily EE, whilst those participants restricting EI experienced a significant decline in total EE. The authors speculated that both metabolic and behavioural adaptations contributed to this change (Redman *et al*, 2009). Exercise has also been observed to increase RMR in overweight women during a period of dietary

restriction, specifically by maintaining and augmenting the metabolic activity of fat free mass (Shinkai *et al*, 1994). In comparison to body mass changes observed in participants restricting EI, changes in exercisers were much smaller; this evidence may suggest that exercise protects against physiological compensatory mechanisms. One study investigating this observed similar body mass changes in EI restriction and exercise groups had no effect on RMR in either group. Kraemer *et al* (1997) observed significant and similar body mass reductions in overweight women who restricted EI, completed endurance training, or completing a combination of resistance and endurance training. There were no changes in RMR in any of the groups, which may indicate that exercise does not have a protective effect on metabolic rate in the medium term. Body mass changes in all groups were approximately 6kg, and it is possible that this change may not have been sufficient to affect RMR. The previously mentioned studies observed a protective effect of exercise, but this was in participants who had minimal change in body mass. This study shows that when changes are equal to those induced by EI restriction, exercise may not exhibit the protective effect on EE that the previous studies described. Study duration was also shorter than the previous two studies which may contribute to discrepancies between results.

Metabolic compensatory adaptations affect overweight and obese seeking to reduce body mass in a way that opposes further energy balance perturbations, and makes maintenance of a lower body mass difficult. These physiological adaptations observed in response to body mass reduction are most probably an evolutionary defence system protecting against adipose tissue depletion, similar to the down-regulation in EE and spontaneous physical activity seen in the participants who were resident in a biosphere for 2 years with limited food supplies (Weyer *et al*, 2000). Such strategies were of use when humans were hunter-gatherers, but have now become a risk factor for body mass gain in the modern environment (Leibel *et al*, 1995). Exercise may provide a means of attenuating these physiological changes and maximising body fat loss in overweight individuals.

Although these intervention studies attempt to detect and quantify EI or EE compensatory responses, no studies exist that measure both EI and total EE

levels in overweight and obese during long term exercise interventions. In order to fully understand how chronic exercise participation affects energy balance, it is necessary to assess both these elements. Such studies would be highly labour intensive and present a demanding workload for participants; however such an intervention would present valuable information if conducted robustly. Evidence suggests that overweight women may be the most relevant group to examine in this context, due to potentially greater susceptibility to compensatory changes. As a result, observation of EI, ExEE, non ExEE, and potential mechanisms contributing to compensatory behaviours in this group is required.

Table 1.17 Evidence regarding the effects of statistically significant body fat reduction, induced via dietary energy restriction and/or exercise participation, on metabolic rate and energy expenditure in overweight and obese men and women.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg/min)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate
Elliot <i>et al</i> (1989)	7 obese women	37.6 ± 6.3 (mean ± SD)	40 ± 10 (mean ± SD)	Not reported	14-16 weeks	Participants completed a protein-sparing modified fast (PSMF) with an energy content of 1254 kJ/day for 6-7 weeks, and then gradually transitioned back into a solid food diet of 4598-5852 kJ/day during the following 9 weeks.	Indirect calorimetry	Δ body mass (kg): -28.3 ± 11.4 (made up of 82% fat mass) (mean ± SD)	There was a significant 22% decrease in RMR as a result of the intervention (p<0.001). RMR remained significantly lower than baseline for 8 weeks after the intervention when body mass was stable (p>0.05).	30%
Froidevaux <i>et al</i> (1993)	10 obese women	29.8 ± 6.5 (mean ± SD)	33 ± 10 (mean ± SD)	Not reported	3 months to 1 year depending on participant motivation	Participants completed a PSMF with EI of 3344-4180 kJ/day	Indirect calorimetry	Δ body mass (kg): -14 ± 8 (mean ± SD)	The intervention resulted in a significant 14% depression of RMR (-1498 ± 1138 kJ/day) (p<0.01), which persisted after the intervention. Lipid oxidation rates was significantly lower after the intervention (p<0.01), and this change in lipid oxidation was found to be predictive of body mass regain	30%
Keim <i>et al</i> (1990)	10 overweight women	Not reported, % body fat ranged from 31-40%	D+EX: 27 ± 3 EX: 25 ± 3 (mean ± SEM)	D+EX: 2.31 ± 0.16 EX: 2.54 ± 0.11 (L/min) (mean ± SEM)	12 weeks	Participants were randomised to two groups, both of which completed treadmill walking exercise on 6 days/week to expend 1470 kJ/session, at an intensity of 65-80% $\dot{V}O_{2max}$. 1. D+EX - reduced EI by 50%, consuming diet of 55% carbohydrate, 18% protein and 27% fat. 2. EX - exercise intervention only.	Indirect calorimetry	<u>Baseline body fat mass (kg)</u> D+EX: 30.1 ± 1.8 EX: 28.1 ± 1.9 <u>Week 12 body fat mass (kg)</u> D+EX: 21.7 ± 1.9 EX: 23.7 ± 2.5 (mean ± SEM)	D+EX experienced a 9% decline in total RMR during the intervention (p<0.05), which was not statistically significant when expressed relative to FFM (p>0.05) RMR relative to FFM was significantly higher in the EX group at weeks 6, 9 and 12. <u>RMR(kJ/kg FFM/day):</u> D+EX EX Baseline 129.3 ± 4.6 131.0 ± 2.5 Week 6 129.3 ± 4.2 137.2 ± 4.6 Week 9 130.5 ± 3.8 137.2 ± 5.0 Week 12 128.0 ± 5.4 138.1 ± 4.6 (mean ± SEM)	Not reported
Kraemer <i>et al</i> (1997)	31 overweight	C: 28.2 ± 4.0	C: 31.0 ± 9.6	C: 28.4 ± 8.1	12 weeks	Participants were randomised to one of	Indirect calorimetry	Δ body fat %: D: -5.8	There were no significant differences in RMR between groups	Not reported

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg/min)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate
	women	D: 27.3 ± 3.1 DE: 28.3 ± 4.2 DES: 30.5 ± 5.1 (mean ± SD)	D: 34.6 ± 10.2 DE: 35.6 ± 8.5 DES: 36.5 ± 7.6 (mean ± SD)	D: 30.4 ± 5.9 DE: 28.2 ± 3.9 DES: 27.8 ± 4.5 (mean ± SD)		four groups: 1. Dietary restriction (D, n=8) 2. Dietary restriction and endurance training (DE, n=9) 3. Dietary restriction combined with endurance and strength training (DES, n=8) 4. Control (C, n=6) Participants in endurance exercise groups completed 3 sessions/week aerobic exercise at 70-80% HRmax. Sessions were 30 mins in duration and gradually increased to a maximum of 50 mins. The strength training programme was progressive and also consisted of 3 sessions/week. Dietary intervention was energy restriction designed to induce 6-9kg body mass reduction during the intervention.		DE: -8.0 DES: -4.3	at baseline or week 12 (p>0.05). There were no significant changes in RMR in any group as a result of the intervention (p>0.05). <u>Δ RMR (kJ/kg FFM/day):</u> C: +8.1 D: -8.0 DE: -4.2 DES: -9.3 (mean ± SD)	
Leibel <i>et al</i> (1995)	18 obese (11 women and 7 men) and 24 lean (7 women and 16 men)	Not reported	Lean: 26 ± 10 Obese: 29 ± 10 (mean ± SD)	Not measured	No set length	Participants were under or over fed in order to gain (n=11 obese, n=13 lean) or lose (n=9 obese, n=11 lean) 10% of initial body mass. After a 14 day period of maintenance EI of participants who gained body mass was altered in order to regain to initial body mass	Indirect calorimetry and doubly labelled water.	Not reported	A 10% reduction of body mass resulted in a significant reduction in resting EE (p<0.05) in lean and obese participants. Resting EE was significantly higher in lean before and after body mass loss (p<0.001). <u>Resting EE (kJ/kg FFM):</u> Baseline 10% loss Lean 121 ± 13 109 ± 13 Obese 142 ± 29 126 ± 17	Not reported

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg/min)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate																											
						levels.			<p>There was no change in resting EE of obese or lean participants who increased body mass, but resting EE was significantly higher in obese compared to lean participants ($p < 0.001$).</p> <p>Resting EE (kJ/kg FFM):</p> <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>10% gain</th> </tr> </thead> <tbody> <tr> <td>Lean</td> <td>117 ± 21</td> <td>126 ± 21</td> </tr> <tr> <td>Obese</td> <td>146 ± 29</td> <td>138 ± 21</td> </tr> </tbody> </table> <p>Total EE significantly increased in all body mass gain participants, and significantly decreased in all body mass reduction participants ($p < 0.05$). Total EE was significantly higher in obese participants before and after body mass change in those who gained body mass ($p < 0.05$).</p> <p>Resting EE (kJ/kg FFM):</p> <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>10% gain</th> </tr> </thead> <tbody> <tr> <td>Lean</td> <td>196 ± 29</td> <td>226 ± 75</td> </tr> <tr> <td>Obese</td> <td>213 ± 29</td> <td>247 ± 25</td> </tr> </tbody> </table> <p>Total EE was significantly higher in obese participants than lean at baseline ($p < 0.001$), and after body mass reduction there were no differences in total EE.</p> <p>Total EE (kJ/kg FFM):</p> <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>10% loss</th> </tr> </thead> <tbody> <tr> <td>Lean</td> <td>188 ± 25</td> <td>163 ± 13</td> </tr> <tr> <td>Obese</td> <td>209 ± 33</td> <td>176 ± 21</td> </tr> </tbody> </table> <p>(mean ± SD)</p>		Baseline	10% gain	Lean	117 ± 21	126 ± 21	Obese	146 ± 29	138 ± 21		Baseline	10% gain	Lean	196 ± 29	226 ± 75	Obese	213 ± 29	247 ± 25		Baseline	10% loss	Lean	188 ± 25	163 ± 13	Obese	209 ± 33	176 ± 21	
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Potteiger <i>et al</i> (2008)	74 overweight	Control: Men: 29.0	Control: Men: 24 ±	Control: Men: 39.5 ±	16 months	Participants completed aerobic exercise on	Indirect calorimetry	<u>Men</u> Control group:	There were no significant changes in RMR in the control participants	43.5%																											

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg/min)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate
	and obese men and women	± 3.0 Women: 29.3 ± 2.3 Exercise: Men: 29.7 ± 2.9 Women: 28.7 ± 3.2 (mean \pm SD)	4 Women: 21 ± 4 Exercise: Men: 22 ± 4 Women: 24 ± 5 (mean \pm SD)	5.7 Women: 32.4 ± 3.1 Exercise: Men: 39.2 ± 5.2 Women: 32.8 ± 4.2 (mean \pm SD)		3 to 5 days of the week, for a duration of 20-45 minutes per session and at an intensity of 60-75% HRR.		-0.5 (SD not reported) Exercise group: -4.9 ± 4.4 Women Control group: $+2.1 \pm 4.8$ Exercise group: -0.3 ± 2.7 (mean \pm SD)	($p > 0.05$). RMR was significantly lower in women compared to men throughout the intervention ($p < 0.05$). RMR increased significantly during the intervention for participants in the exercise group ($p < 0.05$). <u>Δ RMR (kJ/kg FFM/day)</u> Women: 5.4 ± 15.1 Men: 8.4 ± 10.9 (mean \pm SD)	
Redman <i>et al</i> (2009)	48 overweight men (n=21) and women (n=27)	Men: 27.9 ± 1.7 Women: 27.7 ± 1.8 (mean \pm SEM)	Men: 38 ± 7 Women: 38 ± 6 (mean \pm SEM)	Control Women: 32.4 ± 4.1 Men: 39.5 ± 5.7 Exercise Women: 32.8 ± 4.2 Men: 39.2 ± 5.2 (mean \pm SD)	6 months	Participants were randomised to one of four groups: 1. exercise to increase EE by 12.5% and 12.5% EI restriction (CR+EX, n=12) 2. 25% EI restriction restricted (CR, n=12) 3. EI restricted to 3720 kJ day ⁻¹ until body mass was reduced by 15% (LCD, n=11) 4. Control participants maintained their normal lifestyle (C, n=11) Participants were supplied with food for the first 12 weeks, and self-selected food for the last 12 weeks. Exercisers completed structured aerobic exercise 5 days/week.	Doubly labelled water	<u>Δ Body mass:</u> C: -0.8 ± 0.8 CR: -8.3 ± 0.8 CR+EX: -8.4 ± 0.8 LCD: -11.2 ± 0.6 (mean \pm SEM)	There were no significant changes in TEE after the intervention in any of the groups ($p > 0.05$). <u>Δ TEE (kJ/day)</u> C: -54 ± 774 CR: -874 ± 477 CR+EX: $+540 \pm 360$ LCD: -1151 ± 127 (mean \pm SEM) Changes in TEE were significant when data from CR and LCD groups were combined (Δ TEE: -1004 ± 37 kJ/day; $p = 0.01$).	4.2%
Shinkai <i>et al</i> (1994)	32 obese women	26.9 ± 2.1 (mean \pm SD)	53.9 ± 6.7 (mean \pm SD)	E/D: 31.7 ± 3.5	12 weeks	Participants were randomised to one of two groups:	Indirect calorimetry	-3.6 kg (range not reported)	The absolute RMR did not change, but the experimental group showed significant increases in the RMR per	Not reported

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg/min)	Intervention length	Type of intervention	Energy expenditure assessment	Δ body fat mass (kg)	Results	Attrition rate																		
		SD)	SD)	C: 33.5 \pm 5.3 (mean \pm SD)		1. Exercise/Diet - participated in an aerobic training programme, 45-60 min/day at 50%-60% $\dot{V}O_{2max}$, 3-4 days/week, and also adopted a self-regulated energy deficit relative to predicted energy requirements (-1.05 MJ.day ⁻¹ to -1.14 MJ.day ⁻¹) (n=17). 2. Control - no intervention (n=15)			unit of body mass (10%) and per unit of FFM (4%) (p<0.05). Δ RMR (kJ/kg/hour): <table border="0"> <tr> <td></td> <td><u>Baseline</u></td> <td><u>Week 12</u></td> </tr> <tr> <td>E/D:</td> <td>3.5 \pm 0.2</td> <td>3.8 \pm 0.3</td> </tr> <tr> <td>C:</td> <td>3.6 \pm 0.3</td> <td>3.7 \pm 0.3</td> </tr> </table> Δ RMR (kJ FFM/kg/hour): <table border="0"> <tr> <td></td> <td><u>Baseline</u></td> <td><u>Week 12</u></td> </tr> <tr> <td>E/D:</td> <td>5.5 \pm 0.5</td> <td>5.7 \pm 0.4</td> </tr> <tr> <td>C:</td> <td>5.5 \pm 0.5</td> <td>5.6 \pm 0.6</td> </tr> </table> (mean \pm SD) The increase in RMR relative to FFM was not related to $\dot{V}O_{2max}$ (ml/kg FFM/min).		<u>Baseline</u>	<u>Week 12</u>	E/D:	3.5 \pm 0.2	3.8 \pm 0.3	C:	3.6 \pm 0.3	3.7 \pm 0.3		<u>Baseline</u>	<u>Week 12</u>	E/D:	5.5 \pm 0.5	5.7 \pm 0.4	C:	5.5 \pm 0.5	5.6 \pm 0.6	
	<u>Baseline</u>	<u>Week 12</u>																										
E/D:	3.5 \pm 0.2	3.8 \pm 0.3																										
C:	3.6 \pm 0.3	3.7 \pm 0.3																										
	<u>Baseline</u>	<u>Week 12</u>																										
E/D:	5.5 \pm 0.5	5.7 \pm 0.4																										
C:	5.5 \pm 0.5	5.6 \pm 0.6																										

1.4 Impact of exercise and body mass reduction on appetite regulating hormones

1.4.1 Appetite regulating hormones

Appetite is one of the many factors that influence EI in humans, and is physiologically regulated by a series of peptide hormones, secreted locally in the stomach and gut, which signal feelings of hunger, satiety and fullness via the hypothalamus (figure 1.5). Circulating levels of these hormones are responsive to nutrient intake (Essah *et al*, 2007, Kong *et al*, 2009), and levels are often observed to be disturbed in obesity (Maier *et al*, 2008; Carlson *et al*, 2009). Exercise participation and state of energy balance may moderate their secretion and effects (Hagobian *et al*, 2009), and perhaps contribute to compensatory changes in EI (Martins *et al*, 2007b). As a result, these hormones have been of great interest in acute and chronic exercise, and body mass reduction, intervention studies. Past research has often focused on leptin, a hormone secreted by adipose tissue that plays a role in both short and long term energy balance regulation, and levels have been shown to be higher in obese individuals, indicating a resistance to its effects in these individuals (Considine *et al*, 1996). The effects of exercise on leptin are well understood due to the volume of existing research; this hormone does not seem to respond to acute exercise in overweight and obese (Racette *et al*, 1997; Tsofliou *et al*, 2003; Kyriazis *et al*, 2007; Sari *et al*, 2007), and it has been documented that leptin levels respond only to changes in fat mass in the long term, and not exercise participation itself (Perusse *et al*, 1997; Kraemer *et al*, 2002). Research has therefore increasingly focused on other appetite regulating hormones to increase understanding of appetite and energy balance regulation, and to investigate their potential role in exercise induced compensatory responses; ghrelin and peptide YY are two such peptide hormones which are currently the subject of interest.

1.4.1.1 Ghrelin

Ghrelin is a 28-amino acid peptide, which was discovered in 1999 (Kojima *et al*, 1999) following the earlier discovery of the ghrelin receptor in 1996 (Howard *et al*, 1996). It is synthesised primarily in the stomach (Chaudri *et al*, 2008) and exists in two molecular forms, the acylated form; which has an extra serine molecule in position 3, and the deacylated form (Asakawa *et al*, 2005). The acylated form of ghrelin makes up only 10% of total ghrelin concentration (Huda *et al*, 2009) and the acylation process seems to be an important step in the conversion of ghrelin to an active form which can cross the blood brain barrier and stimulate hunger and food intake via the hypothalamus (figure 1.5).

Ghrelin is the only orexigenic, or appetite stimulating, peptide hormone identified thus far (Lim *et al*, 2010). It plays a unique role in meal initiation; levels are highest in the fasted or hypoglycaemic state and fall rapidly in the post-prandial state (Cummings *et al*, 2001). Infusion of ghrelin has also been shown to increase EI, appetite and gastric emptying in humans (Wren *et al*, 2001; Tack *et al*, 2005; Levin *et al*, 2006).

Ghrelin is of interest in the context of exercise induced compensatory mechanisms because it is the only known orexigenic hormone, and has also been shown to be highly sensitive to long term changes in adiposity in women (Foster-Schubert *et al*, 2005; Garcia *et al*, 2006). It is not clear if ghrelin levels in overweight and obese are affected by exercise participation *per se* in the short term; studies are limited in number and observation periods often short in duration (Unick *et al*, 2010; King *et al*, 2011b). There does appear to be a stimulatory effect of exercise on circulating ghrelin levels in overweight women in the medium term, independent of state of energy balance (Hagobian *et al*, 2009). This existing evidence provides significant reason to investigate the acute and chronic effects of exercise on circulating levels of this form of ghrelin in

overweight and obese women, and the possible contribution of changes in concentration to compensatory changes in EI.

1.4.1.2 Ghrelin and Body Composition

Ghrelin has been found to be negatively correlated with body mass and fatness (Ravussin *et al*, 2001, Tschop *et al*, 2001). Circulating concentrations of ghrelin have been found to differ between lean and severely obese women, with pre- and post-prandial levels being significantly lower in the latter (Tschop *et al*, 2001; Marzullo *et al*, 2004). The postprandial decrease in ghrelin concentrations may be delayed in obese individuals, occurring 90 minutes after meal ingestion compared to 30 minutes in lean individuals (Carlson *et al*, 2009). Others have suggested that meal ingestion does not suppress ghrelin secretion at all in obese participants (English *et al*, 2002). Reduction in body mass may also up-regulate ghrelin secretion; a 17% body mass reduction in obese participants has been associated with a 24% increase in circulating ghrelin levels (Cummings *et al*, 2002). These findings suggest disturbed appetite regulation in obesity that may counteract attempts to reduce body mass.

1.4.1.3 Peptide YY

Peptide YY is a 36 amino-acid peptide produced by L-cells in the intestine and secreted alongside glucagon-like peptide 1. It was first isolated in 1980 (Tatemoto & Mutt, 1980); its role in digestion and satiety was discovered later when it was found to be secreted from cells in the small intestine (Adrian *et al*, 1985). There are two forms, PYY₁₋₃₆ and PYY₃₋₃₆, the latter being the main form found in the circulation and the form able to penetrate the blood brain barrier (McGowan & Bloom, 2004). Peptide YY is one of the hormones involved in regulating satiety responses, and is secreted in response to food ingestion with a peak in circulating levels observed approximately 1hr after food ingestion.

Peptide YY ultimately exerts its effects, like ghrelin, through the hypothalamic circuits (le Roux & Bloom, 2005) (figure 1.5).

Evidence regarding peptide YY levels in obesity is conflicting; abnormally low levels have been observed in obese (Batterham *et al*, 2003), whilst others have found no difference between lean and obese (Pfluger *et al*, 2007). There is evidence that impaired cholinergic regulation in obesity may contribute to disturbed levels of peptide YY and ghrelin in obesity (Maier *et al*, 2008). Indeed venous infusion of peptide YY has been shown to inhibit food intake in lean and obese individuals to the same extent (Batterham *et al*, 2003; Small & Bloom, 2004) indicating that the obese are not resistant to its effects. Body mass reduction in obese individuals has been also associated with a decrease in peptide YY levels (Pfluger *et al*, 2007).

Peptide YY may be related to EE; levels have been negatively associated with changes in body mass, resting metabolic rate and 24 hour substrate oxidation in obese men and women (Guo *et al*, 2006), as well as being positively correlated with post-prandial EE in lean women (Doucet *et al*, 2008). As a result, peptide YY is of interest in the context of exercise induced changes in energy balance and possible contributions to compensatory mechanisms. Acute exercise has been shown to lead to a short-term augmentation of peptide YY levels in lean and obese men, and this change appears to have strong links to subjective feelings of hunger which may influence EI (Broom *et al*, 2009; Ueda *et al*, 2009a). However there is currently limited evidence available regarding the acute effects of exercise on peptide YY levels in overweight and obese women, and studies regarding chronic effects are severely limited. As a result this hormone remains of great research interest in this context at present.

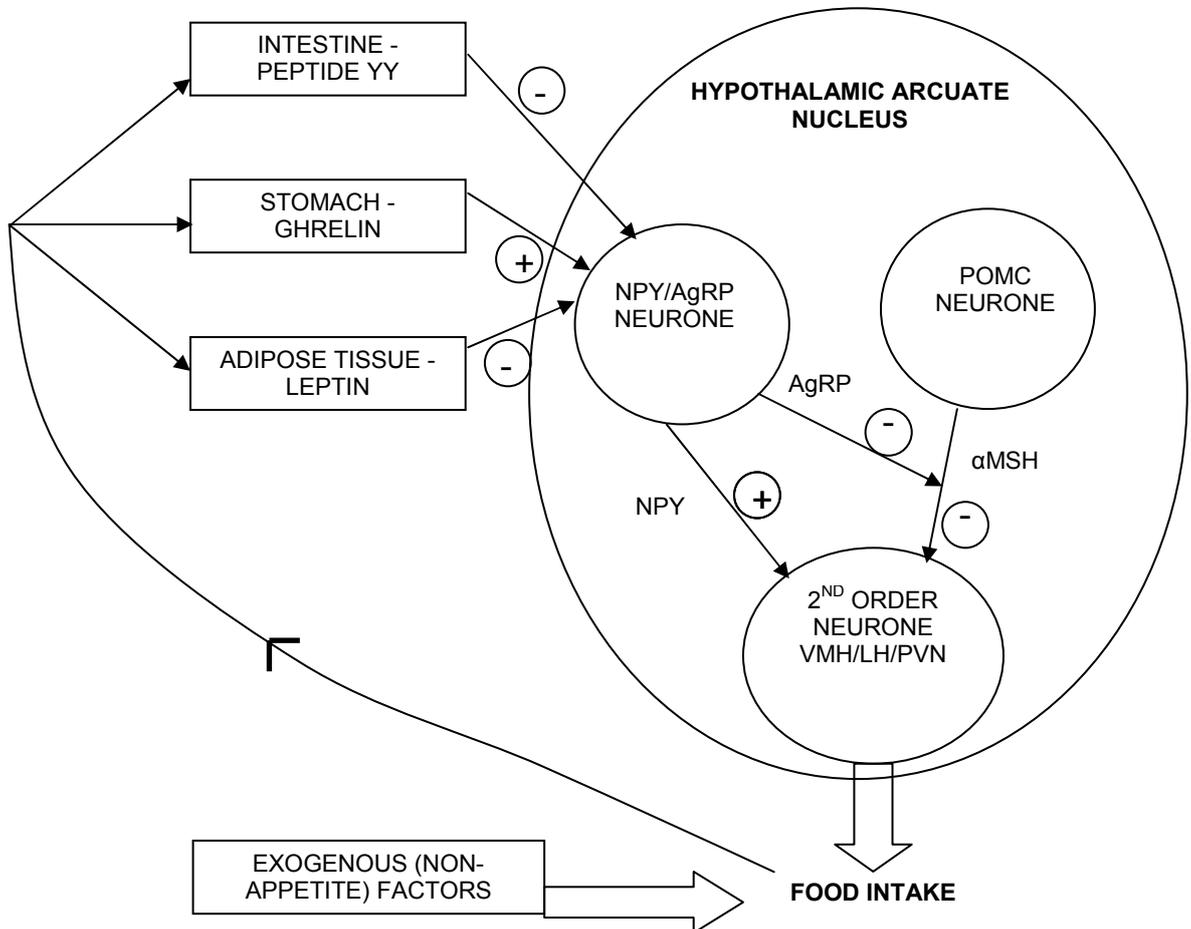


Figure 1.5 Overview of hypothalamic regulation of food intake via peptide YY and acylated ghrelin in humans. Acylated ghrelin and PYY3-36 cross the blood-brain barrier and reach the hypothalamic arcuate nucleus. Acylated ghrelin binds primarily to the GHS-receptor and peptide PYY3-36 to the Y2 receptor in the arcuate nucleus and exert their effects via NPY neurone cells which produce agouti-related peptide (AgRP) and neuropeptide Y (NPY). Binding of acylated ghrelin increases NPY and AgRP production, which leads to a stimulation of food intake via second order neurones located in the lateral (LH) and ventromedial (VMH) hypothalamus and paraventricular nucleus (PVN). AgRP also antagonises the binding of α -melanocyte stimulating hormone (α -MSH), which is produced in pro-opiomelanocortin (POMC) neurones and inhibits food intake through the same second order neurones. PYY3-36 exerts its effects by inhibiting the electrical activity of NPY nerve terminals and thus production of AgRP, resulting in activation of adjacent pro-opiomelanocortin (POMC) neurones and thus inhibition of food intake. Leptin is a peptide secreted by adipose tissue cells; increasing adiposity leads to greater secretion of leptin which inhibits NPY/AgRP neurones and thus food intake. These peptides form only part of the appetite regulating system; food intake is under more complex regulation, including psychological influences, hence food intake is not only motivated by appetite.

1.4.1.4 Assessment of Appetite in Human Acute Exercise Studies

Many studies have examined subjective appetite feelings in conjunction with ghrelin and peptide YY in order to determine if these hormones have a direct effect on perceived appetite. The method used for assessing such feelings in all studies included in this review is the visual analogue scale (VAS) (Flint *et al*, 2000). This questionnaire asks participants to rate the intensity of specific aspects of appetite, such as hunger and fullness, on a blank 100mm line with anchor statements at either end (“I am not hungry at all” and “I have never been more hungry”). The participant marks the line as they feel is appropriate relative to the anchor statements, and a score between 0 and 100 can be ascertained by the placing of the mark. The typical methods of data or statistical analyses used for these data are either area-under-the-curve obtained from a plot of average scores, or analysis of variance (ANOVA) to allow assessment of effects of time and/or trial on appetite. Evidence regarding the reproducibility of scores obtained using this method is mixed; some have reported that the VAS is reliable for use in lean males participating in appetite research studies (Flint *et al*, 2000), whilst others have observed poor reproducibility of this method in small samples of lean men (Raben *et al*, 1995). However, a systematic review of the evidence has argued that reproducibility of this method is good when used in within-subject designs conducted under controlled conditions, and that scores obtained using this method seem sensitive to experimental manipulation (Stubbs *et al*, 2000). The VAS remains the only method available that provides a measurement of these entirely subjective sensations.

1.4.2 Acute Exercise-Induced Changes in Appetite Regulating Hormones in Overweight and Obese

1.4.2.1 Ghrelin

The existing evidence base has focused largely on based active, lean participants and has produced conflicting results; some studies have indicated a short term suppression of ghrelin and appetite occurs with no effect on subsequent EI in lean men (Ballard *et al*, 2009; Broom *et al*, 2007 & 2009; Vestergaard *et al* 2007; King *et al*, 2010a). Some research has indicated an increase in ghrelin levels that may have an effect on subsequent appetite and EI (Erdmann *et al*, 2007; Malkova *et al*, 2008), whilst others have observed a short term suppression of hunger, but not EI, independent of ghrelin concentrations (Martins *et al*, 2007). Others yet have observed no significant acute effects of exercise on ghrelin, appetite or EI (Dall *et al*, 2002; Kraemer *et al*, 2004; Schmidt *et al*, 2004; Zoladz *et al*, 2005; King *et al*, 2010b). Sample sizes are generally small in the majority of these studies ($n < 20$) with no reported power calculations, so it is possible that a lack of statistical power in some studies contributed to the discrepancies between results. Dissimilarity in findings may also reflect differences in type and duration of exercise, as well as differing length of observation periods between studies. Unfortunately, there is far less information regarding the effects of acute exercise on ghrelin levels in overweight and obese, however this is the main population of interest in this thesis and the existing evidence will be reviewed in this section.

Study Selection

The primary outcome of interest in this review was short term exercise-induced changes in plasma ghrelin levels. Inclusion criteria for studies reviewed in this section (summarised in table 1.18) were:

- Published between 1999 and 2011.
- Written in the English language.

- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Experimental conditions must include participants completing aerobic exercise to induce negative energy balance in the short term (<1 week duration), without dietary manipulation.
- Study design including either a control trial or a control group.
- Measurement of total or acylated ghrelin concentrations immediately after exercise as a primary outcome of interest.

Five relevant short term studies with that fit these criteria were identified (table 1.18), of which:

- One observed a statistically significant exercise induced increase in ghrelin levels.
- Three observed no statistically significant effect of exercise on ghrelin levels.
- One observed a statistically significant exercise induced decrease in ghrelin levels.

Please note there are no clinical reference data for plasma ghrelin levels; the magnitude of change in ghrelin which has a clinically/biologically significant impact has not been defined. As such, any significant differences referred to in the context of this hormone refer to statistically significant differences only.

One study including obese participants indicated that maximal exercise suppresses acylated ghrelin levels in lean and obese participants, although no effect on total ghrelin levels was observed in either group (Marzullo *et al*, 2008). Unusually, this study did not specify whether participants were male or female. However, contradictory findings have been reported in both lean and obese males, with no change in total ghrelin observed in response to sub-maximal exercise in men (Ueda *et al*, 2009a). The latter study did not observe a difference in absolute concentrations between lean and obese either, even though abnormally low ghrelin levels are a commonly observed feature of obese

individuals (English *et al*, 2002; Marzullo *et al*, 2004). The discrepancies in these findings raise may be attributable to differences in methodology between studies; it may be that only maximal exercise has an impact on acylated ghrelin, and measurement of total ghrelin may mask the true effects of exercise on the acylated form of the hormone. Duration of observation in both studies was also short, hence it is not clear if the observed suppression would persist and have a significant impact on eating behaviour. Unfortunately, no other evidence of the effects of acute exercise on acylated ghrelin in obese males was identified to corroborate either of these findings.

Investigations with overweight women are also few in number; existing evidence has reported no effect of steady state, moderate walking exercise of approximately 40 minutes duration on acylated ghrelin levels (Unick *et al*, 2010). This short, relatively undemanding, single exercise bout also failed to elicit any compensatory responses in terms of appetite or EI; an encouraging finding since this indicated that the vast majority of participants achieved a state of negative energy balance. Unfortunately there are methodological limitations of this study; the observational period was short, and EI was assessed at just one meal. The observation period may not have been long enough to capture all responses, and compensation may have occurred after the single test meal as a result. As exercise was relatively undemanding and of low ExEE, it is unclear whether a more vigorous exercise bout would elicit a change in acylated ghrelin or appetite and EI in overweight women. A similarly moderate session of 20 minutes brisk walking has previously been observed to increase satiety and fullness in obese women in the short term (Tsofliou *et al*, 2003), although ghrelin levels were not measured in this study. This indicates that walking may have a beneficial impact on appetite for some individuals. It is not clear why there is a discrepancy between appetite results obtained from overweight and obese women in these studies, though participants in the latter study were more obese which may have been a contributing factor.

The effects of exercise on acylated ghrelin may not be entirely dependent on changes in energy balance. Multiple exercise sessions completed over a four consecutive days have been observed to stimulate acylated ghrelin levels in

overweight women, independent of energy balance (Hagobian *et al*, 2009). Such a change could conceivably contribute to compensatory responses. Acylated ghrelin levels were not directly linked to appetite in this study; the male participants in this study reported an inhibition of appetite under neutral energy balance conditions, with no associated changes in acylated ghrelin.

Paradoxically, despite the up-regulation of acylated ghrelin, the women reported no changes in perceived appetite. This may indicate that overweight males regulate appetite more sensitively than women, independent of ghrelin concentrations. This raises the possibility that less sensitive regulation in women could also contribute to a greater susceptibility to compensatory responses. These effects on ghrelin levels and appetite may only be induced by multiple consecutive exercise sessions, or be dependent on adiposity, since these findings are in contrast to a similar study observing the effects of a single exercise session in lean men and women (Burns *et al*, 2007). It is unclear whether these changes in appetite observed in overweight men and women would impact on subsequent EI since this outcome was not measured by Hagobian *et al*. Sample size was relatively small in this study, but exercise sessions were standardised to increase total daily EE by 20% in all participants. Exercise induced effects may also be sex specific, as other studies with obese men have reported no change in 48 hour total ghrelin concentrations following moderate treadmill exercise with standardised ExEE (Kyriazis *et al*, 2007). This is one of the few acute exercise studies that observed ghrelin concentrations for two days post-exercise, but as single measurements were made 24 and 48 hours post-exercise it is possible that changes occurring between the measurements were missed. Alternatively, the assessment of total ghrelin levels only may have masked effects on acylated ghrelin levels that have been observed in other studies with overweight individuals (Marzullo *et al*, 2008).

Table 1.18 Evidence table regarding the acute effects of exercise on energy intake/appetite and ghrelin concentrations in overweight and obese adults

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention(s)	Control trial?	Observation period	Ghrelin results	Energy intake/Appetite results:
Hagobian <i>et al</i> (2009)	18 participants - 9 male, 9 female	Males: 25.7 ± 2.3 Females: 28.0 ± 3.5 (mean ± SD)	Males: 26.8 ± 11.8 Females: 23.3 ± 8 (mean ± SD)	Estimated $\dot{V}O_{2peak}$ Males: 44.9 ± 4.8 Females: 34.9 ± 5.2 (mean ± SD)	Energy Deficit trial (DEF): Exercise on 4 consecutive days without replacement of ExEE. Energy Balance trial (BAL): Exercise on 4 consecutive days with replacement of ExEE.	Yes	120 mins on the morning after the end of each experimental protocol	<u>ExEE:</u> <u>DEF</u> <u>BAL</u> Men 3118 ± 98 3139 ± 418 Women 2508 ± 276 2520 ± 268 (mean ± SD, kJ) No difference in acylated ghrelin response in men between conditions (p>0.05). In women, acylated ghrelin concentrations were significantly higher after DEF and BAL compared to control (p<0.05): Ghrelin AUC: DEF ↑ 32% BAL ↑ 25% In men and women total ghrelin concentrations and AUC were significantly higher after DEF and BAL compared to control (p<0.05).	El was controlled as described in study protocol. El in men and women during both protocols: <u>DEF</u> <u>BAL</u> Men 10.6 ± 1.3 13.7 ± 1.7 Women 8.2 ± 1.0 10.9 ± 1.1 (mean ± SD, MJ)
Kyriazis <i>et al</i> (2007)	15 males: randomised to exercise group (n=8) or control group (n=7)	Control group: 34.5 ± 1.3 Exercise group: 32.5 ± 0.8 (mean ± SE)	Control group: 22.9 ± 1.9 Exercise group: 26.4 ± 1.9 (mean ± SE)	Control group: 31.0 ± 2.1 Exercise group: 33.6 ± 1.9 (mean ± SE)	Treadmill exercise for 60 mins at 50-60% of $\dot{V}O_{2max}$	Yes, control group	48 hours - samples taken before, immediately after, 24 hours after, and 48 hours after intervention.	ExEE: 2370 ± 105 kJ No differences seen in ghrelin concentrations (p>0.05). Ghrelin concentrations: Pre-intervention: 38.4 ± 21.4 Post-intervention: 33.8 ± 11.8 24 hrs post-intervention: 34.6 ± 14.2 48 hrs post-intervention: 38.8 ± 16.9 (mean ± SEM, pg ml ⁻¹) Ghrelin was not measured in control group.	Not measured.

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention(s)	Control trial?	Observation period	Ghrelin results	Energy intake/Appetite results:																		
Marzullo <i>et al</i> (2008)	8 obese participants and 8 lean control participants	Lean: 22.1 ± 1.2 Obese: 33.7 ± 1.5 (mean ± SEM)	Not reported	Not reported	Maximal progressive cycle ergometer exercise test:	No	During test and 40 mins post-exercise	ExEE: not reported Acylated ghrelin concentrations were significantly lower during exercise compared to baseline in both groups (p<0.05). Concentrations of acylated and total ghrelin were significantly higher in lean compared to obese participants (p<0.05). Acylated Ghrelin concentrations: <table border="0"> <tr> <td></td> <td><u>At rest</u></td> <td><u>Peak exercise</u></td> </tr> <tr> <td>Lean</td> <td>410 ± 21</td> <td>326 ± 7</td> </tr> <tr> <td>Obese</td> <td>290 ± 43</td> <td>185 ± 24</td> </tr> </table> (mean ± SEM, pg ml ⁻¹) No changes in total ghrelin levels (p>0.05).		<u>At rest</u>	<u>Peak exercise</u>	Lean	410 ± 21	326 ± 7	Obese	290 ± 43	185 ± 24	Not measured.									
	<u>At rest</u>	<u>Peak exercise</u>																									
Lean	410 ± 21	326 ± 7																									
Obese	290 ± 43	185 ± 24																									
Ueda <i>et al</i> (2009a)	7 lean control participants and 7 obese participants - all males	Lean: 22.4 ± 2.4 Obese: 30.0 ± 3.1 (mean ± SD)	Lean: 22.4 ± 4.2 Obese: 22.9 ± 3.4 (mean ± SD)	Lean: 46.6 ± 3.9 Obese: 34.0 ± 6.3 (mean ± SD)	60 mins cycle ergometer exercise at 50% $\dot{V}O_{2max}$	Yes	180 mins	ExEE: Not reported No differences in ghrelin concentrations (p>0.05).	El and REI were lower in exercise than control trial in both groups (p<0.001). El and REI during exercise trial were significantly lower in obese participants than lean participants (p<0.05). No effect of exercise on appetite (p>0.05). <u>EI & REI:</u> Lean <table border="0"> <tr> <td></td> <td><u>Resting</u></td> <td><u>Exercise</u></td> </tr> <tr> <td>EI:</td> <td>3503 ± 477</td> <td>2893 ± 447</td> </tr> <tr> <td>REI:</td> <td>2642 ± 485</td> <td>819 ± 451</td> </tr> </table> Obese <table border="0"> <tr> <td></td> <td><u>Resting</u></td> <td><u>Exercise</u></td> </tr> <tr> <td>EI:</td> <td>3946 ± 736</td> <td>2567 ± 364</td> </tr> <tr> <td>REI:</td> <td>2767 ± 640</td> <td>-389 ± 468</td> </tr> </table> (mean ± SD, kJ)		<u>Resting</u>	<u>Exercise</u>	EI:	3503 ± 477	2893 ± 447	REI:	2642 ± 485	819 ± 451		<u>Resting</u>	<u>Exercise</u>	EI:	3946 ± 736	2567 ± 364	REI:	2767 ± 640	-389 ± 468
	<u>Resting</u>	<u>Exercise</u>																									
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EI:	3946 ± 736	2567 ± 364																									
REI:	2767 ± 640	-389 ± 468																									

Authors	Participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml.kg ⁻¹ .min ⁻¹)	Intervention(s)	Control trial?	Observation period	Ghrelin results	Energy intake/Appetite results:												
Unick <i>et al</i> (2010)	n = 19 females	32.5 ± 4.3 (mean ± SD)	28.5 ± 8.3 (mean ± SD)	Fitness 6.5 ± 3.3 (submax MET's) (mean ± SD)	Moderate intensity walking for 40mins at 70-75% age-predicted maximum HR.	Yes	120 mins post-exercise	ExEE: 1471 ± 301 kJ (mean ± SD) No significant differences in acylated ghrelin concentrations between trials (p>0.05). Acylated ghrelin AUC: Ex: 12721 ± 6677 Con: 13053 ± 5291 (mean ± SD, pg ml ⁻¹ x 120 mins)	No difference in total EI (p=0.92) or appetite. REI was significantly lower in exercise trial (p<0.001). <table border="0"> <tr> <td></td> <td><u>Control</u></td> <td><u>Exercise</u></td> </tr> <tr> <td>EI:</td> <td>2.29 ± 1.2</td> <td>2.31 ± 1.0</td> </tr> <tr> <td>REI:</td> <td>2.11 ± 1.2</td> <td>0.83 ± 1.1</td> </tr> <tr> <td></td> <td colspan="2">(mean ± SD, MJ)</td> </tr> </table>		<u>Control</u>	<u>Exercise</u>	EI:	2.29 ± 1.2	2.31 ± 1.0	REI:	2.11 ± 1.2	0.83 ± 1.1		(mean ± SD, MJ)	
	<u>Control</u>	<u>Exercise</u>																			
EI:	2.29 ± 1.2	2.31 ± 1.0																			
REI:	2.11 ± 1.2	0.83 ± 1.1																			
	(mean ± SD, MJ)																				

1.4.2.2 Peptide YY

Evidence regarding the acute effects of exercise on peptide YY levels is more limited than that examining ghrelin, but also presents more consistent results. Only one study with obese participants could be identified hence evidence from lean is included in this section. Study selection criteria for this section were as follows.

- Published between 1985 and 2011.
- Written in the English language.
- Adults over 16 years old (no BMI restriction due to limited evidence).
- Experimental conditions must include participants completing aerobic exercise to induce negative energy balance in the short term (<1 week duration).
- Study design must include either a control trial or a control group.
- Measurement of peptide YY concentrations immediately after exercise as a primary outcome of interest.

Eight relevant studies were identified (table 1.19) according to these criteria, of which:

- Seven observed an exercise induced increase in peptide YY levels.
- One observed an exercise induced decrease in peptide YY levels.

Peptide YY levels in lean males seem to be transiently affected by aerobic exercise, and these changes appear to be linked with perceived appetite. Of a small sample of lean males completing aerobic and resistance exercise, only aerobic exercise participation resulted in a significant increase in peptide YY concentrations over the eight hour observation period. This change was accompanied by a suppression of hunger, but it is not clear if these changes affected subsequent EI (Broom *et al*, 2009). The earlier mentioned study of King *et al* (2011b) concurred with these findings and furthermore illustrated that

exercise has a very specific effect on peptide YY levels, independent of energy balance. In this study, circulating peptide YY concentrations were significantly increased over a 9 hour period in response to an exercise induced energy deficit, but significantly suppressed in response to an energy deficit induced by food restriction in these men. Appetite and EI responses agreed with these findings; EI was lower and feelings of satiety increased in the exercise trial, compared to control, whilst the opposite effect on EI and satiety was observed in the food restriction trial. Thus exercise induced negative energy balance seems to have a specific satiety enhancing effect via peptide YY in lean males. The mechanisms for the difference in response between energy deficits induced by exercise and dietary means are not immediately clear, and this may be an area for future investigation. Like many acute studies, sample sizes were small in both studies, and power calculation not reported. However, as there were significant findings these sample sizes are clearly adequate to detect changes in the variables of interest in this population.

Others have reported that the exercise induced stimulation of peptide YY is short lived post exercise in lean men and women (Martins *et al*, 2007b). The transient nature of this peptide YY stimulation has also been documented in obese males completing a comparable moderate aerobic exercise protocol (Ueda *et al*, 2009a). Exercise in these studies was of lower intensity and duration than the aforementioned studies of Broom *et al* and King *et al* which found a longer duration of post-exercise peptide YY suppression; indeed intensity and duration of exercise seem to be important factors in the magnitude of exercise induced stimulation in peptide YY. A greater exercise-induced increase in circulating peptide YY was observed in lean males following high intensity exercise compared to moderate intensity, and this effect only persisted post-exercise after the high intensity session (Ueda *et al*, 2009b). Perceived hunger was suppressed post-exercise irrespective of exercise intensity. Post-exercise stimulation of peptide YY also seems prolonged by medium term exercise. Twenty-four hour peptide YY concentrations were significantly increased in lean men completing two hours of exercise for three consecutive days, although there was no corresponding effect on appetite (Cooper *et al*, 2010). These results

indicate that the apparent link between peptide YY and appetite may only occur in the shorter term. It is not clear if these more prolonged changes in peptide YY over the medium term would subsequently affect EI, and hence body mass regulation.

Other factors, such as timing of exercise relative to meal ingestion may have profound effects on peptide YY levels. Exercise in the fasted state seems to result in lower pre-prandial peptide YY levels, and longer duration of the normal meal-induced increase in circulating concentrations. Interestingly these effects were not seen after exercise completed in the fed state. Appetite changes were contradictory to peptide YY findings; post-exercise hunger ratings were significantly higher after exercise in the fasted state, and apparently unaffected by meal consumption. This effect persisted throughout the seven hour observation period. Other factors, possibly cognitive in nature, may disrupt the link between peptide YY and perceived feelings of appetite (Cheng *et al*, 2009).

Table 1.19 Evidence table regarding the acute effects of exercise on energy intake/appetite and peptide YY concentrations in lean adults.

Authors	No. of participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Intervention(s)	Control trial	Observation period	Peptide YY results	Energy intake/Appetite results
Broom <i>et al</i> , 2009	11 active, lean males	23.1 ± 0.4 (mean ± SEM)	21.1 ± 0.3 (mean ± SEM)	62.1 ± 1.8 (mean ± SEM)	1) Resistance exercise: 90 min weight lifting followed by 6.5hours rest 2) Aerobic exercise - 60 min run followed by 7 hours rest 3) Control: 8 hours rest	Yes	8 hours	1. Resistance ExEE: 1473 ± 114 kJ 2. Aerobic ExEE: 3832 ± 97 kJ Peptide YY was significantly higher in aerobic exercise trial compared to resistance exercise and control trials (p<0.02). Total AUC for PYY concentrations: 1. Resistance: 1381 ± 97 2. Aerobic: 1750 ± 710 3. Control: 1411 ± 110 (mean ± SEM, pg ml ⁻¹)	Hunger was significantly lower during the pre-prandial period (0-2hrs) in the aerobic exercise trial compared to control (p<0.05).
Cheng <i>et al</i> (2006)	12 lean, active males	Not reported Mean body mass: 82.2 ± 11.7kg Mean height: 1.8 ± 0.1 m (mean ± SD)	24.6 ± 4.8 (mean ± SD)	49.2 ± 8.1 (mean ± SD)	50 mins cycle ergometer exercise at 60% $\dot{V}O_{2max}$ performed either before (EM) or after meal (ME) consumption.	Yes	7 hours	ExEE: Not reported PYY was significantly lower prior to meal in EM trial compared to ME and control trials (p<0.05). PYY remained significantly higher than fasting values for 7 hours in EM trial compared to just 3 hours in control trial (p<0.05).	After meal, hunger was significantly decreased in control and ME trials (p<0.05). Exercise lowered hunger prior to meal in EM trial, and prevented increase in hunger during exercise in ME trial (p<0.05).
Cooper <i>et al</i> (2010)	8 lean, sedentary males	22.5 ± 3.3 (mean ± SD)	25 ± 8 (mean ± SD)	40.5 ± 5.1 (mean ± SD)	Participants consumed diets comprising 50% energy from fat and EI prescribed to equal 24 hour EE. Dietary fat intake and exercise levels	Yes	24 hours	ExEE: To raise 24 hr EE to 1.8xRMR Exercise significantly increased 24 hours peptide YY concentrations irrespective of dietary fat intake (p<0.001). 24 hour average PYY concentrations: SE: 118 ± 10	Energy balance: SE: -815 ± 543 UE: -869 ± 531 SS: -146 ± 523 US: -109 ± 443 (mean ± SEM, kJ day ⁻¹) No effect of exercise on appetite ratings (p>0.05).

