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Effects of acute and chronic exercise on appetite regulatory hormones, craving and body weight in sedentary lean and overweight/obese females

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EFFECTS OF ACUTE AND CHRONIC EXERCISE ON APPETITE REGULATORY HORMONES, CRAVING AND BODY WEIGHT IN SEDENTARY LEAN AND OVERWEIGHT/OBESE FEMALES

By

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Abstract

The thesis comprises of two major parts; firstly, the responses of overweight/obese and lean females´ body weight and selected appetite-regulating hormones (fasting and postprandial) to an 8 weeks exercise-training program avoiding bias towards weight loss by internal and external motivation were investigated. A single blind design was conducted with concealed aims and objectives and exclusion of individuals with intention to lose weight. Overweight/obese (OV/OB) (n=23, BMI= 30.27±3.66 kg m^{-2}; age= 23.39±5.70 years) and lean (L) (n=11; BMI= 22.41±2.14 kg m^{-2}; age= 24.55±6.93 years) completed the study. Results: both OV/OB and L groups resulted in no significant changes in body weight. Of the selected appetite hormones, amylin levels were significantly altered in the OV/OB group affecting fasting and postprandial amylin with reductions (-24.2% and -14.1%, respectively); no changes were detected in L group. Multi regression analysis revealed that leptin and postprandial amylin levels were significant predictors of BMI changes after the exercise intervention explaining 45% of the variance in the OV/OB group. Conclusion: The findings suggest that under ad libitum condition, un-biased by internal and external motivation for weight loss, 8 weeks exercise training did not result in weight loss in overweight/obese and lean females.

Secondly, the effects of acute exercise bouts of moderate and high intensity on food craving dimensions, implicit and explicit attitude towards food, and on selected appetite hormones in lean and overweight/obese sedentary females were investigated. In the second part two studies have been conducted, a pilot study with lean sedentary females (n=10, BMI= 21.93±2.16kg m^{-2}, age= 23.50±2.95years) and a trial with 19 sedentary females (lean individuals (L) n=9, BMI= 20.94±2.84kg m^{-2}, age= 21.56±2.66 years; overweight individuals (OV/OB) n=10, BMI= 29.51±4.31kg m^{-2}, age= 23.70±5.05years). In the trial females performed one high (HI) (90% $\dot{V}O_{2peak}$) and one moderate intensity (MI) (50% $\dot{V}O_{2peak}$)

exercise bout on cycle ergometers with matched energy expenditure in a counterbalanced design, followed by a test meal. Selected appetite hormone measures were taken at fasting, pre exercise, post exercise and 1 hour post exercise. Food craving questionnaire-state (FCQ-S for savoury and sweet foods) measures were taken at fasting, postprandial, pre exercise, post exercise, 1 hour post exercise, post-test meal and 30 minutes post-test meal. Results: An acute bout of exercise, at both moderate- and high-intensity, resulted in a significant suppression of appetite for both sweet and savoury foods in OV/OB individuals ($P<0.05$), with no change reported in lean females ($P> 0.05$). With regard to hormonal response; the findings indicated that an acute bout of exercise result in a significant reduction post-exercise in amylin concentration, with the greatest response evident following the high-intensity protocol where amylin was reduced from pre- to 1h post-exercise by 46% in OV/OB group. The study also demonstrated that OV/OB individuals have a greater drive to eat in a postprandial state following the ingestion of a 300 kcal test-meal. Conclusion: OV/OB females experience a larger decline in craving after exercise than lean; otherwise, this might be overcompensated by the increased craving in the postprandial state by OV/OB.

The collective findings of this thesis suggest that the utility of exercise alone as a mediator of weight loss seems unsupported at least in the limitations of gender and exercise duration used for our research design. Our investigations in both lean and overweight females suggest that exercise should be used in conjunction with dietary restriction to maximise the chance of success for weight loss programs. Studies that have formerly reported weight loss in females may be confounded by the participants desire to lose weight during the study, exhibiting increased self-control in their eating behaviour alongside the exercise intervention.

Key words: exercise, appetite, food craving, food intake, amylin, leptin, obesity, weight loss.

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Conference Presentations

From the studies presented in this thesis, a poster entitled “Appetite hormone response and body composition changes after 8 weeks’ exercise training with low and high intensities in overweight/obese females” was accepted and presented on the international conference of international union of physiological sciences (IUPS) 2013, Birmingham, UK, 21-26 July 2013.

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List of Abbreviations

α MSH	α-Melanocyte-Stimulating Hormone
ADL	Activities of Daily Living
AG	Acylated Ghrelin
AgRP	Agouti-Related Peptide
ANOVA	Analysis of Variance
ARC	Arcuate Nucleus
BMI	Body Mass Index
CCK	Cholecystokinin
ChREBP	Carbohydrate-Response-Element-Binding-Protein
CNS	Central Nervous System
CV	Cardiovascular
CSF	Cerebrospinal Fluid
DEXA	Dual Energy X-Ray Absorptiometry
DG	Desacyl Ghrelin
DIT	Diet Induced Thermogenesis
EAQ	Explicit Attitude Test
EB	Energy Balance
ECG	Electro-Cardiography
EDTA	Ethlenediamine Tetra-Acetic Acid
EE	Energy Expenditure
EEEx	Exercise-induced Energy Expenditure
EI	Energy Intake
ELISA	Enzyme-Linked Immunosorbent Assay
EPOC	Excess Post-exercise Oxygen Consumption
F	Fasting
FCQ-S	Food Cravings Questionnaire-State
FCQ-T	Food Cravings Questionnaire-Trait
GLP-1	Glucagon-Like Peptide-1
HF-LF	High Fat-Low Fat
HI	High Intensity
HR	Heart Rate

HR_{max}	Maximum Heart Rate
HS-LS	High Sugar-Low Sugar
IAT	Implicit Association Test
L	Lean
MI	Moderate intensity
NPY	Neuropeptide Y
OFC	Orbital Frontal Cortex
OV/OB	Overweight/ Obese
PMSF	Phenylmethanesulfonylfluoride
POMC	Proopiomelanocortin
PP	Postprandial
PP	Pancreatic Polypeptide
PVN	paraventricular nucleus
PYY	Peptide YY
RAI	Radio Immunoassay
REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
RPE	Rate of Perceived Exertion
RQ	Respiratory Quotient
SD	standard deviation
TXNIP	Thioredoxin-Interacting-Protein
VAS	Visual Analogue Scales
$\dot{V}O_{2PEAK}$	Peak Oxygen Uptake
WHO	World Health Organisation

Chapter 1: General Introduction

Obesity is now a global epidemic, more than 1.9 billion adults are overweight (BMI >25 kg/m^2), and 600 million reported clinically obese (BMI >30kg/m^2) (World Health Organisation, 2015) a threefold prevalence increase in the UK since 1980 (World Health Organisation, 2013). Excess adiposity is associated with an increased incidence of numerous chronic diseases (Bray, 2004), it is estimated that around 2.8 million adults die annually due to complications of being overweight or obese, whereby 44% of the burden of ill health associated with obesity is made up from diabetes, 23% from ischaemic heart disease, and up to 41% from cancer (WHO, 2009; 2013). As the co-morbidities associated with obesity continue to increase, the classification of overweight and obesity has entered the top five and top ten global risk factors for mortality and morbidity, respectively (WHO, 2009). Furthermore, it is predicted that the number of fatalities due to obesity may soon exceed those that caused by smoking (Hennekens & Andreotti, 2013). Obesity is specifically a major issue in the United Kingdom, as predictions from Government Office for Science (2009) are forecasting that 60% of the population will be obese by 2050. In addition to public health concerns, obesity has a significant economic cost of around £5b within the NHS and a total societal cost of £14b annually (Royal College of Physicians, 2013). Reversing this current trend would be of major significance from both a public health and economic perspective.

The growth in obesity is increasing at such an alerting rate globally, exposing the importance of clinical research to study the mechanisms behind obesity, and to tackle the ever-growing prevalence worldwide. The current obesity epidemic has occurred with both a concomitant rise in the consumption of food that is 'energy-dense' and reduced physical activity levels within modern society (WHO, 2003; Varo *et al.*, 2003), a modern day environment which has

been termed obesogenic. An energy imbalance between calories consumed and calories expended over a prolonged period of time may arise through high energy intake, low energy expenditure or a combination of the two. A continued period of negative energy balance is needed to elicit weight loss over time, which theoretically could be achieved by simply reducing energy intake and/or increasing energy expenditure. For this reason, diet and exercise interventions are commonly prescribed for body weight control, however the efficacy varies depending on the type and/or combination of treatment. When an exercise intervention is solely used, most interventions demonstrate minimal weight loss (Franz *et al*., 2007), however, although weight loss is often reported (Ross, Dagnone *et al.,* 2000, Ross, Janssen *et al.*,2004), exercise training studies do not always result in weight loss and often reveal high individual variability in body weight changes (King, Caudwell *et al.,* 2007, King, Hopkins *et al.*,2007, Barwell, Malkova *et al.,* 2009). However, aerobic exercise can still be associated with a reduction in body fat in the absence of overall weight loss, since weight change does not take into account increases in lean mass (Lee *et al*., 2005). The addition of a dietary intervention to an exercise program is associated with greater weight loss (Franz *et al*., 2007). Collectively, the studies highlight the important role of exercise in the maintenance of a healthy body weight and body composition.

One suggested cause of less than expected weight outcomes in response to exercise is a change in an individual's habitual behaviour, including; i) modified levels of unstructured physical activity, and ii) eating behaviour, comprising, frequency, size, and nutrient selection of food (Blundell, 1991, Blundell and Halford, 1994; Church *et al.*, 2009). Mayer (1953) was first to suggest that energy intake may be increased to compensate against the negative energy balance induced by exercise to result in a less than theoretically expected weight loss following exercise programs. However, recent research suggests that a loose coupling exists

between energy intake and energy expenditure following an acute bout of exercise, Blundell and King (1998) propose that in short- medium- period (1-2 days, 7-16 days, respectively) exercisers can tolerate large negative energy deficits, a term that has been named "exercise-induced anorexia" (King *et al*., 1994). Studies in the longer term, however, have shown that energy intake compensates for exercise-induced energy expenditure when exercise is continued in the long-term (Stubbs *et al*., 2002). These findings can be interpreted as evidence that changes in eating behaviour to compensate for energy expenditure takes a number of weeks before the energy deficit is cancelled out, as is demonstrated consistently in the literature (King*et al.*, 2008; Doucet *et al*., 2011).

Recent studies indicate that the weight change response to an exercise intervention is specific to the individual (Hagobian and Braun, 2010). A study by King *et al.* (2008) examined the inter-individual variability in weight loss in a group of overweight and obese male and female volunteers. After 12 weeks of exercising five days per week, body weight was significantly reduced suggesting that individuals did not compensate for the energy expended by increased energy intake. Interestingly, the variability in weight loss observed was large (-9.5 to +2.6kg), and the exercise induced energy expenditure could only account for 36% of the variance in fat mass response, and changes in fasting respiratory quotient (RQ) could only help explain a further 7% of the remaining variance; therefore, a large proportion of variance remained unaccounted for. The group was spilt into compensators and non-compensators, with compensators demonstrating increased hunger and an increase in energy intake in response to exercise compared to non-compensators, thus those that experienced a lower than expected weight loss may simply have compensated for the energy expended through exercise by increasing their energy intake. Furthermore, Finlayson *et al*. (2009) also identified that there are two types of responders; i) non-compensators, who ate similarly after

exercise when compared to non-exercise trials; ii) compensators, who ate more after exercise when compared to non-exercise trials. The compensators demonstrate enhanced implicit wanting for sweet food post-exercise, and subjectively rate their food as more palatable than the non-compensators thus demonstrating how an individual's hedonic response to exercise may undermine the success of an *ad libitum* exercise intervention. Studies such as these highlight the importance of more targeted and individualised weight loss programs and therefore it is important to gather more knowledge of how exercise affects hunger and energy intake and the mechanisms behind inter-individual differences in weight loss.

Appetite is a subjective perception used to explain the control of food intake which predicts normal eating behaviour (King *et al*., 1997). The regulation of appetite is under control of vastly complex systems; i) the hedonic system, associated with the reward aspects of eating, and; ii) the homeostatic system, associated with the maintenance of energy balance (Lutter and Nestler, 2009), both systems may work together, or may act independently, to control eating behaviour (Saper *et al*., 2002). At the homeostatic level, appetite is controlled by central or peripheral regulation. Central regulation of the central nervous system (CNS) includes homeostatic control of the hypothalamic region of the brain, where many receptors for hormones and peptides are important for the control of eating behaviour (Neary *et al*., 2004). Peripheral regulation includes the secretion of a variety of hormones and seemingly redundant numbers of mediators released from the gastro-intestinal (GI) tract, adrenal glands, pancreas and adipose tissue, as well as nutrients (Neary *et al*., 2004), facilitating the body to regulate energy intake and expenditure. The hormones involved in peripheral regulation can be divided into tonic, or phasic signals, which provide important information about the energy status directed to the brain. Satiety signals leptin and insulin, as well as possibly amylin, provide tonic information about energy status, whereas, peptide YY (PYY_{1-36}, PYY_{3-}

$_{36}$), glucagon like-peptide-1 (GLP-1), cholecystokinin (CCK), amylin, insulin, ghrelin and its acylated form provide phasic signals direct to the hypothalamus but also to the hind brain, subsequently controlling eating behaviour (Suzuki, Simpson, Shillito, & Bloom, 2010).

There are many studies investigating the effect of exercise on appetite, hormonal and food intake parameters in male individuals (Fisher *et al*., 2001, Wasse *et al*., 2012, Christ *et al*., 2006 and Elias *et al*., 2000), however, many less studies are conducted with female participants. This may be due to the confounding effect of the female menstrual cycle since such hormonal changes may result in alterations in energy intake and/or expenditure, as well as mood, irritability, depression, bloating and breast tenderness (Endrikat *et al*., 1997). Nonetheless, the studies reported in this thesis sought to examine appetite, hormonal, and food intake responses to exercise in lean and obese female participants. The first study (chapter 4) aimed to investigate the effects of an 8-week exercise training intervention between lean and obese individuals on body composition, endocrine adaptations, and compensatory responses. Within this, this study will be the first to examine the long term effects of exercise on *ad libitum* energy intake of inactive females, where subjects were naïve to the purpose of the study and recruited without wanting to lose weight. This study will also be the first to investigate the long terms of exercise on circulating amylin levels. Study two (chapter 5) aimed to investigate the effects of two exercise bouts of high- and low-intensity conditions of equivalent energy deficits on appetite regulating hormones for up to 1h post-exercise in sedentary lean and obese females. Furthermore, we sought to explore whether divergent changes in appetite regulating hormone responses were associated with subjective feelings of appetite.

Chapter 2: Literature Review

2.1. Introduction

Obesity is a major health risk for a large variety of diseases with high mortality including cardiovascular disease and cancer (Guh *et al*., 2009). A positive energy balance and low physical activity are thought to be causative for the prevalence of obesity. Otherwise, epidemiological studies and meta-analysis work pointed repeatedly towards an importance of macronutrient composition in diet for obesity; in particular, the macronutrient fat was recognised as a major risk factor for obesity (Swinburn *et al*., 2004; Astrup 1999). Fat is more energy dense and it is more suggested that the fattening effect of high fat diets is due to the amount of calories rather than a specific effect of fat (Crowe *et al*., 2004; Astrup *et al*., 2002). In addition, meta analysis work could also show that low fat diet (high in fibres rich carbohydrates and protein) is very successful in reducing weight in normal weight and overweight people (Astrup *et al*., 2000). However, low carbohydrate diets seem to be more successful in the long run than low fat diets (Hession *et al*., 2009).

Recently, more evidence appears to point towards sugar intake as a problematic factor; a study investigating the influence of macronutrient composition on fat distribution and loss during weight maintenance and weight loss revealed that high glycaemic food leads to less favourable outcomes than low glycaemic food (Goss *et al*., 2013). Additionally, a huge contribution of sugar intake and particularly the intake in liquid form – soft drinks are nowadays more generally accepted as problematic (Malik *et al*., 2010, Dhingra *et al*., 2007, Pereira 2006, Gross et al. 2004). Soft drink consumption (including sports drinks and fruit juice) has increased in parallel with obesity closely matching the dynamic of change in obesity numbers (Hu and Malik, 2010); in UK 235 litres of soft drinks were consumed per capita in 2011 (BSDA 2011). Soft drink intake results in low satiety, ‘empty calories’, which may increase the risk of a positive energy balance (DiMeglio and Mattes, 2000). Lately,

research has brought about a change in the view towards a more specific role of sugars, and liquid sugar intake in particular, in the development of obesity and T2DM on multiple levels (Lustig *et al*., 2012). Indeed, sugars can act at various levels, including changes in taste perception, nutrient absorption, metabolism, reward perception and behaviour into directions typically seen in obese and T2DM individuals. Moreover, high fructose content in drinks is shown to be highly associated with the development of obesity in epidemiological studies (Bray *et al*., 2004). In terms of metabolic alterations, the metabolic phenotype of skeletal muscle in obesity and T2DM is associated with an impaired capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose as the primary fuel with a strong preference for glucose (Corpeleijn *et al*., 2009). A recent proteomic study found that enzymes related to glycolysis are upregulated and mitochondrial markers downregulated in insulin resistant and T2DM muscle (Giebelstein *et al*., 2012), confirming earlier studies finding reduced mitochondrial capacity in skeletal muscle of obese and T2DM (Morgensen *et al*., 2007; Befroy *et al*., 2007; Kelley *et al*., 2002). While this could be a later consequence of alterations in fat mass, there is evidence that sugar intake affects muscle and whole body metabolism preceding obesity. A recent study in our laboratory (Sartor *et al*., 2012) found metabolic alterations on mRNA levels induced in skeletal muscle of lean subjects after a 4 weeks sugar sweetened soft drink supplementation matching the findings of Giebelstein *et al*. (2012) in their proteomic study. Moreover, we found that fasting substrate metabolism shifted from fat towards sugar and insulin sensitivity was reduced. Subjects gained fat mass while having the same total caloric intake. Using human and rabbit primary muscle cells and cell lines, it was also shown that increased sugar availability induces metabolic gene expression changes, increased glycolytic and lipogenic as well as reduced mitochondrial gene expression (Hanke et al. 2008; Hanke *et al*., 2011, Sartor *et al*., 2012). Sugar dependent transcription factors, MondoA and MondoB, were mainly responsible for

these effects in skeletal muscle (Stolzman *et al*., 2008; Hanke *et al*., 2011; Sartor *et al*., 2012). MondoA and MondoB seem to be master-regulators of glycolytic and lipogenic genes and are presumably responsible for a wider range of metabolic genes regulation (Sans CL *et al*., 2006; Poupeau & Postic, 2011).

The influence of macronutrient composition on metabolism and appetite are complex and are consequently a confounding factor for *ad libitum* studies not being able to analyse or control the macronutrient intake exactly. Otherwise, metabolic and endocrine responses to macronutrients can vary widely from subject to subject making it even more difficult to draw conclusions from diet records (Breton *et al*., 2015).

The following section will provide a literature review on the food intake and appetite response to both short- and long-term exercise, including appetite regulating hormones amylin, leptin, insulin, peptide tyrosine-tyrosine (PYY), ghrelin, and acylated ghrelin. The current review will begin with a clear definition of appetite, and progress to include a clear explanation of how hunger and satiety are regulated, both homeostatically, and hedonically. Following this, the effect of both short- and long-term exercise on these processes will be reviewed in both lean and overweight/obese populations.

2.1.1. Definition of appetite

Appetite has commonly been defined as "the desire to eat" within the literature, however, it is argued that the desire to eat refers to a psychological drive, and suggest that "the psychological desire to eat or an interest in food" is a more appropriate definition (Rolfes, 1996). Furthermore, the distinction between 'hunger' and 'appetite' to describe the initiation

of eating is emphasized as hunger is based on the physiological drive to eat, whereas appetite reflects a more learned response to food. On the reverse, satiety is defined as a 'feeling of fullness', whilst satiation as 'the process that leads to the termination of eating' (Benelam, 2009). In recent literature, authors have advised that both energy intake and appetite measures are collected since the relationship of appetite and food intake are not matched (Stubbs *et al*., 2000; Gregersen *et al*., 2008).

Subjective appetite is usually obtained by administering visual analogue scales (VAS) within research. Blundell and colleagues (2010) advise that multiple scales are used to capture the multi-dimensional nature of appetite. Participants are required to draw a line on a horizontal axis that is typically 100-150mm in length and anchored with statements such as: 'How hungry do you feel now?' anchored by *very hungry (100)* and *not at all hungry (0)*; 'How full do you feel now?' anchored by *very full (100)* and *not full at all (0)*; prospective food consumption and "How much would you like to eat now?" anchored by *a lot (100)* and *nothing at all (0)*. In addition, the Food Cravings Questionnaire-Trait (FCQ-T) and –State (FCQ-S) (Cepeda-Benito *et al*., 2001) have been developed to measure hunger together with other components of drives towards eating. The FCQ-T intends to measure features of food cravings that are stable across time, whereas the FCQ-S assesses temporary state-dependent cravings.