Authors	No. of participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Intervention(s)	Control trial	Observation period	Peptide YY results	Energy intake/Appetite results
					<p>were manipulated in a 2x2 design</p> <p>2hr cycling exercise at 45% $\dot{V}O_{2max}$ per day for 3 consecutive days (morning and evening).</p> <p>Conditions:</p> <ol style="list-style-type: none"> 1. SE - high saturated fat (22% of energy) with exercise 2. SS - high saturated fat (22% of energy) without exercise 3. UE - high monounsaturated fat (30% of energy) with exercise 4. US - high monounsaturated fat (30% of energy) without exercise 			<p>UE: 109 ± 9 SS: 101 ± 6 US: 99 ± 5 (mean ± SEM, pg ml⁻¹)</p> <p>Post-exercise peptide YY concentrations were significantly higher in SE and UE compared to SS and US, regardless of time of day (p<0.001).</p>	
King <i>et al</i> (2011b)	12 active, lean males	22.8 ± 0.4 (mean ± SEM)	23.4 ± 1.0 (mean ± SEM)	57.3 ± 1.2 (mean ± SEM)	<p>Energy deficit induced by exercise and food restriction:</p> <ol style="list-style-type: none"> 1. Control trial (Con) - EI sufficient to meet energy needs whilst sedentary. 2. Exercise deficit trial (Ex-Def) - EI sufficient to meet energy needs plus 	Yes	9 hours	<p>ExEE: 4715 ± 113 kJ</p> <p>PYY concentrations were significantly lower in food-def trial compared to control (p=0.004) and ex-def (p<0.001) trials. PYY was significantly higher in ex-def compared to control (p<0.05).</p> <p>PYY concentrations: Con: 391 ± 22 Ex-Def: 438 ± 31 Food-Def: 310 ± 34 (mean ± SEM, pmol L⁻¹ 9hrs⁻¹)</p>	<p>Higher ratings of hunger, and prospective food consumption, lower ratings of satisfaction and fullness in food-def trial compared to con and ex-def trials (p<0.001).</p> <p>Buffet meal EI in food-def trial was significantly higher than control trial (p<0.001). There was a tendency for buffet EI to be higher in food-def compared to ex-def trial (p=0.058).</p> <p>Buffet meal EI:</p>

Authors	No. of participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Intervention(s)	Control trial	Observation period	Peptide YY results	Energy intake/Appetite results								
					a 90 min treadmill run at 70% $\dot{V}O_{2max}$ to induce energy deficit. 3. Food deficit trial (Food-Def) - sedentary with energy deficit of 4820 ± 151 kJ (similar to exercise induced deficit) induced by restricting EI at breakfast and lunch meals.				Con: 4004 ± 427 Ex-Def: 4343 ± 653 Food-Def: 6167 ± 318 (mean ± SEM, kJ)								
Martins <i>et al</i> (2007b)	12 lean volunteers - 6 males and 6 females	22.0 ± 3.2 (mean ± SD)	25.9 ± 4.6 (mean ± SD)	Not reported	60 mins cycle ergometer exercise at 65% of estimated HR _{max}	Yes	4 hours	ExEE: 2057 ± 385 kJ Peptide YY was significantly increased (p=0.04) and ratings of hunger significantly suppressed during exercise (p=0.004) but this effect was not sustained post-exercise.	Absolute EI higher in exercise trial than control (p=0.04), REI significantly lower in exercise trial (p=0.04). EI: <table style="margin-left: auto; margin-right: auto;"> <tr> <td style="text-align: center;"><u>Exercise</u></td> <td style="text-align: center;"><u>Control</u></td> </tr> <tr> <td style="text-align: center;">EI: 3816 ± 1517</td> <td style="text-align: center;">3815 ± 1053</td> </tr> <tr> <td style="text-align: center;">REI: 1760 ± 1262</td> <td style="text-align: center;">2362 ± 945</td> </tr> </table> (mean ± SD, kJ)	<u>Exercise</u>	<u>Control</u>	EI: 3816 ± 1517	3815 ± 1053	REI: 1760 ± 1262	2362 ± 945		
<u>Exercise</u>	<u>Control</u>																
EI: 3816 ± 1517	3815 ± 1053																
REI: 1760 ± 1262	2362 ± 945																
Ueda <i>et al</i> (2009a)	7 lean control participants and 7 obese participants - all males	Lean: 22.4 ± 2.4 Obese: 30.0 ± 3.1 (mean ± SD)	Lean: 22.4 ± 4.2 Obese: 22.9 ± 3.4 (mean ± SD)	Lean: 46.6 ± 3.9 Obese: 34.0 ± 6.3 (mean ± SD)	60 mins cycle ergometer exercise at 50% $\dot{V}O_{2max}$	Yes	180 mins	ExEE: not reported Peptide YY levels were significantly increased during the exercise session compared to control (p<0.02); this effect did not persist post-exercise. No difference in peptide YY between lean and obese (p=0.12).	EI and REI were lower in exercise than control trial in both groups (p<0.001). EI and REI during exercise trial were significantly lower in obese participants than lean participants (p<0.05). <u>EI & REI:</u> <table style="margin-left: auto; margin-right: auto;"> <tr> <td style="text-align: center;"><u>Lean</u></td> <td style="text-align: center;"><u>Exercise</u></td> </tr> <tr> <td style="text-align: center;"><u>Resting</u></td> <td style="text-align: center;"><u>Exercise</u></td> </tr> <tr> <td style="text-align: center;">EI: 3503 ± 477</td> <td style="text-align: center;">2893 ± 447</td> </tr> <tr> <td style="text-align: center;">REI: 2642 ± 485</td> <td style="text-align: center;">819 ± 451</td> </tr> </table> Obese	<u>Lean</u>	<u>Exercise</u>	<u>Resting</u>	<u>Exercise</u>	EI: 3503 ± 477	2893 ± 447	REI: 2642 ± 485	819 ± 451
<u>Lean</u>	<u>Exercise</u>																
<u>Resting</u>	<u>Exercise</u>																
EI: 3503 ± 477	2893 ± 447																
REI: 2642 ± 485	819 ± 451																

Authors	No. of participants	Mean BMI (kg/m ²)	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Intervention(s)	Control trial	Observation period	Peptide YY results	Energy intake/Appetite results
									<u>Resting</u> <u>Exercise</u> EI: 3946 ± 736 2567 ± 364 REI: 2767 ± 640 -389 ± 468 (mean ± SD, kJ)
Ueda <i>et al</i> (2009b)	10 lean males	22.5 ± 1.0 (mean ± SD)	23.4 ± 4.3 (mean ± SD)	45.9 ± 8.5 (mean ± SD)	30 mins cycling exercise at 75% (high intensity) and 50% (moderate intensity) $\dot{V}O_{2max}$	Yes	150 mins	Peptide YY concentrations significantly increased in both exercise trials compared to control. Peptide YY concentrations were significantly greater in high intensity trial compared to moderate intensity trial (p<0.001). AUC for PYY concentrations(pmol ml⁻¹ x 60 mins): High: 446.8 ± 187.0 Moderate: 293.4 ± 171.9 Control: 26.7 ± 16.2	EI in both exercise trials was significantly lower than control trial (p<0.01), there were no differences between exercise trials (p>0.05). Hunger was significantly suppressed during and after exercise (p=0.05).

1.4.3 Changes in Appetite Regulating Hormones in Response to Long Term Exercise Participation

Study Selection

The primary outcome of interest in this review was changes in circulating ghrelin and peptide YY levels in response to statistically significant body composition changes. Inclusion criteria for studies (summarised in table 1.20) were:

- Published between 1985 and 2011.
- Written in the English language.
- Adults over 16 years old, classed as overweight and obese (>BMI 25 kg/m²).
- Interventions including an exercise/physical activity element designed to induce negative energy balance and significant body fat reduction (studies including a dietary and other interventions were included providing all groups were subject to the same intervention allowing exercise induced effects to be elucidated).
- Must include body mass and/or body fat measurements.
- Interventions >3 weeks in duration.
- Body mass and/or fat mass changes as a primary outcome of interest.

1.4.3.1 Changes in Ghrelin and Body Mass in Response to Chronic Exercise Participation in Overweight and Obese Individuals

There is convincing, consistent evidence that ghrelin is significantly up-regulated in response to exercise-induced body mass changes in lean and obese individuals. Few studies observe the effects of exercise exclusively, most include some form of dietary intervention. As a result studies with dietary intervention were also included and eight relevant studies were identified, seven of which observed a

significant chronic up-regulation of ghrelin in response to a significant body mass reduction.

Significant reductions in body mass induce counterproductive changes in ghrelin levels in overweight women. Long term studies have consistently shown that ghrelin levels increase as an apparent defensive reaction to the depletion of body fat stores, and not due to the effects of exercise participation *per se*. Significant, progressive increases in circulating ghrelin levels have been observed in overweight and obese women reducing body mass by ≥ 3 kg, achieved through a combination of exercise and EI restriction in interventions (Foster-Schubert *et al*, 2005; Ata *et al*, 2010; Martins *et al*, 2010a). Such changes in acylated ghrelin levels, accompanied by an increase in fasting hunger and greater post-prandial suppression of hunger, have also been observed in women achieving this magnitude of exercise-induced body mass reduction in body mass over twelve weeks (Martins *et al*, 2010a). This was the only study identified which measured both acylated and total ghrelin levels. Unlike a one year exercise only intervention with a much greater sample size (Foster-Schubert *et al*, 2005), no change in total ghrelin was observed in this study; it is possible that this study lacked statistical power which may have prevented detection of changes in total ghrelin. However, results of all these interventions indicate that even a non-clinically significant body mass reduction (<5% change) may result in increased sensitivity of appetite regulation in these women.

The increase in ghrelin levels resulting from body mass reduction may also be threshold dependent. The mean body mass reductions in a large sample of overweight women participating in a one year exercise intervention were modest (-1.4kg), but still sufficient to induce a progressive increase in total ghrelin levels which reached significance after twelve months. Although mean values indicated an increase in ghrelin occurred in the whole group, further analysis revealed that a significant 18% increase in ghrelin was observed only in those who reduced body mass by at least 3kg (Foster-Schubert *et al*, 2005). The authors attempted to prevent dietary changes but commented that the

relatively modest changes in body mass were most probably indicative that these attempts failed, and compensatory changes in EI probably occurred. Since this increase in ghrelin levels is most likely a mechanism to preserve body fat stores in the face of depletion, it is perhaps not surprising that this mechanism is threshold dependent. Further evidence has reported that ghrelin levels normalise after stabilisation of body mass. An 8.5% reduction in body mass, induced by a six month diet and exercise intervention, was associated with an increase in ghrelin levels which returned to baseline values after participants maintained their stable, lower body mass for a further six months after the intervention concluded (Garcia *et al*, 2006). This provides further evidence that these observed increases in circulating ghrelin operate to increase EI and defend body fat stores, since they also diminish once body mass equilibrium is achieved once more.

This defensive up-regulation of ghrelin levels may not occur in more severely obese individuals, and hence may be dependent on initial adiposity. Such individuals have very disturbed ghrelin regulation, characterised by abnormally low ghrelin concentrations and an absence of normal meal-related fluctuations. A three week intervention combining exercise, EI restriction, and counselling which resulted in a clinically significant 5% body mass reduction in a small sample of morbidly obese men and women (Management of Obesity, Scottish Intercollegiate Guidelines Network, 2010) induced no changes in total ghrelin levels after (Morpurgo *et al*, 2003). Additionally, normal ghrelin responses to meal ingestion were not restored either. This may indicate that the severely obese may find it easier, initially, to achieve a significant body mass reduction, since ghrelin does not respond in a counter-regulatory fashion, or could be indicative of a lack of statistical power due to the small sample size. The lack of response may also be a symptom of highly disturbed ghrelin regulation, or an indication that physiological mechanisms do not respond to changes in adiposity when obesity is severe. However it is not clear if ghrelin levels would up-regulate after a certain degree of body mass reduction in these individuals, as has been observed in less obese individuals; larger interventions are required to clarify this issue.

Table 1.20 Evidence regarding the chronic effects of exercise on ghrelin concentrations in lean and overweight/obese men and women.

Authors	No. of participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ ($ml/kg^{-1}/min^{-1}$)	Mean baseline BMI (kg/m^2)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m^2)	Mean body mass change (kg)	Results												
Ata <i>et al</i> (2010)	70 overweight, pre-menopausal women	n=60	Range: 20-45 years	Not reported	29.6 ± 3.2 (mean ± SD)	10 weeks	Dietary restriction composed of 40% carbohydrate, 30% protein and 30% fat. EI of diet was varied depending on individual energy needs. Participants were asked to progressively increase the number of steps taken per day by 1500 until a maximum of 4500 more steps per day than baseline levels was reached.	28.3 ± 3.4 (mean ± SD)	Baseline body mass: 79.3 ± 11.1 kg End body mass: 75.7 ± 11.4 kg (p<0.0001) (mean ± SD)	Ghrelin levels increased by 17% following the intervention (p<0.01). Ghrelin concentrations: Baseline: 2.9 ± 1.7 ng ml ⁻¹ Post-intervention: 3.5 ± 1.7 ng ml ⁻¹ (mean ± SD)												
Foster-Schubert <i>et al</i> (2005)	173 overweight, sedentary, post-menopausal women randomised to exercisers group (n=87) or control stretchers group (n=86)	n=168	Exercisers: 60.7 ± 6.7 Stretchers: 60.6 ± 6.8 (mean ± SD)	Exercisers: 20.0 ± 3.5 Stretchers: 20.4 ± 3.0 (mean ± SD)	Exercisers: 30.4 ± 4.1 Stretchers: 30.5 ± 3.7 (mean ± SD)	12 months	Exercise intervention: 45 mins moderate intensity aerobic exercise - 5 days/week for 12 months. Control intervention: one 45 min stretching session once per week for 12 months	Not reported	After 12 months: Exercisers: -1.4 ± 0.4 Stretchers: 0.1 ± 0.4 (p<0.05) (mean ± SEM)	Ghrelin concentrations progressively increased over the course of the exercise intervention (p<0.05). Ghrelin concentrations: Baseline: 599 ± 38 pg ml ⁻¹ Δ 3 month change: 24 ± 14 pg ml ⁻¹ Δ 12 month change: 32 ± 16 pg ml ⁻¹ Body mass loss >3kg = ↑ 17.7% in ghrelin concentrations after 12 months exercising (p<0.001 compared with baseline; p<0.01 compared with body mass loss <3kg). Changes in ghrelin concentrations: <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>Δ12 months</th> </tr> </thead> <tbody> <tr> <td><0.5kg loss:</td> <td>616 ± 63</td> <td>3 ± 23</td> </tr> <tr> <td>0.5-3kg loss:</td> <td>589 ± 56</td> <td>41 ± 31</td> </tr> <tr> <td>>3kg loss:</td> <td>560 ± 72</td> <td>99 ± 30</td> </tr> </tbody> </table> (mean ± SEM, pg ml ⁻¹) No significant change in ghrelin in stretchers (p>0.05).		Baseline	Δ12 months	<0.5kg loss:	616 ± 63	3 ± 23	0.5-3kg loss:	589 ± 56	41 ± 31	>3kg loss:	560 ± 72	99 ± 30
	Baseline	Δ12 months																				
<0.5kg loss:	616 ± 63	3 ± 23																				
0.5-3kg loss:	589 ± 56	41 ± 31																				
>3kg loss:	560 ± 72	99 ± 30																				
Garcia <i>et</i>	48 Obese	n=48	WL:	Not	WL:	12 months	WL: Lifestyle intervention	WL:	Not	Mean fasting ghrelin concentrations												

Authors	No. of participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ ($ml/kg^{-1}/min^{-1}$)	Mean baseline BMI (kg/m^2)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m^2)	Mean body mass change (kg)	Results												
al (2006)	Mexican-American women randomised to 2 groups: body mass reduction (WL) program group (n=25) or no intervention control (CON) group (n=23)		43.6 ± 7.6 CON: 44 ± 9.6 (mean ± SD)	reported	37.7 ± 7.9 CON: 35.8 ± 5.3 (mean ± SD)		program consisting of administering 120mg Orlistat 3 times a day, classes with dietician aimed at reducing EI by 2092 kJ/day, and classes with fitness instructor aimed at increasing physical activity to 150mins/week, mainly walking. CON: received multivitamin tablets every day, received no instruction.	33.9 ± 1.4 CON: 36.1 ± 1.2 (mean ± SD)	reported	significantly increased after 6 months in WL group only (p<0.05), but concentrations had returned to baseline levels after 12 months (p<0.01 compared with 6 month values). There were no changes in ghrelin concentrations in CON group (p>0.05). Fasting ghrelin concentrations: <table border="1"> <thead> <tr> <th></th> <th>Baseline</th> <th>6 months</th> <th>12 months</th> </tr> </thead> <tbody> <tr> <td>WL:</td> <td>589 ± 52</td> <td>704 ± 64</td> <td>541 ± 45</td> </tr> <tr> <td>CON:</td> <td>595 ± 48</td> <td>580 ± 27</td> <td>472 ± 42</td> </tr> </tbody> </table> (mean ± SD, pg ml ⁻¹) Baseline ghrelin concentrations were significantly correlated to BMI changes at 12 months in the WL group (r=0.33; p=0.04)		Baseline	6 months	12 months	WL:	589 ± 52	704 ± 64	541 ± 45	CON:	595 ± 48	580 ± 27	472 ± 42
	Baseline	6 months	12 months																			
WL:	589 ± 52	704 ± 64	541 ± 45																			
CON:	595 ± 48	580 ± 27	472 ± 42																			
Martins et al (2010a)	22 overweight/obese participants (8 males and 14 females)	n=15 (8 males and 7 females)	36.9 ± 8.3 (mean ± SD)	32.9 ± 6.6 (mean ± SD)	31.3 ± 2.3 (mean ± SD)	12 weeks	Exercise programme consisting of 5 sessions/week of treadmill walking or running at 75% maximal heart rate, in order to expend 2092 kJ per session.	30.1 ± 2.3 (mean ± SD)	Mean pre-intervention body mass: 96.1 ± 12.0 Mean post-intervention body mass: 92.6 ± 11.7 (mean ± SD)	No effect of exercise*time on acylated ghrelin. Fasting acylated ghrelin concentrations were significantly increased post-intervention (p<0.05), and there was a significant 127% increase in the extent of post-prandial suppression of acylated ghrelin levels post-intervention (p=0.009). Fasting acylated ghrelin concentrations: Pre-intervention: 37.2 ± 18.2 Post-intervention: 51.7 ± 26.0 (mean ± SD, pmol L ⁻¹) Post-prandial Δ acylated ghrelin concentrations: Pre-intervention: 12.4 ± 11.1 Post-intervention: 28.1 ± 21.4 (mean ± SD, pmol L ⁻¹) There were no changes in total ghrelin												

Authors	No. of participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Mean body mass change (kg)	Results									
										concentrations (p>0.05). Fasting hunger sensations were increased after the intervention (p<0.01).									
Morpurgo <i>et al</i> (2003)	10 severely obese participants (3 male, 7 female) and 5 healthy, lean controls who did not take part in the intervention.	n=10	35 ± 9.3 (mean ± SD)	Not reported	45.2 ± 10.6 (mean ± SD)	3 weeks	Combination intervention of energy-restricted diet (1200-7531 kJ/day; 21% protein, 53% carbohydrates, 26% lipids), exercise training (30 mins cycle exercise 5 days/week, 50-70mins leisure walking 2 days/week and 30 mins indoor activity 5 days/week), psychological counselling and nutritional education	42 ± 10 (mean ± SD)	-5% body mass loss Fat mass reduction: -4.4kg	At baseline, obese had significantly lower ghrelin levels, and fasting ghrelin levels were not modified by meal ingestion compared to controls (p<0.05). 5% body mass reduction did not significantly affect ghrelin concentrations in obese (p>0.05). Baseline ghrelin concentrations: <table border="0"> <tr> <td></td> <td><u>Pre-prandial</u></td> <td><u>Post-prandial</u></td> </tr> <tr> <td>Lean</td> <td>352.4 ± 176.7</td> <td>199.0 ± 105.2</td> </tr> <tr> <td>Obese</td> <td>110.8 ± 69.7</td> <td>91.8 ± 70.2</td> </tr> </table> Basal ghrelin concentrations: in obese: Pre-intervention: 110.8 ± 69.7 Post-intervention: 126.4 ± 108.6 (mean ± SD, pmol L ⁻¹)		<u>Pre-prandial</u>	<u>Post-prandial</u>	Lean	352.4 ± 176.7	199.0 ± 105.2	Obese	110.8 ± 69.7	91.8 ± 70.2
	<u>Pre-prandial</u>	<u>Post-prandial</u>																	
Lean	352.4 ± 176.7	199.0 ± 105.2																	
Obese	110.8 ± 69.7	91.8 ± 70.2																	
Santosa <i>et al</i> (2007)	42 hyperlipidaemic females	n=35	49.4 ± 6.7 (mean ± SD)	Not reported	31.4 ± 2.8 (mean ± SD)	6 months	Intervention aimed at decreasing EI by 20% and increasing EE by 10%. Participants received dietary advice and instruction from a personal trainer, and had access to gym to complete exercise or could exercise independently.	26.9 ± 2.9 (mean ± SD)	-11.7 ± 2.5 (mean ± SD)	Compared to baseline, ghrelin levels rose significantly by 21.2 ± 26.7% post-intervention (p<0.001). Ghrelin concentrations: Pre-intervention: 1102.0 ± 464.8 Post-intervention: 1307.0 ± 562.5 (mean ± SD, pg ml ⁻¹)									

1.4.3.2 Changes in Peptide YY and Body Mass in Response to Chronic Exercise Participation in Overweight and Obese Individuals

Studies observing peptide YY in response to long term exercise are very limited in number. Only three relevant studies could be identified, summarised in table 1.21, and results of these studies are not in full agreement. A significant reduction in body fat in overweight adolescents, induced by eight months exercise participation, resulted in a 23% increase in peptide YY levels (Jones *et al*, 2009). These results have not been echoed in overweight adults; despite statistically significant reductions in body mass induced over three to six months exercise participation (mean losses -3.1 kg and -1.8kg), no change in peptide YY levels has been witnessed in small samples of overweight men and women (Martins *et al*, 2010a; Turner *et al*, 2010). From the limited evidence that exists no real conclusions can be drawn, but it seems peptide YY levels may be altered by modest body composition changes only in younger individuals. However, a clinically significant body mass reduction induced by a clinical intervention has been shown to result in a decrease in peptide YY levels in obese adults (Pfluger *et al*, 2007), therefore it may be that body mass changes in the exercise studies were not large enough to induce changes in peptide YY. Further investigation is needed to elucidate the effects of exercise and body mass reduction on peptide YY levels on overweight and obese, as current evidence indicates a possible effect, but is too limited to be conclusive.

Table 1.21 Evidence table regarding the chronic effects of exercise on peptide YY concentrations in adults.

Authors	No. of participants	No. of completers	Mean Age (years)	Mean $\dot{V}O_{2max}$ (ml/kg ⁻¹ /min ⁻¹)	Mean baseline BMI (kg/m ²)	Intervention length	Type of intervention	Mean post-intervention BMI (kg/m ²)	Mean body mass change (kg)	Results												
Jones <i>et al</i> (2009)	15 overweight adolescents	n=12	15.3 ± 0.5 (mean ± SD)	Peak $\dot{V}O_2$: 35.2 ± 9.9 (mean ± SD)	31.8 ± 5.2 (mean ± SD)	32 weeks	Three 45min sessions of aerobic exercise at 60-85% peak $\dot{V}O_2$ per week	32.1 ± 5.6 (mean ± SD)	Baseline: 90.9 ± 15.6 Post-intervention: 92.8 ± 17.8 (mean ± SD)	Post-intervention, peptide YY levels had significantly ↑ by 23% (p=0.05). Peptide YY concentrations: Pre-intervention: 171.2 ± 63.2 Post-intervention: 209.8 ± 78.9 (mean ± SD, pg ml ⁻¹)												
Martins <i>et al</i> (2010a)	22 overweight/obese participants; males n=8 and females n=14	n=15; males n=8 and females n=7	36.9 ± 8.3 (mean ± SD)	32.9 ± 6.6 (mean ± SD)	31.3 ± 2.3 (mean ± SD)	12 weeks	Exercise programme consisting of 5 sessions/week of treadmill walking or running at 75% maximal heart rate, in order to expend 2092 kJ per session	30.1 ± 2.3 (mean ± SD)	Mean pre-intervention body mass: 96.1 ± 12.0 Mean post-intervention body mass: 92.6 ± 11.7 (mean ± SD)	No significant effect on peptide YY concentrations (p>0.05). Peptide YY concentrations: Pre-intervention: 10.6 ± 5.5 Post-intervention: 10.3 ± 4.8 (mean ± SD, pmol L ⁻¹) There was no change in recorded EI (p>0.05). EI: Pre-intervention: 9.41 ± 2.38 Post-intervention: 9.31 ± 2.79 (mean ± SD, MJ)												
Turner <i>et al</i> (2010)	54 overweight, middle-aged men	n=29; n=15 exercise group and n=14 control group	55 ± 5 (mean ± SD)	Not reported	28.1 ± 2.7 (mean ± SD)	24 weeks	Aerobic, progressive exercise intervention, beginning with three 30 mins sessions at 50% $\dot{V}O_{2max}$ and working up to four 60 mins sessions at 70% $\dot{V}O_{2max}$ per week.	27.4 ± 2.4 (mean ± SD)	Exercise: -1.8 ± 2.2 Control: +0.2 ± 2.2 (mean ± SD)	There were no significant differences in peptide YY concentrations between groups at baseline, post-intervention, or after 2 weeks of detraining (p>0.05). There was no significant effect of the exercise intervention of peptide YY concentrations (p>0.05). Peptide YY concentrations: <table border="1"> <thead> <tr> <th></th> <th><u>Exercise</u></th> <th><u>Control</u></th> </tr> </thead> <tbody> <tr> <td>Baseline:</td> <td>189 ± 51</td> <td>192 ± 93</td> </tr> <tr> <td>Week 24:</td> <td>171 ± 38</td> <td>199 ± 72</td> </tr> <tr> <td>Week 26:</td> <td>168 ± 72</td> <td>187 ± 67</td> </tr> </tbody> </table> (mean ± SD, pg ml ⁻¹)		<u>Exercise</u>	<u>Control</u>	Baseline:	189 ± 51	192 ± 93	Week 24:	171 ± 38	199 ± 72	Week 26:	168 ± 72	187 ± 67
	<u>Exercise</u>	<u>Control</u>																				
Baseline:	189 ± 51	192 ± 93																				
Week 24:	171 ± 38	199 ± 72																				
Week 26:	168 ± 72	187 ± 67																				

1.4.4 General Limitations

Acute exercise studies are numerous but the evidence base is limited in many ways. Principally, the majority of studies have been conducted on lean males, many of whom are all well-trained and habitually active. There is a lack of information on overweight and obese, sedentary females in particular. Disparities in study designs; differences in mode, intensity and duration of exercise, duration of observation periods, and study timings mean studies are not easily comparable. Many factors may influence ghrelin concentrations, such as the content and timing of meals. Macronutrient intakes have been shown to have an effect on post-prandial ghrelin concentrations (Blom *et al*, 2006; Kong *et al*, 2009; St Pierre *et al*, 2009), therefore this could account for some of the disparity in this field.

A significant problem in the current published evidence is the frequent use of small sample sizes ($n < 20$), with no reported power calculations to justify these samples. Type 2 statistical, or β errors (incorrect acceptance of the null hypothesis) may occur in such studies, resulting in misleading data and incorrect conclusions. Power calculations should be more regularly reported in the literature.

Methods of measuring EI used in exercise intervention studies are also limited in their ability to detect true changes in EI. Post exercise EI is often measured at buffet meals served in the laboratory in an attempt to circumvent the problems associated with indirect dietary assessment methods. However this is a somewhat flawed approach method since the reliability of laboratory based buffet meal method has not been widely investigated. The studies which do exist report high test-retest reliability of EI values obtained using this method (Arvaniti *et al*, 2000, Gregersen *et al*, 2008; Nair *et al*, 2009; Lara *et al*, 2010), but are limited since they largely examine EI at a single meal consumed by lean males at rest. Only one study that tested the buffet meal method in a post-exercise scenario could be found (Laan *et al*, 2010) but this study assessed the

reliability of EI of highly active lean men and women at a single post-exercise meal. These results are not applicable to overweight and/or female participants, and do not suggest that reproducibility of EI over a longer period would be high. Thus it remains unclear whether the buffet meal method is reliable for use in acute exercise studies.

Additionally, many studies observe total ghrelin levels only, which may mask effects on acylated ghrelin resulting in incorrect conclusions being drawn. Few studies observe peptide YY responses, particularly in the long term, and observation of the active form of this hormone PYY₃₋₃₆ has not been carried out in this context either. At present the lack of evidence makes it impossible to reliably draw conclusions about the effect of body mass changes in the long term on peptide YY. Further research is needed into the mechanisms causing up-regulation of ghrelin. More specific observations of the active forms of ghrelin and, in particular, PYY are also needed as it is possible that they play a role in exercise induced compensatory mechanisms.

1.5 Dietary Restraint, Metabolism and Physical activity

1.5.1 The Theory of Dietary Restraint

1.5.1.1 Origins – The “Set Point” Theory

It has been suggested that individual body mass is regulated around a personal “set point” which is dictated by physiological characteristics, such as the number of fat cells in the body. This value varies between individuals and is a constant; when body fat mass is reduced these cells shrink in size but not in number (Stern & Greenwood, 1974; Spalding *et al*, 2008) thus body fatness is a partially inherited characteristic. As a result it has been hypothesised that individual food intake is regulated by the number of fat cells, or the “set point”, to maintain optimum body mass (Nisbett, 1972). In the 1960’s and 70’s it was postulated that that food intake was regulated via the hypothalamus (Nisbett, 1968 & 1972); a theory that later proved true with the discovery of appetite regulating peptide hormones and their mechanisms of action. It was proposed that for some individuals optimum body mass is greater than recommended, and some individuals would achieve body mass equilibrium with a BMI > 25 kg m⁻². Medical guidelines and social pressure dictate that a healthy and preferable BMI be < 25 kg m⁻² therefore individuals with an overweight “set point” may endeavour to chronically restrict food intake to achieve the desired body mass below their natural “set point”

Herman and Mack (1975) conducted some of the first experimental work based on the set-point theory. They introduced the term “biologically underweight” to describe individuals who continuously employ dietary restraint in order to maintain body mass below their “set point”. These individuals were characterised by the specific eating behaviour characteristics they displayed. Individuals with a high level of dietary restraint were observed to increase their intake of ice cream after consuming a milkshake preload, whereas those with low dietary restraint levels showed more sensitive regulation and decreased intake after consuming the same preload (Herman & Mack, 1975). This

phenomenon was described as “counter regulation” and has been shown experimentally as a characteristic behaviour of restrained eaters. Counter regulatory behaviour is usually observed when these individuals consume a food that violates their normal dietary practices (Polivy, 1976; Hibscher & Hermann, 1977). However, not all studies have found evidence of counter regulation in restrained eaters (Martins *et al*, 2008). This discrepancy may be attributable to varying energy content of preload used in experimental trials; there appears to be a level of food and EI above which counter-regulatory behaviour ceases and normal regulation occurs as expected, even in restrained eaters (Herman *et al*, 1987). Individuals with high restraint are also more responsive to food cues; hunger, desire to eat and subsequent food consumption in response to visual food cues seem to be greater than in those with low levels of restraint (Fedoroff *et al*, 1997 & 2003). These experimental studies led to the development of definitions of restrained and unrestrained eaters; restrained eaters are characterised by chronic restriction of food intake which is punctuated by periodic loss of control resulting in “binge eating” episodes, often in response to negative emotions or food cues (Ruderman, 1985). It is important to note that dieting and restraint behaviours are not interchangeable, restraint is a chronic dietary practice where dieting is often more transient, and many restrained eaters do not report dieting to reduce body mass (Lowe & Timko, 2004). Unrestrained eaters do not exhibit the characteristic behaviours of restrained eaters; theoretically they are able to regulate their body mass around their natural “set point” without altering their eating behaviour. Restrained eaters may be described as “biologically underweight”, and are considered to be in a constant state of food deprivation in order to maintain body mass below their “set point”. External and emotional influences can trigger a loss of control in these individuals and result in unrestrained food consumption dictated by their natural “set point”. Therefore this characteristic eating behaviour may not be determined by body mass *per se*, but by the state of relative deprivation, which would be highest in the most “biologically underweight” (Herman & Mack, 1975). Indeed, high levels of circulating triglycerides, a marker of short term starvation, were observed in restrained eaters in one experimental study (Laessle *et al*, 1989). This finding could be attributable to other factors, such as differing dietary intake, or could indicate that the theory is correct and restrained eaters are indeed in a state of relative deprivation.

1.5.1.2 Measurement of Dietary Restraint

There were several early attempts to develop restraint measurement tools, such as the latent obesity questionnaire (Pudel *et al*, 1975) and the restraint scale (Herman & Mack, 1975). These were found to be of limited validity in obese participants (Ruderman, 1983; Stunkard and Messick, 1985). In 1985 the Three Factor Eating Questionnaire/Inventory (TFEQ) was developed by Stunkard & Messick and is still in popular use. This questionnaire measures three aspects of eating behaviour; restraint, disinhibition and hunger. Disinhibition is the term used to describe individual susceptibility to loss of restraint which will likely result in “binge eating”. The internal consistency for all three factors has been found to be high (coefficient α reliabilities 0.90, 0.87 and 0.82 for restraint, disinhibition and hunger respectively) (Stunkard & Messick, 1985), with strong reliability and temporal stability of restraint scores in women ($r=0.82$ for temporal stability) (Laessle *et al*, 1989; Bardone-Cone *et al*, 2007). Critics have argued that this questionnaire does not reflect long term dietary practices accurately (Stice *et al*, 2004, 2007 & 2010), whilst others have also questioned the validity of this inventory in overweight women (van Strien *et al*, 2007). However, the studies raising these criticisms used EI values as a measure of dietary restraint, but this approach is flawed since EI values reflect the amount an individual consumes, but not how much they desire to eat. Since the EI of restrained eaters is determined by level of restraint, and not by personal desire for food, EI may not be highly representative of dietary restraint (van Strien *et al*, 2006).

1.5.2 Characteristics of Restrained Eaters and Unrestrained Eaters

It has been established that the eating behaviour of restrained eaters is characterised by chronic restriction of food intake, punctuated by periods of disinhibition and overconsumption, as well as counter-regulatory behaviour. Experimental work has also elucidated physical differences between restrained and unrestrained eaters. Despite efforts to maintain a desirable body mass,

dietary restraint has proven not to be an effective strategy to manage body mass. The tendency to periodically over indulge results in a higher BMI in restrained eaters compared to their unrestrained counterparts (Tuschl *et al*, 1990; Poehlman *et al*, 1991; Klesges *et al*, 1992; Hays *et al*, 2002; Mulvihill *et al*, 2002; Beisegel *et al*, 2004; Waugh *et al*, 2007). Disinhibition levels may also play a role in these differences as some evidence has suggested that body mass is more strongly associated with disinhibition than restraint (Lawson *et al*, 1995; Lindroos *et al*, 1997). Indeed restrained eaters with high disinhibition levels tend to eat more in response to negative moods and stress (Ruderman, 1985; Yeomans & Coughlan, 2009), and the importance of assessing disinhibition alongside restraint will be discussed later in this chapter.

Differences in self-reported dietary intake between restrained and unrestrained eaters have also been found; restrained eaters have reported greater fruit and vegetable consumption than their unrestrained counterparts (Tepper *et al*, 1996; Beisegel *et al*, 2004). Restrained eaters have also reported lower fat intake and higher carbohydrate intake than unrestrained eaters (de Castro, 1995, Tepper *et al*, 1996), but no difference in total EI. The self-reported nature of this data means it may not be entirely accurate, but it would be logical to expect these kinds of differences in dietary patterns.

1.5.3 Evidence for Physiological Differences between Restrained and Unrestrained Eaters

There is some evidence of physiological differences between restrained and unrestrained eaters indicating that this behaviour may have a biological basis. Higher post-prandial carbohydrate oxidation rates (Keim & Horn, 2004), lower postprandial glucose, and lower post-prandial, overnight, and fasting insulin levels (Pirke *et al*, 1990; Keim & Horn, 2004; Martins *et al*, 2008 & 2009) have been observed in restrained eaters. Higher circulating levels of the orexigenic peptide hormone ghrelin have been observed in restrained eaters and may be indicative of increased appetite (Schur *et al*, 2008). These findings indicate that restrained eating may have a physiological basis, though it is not known if these

characteristics represent a physiological predisposition to restrained eating, or conversely if these differences result from the practice of dietary restraint.

Research has suggested that there are also neurological differences between restrained and unrestrained eaters. Different brain activation patterns have been observed in restrained and unrestrained in response to pictures of foods of varying palatability. Results indicated that, compared to their unrestrained counterparts, restrained eaters experience less intense feelings of hunger in the fasted state, but find foods perceived to be palatable as more appealing when in the fed state (Coletta *et al*, 2009).

It has been shown that exercise may have a beneficial effect on appetite regulation in restrained eaters. There is evidence that even a single session of moderate aerobic exercise may result in improved appetite control and lower EI in lean female restrained eaters, even in the presence of high fat foods (Lluch *et al*, 2000). It is not clear why this difference was observed; neurological appetite regulation in restrained eaters may induce greater desire for palatable foods (Coletta *et al*, 2009) and it possible that exercise improves appetite control on a neurological basis. A possibility for future research would be to investigate the acute effects of exercise on neurological responses to food cues in restrained eaters.

It has been hypothesised that dietary restraint may be a behavioural adaptation resulting from inherently lower energy requirements (de Castro, 1995). Self reported EI has been negatively associated with restraint level in a sample of six hundred men and women (Provencher *et al*, 2003). It has been argued that this finding reflects a greater degree of underreporting in restrained eaters (Bathalon *et al*, 2000), though other studies have found no evidence of an increased prevalence of underreporting in restrained eaters (Tuschl *et al*, 1990). The theory of diminished energy requirements in restrained eaters may be feasible since the characteristic pattern of restriction and overconsumption in restrained eaters may result in frequent “weight cycling”, defined as the repeated loss and gain of body mass due to intermittent dieting. This practice has been shown to result in diminished EE, and could be a mechanism predisposing restrained

eaters to have lower energy requirements. Indeed in a group of lean women who had reported four or more episodes of significant body mass fluctuations (>3.5 kg) in the past year, body mass and percentage body fat were found to be higher compared to controls with stable body mass (Manore *et al*, 1991). As a result, despite greater body mass and similar lean mass, the “weight cycling” participants were found to have a significantly lower relative EE both at rest and during exercise compared to controls. Indeed dieting status appears to have an association with “weight cycling” in restrained eaters; those who report dieting to reduce body mass show a greater frequency of this behaviour than non dieting restrained eaters (Lowe & Timko, 2004).

As a result, many studies have investigated energy requirements in restrained and unrestrained eaters (table 1.22). Many studies have measured metabolic rate and results have been mixed; some have found no evidence of a difference (Lawson *et al*, 1995; Platte *et al*, 1996b; Tepper *et al*, 1996; Van Loan & Keim, 2000; Bathalon *et al*, 2001; Beseigel *et al*, 2004; Keim & Horn, 2004; Waugh *et al*, 2007; Vescovi *et al*, 2008), whilst others have reported a discrepancy in energy requirements between the two groups (Tuschl *et al*, 1990; Manore *et al*, 1991; Poehlman *et al*, 1991; Platte *et al*, 1996a; Mulvihill *et al*, 2002; Martin *et al*, 2007; Laessle & Kikker, 2008). Others have investigated differences in physical activity and total EE requirements; lower total EE has been observed in lean, young, female restrained eaters (Tuschl *et al*, 1990), but not in a group of lean, postmenopausal women (Bathalon *et al*, 2001). As the evidence is mixed it is not clear if restrained eaters do have diminished energy requirements; discrepancies in results may be due to differences in frequency of weight cycling, or these results may be confounded by the presence of two distinct sub-groups of restrained eaters; those with flexible or rigid control of restraint.

1.5.4 Flexible and rigid control of dietary restraint

More recent research has presented evidence indicating that the theory of restraint may be more complicated than first presumed. As noted previously, restrained eaters generally have greater BMI than unrestrained eaters. More

recent experimental work has revealed that restraint is not a homogenous concept, and in fact there are two different sub-types of restraint which can be defined based on disinhibition levels. Westenhoefer and colleagues conducted a series of studies in the 1990's in which they found that restrained eaters with low disinhibition levels reduced EI following an energy dense preload, whereas those with high disinhibition levels consumed the same regardless of the preload. The presence of counter regulatory behaviour, a characteristic of restrained eaters, was thus dependent on disinhibition levels as well as restraint. This work led to the development of two restraint subscales which differentiate between restrained eaters with high and low disinhibition by taking both behaviours into account within one subscale. This allowed the interaction between the characteristics to be assessed, unlike the TFEQ which assesses them separately. These were named the flexible and rigid restraint subscales. Flexible control is associated with low disinhibition and lower frequency of disinhibited eating episodes. Rigid control is characterised by high disinhibition scores, and these individuals show greater frequency of periods of disinhibited eating (Westenhoefer *et al*, 1991 & 1994; Hays *et al*, 2008). These two sub-groups of restrained eaters have been shown to differ in terms of physical characteristics; those with flexible control generally have lower body mass than those with rigid control. A correlation between flexible dietary control and lower body mass ($r=0.65$) has been observed in a large sample of lean and obese men and women. Weak, negative associations between flexible control, body fatness and waist circumference ($r=-0.11$) in women, and between flexible control levels and BMI in obese men ($r=-0.22$) (Smith *et al*, 1999) have also been observed. Correspondingly, a weak but positive association between rigid control and BMI, body mass and body fatness in non-obese women has been documented ($r=0.20$) (Provencher *et al*, 2003). Reliability of the rigid and flexible control scale has been demonstrated to be high in eating disorder patients, with α coefficients of 0.78 and 0.80 respectively (Shearin *et al*, 1994). However many of these associations are weak, and critics of the sub-classification of the restraint subscale have presented evidence showing that these correlations exist with the same strength when only total restraint score is used (McGuire *et al*, 2001; Provencher *et al*, 2001). However, validation studies have found that the flexible and rigid subscales designed by Westenhoefer *et al* (1991) are useful for distinguishing between differing patterns of eating behaviours.

The questionnaire devised by Westenhoefer *et al* (1991) to measure levels of flexible and rigid control of restraint is based heavily on the three factor eating questionnaire; it contains some of the same questions and also includes new questions specially designed for this purpose. Reliability of this questionnaire, and the flexible and rigid subscales has been found to be high (coefficients of 0.77 for rigid and 0.79 for flexible control). These factors were also found to explain a reasonable amount of variability in BMI; flexible control accounted for 24% and 33%, and rigid control for 30% and 42% of the variance in BMI in men and women respectively (Westenhoefer *et al*, 1999). Rigid control has also been directly associated with eating disorder symptoms ($r=0.66$), and mood disturbances ($r=0.26$) in healthy, non-obese women (Stewart *et al* 2002). Rigid control combined with BMI has been used fairly successfully to predict dieting status in a diverse sample of over four hundred women living in the north east region of the USA. In combination, these two factors successfully predicted dieting status in 73.6 % of those who reported dieting to reduce body mass. In the same study a model taking both flexible and rigid control scores into account successfully classified 52.6 % of those who reported dieting to maintain body mass (Timko & Perone, 2006). These findings concur with those of Westenhoefer *et al* (1991) who reported that rigid control was the only significant predictor of dieting to reduce body mass, and this further supports the idea that restraint is not a homogeneous concept.

The flexible and rigid subscales have also been shown to be of use in eating disorder patients. In one such study flexible control was associated with an anorexia diagnosis ($r=0.55$), and inversely associated with BMI ($r=-0.59$), whilst rigid control was associated with bulimia diagnosis and body mass fluctuations (both $r=0.33$). Interestingly, a similar study with binge eating disorder patients found neither flexible nor rigid control was associated with frequency of binge eating, and both variables predicted 13% of the observed variance in BMI (Masheb & Grilo, 2002). Results regarding the association between these subscales and presence of binge eating disorder are mixed. This may seem odd given the characteristics the flexible and rigid control subscales are designed to measure, but although restrained eaters exhibit “binge eating” episodes, this

term is not implied in this case to represent an eating disorder, but rather a characteristic dietary pattern. The findings from clinically diagnosed eating disorder patients cannot be presumed to be applicable to healthy restrained eaters.

It is possible that previous mixed results regarding differences in energy requirements between restrained and unrestrained eaters are attributable to the presence of two distinct, differing sub-groups of restrained eaters. Previous studies have not accounted for the sub-groups and no published studies have investigated these requirements in restrained eaters with flexible and rigid control.