2.1.2. Regulation of appetite and energy intake

Appetite regulation is a complex process under the control of both homeostatic, and hedonic systems that are primarily responsible for the maintenance of energy balance, and rewarding aspect of food, respectively (Lutter and Nestler, 2009). The two systems may interact with

one another to control feeding, or, act independently (Saper *et al*., 2002), for instance, feeding can be initiated by the taste and olfactory sensation of foods even in the presence of complementary signals from the homeostatic system indicating a positive energy balance (Van Vugt, 2010).

2.1.3. Central regulation

The brainstem and hypothalamic region of the brain are thought to be the key sites in the regulation of energy homeostasis, comprising a vast amount of central and peripheral signals that pertain information regarding the acute and chronic nutritional state of the body (Murphy and Bloom, 2006). Neary *et al*. (2004) illustrates the hypothalamus subdivided into several nuclei with each sub-section consisting of neurons, each with their own specific function for energy homeostasis (Figure 2.1.2). The arcuate nucleus (ARC) nucleus is thought to be the primary site for the integration of signals for energy homeostasis, receiving inputs from other nuclei within the hypothalamic region of the brain as well as directly from the circulation via peripheral hormonal signals that cross the incomplete blood brain barrier at the median eminence. These signals contain either the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related peptide (AgRP), or the anorexigenic neuropeptide pro-opiomelanocortin (POMC) (Neary *et al*., 2004). ARC neurone stimulation induces the transmission of neuropeptides to other hypothalamic nuclei, particularly the PVN. Stimulation of POMC neurones increases the expression of the anorexigenic neuropeptide α-melanocyte-stimulating hormone (αMSH), acting on PVN MC3 and MC4 receptors to reduce appetite. Conversely, AgRP blocks the action of α-MSH by exerting an antagonistic effect on MC3 and MC4 (Wynne *et al*., 2005), while NPY stimulates feeding through activating PVN Y1 and Y5 receptors (Neary *et al.*, 2004).

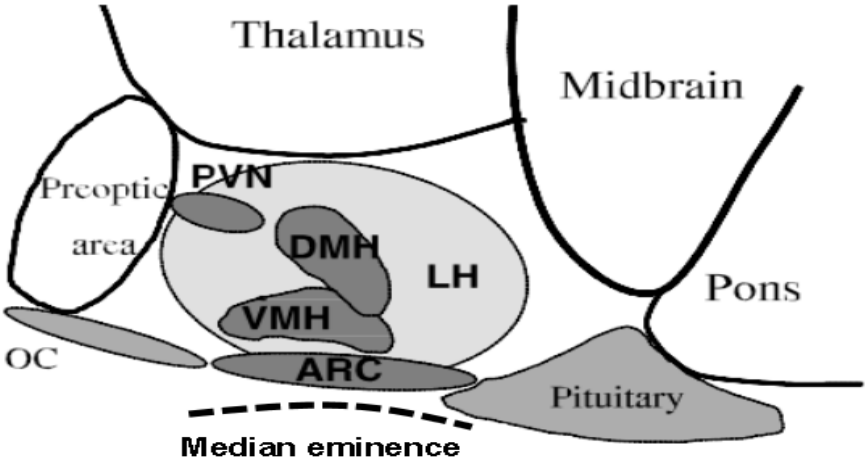

Figure 2.1.2. Hypothalamic nuclei involved in energy homeostasis (lateral view). ARC, arcuate nucleus (known as the infundibular nucleus in humans); PVN, paraventricular nucleus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamic area; OC, optic chiasm (Neary *et al*., 2004).

2.1.4. Peripheral regulation of appetite and energy intake

Peripheral regulation includes the secretion of a variety of hormones from the gastrointestinal tract, adrenal glands, pancreas, and adipose tissue (Neary *et al*., 2004), enabling the body to regulate energy intake and expenditure. These various hormonal signals can be divided into tonic signals which deliver information pertaining to energy reserves such as insulin and leptin, as well as episodic signals, which fluctuate transiently in the acute state in response to a meal including amylin, pancreatic polypeptide (PP), peptide tyrosine-tyrosine (PYY), glucagon like peptide-1 (GLP-1), and appetite stimulating hormone ghrelin.

2.1.5. Tonic signals

Using a rat model, Kennedy (1953) first postulated that the hypothalamic control of food intake was regulated by a circulating factor providing information pertaining body fat stores. The two tonic signals focussed on within this literature review include insulin, and leptin,

with their roles in the chronic regulation of food intake, metabolic homeostasis, and body composition (Porte *et al.*, 2005; Adami *et al*., 2002).

2.1.6. Insulin

Insulin, secreted from *ß*-cells of the islets of Langerhans located in the pancreas in response to changing plasma glucose concentrations, or via the insulinotropic effects of proteins (Akhavan *et al*., 2014), insulin concentrations have also previously been shown to increase with body fat mass even though insulin is not secreted from adipocytes (Woods & Seeley, 1998). Administering insulin elicits a reduction in food intake and weight in rodent models (Air *et al*., 2002). Moreover, insulin administration functions on energy balance homeostasis by not only decreasing appetite, but increasing energy expenditure through sympathetic nervous system activity (Porte *et al*., 2005). Insulin is believed to induce its anorectic effects via the inhibition of NPY and AgRP neurones and concomitant stimulation of POMC neurones in the ARC of the hypothalamus (Murphy and Bloom, 2004). However, the anorexigenic effects of insulin may be reduced in obesity in a state of insulin resistance on the hypothalamus (De Souza *et al*., 2005), as with peripheral insulin resistance caused by excess adiposity (Schwartz & Porte, 2005).

2.1.7. Leptin

Leptin is the product of the *ob* gene and is produced and stored primarily in adipose tissue (Nam *et al*., 2001) acting as an adiposity signal, whereby basal circulating concentrations correlate with adiposity levels (Polonsky *et al*., 1998; Kahn and Flier, 2000). Leptin administration not only decreases appetite, but similar to insulin, increases energy expenditure through sympathetic nervous system activity (Zhang *et al*., 1994; Porte *et al*.,

2005; Farooqi *et al*., 2002). Leptin exerts its anorexigenic effects in the ARC of the hypothalamus via direct inhibition of NPY and AgRP neurones and stimulation of POMC neurones (Sahu, 2003). It has been demonstrated that leptin secretion follows rises in plasma insulin concentrations, and decreases following falls in insulin during fasting (French and Castiglione, 2002). As such, dietary fat and fructose, which do not stimulate insulin secretion, lead to reduced leptin production, suggesting high-fat/ high-sugar diets may increase the susceptibility to weight gain (Havel, 2004).

The importance of leptin in energy homeostasis regulation is emphasised by the observations that individuals with congenital leptin deficiency exhibit extreme childhood obesity and hyperphagia (Montague *et al*., 1997), and that the treatment of these individuals with recombinant leptin reverses this phenotype (Farooqi *et al.*, 2002). Although, it must be noted that this is a very rare condition, and most obese individuals do not exhibit congenital leptin deficiency, but circulating leptin concentrations are within proportional amounts to the degree of fat mass (Considine *et al*., 1996), which may suggest a state of leptin resistance, similar to insulin resistance, thereby reducing the anorexigenic effects of this hormone (Munzberg *et al*., 2005). Nam *et al*. (2001) suggest that an impairment in the ability of leptin to cross the incomplete blood-brain barrier to the central nervous system is the key mechanism behind leptin resistance, putting forward the theory that the ratio of cerebrospinal fluid (CSF) to plasma leptin concentrations shifts in obesity so that an increase in the circulating hormone is not matched by an increase in CSF thus resulting in a reduced efficiency of leptin uptake to the CNS in obese individuals. It is yet unknown whether elevated concentrations of insulin sustained during obesity cause a resistance in the central signalling pathways.

2.1.8. Episodic signals

Episodic hormonal signals function acutely in response to food intake via signalling through vagus nerve or via the perfusion of the incomplete blood-brain barrier of the hypothalamus (Neary *et al.*, 2004). The majority of gut hormones such as (PYY $[_{1\text{-}36}, _{3\text{-}36}]$), GLP-1, CCK and amylin act as satiety signals and suppress appetite (Blundell *et al*., 2008) while ghrelin is the only known episodic hormone released from the gastric cells within the stomach and that stimulates appetite (Cummings *et al*., 2001). Episodic hormones are involved in regulating either initiation or termination of meal; therefore, they determine frequency of meals and meal size.

2.1.9. Ghrelin

The hormone ghrelin is a 28 amino acid peptide that is released into the circulation by cells in the gastric mucosa. The name originates from its ability to simulate growth hormone secretion since "ghre" translates to "grow". As the only recognised orexigenic hormone, ghrelin stimulates increased food intake and meal initiation (Cummings and Foster, 2003), as demonstrated by intravenous (Druce *et al*., 2006) or subcutaneous (Druce *et al*., 2005) infusion increasing food intake during an *ad libitum* buffet test meal, and increasing subjective hunger measured by visual analogue scales (VAS) in lean and obese individuals, when compared to a saline control group (Wren *et al*., 2001). Furthermore, ghrelin seems to influence digestion by stimulating gastric acid secretion and gastric motility, as demonstrated in rats (Masuda *et al*., 2000). Feeding is the most predominant factor in the regulation of ghrelin secretion (Callahan *et al*., 2004), with increased circulating concentrations observed in response to fasting, and decreased circulating concentrations observed following food

ingestion, although the mechanisms mediating this effect are not known (Tschop *et al*., 2000).

Ghrelin exists in two forms, acylated (AG) and desacyl ghrelin (DG), whereby AG only makes up ~10% of total ghrelin but is thought to be the more active appetite stimulating form (Asakawa *et al*., 2005). This acylation is essential for ghrelin to bind to the growth hormone-secretagogue receptor and to cross the blood-brain barrier (Murphy and Bloom, 2006). Acylated ghrelin concentrations are elevated by fasting, and suppressed by inversely proportional amounts to the calorific load of a meal once ingested, indicating that acylated ghrelin regulates the short-term control of the energy balance (Callahan *et al*., 2004).

Ghrelin levels are decreased in human obesity, which may be due to the suppressive effect that insulin plays on ghrelin concentrations (Flanagan *et al*., 2003), since human obesity is a state characterised by hyperinsulinemia (Tschop *et al*., 2001). Furthermore, ghrelin levels are observed to increase following weight loss (Cummings *et al*., 2002), this may be part of a feedback mechanism by which body weight is regulated (McLaughlin *et al*., 2004).

2.1.10. Peptide YY

The hormone peptide tyrosine-tyrosine (PYY), named in reference to the presence of a tyrosine residue which has the amino acid abbreviation Y at each end of its molecular structure, is released into the circulation by endocrine L cells of the small and large bowel (Tatemoto & Mutt, 1980). PYY exists in two known endogenous forms: intact 36 amino acid peptide PYY_{1-36}, and the truncated 34 amino acid peptide PYY_{3-36}, the more predominant

form in the circulation (Neary *et al*., 2004). Feeding is the predominant factor in PYY secretion, released into the circulation in a nutrient-dependent manner, concentrations rise ~15 minutes following ingestion and peak values occur ~1-2 hours after completion of the meal and remain elevated for up to 6 hours (Adrian *et al*., 1985). Interestingly, the initial postprandial rise in PYY concentrations occurs prior to the nutrient sensing of the L-cells in the gastro-intestinal tract, thus suggesting a neural or hormonal mechanism for the initial release (Adrian *et al*., 1985).

The intravenous administration of PYY has demonstrated delayed gastric emptying and elicits anorectic responses suppressing food intake via receptors located in the hypothalamus (Neary *et al*., 2004). Batterham and colleagues (2002) observed a reduction in food intake of 33% when PYY_{3-36} was administered via intravenous infusion, compared to a saline control, over a 24-hour experimental period. It has been observed that obese individuals have lower fasting plasma PYY concentrations than lean subjects (Batterham *et al.*, 2002), however, the same relationship does not occur for PYY_{3-36} (Pfluger*et al*., 2007); therefore, it seems unlikely that a PYY deficiency contributes to the genesis of obesity. The anorectic effects of PYY are thought to be mediated primarily by Y2 receptors (Y2R) in the ARC of the hypothalamus (Karra & Batterham, 2010), a mediating influence that would explain the greater appetite suppressing potency of PYY_{3-36} compared with PYY_{1-36} (Sloth *et al*., 2007). The binding of PYY to Y2R appears to suppress appetite by inhibiting NPY neurones, which decreases orexigenic signalling and disinhibits POMC neurones to increase anorexigenic outputs (Batterham *et al*., 2002; 2006).

Recent research has suggested that PYY may exert influence on appetite and food intake via brain reward centres. Batterham *et al*. (2007) utilised blood oxygen level-dependent magnetic resonance imaging to demonstrate that PYY_{3-36} infusion produced the largest change in brain activity in regions implicated in reward processing, such as in the left caudolateral orbital frontal cortex (OFC). Interestingly the change in OFC signalling explained 77% of the variance in caloric intake after PYY_{3-36} infusion, suggesting that PYY may suppress energy intake by decreasing the rewarding aspects of food.

In addition, PYY appears to influence long-term energy homeostasis. In lean individuals, PYY responds to chronic changes in energy balance with postprandial increases in circulating concentrations, a response blunted in obese individuals, resulting in reduced postprandial satiety levels (Batterham *et al*., 2003; 2006). The mechanisms underlying a blunted PYY response in obesity is unclear, but rodent studies suggest that circulating concentrations are suppressed as a result of impaired postprandial secretion, rather than synthesis, of PYY (leRoux *et al.,* 2006). Regardless of the mechanisms mediating this effect, a blunted PYY and satiety response to meal ingestion may contribute to obesity.

2.1.11. Amylin

Amylin is a 37-amino acid peptide that is co-secreted with insulin from ß-cells of the islets of Langerhans located in the pancreas in response to a meal (Qi *et al*., 2010). Amylin concentrations rise several-fold in response to a meal, peaking after ~1hour and remaining elevated for up to 4hours post-meal (Roth *et al*., 2009). Amylin receptor agonism suppresses appetite (Pullman *et al*., 2006), slows gastric emptying (Smith *et al*., 2008), and reduces postprandial glucagon release in a glucose-dependent manner (Roth *et al*., 2008). Amylin

reduces the rate of glucose release into the bloodstream for insulin to pertain a more controlled glucose homeostasis. Indeed, blockade of administration of the amylin antagonist AC187 increases food intake, elevates circulating glucagon levels, quickens the gastric emptying rate, and elevates glycaemia following oral glucose ingestion (Riediger *et al*., 2004; Gedulin *et al*., 2006).

2.1.12. Appetite hormones and hedonic response

The hedonic system is associated with the rewarding aspects of food involved in the maintenance of energy balance (Lutter and Nestler, 2009). Van Vugt (2010) highlights that even in the presence of homeostatic signalling that indicates satiety has been reached, the smell or taste of food can override these signals to initiate feeding, demonstrating the great importance of the hedonic system in regulating food intake. Furthermore, utilising brain imaging methodology, Gibson *et al*. (2010) demonstrate that ghrelin and can stimulate the sites in the brain that are associated with reward-driven eating. Furthermore, using functional magnetic resonance imaging, Batterham and colleagues (2007) have shown that infusion of typical postprandial concentrations of PYY_{3-36} in normal weight volunteers modulates neuronal activity not just in the hypothalamus but also in regions of the brain involved in reward processing. Thus it is likely that PYY exerts its anorexigenic effects by acting on both homeostatic and hedonic brain circuits (Batterham *et al*., 2007). Collectively, these studies demonstrate the acute regulation of food intake by circulating hormones is not limited to effects on homeostatic brain sites, but also encompass aspects of hedonic regulation of food intake.

2.2. Exercise, appetite and food intake

There has been a surge of studies investigating the effects of exercise in the literature within recent years; exercise may have short-term (acute) and long-term (chronic) effects on the regulation of energy balance including energy intake and energy expenditure which consists of resting metabolic rate, physical activity and thermogenesis via a complex physiological system of afferent signals integrated by peripheral nerves and brain centres (Bilski *et al*., 2009; Stanley *et al*., 2005).

Many researchers have suggested that the efficacy of an exercise bout to promote a negative energy balance may be influenced by the intensity of the exercise performed (Stensel *et al*., 2010). Van Loon *et al*. (2001) demonstrated that although aerobic activity depends almost entirely upon utilisation of substrates fat and carbohydrate, the proportion of energy derived from each source differs in relation to the intensity performed. Healthy male cyclists ($\dot{V}O_{2max}$ 5.48 ± 0.16 l/min^{-}1) utilised equal amounts of fat and carbohydrate sources at a moderate intensity (~55% $\dot{V}O_{2max}$), but increasing the intensity (~75% $\dot{V}O_{2max}$) resulted in a greater proportion of energy derived from carbohydrate than fat (76% and 24%), whereas decreasing the intensity (~40% $\dot{V}O_{2max}$) elicits a greater reliance on fat sources than carbohydrate (55% and 45%) (Van Loon *et al*., 2001). Since decreases in carbohydrate availability have been linked to an increased meal size and frequency (Finlayson *et al*., 2009), it is possible that restoration of muscle glycogen and the homeostatic control of blood glucose is a major priority following high-intensity exercise and more urgent signalling for increased energy intake protects against the negative energy balance.

As such, increasing exercise intensity in young women has shown to elicit an increase in energy intake reported by diet diaries (Stubbs *et al*., 2002), or during a subsequent *ad libitum* buffet test meal (Pomerleau *et al*., 2004) which was almost sufficient to totally compensate for the exercise-induced energy expenditure. In addition, individuals who were classified as high fat oxidizers based on exercise RQ experienced significantly lower post-exercise energy intake than the high carbohydrate oxidizers ($P< 0.05$) (Almeras *et al*., 1995). Paradoxically, a temporary suppression of appetite reported in women is much more pronounced following intense exercise than moderate-low intensity exercise, often with no change following an acute bout of low intensity exercise (Bilski *et al*., 2009), suggesting an intensity threshold for exercise-induced anorexia.

2.3. Acute exercise and appetite hormones

Mayer (1953) first suggested that the efficacy of an exercise intervention is undermined by a compensatory increase in appetite and subsequent energy intake. This has been observed in many long-term studies (Stubbs *et al*., 2002; King *et al*., 2008), however, acute exercise bouts may, unexpectedly, lead to short-term hunger suppression (Broom *et al*., 2009). Although a brief suppression of appetite is observed, this may not necessarily translate into a subsequent reduction in food intake at an *ad libitum* test meal (King *et al*., 1999; Martins *et al*., 2007). This post-exercise state has been termed 'exercise-induced anorexia' (Stensel, 2010). Currently, a sound understanding of how exercise influences appetite is lacking; therefore, it is important to identify the homeostatic and hedonic processes which could be mobilised by exercise to influence food intake.

2.3.1. Leptin and insulin

Acute exercise bouts demonstrate that plasma insulin concentrations are slightly suppressed following continuous cycling protocols of moderate intensity (~60% $\dot{V}O_{2max}$) (Kreisman *et al*., 2000), remain unaffected during moderate-intense exercise (85% $\dot{V}O_{2max}$), but increase at maximal intensities (Marliss *et al*., 1991; Kraemer *et al*., 2002). However, post-exercise observations demonstrate an increase of plasma insulin lasting up to 60min during recovery (Marliss *et al*., 1992; Marliss and Vranic, 2002).

Leptin, conversely, demonstrates no change in circulating concentrations in response to acute exercise (Kraemer *et al*., 2002). However, Elias *et al.* (2000) did demonstrate reduced leptin concentrations in males following a graded treadmill exercise test to exhaustion, but this may be associated with an elevated production of non-esterified fatty acids (NEFA) during the exercise, as shown previously to be inversely correlated with leptin levels (Duclos *et al*., 1999). Furthermore, Kraemer *et al*. (1999) demonstrated reduced leptin levels following 30minutes of high-intensity (80% $\dot{V}O_{2max}$) exercise, but control trials revealed these reductions to be due to circadian rhythm, an effect which may also further explain the reduction in Elias *et al*. (2000) since no control trial was conducted. In addition, Kraemer and colleagues (2001) have demonstrated that decreased leptin in response to a graded test to exhaustion may have been due to changes in hemoconcentration, presenting higher concentrations of leptin to the receptors. Thus, it seems short-term exercise does not acutely effect leptin secretion, regardless of the intensity, but reported changes may be attributable to circadian rhythm fluctuation or changes in hemoconcentration.

2.3.2. Ghrelin

Several studies have attempted to assess whether the acute suppression of hunger following exercise is related to decreased plasma ghrelin concentrations (Dall *et al*., 2002; Burns *et al.,* 2007) with the majority of studies observing a single session of aerobic exercise has no influence on ghrelin concentrations. Although, at least one study has reported a suppression of ghrelin 1h post exercise (Vestegaard *et al*., 2007), whilst another reported increase circulating ghrelin concentrations during 3 hours of aerobic exercise (Christ *et al*., 2006).