Table 1.22 Evidence table regarding energy requirements in restrained and unrestrained eaters. R=restrained eaters, UR=unrestrained eaters, BMR=basal metabolic rate, RMR=resting metabolic rate, TEE=total energy expenditure, REE = resting energy expenditure, BM=body mass

Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results									
Bathalon <i>et al</i> (2001)	60 non-obese post-menopausal females	UR, n=26 R, n=34 Scores of ≤ 5 classed as UR and ≥ 13 as R	UR: 23.6 ± 0.6 R: 24.8 ± 0.5 (mean ± SEM)	UR: 60.3 ± 0.6 R: 50.4 ± 0.6 (mean ± SEM)	Doubly labelled water	Three factor eating questionnaire	No significant differences in total EE (TEE) and resting EE between groups (p>0.05). R had significantly lower physical activity level (PAL = TEE/REE) and significant higher reported duration of heavy activity than UR (p<0.05). <table style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th></th> <th style="text-align: center;"><u>UR</u></th> <th style="text-align: center;"><u>R</u></th> </tr> </thead> <tbody> <tr> <td>Physical activity level</td> <td style="text-align: center;">1.84 ± 0.04</td> <td style="text-align: center;">1.72 ± 0.04</td> </tr> <tr> <td>Duration (mins/day)</td> <td style="text-align: center;">7.3 ± 2.3 (mean ± SEM)</td> <td style="text-align: center;">21.4 ± 4.4</td> </tr> </tbody> </table> Multiple regression analysis showed that restraint status was a significant predictor of non-exercise activity thermogenesis (p=0.03) and there was an interaction between body mass change and restraint status (p=0.05).		<u>UR</u>	<u>R</u>	Physical activity level	1.84 ± 0.04	1.72 ± 0.04	Duration (mins/day)	7.3 ± 2.3 (mean ± SEM)	21.4 ± 4.4
	<u>UR</u>	<u>R</u>														
Physical activity level	1.84 ± 0.04	1.72 ± 0.04														
Duration (mins/day)	7.3 ± 2.3 (mean ± SEM)	21.4 ± 4.4														
Beseigel <i>et al</i> (2004)	65 lean, healthy females aged between 18 and 25 years	UR, n = 24 R, n = 25 Scores of ≤ 6 classed as UR and ≥ 12 as R	UR: 21.0 ± 2.0 R: 21.9 ± 2.0 (mean ± SD)	UR: 20.2 ± 2.4 R: 20.7 ± 2.3 (mean ± SD)	Indirect calorimetry for REE and 7 day physical activity recall questionnaire	Three factor eating questionnaire	No significant differences in REE, or reported physical activity were found between R and UR (p>0.05). Body fat % and fat mass were significantly higher in R than UR (p=0.01). <table style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th></th> <th style="text-align: center;"><u>UR</u></th> <th style="text-align: center;"><u>R</u></th> </tr> </thead> <tbody> <tr> <td>Body fat %</td> <td style="text-align: center;">25.0 ± 4.1</td> <td style="text-align: center;">28.0 ± 5.0</td> </tr> <tr> <td>Fat mass (kg)</td> <td style="text-align: center;">14.5 ± 3.7 (mean ± SD)</td> <td style="text-align: center;">17.0 ± 4.2</td> </tr> </tbody> </table>		<u>UR</u>	<u>R</u>	Body fat %	25.0 ± 4.1	28.0 ± 5.0	Fat mass (kg)	14.5 ± 3.7 (mean ± SD)	17.0 ± 4.2
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Body fat %	25.0 ± 4.1	28.0 ± 5.0														
Fat mass (kg)	14.5 ± 3.7 (mean ± SD)	17.0 ± 4.2														
Keim & Horn (2004)	15 pre-menopausal, overweight/obese females	UR, n = 8; mean restraint score 14.4 ± 0.9 R, n=7; mean restraint score 5.9 ±	UR: 30.8 ± 2.1 R: 28.6 ± 2.8 (mean ± SEM)	UR: 33.1 ± 2.9 R: 36.6 ± 3.1 (mean ± SEM)	Indirect calorimetry	Three-factor eating inventory	REE was not different between groups at baseline, however after 3 days of an energy adequate diet REE increased in R and decreased in UR (p<0.05). This main effect of restraint was significant (p<0.01). <table style="margin-left: auto; margin-right: auto;"> <tbody> <tr> <td style="text-align: center;">R: 0.16 ± 0.07</td> </tr> <tr> <td style="text-align: center;">UR: -0.07 ± 0.07</td> </tr> </tbody> </table> (kJ/min, least squares mean ± SEM, adjusted for fat-free mass). R had significantly lower body mass (kg), BMI and fat free mass (kg) than UR (p<0.05).	R: 0.16 ± 0.07	UR: -0.07 ± 0.07							
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Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results												
		1.1 (mean ± SEM)					<table style="width: 100%; border: none;"> <tr> <td></td> <td style="text-align: center;"><u>R</u></td> <td style="text-align: center;"><u>UR</u></td> </tr> <tr> <td style="text-align: center;">Body mass (kg)</td> <td style="text-align: center;">79.6 ± 8.2</td> <td style="text-align: center;">87.1 ± 6.2</td> </tr> <tr> <td style="text-align: center;">Fat free mass (kg)</td> <td style="text-align: center;">45.7 ± 2.9</td> <td style="text-align: center;">51.2 ± 2.7</td> </tr> <tr> <td></td> <td colspan="2" style="text-align: center;">(mean ± SEM)</td> </tr> </table>		<u>R</u>	<u>UR</u>	Body mass (kg)	79.6 ± 8.2	87.1 ± 6.2	Fat free mass (kg)	45.7 ± 2.9	51.2 ± 2.7		(mean ± SEM)	
	<u>R</u>	<u>UR</u>																	
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Fat free mass (kg)	45.7 ± 2.9	51.2 ± 2.7																	
	(mean ± SEM)																		
Klesges <i>et al</i> (1992)	250 Caucasian adults. Male n=123, female n=127.	Male R, n=68 and male UR, n=73. Men with restraint score ≥ 14 classed as R Female R, n=76 and female UR, n=70. Females with restraint score ≥ 17 classed as R.	Male R: 29.2 ± 4.2 UR: 24.7 ± 2.8 Female R: 27.0 ± 5.0 UR: 22.5 ± 4.5 (mean ± SD)	Male R: 26.7 ± 4.9 UR: 36.7 ± 4.7 Female R: 35.4 ± 4.3 UR: 35.1 ± 4.0 (mean ± SD)	Baecke physical activity questionnaire	Revised restraint scale	<p>There were no significant differences in self-reported activity levels between R and UR (p>0.05).</p> <p>There was a significant direct relationship between restraint scores and body mass in men (r=0.40, p<0.0001) and women (r=0.45, p<0.001).</p> <p>In females only, body mass gain over 1 year was associated with restraint score (r=0.29, p=0.04).</p> <p>BMI was significantly higher in male and female R compared to UR (p<0.01).</p>												
Laessle & Kikker (2008)	33 lean, healthy females	Not reported	21.2 ± 2.1 (mean ± SD)	22.5 ± 4.3 (mean ± SD)	Indirect calorimetry to measure RMR	Three factor eating questionnaire	There was a significant negative relationship between restraint score and RMR (controlled for BMI, partial r= -0.46, p<0.01).												
Lawson <i>et al</i> (1995)	44 pre-menopausal females divided into 4 groups: 1. Low restraint/low disinhibition (LR/LD) 2. High	Restraint score ≤6 = classed as UR. Restraint score ≥ 13 = classed as R. Disinhibition score ≤6 = classed as	Not reported LR/LD: 19.1 ± 2.09 HR/LD: 23.1 ± 2.19 LR/HD: 37.4 ± 2.19	LR/LD: 28.9 ± 2.7 HR/LD: 32.9 ± 2.8 LR/HD: 36.1 ± 2.8	Accelerometer	Three factor eating questionnaire	<p>There were no significant differences in RMR between the groups when controlled for fat and fat-free mass (p>0.05).</p> <p>There were no significant differences between activity levels of R and UR (p>0.05).</p> <p>Participants with high disinhibition were less active than those with low disinhibition (p<0.04).</p> <p style="text-align: center;">Activity EE: High disinhibition: 108.8 Low disinhibition: 119.7</p>												

Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results																
	restraint/low disinhibition (HR/LD) 3. Low restraint/high disinhibition (LR/HD) 4. High restraint/high disinhibition (HR/HD)	low disinhibition, score ≥ 12 = classed as high disinhibition	HR/HD: 27.9 ± 2.29 (mean \pm SEM)	HR/HD: 37.0 ± 2.9 (mean \pm SEM)			(kJ kg ⁻¹ 24h ⁻¹) UR participants with high disinhibition has significantly higher BMI than R participants with high disinhibition (p<0.05). R and UR participants with low disinhibition had significantly lower BMI than the groups with high disinhibition (p<0.05).																
Manore <i>et al</i> (1991)	33 non-obese females; 11 cyclical dieters and 12 non-dieters	Not reported	Dieters: 23.6 ± 0.6 Control: 20.6 ± 0.4 (mean \pm SEM)	Dieters: 23 ± 2 Control: 25 ± 1 (mean \pm SEM)	Indirect calorimetry	Not measured	REE was significantly lower in dieters than controls (p<0.01). <table border="1"> <thead> <tr> <th></th> <th><u>Dieters</u></th> <th><u>Controls</u></th> </tr> </thead> <tbody> <tr> <td>Body mass (kg)</td> <td>65.7 ± 2.2</td> <td>56.7 ± 1.6</td> </tr> <tr> <td>Body fat %</td> <td>26.4 ± 1.2</td> <td>20.6 ± 1.9</td> </tr> <tr> <td>REE (kJ kg⁻¹ day⁻¹)</td> <td>79.9 ± 2.1</td> <td>90.8 ± 2.1</td> </tr> </tbody> </table> (mean \pm SEM) During exercise at 3 different intensities, relative ExEE was significantly lower (4.5-7.4%) in dieters than controls (p=0.0083). Dieters had significantly higher body mass (p=0.004), BMI (p<0.002), and body fat % (p<0.002) than control participants.		<u>Dieters</u>	<u>Controls</u>	Body mass (kg)	65.7 ± 2.2	56.7 ± 1.6	Body fat %	26.4 ± 1.2	20.6 ± 1.9	REE (kJ kg ⁻¹ day ⁻¹)	79.9 ± 2.1	90.8 ± 2.1				
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Mulvihill <i>et al</i> (2020)	64 adolescent females (aged 14-15 years)	Low restraint group (LR): 1.84 ± 0.33 (n=19) Medium restraint group (MR): 7.35 ± 0.27 (n=20)	LR: 19.6 ± 0.5 MR: 22.2 ± 0.6 HR: 23.9 ± 1.2 (mean \pm SEM)	Whole group: 14.9 ± 0.3 (mean \pm SD)	7 day physical activity diary	Dutch eating behaviour questionnaire and three factor eating questionnaire	Girls with higher restraint score had significantly higher TEE (p<0.001), BMR (p<0.01), body mass (p<0.01), and BMI (p<0.001) than those with the lowest scores. <table border="1"> <thead> <tr> <th></th> <th><u>LR</u></th> <th><u>MR</u></th> <th><u>HR</u></th> </tr> </thead> <tbody> <tr> <td>TEE (MJ)</td> <td>8.51 ± 0.22</td> <td>9.12 ± 0.20</td> <td>9.95 ± 0.33</td> </tr> <tr> <td>BMR (MJ)</td> <td>5.84 ± 0.12</td> <td>6.30 ± 0.11</td> <td>6.39 ± 0.17</td> </tr> <tr> <td>BM (kg)</td> <td>52.9 ± 2.1</td> <td>61.2 ± 2.0</td> <td>62.8 ± 3.1</td> </tr> </tbody> </table> (mean \pm SEM)		<u>LR</u>	<u>MR</u>	<u>HR</u>	TEE (MJ)	8.51 ± 0.22	9.12 ± 0.20	9.95 ± 0.33	BMR (MJ)	5.84 ± 0.12	6.30 ± 0.11	6.39 ± 0.17	BM (kg)	52.9 ± 2.1	61.2 ± 2.0	62.8 ± 3.1
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Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results
		High restraint group (HR): 13.5 ± 0.53 (n=19) (mean ± SEM)					
Platte <i>et al</i> (1996a)	24 lean females aged 18-20 years	Score of ≤ 3 on restraint subscale classed as UR (n=12), score ≥ 10 classed as R (n=12)	UR: 20.0 ± 1.4 R: 20.1 ± 1.6 (mean ± SD)	UR: 24.7 ± 2.5 R: 23.8 ± 4.0 (mean ± SD)	Indirect calorimetry	Three factor eating questionnaire	RMR was significantly lower in R than UR (p=0.02). R: 5163 ± 485 UR: 5678 ± 464 (mean ± SD, kJ day ⁻¹) Body mass (r=0.61; p<0.01) and lean body mass (r=0.71; p<0.001) were significantly associated with RMR in UR only.
Platte <i>et al</i> (1996b)	24 lean females - all restrained eaters, divided into 2 groups: 1. Weight stable R (WS; n=12) 2. Weight cycling R (WC; n=12)	Score ≥ 10 on restraint subscale classed as R.	WS: 21.2 ± 1.6 WC: 22.1 ± 1.8 (mean ± SD)	WS: 22.3 ± 2.5 WC: 22.3 ± 2.3 (mean ± SD)	Indirect calorimetry	Three factor eating questionnaire	There were no significant differences in RMR or diet-induced thermogenesis between the groups (p>0.05). Correlations between lean body mass (r=0.87, p<0.001) and body mass (r=0.91, p<0.001) with RMR were significant only in the WS group.
Poehlman <i>et al</i> (1991)	44 non-obese females	Whole group mean: 11 ± 5 (mean ± SD)	Not reported Mean body mass: 59 ± 7.3 kg Mean body fat %: 18 ± 5.2 (mean ± SD)	28 ± 5.9 (mean ± SD)	Indirect calorimetry	Three factor eating questionnaire	Mean RMR in the whole group was 3.93 ± 0.46 (kJ min ⁻¹). High levels of dietary restraint were associated with higher levels of body fat (r=0.31; p<0.05) and a lower resting metabolic rate (r=-0.29; p=0.07), even after controlling for fat free mass.

Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results												
			SD)																
Tepper <i>et al</i> (1996)	249 lean participants: Females (n=138) and males (n=111)	R females (n=79): 14.0 ± 0.3 UR females (n=59): 6.0 ± 0.3 R males (n=53): 11.3 ± 5.0 UR males (n=58): 3.2 ± 0.3 (mean ± SEM)	R females: 22.5 ± 0.04 UR females: 21.6 ± 0.05 R males: 23.2 ± 0.04 UR males: 22.7 ± 0.04 (mean ± SEM)	Whole group: 22.1 ± 7.9 (mean ± SD)	Physical activity questionnaire	Three factor eating questionnaire	Males were more physically active than females (p<0.001). There was no significant main effect of restraint or restraint by gender interaction on physical activity (p>0.05).												
Tuschl <i>et al</i> (1990)	23 lean females	Score of ≤ 3 on restraint subscale classed as UR (n=11), score ≥ 10 classed as R (n=12)	UR: 20.0 ± 1.3 R: 21.1 ± 1.3 (mean ± SD)	Females aged between 18-30 years.	Doubly labelled water	Three factor eating questionnaire	R expended 2594 kJ/day less than UR after adjusting for body composition and height (p<0.005). R also consumed 1714 kJ/day less than UR after adjusting for body composition and height (p<0.002). R had a significantly higher BMI than UR (p<0.03).												
Van Loan & Keim (2000)	185 pre-menopausal females	UR (n=89): 4.7 ± 2.0 R (n=96): 13.4 ± 3.0 (mean ± SD)	UR: 22.8 ± 3.6 R: 23.8 ± 3.1 (mean ± SD)	UR: 30.7 ± 7.2 R: 30.8 ± 7.7 (mean ± SD)	Paffenbarger questionnaire	Three factor eating questionnaire	There were no significant differences in physical activity levels or METs per week between groups (p>0.05). <table style="margin-left: auto; margin-right: auto;"> <tr> <td></td> <td style="text-align: center;"><u>UR</u></td> <td style="text-align: center;"><u>R</u></td> </tr> <tr> <td>METs (per week)</td> <td style="text-align: center;">84.1 ± 143.4</td> <td style="text-align: center;">53.9 ± 80.1</td> </tr> <tr> <td>PA (hrs/week)</td> <td style="text-align: center;">6.4 ± 5.7</td> <td style="text-align: center;">5.4 ± 5.0</td> </tr> <tr> <td></td> <td colspan="2" style="text-align: center;">(mean ± SD)</td> </tr> </table>		<u>UR</u>	<u>R</u>	METs (per week)	84.1 ± 143.4	53.9 ± 80.1	PA (hrs/week)	6.4 ± 5.7	5.4 ± 5.0		(mean ± SD)	
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Vescovi <i>et al</i> (2008)	84 physically active, lean, pre-menopausal women	UR (n=46): 4.9 ± 0.3 R (n=38):	UR: 21.6 ± 0.3 R: 21.4 ± 0.3	UR: 24.7 ± 0.7 R:	Indirect calorimetry	Three factor eating questionnaire	There were no significant differences in REE in absolute terms (p=0.26) or when controlled for fat free mass (p=0.12). <table style="margin-left: auto; margin-right: auto;"> <tr> <td></td> <td style="text-align: center;"><u>UR</u></td> <td style="text-align: center;"><u>R</u></td> </tr> <tr> <td>REE (kJ day⁻¹)</td> <td style="text-align: center;">5584.1 ± 117.2</td> <td style="text-align: center;">5405.2 ± 100.5</td> </tr> </table>		<u>UR</u>	<u>R</u>	REE (kJ day ⁻¹)	5584.1 ± 117.2	5405.2 ± 100.5						
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Authors	Participants	Dietary restraint scores	Mean BMI (kg/m ²)	Mean age (years)	Method of measuring PA/EE	Method of measuring dietary restraint	Results
		13.5 ± 0.5 (mean ± SEM)	(mean ± SEM)	23.1 ± 0.7 (mean ± SEM)			REE (kJ kg FFM ⁻¹) 128.1 ± 2.1 (mean ± SEM) 123.1 ± 2.1
Waugh <i>et al</i> (2007)	189 healthy, young females	Lowest tertile (score<5; n=76): 3.5 ± 1.2 Middle tertile (n=70): 7.1 ± 1.3 Highest tertile (score>9.4; n=79): 12.1 ± 2.4 (mean ± SD)	Lowest tertile: 21.2 Middle tertile: 23.5 Highest tertile: 25.2	Lowest tertile: 34 Middle tertile: 33 Highest tertile: 34	Baecke questionnaire	Three factor eating questionnaire	Women in the highest tertile of restraint had significantly higher BMI (p<0.03) and higher Baecke sport index (p=0.006), but not higher total Baecke score (p=0.08), than those in the middle and lowest tertiles. Restraint scores were significantly correlated with BMI (r=0.27, p<0.0001), Baecke PS score (r=0.16, p=0.01) and Baecke sport index score (r=0.22, p=0.0005).

1.6 Aims

The prevalence of obesity has risen dramatically in the last 40 years (Cole, 2003) and has now become a global epidemic (Popkin & Doak, 1998). Many developed countries are considered to have what is termed an “obesogenic” environment, with energy dense foods widely available and sedentary lifestyles contributing to the increasing prevalence of obesity (Lake & Townshend, 2006). Treatment of obesity is a complex proposition; individual energy balance is regulated and influenced by a myriad of physiological, psychological and environmental factors. A significant reduction in body fat mass requires the individuals to be in a chronic state of negative energy balance, and significant behavioural changes in eating and activity behaviour are needed in order to achieve this state. Changes in physical activity levels may be counterproductive; some overweight and obese seem prone to compensatory changes in EI and non activity EE in response to regular exercise participation (King *et al*, 2008; Manthou *et al*, 2010); mechanisms have not been identified and there are still gaps in this field of literature which require further investigation. Appetite regulating hormones such as ghrelin and peptide YY have been investigated as a possible mechanism behind such changes; although the former has been shown to up-regulate in response to body mass reduction in overweight women (Foster-Schubert *et al*, 2005), it is not clear if such changes contribute to compensatory mechanisms. Evidence regarding peptide YY is limited and even less is known about the effect of exercise on this hormone, and the role it may play in compensatory changes. Furthermore it is possible that differences in these hormones contribute to differences in individual susceptibility to compensatory changes; this has not been widely investigated and further research is required to investigate this possibility. Short term observation of these hormones and EI responses to a single exercise session have also been of interest in order to understand the impact of exercise participation on short term energy balance regulation. Research has focused largely on lean individuals, particularly men, and it is still of interest to investigate these short term responses in overweight women. Existing evidence has indicated no effect (Unick *et al*, 2010), but sample sizes are small and the methodology used to measure EI has not been validated with this population so these findings are not definitive. Effects of exercise on

peptide YY are relatively poorly understood in the short term as well as the long term, particularly in overweight women.

Behavioural factors may also play a role in energy balance and body mass regulation. Dietary restraint levels are one such factor and have been positively associated with body mass and body mass gain over time in women (Klesges *et al*, 1992). Restraint level may also have a physiological basis as it has been linked with glucose regulation (Keim and Horn, 2004) and circulating levels of ghrelin (Schur *et al*, 2008). The hypothesis that diminished energy requirements are linked with high dietary restraint has not been definitively proven or disproven; findings in the literature are mixed (Tuschl *et al*, 1990; Poehlman *et al*, 1991; Platte *et al*, 1996a; Martin *et al*, 2007; Laessle & Kikker, 2008). Restraint has been shown to have an important interaction with disinhibition levels (Westenhoefer *et al*, 1991 & 1994) and body mass (Lawson *et al*, 1995). The construct of dietary restraint has been sub-classified into two distinct types based on this interaction; flexible and rigid control of restraint (Westenhoefer *et al*, 1999). There is relatively little evidence regarding the differences between these two sub-groups and, and it is not know if they differ in either physiological or activity energy requirements.

Many interlinked biological and physiological factors influence total EE, and hence there are many possible mechanisms responsible for exercise induced compensatory behaviours. There is much that is still not known about these behaviours however, and this thesis aims to some explore these factors further. Potential behavioural and physiological contributing factors will be examined in an attempt to provide better understanding of energy balance regulation in overweight and obese, sedentary women.

The main research questions this thesis sought to answer were:

- Do acute and chronic exercise-induced compensatory responses in energy balance occur in overweight and obese, sedentary, pre-menopausal women?

- Does individual variability in exercise-induced body fat reduction have an association with changes in appetite, EI and/or EE in overweight women?
- Does acute and chronic exercise participation affect circulating acylated ghrelin and peptide YY levels, and do these hormones play a role in compensatory changes in appetite, EI and EE?
- Are energy intake values obtained from the laboratory based buffet meal method reproducible at rest and post-exercise in overweight and obese, sedentary, pre-menopausal women?
- Does dietary restraint have an association with energy expenditure and physical activity levels?
- Do restrained eaters defined as having flexible or rigid control differ in their physiological energy requirements and physical activity patterns?

In order to answer these research questions, the four separate studies detailed in this thesis were carried out.

Chapter 3 details a methodological investigation which was conducted last, chronologically, but has the greatest impact on the existing field of literature of all four studies in this thesis. This study was borne out of the findings of the work detailed in chapter 4, which investigated the acute effects of a single moderate exercise session on appetite, EI, or appetite hormone levels in twenty-nine overweight and obese women. Evidence regarding the impact of exercise on short term appetite and energy balance regulation in overweight women is currently very limited, and it is not clear if exercise is associated with short term compensatory responses in these women. Findings from chapter 4 indicated that a single walking-based exercise session did not alter energy balance regulation or induce compensatory response, but small sample sizes and methodological limitations means that these data are not conclusive. EI levels were assessed using the buffet meal methodology; a means often utilised in acute exercise studies in an attempt to bypass the difficulties of self reported EI assessment methods. Many studies using this method fail to observe any change

in EI, and it is possible that other factors may introduce bias therefore affecting EI values obtained in this setting (Herman & Polivy, 2005). Two studies in this thesis were also designed using this method of EI assessment as data collected from seven day self-reported had to be discarded due to a large degree of under-reporting. Acute EI data obtained from buffet meals were found to be highly variable between individuals, and this raised questions regarding the reliability of this method. Further investigation showed that the test-retest reliability of this method was unproven under post-exercise conditions, and in overweight individuals. A study to examine test-retest reliability of twenty-four hour EI values obtained from this method was carried out with fourteen overweight and obese women. This investigation potentially undermined the data of the earlier studies in this thesis; however these methodological considerations warranted further investigation. Results of this study indicated that test-retest reliability of this method was poor in these women, under both control and exercise conditions. This finding impacts not only the acute study of chapter 4, but potentially much of the published literature regarding acute responses to exercise as well.

Additionally, though evidence consistently indicates that body mass reduction induces an up-regulation of ghrelin levels (Foster-Schubert *et al*, 2005; Garcia *et al*, 2006), it is still not clear if acylated ghrelin plays a role in chronic exercise-induced compensatory mechanisms. These mechanisms seem to be of a highly individual nature, and are linked to subsequent changes in EI and appetite (King *et al*, 2008 & 2009), but a mechanism has still to be defined for these responses. As a result the chronic effects of exercise on appetite hormones, measures of appetite and EI were all of interest in this thesis. The effects of 16 weeks exercise participation on these factors was investigated in fifteen overweight and obese, sedentary women in chapter 5. Due to limited sample size and statistical power, no compensatory changes in energy balance or potentially mechanistic changes in appetite hormone levels were apparent.

Chapter 6 detailed an observational study which investigated energy requirements in 21 sedentary, overweight and obese women classed as restrained eaters. These women were further subdivided into those with rigid

and flexible control of restraint and a novel investigation of energy requirements in these subgroups was carried out. No evidence of differences in energy requirements between restrained and unrestrained, or between flexible and rigid restrained eaters; the latter finding was inconclusive due to small participant numbers in these sub-groups. This would indicate that variability in body fatness, and hence energy balance, may be attributable to differences in dietary intake, at least between restrained and unrestrained eaters.

CHAPTER 2: General methods

2.1 Recruitment and Ethical Approvals

Ethical approvals for all studies were obtained from the Faculty of Medicine Ethics Committee, University of Glasgow. Participants were recruited via advertisements placed in the university and local NHS staff newsletters, on the university website and on a local community website. Posters were also placed around campus, and in several local hospitals; the Western Infirmary, Glasgow Royal Infirmary, Gartnavel hospital and Royal Hospital for Sick Children (appendix I).

Potential participants expressed interest via phone or email. Study procedures were explained verbally, either over the phone or in person, and participants were then given a written information sheet to read (appendix II). If participants then chose to participate they gave written consent (appendix III) and completed several preliminary questionnaires; a health screening questionnaire (appendix IV), the international physical activity questionnaire (IPAQ) (appendix V) to assess physical activity levels, and a personal information and food preference questionnaire (appendix VI). All personal information was anonymised and stored securely on University of Glasgow servers. Both participant and researcher signed and dated a written consent form.

Inclusion criteria for all studies in this thesis were:

- Female
- Pre-menopausal
- BMI of $\geq 25 \text{ kg/m}^2$
- Healthy (not taking any medication except for oral contraceptives, and blood pressure $<160/90\text{mmHg}$)
- Non-smokers
- Currently sedentary (<2 hours planned exercise per week)

- Not currently dieting to reduce body mass
- Not currently pregnant

2.2 Anthropometry and physical characteristics

2.2.1 Height and Body mass

Standard procedures were used for measurement of basic anthropometry. Height was measured to the nearest 0.1cm using a stadiometer (Seca 213, Seca, Birmingham, UK) and body mass was measured to the nearest 0.1kg in the fasted state using digital scales (TANITA TBF-300, Tanita B.V, Hoofddorp, The Netherlands) and taken to the nearest 0.1kg. Participants were measured and weighed in bare feet with light clothing; a correction of 0.5kg was entered before weighing to allow for the weight of clothing.

2.2.2 Body Mass Index (BMI)

Body mass index was calculated from measured height and body mass using the standard formula ($BMI = \text{body mass (kg)}/\text{height (m}^2\text{)}$) (Keys *et al*, 1972).

2.2.3 Waist and hip circumference

Waist and hip circumference were measured over light clothing and after exhalation; participants stood with feet together and arms at their side for the measurement. Waist measurements were taken at the midpoint between the lower rib margin and the iliac crest in the horizontal plane. Hip measurements were taken at the maximum circumference around the buttocks, below the iliac crest. Measurements were taken to the nearest cm and waist:hip ratio was calculated by dividing waist measurement by hip measurement (World Health Organisation. Measuring obesity: classification and description of anthropometric data. Copenhagen: WHO, 1989)

2.2.4 Body composition

2.2.4.1 Bio-impedance

Body composition measurements were obtained from bio impedance scales (TANITA TBF-300, Tanita B.V, Hoofddorp, The Netherlands) in all studies. Bio impedance is measured by passing a small electrical signal (50 KHz, 800 μ A) through the footplates of the scales. Impedance is defined as the strength and speed of an electrical signal travelling through the body and is measured in ohms. Since fat free mass acts as a conductor due to its high water and electrolyte content whilst fat mass is a resistor, the impedance measurement is representative of the proportions of each type of tissue present. This data is combined with age, sex, height, and fitness level to given an estimate of body fat %.

Led-to leg bio impedance measurements (Tanita TBF-300, Tanita B.V, Hoofddorp, The Netherlands) were made in the fasted state with the participants barefoot, in light clothing, standing straight with arms by their sides. Before measurement, sex, body type (athletic or average), age, height were entered. Body type of each participant was determined according to manufacturers specifications; “athletic” setting is only appropriate if the participant engages in >10 hours/week intense physical activity and has a resting heart rate of < 60 beats per minute, therefore all participants were measured using the “average” setting.

2.2.4.2 Air Displacement Plethysmography

Air displacement plethysmography was used to assess body composition. This method is based on a two compartment model of body composition, which assumes that body mass is made up of two types of tissue, fat mass and fat-free mass (including protein, water, mineral and glycogen). This method determines body volume from pressure measurements via Poisson’s Law:

$$P1/P2 = (V2/V1)^\lambda$$

Where P = pressure, V = volume and λ = the ratio of the specific heat of the gas at constant pressure compared to that of constant volume. Since the device used for this measurement is constructed of two chambers with a diaphragm system that produces small volume and pressure perturbations of equal and opposite magnitude in order to obtain body volume, the letters 1 and 2 denote pressure and volume in each of these chambers.

Body density is then calculated using the following equation:

$$D = M / (V_{\text{brow}} + 0.40 V_{\text{tg}} - \text{SAA})$$

Where M = body mass (kg), V_{brow} = raw body volume (L), V_{tg} = thoracic gas volume (L), and SAA is the surface area artefact (L); this variable is automatically computed by the software of the device, and accounts for the presence of iso thermic air in the chamber which can affect accuracy of the measurement.

Since it is known that lean mass (1.06 kg/L) is denser than fat mass (0.9196 kg/L) (Lukaski, 1987), the following equation (Siri, 1961) is used to calculate percentage body fat from body density:

$$\% \text{ fat} = (495 - \text{Density}) - 450$$

As this method is based on a two-compartment model the remaining proportion is assumed to be entirely fat free mass and this can be simply calculated by subtracting the value obtained for fat mass from total body mass.

This procedure was carried out in the laboratory using the Bodpod® apparatus (Life measurement Inc., Concord, CA USA) (figure 2.1). Before each measurement, the Bodpod® was calibrated empty and then against a 49.998L cylinder. Participants were asked not to eat a heavy meal or engage in exercise for at least 2 hours before the test. Tests were conducted in underwear or swimming costume and cap, reducing the likelihood of air pockets underneath loose clothing affecting accuracy of the measurement. Age, sex, and height of

the participant were entered before beginning measurement. Body mass was measured directly, to the nearest 0.01 kg, using the integrated scales. Participants were instructed to sit quietly with their hands in their lap, avoid touching the sides of the pod, and breathe normally during measurement. They were shown how to exit the pod if they felt uncomfortable at any point during the test. Two measurements were made and averaged in order to obtain accurate values. If values obtained from the first two measurements were not within 5% agreement a third measurement was automatically carried out to ensure accuracy of results.

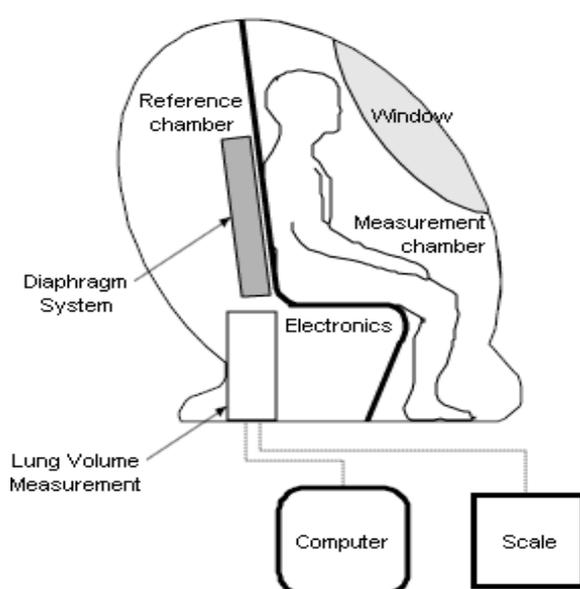


Figure 2.1 Schematic of the Bodpod® apparatus (Source: Baylor College of Medicine).

2.2.4.3 Dual X-Ray Absorptiometry (DEXA)

Body composition was determined by dual X-Ray absorptiometry (DEXA) scan. This approach is one of the most accurate methods of assessing body composition (Mazess *et al*, 1990). In this method a constant potential energy source of 76kV and a dose efficient K-edge filter are used to create a congruent beam of dual energy radiation; these energies are attenuated to different degrees by bone mineral content, and fat and lean mass content. The differences in attenuation of the dual energy radiation allow determination of body composition.

All scans were conducted at the Royal Hospital for Sick Children, Glasgow, UK, by a trained and qualified operator using a Lunar Prodigy scanner (GE Medical Systems, Waukesha, Wisconsin, US) with Lunar enCORE 2004 software (Version 8.80.001, GE Medical Systems, Wisconsin, US). Before commencing measurement, body mass was measured to the nearest 0.1kg with the use of an electronic column scale (Weylux 824, 160kg x 100g, H Fereday and Sons, London, UK), and height was recorded to the nearest 0.1cm with a stadiometer (Raven Equipment Ltd., Essex, UK). Participants wore a light hospital gown and removed all metallic objects prior to beginning the scan. Scans took approximately 5 minutes to complete and results were available immediately.

Results obtained from the scan include total body mass, body fat % and mass, lean mass (excluding bone mass) and information about central fat distribution, specifically % fat in the android and the gynoid region. Android fat % describes extent of abdominal adiposity, whilst gynoid includes fat deposited lower around in the pelvic region, as illustrated in figure 2.2.

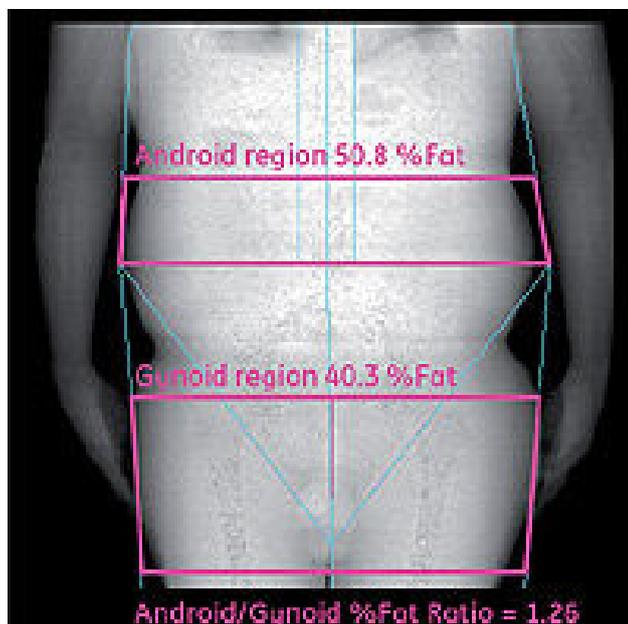


Figure 2.2 Definition of android and gynoid areas in DEXA body composition analysis (Source: GE Medical systems lunar densitometry).

2.2.5 Blood pressure measurements

Blood pressure was measured during screening to ensure participants had no indication of hypertension, which would exclude them from participation.

Measurements were made using an automated monitor (Omron Healthcare UK Limited, Milton Keynes, UK) and conducted according to the European Society of Hypertension's guidelines (Parati *et al*, 2008). Specifically, all measurements were preceded by at least five minutes seated rest in a quiet room maintained at a comfortable temperature. Values were obtained with the participant seated with legs uncrossed and back supported, the cuff positioned at heart level on a supported arm, and comfortable environmental conditions. Participants were asked not to talk during the measurement in order to minimise disturbances during the measurement.

2.3 Sub-maximal exercise test

Participants completed an incremental sub maximal exercise test to establish $\dot{V}O_{2max}$. Participants were allowed to complete this test on either a treadmill or bicycle ergometer (Ergomedic 873, Monark, Sweden) depending on the participants' preference.

All submaximal tests were conducted within air conditioned exercise laboratories within the West Medical Building, University of Glasgow. Unlimited water and towels were provided, a chair was positioned nearby, and participants were free to stop the test at any time for any reason. Submaximal tests were supervised by one person who was present at all times and had been trained in first aid. Tests were terminated either when the participant felt they could no longer continue, or when the heart rate reached 85% of age-predicted maximum (HR_{max}), defined as $220 - \text{age}$ (Fox & Haskell, 1970).

2.3.1 Treadmill protocol

Tests were incremental and each stage lasted 5 minutes. Participants began the test at a walking speed of 5-6 km/hr and the speed was increased by increments of 0.5-0.8 km/hr depending on participants' fitness and heart rate response. In the last minute of each stage heart rate was recorded and an expired air sample collected using standard Douglas bag technique to allow determination of the rate of oxygen consumption (Consolazio, 1963).

2.3.2 Bicycle ergometer protocol

The protocol and timing of samples was identical for the bicycle ergometer. Participants were asked to find a comfortable cadence they felt they could maintain (usually 50-60 rpm) and resistance was then adjusted to begin the test at workload of 50-60W, again depending on fitness level and heart rate response. Each stage lasted 5 minutes and workload was increased by 15-25W after completion of each stage. Measurements, timings, and criteria for termination of the test were identical to those described for treadmill protocol.

2.3.3 Expired Air Analysis

All air samples collected in Douglas bags were analysed for oxygen and carbon dioxide content using a gas analyser (Servomex 4000 series, Servomex Group Ltd., East Sussex, UK). Before commencing tests, the gas analyser was calibrated against reference gases of a known concentration (BOC Gases, BOC Limited, Surrey, UK). Barometric pressure was measured and all measurements were corrected for room temperature and dry gas pressure. Douglas bags were connected to the analyser using the Douglas bag sampling port and 0.6L air, controlled by a flow meter, was sampled over a two minute period. Oxygen and carbon dioxide content of the expired air samples was determined as a percentage. The volume and temperature of expired air was then measured using an extraction unit with a digital dry gas meter (Harvard Apparatus Ltd, Kent, UK) with an integrated digital thermometer (Cranlea & Company, Birmingham, UK). Volume of 0.6L was added to total gas volume to correct for the earlier gas analyser sample. $\dot{V}O_2$ and $\dot{V}CO_2$ were subsequently calculated using the Haldane transformation of the Fick equation (Wilmore & Costill, 1973):

$$V_I = \text{ESTPD} \times (100 - \text{Exp. Fraction } O_2 - \text{Exp. Fraction } CO_2) / 79.04$$

$$\dot{V}O_2 \text{ (l.min}^{-1}\text{)} = V_I \times 0.2093 - (\text{VESTPD} \times \text{Exp. Fraction } O_2 / 100)$$

$$\dot{V}CO_2 \text{ (l.min}^{-1}\text{)} = (\text{VESTPD} \times \text{Exp. Fraction } CO_2 / 100) - V_I \times 0.0003$$

Where I = Volume inhaled, E= Volume exhaled, STPD = dry gas pressure.

2.3.4 Prediction of $\dot{V}O_{2max}$

$\dot{V}O_{2max}$ was predicted by plotting the linear relationship between HR and $\dot{V}O_2$ obtained during the sub-maximal fitness test. Predicted $\dot{V}O_{2max}$ was calculated by extrapolating $\dot{V}O_2$ to HR_{max} (American College of Sports Medicine, 1995).

$\dot{V}O_{2max}$ was finally expressed relative to body mass ($ml\ kg^{-1}min^{-1}$)

2.4 Metabolic rate measurements

Metabolic rate measurements were conducted by indirect calorimetry via a ventilated hood system. Participants were asked to arrive at the laboratory by automated means of transport prior to the measurement. Participants lay supine with legs flat and arms by their side in a darkened, thermo-neutral environment for metabolic rate measurement. The hood was placed over the participants head and expired air was sampled every 60 seconds for 25 minutes (Oxycon Pro, Carefusion, San Diego, CA 92130, USA). Participants were monitored by the experimenter for the entirety of the measurement to ensure they remained still and quiet. $\dot{V}O_2$ and $\dot{V}CO_2$ were determined from expired air samples by the hood system, and substrate oxidation and metabolic rate were subsequently determined using the equations of Frayn & McDonald (1997):

$$\text{Rate of fat oxidation (g.min}^{-1}\text{)} = (\dot{V}O_2 - \dot{V}CO_2)/0.57$$

$$\text{Rate of carbohydrate oxidation (g.min}^{-1}\text{)} = (1.4 \times \dot{V}CO_2 - \dot{V}O_2)/0.30$$

$$\text{Rate of EE (kJ.min}^{-1}\text{)} = [\text{rate of carbohydrate oxidation} \times 15.6] +$$

$$[\text{rate of fat oxidation} \times 39]$$

The first 10 minutes of each measurement was discarded to ensure only stable resting values were recorded. Stable values were averaged to determine mean metabolic rate and substrate oxidation. Fasting metabolic rate measurements were carried out in the morning after participants had fasted for at least 10

hours. All other measurements were conducted during trials in the non-fasted state.

2.5 Measurements of free-living energy expenditure

Total EE was calculated according to the method of Moon & Butte (1996) in which HR during various activities is related to $\dot{V}O_2$ and $\dot{V}CO_2$, and thus rate of EE, via linear regression equations. This method has shown good agreement with measurements obtained via a whole-body calorimeter and mean error rates for estimates of $\dot{V}O_2$ and $\dot{V}CO_2$ obtained via this method were $-3.4 \pm 4.5\%$ and $-4.6 \pm 3.6\%$ respectively (Moon & Butte, 1996). This method was chosen because of ease of use for free-living EE assessment and because it allows characterisation of activity patterns. Accuracy of this method is high; HR has been shown to be highly correlated with EE ($r=0.87$) (Strath *et al*, 2000). Indeed this method has proven to be most reliable of available heart rate monitoring methods (Treuth *et al*, 1998), and has previously been utilised in obese and non-obese adolescents (Lazzer *et al*, 2003). According to this method, activities are classed as sleeping, inactive (sitting or lying down) and active (any activity performed unseated). By continuously monitoring HR and keeping a record of all free-living activities this method can estimate free-living EE. This method has the advantage of compensating for the loss of linearity in the relationship between HR and $\dot{V}O_2$ that is observed in the inactive state. This method also enables categorisation and measurement of active and inactive sub-components of total EE, which is more informative in this context than methods such as the “gold standard” doubly labelled water which enables assessment of total EE only (Schoeller, 1988). Calibration of individual relationships between HR, $\dot{V}O_2$ and $\dot{V}CO_2$ whilst inactive and active was conducted with each participant.

2.5.1 Physical Activity Diary

Participants were asked to keep an activity diary for five weekdays and two weekend days (appendix VII). Participants were instructed about correct completion of the physical activity diary before beginning. The activity diary was designed previously and utilised for similar research within the university

(Barwell *et al* 2009, Manthou *et al*, 2010). Participants reported the type of activities under the headings of sleeping, sitting, standing, walking, self care, driving, exercise, and a column to record activities that did not fit into these categories was also included. Participants recorded the time of day of each activity and duration of each activity as accurately as possible (to the nearest ten minutes or less) throughout the course of the day.

2.5.2 Heart rate monitoring

HR was monitored and recorded at 1 minute intervals by short-range telemetry (Polar RS400, Polar Electro, Finland) in the laboratory and during the week of free-living measurement. Participants were given written and verbal instructions in the use of the heart rate monitor and were instructed to monitor heart rate for five week days and two weekend days. Participants were asked to wear the monitor from waking till going to bed, however participants were permitted to remove the monitor earlier in the day if the chest band caused discomfort. In the event of early removal of the monitor participants continued to record activities in the diary to allow calculation of total EE for that day.

Treatment of missing or erroneous data

Any short periods of HR data (≤ 10 minutes) that were missing or obviously erroneous were replaced with the last recorded feasible value. Any longer gaps in the HR data (≥ 10 minutes) were not replaced with prior HR values; information recorded in the physical activity diary by the subject was used to classify activities performed during that time period.

2.5.3 Calibration of heart rate to $\dot{V}O_2$ and $\dot{V}CO_2$

2.5.3.1 Inactive

Values of HR, $\dot{V}O_2$ and $\dot{V}CO_2$ were collected by indirect calorimetry (Oxycon Pro, Carefusion, San Diego, CA 92130, USA) whilst participants were supine in a thermo-neutral environment. Values were collected at one minute intervals for 15 minutes. Values of $\dot{V}O_2$, $\dot{V}CO_2$ and the cubed value of the HR were plotted to

establish the inactive relationships, and regressions equations of the form $y=bx-a$ were obtained for each relationship (where x represents heart rate cubed, and y represents $\dot{V}O_2/\dot{V}CO_2$, b represents the gradient, and a represents the y-intercept value). Sleeping EE was assumed to be equivalent to 95% of resting metabolic rate values (Goldberg *et al*, 1988).

2.5.3.2 Active

For the active calibration, expired air was collected and HR recorded whilst participants were standing still, swaying, and walking at self-selected moderate and vigorous paces. These values and those obtained during the fitness test were plotted and used to calculate regression equations with coefficients relating HR to $\dot{V}O_2$ and $\dot{V}CO_2$ individually. These relationships were expressed in the form $y=bx-a$, where x represents heart rate, and y represents $\dot{V}O_2/\dot{V}CO_2$, b represents the gradient, and a represents the y-intercept value.

2.5.4 Calculation of total energy expenditure

Total EE was calculated from recorded HR data and the physical activity diary for each day recorded. HR data was downloaded into a spreadsheet in and grouped in ten minute intervals, and average values for each ten minute interval were taken. Based on reported times and durations of activities recorded in the physical activity diary, the corresponding average HR during each ten minute period was classified as either inactive or active. By classifying HR data in this fashion an average active and inactive HR value could then calculated for each day. These average values were related back to $\dot{V}O_2$ and $\dot{V}CO_2$ values using the individual regression equations established during calibration testing. Active and inactive rate of fat and carbohydrate oxidation, and subsequently average rates of EE, was then calculated using validated equations (Fraysn & McDonald, 1997) as follows:

$$\text{Rate of fat oxidation (g/min)} = (\dot{V}O_2 - \dot{V}CO_2) / 0.57$$

$$\text{Rate of CHO oxidation (g/min)} = (1.4 \times \dot{V}CO_2 - \dot{V}O_2) / 0.3$$

$$\text{Rate of EE (kJ/min)} = (\text{Rate of CHO oxidation} \times 15.6) + (\text{Rate of fat oxidation} \times 39)$$

Sleeping EE was calculated by multiplying reported time spent sleeping by 95% of resting metabolic rate. The daily time spent being active, inactive and sleeping was recorded in the diary and these durations were used to multiply up the respective calculated rates of EE for each type of activity to obtain total active, inactive and sleeping EE, and hence total EE for each day. Values from all seven recorded days were then averaged to obtain mean EE values for all participants. A worked step-by-step example of this calculation is detailed in appendix VII.

2.6 Measurements of compliance and energy expenditure

Compliance to the exercise programme was measured via heart rate monitoring. All participants were provided with an HR monitor (Polar RS400, Polar Electro, Finland) which they were asked to wear during all exercise sessions. For each exercise session average HR was calculated and, using the previously established relationships between active HR, $\dot{V}O_2$ and $\dot{V}CO_2$ (of the form $y=bx-a$, where x represents heart rate and y represents $\dot{V}O_2/\dot{V}CO_2$), the mean $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated for each session. These values were then used to calculate mean substrate oxidation rate and rate of EE for the exercise session (Frayn & McDonald, 1997). Rate of EE was multiplied by recorded length of exercise session to obtain total session EE. Total compliance was calculated as a percentage of prescribed ExEE as follows:

$$\text{Compliance} = (\text{Actual ExEE} / \text{Prescribed ExEE}) \times 100$$

2.7 Measurement of appetite

Subjective feelings of appetite were measured throughout test meal days using the visual analogue scale (VAS) (appendix VIII). Each questionnaire consisted of 5 questions on a single page to measure different aspects of appetite; hunger, satiety, fullness, prospective food consumption and desire to eat. Questions

were phrased to measure extent of feeling at that time (e.g. how hungry do you feel now?) Participants were required to rate the intensity of their appetite by marking a 100mm line, relative to two anchor statements placed at either end of the line (e.g. I have never been more hungry, or I am not hungry at all). Questionnaires were scored by measuring the placing of the mark, which gave a score between 1 and 100 representing intensity of sensation. These values are plotted over time and time-averaged area-under-the-curve (AUC) calculated in order to express subjects appetite feelings relative to the time period in which they are observed.

2.8 Measurement of energy and macronutrient intake

EI was directly observed in the laboratory via buffet style meals. Before beginning the trials participants were asked about food preferences, dislikes or allergies to ensure all food presented to them was considered palatable for that individual. Three meals were served to participants during each trial; an evening meal on day 1 (figure 2.3), and breakfast (figure 2.4) and lunch on day 2 (figure 2.5). All meals were served in one of the metabolic suites in the West Medical Building, University of Glasgow, which provided light, airy and pleasant surroundings. These suites were separate from where exercise took place and large enough to create a separate space solely for participants to consume meals in. Meals were served at a table facing the window and care was taken to ensure that participants were not disturbed whilst eating.

Buffet meals included a selection of commercially prepared foods (table 2.1) which were identical between trials. The amount of food presented at each meal was in excess of amount the participant was expected to consume to ensure the possibility to eat to satiety. Participants consumed each meal alone and were instructed to eat as much as they desired before the experimenter returned after 30 minutes to remove all remaining foods. All foods were weighed before and after consumption of the buffet meal to enable calculation of EI using nutrient analysis software (Windiets 2005, Robert Gordon University, Aberdeen, UK) based on current UK food composition table (The Composition of Foods 6th edition, McCance & Widdowson 2002). Participants were unaware that EI was

being monitored in an attempt to minimise potential adverse effects influences on eating behaviour (Herman & Polivy, 2005).



Figure 2.3 Evening buffet meal



Figure 2.4 Breakfast buffet meal



Figure 2.5 Lunch buffet meal

Table 2.1 Foods served at buffet meals

<i>Meal</i>	<i>Foods</i>
Breakfast	Croissants Jam 1 Breakfast cereal, (e.g. Special K, Crunchy Nut Cornflakes - type dependent on participants preference) Semi-skimmed milk Whole milk yogurt Cut fresh fruit Pure orange juice Coffee or tea
Lunch	Sandwiches (white and wholemeal bread) Sandwich filler Vegetable or Tomato Soup Salad French dressing Cakes/Biscuits Cut fresh fruit Whole milk yogurt Pure orange juice Coffee or tea
Dinner	Ready meal (pasta based) White and wholemeal bread/toast “Flora” margarine spread Salad French dressing Cakes/Biscuits Cut fresh fruit Whole milk yogurt Pure orange juice Coffee or tea

2.9 Measurement of Eating Behaviour

2.9.1 Measurement of Dietary Restraint

The Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985) was administered to each participant on two separate occasions, before and after the week of activity monitoring, to determine dietary restraint. The three factor eating questionnaire measures three aspects of eating behaviour, dietary restraint, disinhibition, and hunger.

Validation studies have shown the TFEQ is effective for identification of current dieting status in females; individuals scoring highest on the restraint subscale report lowest EI and sweet consumption (French *et al*, 1995). The three subscales of the questionnaire have been shown to produce reliable results; temporal stability of restraint scores over a 5 month period has been quantified as 0.82 in a sample of one hundred and seventy nine women (Bardone-Cone *et al*, 2007). Coefficient α reliabilities representing internal consistency of for restraint, disinhibition and hunger scores have also been shown to be high in combined samples, as well as in populations of restrained and unrestrained eaters (Stunkard & Messick, 1985; table 2.2).

Table 2.2 Internal consistency estimates for the factor scales (Source: The Eating Inventory Manual, Stunkard & Messick, 1988).

Sample	N	Cognitive Restraint	Disinhibition	Hunger
Combined sample	98	0.93	0.91	0.85
Restrained Eaters	53	0.79	0.84	0.83
Unrestrained eaters	45	0.92	0.84	0.87

The standard error of measurements (SEM) was calculated from this same population, and were reported to be 1.16, 0.93, and 1.16 for restraint, disinhibition, and hunger respectively, indicating that the magnitude of error associated with these scores is reasonably low. The TFEQ has also been reported to be useful for prediction of eating response in a laboratory settings, with disinhibition score being quite highly correlated ($r=0.77$) with overeating in this situation, as well as in everyday life (Stunkard & Messick, 1988).

2.9.2 Measurement of flexible and rigid control of results

Flexible and rigid control of restraint were assessed using a questionnaire specifically designed for this purpose (appendix IX) (Westenhoefer *et al*, 1994). This questionnaire is based on the TFEQ and contains some of the same questions, as well as questions not featured in the TFEQ to assess type of restraint. The twenty eight questions are a mixture of true/false and multiple

choice formats. Twelve questions assess level of flexible control, whilst sixteen assess rigid control. This questionnaire is scored based on a point system devised by the designers; the section in which an individual scores highest indicates the predominant form of restraint.

This questionnaire in its present form was developed and validated in over nearly two thousand individuals by Westenhoefer *et al* (1999); internal consistency of flexible restraint scores in women was 0.82 and 0.80 for rigid control. This study found no association found between restraint and BMI until the two subgroups were considered; rigid control was associated with higher BMI than flexible control. This questionnaire has also proved useful in identifying type of disturbed eating in female eating disorder patients; rigid control was significantly associated with bulimia ($r=0.33$) and anorexia diagnosis ($r=0.48$), whereas flexible control was significantly associated only with anorexia ($r=0.55$). Flexible control was associated with current, maximum and minimum BMI ($r=0.45$, 0.37 , 0.59 respectively) and rigid control associated with minimum BMI ($r=0.34$). Reliability coefficients for flexible and rigid control scores were similar to those reported in Westenhoefer *et al* (1999); 0.80 and 0.78 respectively (Shearin *et al*, 1994). Other studies have supported the construct validity of this questionnaire, reporting a lack of disturbed eating behaviour and an inverse association between BMI and flexible control, whereas greater occurrence of eating and mood disturbances and a positive association with BMI were observed for rigid control (Smith *et al*, 1999; Stewart *et al*, 2002; Provencher *et al*, 2003).

2.9.3 Assessment of eating habits

Eating habits were assessed using the dietary targets monitor, a food frequency questionnaire which has been validated extensively in Scottish adults (appendix X) (Lean *et al*, 2003). This FFQ assesses frequency of consumption of 23 different categories of food and has an ordinal scoring scale, frequency categories in this scale range from 6+ times per day to less than once a month. Average weekly consumption data was calculated from this FFQ for each participant.

2.10 Blood sampling and analyses

2.10.1 *Blood sampling and plasma preparation*

Blood samples were taken via a cannula inserted into the antecubital vein by an experienced research nurse. The cannula was flushed with 0.2% sterile saline every thirty minutes during trials to prevent clotting, and residual saline was removed with a 2ml syringe before each blood sample was taken. Samples were collected in 9ml vacutainers containing EDTA (ethylenediaminetetraacetic acid) to prevent coagulation (Greiner Bio One, Stonehouse, UK).

Blood samples intended for acylated ghrelin determination were first treated with 10 μ l (per ml of blood) of a 100 mM PMSF (phenylmethanesulfonylfluoride) solution to prevent protease degradation (Hosoda *et al.*, 2004), and centrifuged at 14000 rpm for 5 minutes. The plasma supernatant was then added to 100 μ l (per ml of plasma) of 1 M hydrochloric acid per ml of supernatant and centrifuged at the same rate. Samples intended for peptide YY determination were treated with 80 μ l (per ml of blood) aprotinin solution (3-7 TIU/mg protein) and centrifuged for 5 minutes at 14000 rpm. Plasma supernatant from all samples was immediately aliquoted into 2x250 μ l samples upon final removal from centrifuge and stored at -80 °C.