In studies investigating acylated ghrelin however, exercise in the short term has been shown to suppress concentrations for up to 3h following acute aerobic (Broom *et al*., 2007) and resistance (Broom *et al*., 2009) exercise. Moreover, Broom *et al.* (2007) demonstrated a significant correlation between plasma acylated under-the-curve values and hunger over the first 3h of the exercise trial, whereby lower hunger sensations were reported in those with lower acylated ghrelin concentrations, which suggests the important role acylated ghrelin may play in appetite suppression during and immediately after exercise.

2.3.3. PYY

Research studies investigating the effects of acute exercise on circulating PYY concentrations have found that exercise results in an increase in total PYY. For example, Martins *et al*. (2007) observed increases in plasma PYY concentrations during and upon completion of exercise (65% HR_{max}) for 60 minutes in healthy men and women. The increase in plasma PYY occurred with a concomitant reduction in hunger, both of which returned to control values within 30 minutes post-exercise, a result that is consistent in the literature (Ueda *et al*., 2009; Wasse *et al*., 2012), suggesting that perhaps PYY plays a role in the reported 'exercise-

induced anorexia'. The aforementioned studies all investigated concentrations of both PYY_{1-36} and PYY_{3-36}, however, as discussed earlier, the main appetite suppressing effects of PYY are through the action of PYY_{3-36} specifically. Despite a strong correlation between changes observed in total PYY and PYY_{3-36} (Tsilchorozidou *et al*., 2008), it is much more beneficial to directly measure PYY_{3-36} responses in relation to appetite. The studies investigating PYY_{3-36} show, similar to total PYY, increased concentrations are present during aerobic exercise in lean (Martins *et al*., 2007) and obese (Ueda *et al*., 2009) participants, and the effect returns to control values 1h post-exercise. Again, this effect is consistent in the literature, with King *et al*. (2011) demonstrating that 90 minutes of running (70% $\dot{V}O_{2max}$) stimulated an increase (↑27%) in circulating PYY_{3-36} concentrations.

2.3.4. Amylin

There are very limited research studies investigating the response of amylin concentrations following exercise. One study by Kraemer and colleagues (2002), utilised a progressive intermittent based aerobic exercise protocol of moderate to maximal intensities (60%, 75%, 90%, and 100% $\dot{V}O_{2max}$) in well trained individuals ($\dot{V}O_{2max}$ 61.01±2.37 mL/kg/min) and observed no change in amylin values following the 60% and 75% $\dot{V}O_{2max}$ bouts, but increased (58%) after 90% $\dot{V}O_{2max}$ to peak after 100% $\dot{V}O_{2max}$, and remained elevated during the 1h recovery from exercise. More recently, Kraemer and colleagues (2011), again, demonstrated no change in amylin levels during 90 minutes of moderate-intensity exercise in comparison to the control trial, suggesting that there is a high intensity threshold for changes in amylin concentrations.

2.4. Long-term exercise and appetite hormones

2.4.1. Leptin

Leptin concentrations do not seem to be influenced by chronic exercise bouts in the absence of weight loss. In the long-term, Kraemer *et al*. (1999) investigated 9 weeks of aerobic exercise training, the findings from both studies confirmed that the exercise had no influence on fasting leptin concentration. These findings however are not surprising considering the exercise program did not elicit a reduction in adiposity. Indeed, Ozcelik *et al.* (2004) utilised a 12-week aerobic exercise period which did result in decreased fat mass, with a concomitant reduction of plasma leptin of the same relative magnitude. These studies indicate that decreases in leptin following exercise programs are dependent upon fat mass loss, however, it has been proposed that an exercise intervention that causes improvements in insulin sensitivity may alter leptin levels independently of changes to fat mass (Martins *et al*., 2008) due to changes in insulin and cortisol levels which are known to modulate leptin synthesis (Considine, 1997), however, this is not a consistent finding in the literature (Hagobian *et al*., 2009).

2.4.2. Ghrelin

In long-term exercise studies, fasting total ghrelin concentrations are shown to increase providing weight loss occurs, with an inverse correlation with body weight TG (Cummings *et al*., 2002). For instance, Forster-Schubert *et al*. (2005) demonstrated that weight loss induced by a 1-year exercise intervention (45min, 5d/wk, 60-75% HR_{max}) significantly increased ghrelin concentrations, suggesting an adaptive response to weight loss acting in a negative feedback loop to regulate body weight. Indeed, it is accepted that in the absence of weight loss exercise has no significant effect on the fasting plasma levels of total ghrelin in lean

(Zoladz *et al*., 2005) or overweight (Borer *et al*., 2005) individuals, whereas acylated ghrelin has been shown to remain unchanged following long-term exercise interventions independent of weight loss (Kim *et al*., 2008).

2.4.3. PYY and amylin

In long-term exercise studies investigating circulating amylin concentrations, a 14-day combined diet and exercise intervention resulted in a reduction of amylin in obese children (Izadpanah *et al*., 2012). However, to our knowledge, there are currently no studies investigating specifically the training effects of exercise on amylin without diet induced weight loss. Several studies have demonstrated declined PYY concentrations following long-term exercise interventions which induce weight loss (Jones *et al*., 2009; Roth *et al*., 2005). However, very few studies have been conducted into PYY_{3-36}, and the information is currently limited.

2.5. Hedonic appetite regulation and exercise

The importance of identifying both homeostatic and hedonic processes in regulation of appetite should be underlined (Finlayson *et al*., 2009), and therefore research studies have sought to examine the effects of exercise on hedonic aspects of appetite regulation. Finlayson and colleagues (2009) suggest that exercise may mediate changes in food preference for foods with specific sensory and/or nutrient properties. To distinguish the hedonic processes that are either explicitly affective, or implicitly motivation, the terms ‘liking’ and ‘wanting’ were assigned, respectively (Berridge, 1996). The two terms represent dual components of food preference which can be altered by exercise-induced energy expenditure, resulting in behavioural changes of over-compensatory eating (Finlayson *et al*., 2009). Food craving, a

wanting state generally defined as an intense desire to ingest a specific food item (Weingarten and Elston, 1990), is thought to mediate uncontrolled eating behaviour such as that prevalent in obesity, and as such has been identified to be related to body weight, suggesting a ubiquitous role of craving in food consumption (Franken and Muris, 2005). In order to assess food cravings, two self-report questionnaires have been developed, the Food Cravings Questionnaire-Trait (FCQ-T) and –State (FCQ-S) (Cepeda-Benito *et al*., 2001). The FCQ-T intends to measure features of food cravings that are stable across time, whereas the FCQ-S assesses temporary state-dependent cravings. Both questionnaires have previously shown to possess good reliability and validity in measuring food cravings (Cepeda-Benito *et al*., 2000). More recently, Nijs *et al*. (2007) produced more generalised questionnaires in the belief that a general urge to eat (rather than a desire to eat a specific food) may mediate excessive eating patterns prevalent in obesity.

The expression of habitual eating behaviour involves the co-ordination and interaction of both homeostatic and hedonic signals (Finlayson, King, & Blundell, 2008). Recent research has focused on the hedonic determinants of eating behaviour, and Finlayson *et al*. (2007) have highlighted the importance of distinguishing liking (i.e. the perceived pleasurable sensory properties of food) from wanting (i.e. the attraction towards a specific food over available alternatives), both of which are thought to act in parallel to facilitate eating behaviour (Finlayson *et al*., 2008). Moreover, current advances in neurobiology are demonstrating the emergence of a new conceptual approach to reward, where affect and motivation (i.e. liking and wanting) can be seen as the major force in guiding human eating behaviour. Food reward seems to be represented functionally and structurally by distinct components, Berridge and colleagues (1996) focus on opioid neurotransmission in the nucleus accumbens shell and the mesolimbic dopamine system, their research has shown that

core processes of 'liking' and 'wanting' can be separately manipulated in rodent models to produce patterns of behaviour which are either exclusively affective or motivational in conjunction with a food stimulus. This concept should be emphasised in the light of the current obesogenic environment where food is plentiful, cheap, energy-dense and enticing, and habitual physical activity is reducing (Finlayson *et al*., 2007). It is suggested by Peters *et al*. (2002) that throughout time weight control has been converted from a highly regulated and instinctual system, to include a high degree of intensive cognitive processes. Furthermore, it is suggested that the current obesogenic environment may predispose overweight/obese individuals to weight regain following a weight loss program, due to the hedonic drive being encouraged and exploited (Ikeda *et al*., 2005).

Berridge and Robinson (2003) describe how through separate examination of specific neural substrates in the brain, implicit behavioural measures of liking and wanting reflect core processes that can operate without conscious awareness concomitant with their explicit counterparts which express themselves subjectively in the form of hedonic feelings from the ingestion of a specific food (conscious liking) and the intent or desire to consume a specific food (conscious wanting). Finlayson *et al*. (2007) emphasises that understanding how liking and wanting may influence behaviour towards eating since they operate at implicit and explicit levels of conscious awareness. Similarly, Brug *et al*. (1995) highlight that an individual's attitude towards food could be an important factor contributing to obesity, controlled by two modes, implicit attitude, which can impact behaviour in an affective and instinctive way, irrespective of any consideration towards the advantages or disadvantages of that particular behaviour, and explicit attitude, which may guide behaviour through a deliberate and conscious analysis of the risk and reward of that behaviour (Craeynest *et al*.,

2005). Finlayson and colleagues (2008) suggest that these two contrasting processes may operate together, or independently to facilitate eating behaviour.

There are many barriers for interpreting 'liking' and 'wanting' into constructs amenable to the study of behavioural eating in research, for example, a person's subjective feelings may not represent the complexity of underlying processes. Berridge (1996) warns of the distorting potential of excessive cognitive processing on affect, suggesting that the more someone is encouraged to consider how they feel, the less reliable their data may become. Implicit attitudes are frequently measured indirectly by using latency based tasks such as the Implicit Association Test (IAT) which measures automatic associations between an attitude object and a certain valence (Craeynest *et al*., 2008), whereas explicit attitudes are assessed via self-reported questionnaires (Craeynest *et al*., 2005). It is believed that implicit cognition discovers information that an individual may otherwise be unwilling to share due to contradictions with their beliefs or values, or it may have perceived negative social consequences, and therefore implicit attitude may prove more relevant than explicit attitude (Nozek *et al*., 2007). As such, Berridge (2004) provide evidence that implicit wanting could be a more compelling influence on behaviour than explicit wanting. In one study, activation of the dopamine system caused a reward cue for sucrose to become 'hyper incentive', temporarily more dominant than sucrose presented alone or with an irrelevant cue (Whyvell and Berridge, 2000).