2.10.2 *Biochemical analysis*

Acylated ghrelin and total peptide YY (PYY) were analysed by radioimmunoassay (RIA) kit (Linco Research, Inc., MO, USA). Samples treated with PMSF and HCl were analysed for acylated ghrelin and samples treated with aprotinin were involves a labelled tracer antigen and an unlabelled antigen (plasma sample) which are both incubated with a fixed dilution of antiserum. Since there are a fixed number of binding sites associated with this antiserum, the number of binding sites for the labelled antigen in plasma are limited. The amount of bound, labelled antigen will therefore decrease as unlabelled antigen increases. By measuring the radioactivity of one, or both, of these antigens using a gamma counter, the plasma concentration of acylated ghrelin/peptide YY can be

determined by comparison to a standard curve. The standard curve is created by measuring the radioactivity of solutions with known concentration of the relevant antigen, thus allowing radioactivity to be related to concentration. Standard solutions of varying concentrations were supplied with the RIA kit. The acylated ghrelin assay utilises ^{125}I -labelled acylated ghrelin and an acylated ghrelin antiserum, whilst the peptide YY assay utilises ^{125}I -labelled peptide YY and a peptide YY antiserum.

All plasma samples were kept at $-80\text{ }^{\circ}\text{C}$ and defrosted immediately before testing. Each sample was measured in duplicate within the same assay, and any samples that did not give a value, or returned an anomalous value, were re-measured where possible. All assays were performed by the same individual who had completed the Radiation Protection Certificate at the University of Glasgow and a laboratory induction course. All assays were performed in designated radioactive work areas within the biochemistry laboratories at the Royal Hospital for Sick Children, Glasgow, and relevant safety procedures were followed.

2.10.3 Accuracy and precision of assays

Acylated ghrelin and peptide YY were determined by radioimmunoassay (Millipore, Watford, UK). The average within-batch coefficients of variation for the assays were 9.8 % for acylated ghrelin and 6.1 % for peptide YY. All samples were measured in duplicate and samples from each participant were run in the same assay to minimise effects of inter-assay variation.

2.11 Statistical Methods

All data were tested for and found to be normally distributed before analysis using the Anderson-Darling normality test. This test was chosen as it is considered one of the more sensitive normality tests, there are no limitations on the sample size it can be used with, it is more sensitive to discrepancies in the tails of the sample distribution than other methods, and is suitable for normality testing of continuous data obtained from small sample sizes (Stephens, 1974;

Lesaffre, 1983). Statistical significance was set at 5 % for all studies. Data were analysed using Minitab 14 (Minitab Ltd., Coventry, UK), and IBM SPSS Statistics 19 was additionally used for the statistical analysis of data detailed in chapter 3 (IBM corporation, Somers, NY, USA).

2.11.1 Chapter 3 Statistical Analyses

Total and time-averaged AUC for all appetite sensations was calculated using the trapezoidal rule. Paired t-tests were used to compare characteristics of the exercise sessions and total area-under-the curve between pairs of trials. Repeated measures ANOVA were used to assess differences in appetite, EI and body composition between trials.

Bland-Altman plots were used to visually assess agreement in EI measurements made in the control and exercise trials. The Bland-Altman plot is commonly used in method comparison studies and allows visual judgement of the extent of agreement and any potential bias between two measurements. The two-way mixed effects interclass correlation coefficients (r_i) was used to quantify the test-retest reliability of EI and appetite values. This coefficient estimates the average correlation between all possible pairs of observations giving a measurement of reliability (Bland & Altman, 1996). This statistic is commonly used in similar reliability studies and gives a quantitative assessment of test-retest reliability alongside the visual representation of the Bland-Altman plots (Arvaniti *et al*, 2000; Nair *et al*, 2009; Laan *et al*, 2010). All results are presented as mean (95% Confidence Interval (CI)) in this chapter unless specified otherwise.

2.11.2 Chapter 4 Statistical Analyses

Time averaged AUC was calculated for acylated ghrelin and peptide YY concentrations using the trapezoidal rule. Paired t-tests were used to assess differences in EE during the intervention, metabolic rate, total EI and time averaged AUC of peptide hormones. A repeated measures analysis of variance (ANOVA) was used to investigate effects of trial and time on acylated ghrelin, peptide YY, EI and appetite. Repeated measures ANOVA was selected for this

analyses as it allows multiple comparisons of data collected at different time points to be drawn whilst minimising the type 1 error rate associated with using multiple t-tests for comparisons (Kao & Green, 2008). Post-hoc tukey comparisons were then used to identify specific significant differences; the Tukey test allows comparisons of all pairs of means to be drawn, and provides confidence intervals of mean differences. The Tukey test is useful when all pair wise comparisons are of interest and has the advantage of providing smaller confidence intervals than other available multiple comparison tests (Kao & Green, 2008). Pearson correlation coefficients were calculated for associations between appetite, acylated ghrelin and peptide YY. All results in this chapter are presented as mean \pm SEM unless specified otherwise.

Due to the large degree of variability in exercise intervention EE, analysis to identify potential outliers was carried out for this study after all data were collected. Box plots and a histogram with an overlaid normal curve were plotted for ExEE data; these plots graphically summarise the statistical distribution of data, and highlight any values which lie out with this distribution (Williamson *et al.*, 1989).

2.11.3 Chapter 5 Statistical Analyses

Time-averaged AUC was calculated for appetite measures using the trapezoidal rule and differences were assessed using paired t test. This test was also used to assess differences in total and relative EI, metabolic rate, and exercise responses between trials. Repeated measures ANOVA was used to assess differences in body composition and cardiovascular fitness levels over time, and to assess the interactive effect of time*trial on appetite ratings, macronutrient intake, EI, and acylated ghrelin and peptide YY levels. Post-hoc Tukey test of multiple comparisons was then used to identify specific significant differences. All results are presented as mean \pm SEM in this chapter unless specified otherwise.

2.11.4 Chapter 6 Statistical Analyses

Two sample t-tests were used to compare differences between restrained and unrestrained eaters, and between the two subgroups of restrained eaters. Pearson correlation coefficients were calculated between EE and physical and anthropometric characteristics. Statistical significance was set at $p < 0.05$. Values are mean \pm SEM unless otherwise stated.

2.12 Sample Size and Power Calculations in Acute Exercise Studies

Given that very few of the studies reviewed in this chapter report a power calculation, a discussion of appropriate methods of conducting power calculations in acute exercise studies seems prudent. Using data from this thesis, worked examples will be used in the discussion.

The nature of power calculations is to minimise the chance of a type 1 or 2 error occurring. The probability of these errors in a given sample is represented by the statistical values α and β . Many studies in this field find non-significant results; these findings can simply be the result of inadequate power; a classic example of a type 2 error - the “false negative”. The probability of this type of error is quantified by the value β , which is related to statistical power in the following way:

$$\text{Power} = 1 - \beta$$

Thus β must be minimised in order to maximal statistical power in any given study; power calculation of appropriate sample size is necessary to achieve this (Altman, 1990).

The value of α in power calculations must also be considered; α is related to β and quantifies the probability of rejecting the null hypothesis when it is in fact

true. This is known as a type 1 error - the “false positive”. The value of α is usually set at 5%, which relates to the widely accepted value of 0.05 taken to indicate statistical significance.

Studies that find a statistically significant result can be assumed to have adequate power and not susceptible to type 2 errors. Such studies could still be subject to type 1 errors, but this will not be discussed as this section will focus specifically on type 2 errors.

Power calculations are usually done prospectively, based on pilot data, findings of a similar study, or sometimes simply a good guess as to how variable the population is likely to be. There is also value in conducting power calculations retrospectively as they can provide valuable information for future studies, and give more accurate details of the effect size that can be detected. Retrospective power analysis was carried out on the acylated ghrelin data reported in chapter 3. In this study acylated ghrelin data for 15 overweight, pre-menopausal, female participants was available. A prospective power calculation was not carried out for this study, since it was part of the larger study detailed in chapter 4, and the original power calculation was conducted for outcomes of the larger study. As a result a retrospective power calculation was conducted on the chapter 4 data.

2.12.1 *Using the Correct Standard Deviation*

In the majority of studies which have examined the acute effects of exercise on acylated ghrelin, measurements are taken over a period of hours, pre- and post-intervention and the average difference in ghrelin concentrations over a period of hours is assessed. The study used as an example was complicated as it consisted of two observational periods; afternoon on day 1 and morning on day 2, incorporating a 16 hour overnight period spent at home. Each observational period was thus treated discretely for the purpose of power calculations due to the length of time separating them.

Before any calculations could be carried out it was necessary to decide where a difference in ghrelin was most likely to be present in order to elucidate the appropriate SD. It was reasoned that since day 1 observations included the intervention period, a difference would most feasibly be seen here (although the method described is applicable to data from either day). On day 1, a series of 5 measurements was made before the evening meal, and there was a steady, slight rise in acylated ghrelin over this period as would be expected. If exercise affected ghrelin it may be expected that this rise would be either attenuated or stimulated, hence the power calculation was constructed to detect a difference in this day 1 change in ghrelin concentrations between trials. Firstly, the change between the first and last measurements on day 1 was examined to obtain the necessary SD:

- For each participant the difference between the baseline and end values (time points 210-0) of acylated ghrelin concentrations during day 1 was calculated.
- The mean and standard deviation of these differences for each trial were then calculated; giving an average change in acylated ghrelin concentrations for both control and exercise trials over the course of day 1 (Control trial: Mean 36.9 SD 110.2 pg ml⁻¹; Exercise trial: Mean 35.0 SD 81.2 pg ml⁻¹).
- A paired t test was then carried out on these data. This provided the mean and standard deviation of the difference between the average day 1 change in ghrelin concentrations in the control and exercise trial. Results of this t-test are shown below:

Paired T for CON Δ 210-0 - EX Δ 210-0

	N	Mean	StDev	SE Mean
CON Δ 210-0	15	36.94	110.21	28.45
EX Δ 210-0	15	35.04	81.15	20.95
Difference	15	1.90	100.86	26.04

95% CI for mean difference: (-53.95444, 57.75444)

T-Test of mean difference = 0 (vs. not = 0): T-Value = 0.07 P-Value = 0.943

There are clearly no differences in the day 1 change in ghrelin concentrations between trials (i.e. the difference between first and last ghrelin measurements on day 1 is approximately 36 pg ml^{-1} for both trials and the mean difference between these two trials 1.9 pg ml^{-1}). In order to determine the exact effect size which can be excluded in this study the SD of the difference must be utilised in a retrospective power calculation; 100.9 pg ml^{-1} . The large value of the SD shows that variability in acylated ghrelin concentrations in these particular participants was huge.

2.12.2 *Calculating the Existing Statistical Power of a Study*

The first step involved in retrospective power calculation for a study is to calculate the power to detect the observed difference (1.9 pg ml^{-1} in this case). This requires the use of the power analysis function in a statistical analysis package (Minitab 14, Minitab Ltd., Coventry, UK). In this case a 1-sample t test power calculation for paired data was used; the method of calculating power, including values of α and β , is detailed below:

FORMULAS

Values Specified by the User

n = sample size

σ = estimated/approximated standard deviation

δ = difference between true mean and hypothesized mean

α = significance level

Derived Values

v = degrees of freedom for error = $n - 1$

λ = non-centrality parameter for t

t_{α} = one-sided critical value (upper α point of the t distribution with v degrees of freedom)

$t_{\alpha/2}$ = two-sided critical value (upper $\alpha/2$ point of the t distribution with v degrees of freedom)

Non-centrality Parameter

$\lambda = \text{SQRT}(n) * \delta / \sigma$

One-sided Power

For the $>$ alternative,

$$\text{Power} = 1 - t(\alpha; \nu, \lambda)$$

For the $<$ alternative,

$$\text{Power} = t(-\alpha; \nu, \lambda)$$

Two-sided Power

$$\text{Power} = 1 - t(\alpha/2; \nu, \lambda) + t(-\alpha/2; \nu, \lambda)$$

The information which must be provided by the user for this formula is the study sample size (15), the observed actual difference in the data (1.9), and the SD of that difference (100.9). Thus the power that the study had to detect the difference that actually exists between trials is calculated. The results of this calculation are shown below.

1-Sample t Test

Testing mean = null (versus not = null)

Calculating power for mean = null + difference

Alpha = 0.05 Assumed standard deviation = 100.9

Difference	Size	Power
1.9	15	0.05

So this shows there is only 5% power to detect the actual difference observed in acylated ghrelin concentrations between exercise and control trials when $\alpha = 0.05$. This means that if there is a true difference of 1.9 pg ml^{-1} then a hypothesis test on the data will not be able to detect it and the p value of such a test will be greater than 0.05.

In the present study β is 95%, therefore if the observed difference were a true difference, and not simply the result of normal variation, there is a 95% chance it would not be detected. In other words, a 95% chance of a “false negative”, or type 2 error exists in this study. In reality it is unlikely that 1.9 pg ml^{-1} would be a truly biologically significant difference, but this calculation provides a greater understanding of inherent error rates. A larger sample size would be needed to reduce the possibility of a type 2 error occurring to an acceptable level

(typically 80% power is considered adequate for most power calculations, this means the chance of a type 2 error occurring is 20%).

2.12.3 Post-hoc power calculations

There is no reference value for the magnitude of change in ghrelin levels that would be considered biologically or clinically significant; previous studies have found differences of 20-40 pg ml⁻¹ to be significant and thus two power calculations were carried out to calculate the sample size that would be needed in the study to detect these change of 20 and 40 pg ml⁻¹. This involves conducting a 1 sample t test power calculation for paired data and three values are required for this; the desired difference (20 or 40), the observed SD (100.9), and desired level of power (80%). The results of the calculations are shown below:

Power and Sample Size calculation 1

1-Sample t Test

Testing mean = null (versus not = null)

Calculating power for mean = null + difference

$\alpha = 0.05$ Assumed standard deviation = 100.9

Difference	Size	Power	Actual Power
20	202	0.8	0.80

Power and Sample Size calculation 2

1-Sample t Test

Testing mean = null (versus not = null)

Calculating power for mean = null + difference

$\alpha = 0.05$ Assumed standard deviation = 100.9

Difference	Size	Power	Actual Power
40	52	0.8	0.80

Thus 52 participants would be required to detect a difference of 40 pg ml⁻¹ and 202 participants would be needed to detect a difference of 20 pg ml⁻¹ in this data set with 80% power and $\alpha = 0.05$.

2.12.4 Power curves

Another function available in statistical software (Minitab 14) is the construction of a power curve, this helps the researcher determine the minimum effect that can be either excluded or detected at 80% power, α of 0.05, and the existing sample size. Once again a 1 sample t test power calculation is used for this type of paired data. This time the required power (i.e. 80%), the relevant standard deviation, and the α value, and the range of values we wish to be able to detect or exclude are entered (i.e. if the values 50:100/0.05 are entered this creates a power curve telling us how many participants are needed to detect a range of effects of 50 pg ml⁻¹ up to 100 pg ml⁻¹). The range of effect sizes and the number of participants required to detect this effect is then produced. For the existing samples size the power curve output is shown below (by increments of 0.5 pg ml⁻¹):

Power and Sample Size

1-Sample t Test

Testing mean = null (versus not = null)

Calculating power for mean = null + difference

$\alpha = 0.05$ Assumed standard deviation = 100.8

Difference Size Power Actual Power

<u>78.50</u>	15	0.8	0.800749
79.00	15	0.8	0.805677
79.50	15	0.8	0.810529
80.00	15	0.8	0.815307
80.50	15	0.8	0.820008
81.00	15	0.8	0.824633
81.50	15	0.8	0.829182
<u>81.60</u>	15	0.8	0.830082

Therefore for the current data set the minimum effect size we have power to detect or exclude is 78.5 pg ml^{-1} with 80% power, and the maximum is 81.6 pg ml^{-1} with 83% power.

Thus power calculations are useful not only for determining sample size prospectively, but can also be a useful retrospective tool that aids interpretation of a data set. For instance, with these calculations the conclusion of the study in question can be more informative than simply saying there was no significant difference. Instead the author and reader can be informed that the study excludes a difference in ghrelin levels between trials of 78.5 to 81.6 pg ml^{-1} ; smaller effects may occur and escape detection. This knowledge gives more informative conclusions and highlights the importance of power calculations. Papers published without reporting power calculations in any manner are difficult to interpret in comparison.

CHAPTER 3: Low test-retest reliability of twenty-four hour post-exercise assessment of energy intake and appetite measures in overweight and obese women obtained using current methodology.

3.1 Participants and Study Design

3.1.1 Participants

Fourteen healthy women were screened as described in section 2.1 and gave written, informed consent to participate (appendix III), and there was no attrition from this study.

3.1.2 Study Design

Each participant completed a sub-maximal fitness test before completing two sets of trials. Participation involved a total of four trials - two exercise and two control. Participants were asked to avoid alcohol and standardise their food intake prior to each trial. All trials were timed to ensure they were completed in the same phase of the menstrual cycle for each participant, in practice trials were typically approximately four weeks apart; exact timings depended on the individual participants' cycle. The first set of trials, one exercise and one control, were completed in a randomised, counter-balanced fashion. Thereafter the second set of trials was completed in reverse order in order to minimise potential bias effects of order.

Each trial lasted 24 hours, spanning over 2 days (figure 3.1); observation was carried out in the afternoon of day 1, and the morning of day 2. Participants attended the laboratory on day 1 at ~2pm and remained for four hours, during which the intervention (exercise or control) period was completed. Participants fasted overnight at home and returned to the laboratory the next morning, remaining for a further five hours. Body composition was measured via bio-impedance in the fasted state at the beginning of day 2 of all trials (TANITA TBF-300, Tanita B.V, Hoofddorp, The Netherlands). Appetite assessment was carried out a total of eleven times; five on day 1 and six on day 2 using a visual analogue scale (VAS) as described in section 2.7 (appendix VIII) (Flint *et al*, 2000). Three ad-libitum buffet meals were served during each trial; evening meal on day 1, and breakfast and lunch on day 2.

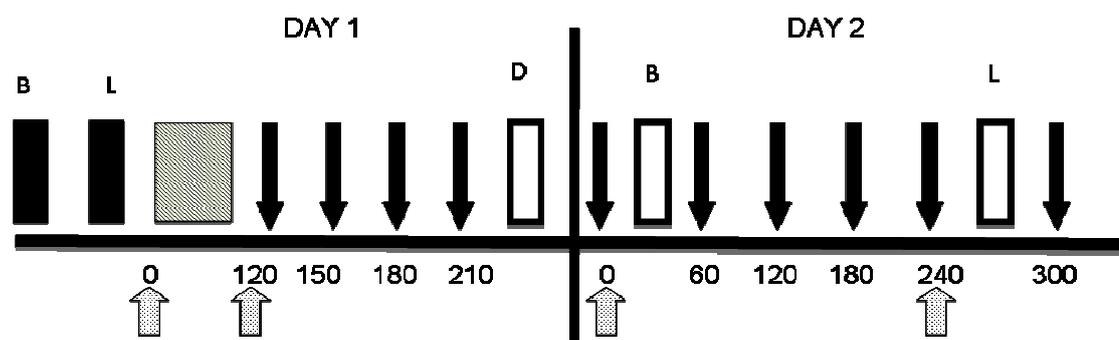


Figure 3.1 Schematic representation of study protocol. Grey rectangle represents intervention period, black arrows represent appetite assessments, grey arrows represent metabolic rate measurements, outlined rectangles represent buffet meal while solid black rectangles represent normal meal at home/work.

3.1.3 Sub-maximal Fitness Test

Participants completed a graded, sub-maximal fitness test on the treadmill before beginning trials in order to determine intensity of exercise sessions. Fitness tests were carried out according to the protocol described in section 2.3.

3.1.4 Intervention sessions

During the exercise trials, a moderate ($65\% \dot{V}O_{2max}$) intensity treadmill walking session was carried out in the afternoon of day 1 to expend 1.65MJ; an EE similar to that recommended for individual sessions for long term body mass control (Donnelly *et al*, 2009). At all other times during the trials participants were sedentary. The control trials were identical except that participants remained sedentary during the intervention period on day 1.

3.1.5 Ad-libitum Buffet meals

During each trial participants were served 3 buffet meals; evening meal on day 1, and breakfast and lunch on day 2. Dinner was served at the end of the day 1 observation period before participants returned home to fast, breakfast was served after fasting measurements were made on the morning of day 2, and lunch was served 4 hours after the breakfast meal. After the lunchtime meal final measurements were made before the trial concluded. Meals were prepared and served as described in section 2.8.

3.2 Results

3.2.1 Participant characteristics

Fourteen participants completed the study with no attrition. Participants had mean age of 35.7 ± 8.7 years, height 162 ± 6 cm, body mass 78.6 ± 14.3 kg, BMI 30.0 ± 5.1 kg m⁻², body fat 38.7 ± 5.5 %, and $\dot{V}O_{2\max}$ 30.9 ± 7.1 ml⁻¹ kg⁻¹ min⁻¹ (mean \pm SD). There were no significant differences in body mass, BMI, or body composition between trials ($p > 0.05$).

3.2.2 Exercise responses

There were no significant differences in intensity (60.5 ± 3.4 vs. 61.0 ± 3.5 % $\dot{V}O_{2\max}$), duration (67.7 ± 3.3 vs. 66.4 ± 3.3 minutes) or EE (1.65 ± 0.10 vs. 1.66 ± 0.10 MJ) of the two exercise sessions conducted during exercise trials (mean \pm SEM, all $p > 0.05$).

3.2.3 Energy and macronutrient intake

Paired t-test showed that although there was no difference in EI between exercise trials ($p > 0.05$), there was a tendency for control trial EI to differ ($p = 0.08$; table 3.1). Repeated measures ANOVA comparison of EI in all trials showed that there was a significant main effect of meal; EI was significantly lower at the breakfast meal than at either lunch or dinner in all trials ($p = 0.0003$).

The r_i for the control trial EI was 0.50 (0.03, 0.80) ($p = 0.02$), and for exercise trial EI was 0.04 (-0.53, 0.55) ($p = 0.45$). The r_i value for the difference in exercise and control trial EI was -0.05 (-0.54, 0.48) ($p = 0.57$).

Macronutrient intake was not different between exercise trials ($p>0.05$) but total protein ($p=0.004$) and fat intake ($p=0.02$) were significantly different between control trials (table 3.1). Repeated measures ANOVA of macronutrient intakes showed a significant main effect of meal for all four trials; carbohydrate and protein intake was significantly lower at breakfast compared to lunch or evening meal in all trials ($p<0.05$), intake of these macronutrients at lunch was also significantly lower than evening meal intake ($p<0.05$). Fat intake was significantly lower at breakfast in comparison to lunchtime meal only ($p<0.05$).

The r_i values for macronutrient intake in control and exercise trials are summarised in table 3.2.

Table 3.1 Total energy and macronutrient intake assessed by buffet meals during exercise and control trials (n=14). Values are mean (95% CI).

	<i>Control trial</i>	<i>Control trial</i>	<i>Exercise trial</i>	<i>Exercise trial</i>
	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
Energy intake (MJ)	10.4 (9.2, 11.6)	9.2 (7.6, 10.8)	10.7 (9.5, 12.0)	10.3 (8.7, 11.8)
Carbohydrate Intake (g)	363 (307, 418)	315 (271, 359)	385 (325, 445)	350 (297, 402)
Protein intake (g)	100 (82, 118)	77 (65, 90)	101 (83, 119)	84 (73, 95)
Fat intake (g)	110 (79, 140)	79 (57, 101)	168 (63, 274)	90 (71, 109)

Table 3.2 Intraclass correlation coefficients (r_i) representing reproducibility of total macronutrient intake in control and exercise trials. Values are mean (95% CI), * = $p<0.05$.

	<i>Control trials</i>	<i>Exercise trials</i>
Carbohydrate intake (g)	0.16 (-0.31, 0.60)	0.08 (-0.45, 0.57)
Protein intake (g)	0.42 (-0.08, 0.77)*	-0.09 (-0.50, 0.42)
Fat intake (g)	0.50 (0.003, 0.81)*	-0.06 (-0.50, 0.44)

3.2.4 Bland Altman Analysis

Bland Altman plots for control trials (figure 3.2), exercise trials (figure 3.3), and the change in EI between sets of exercise and control trials (figure 3.4) showed no pattern, indicating a high variability of EI values under control and exercise conditions, and in the direction of change in EI between control and exercise trials.

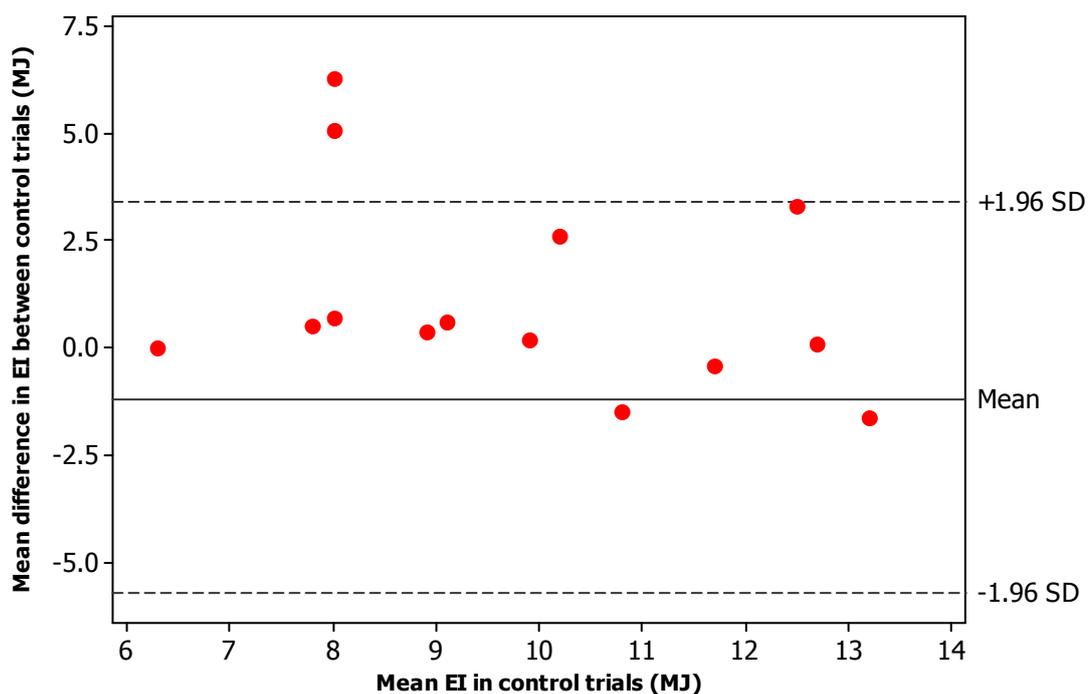


Figure 3.2 Bland Altman analysis of control trial energy intake (n=15).

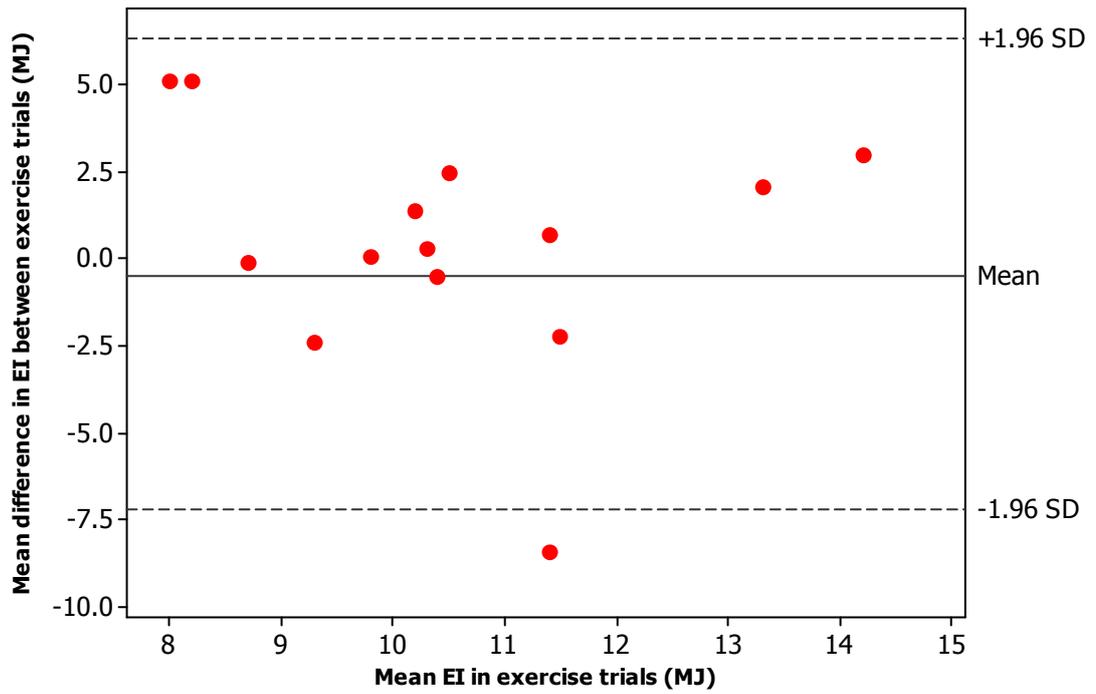


Figure 3.3 Bland Altman analysis of exercise trial energy intake (n=15).

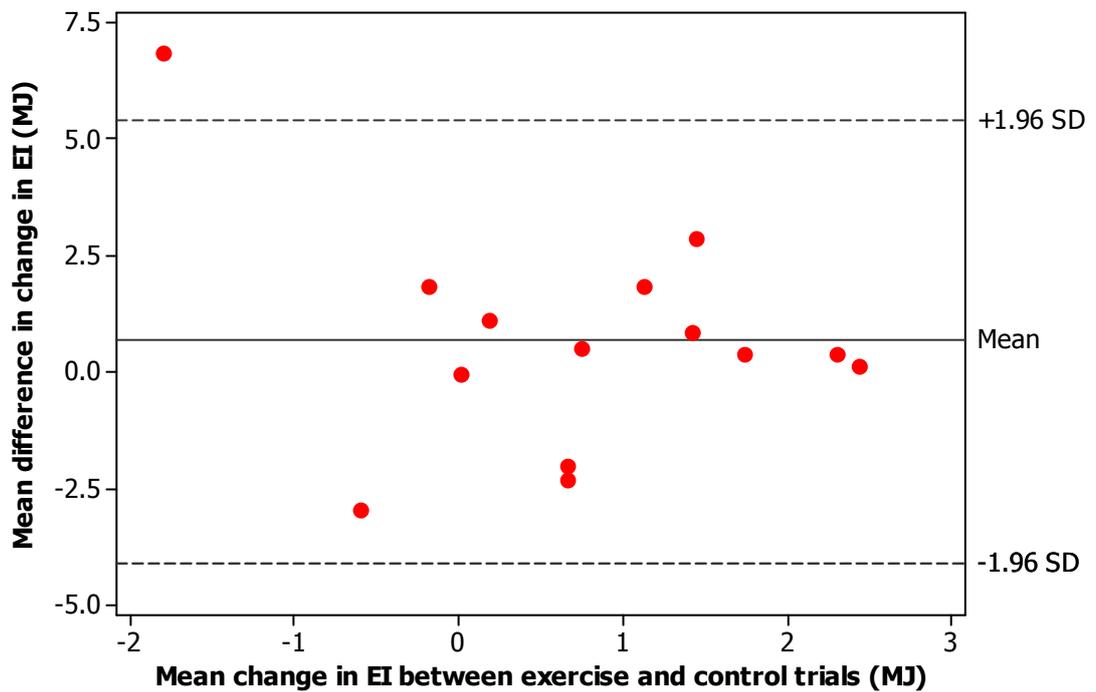


Figure 3.4 Bland Altman analysis of the difference in energy intake between exercise and control trial energy intakes (n=15).

3.2.5 Appetite

Paired t-test showed no differences between the control trials or exercise trials in total or time-averaged AUC values for appetite ratings (table 3.3). Repeated measures ANOVA found no interaction effect of time*trial ($p>0.05$), but there was a significant effect of time for all sensations ($p<0.001$) in control and exercise trials. There was also a significant effect of the control trials on hunger ratings ($p=0.005$); scores were significantly lower in the first trial compared to the second. No other effects of trial on appetite scores were found ($p>0.05$). Reproducibility of appetite scores was variable; r_i for total AUC values are summarised in table 3.4.

Table 3.3 Time-averaged AUC for appetite during exercise and control trials (n=14). Values are mean (95% CI), PFC = prospective food consumption, DTE = desire to eat. ^a and ^b represent significant differences ($p<0.05$).

	<i>Control trial</i>	<i>Control trial</i>	<i>Exercise trial</i>	<i>Exercise trial</i>
	1	2	1	2
Hunger (mm.min ⁻¹)	32.0 (26.8, 37.3) ^a	36.2 (29.2, 43.2) ^b	37.3 (32.4, 42.1)	35.8 (29.5, 42.2)
Satiety (mm.min ⁻¹)	63.5 (55.0, 72.1)	60.5 (54.6, 66.4)	54.0 (42.2, 60.8)	57.7 (52, 63.3)
Fullness (mm.min ⁻¹)	59.1 (52.2, 66.0)	59.5 (52.9, 66.1)	54.1 (46.8, 61.4)	57.4 (51.9, 63.0)
PFC (mm.min ⁻¹)	38.1 (31.7, 44.5)	39.6 (32.1, 47.1)	42.8 (37.3, 48.2)	39.0 (32.4, 45.5)
DTE (mm.min ⁻¹)	31.9 (26.8, 36.9)	35.1 (28.1, 42.2)	37.4 (31.6, 43.2)	37.4 (30.3, 44.6)

Table 3.4 Intraclass correlation coefficients (r_i) representing reproducibility of total AUC of subjectively rated appetite sensations under control and exercise conditions. Values are mean (95% CI). PFC = prospective food consumption, DTE = desire to eat, * = $p < 0.05$.

	<i>Control trials</i>	<i>Exercise trials</i>
Hunger	0.29 (-0.23, 0.69)	0.65 (0.20, 0.87)*
Satiety	0.56 (0.07, 0.83)*	0.44 (-0.07, 0.77)*
Fullness	0.71 (0.30, 0.90)*	0.31 (-0.23, 0.71)
PFC	0.40 (-0.17, 0.76)	0.54 (0.07, 0.82)*
DTE	0.43 (-0.09, 0.77)*	0.58 (0.08, 0.85)*

3.3 Discussion

This study aimed to evaluate the test-retest reliability of EI estimates obtained from the laboratory based buffet meal in overweight and obese women both at rest and post-exercise. The main finding of this study was that this method of assessing EI is not reliable for assessment of 24 hour food intake in overweight and obese women. Test-retest reliability of subjective ratings of appetite, assessed by a visual analogue scale (VAS), was variable in these women under both resting and exercise conditions.

Bland Altman plots were used to visualise the data for exercise and control trials, as well as for the change in EI between control and exercise trials. The Bland-Altman plot for the control trials showed a scattering of points around the mean line, which indicates that values from these trials may be in agreement. The scattering of points in the plots for the exercise trials and the change in EI between control and exercise trials was random and showed poor agreement in values obtained under these conditions. These data were explored further and test-retest reliability of these results was assessed statistically using the intraclass correlation coefficient (r_i). The r_i for exercise trial EI was 0.04 and not significant, which indicates low test-retest reliability of post-exercise EI measurements conducted under identical conditions. Given the popularity of the

buffet meal method in acute exercise studies, it is surprising that such an investigation has only been conducted once prior to this work. Analysis of macronutrient intake found r_i values for carbohydrate, fat and protein intake were all non-significant and <0.1 . Neither EI nor macronutrient intake assessed via this method is reproducible in acute exercise studies; conclusions from those studies that involve overweight and obese women participating in a moderate exercise session may be misleading. The exercise session in this study was of moderate intensity and ExEE, it is possible that higher intensity exercise may produce more reliable results. Given the extremely low r_i in the present study it does seem unlikely that test-retest reliability in these women would be significant at any intensity of exercise.

The r_i for control trial EI was significant but low; the value of 0.50 represents only 50% agreement between the two assessments, and r_i coefficient values <0.60 are considered to represent rather weak test-retest reliability (Laan *et al*, 2010). The confidence intervals associated with this value must also be taken into account when interpreting correlation values (Hebert & Miller, 1991); spanning from 0.03 to 0.80, the confidence interval of the control trial r_i indicates large variability in the degree of test-retest reliability. The only existing study assessing test-retest reliability of food intake in 19 overweight women at rest observed higher values; test-retest r values of food intake in grams ranged from 0.47 to 0.81 (Barkeling *et al*, 1995). This study assessed intake at a single meal on five separate occasions, and served an excess of a single food as opposed to a buffet, therefore these findings are not directly comparable with those of the present study. The r_i values for protein (0.43) and fat intake (0.50) in control trials were significant but did not indicate high test-retest reliability. Confidence intervals of both extend from zero to 0.80; this level of variability is not surprising given findings regarding total EI. The high level of variability observed in the present work somewhat calls the reliability of previous EI results obtained from overweight and obese women under resting conditions into question, i.e. those obtained from preload studies. Indeed the magnitude of variability in day-to-day macronutrient intake is also highly individual (Tarasuk & Beaton, 1991), and this is probably reflected in the test-retest reliability values observed. Considering the non-significant r_i values for macronutrient intake in exercise trials the implications of these findings are that

the buffet style method is not a reliable method of assessing exercise induced effects on food preferences of overweight and obese women either.

Acute exercise studies often compare EI under control conditions to post-exercise EI to ascertain if exercise induces acute EI compensation (Unick *et al*, 2010). The r_i for the difference in EI between the sets of exercise and control trials in the present study was -0.05 and non significant. The effect of exercise on EI in these women was therefore not uniform or consistent making it impossible to assess existence of acute compensatory mechanisms via this method. This too may be attributable to normal daily variation in EI, or this may indicate that the hypothesised phenomenon of acute compensatory responses to exercise simply does not exist in overweight and obese women.

Assessing appetite is a difficult task since it is entirely subjective (Stubbs *et al*, 2000). The VAS is the most commonly used method for these measurements and has been found to yield reproducible results under resting conditions in lean men (Flint *et al*, 2000). No existing studies assessing test-retest reliability of post-exercise scores obtained from overweight women could be found. The stability of appetite ratings of obese women served a single test meal on several occasions has been shown to be variable (Barkeling *et al*, 1995), and the present study observed similar variability in test-retest reliability of appetite ratings. Significant r_i values were found for satiety, fullness and desire to eat in control trials, and for hunger, satiety, prospective food consumption and desire to eat in exercise trials. However, only two of these coefficients could be considered to indicate a high level of test-retest reliability; coefficient for hunger ratings in the exercise trials was 0.65 (0.20, 0.87) and for fullness in the control trials coefficient was 0.71 (0.30, 0.90). As with EI in control trials, r_i values were significant but associated confidence intervals large, indicating high level of individual variability in reliability of these scores. The remaining r_i for appetite ratings range from 0.43 to 0.58 and associated confidence intervals of these are also very expansive; from 0.07 up to 0.85. Subjective appetite scores obtained using a VAS may be rather unreliable in some overweight and obese women and on a group level results may even be misleading.

To our knowledge, there is no evidence regarding reliability of this method for assessing EI in overweight and obese females with which to compare these results. Thus this study is novel in examining the test-retest reliability of EI measures obtained using this method, and in assessing the suitability of this method for investigating acute exercise induced effects on EI in this population. The main strength of this study is that 24 hour EI was measured; this provides a longer observation period with EI assessed at multiple meals and is more informative than single meal EI assessments which may not be representative of intake over a longer period. Buffet meals consisted of a variety of everyday foods, whereas other studies have measured intake of a single food (Gregersen *et al*, 2008; Ueda *et al*, 2009a). Cessation of eating episodes could be related to familiarity and diminishing appeal of the food in this situation, particularly when multiple trials are conducted in a short space of time with the same food. Food consumption may not be representative of normal daily intake where a wide variety of foods are commonly available, and the present study presented a variety of foods to minimise the likelihood of food “fatigue” affecting measured consumption. It is important to note that these results are applicable only to overweight and obese women and previous work suggests that the buffet meal method is far more reliable in lean males under sedentary conditions (Gregersen *et al*, 2008; Nair *et al*, 2009). Further investigation is needed to clarify the reliability of this method for post-exercise EI assessment in lean males and other groups.

3.3.1 Limitations

The findings of this study should be considered in the context of some minor limitations. Like other available methods, the laboratory based buffet meal method also has inherent limitations which may also have contributed to poor test-retest reliability. Food is served in an unnatural environment and this may affect eating behaviour. Although participants were not informed that EI was being measured, they were probably aware that the researcher preparing food and removing leftovers can observe their intake, and this perceived social pressure is known to affect EI (de Castro *et al*, 1990; Herman & Polivy, 2005). Dietary restraint of participants was not assessed in this study and this may have contributed to the large degree of variability observed. Additionally given the

design of the study, including an overnight fast at home, total compliance to instructions cannot be assured and this could have an impact on these findings. Finally, due to the nature of this study, a prospective power calculation was not carried out. However, we consider fourteen participants to be a relevant sample size as this number is typical of many short term exercise studies that utilise this method of EI measurement (King *et al*, 2010a,b; King *et al*, 2011a,b) and of reliability studies with lean males (Arvaniti *et al*, 2000; Nair *et al*, 2009).

Since trials were separated by 28 day intervals in order to control for the effects of the menstrual cycle, this study typically took approximately four months to complete. Some participants in this study were individuals who had already completed one control and one exercise trial as part of the long term exercise intervention presented in chapter 5. These participants returned to complete a further two trials for the purposes of this investigation, resulting in a period of a year or more between the first and second set of trials for some participants. Although these participants were screened to ensure there were no significant changes in physical characteristics that had taken place during this time, there may have been effects of time and seasonality that impacted food choice at buffet meals. Food preferences may have changed slightly over time for the returning participants, and seasonality has been shown to have a modest impact on EI in women (Fyfe *et al*, 2010). This may have contributed to the low test-retest reliability associated with the buffet meal method.

This study has shown that the laboratory based ad-libitum buffet meal method does not provide a reliable, reproducible estimate of EI in pre-menopausal, overweight and obese women under either control or exercise conditions. The test-retest reliability of this method varies greatly between individuals and hence this method of EI assessment is not suitable for use in this population.

CHAPTER 4: Effect of a walking based exercise session on appetite, energy intake, plasma acylated ghrelin and peptide YY concentrations in overweight and obese pre-menopausal women

4.1 Participants and Study Design

4.1.1 Participants

Forty-two healthy women were screened as described in section 2.1 and gave written, informed consent to participate (appendix III). Thirteen women chose to withdraw from the study before completion reporting their reason as a lack of time. Twenty-nine women completed this study; fifteen participants fell into the overweight category and fourteen were obese. Characteristics of completing participants are summarised in table 4.1.

Table 4.1 Characteristics of the participants(n=29).

<i>Characteristic</i>	<i>Mean</i>	<i>SD</i>
Age (years)	33.9	9.1
Height (cm)	160.7	5.9
Body mass (kg)	77.8	13.2
BMI (kg m ⁻²)	30.1	4.4
Body fat %	39.0	5.1
Predicted maximal oxygen uptake (ml kg ⁻¹ min ⁻¹)	30.4	6.0

4.1.2 Study Design

Participants completed two 24-hour trials (exercise and control) in a randomised, counter-balanced fashion. Participants were asked to keep a

weighed record of free-living food intake, to avoid alcohol and maintain habitual activity levels for the 2.5 days prior to the first trial. These conditions were replicated before subsequent trials. Trials were separated by at least 3 weeks and were usually approximately 28 days apart to ensure both trials were completed in the same phase of the menstrual cycle.

Trial duration was 4 hours on day 1 and 5 hours on day 2. Each began in the afternoon of day 1 (2pm) after participants consumed their normal lunchtime meal. Upon arrival at the laboratory initial measurements were made before participants completed the intervention period (exercise or control), observation continued for a further 90 minutes post-intervention. At the end of day 1 participants were served a buffet evening meal and were instructed to consume food until satiation, before returning home to fast overnight. Participants attended the laboratory once more on the morning of day 2 in the fasted state (9am), observations continued whilst participants were sedentary for a further 5 hours. During this period a buffet breakfast and lunch meal were served to participants. EI at all meals was covertly monitored.

Assessment of appetite, venous blood sampling, and metabolic rate and substrate oxidation measurements were also carried out at several points throughout each trial (figure 4.1).

The exercise intervention consisted of treadmill walking exercise conducted at an intensity of 65% maximal oxygen consumption ($\dot{V}O_{2max}$) for a duration sufficient to expend 1.7 MJ. Expired air samples were taken every 15 minutes to enable calculation of EE. Heart rate (HR) was continuously monitored using short-range telemetry (Polar RS400, Polar Electro, Finland). At all other times during the trials participants were sedentary and sat quietly reading or watching television. The control trial was identical to the exercise trial except that participants remained sedentary during the intervention period on day 1, for the same duration as the exercise session. Estimated EE for the control period was calculated from resting metabolic rate measurements made directly prior to the intervention session (Control session EE = resting metabolic rate (kJ min^{-1}) x duration of intervention (minutes)).

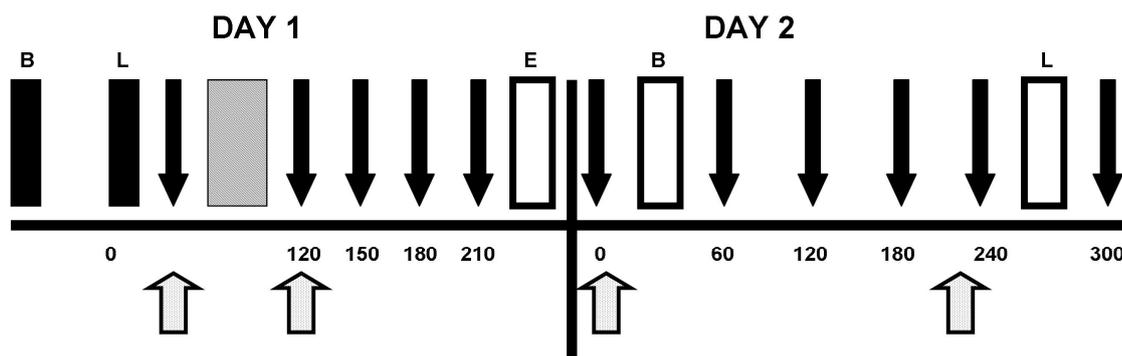


Figure 4.1 Schematic representation of study protocol. Grey rectangle represents intervention period, black arrows represent appetite assessments by visual analogue scale, grey arrows represent metabolic rate measurements, outlined rectangles represent buffet meal while solid black rectangles represent normal meal at home/work. B denotes breakfast, L denotes lunch and E denotes evening meal. All time points are expressed in minutes.

4.1.3 Sub-Maximal Exercise tests

A sub-maximal treadmill fitness test was carried out to determine predicted $\dot{V}O_{2max}$, and thus intensity of subsequent exercise sessions. All tests were carried out according to the procedure described in section 2.3.

4.1.4 Ad-libitum Buffet meals

During each trial participants were served 3 buffet meals; evening meal on day 1, and breakfast and lunch on day 2. Dinner was served at the end of the day 1 observation period before participants returned home to fast. Breakfast was served after fasting measurements were made on the morning of day 2, and lunch was served 4 hours after the breakfast meal. After the lunchtime meal final measurements were made before the trial concluded. Meals were prepared and served as described in section 2.8.

4.1.5 Assessment of appetite

A visual analogue scale (appendix VIII) was used to assess appetite (Flint *et al*, 2000) as described in section 2.7.

4.1.6 Metabolic rate and substrate oxidation

Metabolic rate was determined at 4 separate time points during each trial; immediately before and after the intervention period on day 1, and before each meal on day 2, including a fasting measurement. Measurements were carried out using the indirect calorimetry method (Oxycon Pro, Care fusion, San Diego, CA 92130, USA) as described in section 2.4. Metabolic data was only available for twenty-two participants due to mechanical failure of equipment.

4.1.7 Blood sampling and analyses

Venous blood samples were collected via a cannula (BD Venlo, Becton Dickinson UK, Oxfordshire, UK) at time points 0, 120, 150, 180 and 210 minutes on day 1 and 0, 60, 120, 180, 240 and 300 minutes on day 2. Collection, processing, and analysis of blood samples for acylated ghrelin and peptide YY was carried out as described in section 2.9.

4.2 Results

4.2.1 Baseline participant characteristics

Twenty-nine participants completed the study and there were no differences in participant characteristics between trials at baseline (time point 0 on day 1); no significant differences were found in plasma acylated ghrelin concentrations, peptide YY concentrations, metabolic rate, substrate oxidation, or in subjectively rated appetite sensations ($p > 0.05$; table 4.2).

Table 4.2 Baseline hormones, metabolic and appetite measurements in control and exercise trials. Values are mean \pm SEM (n=15 for acylated ghrelin, n=10 for peptide YY and n=29 for all other parameters). PFC = prospective food consumption.

	<i>Control Trial</i>		<i>Exercise trial</i>		<i>P value</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Acylated ghrelin (pg ml ⁻¹)	79.8	23.9	63.8	11.7	0.53
Peptide YY (pg ml ⁻¹)	136.8	20.5	149.2	12.4	0.61
Metabolic rate (kJ min ⁻¹)	4.57	0.17	4.63	0.15	0.63
Hunger (1-100)	22.1	3.6	24.4	3.6	0.16
Satiety (1-100)	61.5	4.9	60.3	4.7	0.39
Fullness (1-100)	57.4	5.5	59.9	4.7	0.24
PFC (1-100)	34.1	4.3	31.3	3.6	0.19
Desire to eat (1-100)	23.1	4.0	24.7	4.3	0.37

4.2.2 Exercise responses

The net EE and metabolic characteristics of the exercise session are summarised in table 4.3. Net total exercise EE ranged from a minimum of 0.71 to a maximum value of 2.20 MJ; mean EE of exercise intervention was significantly greater than that of control intervention. Exercise was completed at a mean speed of 6.3 ± 0.2 km hr⁻¹, for a mean duration of 69.6 ± 2.6 minutes, and at a mean heart rate of 135 ± 3 beats min⁻¹. Mean oxygen consumption during exercise was 1.41 ± 0.07 L min⁻¹; this was equivalent to $61.5 \pm 2.6\%$ of $\dot{V}O_{2max}$.