Recent investigations have extended our knowledge of the how the reward system can interact with homeostatic mechanisms on neural and molecular levels in the regulation of appetite. Cannabinoids, a class of diverse chemical compounds that act on cannabinoid

receptors, and their endogenous ligands (i.e. anandamide) are implicated in the reward system (Saper *et al*., 2002). The central and peripheral administration of anandamide has increased appetite in rodents which seemed to be related to alterations in incentive desire for palatable foods (Kirkham and Williams, 2001). Furthermore, the cannabinoid system interacts with homeostatic processes in many ways, leptin signalling becomes defective when hypothalamic endocannabinoid levels are high (Marzo *et al*., 2001), furthermore cannabinoid receptors can be found on adipocytes, which may act to directly increase lipogenesis (Cota *et al*., 2003). Moreover, the reward system works together with appetite-controlling neurones that upregulate the expression of peptides that drive hunger such as NPY and orexins while concomitantly inhibiting signals from satiety peptides from circulating hormones insulin, leptin and cholecystokinin, which results in the ability of highly palatable food to initiate eating via a reward system, rather than by a biological need for energy (Finlayson *et al*., 2007). In addition, direct and indirect projections from the accumbens to the hypothalamus may explain the ability for mesolimbic processes triggered by environmental cues and incentives to hijack the homeostatic regulatory circuits and up-regulate energy intake (Berthoud, 2004). The above studies are clear documentation that homeostatic and hedonic systems are inseparable, rather than separate processes.

Changes in food reward have been implicated in exercise-induced compensatory eating behaviour. Finlayson and colleagues (2009) observed that following 50 minutes of cycling, lean women who overconsumed relative to the energy cost of exercise exhibited increased wanting for food compared to those who did not exhibit post-exercise compensatory eating. However, the underlying mechanisms of food reward, and the physiological correlates of exercise-induced changes in food reward, are poorly understood. Most research studies investigating differences in explicit and implicit attitudes towards food in lean and obese

individuals have reported no difference, and report that both groups demonstrate negative implicit attitudes towards high fat and calorie foods (Maison *et al*., 2001; Roefs & Jansen, 2002). However, simply labelling foods as 'low calorie' or 'high calorie' does not enable the researcher to differentiate between the specific macronutrients of food (i.e., fat and sugar). Indeed, heightened liking and wanting for food have been noted in overweight and obese individuals (Nijs *et al*., 2010) and those who demonstrate binge eating (Davis *et al*., 2009).

2.6. Exercise and weight loss

Although much attention has been given to the influence of exercise in weight reduction, the effectiveness of exercise alone is still unclear (Pi-Sunyer & Woo, 1985; Gwinup, 1987; Garrow & Summerbell, 1995; Miller *et al.*, 1997; Grundy *et al.*, 1999; Wing *et al.*, 1999; Donnelly & Smith, 2005; Wareham *et al.*, 2005; Franz *et al.*, 2007). In fact, an exercise only approach has previously been cited as being largely unsuccessful (Franz *et al.*, 2007) and having a considerably smaller effect when compared to a combination of dietary restriction and exercise (Pi-Sunyer & Woo, 1985). Further to this argument, the prescription of exercise in these interventions has varied greatly, in terms of intensity, duration, mode and length, all of which could hold an important effect on any potential weight loss.

2.6.1. Effects of exercise intensity

A chronic negative energy balance is required to achieve weight loss, providing energy intake and basal metabolism remain consistent, an increase in exercise expenditure would be sufficient to achieve this, however, the role intensity plays is poorly understood with many conflicting studies. As reported recently, high intensity exercise training can alter fat oxidation rates more greatly post exercise and promote greater change in body composition

as a result (Yoshioka *et al.*, 2001). For this reason, it seems logical that those who partake in vigorous exercise more regularly display lower levels of subcutaneous adiposity (Tremblay *et al.*, 1990). However, when energy expenditure is matched between moderate and vigorous intensity exercise interventions, little difference in the effects on weight loss has previously been observed (Nicklas *et al.*, 2009). Although, where no differences were detected in weight loss, considerable and significant improvements in cardiovascular fitness following high intensity exercise but not low intensity were seen (Nicklas *et al.*, 2009). Greater benefits of high intensity exercise have also been reported in clinical populations, in a study with obese women with metabolic syndrome, high intensity exercise was found to more effectively reduce abdominal fat, subcutaneous fat and abdominal visceral fat (Irving *et al.*, 2008). In a further study with overweight elderly adults, no change in body mass was present but a significant reduction in visceral adiposity was still observed following a 12-week high intensity intervention (Coker *et al.*, 2009). In contrast, no change was seen in the low intensity group giving further evidence towards the greater health benefits of high intensity exercise.

Despite the above benefits of high-intensity exercise, lower attrition rates have been reported in lower intensity exercise interventions. Nicklas *et al*. (2009) observed group attrition rates of 11.1% and 26.7%, following a moderate and vigorous intensity exercise intervention respectively, demonstrating a greater level of compliance in those participants following the lower intensity programme. This finding is important as it illustrates that sedentary, overweight/obese individuals are more likely to participate in a low intensity exercise programme for longer, leading to a healthier and more balanced active lifestyle in the long run. In addition, low intensity exercise has been associated with higher rates of fat oxidation during exercise, promoting changes in body composition by increasing fat loss. In a 3-week

residential weight management study with obese adolescents and energy restriction, participants following a low intensity exercise program experienced significantly greater losses in body mass and fat mass than those following the high intensity program due to increased fat oxidation (Lazzer *et al.*, 2011). Although these results help to promote low intensity exercise, this study was just 3 weeks long and in studies with longer interventions and obese adults these findings have not been replicated (Kanaley *et al.*, 2001; Potteiger *et al.*, 2008), suggesting these effects may only be present at the onset of exercise or perhaps limited to the younger population.

Additionally, a limitation with the research concerning exercise intensity is the variation in classification of intensities, lactate threshold, VO_{2max} and HR_{max} are all used interchangeably between studies. Moreover, the use of energy restriction in some studies and not in others can make drawing conclusions problematic. Overall, taking into account the benefits of high intensity exercise and the important issue of compliance, exercise interventions for the sedentary, overweight/obese must balance these factors to achieve the greatest health benefits and compliance of participants.

2.6.2. Effects of exercise duration

Another important aspect of exercise interventions which must be taken into account is duration, where exercise volume and energy expenditure can be altered to influence body weight regulation. In many cases, a dose-response relationship between exercise duration and weight loss has been documented in healthy individuals (Ross & Jansen, 2001; Ohkawara *et al.*, 2007), it is clear that by increasing exercise duration, more change can be expected in body composition. This relationship has also been observed in intervention studies with

overweight individuals, regardless of age, gender and exercise intensity (Grediagin *et al.*, 1995; Jeffrey *et al.*, 2003; Irving *et al.*, 2008; Church *et al.*, 2009; Coker *et al.*, 2009; Niklas *et al.*, 2009; Jakicic *et al.*, 2011; Lazzer *et al.*, 2011). Furthermore, a dose-response relationship is also seen in clinical populations independent of intensity, greater body fat reduction was observed after high volumes of activity in overweight patients with dyslipidaemia (Slentz *et al.*, 2004).

Of course, the dose-response relationship is dependent on energy intake. An increase in energy intake and an associated decrease in energy expenditure outside of exercise intervention has previously been reported in overweight women. Church *et al.* (2009) go as far as to suggest high volumes of vigorous activity can be counterproductive, inducing less change in body composition than those including far less volumes of exercise because of such compensatory mechanisms. However, it is often difficult to detect compensatory responses to exercise due to the inherent problems associated with energy intake and self-reporting. Nevertheless, it is evident that increasing exercise duration will inevitably promote weight reduction but one must be cautious of the possible compensatory responses often seen in overweight or obese populations, to yield positive results.

2.6.3. Types of exercise

Modality is a further consideration which may alter the effect of an exercise intervention, exercise can be prescribed in either a continuous or intermittent fashion, where overall volume is kept constant. In a study with overweight females, where continuous and intermittent exercise interventions were directly compared and duration was matched over 12 weeks, no difference in weight loss was observed (Schmidt *et al.*, 2001). Additionally, after 9

months taking part in continuous and intermittent exercise, obese women displayed similar weight loss, however, 18 months after the intervention, continuous exercisers continued to reduce body mass, unlike intermittent exercisers whose body weight returned to pre intervention levels (Jakicic *et al.*, 1999; Donnelly *et al.*, 2000). Individual variation may also be an important factor in the effectiveness of these different types of intervention. In a 32-week intermittent intervention with obese women, body mass reduction was not observed in all participants, some participants reduced body mass as expected but others actually displayed an increase (Snyder *et al.*, 1997). In line with these findings, King *et al.* (2008) also observed similar results in individual variability following 12 weeks' continuous exercise intervention, they suggest individuals who did not experience weight loss or lower than expected, did so because of their compensation responses.

Even though most evidence seems to point towards continuous exercise being more effective at reducing weight than intermittent, as with intensity, compliance is an important issue. Greater compliance to intermittent exercise interventions has previously been demonstrated (Jakicic *et al.*, 1995). This is likely the case as sedentary individuals favour more frequent, shorter periods of exercise, leading to greater exercise energy expenditure, lower attrition rates and greater weight loss. In a further study, Jakicic *et al*. (1999) provided participants with treadmills, to perform intermittent exercise in their own homes; this resulted in the greatest weight loss more so than either intermittent or continuous exercise in a lab based environment. This group displayed the lowest attrition rate and elicited the greatest exercise response, likely due to the more convenient and realistic nature of the intervention, further outlining the importance of compliance in exercise interventions and weight loss.

2.6.4. Length of interventions

As previously mentioned, a dose-response relationship exists between exercise and body weight reduction (Ross & Janssen, 2001; Ohkawara *et al.*, 2007) and in most medium term interventions this is clearly demonstrated, however, the two do not seem to be as strongly linked following long term interventions (Miller *et al.*, 1997). Statistically significant weight loss is often achieved by medium term interventions but in most cases, it is not large enough to be meaningful to clinical populations. Moreover, longer-term interventions often have issues with compliance making body weight reduction difficult to achieve. For this reason, most intervention studies are shorter in duration and focus more on moderately obese, middle-aged participants, as these are less likely to dropout (Miller *et al.*, 1997). Additionally, long term interventions are far less in number because of their intensive nature and high attrition rates, of which, 20% or more is frequently reported (Franz *et al.*, 2007).

Medium term exercise interventions with the overweight/obese, often 3-4 months in length and with high amounts of aerobic exercise, can result in relatively large losses in body weight (Leon *et al.*, 1979; Bouchard *et al.*, 1990). Although, upon closer inspection the predicted weight loss, calculated from exercise energy expenditure, can be far greater than the actual weight loss, only 50% of predicted where no compensatory rise in self-reported energy intake was witnessed (Leon *et al.*, 1979). However, when energy intake is more tightly controlled, with the use of a residential experimental design, 100% of predicted weight loss can be achieved (Bouchard *et al.*, 1990). The increased likelihood of weight loss comes at a price nonetheless, with the cost of such experiments, only very small sample sizes are feasible. In contrast, where are more realistic approach is used higher samples can be obtained but the attrition rate can also be higher.

Medium term interventions do not always result in body fat/weight loss, even where exercise energy expenditure was sufficient to achieve negative energy deficit. In spite of this, self-reported energy intake is still usually reduced in these studies (Barwell *et al.*, 2009), pointing towards inaccurate energy intake data. Moreover, further interventions studies which failed to achieve weight loss, report alterations in physical activity outside of the exercise intervention, once again preventing positive outcomes from exercise (Manthou *et al.*, 2010). Both factors seem to add evidence to the presence of compensatory mechanisms present in overweight/obese populations, which obstruct any potential weight loss from changes in physical activity. Consequently, evidence suggests that medium term interventions do display moderate success in promoting body weight reduction but the responses to exercise can have a high degree of individual variability.

Long-term interventions, in excess of 6 months and often a year or more in length, in contrast to medium term interventions, do not portray exercise as a very effective method of weight loss, often very little change in body composition is reported. In a 6-month study with obese men and women, fat mass was reduced by only 3kg, following a low intensity intervention (Bjorntorp *et al.*, 1973). However, exercise energy expenditure was not measured so results cannot be compared to expected change. In a further study with overweight men and a 6-month exercise intervention, energy expenditure was tracked this time but once again only modest weight loss was seen, 40% of expected values in fact (Turner *et al.*, 2010). Additionally, following a moderate intensity exercise intervention with overweight women, body mass declined by only 0.5kg in 3 months and 1.4kg after 12 months (Foster-Schubert *et al.*, 2005). Even when a higher volume of exercise was used in the same population, similar findings were observed (Bjorntorp *et al.*, 1973). Moreover, in a study where body mass reduced but not significantly after 3 months of exercise, after an additional 6 months this

small reduction was regained. Further investigation by the authors revealed that the participants with the lowest body fat, demonstrated the greatest fat loss and participants with the highest actually gained body fat (Spalding *et al.*, 2008).

However, outcomes following long-term interventions are not always so bleak, significant fat loss and wider health improvements have been reported. A yearlong moderate intensity exercise intervention with overweight men, significantly reduced fat mass by approximately 4kg, in two studies performed by the same research group (Fortmann *et al.*, 1988; Wood *et al.*, 1988). Although this fat loss may seem modest, participants also displayed significant improvements in blood pressure and lipid profile. In addition, similar improvements were seen in health markers when energy restriction was employed in place of exercise, suggesting benefits are primarily associated with reductions in fat mass not by exercise alone. Furthermore, significant fat loss after one year was observed in lean and overweight men and women following an exercise intervention, where exercise energy expenditure was monitored and designed to bring about a deficit of 20% by the end of the intervention (Weiss and Holloszy, 2007). In parallel to the exercise intervention, a separate energy restriction trial was run and although similar fat losses were reported, a concomitant loss of lean mass was observed. The relative success of this intervention is likely due to the measurement of energy expenditure, which induced a controlled energy deficit and along with the maintenance of lean mass, outlines some key recommendations for lifestyle interventions.

2.7. Exercise in lean versus obese individuals

It has been acknowledged that the obese population tend to have an impaired ability to oxidize fat during rest and exercise. A study by Ranneries *et al*. (1998) comparing formerly-

obese (FO) women with weight equivalent never-obese women found that the FO had lower fat-oxidation during rest (1.32 vs. 3.70 kJ/min^{-1}, P<0.05), higher respiratory quotient (RQ) at rest (0.84 and 0.74, P<0.05), and a lower resting energy expenditure (REE) (3.77 and 4.88 kJ/min^{-1}, P <0.05) than the never-obese individuals. These factors indicate an impaired uptake and utilisation of fat sources in formerly-obese individuals, which may contribute to a positive energy balance, subsequent weight gain, and consequently obesity. In addition, Perez-Martin *et al*. (2001) compared substrate utilisation after a 12 hour fast, during a range of exercise intensities in both obese and matched-lean individuals (matched for age and maximal aerobic power). The findings show obese individuals have significantly lower maximal fat oxidation intensity than matched-lean individuals, also the fat oxidation was lower for all intensities assessed (30, 40, 50, and 60% $\dot{V}O_{2max}$). In addition, the substrate crossover point was significantly lower in the obese vs. matched-lean individuals (33.3% and 50.1% $\dot{V}O_{2max}$, P< 0.001). These findings represent an impairment of fat oxidation ability and a sooner carbohydrate dependency during exercise in the obese.

A lower capacity for fatty acid oxidation may be related to an altered mitochondrial function, caused by an increased fatty acid transport and intramuscular lipid accumulation documented in obese muscle, resulting in a greater amount of FAT/CD36 permanently at the plasma membrane (Hawley and Zierath, 2008). A greater amount of these proteins at the plasma membrane decreases the amount located nearby to the mitochondrion, as is normal in rodent (Campbell *et al*., 2004) and control human muscle (Bezaire *et al*., 2006; Holloway *et al*., 2006). Evidence for the role of FAT/CD36 in this pathology has been documented by research blocking FAT/CD36 in isolated mitochondria, resulting in a reduced long-chain, but not short-chain, fatty acid oxidation (Bezaire *et al*., 2006), furthermore (Campbell*et al*., 2004; Holloway *et al*., 2006). Although the mechanisms of how FAT/CD36 supports fatty acid

oxidation at the mitochondrion is unknown, it appears that these proteins are involved in not only transporting long-chain fatty acids into the muscle cell, but also into the mitochondrion, as highlighted by the good correlation between FAT/CD36 at the mitochondrion and the rate of fatty acid oxidation during exercise (Holloway *et al*., 2006).

Chapter 3: General Methods

The following chapter describes the general methodology commonly applied in the studies within this thesis. The current thesis comprises two experimental studies. Methods specific to each study are not reported here and they will be presented separately in each of the pertinent studies.

3.1. Participant recruitment and subjects

All participants were recruited from within Bangor University email system intranet (emails, discussion forums and the notice board) and the general public by posters, leaflets and/or word of mouth (Appendix 1 for Chapter 4; Appendix 2 for Chapter5). Full written information was given to potential study participants, who expressed an interest in taking part in the study via phone or email; this provided information regarding the purpose of the study, the experimental procedures and any potential risks or discomfort that could be experienced (Appendix 3 for Chapter 4; Appendix 4 and 5 for Chapter 5). Following this, participants were given verbal explanation of the experimental procedures and provided with the opportunity to ask any questions. Each participant then signed a statement of informed consent (Appendix 6 for Chapter 4; Appendix 7 for Chapter 5) and completed a health screening and physical activity questionnaire (Appendix 8 for Chapter 4; Appendix 9 for Chapter 5) for the assessment of our inclusion criteria. In both studies, after completion of baseline measures, L and OV/OB participant were primarily assigned to either a HI or a MI group in a random order. Randomisation was performed by allocating a particular number to each participant, and the numbers were placed in a sealed envelope. Then the numbers were singled out haphazardly. We had an equal distribution of HI and MI in each group. Participants were recruited to the study only if they met the following criteria:

i) Female sedentary, here defined as not being involved in regular strenuous physical activity more than one time per week or light exercise more than 20 minutes a day, 3 days per week (Martins *et al*., 2010).

ii) Aged 18-40 years old.

iii) Not pregnant.

iv) Non-smoker.

v) Without any cardiovascular, metabolic, or pulmonary disease, such as diabetes mellitus, hypertension etc. CVD or DM absence was determined by self-report health questionnaire.

vi) Not on weight reducing diet/therapy/nutritional supplements. Nutritional supplements as protein, creatine, or specific diet products (For Chapter 4).

vii) Participants who express no intention to lose weight (For Chapter 4).

viii) Classified as either lean (BMI: 18 to 24.9 kg/m^2) or overweight/obese (BMI: 25 to 39.9 kg/m^2).

Grouping of lean and overweight/obese subjects was performed according to generally accepted BMI criteria (Shah and Baverman, 2012). The mean BMI of the overweight/obese group was 30.27±3.66; therefore, according to the BMI criteria, our mean represents an obese subject group. The term overweight/obese is used due to the inclusion of participants who have been close to the border of BMI 30; therefore, we use the broader term.

3.2. Anthropometry

All measurements were conducted in the physiology and psychology laboratories in the School of Sport, Health & Exercise Sciences. Measurements for height, weight, body mass index, and body composition were made as follows;

i) Height – measured to the nearest 1 cm using a wall-mounted stadiometer (Bodycare Products, Southam, United Kingdom). Participants removed their footwear and stood flat footed with their heels against a back plate.

ii) Weight – measured to the nearest 0.01 kg using a calibrated balance beam (Seca, Hamberg, Germany). Participants removed footwear, wore light clothing, and removed items from their pockets before stepping onto the scale.

iii) Body mass index (BMI) – calculated by dividing the participants weight in kilograms by the square of their height in metres ($kg.m^{-2}$).

iv) Body composition, for the 8weeks study, was measured using Dual Energy X-ray Absorptiometry (DEXA) (QDR-4500, Hologic, Inc., Waltham) for lean mass, fat mass, percentage fat mass, and bone mineral density by a trained professional. Participants removed footwear, wore light clothing without metal items, and removed all objects from their pockets before completing a pre-scan questionnaire and consent form. DEXA scans are a validated method for measuring body composition (Pietrobelli, Formica *et al.*, 1996, Prior, Cureton *et al.*, 1997). But regarding body composition for the bout studies, it was measured by bioelectrical impedance analysis (InBody 230, GE Healthcare, USA), giving rise to percentage fat and lean mass. This change was necessary due to alterations in the radiation policies of governance.

3.3. Cardiorespiratory fitness assessment

For the determination of maximal oxygen uptake ($\dot{V}O_{2max}$; ml.kg^{-1}.min^{-1}) participants performed a progressive exercise test using an electronically braked cycle ergometer (Corival 400, Lode, Groningen, Netherlands). The test commenced with a 3 minute seated measurement followed by a minute cycling at a workload of 25watts (W) which increased to 50W, the resistance then increased 20W every minute until the voluntary experienced volitional exhaustion. Heart rate (Polar F1 - Polar Electro Oy, Kempele, Finland), using short range telemetry, and ratings of perceived exertion (RPE), using the Borg Scale of 6 (indicating no exertion) to 20 (indicating maximal exertion) (Borg, 1998), were recorded during the last 15 seconds of each stage. Expired gas fractions of oxygen and carbon dioxide were measured continuously throughout the incremental test, using online spirometry analysis (Oxycon Pro, Erich Jaegar, Germany). Previously the system was validated as an accurate tool for measurements of metabolic parameters during low and high-intensity exercise (Rietjens, Kuipers *et al.*, 2001). In addition, the aforementioned data was analysed to determine RER and energy expenditure (kcal.min^{-1}) via the online spirometry system. Since the participants were sedentary and untrained, all voluntaries ceased cycling before achieving a true $\dot{V}O_{2max}$; therefore, $\dot{V}O_{2Peak}$ was used as a valid measure of aerobic power. $\dot{V}O_{2Peak}$ was realized when either of the following criteria was satisfied: a final respiratory exchange ratio (RER) greater than 1.1, RPE of 20 or cadence less than 60rpm.