4.2.3 Energy intake

Absolute EI on day 1 (Control: 4.05 ± 0.22 ; Exercise: 4.23 ± 0.26 MJ, $p=0.32$) and 2 (Control: 6.75 ± 0.39 ; Exercise: 6.72 ± 0.36 MJ, $p=0.90$) was not different between trials. Total 24 h EI was not significantly different between trials ($p=0.63$; table 4.3).

ANOVA revealed a significant effect of meal on EI, with breakfast meal EI being significantly lower than lunch or evening meal EI in both trials ($p<0.003$). There

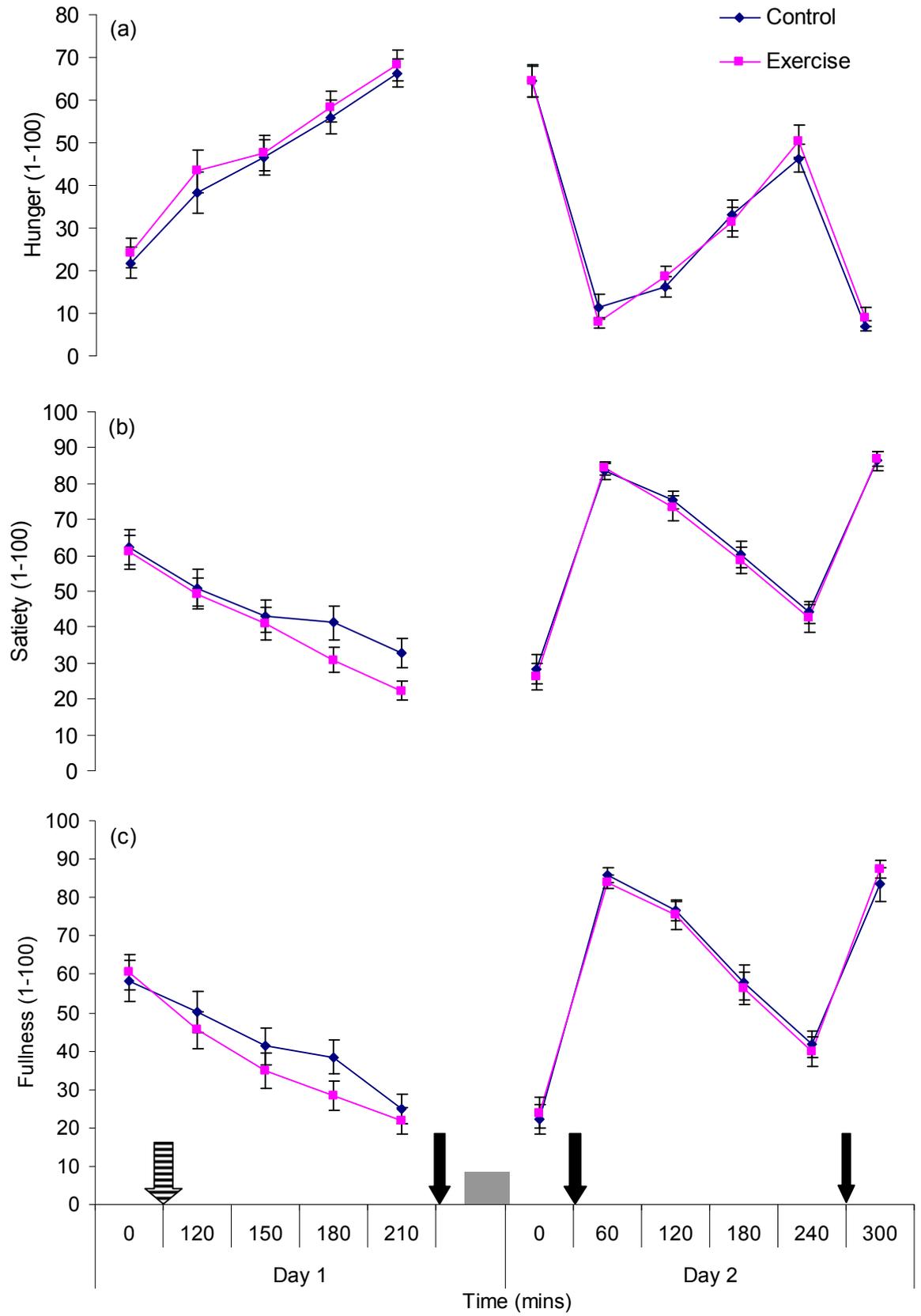
were no significant differences in carbohydrate, protein or fat intake between trials ($p>0.05$) (table 4.3).

Table 4.3 Characteristics of intervention periods and energy and macronutrient intake in control and exercise trials(n=29).

	<i>Control trial</i>		<i>Exercise trial</i>		<i>p-value</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Energy expenditure					
(kcal)	70	2	407	16	<0.0001
(MJ)	0.29	0.01	1.70	0.06	<0.0001
Total energy intake					
(kcal)	2582	130	2620	130	0.63
(MJ)	10.8	0.5	10.9	0.5	0.63
Carbohydrate intake (g)	366	21	374	21	0.45
Protein intake (g)	84	4	83	4	0.71
Fat intake (g)	97	7	122	24	0.34

4.2.4 Appetite

Two factor, repeated measures ANOVA revealed a main effect of time (all $p<0.0001$) on all appetite sensations (hunger, satiety, fullness, prospective food consumption and desire to eat). There was no significant main effect of trial or a trial * time interaction effect on any of the appetite sensations ($p>0.05$; figure 4.2).



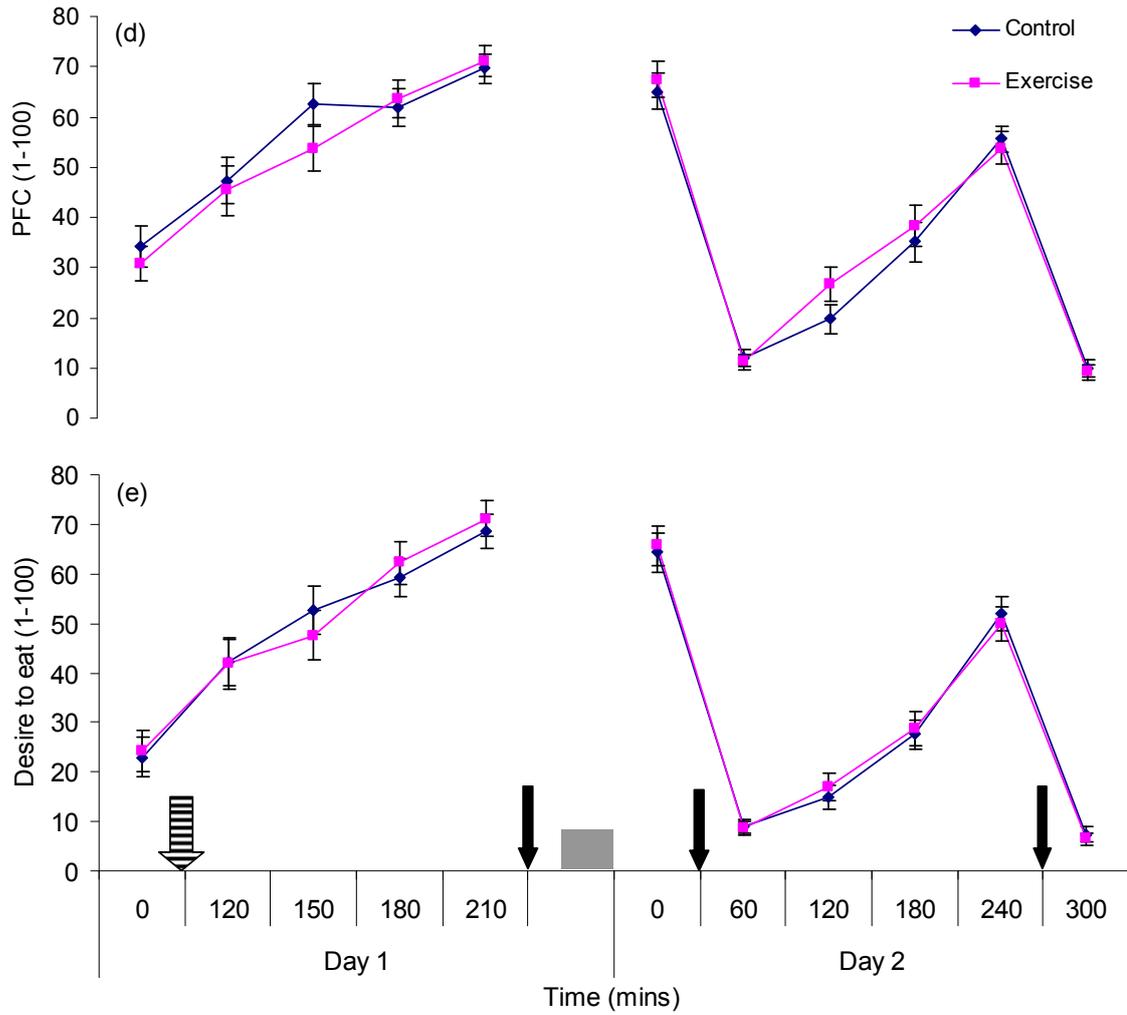


Figure 4.2 Subjectively rated sensations of hunger (a), satiety (b), fullness (c), prospective food consumption (d) and desire to eat (e) during the control and exercise trials. Values are mean \pm SEM (n=29). Shaded arrow represents intervention period, black arrow represents a buffet meal and grey rectangle represents overnight fast at home.

4.2.5 Acylated Ghrelin and Peptide YY concentrations

There were also no differences in time-averaged AUC of acylated ghrelin and peptide YY responses between days 1 and 2 of each trial ($p > 0.05$; table 4.4).

Fasting acylated ghrelin (Exercise: 67.5 ± 12.1 , Control: 97.8 ± 21.4 pg ml^{-1} ; $p = 0.10$) and peptide YY concentrations (Exercise: 86.5 ± 7.2 , Control: 90.9 ± 10.6 pg ml^{-1} ; $p = 0.64$) were not significantly different between trials. Two factor ANOVA revealed a significant main effect of time, but no main effect of trial or trial * time interaction effect on either acylated ghrelin or peptide YY concentrations ($p > 0.05$; figure 4.3).

Table 4.4 Time averaged area-under-the curve values (pg ml^{-1}) for acylated ghrelin (n=15) and peptide YY (n=10) concentrations during days 1 and 2 of control and exercise trials.

	<i>Day 1</i>		<i>Day 2</i>		<i>P value</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Acylated Ghrelin:					
Control trial	78.7	10.3	62.2	11.4	0.18
Exercise trial	73.2	9.8	57.3	9.8	0.12
Peptide YY:					
Control trial	119.8	16.4	128.7	10.4	0.57
Exercise trial	123.9	9.4	128.7	9.9	0.74

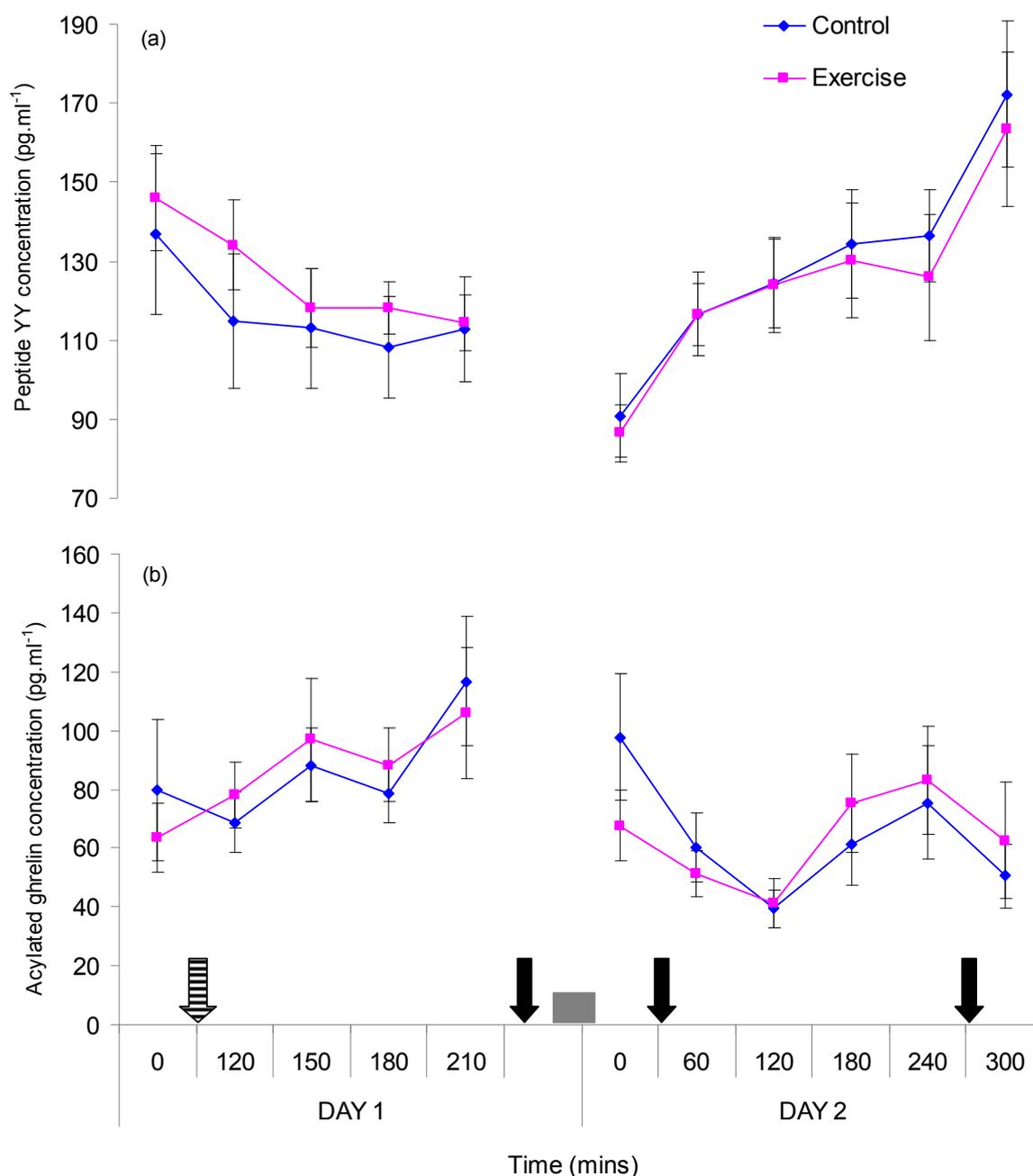


Figure 4.3 Plasma peptide YY (a, n=10) and acylated ghrelin (b, n=15) concentrations in control and exercise trials. Values are mean \pm SEM. Shaded arrow represents intervention period, black arrow represents a buffet meal and grey rectangle represents overnight fast at home.

4.2.6 Metabolic rate and Substrate Oxidation

There were no significant differences in metabolic rate between trials at any time point ($p > 0.05$), although post-intervention (time point 120) metabolic rate measurements (time point 120 on day 1) were approaching significance for a

difference between trials (Exercise: 4.60 ± 0.17 , Control: 4.36 ± 0.16 kJ min⁻¹; $p=0.07$).

Fasting fat oxidation rate (Ex: 0.09 ± 0.01 , Con: 0.08 ± 0.00 g min⁻¹; $p=0.01$) was significantly higher and fasting carbohydrate oxidation rate (Ex: 0.04 ± 0.01 , Con: 0.08 ± 0.01 g min⁻¹; $p=0.01$) significantly lower in the exercise compared to control trial.

Immediately post-intervention, fat oxidation rate was significantly higher (Ex: 0.11 ± 0.01 , Con: 0.08 ± 0.01 g min⁻¹; $p=0.0001$) and carbohydrate oxidation rate (Ex: 0.01 ± 0.01 , Con: 0.07 ± 0.01 g min⁻¹; $p=0.0001$) significantly lower in exercise compared to the control trial.

Prior to the lunch meal on day 2 of trials, fat oxidation rate was significantly higher (Ex: 0.07 ± 0.01 , Con: 0.06 ± 0.00 g min⁻¹; $p=0.05$) compared to the control trial (figure 4.4).

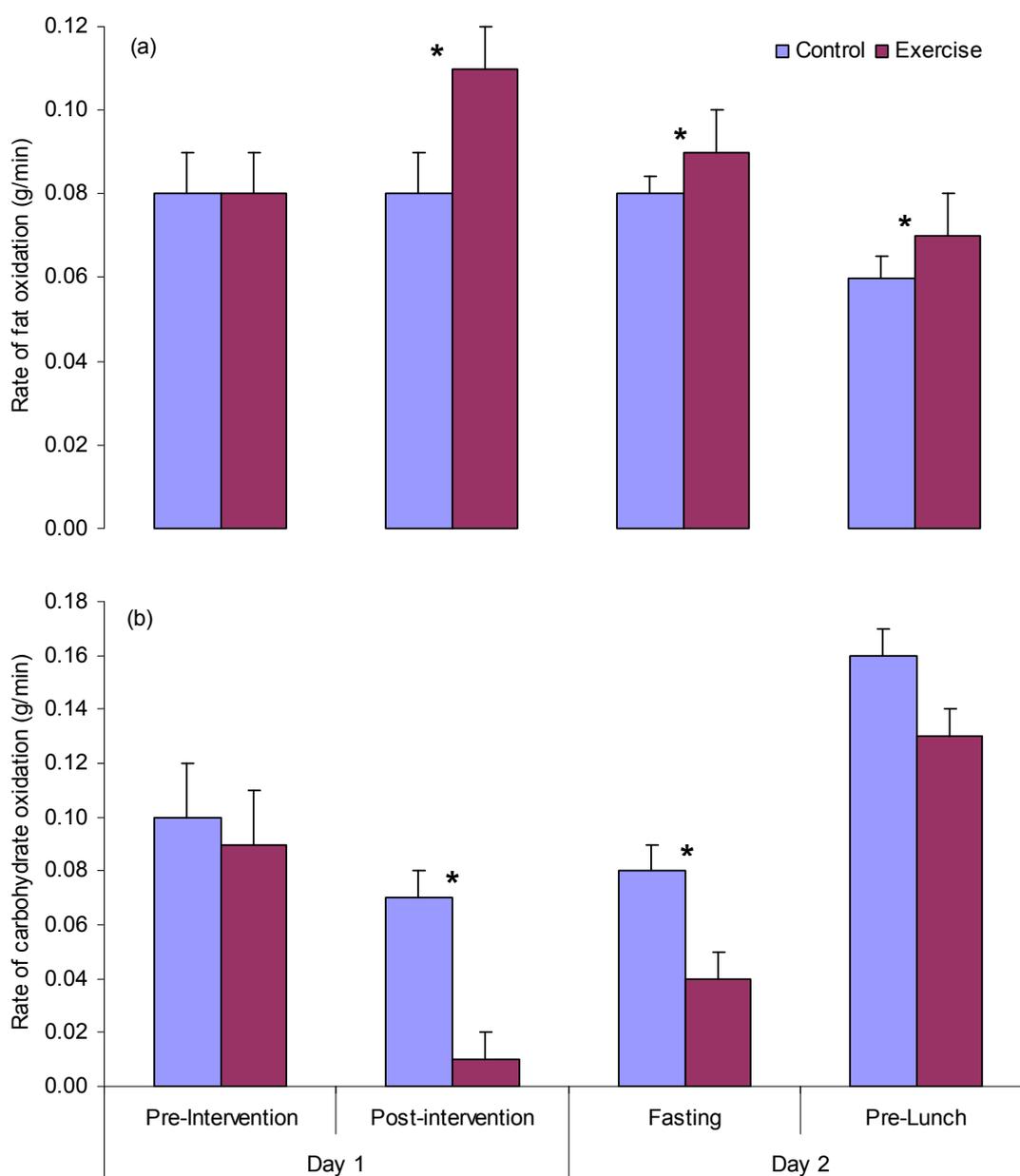


Figure 4.4 Fat (a) and carbohydrate (b) oxidation rates (g min^{-1}) during the control and exercise trials. Values are mean \pm SEM ($n=22$).

4.2.7 Appetite Correlations

Day 1 AUC for peptide YY concentrations was significantly correlated with day 1 AUC for satiety ($r=0.66$, $p=0.04$) and fullness ($r=0.65$, $p=0.04$) ratings in control trial only. There were no correlations between peptide YY and appetite AUC on day 2 of control trial or on either day of exercise trial. There were no significant correlations between AUC of ghrelin and appetite ratings.

There was a tendency for the change in total EI between trials to be negatively associated with the change in fasting acylated ghrelin concentrations between trials (Exercise-Control values: $r=-0.48$, $p=0.07$). There were no associations between peptide YY and EI ($p>0.05$).

4.2.8 Power calculations

A post-hoc power analysis was carried out to determine the minimum effect size on acylated ghrelin and peptide YY concentrations that could be detected in this study, as described in section 1.5. Blood samples for acylated ghrelin were only available for 15 participants, and for peptide YY 10 participants, due to problems obtaining blood samples from some participants. Acylated ghrelin and peptide YY both show normal daily variation in concentrations. This study sought to determine if concentrations were attenuated or stimulated by exercise beyond normal daily variation, thus the difference between average concentrations at time point 210 and time point 0 on day 1 was calculated separately for control and exercise trials. These values were then compared by paired t test to obtain the SD of difference between trials; subsequent analyses determined that the minimum effect size that could be detected, or excluded, with these participant numbers and 80% power was 78 pg ml^{-1} for acylated ghrelin concentrations, and 55 pg ml^{-1} for peptide YY concentrations.

4.3 Discussion

The purpose of this study was to investigate the short term regulation of appetite, EI, acylated ghrelin and peptide YY in response to a moderate exercise-induced energy deficit in overweight and obese females. This study did not find a change in appetite, EI or appetite regulating peptide hormones in the 24 hours following an exercise session, which induced a net EE of 1.7 MJ. Findings from this study agree with other studies conducted with overweight and obese females (George & Morgenstein 2003; Schneider *et al*, 2009; Unick *et al*,

2010); observation of responses was carried out for a few hours post-exercise and activity duration ranged from as little as 3 minutes up to 1 hour in these studies. Hence the present study is the first to observe appetite regulating hormones and EI responses in overweight women over a 24 hour period, spanning two days, and find that exercise does not induce a compensatory up-regulation of appetite in response to a moderate, aerobic exercise session in this time period.

There was no effect of exercise on subjectively rated appetite in this study, which indicates acute automatic up-regulation did not occur. This is in agreement with other findings from overweight and obese females (Unick *et al*, 2010) obese males (Ueda *et al*, 2009a), and lean males completing a similar bout of treadmill walking exercise (King *et al*, 2010b). An afternoon treadmill walking session has also previously been observed to reduce appetite in obese women (Tsofliou *et al*, 2003); duration of exercise was shorter and participants more obese than in the current study which may explain disparities in results. An exercise-induced transient decrease in hunger has been observed in active, lean males (King *et al*, 1997b); this phenomenon has not been reliably reported in overweight individuals thus it is not unexpected that this effect was not observed. Discrepancies in findings regarding appetite may be attributable to differences in study design; in particular the timing of food consumption relative to exercise may influence feelings of appetite (Cheng *et al*, 2009).

A transient exercise induced up-regulation of peptide YY, with concurrent change in satiety, has been observed in lean and obese men (Ueda *et al*, 2009a,b). The present study did not find any such changes in peptide YY, and this lack of a change may explain why satiety was unaffected by exercise participation in the present study. Peptide YY has previously been associated with postprandial satiety in overweight women (Guo *et al*, 2006); the present study only found such associations on day 1 of the control trial. Potentially this could be attributable to some time-of-day dependent effect, or it may be that exercise disrupts the link between perceived appetite and peptide YY, but this remains unclear. Peptide YY was not associated with EI, a finding previously reported in lean males (Ueda *et al*, 2009b). Similar to ghrelin there are two sub-

types of the peptide YY molecule which differ slightly in structure, PYY₁₋₃₆ and YY₃₋₃₆. Total peptide YY levels were measured in these acute exercise studies, but it is only the latter form which is able to cross the blood barrier and bind to hypothalamic receptors to exert effects on satiety (McGowan & Bloom, 2004). Future studies should concentrate specifically on measurement of peptide YY₃₋₃₆ to investigate exercise induced effects on the active form of this peptide.

No effect of exercise on acylated ghrelin concentrations was observed in this group, which agrees with previous data obtained from obese women (Unick *et al*, 2010). Acylated ghrelin levels have previously been observed to up-regulate to a similar degree when multiple exercise bouts are performed over a four day period, with and without energy replacement (Hagobian *et al*, 2009). Newer evidence has shown that acylated ghrelin and peptide YY are responsive only to a food induced energy deficit in lean men; exercise induced deficits had no such effect (King *et al*, 2011b). Thus these regulatory peptides seem unresponsive to exercise participation *per se*, responding most strongly to some as yet unidentified signal induced by food restriction. Significant up-regulation of ghrelin only seems to occur in the longer term in response to depletion of body fat reserves; changes in ghrelin have been positively correlated with extent of body mass reduction (Hansen *et al*, 2002), and may occur only in response to losses >3 kg (Foster-Schubert *et al*, 2005). Up-regulation of ghrelin is observed in response to significant body mass reduction regardless of the method(s) used to achieve this loss (Ata *et al*, 2010) therefore this change in ghrelin is not exercise-dependent. It seems that ghrelin reacts only to defend body fat stores in response to some degree of depletion, in which case it is logical that acute studies would observe no attenuation in ghrelin or appetite.

Acylated ghrelin concentrations were not associated with subjectively rated appetite at any point, which is perhaps surprising since ghrelin contributes to feelings of hunger. This lack of association disagrees with findings from lean males (King *et al*, 2010b), but agrees with others that find post-exercise changes in appetite are not attributable to ghrelin (Malkova *et al*, 2008). It seems that in these women ghrelin does not directly influence feelings of hunger; it is possible that this appetite regulation is disrupted in overweight and obese individuals

since ghrelin levels are abnormally low in obesity (Tschop *et al*, 2001). Ghrelin responses in the participants showed normal meal-related fluctuations, with peaks reached prior to meal consumption and nadir reached ~90 minutes after food ingestion, illustrating that post-prandial ghrelin regulation is not disturbed in obese individuals as has been suggested (English *et al*, 2002).

Consistent with findings regarding appetite, total EI was also unaffected by exercise and compensatory responses to exercise participation were not observed in these women. Since EI was also observed the day after exercise participation in this study, this evidence would indicate that delayed compensatory responses did not occur in these women, which to our knowledge is a novel finding in this group. Unfortunately, these results may not be reliable given the findings reported in chapter 5; a study conducted after the present work in order to test reliability of the buffet meal method in this participant group. The present study led to the work detailed in chapter 5 as the buffet meal method had not previously been tested for reliability with this participant group. In light of these findings it seems that EI values are not sufficiently reliable in this group.

Emerging evidence has indicated that individual susceptibility to acute post-exercise compensatory responses varies in lean females; those who are prone to post-exercise increases in EI have been observed to do so because of an increase in the perceived palatability of, and implicit desire for, food (Finlayson *et al*, 2009). Such acute exercise-induced changes may occur similarly in overweight and obese men and women; increased liking for food, and an increase in perception of reward value of food following a single exercise session was later associated with subsequent failure to achieve a significant body mass reduction after 12 weeks exercise participation (Finlayson *et al*, 2011). Potentially this could be moderated by increased sensitivity to reward in some individuals, according to the reinforcement sensitivity theory of Gray (1987) which states that a neurological system named the behavioural approach system controls appetitive motivation and the pursuit of reward. Individuals with a highly sensitive behavioural approach system have corresponding heightened sensitivity to rewards, and since individual food consumption is moderated by the

perceived reward value of food may play a role in regulation of food intake; indeed a higher BMI has been associated with increased sensitivity to the reward value of food (Franken & Muris 2005), and these innate differences may explain the findings of Finlayson *et al* (2011). Palatable food intake induces a neurological reward response by stimulating dopamine secretion in the striatum (Schultz, 1998); obese individuals may have higher reward sensitivity due to the presence of a hyper-reactive dopamine secretion system (Franken & Muris, 2005), or lower levels of dopamine receptors in the brain (Wang *et al*, 2001). Indeed it has been shown that 6 month body mass gain in women was associated with a reduced dopamine response to food consumption compared to women with stable body mass during the same period (Stice *et al*, 2010). Evidence regarding exercise-induced changes in dopamine activity is limited; existing studies have found no change in dopamine secretion in lean individuals after 30 minutes treadmill running or completion of a triathlon (Sagnol *et al*, 1990; Wang *et al*, 2000). Further investigation of dopamine activity and reward sensitivity post-exercise in obese may thus be warranted as it is not clear how exercise affects those individual with heightened reward sensitivity. Exercise-induced changes in food preference may thus play a role in initiating compensatory responses to exercise. It has not yet been shown that increased desire for energy dense foods actively translates into increased consumption of these foods; in the present study whole group macronutrient consumption was unchanged by exercise hence we could find no evidence of this effect. The work presented in chapter 3 also showed weak test-retest reliability of macronutrient intake values obtained via the buffet meal method; the findings of this work and that of Finlayson *et al* (2009, 2011) may thus be limited by poor test-retest reliability of the methodology.

Participation in a moderate exercise session significantly increased fat and decreased carbohydrate oxidation during the following 24 hours with no overall change in 24 hour EE; a finding that also been documented in obese men (Saris & Schrauwen, 2003). A transient, acute exercise-induced shift towards fat oxidation is well documented in both lean and obese adults (Ezell *et al*, 1999; Marion-Latard *et al*, 2003; Pillard *et al*, 2007). Findings regarding post-exercise fat oxidation rate over a 24 hour period are mixed; findings from lean and obese men completing a similar duration and intensity of exercise agree with this study

(Jamurtas *et al*, 2004; Burton *et al*, 2008), whilst others find both aerobic and resistance exercise induces an increase in carbohydrate oxidation and subsequently 24hr EE in lean men and women (Melanson *et al*, 2002 a&b). Discrepancies in findings may be due to timing of exercise relative to food intake (Bennard & Doucet, 2006) or post-exercise macronutrient intake (Dionne *et al*, 1999). Fat oxidation and EE in response to a single aerobic exercise session are poorly investigated in sedentary, overweight females; the present study finds that acute exercise alters substrate oxidation in the following 24 hours but has no effect on EE. A single walking exercise session does not induce metabolic compensatory adaptations in overweight and obese women.

On an individual basis, some women may be prone to overconsumption following completion of an exercise session (Unick *et al*, 2010) but it is possible that non-physiological mechanisms induce such responses. Indeed increases in food consumption have been observed only in overweight women reporting increased negative mood post-exercise (Schneider *et al*, 2009), or change in food preference, which could be psychologically motivated, may be involved (Finlayson *et al*, 2011). The psychological factors potentially mediating post-exercise compensatory responses deserve greater attention in the literature, particularly as these factors may be modifiable where physiological factors are not.

As mentioned in chapter 3, the assessment of EI at multiple meals over a 24 hours period is a strength of this study, providing a longer observation period compared to similar studies conducted with overweight and obese women (Unick *et al*, 2010). The exercise intervention in this study induced a higher ExEE than that of Unick and colleagues, which was in line with single session ExEE recommendations for body mass control (Jakicic *et al*, 2001; Donnelly *et al*, 2009). This study allows reflection on potential impact of exercise guidelines on short term appetite responses in overweight and obese women. Metabolic measurements throughout the trials also allowed accurate assessment of ExEE and post-exercise metabolic responses. Additionally, as total ghrelin levels have been observed not to reflect exercise induced effects on acylated ghrelin concentrations (Marzullo *et al*, 2008), the decision to measure the latter form of

ghrelin increased the possibility of detecting exercise induced effects. There is a lack of information on post-exercise peptide YY responses in overweight and obese women, thus this investigation provided information on a relatively less well understood appetite regulating hormone.

4.3.1 Limitations

The findings of this study should be appreciated in the context of certain limitations. As previously mentioned, the work detailed in chapter 3 of this thesis called into question the reliability of the buffet meal EI measures used in this study; in light of the poor test-retest reliability of EI values observed in a subset of the women who also took part in this study, reliable conclusions about potential compensation responses cannot be drawn. For the most part, appetite ratings were found to be significantly reproducible, though such ratings are always entirely subjective and thus difficult to measure accurately.

The range of ExEE was also larger than intended due to poor level of fitness in some participants, making the intervention in this study less standardised than intended. Differing ExEE between participants may have had an impact on individual EI responses since the impact of the exercise on energy balance was not homogeneous in these women. Due to the large degree of variability in these results, analysis to identify potential outliers was carried out for this study as detailed in section 2.11.1.2. These plots revealed that the participant with the lowest ExEE value of 0.71 MJ was a potential outlier as it lay beyond the lower whisker of the box plot, and was therefore out with the normal spread of the group values. No biochemical or metabolic data was available for this participant, and EI and appetite results were analysed without this participant to identify the possible impact on the results. Findings were not altered by the inclusion of this participant therefore data from the full participant group were reported. The EI findings were also supported by previous work involving overweight and obese women (Unick *et al*, 2010) indicating the range of ExEE values may not have adversely affected results.

Participants were permitted to complete the preliminary sub-maximal fitness test and the exercise intervention session on either the treadmill or bicycle ergometer depending on preference, which is a potential limitation as it is possible that the mode of exercise may have had an effect on various outcomes such as EI, EE, RMR and appetite. However, it was felt necessary to offer the option of the bicycle ergometer as some participants were not confident using a treadmill, and expressed a desire for an alternative option. It was reasoned that accuracy of measurements may also have been affected adversely if participants felt uncomfortable and tense during exercise, and it was important to ensure safety of all participants during exercise sessions; some sedentary participants were quite unaccustomed to treadmills and anxious as a result of a concern about slipping. In order to minimise potential effects of these differences, all participants completed submaximal fitness tests and exercise intervention using the same mode of exercise; participants selected their method of exercise at the preliminary fitness test, and the exercise intervention was carried out using the same manner of exercise. It should be noted that the majority of participants were happy to complete treadmill exercise, only twelve of the twenty-nine participants chose to complete bicycle ergometer exercise. It may be that bicycle ergometer exercise is more feasible for sedentary participants and future work may benefit from using only this type of exercise with this population if treadmill exercise is likely to be difficult for some in order to improve comparability of results and minimise potential adverse effects.

It is possible that effect sizes smaller than those identified in the power analysis may have occurred in acylated ghrelin and peptide YY and have escaped detection in this study. In light of the fact that neither appetite nor EI is affected in this study any smaller, undetected changes in these peptides could not be considered biologically important. Variability in acylated ghrelin concentrations in these women was large, and power calculations revealed that large sample sizes would be needed to detect an effect on acylated ghrelin and peptide YY. A 20 pg/ml change in acylated ghrelin has previously been reported (Burton *et al*, 2010); based on the standard deviation of mean acylated ghrelin concentrations in this study, a sample size of two hundred women would be necessary to detect this magnitude of change. Calculations showed that as many as two hundred women would be necessary to detect changes smaller than this,

if the variability in acylated ghrelin concentrations was similarly large in other overweight and obese, female populations. Attrition levels can be high in these types of study, and greater numbers of women than the power calculations suggest would need to be recruited to account for this. The feasibility of conducting this size of acute exercise study is questionable; recruitment on this scale would likely be difficult in this context and an investigation of that size would be very time-consuming and expensive to complete. Unless assured of a sample size adequate to provide definitive results, for example if an acute investigation was incorporated into a large scale long term intervention with significant resources, it does not seem entirely worthwhile investing resources in a similar study on a much larger scale.

Additionally, it is also possible that compensatory changes occurred after the observational period and were not captured in this study, although the observation period in this study is longer than many others and also has the advantage of observing responses the following day. Studies observing EI for 2 days post-exercise have also failed to find an effect (King *et al*, 1997b) thus there may have been no benefit of extending observation. Lastly, the participants were asked to fast overnight at home between day 1 and day 2 of each trial, and to standardise free-living food intake and activity levels before trials. We cannot be sure of full compliance with these instructions, but there were no baseline differences in appetite or metabolism between trials hence good compliance to these instructions is inferred.

In conclusion, this study did not find evidence of an automatic compensatory response in appetite or EI in the 24 hours following a moderate intensity, aerobic exercise session in overweight and obese women. Acylated ghrelin and peptide YY concentrations were not affected by exercise participation, indicating that physiological appetite regulating systems do not seem to be influenced in the short term by a small exercise induced energy deficit. However the small sample size, limited statistical power, and the use of the buffet meal method in this study means that EI and biochemical results are not conclusive. As a result it is not clear from these data if overweight and obese women acutely compensate

for ExEE, or if appetite regulating hormones are acutely affected by exercise participation, as it is not possible to rule out the possibility that these responses went undetected.

CHAPTER 5: Effect of a 16 week exercise programme on body composition, appetite and energy balance in overweight and obese women.

5.1 Aims

- To quantify the effect of sixteen weeks aerobic exercise training on body composition in pre-menopausal, sedentary, overweight and obese women.
- To ascertain if compensatory changes in appetite, EI and EE occur after eight weeks of exercise training.
- To determine if compensatory changes in EI and/or EE after eight weeks of exercise training are regulated by acylated ghrelin and/or peptide YY.

5.2 Participants and Study Design

5.2.1 Participants

Participants in this study replied to poster or internet advertisements (appendix I). This study was marketed at overweight, sedentary, pre-menopausal women seeking to improve fitness and reduce body fat, and was advertised as an activity and “weight loss” intervention with free gym access and body composition measurements provided. Women responding to these advertisements were motivated by their interest in increasing fitness levels and reducing body mass. Fifty-six participants contacted the researcher by phone or email, and were screened for participation as described in section 2.1 after initial explanation of the study protocol. As an incentive, participants were also informed at this stage that they would receive a further two months of free access to the University of Glasgow gym upon successful completion of the study. Twenty-one women did not meet the participant inclusion criteria, therefore thirty-five women gave written, informed consent (appendix III) and commenced the study. Twenty participants chose to withdraw from the study within the first 8 weeks due to unrelated health concerns, relocation, or lack of

time. Participant flow in this study is represented in figure 5.1. One participant completed 8 weeks of exercise before withdrawing thus data is available for fifteen participants at week 8 and fourteen at week 16. Of the completing participants, seven were overweight and eight obese. Baseline characteristics of completing participants are summarised in table 5.1.

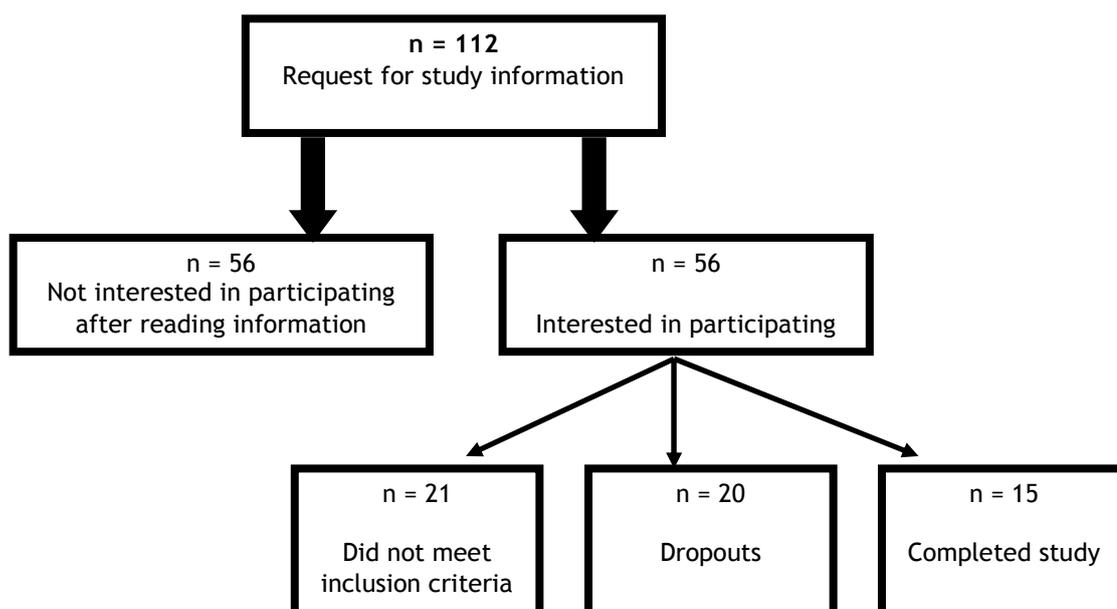


Figure 5.1 Study participant flow diagram.

Table 5.1 Baseline participant characteristics(n=15).

<i>Characteristic</i>	<i>Mean</i>	<i>SD</i>
Age (years)	34	8
Height (cm)	160	7
Body mass (kg)	76.3	10.8
BMI (kg m ⁻²)	30.0	3.4
Body fat %	44.3	2.5
Predicted maximal oxygen uptake (ml kg ⁻¹ min ⁻¹)	29.7	5.3

5.2.2 Study Design

Participants took part in a 16 week progressive, aerobic exercise intervention. Appetite, EI and expenditure, and metabolic rate were assessed during a 24hr exercise trial conducted at baseline and after 8 weeks. Body composition and predicted $\dot{V}O_{2max}$ were assessed at baseline, and after 8 and 16 weeks of exercise. Study design is summarised in figure 5.2.

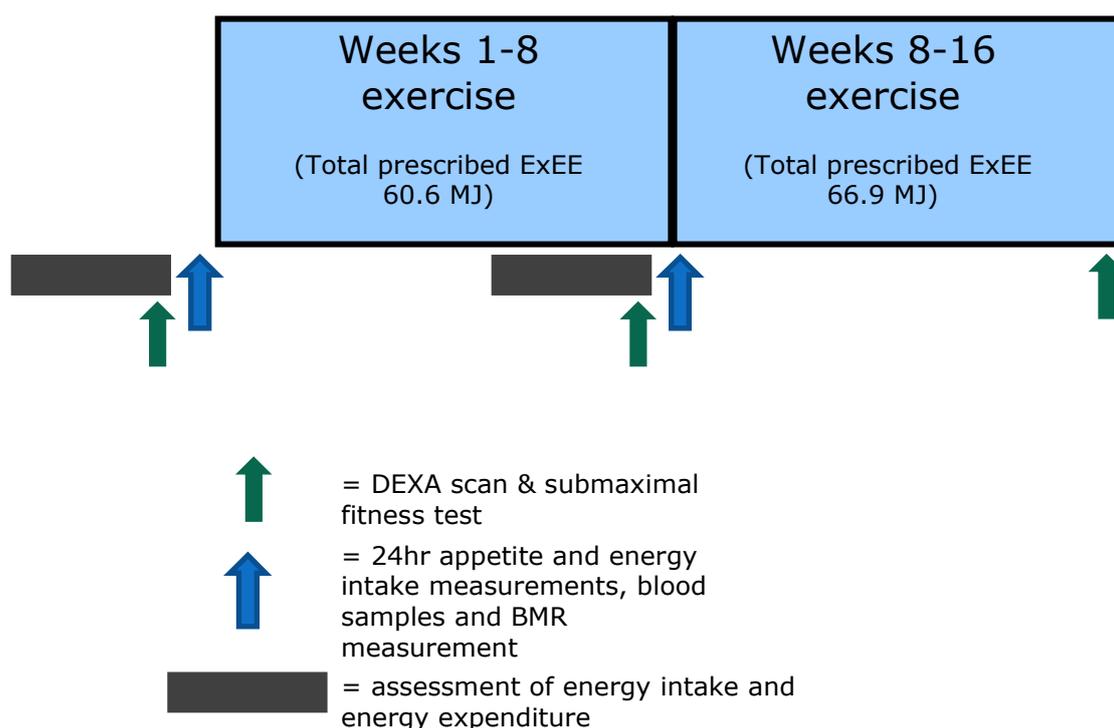


Figure 5.2 Schematic representation of study design.

5.2.3 Sub-Maximal Fitness tests

A sub-maximal fitness test was carried out to determine predicted $\dot{V}O_{2max}$, and thus intensity of subsequent exercise sessions. All tests were carried out according to the procedure described in section 2.3.

5.2.4 Energy expenditure assessment

Mean EE was assessed by physical activity diary (appendix VII) and heart rate monitoring according to the method of Moon & Butte (1996), as described in section 2.5. Participants recorded all free-living activities and wore a heart rate monitor during waking hours for seven days (5 week days and 2 weekend days) at baseline and week 8 of the intervention. Total, sleeping, active and inactive daily energy expenditure was subsequently calculated for each participant.

5.2.5 Exercise trial protocol

Participants completed two 24 hour exercise trials; the first at baseline and the second after 8 weeks of exercise. Participants were asked to keep a record of free-living food intake, and to avoid alcohol for 2.5 days immediately before the first trial. These conditions were replicated before the week 8 trial.

Trials took place over 2 days; each began in the afternoon of day 1 (~2pm) after participants consumed their normal lunchtime meal. Upon arrival at the laboratory initial measurements were made before participants completed the intervention session. Participants were observed at rest for a further 90 minutes after this, thus observation lasted 4 hours in total on day 1. Participants returned home to fast overnight and attended the laboratory once more in the morning of day 2 (~9am), where observations continued whilst participants were in a sedentary state for a further 5 hours. The protocol of exercise trials is summarised in figure 5.3.

The exercise intervention session consisted of treadmill walking exercise conducted at an intensity of 65 % $\dot{V}O_{2max}$, for a duration sufficient to expend 1.65 MJ. Expired air samples were taken every 15 minutes during the session to enable calculation of ExEE. HR was continuously monitored using short-range telemetry (Polar RS400, Polar Electro, and Finland). At all other times during the

trials participants were sedentary. The control trial was identical to the exercise trials except that participants remained sedentary during the intervention period on day 1, for the same duration as the exercise session. Estimated EE for the control period was calculated from resting metabolic rate measurements made directly prior to the intervention session (Control session EE = resting metabolic rate (kJ min^{-1}) x duration of intervention (minutes)).

Assessment of appetite, metabolic rate, and venous blood sampling were also carried out throughout each trial (figure 5.3 for timings).

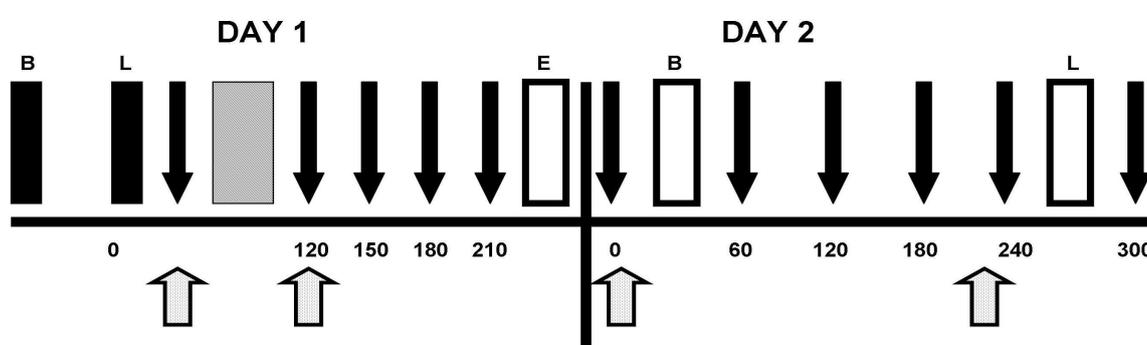


Figure 5.3 Schematic representation of study protocol. Grey rectangle represents intervention period, black arrows represent blood samples and appetite assessments, grey arrows represent metabolic rate measurements, outlined rectangles represent buffet meal while solid black rectangles represent normal meal at home/work.

5.2.6 Energy intake assessment

Three buffet meals were served during trials to allow covert assessment of EI. At the end of day 1 participants were served a buffet evening meal and during day 2 a buffet breakfast and lunch were served 4 hours apart. Meals were prepared and served as described in section 2.8.

5.2.7 Assessment of appetite

A visual analogue scale (appendix VIII) was used to assess subjective feelings of appetite during trials.

5.2.8 Metabolic rate and substrate oxidation

Metabolic rate was determined at 4 separate time points during each trial; immediately before and after the intervention period on day 1, and before each meal on day 2, including a fasting measurement. Measurements were carried out using the indirect calorimetry method (Oxycon Pro, Carefusion, San Diego, CA 92130, USA) as described in section 2.4. Metabolic data was only available for twelve participants due to mechanical failure of equipment.

5.2.9 Blood sampling and analyses

Venous blood samples were collected via a cannula inserted into an antecubital vein (BD Venflon, Becton Dickinson UK, Oxfordshire, UK). Sample collections were carried out at time points 0, 120, 150, 180 and 210 minutes on day 1 and 0, 60, 120, 180, 240 and 300 minutes on day 2 of trials. Collection, processing, and analysis of blood samples for acylated ghrelin and peptide YY was carried out as described in section 2.9. Due to technical problems data for acylated ghrelin is only available for 13 participants and peptide YY for 11 participants.

5.2.10 Exercise Intervention

During the intervention, participants exercised unsupervised for a duration to expend 2090 kJ per session. In week 1 two sessions were completed, and this was increased to a maximum of four sessions, and an ExEE of 8360 kJ week⁻¹, in week 3. Any form of aerobic exercise was permitted, including treadmill walking/running, the cross trainer, stationary bicycle, rowing ergometer, group aerobics classes and outdoor running. Compliance was assessed via heart rate monitoring and participants were asked to maintain normal dietary intake throughout the intervention period.

Participants were granted free access to the University of Glasgow gymnasium to complete all exercise sessions. Participants received instruction on how to operate all gym equipment, and the first exercise session was completed under the experimenter's direction and supervision to ensure participants were comfortable.

5.3 Results

5.3.1 Whole Group Body Composition Changes

Week 8:

Changes in body composition at week 8 were not significant ($p > 0.05$); mean changes in body and fat mass were -0.7 ± 0.6 kg and 0.9 ± 0.4 kg respectively (table 5.2). These changes were equivalent to a -0.8 ± 0.7 % change in body mass and a -2.5 ± 1.2 % change in fat mass.

Week 16:

Compared to baseline, mean changes in body and fat mass after 16 weeks of exercise were -1.8 ± 0.8 kg and -1.7 ± 0.7 kg respectively. These changes were equivalent to a -2.2 ± 1.0 % change in body mass and a -4.8 ± 1.8 % change in fat mass. Changes in body mass, BMI, % body fat, total fat mass and gynoid fat % after 16 weeks were significant compared to baseline ($p < 0.05$), and there were no changes in lean mass or % android fat (table 5.2).

Table 5.2 Aerobic fitness, body composition and fat distribution measured at baseline and after 8 and 16 weeks of exercise. $n=15$ for all week 8 values and $n=14$ for body composition and $n=11$ for $\dot{V}O_{2\max}$ values at week 16. * symbol denotes a value that is significantly different from baseline ($p < 0.05$).

	Week 0		Week 8		Week 16	
	Mean	SEM	Mean	SEM	Mean	SEM
$\dot{V}O_{2\max}$ (ml kg ⁻¹ min ⁻¹)	29.7	1.4	32.9	1.0*	32.8	1.3
Body mass (kg)	75.1	2.7	74.4	2.6	72.1	2.3*
BMI (kg m ⁻²)	30.0	0.9	29.7	0.8	29.1	0.8*
Body fat %	44.3	0.7	43.5	0.6	42.9	0.6*
Body fat mass (kg)	32.3	1.5	31.5	1.4	30.0	1.2*
Lean mass (kg)	40.3	1.2	40.5	1.2	39.8	1.2
Android region % fat	49.8	0.9	49.4	1.2	48.6	1.0
Gynoid region % fat	51.1	0.8	50.1	0.9	49.6	0.7*

5.3.2 Individual Body Composition Changes

There was a large degree of individual variation in body composition changes at weeks 8 and 16. Compared to baseline, individual changes in body and fat mass after eight weeks of exercise ranged from +2.2 to -7.7kg and +1.5 to -4.7 kg respectively. At week 16, body and fat mass changes ranged from +2.0 to -8.9 kg and +1.8 to -6.6 kg respectively (figures 5.4 & 5.5).

Changes in lean mass were similarly variable; relative to baseline, changes ranged from +1.9 to -3.0 kg and +1.0 to -2.3 kg at weeks 8 and 16 respectively.

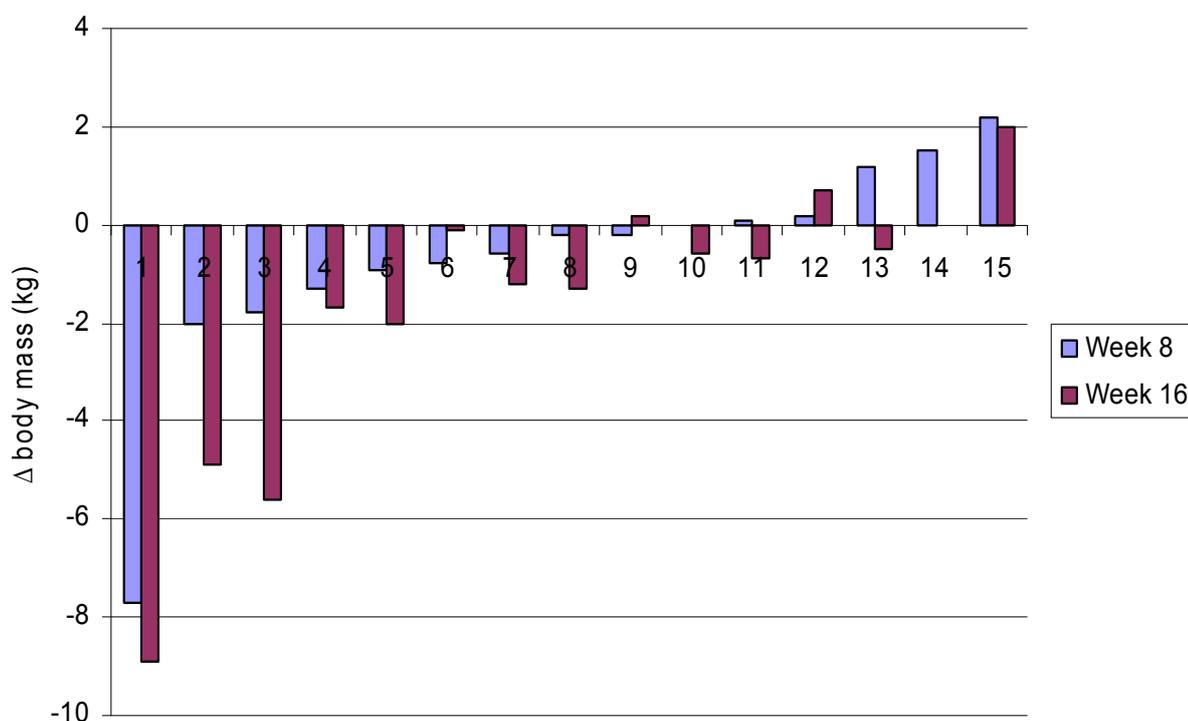


Figure 5.4 Inter individual body mass changes after 8 and 16 weeks of the exercise intervention, as compared to baseline. Each pair of histograms represents changes for one participant.

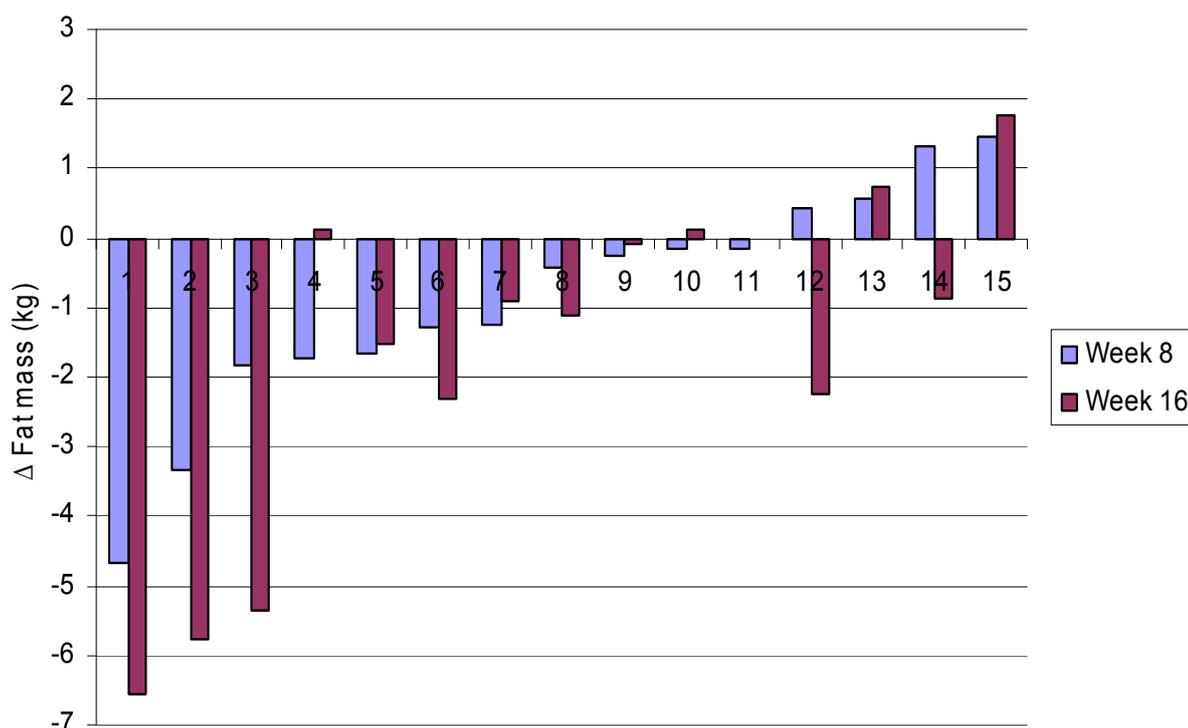


Figure 5.5 Inter individual fat mass changes after 8 and 16 weeks of the exercise intervention, as compared to baseline. Each pair of histograms represents changes for one participant.

5.3.3 Compliance and Exercise Energy Expenditure

Week 8:

Mean compliance in the first half of the intervention, based on volume of prescribed exercise completed, was 97.5 ± 24.3 %. Participants did not always maintain desired intensity of exercise during sessions, thus compliance based on achievement of prescribed total ExEE was lower, 42.8 ± 16.7 MJ, which was equivalent to 70.6 ± 27.4 % of prescribed ExEE (all values expressed as mean \pm SD).

Week 16:

Mean compliance to prescribed exercise over the whole 16 week intervention, based on the volume of prescribed exercise completed, was 93.2 ± 20.9 %. Participants did not always maintain desired intensity of exercise during sessions, thus compliance based on achievement of prescribed total ExEE was lower, 80.8 ± 29.8 MJ, which was equivalent to 63.4 ± 23.4 % of prescribed

values (all values expressed as mean \pm SD). Compliance based on duration and ExEE was highly variable between individuals, ranging from 58% to 118%, and 20 to 144 MJ respectively. Compliance decreased greatly in the second half of the intervention for some participants; two exercisers recorded only 45% compliance in this period based on duration of exercise completed. Mean compliance for the whole group did not differ between the first and second half of the intervention ($p=0.26$).

5.3.4 Cardiovascular Fitness

$\dot{V}O_{2\max}$ significantly increased between baseline and week 8 ($p=0.03$). There was a tendency for $\dot{V}O_{2\max}$ to be higher after 16 weeks of exercise compared to baseline ($p=0.09$) but there was no significant difference in predicted $\dot{V}O_{2\max}$ between weeks 8 and 16 of the intervention ($p=0.99$; table 5.2).

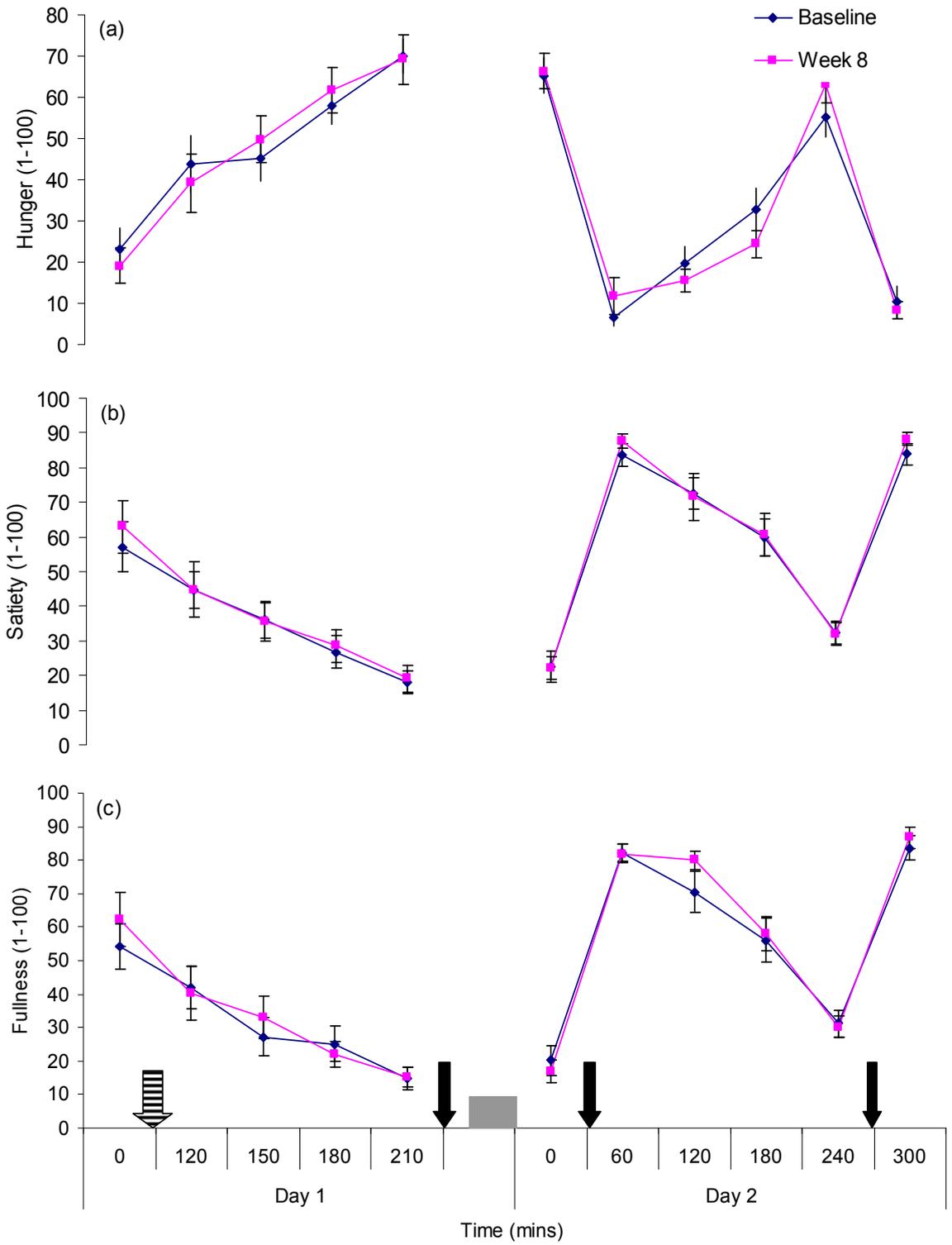
5.3.5 Exercise trial responses

ExEE during trials was not significantly different between baseline and week 8 trials (1688 ± 80 vs. 1613 ± 61 kJ; $p=0.72$), and there was no difference in mean heart rate recorded during these sessions ($p=0.68$). Rate of carbon dioxide production ($\dot{V}CO_2$), relative to body mass, was approaching significance for being higher at week 8 ($p=0.06$). Carbohydrate oxidation rate and respiratory exchange ratio tended to be lower at week 8 compared to baseline ($p=0.07$). Oxygen consumption ($\dot{V}O_2$) (18.5 ± 1.0 vs. 19.9 ± 0.5 ml kg min⁻¹; $p=0.01$), fat oxidation (0.25 ± 0.08 , 0.35 ± 0.05 g min⁻¹; $p=0.02$) and rate of EE (24.8 ± 2.0 vs. 25.8 ± 1.4 kJ min⁻¹; $p=0.05$) were all significantly higher during the week 8 exercise session compared to baseline.

5.3.6 Appetite

Repeated measures ANOVA revealed no significant main effect of trial, and no interaction effect of time*trial on any of the five appetite sensations (hunger, satiety, fullness, prospective food consumption and desire to eat) measured during baseline and week 8 exercise trials ($p>0.3$ for all; figure 5.6). There were no differences in time averaged area-under-the curve (AUC) for measures of appetite between baseline and week 8 (figure 5.7).

Paired t test comparison of individual measurements revealed only one significant difference; prospective food consumption ratings on day 2 at time point 120 were significantly higher at baseline compared to week 8 ($p=0.05$).



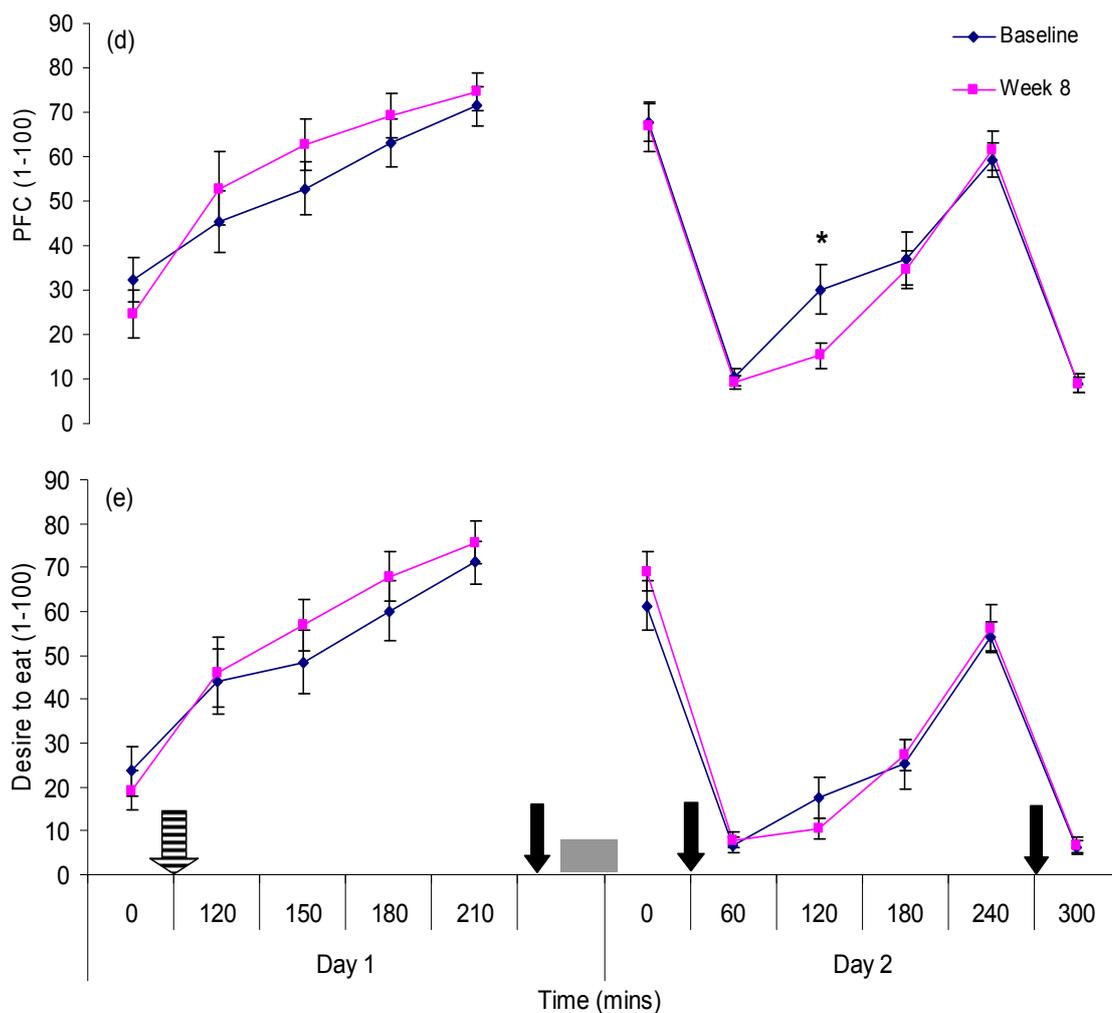


Figure 5.6 Subjectively rated sensations of hunger (a), satiety (b), fullness (c), prospective food consumption (d) and desire to eat (e) during the baseline and week 8 exercise trials. Values are mean \pm SEM (n=15). Shaded arrow represents intervention period, black arrow represents a buffet meal and grey rectangle represents overnight fast at home. * represents p value of 0.05 obtained from paired t-test.

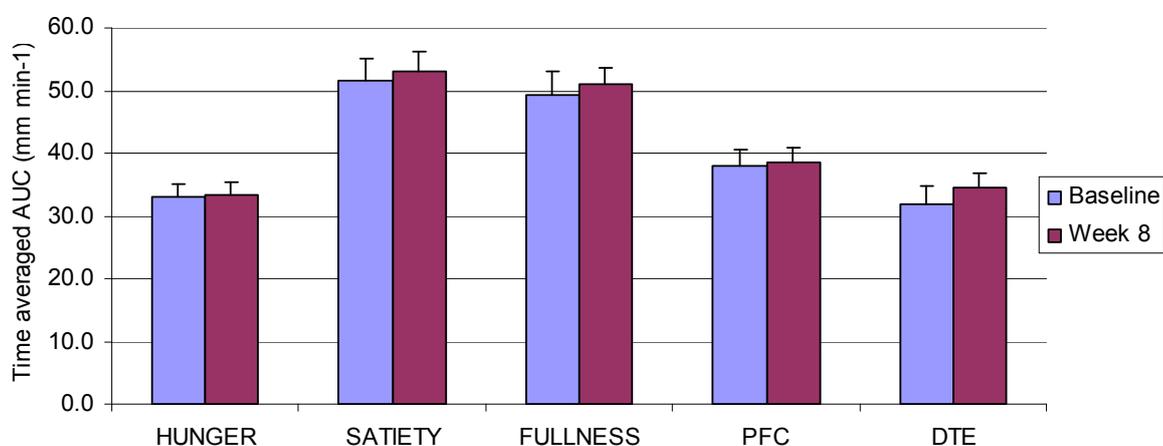


Figure 5.7 Time averaged AUC for subjectively rated appetite after a single exercise session at baseline and after 8 weeks of exercise. Values are mean \pm SEM (n=15). PFC = prospective food consumption, DTE = desire to eat.

5.3.7 Energy intake

Paired t-test found no significant differences in total energy or macronutrient intake (table 5.3; $p>0.05$), and no difference in macronutrient intake or EI at any single meal, between trials ($p>0.1$). Repeated measures ANOVA revealed no effect of trial or interaction effect of meal*trial on EI ($p>0.05$). There was a significant effect of meal; EI at the breakfast meal was significantly lower than that of lunch ($p=0.03$) and evening meal ($p<0.0001$) and lunch EI was also significantly lower than evening meal ($p=0.03$).

There was no effect of trial, or interaction effect of meal*trial, on macronutrient intake ($p>0.05$). A significant effect of meal on protein and fat intake was detected; protein and fat intake at breakfast was significantly lower than at lunch ($p<0.03$) or evening meal ($p<0.0001$). Paired t-test showed there was no difference between trials in macronutrient or EI at any single meal ($p>0.05$).

Table 5.3 Energy and macronutrient intake at baseline and after 8 weeks of exercise, assessed at buffet meals trials (n=15).

	<i>Baseline</i>		<i>Week 8</i>		<i>p-value</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Total energy intake (kcal)	2722	194	2583	144	0.40
(MJ)	11.4	0.8	10.8	0.6	0.40
Carbohydrate intake (g)	371	29	355	19	0.58
Protein intake (g)	86	7	86	7	0.86
Fat intake (g)	115	10	107	8	0.28

5.3.8 Energy Expenditure

Paired t-test found no significant difference in mean daily total EE ($p=0.18$), sleeping EE ($p=0.94$) or inactive EE ($p=0.73$) between baseline and week 8 (figure 5.8), either expressed as absolute values or relative to body mass. There was a tendency for mean daily active EE to increase between baseline and week 8 when expressed in absolute values ($\Delta +0.53 \pm 0.61 \text{ MJ } 24\text{hrs}^{-1}$; $p=0.06$), and this difference became significant when active EE was expressed relative to lean mass ($\Delta +14.7 \pm 13.8 \text{ kJ kg}^{-1} 24\text{hrs}^{-1}$; $p=0.05$).

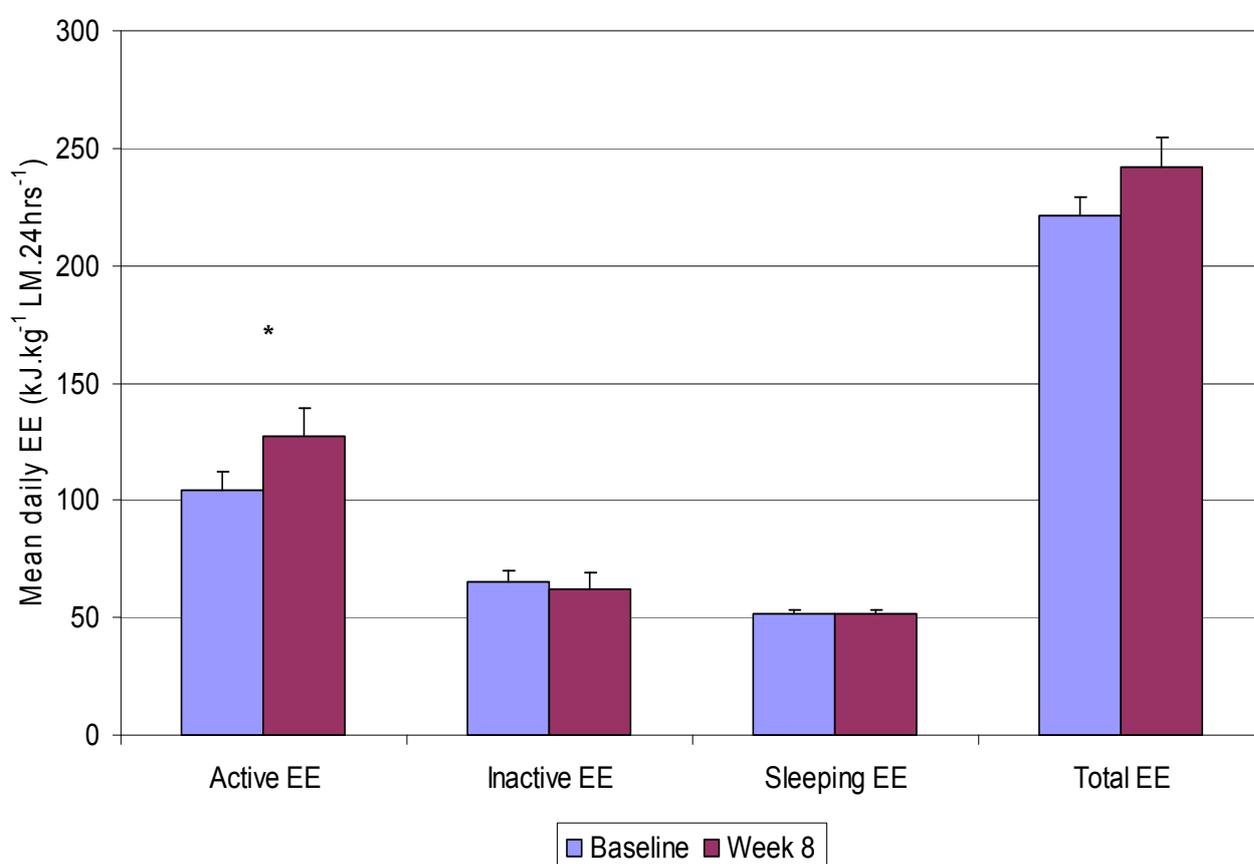


Figure 5.8 Mean daily active, inactive, sleeping and total energy expenditure ($\text{kJ kg}^{-1} \text{LM } 24\text{hrs}^{-1}$) at baseline and week 8 of intervention. Values are mean \pm SEM ($n=15$), * denotes p value of 0.05.

5.3.9 Acylated Ghrelin and Peptide YY concentrations

There was a significant effect of time on acylated ghrelin and peptide YY concentrations ($p < 0.003$) but no significant effect of trial, or interaction effect of time*trial, could be found ($p > 0.1$) (figure 5.9).

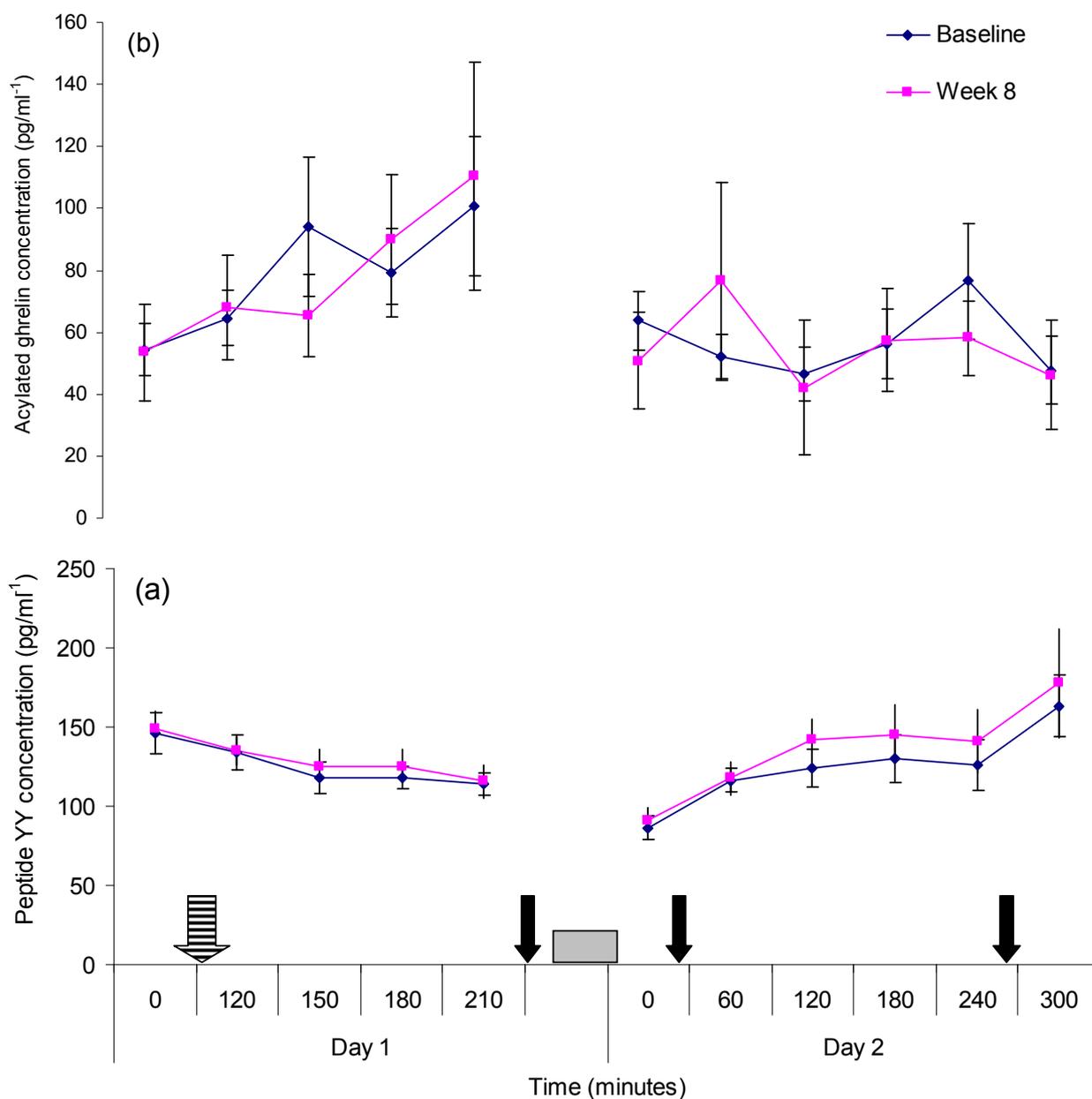


Figure 5.9 Plasma concentrations of and peptide YY (a), and acylated ghrelin (b) concentrations during baseline and week 8 exercise trials. Values are mean \pm SEM, $n=13$ for acylated ghrelin, $n=11$ for peptide YY. Black arrow represents buffet meal, grey box represents overnight at home and shaded box represents exercise session.

5.3.10 Metabolic rate and Substrate Oxidation

Week 8 fasting metabolic rate was significantly higher than baseline (table 5.4), both in absolute terms and when expressed relative to body mass, fat mass, and lean mass ($p < 0.01$). Additionally, post intervention metabolic rate was significantly higher at week 8 when expressed relative to body and fat mass ($p < 0.04$), and pre-lunch metabolic rate on day 2 was significantly higher at week 8 when expressed relative to fat mass ($p = 0.04$; table 5.4). There were no differences in fat and carbohydrate oxidation rates between baseline and week 8 trials ($p > 0.05$).

Table 5.4 Metabolic and substrate oxidations rates during baseline and week 8 exercise trials (n=12). Superscript letters denote $p < 0.04$ for difference between trials; BM = body mass, FM = fat mass and LM = lean mass.

	Day 1				Day 2			
	Pre-intervention		Post-intervention		Fasting		Pre-lunch	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Baseline trial:								
Metabolic rate:								
kJ kg ⁻¹ BM 24hrs ⁻¹	0.062	0.002	0.060 ^a	0.002	0.053 ^a	0.002	0.062	0.001
kJ kg ⁻¹ FM 24hrs ⁻¹	0.146	0.006	0.141 ^a	0.006	0.126 ^a	0.006	0.146	0.004
kJ kg ⁻¹ LM 24hrs ⁻¹	0.118	0.003	0.114	0.003	0.102 ^a	0.004	0.118	0.003
Fat Oxidation g min ⁻¹	0.08	0.01	0.12	0.01	0.09	0.01	0.07	0.01
Carbohydrate oxidation g min ⁻¹	0.10	0.03	0.001	0.02	0.04	0.01	0.14	0.02
Week 8 trial:								
Metabolic rate:								
kJ kg ⁻¹ BM 24hrs ⁻¹	0.064	0.002	0.064 ^b	0.002	0.058 ^b	0.002	0.066	0.002
kJ kg ⁻¹ FM 24hrs ⁻¹	0.153	0.008	0.153 ^b	0.008	0.138 ^b	0.006	0.158	0.006
kJ kg ⁻¹ LM 24hrs ⁻¹	0.120	0.004	0.120	0.004	0.108 ^b	0.003	0.124	0.004
Fat Oxidation g min ⁻¹	0.10	0.01	0.11	0.01	0.10	0.01	0.08	0.01
Carbohydrate oxidation g min ⁻¹	0.07	0.02	0.03	0.02	0.04	0.02	0.13	0.02

5.3.11 Correlations

Baseline fasting acylated ghrelin concentrations were significantly correlated with body mass reduction after 8 weeks ($r=0.55$, $p=0.05$) and there was also a tendency for an association with fat mass reduction at 8 weeks ($r=0.53$, $p=0.06$). There were no correlations reaching statistical significance between body composition changes and peptide YY concentrations at any point ($p>0.05$).

Fat mass loss at 8 weeks was significantly correlated with fasting fat oxidation rate at week 8 ($r=-0.61$, $p=0.04$) and body mass loss at week 8 was significantly correlated with fasting fat oxidation at baseline ($r=-0.64$, $p=0.003$) and at week 8 ($r=-0.77$, $p=0.003$).

Mean ratings for desire to eat were significantly correlated with mean acylated ghrelin concentrations on day 2 of the week 8 trial ($r=-0.74$, $p=0.02$). There were no other significant correlations between appetite ratings and either acylated ghrelin or peptide YY.

Neither body nor fat mass changes were significantly correlated with changes in EI after eight weeks exercise, or with changes in EE at any time point (table 5.5).

Table 5.5 Correlations between body composition changes and changes in EI and EE after eight and sixteen weeks of exercise.

	Δ EI (kJ)	Δ EE (kJ)
Δ Body mass (kg):		
Week 8	0.30	-0.04
Week 16	-	0.16
Δ Fat Mass (kg):		
Week 8	0.16	-0.27
Week 16	-	0.16

5.3.12 Power analysis

Post-hoc power analysis was conducted due to the high attrition rate and smaller than intended sample size. Calculations indicated that with 15 participants, 80% power and α of 0.05, a 1.8kg and 2.6kg change in body mass, and a 1.3kg and 1.9kg change in fat mass could be detected after 8 and 16 weeks of exercise respectively.

Post-hoc power analyses indicated that, with the same parameters, a 2.0 MJ and a 1.8 MJ difference in total daily EI and EE respectively could be detected between baseline and week 8.

5.4 Discussion

The study found that that 16 weeks of aerobic exercise, with a net ExEE of 80.8 MJ, produced a mean body mass (BM) reduction of 1.65 kg, or -2.0%, and a mean fat mass (FM) reduction of 1.60 kg, or -4.5%, in overweight women. Eight weeks of exercise was not sufficient to induce any significant changes in body composition, despite a net ExEE of 42.8 MJ. There was a large degree of individual variability in the magnitude and direction of these values at both time points, in agreement with the findings of King *et al* (2008). Some participants experienced very modest body fat loss, some actually increased fat mass, and there were three participants who achieved fat losses well beyond expected (>5 kg) at weeks 8 and 16, indicating that compensatory behaviours are also highly individual. King *et al* (2008) also reported individual variability in EI changes during a 12 week exercise intervention which accounted for variability in body composition changes; no explanatory changes in either EI or EE were observed in the present study, and body composition changes were not significantly correlated with changes in either EI or EE at either week 8 or 16. It is probable that compensatory changes were not observed in the present work due to small sample size and limited statistical power; the minimum change in EI that could be detected in the present study was 2 MJ, a value much larger than the changes reported by King and colleagues. As reported in chapter 3, the buffet meal method used to measure changes in EI after 8 weeks of exercise has low test-

retest reliability in this population and as a result changes in EI are likely to have escaped detection; the lack of significant body composition changes at this point indicate that compensatory changes did occur. Unfortunately EI measurements were not made at week sixteen; the original study design was planned to allow identification of potential compensatory EI responses after eight weeks of exercise. It was originally intended that participants exhibiting these behaviours would be randomised to a behavioural intervention designed to modify these behaviours in the latter eight weeks of the intervention. This original design was demanding for participants and had to be altered and scaled back due to high attrition and poor recruitment rates, however the timing of EI measurements could not be changed at this point as it was necessary to ensure results from all participants were comparable. As a result of this it is not clear if compensatory changes in EI occurred in the latter half of the intervention, although mean body composition changes were less than predicted. It is likely that the limitations associated with the small sample size and methodology would also have resulted in potential changes escaping detection even if measurements had been made at this point.

Manthou *et al* (2010) detected a compensatory change in non-exercise EE in overweight and obese women completing an 8 week exercise intervention. It is not clear if compensatory change in non-exercise EE occurred in the present study since changes in daily EE of less than 1.8 MJ were not detectable with this sample size, and expected changes in daily EE were approximately +0.76 MJ. Total daily EE was unchanged between weeks 0 and 8 which may in itself indicate an increase in time spent being sedentary during the intervention, since the expected increase was not observed.

Clinical guidelines specify that a 5% body mass change is the minimum change necessary to reduce metabolic and cardiovascular disease risk % (Clinical Guidelines 43: Obesity, National Institute for Health and Clinical Excellence, 2006; Management of Obesity, Scottish Intercollegiate Guidelines Network, 2010). Neither eight nor 16 weeks of exercise was sufficient to induce a clinically significant change, though the change in body fat % approached this value at sixteen weeks. Based on actual ExEE and the assumption that a kg of fat

is equivalent to 32.2 MJ (Forbes *et al*, 1982), changes at week 8 were not expected to be clinically significant; a mean 1.3 kg, or 4.0 % reduction in fat mass was expected. Achievement of a clinically meaningful change in body fat was not expected at this point. A much higher ExEE would be necessary to induce such changes; the exercise protocol in the present study was highly demanding, and increasing ExEE to induce health benefits over a shorter time may not be feasible in sedentary participants. Eight weeks of exercise could not be expected to produce clinically meaningful changes as a result. After 16 weeks expected reductions did exceed this threshold; a clinically significant mean fat mass reduction of 2.5 kg, equivalent to a 7.7% reduction, was predicted. Observed mean changes did not reach this value, with an actual fat mass reduction of 1.7 kg, or 4.8%. Based on these findings sixteen weeks of aerobic exercise may not be expected to confer significant health benefits due to negation of ExEE preventing achievement of a clinically significant body composition change. These findings are in agreement with exercise intervention studies of similar duration and intensity which witnessed equally modest losses (Andersson *et al*, 2001; Martins *et al*, 2010a). Individual comparison of actual fat mass losses to predicted values revealed that only four participants achieved or exceeded expected losses at both week 8 and 16. There were no explanatory differences in average compliance (calculated by comparing actual duration of exercise completed, as recorded by HR monitors, compared to prescribed durations) to the intervention between these four participants and the remaining ten (92.2 vs. 95.9%; $p=0.77$), therefore it seems likely that at least ten of these participants were compensating for the increased ExEE via changes throughout the intervention. Due to the small sample size these changes cannot be detected in the present work, but their presence can be inferred from body mass changes and compliance data.

This study also showed that body fat was specifically reduced in the gynoid region of these women after sixteen weeks of exercise, in agreement with findings from similar interventions (Janssen *et al*, 2002; Irwin *et al*, 2003). Adipose tissue in the abdominal android region was unchanged which agrees with similar data from other exercise intervention studies (Wallman *et al*, 2009). Given the association between excess abdominal adipose tissue and an increased risk of chronic diseases, it is perhaps disappointing that no significant reduction

was seen in this area. Exercise may be more effective for reducing fat deposits around the pelvic region in women. The smaller body fat change observed after eight weeks of exercise resulted in no changes in regional body composition, and it seems that longer periods of exercise are needed to attain such benefits in overweight, sedentary women.

An increase in hunger and satiety responses to food (King *et al*, 2009) and an exercise induced increase in liking and relative preference for energy dense, high-fat, sweet tasting foods (Finlayson *et al*, 2011) have both been observed in overweight and obese volunteers who did not achieve predicted losses in a 12 week intervention. The present study found no change in appetite or macronutrient intake after 8 weeks of exercise, and cannot corroborate these findings. Increased preference for high-fat, sweet foods has been identified as an acute effect of exercise but it is not clear if this translates to a change in food intake; the present study did not measure preferences, and neither acute nor chronic exercise participation affected food selection. It is possible that changes in hunger and satiety observed in the previous work of King *et al* (2009) were not observed in this work because no significant body composition changes had occurred when these measurements were made (8 weeks). Reassessment at 16 weeks may have elucidated different results regarding hunger and satiety.

This study measured acylated ghrelin responses after 8 weeks of exercise participation and found no significant change in circulating levels, although this finding is not conclusive due to low statistical power. Acylated ghrelin samples were available from 13 participants and a *post-hoc* power analysis indicated that the minimum change in ghrelin that could be detected was 101 pg ml⁻¹. This value represents a larger change in acylated ghrelin levels than is generally observed in similar studies (Martins *et al*, 2010a), and it is quite possible that real effects were simply not detected in these women. This may explain why these findings disagree with those of similar interventions conducted with overweight and obese adults who observed a significant up-regulation of ghrelin (Foster-Schubert *et al*, 2005; Martins *et al*, 2010a). Similar up-regulation has also been observed in response to combined dietary and exercise interventions (Hansen *et al*, 2002; Garcia *et al*, 2006; Santosa *et al*, 2007; Ata *et al*, 2010);

ghrelin stimulation seems to occur in response to depletion of body fat stores and not to exercise participation *per se*. It has been suggested that this change is threshold dependent, occurring only in those achieving body mass reduction ≥ 3 kg (Foster-Schubert *et al*, 2005). This may partially explain the lack of effect on acylated ghrelin observed in the present study since mean body and fat mass reductions were < 3 kg. It is possible that acylated ghrelin levels may have been affected by the body fat reduction observed after 16 weeks of exercise, but these measurements were not made at this time point. It is also possible that sub-classification of participants based on achieved compared to predicted body fat loss, as in King *et al* (2008), would elucidate individual changes in ghrelin levels after 8 weeks. Baseline fasting acylated ghrelin levels were significantly correlated with fat mass loss at week 8, thus there is the possibility that ghrelin concentrations may act as a predictor of efficacy of exercise in the individual.

There were no significant changes in peptide YY concentrations after 8 weeks of exercise in the present study. Existing data regarding long term exercise participation and peptide YY is limited but largely finds no effect in overweight men and women (Moran *et al*, 2007; Martins *et al*, 2010a; Turner *et al*, 2010), a finding this study corroborates. Peptide YY samples were only available from ten participants; *post-hoc* power analyses indicated that an effect size of 43.5 pg ml^{-1} could be detected with 80% power and $\alpha 0.05$. Smaller changes may have escaped detection, although previous studies have reached the same conclusions. There were no significant correlations between peptide YY and body composition changes or appetite ratings indicating peptide YY may not be directly linked to these variables. Peptide YY measurements were not made at week 16 therefore it is unclear if a statistically significant body fat reduction would affect peptide YY levels. It is also possible that peptide YY does not play a role in compensatory responses to exercise and is not affected by body mass changes.

In agreement with findings regarding appetite regulating hormones, no effect of exercise was seen on subjectively rated appetite. Effect of long term exercise on appetite is not widely studied; this study agrees with previous findings from lean individuals (Whybrow *et al*, 2008), and with those of a combined dietary and

exercise intervention in obese women (Wadden *et al*, 2008), but disagrees with a 12 week intervention which did observe an increase in fasting hunger (Martins *et al*, 2010a). King *et al* (2008) observed that exercise induced changes in appetite may be subject to the same variability as body composition changes; whole group analysis may conceal these differences in other studies and it may be advisable for future studies to analyse those who do and do not achieve predicted body mass loss separately.

This study found no evidence of metabolic compensatory adaptations; 8 weeks of exercise significantly increased fasting metabolic rate in these women. This agrees with findings of a 9 month exercise intervention with overweight adults (Potteiger *et al*, 2008), and with shorter interventions (King *et al*, 2008). A 7 week intervention conducted with overweight females found a similar increase in RMR, which was attributable to an increase in fasting fat oxidation rate (Barwell *et al*, 2009). No change in fat or carbohydrate oxidation was observed in the present study to explain the increase in metabolic rate. Changes in fat oxidation rate have been found to be associated with extent of exercise induced fat loss (Marra *et al*, 2004; Barwell *et al*, 2009); in this study body fat loss was significantly positively correlated with fasting fat oxidation rate at baseline and at week 8. Genetics, dietary intake, age and sex all influence substrate oxidation rates in the individual (Ravussin & Bogardus, 1989; Weyer *et al*, 1999) and such factors may also contribute to inter-individual variability in body composition changes. Indeed a low rate of fat oxidation has been associated with body mass (re)gain (Zurlo *et al*, 1990; Blaak & Saris, 2002). It is debatable whether exercise training alters fat oxidation (Kanaley *et al*. 2001; Goodpaster *et al*, 2003), and dietary strategies appear to have no effect on fat oxidation (Hawley *et al*, 1998), thus fat oxidation rates may be inherent and play a role in determining efficacy of exercise for body mass reduction in the individual.

5.4.1 Limitations

These results should be appreciated in the context of certain limitations. Firstly, participant numbers were smaller than desired which limited statistical power to detect small changes in all variables. The demanding nature of this study may

also have led to a biased participant sample; this intervention required significant commitment over several months, with a high volume of exercise and many time consuming assessments. Recruitment was carried out around the university campus via posters, and via adverts on local community website. As with the majority of intervention studies, this meant that participants in this study were essentially self-selected as they volunteered themselves for participation. Retention in this study was also a problematic issue, with a high drop out rate of over 50%. This was perhaps surprising given the fact that participants volunteered themselves. It is possible that this attrition rate was a reflection of the considerable commitment required for full participation in the study, and any future studies should be carefully designed to minimise this effect. The small group of women that completed the study may simply have been possessed of higher intrinsic motivation than the non-completers; behavioural differences such as these have the potential to introduce an element of bias into any intervention volunteer group. Although a homogeneous sample is preferable for intervention studies, completers of demanding interventions may not be representative of the general population of sedentary, overweight individuals who are unwilling to commit to such an intervention. However, due to the nature of exercise intervention studies, participants must be volunteers, therefore it is not possible for any researcher to circumvent such issues.

Analysis of successful and non-successful participants, as in King *et al* (2008), was also not possible; individual differences may have been detected if this had been feasible. Low participant numbers were exacerbated by a high attrition rate; twenty volunteers did not complete the initial 8 weeks of the intervention which represents a 57% attrition rate. Other ladies expressed interest in the study but did not agree to participate once further information was supplied, most likely because this was a demanding and time-consuming study. As an example of problems resulting from high workload for volunteers, participants were asked to keep a week long weighed food record in order to provide a secondary measure of EI, however the poor quality of these records resulted in unreliable and unusable data therefore it was not included in this analysis.

Although the full intervention was 16 weeks, many key measurements were made after 8 weeks. These tests were originally planned at this point in order to detect compensatory responses on an individual basis, with the intention of implementing a behavioural intervention in the subsequent eight weeks to try and moderate these responses. Due to significant recruitment and retention problems, the initial study design had to be altered in an attempt to improve participant numbers, and this intervention was scaled back and an additional behavioural intervention was omitted. As mentioned previously, timing of measurements remained unchanged to ensure data from subsequent participants would be comparable. Many key outcome assessments in this study were thus made before a significant body mass reduction was achieved, and as participant numbers were low eight weeks of exercise may not have been sufficient to induce detectable changes; particularly if compensatory EI responses are partial and gradual as has been previously suggested (Stubbs *et al*, 2002b & 2004; Whybrow *et al*, 2008). It is possible that there would have been a greater possibility of detecting compensatory responses after sixteen weeks of exercise, particularly as body fat mass reduction became significant only at this time point.

As mentioned previously, the original intention of this study was to identify those participants demonstrating compensatory responses after eight weeks of exercise. It was originally planned to randomise these individuals to a control group or a behavioural intervention group. As this intervention was not carried out there is no control group in the study; if this work had originally been planned as it was eventually carried out it is likely a control group would have been included in the design to strengthen these findings. The randomisation of participants to either control or exercise groups would more clearly elucidate the effects of exercise participation; research participants often modify aspects of behaviour purely due to the knowledge that they are being observed and measured, known as the Hawthorne effect. Thus without a control group it is not possible to conclude that these results are solely attributable to exercise participation, as participants may also have altered other aspects of behaviour.

This intervention study did record a high level of compliance to an unsupervised exercise programme (93%). Compliance was highly variable between individuals; some performed less than prescribed, whilst others exercised in excess of study requirements, hence compliance ranged from 58 to 118%. For some, support and flexible access to good facilities and equipment maintains higher compliance than unsupervised interventions (Jakicic *et al*, 1999).

Blood samples were not obtained from all participants due to difficulties obtaining venous blood samples; difficulties situating the cannula were experienced with some participants which meant that blood sample collection was not possible. Cannulation can be a particular problem with overweight and obese women as subcutaneous fat deposits can make it very difficult to visualise and place a cannula in a superficial vein.

EI assessment is always associated with significant difficulties regardless of the method utilised (Macdiarmid & Blundell, 1998). Direct observation of intake at ad-libitum buffet meals was carried out in this study but the test-retest reliability of method has not been investigated in this context or in overweight women; this realisation led to the work detailed in chapter 3 which has cast aspersions on reliability of EI estimated obtained by this method. Between this issue and the low participant numbers EI data is not conclusive, and it cannot accurately be stated that EI was unaffected by exercise in these women. Additionally, as discussed in chapter 4, participants completed submaximal fitness tests and supervised exercise sessions completed during the baseline and week 8 trials by either bike or treadmill. Although the mode of exercise was kept constant for each individual participant throughout the intervention, differences between participants in exercise mode completed in the laboratory may potentially have affected related measurements adversely.

A strength of this study is that changes in EE, EI, RMR, and appetite regulating hormones were investigated in response to 8 weeks of exercise. This array of measures was designed to assess the existence of compensatory changes in EI, EE and RMR simultaneously, as well as investigating a potential mechanism for such changes. Most studies do not measure all of these variables concurrently,

concentrating on either EI or EE in some cases, or investigating changes without an interest in potential mechanisms. The scale of this study was an ambitious undertaking, and unfortunately the aims of this study were not met as intended due to the small sample size. This work may still serve as a feasibility study. Demanding exercise protocols, combined with a number of time-consuming and demanding measurements, were most likely the major reason for the high attrition rate in this study. It may be that a protocol this demanding is not an entirely feasible undertaking and a high attrition rate may have been inevitable, as seems to be the case with many exercise intervention studies. It is also possible that attrition would have been decreased if additional resources had been available. This study was conducted largely by a single researcher, and a larger team or researchers sufficient to fully supervise all exercise sessions and provide greater individual support to participants would have improved the participant retention rate. Provision of exercise equipment at home has resulted in low attrition rates for some studies (Jakicic *et al*, 1999) and similar measures may also have improved retention rate in the current work, had sufficient resources to do so been available. The methodology of this study could also have been altered to lessen the workload for participants, such as using doubly labelled water instead of physical activity diaries to measure EE, if a greater budget had been available. Measures such as this may have made the intervention less laborious and perhaps reduced attrition.

This study is one of very few studies that observe peptide YY levels in response to long term exercise training, and there is a lack of information regarding these effects in overweight and obese women in particular. Findings agree with those obtained largely from overweight men (Martins *et al*, 2010a; Turner *et al*, 2010), which also reported no effect. Further research is required to clarify whether peptide YY is truly unaffected by long term exercise participation, and measurement of peptide YY₃₋₃₆ may be particularly relevant since it is this form that is able to cross the blood-brain barrier. However there is currently no data regarding the effects of exercise on this form of peptide YY available. This study also provides valuable information regarding exercise induced changes in regional adiposity in overweight and obese women. The use of DEXA scanning to assess body composition was a particular strength of this study due to the detailed information this method provides about fat distribution. The individual

variability reported by King *et al* (2008) was further corroborated by this work, and increasing evidence of such variability strengthens the evidence that future studies should analyse groups based on their achievement of expected body fat loss to elucidate compensatory responses.

This study concludes that sixteen weeks aerobic exercise in overweight and obese women produces a small, but statistically significant, reduction in body and fat mass. Eight weeks exercise participation was not sufficient to induce a significant change in body composition, though the efficacy of exercise as a body mass reduction method varied greatly between individuals after both eight and sixteen weeks. There was no evidence of compensatory changes in EI or EE after eight weeks of exercise, and similarly no evidence of an effect on subjective appetite ratings or acylated ghrelin and peptide YY concentrations at this point. Unfortunately, limited participant numbers, lack of a control group and low reliability of the method used to assess EI may have prevented detection of effects thus the evidence produced by this study is inconclusive.

CHAPTER 6: Resting energy expenditure and physical activity levels of overweight and obese pre-menopausal women classed as restrained eaters with flexible or rigid control.

6.1 Aims

1. To confirm if energy requirements vary between overweight, pre-menopausal female restrained and unrestrained eaters.
2. To identify potential differences in the energy requirements of restrained eaters with flexible and rigid control.

6.2 Participants and Study Design

6.2.1 Participants

Participants were recruited and screened as described in section 2.1. Forty-six women were recruited for this study but five did not complete the study due to lack of time or unrelated health problems, thus forty-one participants were available for analysis in this study. Overweight and obese women were studied since dietary restraint is reported to be highest in this group (Klesges *et al*, 1992; Provencher *et al*, 2003).

6.2.2 Study Design

Participants participated in an observational study during which they were instructed to maintain normal lifestyle. Participants attended the laboratory in the fasted state for the first visit and a resting metabolic rate measurement was carried out as outlined in section 2.4. Participants subsequently completed an incremental submaximal fitness test on the treadmill as described in section 2.3, and then recorded all free-living activities in a diary (appendix VII) for 7 days in conjunction with continuous heart rate monitoring (section 2.5). Diaries and heart rate monitors were returned on the second laboratory visit, when a body composition assessment and second fasted metabolic rate measurement were

carried out. Each participant completed the three-factor eating questionnaire (TFEQ) on two separate occasions and participants were classified as restrained or unrestrained based on their mean restraint score obtained from both completed questionnaires. Median restraint values of the entire sample group were calculated to obtain a cut-off score for classification of restrained eaters relative to this specific population. Participants were classed as restrained eaters only if their mean restraint score calculated from the two separate questionnaires was above this median cut-off score, an approach adopted in other studies due to the lack of established clinical cut-off values (Tepper *et al*, 1996; Van Loan & Keim, 2000; Rideout & Barr, 2009).

Restrained eaters also completed the flexible and rigid control of restraint subscale (appendix IX) on the second visit, and these participants were classed as having either predominantly flexible or rigid control of restraint based on which subscale in which they achieved the highest score.

6.2.3 Measurement of free-living EE

EE was calculated according to the method of Moon & Butte (1996) as detailed in section 2.5. Data from the physical activity diaries and heart rate monitors was used to calculate daily mean total, active, inactive and sleeping EE taken as an average of values obtained from all seven days.

6.2.4 Power Calculation

Prospective power calculation was carried out to detect difference in RMR between restrained and unrestrained eaters since no data regarding these variables in flexible and rigid restrained eaters was available. Based on previously published data of Platte *et al* (1996) a 560 kJ day⁻¹ difference in RMR may be expected between restrained and unrestrained; it was found that 17 participants in each group would be required to detect this difference with 90% power and α of 0.05.

Post-hoc power analysis showed that differences of 1163 kJ/day and 3865 kJ/day in RMR and TEE respectively can be detected with 14 rigid and 7 flexible restrained eaters, 80% power and α of 0.05.

6.3 Results

6.3.1 Whole Group

6.3.1.1 Participant characteristics

Participants had mean age of 35 ± 9 years, body mass of 77.8 ± 14.0 kg, BMI of 28.5 ± 4.5 kg m⁻², body fat of 40.1 ± 8.1 %, and predicted $\dot{V}O_{2\max}$ of 30.7 ± 7.1 ml kg⁻¹ min⁻¹ (mean \pm SD). Median restraint score was 10.5, and twenty one participants were classed as restrained eaters, the remaining twenty were unrestrained.

6.3.1.2 Correlations

Table 6.1 shows correlations between anthropometric and biological variables and components of EE for the whole group. Dietary restraint was not significantly associated with any variables ($p > 0.05$). RMR was significantly associated with $\dot{V}O_{2\max}$ ($r = 0.39$, $p = 0.01$), body mass ($r = 0.74$, $p < 0.001$), fat mass ($r = 0.58$, $p < 0.001$) and lean mass ($r = 0.62$, $p < 0.001$). $\dot{V}O_{2\max}$ was also significantly associated with lean mass ($r = 0.67$, $p < 0.001$) and total EE ($r = 0.66$, $p < 0.001$).

Table 6.1 Correlations between dietary restraint, RMR, components of EE, $\dot{V}O_{2max}$ and body composition in overweight and obese women (n=41).

	<i>Restraint</i>	<i>RMR</i>	$\dot{V}O_{2max}$	<i>AEE</i>	<i>IAEE</i>	<i>SEE</i>	<i>TEE</i>	<i>BM</i>	<i>BMI</i>	<i>FM</i>	<i>LM</i>
Restraint	-	-0.15	0.24	0.08	-0.19	-0.13	-0.04	-0.16	-0.17	-0.16	-0.07
RMR		-	-0.16	0.15	0.51*	0.84*	0.50*	0.74*	0.62*	0.58*	0.62*
$\dot{V}O_{2max}$			-	0.48*	0.12	-0.33*	0.38*	-0.44*	-0.55*	-0.61*	0.17
AEE				-	0.07	0.05	0.85*	0.05	-0.16	-0.13	0.39*
IAEE					-	0.23	0.56*	0.26	0.16	0.11	0.40*
SEE						-	0.32*	0.69*	0.65*	0.59*	0.49*
TEE							-	0.28	0.06	0.05	0.59*
BM								-	0.89*	0.91*	0.59*
BMI									-	0.93*	0.28
FM										-	0.21
LM											-

$\dot{V}O_{2max}$ expressed in ml.kg.min⁻¹. AEE = active EE, IAEE = inactive EE, SEE = sleeping EE, TEE = total expenditure, BM = body mass (kg), FM = fat mass (kg) and LM = lean mass (kg). * denotes p<0.05

6.3.2 Restrained and Unrestrained eaters

6.3.2.1 Participant characteristics

There were no significant differences in physical characteristics, body composition or cardiovascular fitness between restrained (R) and unrestrained (UR) eaters ($p > 0.05$); characteristics of R and UR are summarised in table 6.2.

Table 6.2 Physical characteristics and three factor eating questionnaire (TFEQ) scores of restrained and unrestrained eaters.

	<i>Restrained (n=21)</i>		<i>Unrestrained (n=20)</i>		<i>p value</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Age (years)	34	2	36	2	0.41
Height (cm)	165	2	165	1	0.83
Body mass (kg)	75.2	2.7	80.5	3.4	0.23
BMI (kg m ⁻²)	27.5	0.8	29.5	1.1	0.14
Body fat %	38.9	1.6	41.2	2.0	0.38
Predicted $\dot{V}O_{2max}$ (ml kg ⁻¹ min ⁻¹)	32.2	1.5	29.1	1.6	0.18
TFEQ scores:					
Restraint	13.5	0.5	5.7	0.6	<0.0001
Disinhibition	11.0	0.9	8.7	0.7	0.05
Hunger	6.0	0.7	6.8	0.9	0.49

6.3.2.2 Metabolic rate

Between R and UR there were no differences in fasting metabolic rate (R: 6044 ± 158 , UR: 6295 ± 213 kJ day⁻¹; $p=0.35$) or respiratory quotient (RQ) (R: $0.77 \pm$

0.02, UR: $0.77 \pm 0.01 \text{ g min}^{-1}$; $p=0.95$), and no differences in active and inactive fat and carbohydrate oxidation rates were observed ($p>0.05$).

6.3.2.3 Physical activity EE

Mean physical activity level (PAL), defined as total EE/resting EE, was not significantly different between R and UR (R: 1.57 ± 0.08 , UR: 1.52 ± 0.06 ; $p=0.58$). There were no differences in total, active, inactive, or sleeping EE between R and UR ($p>0.05$; figure 6.1). Additionally, there were no differences in average daily time spent being active, inactive and sleeping between restrained and unrestrained ($p>0.05$; figure 6.2).

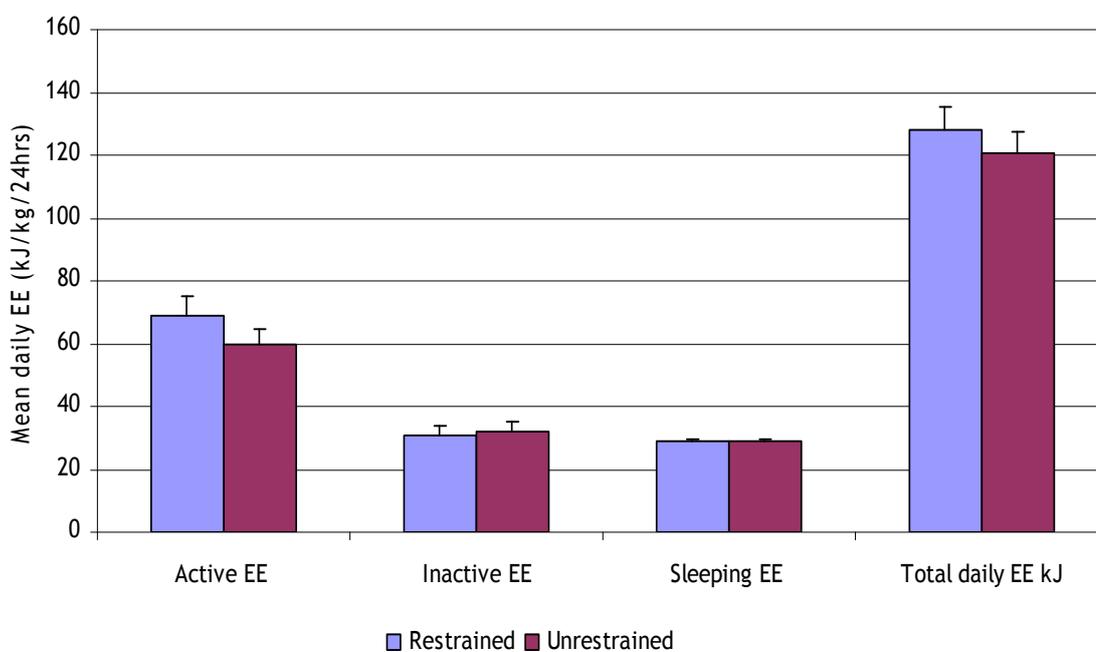


Figure 6.1 Mean 24 hour active, inactive, sleeping and total EE in restrained and unrestrained eaters expressed relative to body mass. Values are mean \pm SEM.

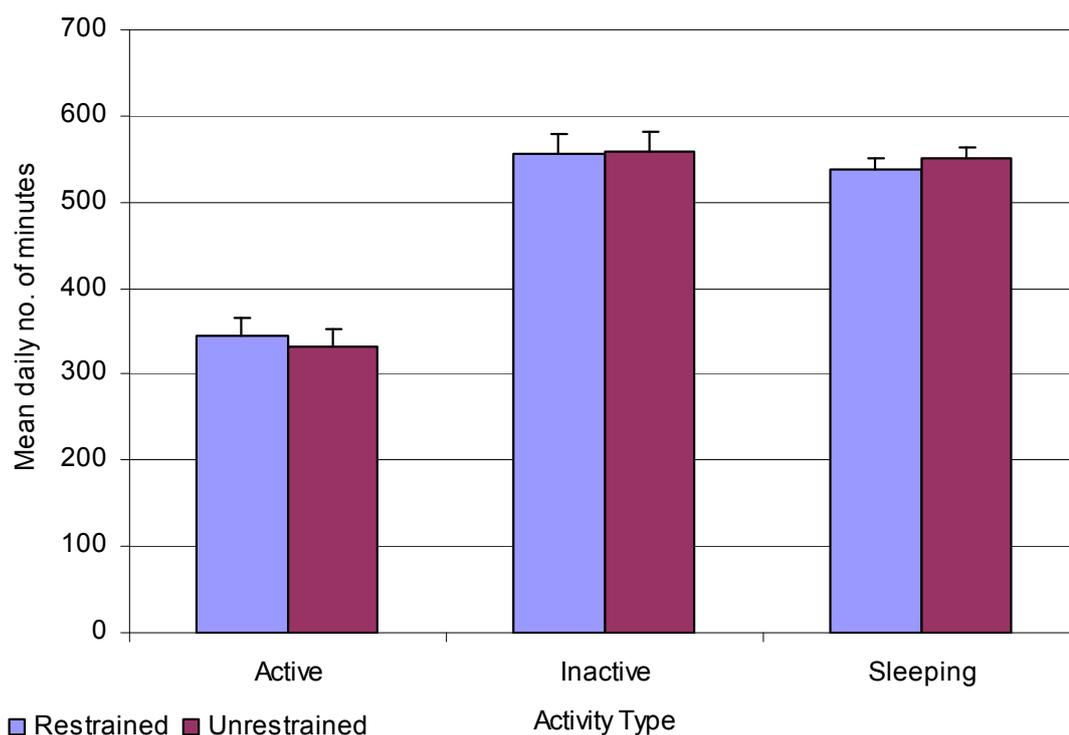


Figure 6.2 Mean total duration of sleeping, active and inactive activities per 24 hours in restrained and unrestrained eaters. Values are mean \pm SEM.

6.3.2.4 Eating patterns

Restrained eaters reported eating significantly more portions of cooked green vegetables, and significantly less sweets/chocolate and cakes (all $P < 0.05$), per week than unrestrained eaters. There was also a tendency for restrained eaters to eat more raw vegetables/salad, and significantly less high fat dairy foods ($p = 0.08$ for both) per week than unrestrained eaters. Weekly food intakes of restrained and unrestrained are described in table 6.3.

Table 6.3 Average weekly food intake (servings per week) of restrained and unrestrained eaters, assessed by FFQ. Superscript letters denote $p < 0.05$, * represents $p = 0.08$.

	<i>Restrained (n=21)</i>	<i>Unrestrained (n=20)</i>
Cereal	4.3	3.6
Fruit	12.6	8.4
Cooked green vegetables	6.5 ^a	4.3 ^b
Raw vegetables/Salad	6.0*	4.0*
Chips	0.9	1.3
Potatoes/pasta/rice	4.9	4.5
Red meat	2.6	1.4
Poultry	3.7	3.0
White fish	1.3	0.9
Oily fish	2.0	1.2
High fat dairy	1.6*	3.1*
Low fat dairy	6.0	5.1
Beans/Pulses	2.6	1.4
Sweets/Chocolate	2.8 ^a	6.9 ^b
Ice Cream	0.5	0.7
Crisps	2.3	3.0
Fruit juice	1.5	2.0
Soft drinks	6.4	4.6
Cakes/pastries etc	1.6 ^a	3.5 ^b

6.3.3 Restrained eaters – Flexible and rigid control

6.3.3.1 Participant characteristics

Of the 21 restrained eaters, 7 scored as having flexible control of restraint, and 14 scored as having rigid control. Characteristics of restrained eaters are summarised in table 6.4.

Table 6.4 Physical characteristics, three factor eating questionnaire (TFEQ) and flexible and rigid control scores of restrained eaters.

	<i>Flexible control</i>		<i>Rigid control</i>		<i>p</i> <i>value</i>
	<i>(n=7)</i>		<i>(n=14)</i>		
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	
Age (years)	30	3	35	3	0.22
Height (cm)	166	3	165	2	0.81
Body mass (kg)	71.1	3.4	77.2	3.7	0.24
BMI (kg m ⁻²)	25.9	1.2	28.3	1.1	0.15
Body fat %	34.6	1.9	41.1	2.0	0.03
Predicted $\dot{V}O_{2max}$ (ml kg ⁻¹ min ⁻¹)	32.9	1.4	31.8	2.1	0.66
TFEQ scores:					
Restraint	14.5	0.9	13.0	0.6	0.19
Disinhibition	8.6	1.8	12.2	0.9	0.10
Hunger	4.5	0.9	6.8	0.9	0.09
Flexible control of restraint	9.7	0.6	6.3	0.7	0.001
Rigid control of restraint	7.9	0.8	11.4	0.8	0.01

6.3.3.2 Metabolic rate

Between restrained eaters with rigid and flexible control there were no differences in fasting metabolic rate (Rigid: 6038 ± 184, Flexible: 6054 ± 322 kJ day⁻¹; p=0.97) or RQ (Rigid: 0.78 ± 0.03, Flexible: 0.76 ± 0.02 g min⁻¹; p=0.70), and no differences in active and inactive fat and carbohydrate oxidation rates (p>0.05).

6.3.3.3 Physical activity EE

Mean PAL was not different between the two subgroups of restrained eaters (Flexible: 1.65 ± 0.13 , Rigid: 1.53 ± 0.10 ; $p=0.46$). There were no differences in total, active, inactive, or sleeping EE between restrained eaters with flexible or rigid control ($p>0.05$); figure 6.3). There were also no differences in average daily time spent being active, inactive and sleeping between these two subgroups of restrained eaters ($p>0.05$; figure 6.4).

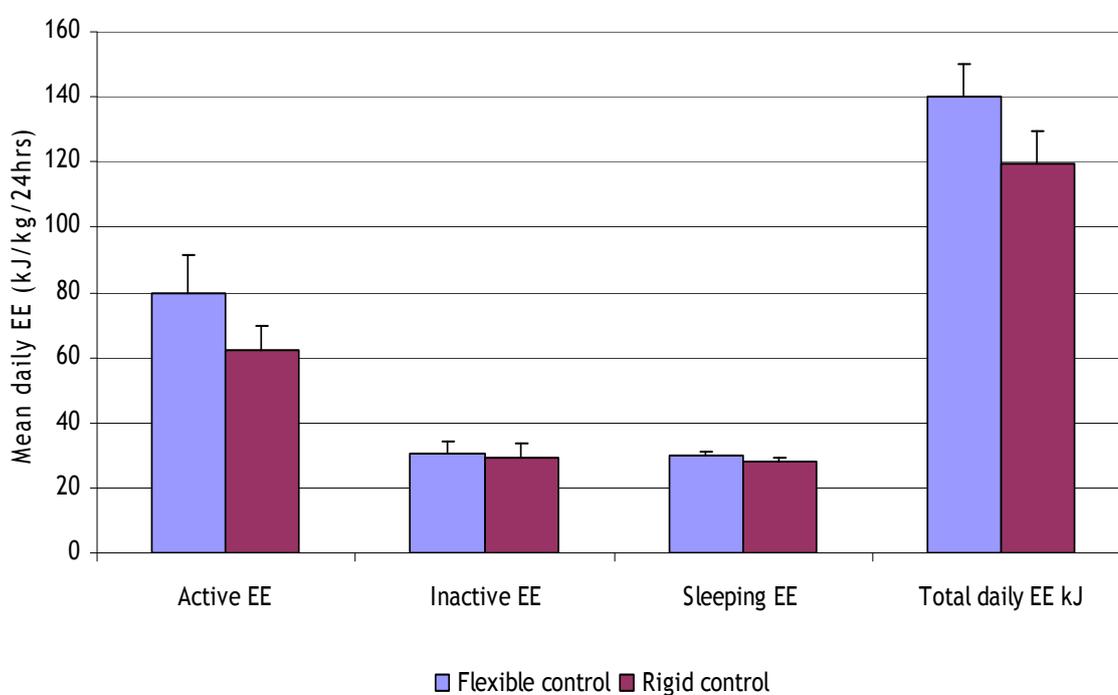


Figure 6.3 Mean 24 hour, inactive, sleeping and total EE in restrained eaters with flexible and rigid control expressed relative to body mass. Values are mean \pm SEM.

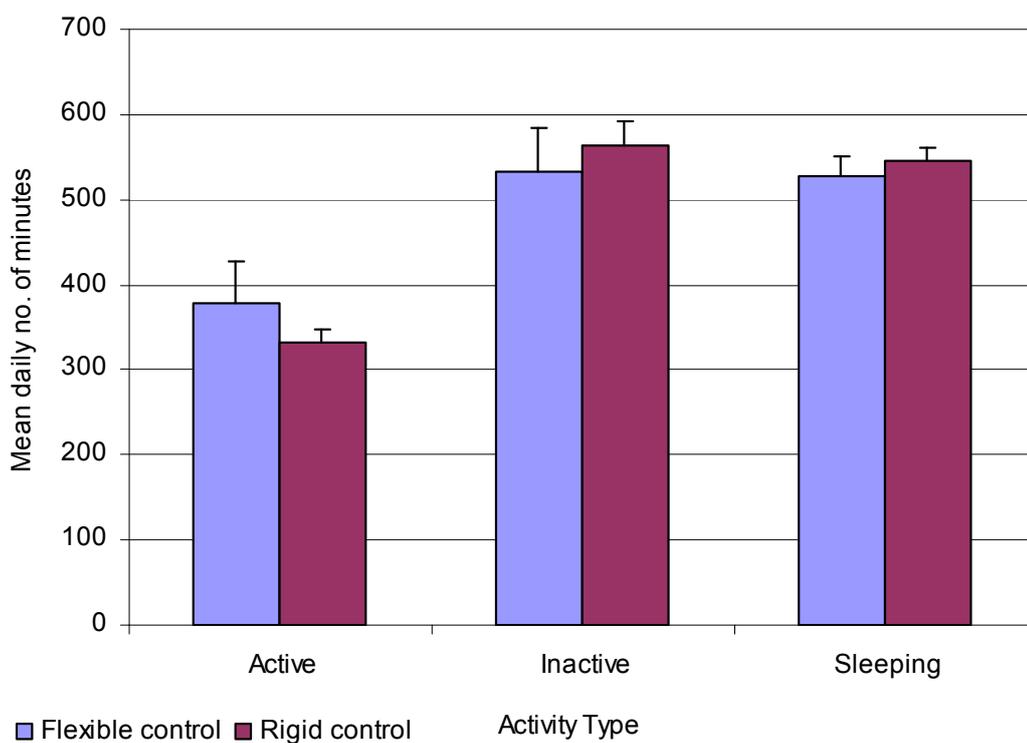


Figure 6.4 Mean total duration of sleeping, active and inactive activities per 24 hours in restrained with flexible and rigid control. Values are mean \pm SEM

6.3.3.4 Eating Patterns

Restrained eaters with flexible control reported consuming significantly more portions of cooked green vegetables and ice cream per week than those with rigid control (both $p < 0.05$). There was also a tendency for flexible restrained eaters to eat less low fat dairy products ($p = 0.09$) than their rigid counterparts. Weekly food intakes of flexible and rigid restrained eaters are described in table 6.5.

Table 6.5 Average weekly food intake (servings per week) of restrained eaters with flexible and rigid control, assessed by FFQ. Superscript letters denote $p < 0.05$, * represents $p = 0.09$.

	Restrained/Rigid (n=14)	Restrained/Flexible (n=7)
Cereal	3.9	5.2
Fruit	12.0	13.9
Cooked green vegetables	5.1 ^a	9.4 ^b
Raw vegetables/Salad	5.1	7.9
Chips	1.1	0.4
Potatoes/pasta/rice	4.2	6.3
Red meat	2.3	3.1
Poultry	4.4	2.3
White fish	0.4	3.1
Oily fish	1.4	3.3
High fat dairy	1.3	2.3
Low fat dairy	7.3*	3.6*
Beans/Pulses	2.7	2.3
Sweets/Chocolate	2.3	3.7
Ice Cream	0.3 ^a	0.9 ^b
Crisps	2.5	1.9
Fruit juice	1.6	1.3
Soft drinks	7.2	4.9
Cakes/pastries etc	1.6	1.6

6.4 Discussion

6.4.1 Whole Group

Whole group correlations show that restraint is not associated with either anthropometric or metabolic variables, or free-living EE. Restraint has been associated with body fatness in lean women but this does not hold true for these overweight and obese women. RMR is significantly positively associated with

inactive, sleeping, and total EE, as well as body fat and lean mass in the present study. $\dot{V}O_{2max}$, relative to body mass, was significantly positively associated with active and total EE, and negatively associated with sleeping EE. TEE itself was significantly positively correlated with RMR, active, inactive, and sleeping EE, $\dot{V}O_{2max}$ and lean mass; this finding is in agreement with a study of lean and obese females (Butte *et al*, 2003). Thus, independent of restraint, total EE seem to be positively associated with lean mass, RMR and cardiovascular fitness in these women.

6.4.2 Restrained and Unrestrained eaters

The findings of this study refute the claim that dietary restraint is a behavioural adaptation to inherently lower energy requirements (De Castro, 1995). This is in agreement with findings from several studies (Klesges *et al*, 1992; Lawson *et al*, 1995; Bathalon *et al*, 2001; Beseigel *et al*, 2004), but in disagreement with others (Poehlman *et al*. 1991; Platte *et al*, 1996; Laessle & Kikker, 2008; Tuschl *et al*, 1990). Discrepancies in findings between studies could be attributable to differences in study populations; studies that report finding a difference between restrained and unrestrained investigated mostly lean, pre-menopausal women, whilst those that did not find a difference included obese and postmenopausal female participants. Only one study including pre-menopausal lean women did not observe a difference in energy requirements between restrained and unrestrained eaters (Beseigel *et al*, 2004). It may be that restraint dependent differences in energy requirements are reliant on body mass, and a difference exists only in lean women. MRI studies in lean women have shown neurological differences between unrestrained and restrained indicating that restrained eaters find palatable foods more appealing in the fed state (Coletta *et al*, 2009); it would be interesting to investigate if lower energy requirements in restrained, lean women are associated with this increased desire for food.

Restrained eaters are generally found to have higher body fatness than unrestrained (Poehlman *et al*, 1991; Beseigel *et al*, 2004), but no differences in body mass or composition were found in the present study. Restraint scores indicate large differences between the groups, however both were similarly overweight. It is not clear why the participants in this study do not exhibit the expected differences in body composition.

Various methods have been used to assess EE in previous studies, and differences between methods are likely to play a role in the discrepancies between findings; indirect calorimetry, physical activity questionnaires, and doubly labelled water are all utilised in the literature and these methods have very different levels of accuracy. Questionnaires are reliant on accurate reporting by participants, and are therefore subject to greater sources of error than objective measurements. The gold standard method of measuring EE, doubly labelled water, is an entirely physiological method and though it can only distinguish total EE it is considered to be the most accurate method of measurement currently available (Schoeller, 1988).

Physical activity levels appear to affect the relationship between restraint and BMI; high restraint has been associated with significantly lower BMI in highly active women, whereas lower levels of hunger have been associated with significantly lower BMI in sedentary women (Riou *et al*, 2011). No association between external hunger and BMI was identified in the present study, however the PAL level of sedentary women in the sample of Riou *et al* was not reported, and whilst the PAL level (1.55) of women in the current study represents a relatively sedentary population on average there is variability in activity levels in these women. In addition it is difficult to compare the sedentariness of women in this study and that of Riou *et al*; observation of a more active or more sedentary population may produce results concordant with the latter work.

The average PAL of sedentary women reported in a review which looked at many large, prospective, international studies was 1.4-1.5 (Saris *et al*, 2003). PAL level of restrained eaters in the present study is higher (1.57), although EE may be somewhat overestimated in these participants. Based on measured metabolic

rate, and assuming a PAL of 1.4-1.5, restrained eaters may theoretically be overestimating EE by anywhere from 485 to 1091 kJ day⁻¹. Reported PAL of unrestrained eaters in this study is slightly lower than restrained, and performing the same calculation indicates that the unrestrained eaters may have overestimated EE by 418 to 673 kJ day⁻¹ at the most. Restrained eaters may be prone to overestimating EE of activities (Visona & George, 2002) and it is possible that inaccurate reporting of restrained eaters affected the results in the present study, resulting in a type 2 statistical error.

Restrained eaters are generally found to have higher percentage body fat than unrestrained (Poehlman *et al*, 1991; Beseigel *et al*, 2004), however no differences in body composition were found between R and UR in the present study. Eating patterns assessed by the FFQ supports the classification of these participants; restrained eaters reported consuming less high fat foods such as sweets, chocolate, cakes and dairy, as well as consuming more green vegetables than unrestrained eater. These eating patterns are in agreement with those reported in a similar study of young women (Beseigel *et al*, 2004). Given the characteristic behaviours of restrained and unrestrained reported previously, these patterns of intake would be logical and expected.

6.4.3 Restrained eaters with flexible and rigid control

There were no differences in RMR, PAL, active, inactive, sleeping or total EE between flexible and rigid restrained eaters; it seems these sub-groups can be characterised only by eating behaviour and BMI and not by physical activity levels. Although this investigation is novel, and finds no difference between these groups, it is not possible to draw conclusions due to small participant numbers and the associated limited statistical power to detect a difference. It is not possible to gauge if previous studies reporting conflicting results regarding the energy requirements of restrained and unrestrained eaters were confounded by the presence of these two sub-groups. Discrepancies may also be partially

attributable to differences in methodology and participants in these previous investigations.

Body fat percentage was significantly lower in restrained eaters with flexible control compared to those with rigid control. This is not unexpected since flexible restrained eaters are characterised by a lower frequency of binge eating episodes (Westenhoefer *et al*, 1999; Smith *et al*, 1999). This finding is in agreement with previously published findings regarding body composition differences between flexible and rigid restrained eaters (Westenhoefer *et al*, 1999; Provencher *et al*, 2003). Frequent body mass fluctuations may also contribute to this difference; restrained eaters are characterised by periods of restraint interrupted by periods of loss of control and binging, resulting in more frequent body mass fluctuations which in turn leads to a decrease in muscle mass and increase in fat content in comparison to those with stable body mass (Manore *et al*, 1991). Thus the greater percentage body fat of rigid restrained eaters may be an indication of more frequent body mass fluctuations in this group.

Dietary restraint, disinhibition, or hunger scores did not differ between groups, although scores on the flexible and rigid subscales were significantly different, as anticipated. Body fatness was significantly lower in flexible restrained eaters compared to rigid as expected (Westenhoefer *et al*, 1999), however there were no differences in body mass, BMI or lean mass between the two groups. Despite body mass being similar between these subgroups, rigid restrained eaters have a greater proportion of adipose tissue which could indicate that this group are indeed more prone to “weight cycling” behaviour (Manore *et al*, 1991).

Food intake assessed by FFQ illustrated interesting differences in eating patterns between the two subgroups; flexible restrained eaters ate more cooked green vegetables, and also more ice cream servings per week. There was also a tendency for this group to eat less low fat dairy. Flexible restrained eaters are characterised by a less rigid approach to dietary restraint, resulting in better regulation of intake and lower disinhibition. It may be logical then that flexible restrained eaters report consuming less low fat foods; occasional consumption of

these foods may prevent loss of control (disinhibition) which results in the binge eating episodes that characterise rigid restrained eaters. Measures of actual EI were not made in this study due to the high prevalence of underreporting in overweight and obese women (Macdiarmid & Blundell, 1998) and in restrained eaters (Asbeck *et al*, 2002; Rennie *et al*, 2006), as such it is unlikely that any direct measure of EI would be accurate in this population.

6.4.4 Limitations

These findings must be considered in the context of certain limitations. The method used to assess physical activity relied on an element of self-reporting from participants; this can lead to inaccuracies in total EE estimates if reporting was not carried out to a high enough standard. This study also has uneven numbers of flexible and rigid restrained eaters; classification of participants takes place after recruitment and as a result it is difficult to actively recruit flexible and rigid restrained eaters to ensure even participant numbers in each group. As a result it was found that the minimum differences that could be detected in RMR and TEE were 1162 kJ/day and 3865 kJ/day respectively; differences of these magnitudes are highly unlikely to exist, particularly between two groups of sedentary women. Previous studies which have found significant differences between restrained and unrestrained eaters have reported much smaller differences (Tuschl *et al*, 1990; Platte *et al*, 1996). The possibility of a type 2 statistical error, or false negative result, occurring in these data is thus high and it is possible that this study has failed to detect an existing difference due to the insufficient sample size. Unfortunately it cannot reliably be concluded that TEE does not differ between flexible and rigid restrained eaters in the current work. It may be of interest to carry out this investigation in a larger sample size in future, and doubly labelled water measurements of EE would also increase accuracy of values and this may also increase the possibility of detecting difference.

Restrained eaters in this study were defined as those individuals whose mean restraint score exceeded the group median value, thus classification was based purely on statistics and distribution which may have led to misclassification of

participants with borderline and misleading findings. This approach was adopted as there are no clinically defined cut-off values for classification of restrained eaters; TFEQ scores are interpreted on an individual basis in clinical settings where classification of individuals as restrained or unrestrained is not as relevant. The use of a median value provides a normative value for classification specific to the population, and other research studies have adopted this classification approach (Tepper *et al*, 1996; Van Loan & Keim, 2000; Rideout & Barr, 2009). The importance of applying professional judgement to the interpretation of these scores is stressed in the accompanying manual due to the lack of established cut-off values (Stunkard & Messick, 1988). A more relevant method of classification in research settings is not apparent and this was considered the most valid approach in these circumstances; some studies apply an arbitrary cut-off value for this purpose (Lluch *et al*, 1998 & 2000), whereas the use of the group median value has the advantage of providing a measure relative to the specific population. A large scale standardisation study may provide useful for providing cut-off values for research purposes, but this approach is also likely to be based on statistical calculation.

This study concludes that energy requirements do not differ between overweight and obese female restrained and unrestrained eaters. Flexible and rigid restrained eaters do not seem to differ in energy requirements but due to insufficient sample size and statistical power it is not possible to reliably conclude that no difference exists between these subgroups in the current work. Findings from restrained and unrestrained eaters suggest that dietary restraint is not a behavioural adaptation reflecting diminished physiological energy needs in overweight and obese women; cognitive factors are likely to contribute greatly to the practice of restrained eating behaviour

CHAPTER 7: General Discussion

7.1 Introduction

As the prevalence of obesity and related chronic diseases rises worldwide, the identification and development of effective body mass reduction methods has become a priority for public health strategies. Treatment of obesity is a complex proposition; individual energy balance is regulated and influenced by a myriad of physiological, psychological and environmental factors and the alterations in this balance necessary to treat obesity require significant behavioural change. Environmental conditions in many developed countries have been termed “obesogenic”; energy dense foods are widely available and opportunities to live a sedentary lifestyle more widespread in such environments, which contributes to the increasing prevalence of obesity (figure 7.1; Lake & Townshend, 2006).

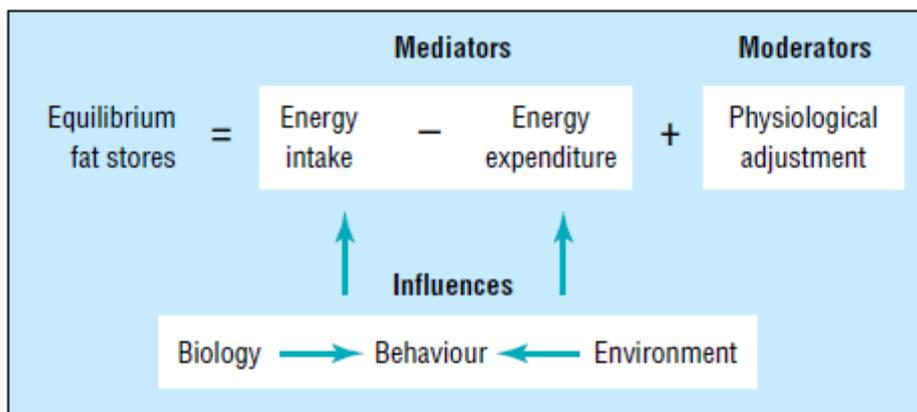


Figure 7.1 Factors affecting energy balance and adiposity (Source: Egger & Swinburn, 1997).

Research has indicated that exercise is one of the least effective available methods for reducing body mass. It is still not clear why this is the case, and although causes and mechanisms have been proposed the body of evidence is not firmly conclusive. Additionally, there are still relatively limited numbers of investigation conducted with the target overweight and obese adult population. This thesis sought to investigate potential compensatory responses to acute and

chronic exercise in overweight and obese women, as well as validating methodology commonly used in this field. It is also recognised that cognitive factors have a profound influence on lifestyle behaviours and therefore body mass. Investigation of the impact of one such cognitive factor, dietary restraint, on physical activity levels was also carried out in order to assess the potential association between cognition and behaviours which influence energy balance. As a result this thesis includes a variety of data on some of the cognitive and physiological factors which impact on body mass, as well as considering some of the methodological problems encountered in obesity research.

7.2 Conclusions

The main research questions this programme of work sought to answer in overweight and obese sedentary women were:

- Are energy intake values obtained from the laboratory based buffet meal method reliable at rest and post-exercise?

The buffet meal methodology was used to assess EI in the studies detailed in chapters 4 and 5. This method was implemented in the work of this thesis because it has been commonly used to assess EI in exercise intervention studies and self-reported EI data were deemed highly unreliable. This method also provided the necessary estimate of EI, not just eating patterns, which was required for these research studies. A high degree of variability in EI values obtained using this method in the studies of chapters 4 and 5 led to the development of the work in chapter 3; a methodological reliability study which utilised the same twenty-four hour design as the acute exercise study in chapter 4. Test-retest reliability of twenty-four hour EI values obtained under resting and post-exercise conditions was found to be poor; though intra-class correlation coefficients did reach significance under control conditions, values indicated weak reliability and the associated confidence intervals indicated there was a large degree of associated individual variability. This study concluded that the buffet meal method does not produce reliable twenty-four hour EI estimates in sedentary, overweight and obese women, under either control or exercise conditions. These findings imply that that this method may unreliable for use in

other populations, almost certainly the overweight and obese. The impact of this could be profound; much existing evidence regarding dietary compensation response to exercise may be based on flawed methodology. Further methodological work is required to establish test-retest reliability of this method.

- Do acute and chronic exercise-induced compensatory responses in energy balance occur?

- Does individual variability in exercise-induced body fat reduction have an association with changes in appetite, EI and/or EE?

- Does acute and chronic exercise participation affect circulating acylated ghrelin and peptide YY levels, and do these hormones play a role in compensatory changes in appetite, EI and EE?

The work detailed in this thesis cannot provide conclusive evidence to answer these research questions due to smaller than intended sample sizes and the resulting limited power to detect statistically significant changes (chapters 4 and 5). Chapter 4 attempted to assess the extent of compensatory responses in EI, appetite, and the appetite regulating hormones acylated ghrelin and peptide YY in response to a single walking based exercise session, sufficient to expend 1.7 MJ. The effect of 16 weeks of exercise on these same outcomes and total EE levels was examined (chapter 5). This study aimed to investigate the existence and variability of chronic compensatory adaptations in EI and EE, as well as the potential associated changes in acylated ghrelin and peptide YY, in overweight and obese women. No evidence of either acute or chronic compensatory changes was found, indicating that overweight and obese, sedentary women do not compensate for ExEE. Unfortunately these results are inconclusive due to limited statistical power and a low test-retest reliability of the methodology used to assess EI. Compensatory responses may thus have escaped detection. However, there may not have been any compensation in the study of chapter 4, given that the energy deficit was so small it was perhaps unlikely that EI would up-regulate in a twenty four hour period. Chapter 5 observed a modest impact of chronic exercise participation on body fatness, with actual losses below predicted values based on known ExEE. This indicated that some form of compensatory response

may indeed have occurred. Body composition changes were highly variable between individuals, but it could not be determined if similarly individual compensatory responses occurred with the existing number of participants. Body mass changes may have been insufficient to induce a change in acylated ghrelin; levels of which may only up-regulate in response to >3kg body mass loss (Foster-Schubert *et al*, 2005), well in excess of mean changes achieved in this study. However, although the body composition results suggest the presence of compensatory changes, this work cannot provide robust evidence from which to draw firm conclusions.

- Is dietary restraint associated with energy expenditure and physical activity levels?

The final study in this thesis investigated EE in restrained and unrestrained eaters; it has been suggested that restrained eaters have diminished energy requirements and existing evidence is mixed (Tuschl *et al*, 1990; Platte *et al*, 1996; Bathalon *et al*, 2001). No association between dietary restraint and RMR or EE was observed in a sample of forty-one women, and neither RMR nor EE differed between restrained and unrestrained overweight and obese women in this study. This study found no evidence to support the hypothesis that energy requirements differ between restrained and unrestrained eaters. Differences in eating behaviours cannot be linked to individual energy needs in either metabolic rate or activity EE.

- Do restrained eaters defined as having flexible or rigid control differ in their physiological energy requirements and physical activity patterns?

It was hypothesised that mixed results regarding differences in energy requirements between restrained and unrestrained may be attributable to the presence of two distinct sub-groups of restrained eaters; those with flexible and rigid control of restraint. This initial hypothesis was not supported as no evidence of a difference in energy requirements was found between restrained and unrestrained eaters, or between the two types of restrained eaters, in chapter 6. Unfortunately numbers of flexible and rigid restrained eaters were below that anticipated for this study. Post-hoc power calculations indicated that the smallest differences that could be detected were 1163 kJ/day and 3865

kJ/day in RMR and TEE respectively. These are greater differences than could realistically be expected hence limited statistical power prevents this evidence from being conclusive.

7.3 Clinical Implications

Some of the evidence detailed in this thesis may have clinical relevance to a weight management setting. Chapter 5 assessed the efficacy of exercise as a method of body mass reduction in overweight and obese, sedentary females and found it to be poor. Both eight and sixteen weeks of exercise failed to induce a clinically significant 5% mean reduction in the whole group, although some individuals did achieve larger changes (changes in body mass ranged from +2.0 to -8.9kg after 16 weeks). The high attrition rate (52%) and large degree of variability in compliance (58-118%) to the intervention indicates that, although in line with public health exercise recommendations for reducing body mass (Donnelly *et al*, 2009), many of the participants found this exercise intervention too demanding. This highlights the importance of considering the level of exercise that is achievable and sustainable on an individual basis. Higher volumes of exercise may induce greater reductions in body fatness, but sedentary individuals may struggle to complete such regimes and therefore achieve any change. It is important to consider the optimum exercise volume which will encourage compliance and confer health benefits in overweight and obese individuals in a clinical setting. Exercise induced body mass changes also vary independent of compliance, and changes in other factors such as dietary intake may also impact energy balance and hence achievement of body fat loss. Delivery of dietary counselling in combination with exercise regimes may strengthen clinical approaches. It would be beneficial if a means of identifying individuals most susceptible to compensation were developed; this would allow clinical interventions to be tailored and targeted to produce optimum changes on an individual basis.

Energy balance and body mass is, in large part, regulated by behavioural and psychological factors (figure 7.1). The characteristic of dietary restraint is one such factor, which describes the extent to which an individual consciously restricts EI. Eating patterns and body mass have been demonstrated to differ depending on restraint and disinhibition levels (Beisegel *et al*, 2004). Additionally, exercise seems to improve appetite control in those with a high restraint level (Lluch *et al*, 1998, 2000). It is important to understand the interactions between different behaviours in order to understand the potential impact on adiposity, particularly in a clinical obesity treatment setting. Questionnaires such as the TFEQ may serve as a tool to assess these factors and the information gathered may allow tailoring of intervention programmes to individual needs. Information gathered by the TFEQ may also be a predictive of intervention outcomes. Changes in restraint, disinhibition, and hunger during an intervention can also be indicative of the success and likelihood of sustaining behaviour changes necessary to reduce and maintain body mass over the long term. Decreases in disinhibition and increases in restraint levels may be promising signs for some individuals for example, they illustrate a change in eating behaviour that may aid attempts to achieve and sustain body mass reduction. The data presented in chapter 6 indicates that there is no physiological difference in energy metabolism or activity levels that would prevent a successful outcome of a clinical intervention with overweight and obese patients. This may be an indicator that restrained and unrestrained individuals would be equally receptive and responsive to an exercise intervention. Since there is no difference in activity level, differences in body mass between the two groups are likely to be largely attributable to dietary intake. These data indicate therefore that dietary counselling may be of the greatest importance for restrained individuals. Assessment of the main type of control exercised by restrained eaters could also be valuable in a clinical setting; flexible restrained eaters usually exhibit a lower BMI (Westenhofer *et al*, 1999), and exhibit better regulation of energy balance. This may allow further targeting of body mass management strategies; this subscale has also been shown to be associated with eating disorder symptoms (Shearin *et al*, 1994; Stewart *et al*, 2002) and dieting status (Timko & Perone, 2006). Since no evidence of differences in metabolic or activity energy requirements was found between these two subgroups, it seems that main differences between these

sub-groups are also attributable to differences in dietary intake, and exercise and dietary counselling may be beneficial for both types of restrained eaters. Since body mass reduction and maintenance is dependent on significant behavioural lifestyle changes in most cases, this type of information could be of use to aid the design of more effective and individualised interventions.

7.4 Recruitment and Attrition

Significant problems with participant recruitment and attrition were encountered whilst carrying out the research detailed in chapters 4 and 5 in this thesis. As a result these studies had smaller than intended sample sizes, and definitive conclusions could not be drawn due to limited statistical power. Participants for all studies were recruited via poster advertisements around the University of Glasgow campus and local hospitals, and advertisements on community websites. The studies of chapters 4 and 5 were advertised together as the acute assessment of post-exercise EI in chapter 4 formed the preliminary part of the chronic exercise intervention in chapter 5. The response to the advertisements for this study was high, with one hundred and twelve women expressing interest, but upon receiving further information about the study protocol fifty-six women declined to participate. Most reported that this decision was due to the considerable time commitment required by this study. Of the women who chose to participate, the attrition rate was high during the preliminary stages and the initial four weeks of the exercise intervention; overall attrition rate was 52.4%. Retention of participants in this intervention was challenging, and demanding for a single researcher to coordinate. It is possible that retention would have been improved had resources been available to offer more individualised support to participants during the intervention. As discussed in chapter 5, other measures such as provision of home based exercise equipment and use of physiological assessment methods such as doubly labelled water instead of demanding self-reporting measures may have lessened participant workload and improved retention. Material incentives can also act as a motivating factor; the methodological study of chapter 3 offered a £40 gift voucher upon successful completion of the study and had a 0% attrition rate,

despite requiring a considerable time commitment. A greater contributing factor to this success may have been the rapport between the researcher and participants; the majority of women in this study were previous participants in the other studies reported in this thesis. Attempts to offer material incentives in the chronic study were less successful; a further 2 months free gym membership was offered to participants upon completion in an attempt to reduce attrition, but this did not appear to be an effective or attractive incentive as it did not have the desired effect on attrition. This exercise intervention, though achievable and suitable for the population under investigation, required significant and sustained commitment and motivation, and external incentives appear not to have been an effective motivator because of this. Supervision of exercise sessions may have increased participant retention, and compliance but this measure was impractical due to limited availability of researcher time; the studies of chapters 3, 4, and 6 were run concurrently for a time and placed significant limitations on the researcher's time. Personalised feedback appeared to be a power motivator for some; informative measures such as the DEXA scans implemented in chapter 5 were of particular interest (to some of the women). Additionally, participants in the study of chapter 6 received detailed feedback information about their PA levels, metabolism, and body composition. Participant numbers were highest in this study, though this may be in large part because this study was shorter and less time consuming and than the other works in this thesis. Attrition was not as great a problem in this work, but sample sizes were still smaller than intended.

All studies were advertised in the same settings, around the university campuses and local hospitals, and on community websites. Many similar research projects are also advertised to these communities, and there may have been a problem of over-saturation of research studies within the targeted recruitment area, which was itself quite small. Advertising was expanded to other areas and hospitals within Glasgow after experiencing initial recruitment and attrition problems in an attempt to increase recruitment, but these initiatives were unsuccessful, possibly because they were aimed at people out with the area in which the University and experimental facilities were located. Recruitment strategies should be carefully considered and targeted at specific populations if possible. Factors such as participant workload, choice of methodology, appropriateness of

incentives, and availability of resources should all be taken into consideration in order to maximise participant recruitment and retention.

The research work in this thesis proved challenging and ambitious. These experiences provided a valuable education in the difficulties of conducting exercise intervention studies, and the lessons learned from the earlier studies led to improved recruitment strategies and minimal or no attrition in the later studies, detailed in chapters 3 and 6. The work of chapters 4 and 5 also proved an interesting feasibility exercise of complex intervention strategies designed to detect compensatory behaviours. Various strategies were implemented in an attempt to improve recruitment and retention, which gave greater understanding of this process and the factors which motivate research participants.

7.5 Detection of compensatory mechanisms

An ongoing challenge in interventions attempting to detect compensatory mechanisms is the poor sensitivity of most available methods of measuring EI; it is difficult to be certain that results are representative and conclusions accurate, particularly in the case of studies reporting no significant changes. True effects may not be detected. Although EE can be measured objectively with physiological methods such as doubly labelled water, the available methods for measuring EI are less objective, and usually rely on an element of self-reporting. Misreported data is common with these methods (Westerterp & Goris, 2002). Various observational methods have been utilised in some studies in an attempt to bypass the difficulties associated with self-reported EI data (Durrant *et al*, 1982; Donnelly *et al*, 2003a,b; George & Morgenstein, 2003; Visona & George 2003). These methods are not without problems; eating episodes usually occur in unfamiliar settings and observing food intake may adversely affect participants eating habits (Herman & Polivy, 2005) even if the researcher tries to conceal the real purpose of such observation. Some studies observe EI over periods of a week or more, but in the case of free-living participants there is no way to ensure that all food intake out with observation times is reported

accurately (Donnelly *et al*, 2003a,b). There is also evidence that compensatory changes are partial and gradual (Stubbs *et al*, 2004; Whybrow *et al*, 2008); and over shorter periods of time small changes may not be of a detectable magnitude.

These types of issues were encountered in the work of chapters 4 & 5; attempts to collect EI data from seven-day food diaries in the work of chapter 5 were aborted because 70% of participants reported EI less than 1.4 times measured metabolic rate, indicated a large degree of underreporting. The buffet meal method was heavily relied on in its stead, but later work revealed this method to have poor test-retest reliability in this population. As a result, it was not possible to conclude that compensatory responses did not occur. The most convincing evidence of exercise-induced compensation published thus far detected compensatory responses only when participants were grouped and analysed based on their achievement of predicted body mass changes (King *et al*, 2008, 2009). This approach is only applicable to long term exercise interventions, and sample sizes were too modest in the chronic intervention of chapter 5 undertake this type of analysis.

As detection of EI compensation is such a prominent issue, and the buffet meal method does not appear reliable for use with overweight and obese women, it is relevant to reflect on possible alternative methods of identifying compensatory responses in this population. No clear answer presents itself for acute exercise studies; if buffet meals do not supply accurate results then other observational methods, such as the use of vending machines, would likely be subject to the same problems. Free-living EI measures are largely inaccurate due to misreporting, and food frequency questionnaires do not provide a measure of EI. Use of multiple dietary assessments does not seem to be an effective means of increasing accuracy of EI values as it has been suggested that underreporting is an characteristic tendency for some individuals, regardless of the method employed (Black & Cole, 2003). In these individuals, any dietary assessment would likely produce inaccurate findings. It is hard to conceive of a method that would substantially improve reliability of EI values in this context, particularly if such changes are gradual in overweight and obese women as well as lean (Stubbs

et al, 2004). It may be more feasible to assess desire and implicit wanting for food as an alternative indicator of the effect of exercise on eating behaviour; this approach has provided interesting results with overweight and obese (Finlayson *et al*, 2009; 2011). Over the long term, changes in EE and body composition can be objectively measured using purely physiological methods, and if expected exercise induced body fat changes can be predicted these data can give indirect evidence of chronic EI compensatory responses. Individual analysis of participants based on achievement of predicted losses may also be more effective for elucidating compensatory changes. The use of specially designed equipment, such as Petra scales which record the type and weight of foods eaten (Bingham *et al*, 1987), could also be a way to improve the accuracy of self-reported EI data, although this would still be subject to inaccuracies if participants do not weigh all food eaten. It has also been reported the use of these scales is problematic due to poor portability, fragility of the equipment, making it prone to damage, and poor compliance of participants using the scales (Harbottle & Duggan, 1994). Given the problems associated with all methods of assessing EI, and the high prevalence of misreported EI data, it may be that the best approach is indeed to use physiological data as indirect evidence of EI compensation. It has been suggested that dietary assessment methods should be combined with physiological measurements such as double labelled water or 24 hour urinary nitrogen output in order to validate results (Bingham, 1991). A combination of objective methods and individual analysis seems likely to provide more reliable evidence of compensatory changes than any attempts to measure EI directly, and would strengthen the evidence in this field as at present many studies utilising self-reported EI data report non-significant results which have a large degree of uncertainty associated with them.

7.6 Overall Conclusions

No evidence of acute or chronic exercise-induced compensatory responses was found in overweight and obese, sedentary women. However, it is quite possible that compensation occurred in these women and was not detected therefore this

work cannot rule out the presence of such responses. It remains a challenge to obtain accurate EI values in research studies and hence determine if compensation occurs, particularly as the evidence in this thesis indicates that the observational buffet meal method does not produce reliable results in this population. Furthermore, eating and PA levels are influenced by other cognitive factors, and may impact on overall energy balance. Dietary restraint is one such factor, and although it seems to be associated with differences in eating behaviour and patterns, the evidence in this thesis indicates that there is no relationship with PA levels in overweight and obese women.

Energy balance regulation is complex and influenced by many cognitive, environmental, and physiological factors. As a result it is difficult to isolate the effect of one factor on this regulation, as other influences may confound results, and methods available for EI measurement are limited in the accuracy. The existence of exercise induced compensatory mechanisms, and the influence potential contributing factors, remains debatable due to limitations of the evidence, both in this thesis and the published literature.

7.7 Directions for Future Research

The work in this thesis has resulted in several recommendations for future directions for research. In summary these recommendation are as follows:

- The test-retest reliability of the buffet meal method should be explored further in this and other participant groups (e.g. sedentary, overweight males), over single and multiple meals, in order to ascertain if this method is at all suitable for use in exercise intervention studies. This may justify the need to develop a more reliable method of quantifying post-exercise EI.
- Exercise interventions, particularly long term studies, measuring peptide YY are relatively few in number. Similar to ghrelin, there are two distinct molecular forms of peptide YY with different properties; peptide YY₁₋₃₆ and peptide YY₃₋₃₆. It is only in the latter form that peptide YY is able to cross the

blood brain barrier and bind to hypothalamic receptors to induce satiety responses to food (McGowan & Bloom, 2004), hence future studies should aim to specifically measure peptide YY₃₋₃₆.

- Future acute exercise studies should extend observation periods in order to rule out the occurrence of delayed compensatory changes in appetite and energy balance. Compensatory changes are not apparent in the initial twenty-four hours post-exercise, but may take place in later stages, since compensatory responses may be gradual (Whybrow *et al*, 2008), and matching of EI to EE may be subject to a lag-time of 3-4 days (Bray *et al*, 2008).
- Many peptide hormones are secreted in the gut and contribute to appetite regulation, however very few have been investigated in this context in any detail. Future studies may wish to investigate other hormones as potential mechanisms, such glucagon-like peptide 1 and cholecystokinin, since there is some evidence that GLP-1 is suppressed by a single bout of walking exercise (Unick *et al*, 2010). Studies thus far have focused heavily on ghrelin, however this is just one of many appetite regulating factors.
- Exercise may acutely improve appetite control in restrained eaters (Lluch *et al*, 1998 & 2000), but no evidence exists regarding the effect of exercise on appetite and EI control in flexible and rigid restrained eaters. This may be a direction for future studies to explore and may provide further information about psychological influences on appetite.
- Studies utilising magnetic resonance imaging (MRI) technology have found that brain activation patterns in restrained and unrestrained eaters may differ in response to food stimuli, resulting in an increased desire for food in the latter (Coletta *et al*, 2009). Further investigation into neurological responses to food could provide valuable information regarding individual differences in appetite regulation, since it is quite possible that compensatory responses are neurologically regulated. Additionally, a similar investigation in flexible and rigid restrained eaters would be entirely novel.

- There is evidence that some individuals have a heightened food reward sensitivity, which may be linked to the dopaminergic signalling system (Franken & Muris, 2005). Mechanisms inducing compensatory responses are yet to be conclusively elucidated, but evidence has emerged that exercise may acutely increase hedonic response and implicit desire for food in individuals who subsequently emerged as compensators in response to long term exercise (Finlayson *et al*, 2009). Neurological mechanisms such as dopaminergic circuitry may be a possible contributing factor to differences in individual susceptibility to compensatory responses and this area merits some research attention.

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APPENDICES

Appendix I a,b,c Recruitment Posters



WOULD YOU LIKE TO BECOME MORE PHYSICALLY ACTIVE?

We are looking for female volunteers to participate in a research study investigating the effects of an exercise programme on changes in body weight.

If you are:

- a woman aged between 18 and 45 years
- heavier than you would like to be
- healthy but not a regular exerciser
- a non-smoker
- not currently dieting
- and would like to increase your level of physical activity, with a personalised 16-week programme in a supportive environment and free gym access!

Then you might like to take part in our study

If you think that you might be interested or would like more

information, without any obligation to participate, please contact:

Gemma Brown E-mail : gemindina@googlemail.com

Tel: 07923 508211



University of Glasgow | Section of Human Nutrition

Would you like to know about your fitness, metabolism and how exercise affects your appetite?

We are looking for overweight, healthy, non-smoking, pre-menopausal female volunteers who are not currently dieting to take part in a University of Glasgow study looking at the reproducibility of the effects of a single exercise session on appetite.

This will involve:

- Assessment of fitness, metabolic rate, and body composition.
- Attending the lab to complete 4 trials.
- Completing an exercise session under supervision in 2 of the trials.

You will be given a £40 Marks & Spencer voucher for your participation. We will also provide a health and fitness feedback for you.

If you are interested and would like more information please e-mail postgraduate researcher Gemma Brown at gemindina@googlemail.com or phone 07923 508211.



University of Glasgow | Section of Human Nutrition

Would you like to have your fitness and metabolism measured?

Would you like to know how many calories you actually need to eat?

Participate in our study and we will assess this, and more, for you!

If you are:

- A pre-menopausal woman
- Overweight
- Healthy and not on any medication (except contraceptive medication)
- Do not smoke
- Not currently dieting or having undergone a recent weight change

Then would you like to take part in a University of Glasgow study looking at differences in energy requirements and activity levels?

You will receive personalised feedback on your fitness and activity levels, current diet and daily energy requirements.

If you are interested and would like more information please e-mail postgraduate researcher Gemma Brown at gemindina@googlemail.com or phone 07923 508211.

Gemma Brown
gemindina@gmail.com

Appendix II a,b,c Volunteer Information Leaflets



VOLUNTEER INFORMATION SHEET

Project title: The effect of a 16 week exercise programme on body fat loss and energy balance in overweight women.

Lay title: **Effects of exercise on weight loss.**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

Exercise is an important means of maintaining a desirable body mass, but sometimes exercise alone is not effective for weight loss regimes and further study is needed to discover why this is. This study aims to assess the effect of a 16 week exercise programme on body fat loss and energy balance. This study will require you to exercise for 16 weeks in total, progressing to the intensity recommended by physical activity guidelines. There will also be a period of observation of your normal lifestyle before beginning exercise and some preliminary tests conducted so your involvement in this research study may last for up to 17 weeks in total.

Why have I been chosen?

You have been chosen because you are a healthy, pre-menopausal adult woman who is somewhat heavier than the ideal weight for your height. We will be aiming to study 35 subjects throughout the entire study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

1) In the first instance will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history, physical activity and diet to ensure that it is perfectly safe for you to participate in this study.
- measure your height, body mass and blood pressure
- provide an opportunity for you to ask questions

After this visit you will be assigned ready to begin the tests detailed below and start your exercise programme.

2) We will then ask you to undertake a number of tests. These will include:

- **Fitness tests** – These tests will be performed at the start and end of the programme and will involve you performing various activities such as sitting, standing and slow walking followed by quicker walking on a treadmill at different speeds. During this we will monitor your heart rate and collect the air you breathe out to determine your fitness level and calculate how many calories you burn during different activities. You should allow about 2 hours to complete these laboratory tests.
- **Assessment of body composition** – we will measure your weight and height and measure around your waist and your hips. We will also measure your body composition by a method called DEXA scanning. DEXA scanning measures the amount of body fat, muscle and bone in your body. This test requires you to attend Yorkhill hospital and you will simply need to lie on a couch for around 10mins while the machine scans you. This test will be done twice – at the start and the end of the exercise programme and will allow us to accurately monitor changes in your body fat proportion.
- **Measurement of Resting Metabolic Rate** – This test will be performed before the start of the exercise programme and once more at the end during the 24hr metabolic trials that are mentioned in more detail further down this list. This test will involve you coming into the lab after an overnight fast and lying comfortably on a couch for about 25 minutes with a clear canopy (like a large spaceman's helmet) over your head. Most people find this quite relaxing. The air that you breathe out will be monitored and from this the number of calories and the amount of fat and carbohydrate that your body is burning will be assessed.
- **Monitoring physical activity and diet** – we will ask you to weigh and record everything that you eat and drink for a week in a diary. We will provide you with scales and instruct you how to use them accurately. We will also ask you to record all your activities in a separate diary and wear a heart rate monitor for a week (this may be the same week or a separate week as the food diary depending what is easiest for you) as we use this information to calculate how many calories you burn during normal daily living. We will ask you to complete these tasks twice – during the week before beginning exercise and during the last week of the exercise programme.
- **24 hour metabolic trial** – we will measure your appetite over a 24hr period (including overnight sleep at home) and take blood samples to measure biomarkers of appetite. We will require you to take part in these trials either 2 or 3 times (some volunteers may have difficulty giving blood in which case we will conduct only 2 trials and forgo taking blood samples). Two of these trials are conducted before beginning the exercise programme (this will be reduced to only 1 if we cannot obtain blood samples) and the last one will be after 8 weeks of exercise have

been completed. Each time we will ask you to attend the lab over 2 consecutive half days – on the first day we will ask you to attend in the afternoon and on the second we will ask you to attend in the morning

- **Day 1** – You will be asked to record the type and amounts of food you consume for two days prior to each trial. We will require you to replicate what you eat for these two days before every trial. On the day of the trial you will consume breakfast and lunch at home or work as normal. You will then come to the laboratory at ~2.00pm and will stay with us for 4hrs. We will collect expired air samples, blood samples (7.5ml at a time) taken via a cannula (tiny sterile plastic tube) in your arm which will be inserted by a qualified and experienced person and also assess your appetite using a short questionnaire at regular intervals during this period. During the second and third of these trials we will also ask you to complete an exercise session under our supervision. We will provide you with a buffet style dinner composed of a variety of foods at the end of this day and ask you to consume the food according to your appetite. You will then return home and we will request you do not eat again until you return to the laboratory the next day.
- **Day 2** – You will come back to the laboratory at ~9.00am and stay with us till just after lunchtime (approx. 2pm). You will again have a cannula inserted into a vein in your arm as before which we will use to take more blood samples. During the day we will provide you with a buffet style breakfast and lunch. These buffets will again be composed of a variety of foods and we will request you eat according to your appetite. Samples of expired air and blood will be taken before and after each meal and every 60mins between meals. Following each blood sample we will ask you to fill in a short appetite questionnaire.

3) You will then undertake the exercise programme, which will last for 16 weeks. The intensity of the exercise sessions will be individually tailored and will increase progressively throughout the programme. You will start by completing 2 exercise sessions in week 1 and will build up the number of sessions by 1 per week until you reach the target of 4 sessions per week. Exercise sessions will be of a duration calculated to expend 500 calories, usually around 50-90mins per session. You may spread the prescribed duration of exercise over 2 sessions if you wish as long as they are completed on the same day. We will give you a choice of exercising on a bike, on a treadmill (running or walking uphill according to preference) or on a rowing machine, or a combination of these if you wish.

You will be given a free pass allowing you access to the University of Glasgow gym in the Stevenson building for the duration of the study. We will show you around the gym and ensure you are comfortable with the use of the equipment before you begin. You will then complete your first exercise session under our supervision if you wish. We will require you to wear a heart rate monitor during all exercise sessions to monitor your progress, and these will be collected by the researcher weekly which also enables you to discuss any difficulties you may be having. If you successfully complete all 16 weeks of exercise we will obtain another month's free entry to the gym for you to continue exercising if you wish to do so.

What else do I have to do?

Other than the specific tasks described above, we ask you to maintain your usual lifestyle, with the exception of avoiding alcohol for 2 days before each appetite trial when you attend the lab for 24hr appetite measurements and blood sampling.

What are the possible disadvantages and risks of taking part?

- Exercise testing will not be at a maximal level but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.
- Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- The DEXA scan we use to assess your body composition uses a very small dose of radiation. The radiation level involved in each scan is equivalent to about a twentieth of a chest X-ray or the amount of natural background radiation we are exposed to in 4 hours.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high blood pressure. If such a problem is revealed we will inform you.

What are the possible benefits of taking part?

There may be no benefits to you but as a result of taking part in this study you will receive information about your level of health and fitness and the opportunity to participate in a controlled, supervised exercise programme. The findings of this study will be published in scientific journals so that understanding about how exercise can help people to maintain a healthy body weight can be increased. This information may help make up better exercise guidelines.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the University will have your name and address removed so that you cannot be recognised from it.

What will happen to my samples after the study has finished?

The blood samples that you provide for this study may be useful for future research into the prevention and treatment of excess body weight gain and related conditions such as diabetes and heart disease; this may involve analysis of certain genes associated with these diseases. Any use of your samples for future research will require further approval from a Research Ethics Committee and samples will be analysed in such a way that the results will not be directly traceable to you. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has funded the research?

This research is funded by the University of Glasgow.

Who has reviewed the study?

This study has been reviewed and approved by the Faculty of Medicine Ethics committee at the University of Glasgow.

Contact for Further Information

Thank you for choosing to take part in our study. Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting any of the following:

Gemma Brown, tel: 07923 508211 e-mail: gemindina@googlemail.com

You will be given a copy of this information sheet and a signed consent form to keep for your records.



VOLUNTEER INFORMATION SHEET

(Version 1, 2 August 2007)

Project Title: Reproducibility of appetite response to a single exercise session

(Lay title: Reproducibility of appetite responses)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

The effects of a single session of exercise are of interest because of implications for weight control. Many studies have looked at these responses but we are aiming to expand on previous research in order to find out if subjective feelings of appetite consistently respond in the same way to a single exercise session. This will give us greater understanding of the way exercise affects how hungry we feel and could have implications on our understanding of long term weight control.

Why have I been chosen?

You have been chosen because you are a healthy adult woman aged between 18-45 years who is somewhat heavier than the ideal weight for your height.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

1) In the first instance will be asked to meet us for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, diet and physical activity patterns to ensure that it is perfectly safe for you to participate in this study
- measure your blood pressure, height and weight to enable us to determine whether you fall into the group of people we wish to study

- give you digital scales and diet diaries and instructions on how to use them
- provide an opportunity for you to ask questions

2) We will then ask you to undertake a number of preliminary tests consisting of fitness test, body composition measurements and dietary assessment. These will include:

- **Fitness Test.** This test will be performed on a treadmill. The test will be of ~25 minutes duration and will consist of 3-5 stages of increasing intensity. During the test your heart rate will be measured using a monitor attached to your chest and we will collect samples of the air that you breathe out via a mouthpiece. This will enable us to determine your fitness level and calculate the intensity for your later exercise session. The test will not involve a maximal effort.
- **Measurement of Resting Metabolic Rate** – This test will be performed periodically end during the 24hr metabolic trials that are mentioned in more detail further down this list. This test will involve you coming into the lab after an overnight fast and lying comfortably on a couch for about 25 minutes with a clear canopy (like a large spaceman's helmet) over your head. Most people find this quite relaxing. The air that you breathe out will be monitored and from this the number of calories and the amount of fat and carbohydrate that your body is burning will be assessed.
- **Body composition measurements.** This will involve measurements of height and body mass and body fatness by using body composition scales. These scales send a very low, safe electrical current through the body, which meets resistance from fat tissue but passes freely through lean tissue and thus assess body composition from the level of resistance met. This is completely painless. We will also measure round your waist and hips.
- **Food diary.** You will be given a food diary with written instructions and will be asked to keep records of all foods and drinks consumed for two days prior to your first trial. Two days before your next visit you will be asked to repeat this diet.

3) After the preliminary tests you will be ready to participate in the main experimental trials, of which there are two types - one exercise and another control. As this study is looking at reproducibility each type of trial will be done twice, meaning that **in total you will complete 4 trials with us**. Each of the trials will take place **over two days** and last for approximately 24 hours (including overnight sleep at home).

Day 1 – You will be asked to consume breakfast and lunch at home. On the afternoon of Day 1 you will be asked to come to laboratory at approx. 2pm and either complete an exercise session at a moderate intensity for ~60 minutes (exercise trial) or rest quietly for the same duration (control trial). At the start and end of exercise and at the equivalent times during the control trial, samples of expired air will be taken. You will also be asked to

fill a short appetite questionnaire. Then you will be provided with buffet style dinner which will be composed of a variety of foods. We will ask you to consume food from this buffet according to your appetite. You will then have your overnight rest at home.

Day 2 – You will arrive in laboratory at ~9am fasted and stay with us until ~2pm. During the day you will be provided with buffet style breakfast and lunch. The buffets will be composed of a variety of food that you can consume to appetite. Personal preferences will be taken into consideration. Samples of expired air will be taken before and after each meal. Following each blood sample you will be asked to fill in a short appetite questionnaire.

What else do I have to do?

Other than the specific tasks described above, we ask you to maintain your usual lifestyle but for two days prior to each trial refrain from alcohol and exercise and keep dietary diaries which you will be asked to replicate before the next trial.

What are the possible disadvantages and risks of taking part?

- Fitness testing will not be at a maximal level but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heart beat.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high blood pressure. If such a problem is revealed, we will inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?

There may be no benefits to you but as a result of taking part in this study you will receive information about your level of health and fitness as well as your metabolic rate and body composition, in addition we can provide advice on aspects of diet and exercise levels and how to improve them. The findings of this study will be published in scientific journals so that understanding about how exercise can affect appetite. This information may help make up better exercise guidelines.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you. In addition you will receive a £40 M&S voucher at the end of the study as a reward for your participation.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the University will have your name and address removed so that you cannot be recognised from it.

Who has reviewed the study?

This study has been reviewed and approved by the Faculty of Biomedical and Life Sciences Ethics committee at the University of Glasgow.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Gemma Brown at gemindina@gmail.com or 07923 508211.

You will be given a copy of this information sheet and a signed consent form to keep for your records.



VOLUNTEER INFORMATION SHEET

(Version 1, September 2009)

Project title: Resting EE and physical activity levels of premenopausal women classed as restrained eaters with flexible or rigid control.

Lay title: Resting EE and physical activity level of premenopausal women with different eating habits.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this study is to investigate whether there are differences in resting EE and physical activity levels according to a person's eating habits.

Why have I been chosen?

You have been chosen because you are a healthy pre-menopausal adult woman who is somewhat heavier than the ideal weight for your height. We will be aiming to study 54 volunteers throughout the entire study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

1) In the first instance you will be asked to attend for a screening visit in which we will:

- Discuss with you and complete confidential questionnaires regarding your health, family history, physical activity, and diet and eating habits to ensure that it is perfectly safe for you to participate in this study.
- measure your height, body mass and blood pressure
- provide an opportunity for you to ask questions

After this visit you will be assigned ready to begin the tests detailed

- **Fitness test** – This will involve you performing various activities such as sitting, standing and walking followed by either cycling on an exercise bike or walking quickly on a treadmill at different speeds.

During this we will monitor your heart rate and collect the air you breathe out to determine oxygen consumed and carbon dioxide produced. This will allow us to calculate how many calories you burn during different activities and evaluate your cardiorespiratory fitness. You should allow about 2 hours to complete these tests.

- **Assessment of body composition** – we will measure your weight and height and measure around your waist and your hips. We will also measure your body composition by a method called air displacement, this will require you to sit still in a small chamber for 5-10 minutes (preferably in swimming costume or similar but this is not essential) which the machine measures your body volume by measuring the air displacement around you.
- **Measurement of Resting Metabolic Rate** – This test will be performed on two separate occasions. The test will involve you coming into the lab in the morning after an overnight fast and lying comfortably on a couch for about 25 minutes with a clear canopy (like a large spaceman's helmet) over your head. Most people find this quite relaxing. The air that you breathe out will be monitored and from this the number of calories and the amount of fat and carbohydrate that your body is burning will be assessed.
- **Monitoring physical activity**—we will ask you to record all your activities for seven consecutive days in a diary and also wear a heart rate monitor during these days (this consists of a watch and a band worn around your ribcage). We use this information to calculate how many calories you burn during normal daily living.

3) Therefore in total we require you to make 2 visits (this can be divided into 3 shorter visits if required); at least one visit - but ideally both - should be conducted first thing in the morning after an overnight fast:

- **Visit 1** – screening and completion of questionnaires, metabolic rate measurement and fitness test (**approx. 2.5 – 3 hours duration**)
- **Visit 2** – 2nd metabolic rate measurement, body composition measurement and return of activity diary and heart rate monitor (**approx. 30 mins to 1 hour duration**).

What are the possible disadvantages and risks of taking part?

- Exercise testing will not be at a maximal level but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include increased blood pressure, fainting or a change in the normal rhythm of the heartbeat.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high blood pressure. If such a problem is revealed we will inform your GP so this can be investigated further.
- This study will require you to give up some of your time and may incur travel expenses travelling to campus. However, visits to the university are few and of generally short duration. We can also combine tests to reduce the number of visits you need to make in addition to conducting tests out of hours where required to suit your availability.
- Wearing the heart rate monitor for 7 consecutive days may cause you some mild discomfort as this involves the wearing of a band around your ribcage that must be reasonably tight to pick up your heart rate accurately.

What are the possible benefits of taking part?

We will provide personalised feedback on your diet, physical activity and fitness level and advise how to improve your diet and achieve desirable level of physical activity and fitness.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the University will have your name and address removed so that you cannot be recognised from it. However in the event that a possible pre-existing health problem is picked up we will contact your GP about this in case further investigation is required by your healthcare providers. However, only your registered GP will be made aware of any potential problems and all other information will be confidential.

Who has funded the research?

This research is funded by the University of Glasgow.

Who has reviewed the study?

This study has been reviewed and approved by the Faculty of Medicine Ethics committee at the University of Glasgow.

Contact for Further Information

Thank you for choosing to take part in our study. Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting any of the following:

Gemma Brown, tel: 07923 508211 e-mail: gemindina@googlemail.com

Thank you for reading this.

Appendix III a,b,c Consent forms



CONSENT FORM

Title of Project: The effect of a 16 week exercise programme on body fat loss and energy balance in overweight women

Name of Researcher: Gemma Brown

Please initial box

- | | | |
|--|---|--|
| 1. I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions. | <input type="checkbox"/> | |
| 2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. | <input type="checkbox"/> | |
| 3. I agree to take part in the above study. | <input type="checkbox"/> | |
| 4. I agree for my samples to be used for future research into the prevention and treatment of excess body weight gain and related conditions, such as diabetes and heart disease. This may involve analysis of genes associated with these diseases. | Yes <input type="checkbox"/>
No <input type="checkbox"/> | |

Name of Volunteer

Date

Signature

Researcher

Date

Signature

1 for volunteer; 1 for researcher



CONSENT FORM

Title of Project: Reproducibility of appetite response to a single exercise session

Name of Researcher: Gemma Brown

Please initial box

1. I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3. I agree to take part in the above study.
4. I agree that my registered GP can be contacted in case of discovery of a possible pre-existing health problem in the course of my participation in this study.

Name of Volunteer

Date

Signature

Researcher

Date

Signature

1 for volunteer; 1 for researcher



CONSENT FORM

Title of Project: Resting EE and physical activity levels of premenopausal women classed as restrained eaters with flexible or rigid control.

Name of Researcher: Gemma Brown

Please initial box

- | | |
|---|--------------------------|
| 1. I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions. | <input type="checkbox"/> |
| 4. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. | <input type="checkbox"/> |
| 5. I agree to take part in the above study. | <input type="checkbox"/> |
| 5. I agree that my registered GP can be contacted in case of discovery of a possible pre-existing health problem in the course of my participation in this study. | <input type="checkbox"/> |

Name of Volunteer

Date

Signature

Researcher

Date

Signature

1 for volunteer; 1 for researcher

Appendix IV Health Screen Questionnaire

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name:

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

- | | | |
|---|----------------------------------|---------------------------------|
| (a) on medication, prescribed or otherwise | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (b) attending your general practitioner | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (c) on a hospital waiting list | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |

2. In the past two years, have you had any illness which required you to:

- | | | |
|---|----------------------------------|---------------------------------|
| (a) consult your GP | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (b) attend a hospital outpatient department | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (c) be admitted to hospital | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |

3. Have you ever had any of the following:

- | | | |
|--|----------------------------------|---------------------------------|
| (a) Convulsions/epilepsy | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (b) Asthma | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (c) Eczema | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (d) Diabetes | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (e) A blood disorder | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (f) Head injury | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (g) Digestive problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (h) Hearing problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (i) Problems with bones or joints | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (j) Disturbance of balance/co-ordination | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (k) Numbness in hands or feet | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (l) Disturbance of vision | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (m) Thyroid problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (n) Kidney or liver problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (o) Chest pain or heart problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
| (p) Any other health problems | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |

4.

- | | | |
|--|----------------------------------|---------------------------------|
| (a) Are you pregnant or think that you might be pregnant | yes [<input type="checkbox"/>] | no [<input type="checkbox"/>] |
|--|----------------------------------|---------------------------------|

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002) SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**
_____ **minutes per day**

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**
_____ **minutes per day**

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking → ***Skip to question 7***

How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

*Appendix VI Personal Information and Food Preference
Questionnaire*

PERSONAL INFORMATION

Name:

Date of Birth:

Contact Address:

Home Telephone No:

Mobile Telephone No:

Personal E-mail:

Work/Uni E-mail:

What days are you available for appetite trials? (2 consecutive days)

What do you usually eat for:

Breakfast:

Lunch:

Dinner:

Which of your meals is the biggest?

Do you have any particular food preferences, allergies or dislikes?

Appendix VII Physical Activity Diary and Example Energy Expenditure Calculations

Physical activity Diary

Thank you very much for agreeing to take part!

The first few pages include instructions as to how best to fill in the diary, an example diary page, and heart rate monitor instructions.

Please read all these pages before you begin as the points below are quite important and will help you to complete the diary and provide us with the best possible data.

Thanks, Gemma

INSTRUCTIONS:

- Please remember to write the date at the top of each page in the space given.
- Try to be punctual with the time you start and finish an activity.
- Do not forget to write down the time you go to sleep, the time you wake up and the time you wear your heart rate monitor.
- Please try and record 5 week days and 2 weekend days that are typical of your normal lifestyle, they do not have to be consecutive days.
- Your data then will be collected and matched with your heart rate.
- Please be as detailed as possible as any gaps in the information mean the results we get from this will be inaccurate.
- We appreciate that you may wish to take the heart rate monitor off in the evening if you find the belt a little uncomfortable, this is fine if you have recorded most of that days heart rate data, however it is very important that if you do take it off early you continue recording activities in the diary until you go to bed.
- This is because any gaps in the diary will probably result in hugely inaccurate final energy expenditure data, which also means your personal feedback, will be inaccurate; and may also mean that your data is unsuitable for inclusion in our final analysis.

Guidelines for using POLAR Heart Rate Monitors

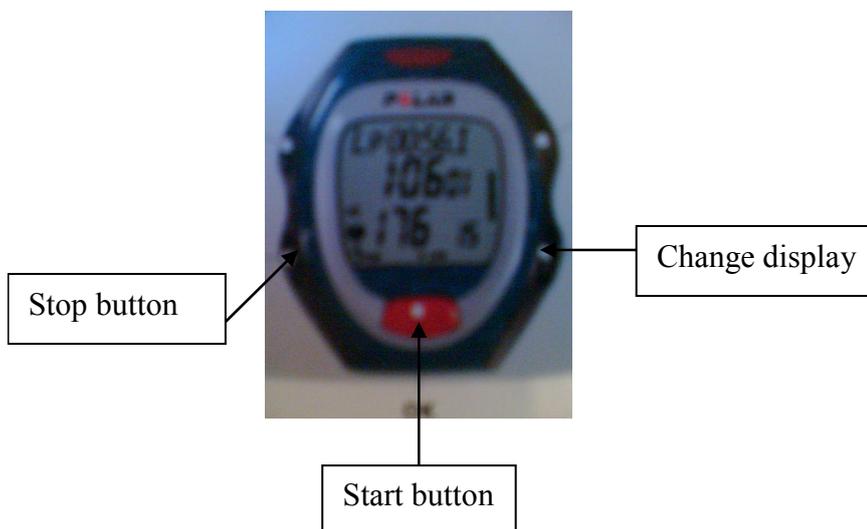
The Polar Heart rate monitors you will use for monitoring your exercise sessions and your continuous heart rate in your monitoring week are very easy to use. They have lots of different functions and capabilities, but we are only interested in monitoring your heart rate over one week period.

For use of the POLAR system during your week of monitoring:

1. Put the belt around your ribcage making sure the transmitter is at the front and the belt is reasonable snug.
2. **To begin recording, press the large red button in the middle of the watch twice. If you do not press it twice the heart rate will not be recorded to memory and no data will be available for later download.**
3. The stop watch (or calorie counter) will then start running (this indicates the watch is recording to memory) and the monitor will start to assess your heart rate, at first the display will look like this [] and then after a few moments the little heart symbol should start flashing and the watch should pick up the heart rate.
4. If there is no heart rate reading try moistening the transmitter on the belt. You may need to stop and start the watch again if it does not pick up but please try not to restart the watch many times, sometimes it will pick up the heart rate on its own after a few minutes.
5. At the end of the day, press the stop button twice till you return to the normal screen displaying the time, and take off the watch and monitor.

Important: Once the watch is displaying the heart rate and recording to memory (stopwatch or calorie counter is running) please do not press stop again until you are ready to stop monitoring and remove the watch and belt. Pressing stop even once before you are finished using the monitor will mean no data is recorded to the watches memory.

N.B: Some electrical devices interfere with your heart rate pick up by the watch- e.g. laptop computers. You may notice that if you are very close to some electrical equipment that your heart rate appears to be very high. Do not worry about this. Provided the heart rate drops when you move away from the device, the monitoring will not be affected.



You should not need to press any of the other buttons. However if you accidentally press other buttons and enter the menu simply press the stop button until the watch returns to the main screen with displaying the time, and then restart again by pressing the red button twice.

- **If any of the buttons on the right hand side get pushed accidentally the display may change to lap time or stopwatch, but in this instance it is still recording and you don't need to worry.**
- **However if you push one of the right hand buttons twice you will get back to the normal heart rate display with calorie counter and stopwatch.**
- **(When the monitor is recording there will always be either a stopwatch or calorie counter, if the display is changed there may not be both but as long as there is one of these things showing it is still recording to memory.)**

Any problems with the monitor you can email me or give me a phone or text on 07923 508211 or 07800 816908.

EXAMPLE CALCULATION OF TOTAL ENERGY EXPENDITURE

Step 1: Calculate average heart rates at ten minute intervals. Match these mean heart rates with activity information recorded by subject in diary. Classify mean heart rates on the right hand side as active or inactive based on the activity being performed at the time, as reported in the diary.

Step 2: After all heart rate data has been classified, calculate mean active and inactive heart rates for the day (in practice the average heart rates from each day are used to work out the mean values calculated from all seven monitoring days, however in this worked example only data from one day is shown).

Time	+0:00	+1:00	+2:00	+3:00	+4:00	+5:00	+6:00	+7:00	+8:00	+9:00	ACTIVITY	Active	Inactive	Mean HR <u>Step 1</u>
12-8.10am	sleeping													
8.10am	54	54	58	56	58	47	49	45	45	67	sitting		/	53.3
20	71	70	66	60	54	57	69	74	64	69	"		/	65.4
30	71	70	77	78	73	69	70	76	70	66	standing	/		72
40	69	68	60	61	59	66	66	67	71	66	"	/		65.3
50	63	60	61	64	63	60	63	65	78	82	"	/		65.9
9am	81	84	85	89	92	90	88	75	88	91	self care	/		86.3
10	90	84	82	89	78	71	76	78	79	82	"	/		80.9
20	85	79	71	75	77	86	78	80	88	93	"	/		81.2
30	87	89	86	96	91	86	90	93	102	105	walking	/		92.5
40	107	104	102	107	105	102	105	91	95	104	"	/		102.2
50	101	101	110	98	99	99	106	109	107	98	"	/		102.8
10am	115	119	126	103	85	82	87	86	79	83	"	/		96.5
10	89	76	76	68	69	79	64	65	87	64	sitting		/	73.7
20	65	66	67	67	65	65	64	65	64	65	"		/	65.3
30	66	61	60	62	61	64	60	58	61	58	"		/	61.1

40	65	60	67	64	61	64	64	72	68	65	"	/	65
50	63	66	68	64	69	58	59	60	73	66	"	/	64.6
11am	62	63	61	62	59	63	67	61	67	67	"	/	63.2
10	61	61	61	73	81	77	75	76	74	68	"	/	70.7
20	67	72	73	79	84	62	68	67	69	66	"	/	70.7
30	57	53	53	57	66	56	57	55	53	53	"	/	56
40	54	53	54	54	55	53	53	52	54	55	"	/	53.7
50	54	52	52	53	54	55	51	52	53	51	"	/	52.7
12pm	57	55	52	51	53	51	52	52	55	50	"	/	52.8
10	50	50	51	53	54	54	57	51	50	49	"	/	51.9
20	51	53	50	52	51	51	51	52	53	52	"	/	51.6
30	52	53	50	50	54	51	50	54	51	53	"	/	51.8
40	57	56	57	54	56	52	63	69	63	77	walking	/	60.4
50	79	56	51	51	55	56	73	62	61	63	sitting	/	60.7
1pm	65	58	56	59	60	61	60	58	61	62	"	/	60
10	62	66	64	67	70	63	63	59	63	66	"	/	64.3
20	63	66	64	65	61	64	67	63	75	77	"	/	66.5
30	69	68	64	61	58	59	68	64	68	65	"	/	64.4
40	64	66	63	63	64	65	67	73	80	82	walking	/	68.7
50	85	84	82	84	90	94	72	71	71	72	sitting	/	80.5
2pm	69	78	68	62	63	63	64	60	60	62	"	/	64.9
10	59	60	59	61	58	62	60	58	59	59	"	/	59.5
20	61	60	58	60	60	59	60	65	62	61	"	/	60.6
30	59	60	60	60	59	59	60	60	63	58	"	/	59.8
40	59	58	62	58	59	60	59	60	59	65	"	/	59.9
50	64	62	59	63	62	66	60	61	62	64	"	/	62.3
3pm	65	63	64	63	62	62	63	62	73	65	"	/	64.2
10	64	69	69	67	60	62	61	60	63	60	"	/	63.5

20	61	60	60	65	57	61	59	59	59	59	"	/	60
30	60	59	57	58	58	60	57	59	61	57	"	/	58.6
40	58	58	56	57	56	57	63	59	58	58	"	/	58
50	57	59	60	62	60	62	60	59	61	60	"	/	60
4pm	61	63	60	60	62	62	59	63	56	57	"	/	60.3
10	58	58	56	59	59	60	56	57	58	57	"	/	57.8
20	57	66	74	83	88	71	83	64	72	75	"	/	73.3
30	72	75	90	82	69	67	66	62	63	69	"	/	71.5
40	64	75	82	84	86	97	95	86	75	74	walking	/	81.8
50	71	72	68	66	70	68	68	68	66	64	sitting	/	68.1
5pm	66	66	70	65	68	65	66	65	65	60	"	/	65.6
10	58	57	60	59	61	63	65	57	58	64	walking	/	60.2
20	65	61	62	62	60	58	60	60	63	58	"	/	60.9
30	58	58	58	60	58	58	61	60	63	59	"	/	59.3
40	61	59	60	61	60	78	85	74	80	77	"	/	69.5
50	79	79	80	81	83	85	90	84	86	84	"	/	83.1
6pm	80	85	85	90	88	85	85	88	88	92	"	/	86.6
10	99	94	80	80	77	79	80	82	85	86	"	/	84.2
20	107	101	89	96	98	94	91	105	130	94	walking	/	100.5
30	74	79	84	79	73	71	78	80	78	74	standing	/	77
40	78	78	75	72	69	74	75	76	71	70	"	/	73.8
50	62	66	73	70	74	81	68	58	62	62	"	/	67.6
7pm	60	68	72	69	61	60	62	60	59	60	sitting	/	63.1
10	63	60	59	67	61	61	59	58	60	70	"	/	61.8
20	80	71	62	58	59	62	61	59	58	57	"	/	62.7
30	65	77	75	79	69	64	64	64	68	66	"	/	69.1
40	67	62	63	58	71	77	71	68	77	60	"	/	67.4
50	62	60	56	57	61	58	59	57	57	61	"	/	58.8

8pm	60	56	55	60	56	55	56	57	57	57	"	/	56.9
10	62	58	58	59	59	60	58	57	59	59	"	/	58.9
20	58	59	57	58	58	60	59	60	61	61	"	/	59.1
30	58	55	55	56	56	56	57	56	54	55	"	/	55.8
40	55	58	56	58	69	58	59	57	56	54	"	/	58
50	61	62	56	58	57	57	57	55	58	55	"	/	57.6
9pm	55	52	59	56	56	53	61	58	55	58	"	/	56.3
10	56	53	53	58	54	56	54	55	53	59	"	/	55.1
20	60	50	49	49	48	53	80	72	65	71	"	/	59.7
30	68	65	53	53	56	56	56	54	56	52	"	/	56.9
40	55	51	50	51	52	50	54	53	54	52	"	/	52.2
50	55	52	52	52	53	53	54	55	53	54	"	/	53.3
10pm	56	55	55	55	53	54	62	57	55	54	"	/	55.6
10	55	55	55	58	53	57	60	53	54	52	"	/	55.2
20	53	52	55	52	52	51	52	53	51	55	"	/	52.6
30	51	54	54	54	53	53	54	50	51	51	"	/	52.5
40	48	49	49	49	52	50	52	49	50	57	"	/	50.5
50	53	53	54	52	50	48	48	49	50	49	"	/	50.6
11pm	48	50	49	54	50	51	51	52	53	53	"	/	51.1
10	50	50	50	51	52	50	56	51	50	49	"	/	50.9
20	50	51	50	52	49	49	49	49	49	51	"	/	49.9
30	49	49	59	68	57	66	67	75	68		self care	/	62

Mean Active	77.6
Mean Inactive	60.0

Step 2

Step 3: Calculate total duration of sleeping, and active and inactive activities as recorded in the diary.

Step 4: Use the coefficients a and b (derived from the individual laboratory calibration measurements of the relationship between HR and EE of active and inactive activities) to calculate active and inactive oxygen consumption and carbon dioxide production from the relevant mean heart rate values calculated in step 2.

14/10/2009						
<u>Step 4</u>	Inactive Calibration			<u>Step 3</u>		
		a	b	Minutes asleep	500	BMR (kJ/day) 6113
	$\dot{V}O_2$	0.1973	-3E-08	Minutes awake		SEE (kJ/day) 2016.0
	$\dot{V}CO_2$	0.1615	-3E-08	Inactive Minutes	690	
				Active Minutes	250	
	Active Calibration					
		a	b			
	$\dot{V}O_2$	0.6648	0.0177			
	$\dot{V}CO_2$	0.6396	0.0167			
	Fat Oxidation (g/min)	CHO oxidation (g/min)	EE (KJ*min)	EE(KJ/day)	TEE(kJ/day)	
Inactive	0.062807018	0.095997345	3.947032268	2723.452265		
Active	0.106466165	0.857302857	17.52610502	4381.526256	<u>9121.0</u>	

Step 5: Use these calculated active and inactive mean oxygen consumption and carbon dioxide production rates (step 4) to calculate rates of fat and carbohydrate oxidation, and consequently rate of energy expenditure, whilst active and at rest.

14/10/2009																					
Inactive Calibration																					
	<table border="1"> <thead> <tr> <th>a</th> <th>b</th> <th></th> </tr> </thead> <tbody> <tr> <td>$\dot{V}O_2$</td> <td>0.1973</td> <td>-3E-08 0.197</td> </tr> <tr> <td>$\dot{V}CO_2$</td> <td>0.1615</td> <td>-3E-08 0.161</td> </tr> </tbody> </table>	a	b		$\dot{V}O_2$	0.1973	-3E-08 0.197	$\dot{V}CO_2$	0.1615	-3E-08 0.161											
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Step 6: Multiply these rates of EE by total reported durations of active and inactive activities for that day, to obtain total active and inactive EE.

14/10/2009					
Inactive Calibration			Minutes asleep	500	BMR (kJ/day) 6113
	a	b	Minutes awake		SEE (kJ/day) 2016.0
$\dot{V}O_2$	0.1973	-3E-08	Inactive Minutes	690	
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Step 6

Step 7: Calculate sleeping EE as 95% of BMR multiplied by reported duration of sleep for that day.

Step 8: Finally, sum the components of energy expenditure to obtain total expenditure.

14/10/2009					
Inactive Calibration				Minutes asleep	500
	a	b		Minutes awake	690
$\dot{V}O_2$	0.1973	-3E-08	0.197	Inactive Minutes	690
$\dot{V}CO_2$	<u>0.1615</u>	<u>-3E-08</u>	0.161	Active Minutes	250
Active Calibration					
	a	b			
$\dot{V}O_2$	0.6648	0.0177	0.855		
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Active	0.106466165	0.857302857	17.52610502	4381.526256	
					TEE(kJ/day)
					9121.0

<u>Step 7</u>	
BMR (kJ/day)	6113
SEE (kJ/day)	2016.0

<u>Step 8</u>
TEE(kJ/day)
9121.0

Appendix VIII Visual Analogue Scale

APPETITE QUESTIONNAIRE

Name: _____ **Date:** ____/____/____ **Trial:**_____

Please answer the following questions by placing a vertical mark through the line for each question.

Regard the end of each line as indicating the most extreme sensation you have ever felt and mark how you feel **NOW**.

-

—

Time: _____

1. How **hungry** do you feel (now)?

I am not hungry at all	_____	I have never been more hungry
------------------------------	-------	-------------------------------------

2. How **satisfied** do you feel (now)?

I am completely empty	_____	I cannot eat another bite
-----------------------------	-------	------------------------------

3. How **full** do you feel (now)?

Not at all full	_____	Totally full
--------------------	-------	--------------

4. How **much** do you think you **can eat** (now)?

Nothing at all	_____	A lot
-------------------	-------	-------

5. How strong is your **desire to eat** (now)?

Not at all Strong	_____	Very strong
----------------------	-------	-------------

Appendix IX Flexible and Rigid Control of Restraint Questionnaire

Name: _____

Date: _____

EATING HABITS QUESTIONNAIRE 2

Please indicate your answer by circling the correct response in the case of the true/false questions, or by entering the letter that represents your answer into the box in case of the frequency questions.

PART A:

1. When I have eaten my quota of calories, I am usually good about not eating any more.

TRUE / FALSE

2. I deliberately take small helpings as a means of weight control.

TRUE / FALSE

3. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.

TRUE / FALSE

4. I consciously hold back at meals in order not to gain weight.

TRUE / FALSE

5. I pay a great deal of attention to changes in my figure.

TRUE / FALSE

6. How conscious are you of what you are eating?

- A) NOT AT ALL
- B) SLIGHTLY
- C) MODERATELY
- D) EXTREMELY

7. How likely are you to consciously eat less than you want?

- A) UNLIKELY
- B) SLIGHTLY UNLIKELY
- C) MODERATELY LIKELY
- D) VERY LIKELY

8. If I eat a little bit more on one day, I make up for it the next day.

TRUE / FALSE

9. I pay attention to my figure, but I still enjoy a variety of foods.

TRUE / FALSE

10. I prefer light foods that are not fattening.

TRUE / FALSE

11. If I eat a little bit more during one meal, I make up for it at the next meal.
TRUE / FALSE

12. Do you deliberately restrict your intake during meals even though you would like to eat more? (always – often – rarely – never)

- A) ALWAYS
- B) OFTEN
- C) RARELY
- D) NEVER

PART B:

1. I have a pretty good idea of the number of calories in common food.
TRUE / FALSE

2. I count calories as a conscious means of controlling my weight.
TRUE / FALSE

3. How often are you dieting in a conscious effort to control your weight?
RARELY
SOMETIMES
USUALLY
ALWAYS

4. Would a weight fluctuation of 5 lb affect the way you live your life?
NOT AT ALL
SLIGHTLY
MODERATELY
VERY MUCH

5. Do feelings of guilt about overeating help you to control your food intake?
A) NEVER
B) RARELY
C) OFTEN
D) ALWAYS

6. How frequently do you avoid “stocking up” on tempting foods?
A) ALMOST NEVER
B) SELDOM
C) USUALLY
D) ALMOST ALWAYS

7. How likely are you to shop for low calorie foods?
A) UNLIKELY
B) SLIGHTLY UNLIKELY
C) MODERATELY LIKEY

D) VERY LIKELY

8. I eat diet foods, even if they do not taste very good.

TRUE / FALSE

9. A diet would be too boring a way for me to lose weight.

TRUE / FALSE

10. I would rather skip a meal than stop eating in the middle of one.

TRUE / FALSE

11. I alternate between times when I diet strictly and times when I don't pay much attention to what and how much I eat.

TRUE / FALSE

12. Sometimes I skip meals to avoid gaining weight.

TRUE / FALSE

13. I avoid some foods on principle even though I like them.

TRUE / FALSE

14. I try to stick to a plan when I lose weight.

TRUE / FALSE

15. Without a diet plan I wouldn't know how to control my weight.

TRUE / FALSE

16. Quick success is most important for me during a diet.

TRUE / FALSE

- do not drink milk

--

5. Do you usually take sugar in:

(a) tea

(b) coffee

DO NOT DRINK TEA/COFFEE

YES	NO

6. At table do you:

- generally add salt to food without tasting first

- taste food and then generally add salt

- taste food but only occasionally add salt

- rarely or never add salt at table

YES	NO

7. Which type of breakfast cereal do you normally eat?

- high fibre (e.g. All Bran, Branflakes, Shredded Wheat, Muesli, Porridge, Weetabix)

- other (e.g. Cornflakes, Rice Krispies, Special K, Sugar Puffs, Honey Snacks)

- no usual type

- do not eat breakfast cereal

9. In summary:

(a) how many times do you eat fruit and vegetables or pure fruit juice

per day OR per week OR per month

(b) how many times do you eat oil rich fish

per day OR per week OR per month

(c) how many times do you eat sweets, chocolates, cakes, scones, sweet pies, pastries or biscuits

per day OR per week OR per month