3.4. Resting metabolic rate respiratory exchange ratio

Both resting metabolic rate (RMR) and respiratory exchange ratio (RER) measurements were carried out in the physiology laboratory in the School of Sport, Health & Exercise using the Oxycon system (Jaeger ER500, VIASYS Healthcare GmbH, Hoechberg, Germany). Testing

was conducted in the early morning (between 7.00 am and 9:30 am) following 12 hours overnight fast, controlled temperature (19-20°C), with minimal ambient noise interference to provide a calm and quiet atmosphere, while the participant listened to classical music via headphones. The measurement was performed in a supine and comfortable position for 30-mins while electro-cardiography (ECG) was monitored during the test for preliminary screening purposes, (heart rate, heart rhythm) and not recorded. Additionally, resting heart rate (HR) values were recorded throughout the test. Prior to the testing participants were fitted with a polar coded transmitter and receiver (Polar F1 - Polar Electro Oy, Kempele, Finland). Furthermore, the participants were monitored throughout the measurement to ensure that sleeping did not occur.

3.5. Test meal

A test meal was conducted to measure postprandial response of glucose and appetite-related hormones for the 8 week investigation (Study 1) along with subjective appetite rate response for the bout investigation (Study 2). Participants were given a liquid test meal (Resource® Energy, Nestle Healthcare Nutrition) consisting of 300 kcal, 42g of carbohydrates (of which: sugars 11.4g; lactose <1.0g), 10g of fat (of which: saturates 1.4g; monosaturates 3.8g; polyunsaturates 4.6g), and 11.2g of protein. This test meal was chosen to avoid variability in intake composition and processing known from other test meal paradigms. Participants were informed to consume the test meal as soon as possible and the exact time of consumption was recorded for subsequent measurements.

3.6. Blood sampling and storage

Venous blood samples were drawn from an antecubital vein using standard venepuncture techniques (Vacutainer Systems™, Becton, Dickinson) by a qualified phlebotomist. Samples were collected into two vacationers (2 x 6mL) containing ethylenediaminetetraacetic acid (EDTA). Whole blood was immediately analysed for measuring fasting and postprandial glucose (except for the main bout study in which merely fasting glucose was measured) (Accu-Chek Aviva, Mannheim, Germany) and blood lipid profiles including total cholesterol, triglycerides and high-density lipoproteins, via the reflotron plus system (Reflotron, Roche, Germany). Low-density lipoproteins were calculated using the formula LDL= TC – HDL – (TG/2.17) (Friedewald, 1972). The samples were then centrifuged for 10min at 3000rpm, 4^0C (Eppendorf Centrifuge 5810R; Hamburg, Germany) to separate plasma and frozen at -80°C until further analysis.

3.7. Blood parameters

Upon completion of data collection, the frozen plasma samples were analysed to determine appetite-related hormones concentrations of amylin, total ghrelin, acylated ghrelin, leptin, insulin and total PYY. Enzyme-linked immunosorbent assay (ELISA) and kits plate reader (Fluostar Omega, BMG Labtech, Germany) were used to measure the hormones. ELISA measurements of amylin (Linco Research, St Charles, MO, USA), total ghrelin (Millipore; St. Charles, MO, USA), acylated ghrelin (BioVendor , Heidelberg, Germany), insulin (Mercodia, Uppsala, Sweden), Leptin (Biovendor, Heidelberg, Germany), total PYY (Millipore Corporation, Billerica, MA, USA) were carried out as instructed in kit manuals. The Homeostasis Model Assessment (HOMA) (www.dtu.ox.ac.uk/homacalculator/) was conducted to estimate steady state beta cell function (%B), insulin resistance (IR) and insulin

sensitivity (%S) by calculating fasting plasma insulin (µIU/mL) x fasting plasma glucose ($m.mol^{-1}$) (Wallace*et al*., 2004). All sample measurements were performed in duplicate. Any values out of range, meaning either, a) samples contained no, or below detectable levels; or, b) samples containing concentrations greater than the highest standard point available, were discounted from the final data analysis

It should be noted PYY_{3-36} concentrations were measured using ELISA kit (Phoenix Pharmeceuticals; Karlsruhe, Germany). The measured values were all outside the expected range being above the expected value of measurements taken from alternative testing kits (King *et al*., 2011 and Deighton *et al*., 2013). Therefore, cross reactivity of the assay with other plasma proteins could not be excluded and the data are not reported in this thesis accordingly.

3.8. Preparation of plasma for ghrelin analysis

Plasma samples intended for total and acylated ghrelin determination were removed from -80 oC storage. After thawing plasma samples (200 µl plasma for each) were treated with 2.5µl PMSF (phenylmethanesulfonylfluoride) solution (250 µM) to prevent protease degradation (Hosoda, Doi et al. 2004). Following this, 200 µl of treated plasma was added to new vials with 10 and 20 µl HCL for measurement of total and acylated ghrelin respectively. After the addition of HCL all samples were centrifuged for two minutes at 19064 x g. Finally, total ghrelin samples were ready to use but acylated ghrelin samples were further diluted 1:5 with assay buffer (50 µl plasma 200 µl assay buffer) prior to following the rest of standardised ELISA procedures.

3.9. Implicit association task (IAT)

The implicit association task (IAT) is a computer-based mechanism to measure (indirectly) the fortitude of unions amongst ideas. The traditional implicit association task IAT is one of the most reliable and preferred measures of implicit attitude, expanded by (Greenwald, McGhee *et al.*, 1998) and altered by Dr Dave Markland, Bangor University. The task typically comprises four classes of stimuli, two target categories – being concept stimuli, for instance names of flowers/insects and the remaining couple being attribute stimuli, for example negative/ positive words. Individuals were required to classify the stimuli according to a target dimension (e.g., high-sugar foods vs. low-sugar foods) or an attribute dimension (e.g., positive vs. negative) by depressing one of the return button as quickly and correctly as feasible. Each return button is aligned to one target and one attribute category. Combination phases of the IAT was critical phase of the IAT where participants perform a double categorisation test; one from the target dimension and one from the attribute dimension (e.g., depress the left response keys for high-sugar foods and positive words; press the right response keys for low-sugar foods and negative words). In the other combination phase, the response assignment is reversed for the target dimension (e.g., press the left response keys for low-sugar foods and positive words; press the right response keys for high-sugar foods and negative words). The IAT test therefore assumes that individuals are able to respond quicker in a behavioural way (pressing a key) when presented with concepts with a stronger association rather than a weaker one. For example, food that contains low sugar food and

positive words have a stronger link than high sugar foods and positive words, therefore respondents are more likely to respond quicker to the stronger association.

3.10. Stimuli and measuring IAT

In the present study, two IATs including high fat-low fat (HF-LF) and high sugar-low sugar (HS-LS) (presented together) were used to assess subject's implicit attitude toward sugary and fatty food. Two target categories were sugary (high sugar, low sugar) and fatty food (high fat, low fat) and two attribute concepts, which were negative (e.g. horrible, bad) and positive (e.g. pleasant, lovely) words. The visual food stimuli, which was used for this test, were selected from a database of photographs gathered from the Internet. The concept stimuli contained 24 colour photographs classified into two divisions including sugary food (high sugar, low sugar) and fatty food (high fat, low fat). Each category was represented by 12 different foods (Appendix 10). Furthermore, "Good" and "Bad" were used as attribute concepts in the tests which are made of 8 positive words (e.g. pleasant, lovely) and 8 negative words (e.g. horrible, bad) (Appendix 11). In order to generate the IAT, the inquisit 3.0 software suite (millisecond Software, 2008) which presents stimuli on a computer screen and records response behavior to pressing a keyboard – with accuracy to the millisecond. The IAT used in the current study consisted of seven blocks (Table 3.10), three of which (block 1, 2 and 5) were practice blocks (20 trials each) to acquaint participants with the categorization rules. The third and fourth blocks, which are labelled the incompatible blocks, numbers 20 and 40 respectively, are the critical test blocks where high fat food pictures were coupled with positive words labeled incompatible blocks, where high fat food pictures were paired with positive words on one response key and the low fat food pictures with the negative words on another response key, and the sixth and seventh blocks (20 and 40 trials

respectively), labeled compatible blocks, in which these pairings were reversed. Prior to each block, the instructions for the classification task were presented on the screen. If participants pressed the incorrect key, a red "X" appeared on screen and remained until a correct response had been made by the participants before moving on to the next stimulus presentation. The pictures and words stimuli were presented randomly without replacement within blocks, independently for each participant. In both IATs; order of presentation of compatible and incompatible blocks were counter-balanced between participants. Trials were separated by a 250ms inter-trial interval. The D-score algorithm for IAT data (Greenwald, Nosek *et al.,* 2003) was used to determine an IAT score from the difference between performance on compatible and incompatible test trials, gathered by recording response latencies. Upon the completion of the experiment a score was indicated, they may be positive or negative, depending on an individual's attitude towards a specific food, for example 0 represents a neutral attitude, in this case (high fat, low fat or high sugar, low sugar) and positive and negative scores indicating positive and negative implicit attitude toward (high fat, low fat or high sugar, low sugar). The mentioned IATs were used to assess the implicit attitudes of the participants towards all four food categories. Prior to the actual experiment a pilot testing was conducted with volunteers from the School of Health and Sport Science to make sure the mentioned food pictures were recognizable and the IATs enabled to measure the implicit attitudes of the participants towards four food categories.

Table 3.10. Experimental procedure of both IATs (counter-balanced order; based on Greenwald *et al.*, 2003).

BLOCK	Function	Trials	Items accredited to left-key Response (E)	Items accredited to right-key Response (I)
1	PRACTICE	20	High sugar/fat foods	Low sugar/fat foods
2	PRACTICE	20	Positive words	Negative words
3	Measure	20	High sugar/fat foods+ Positive words	Low sugar/fat foods+ Negative words
4	Measure	40	High sugar/fat foods+ Positive words	Low sugar/fat foods+ Negative words
5	PRACTICE	20	Low sugar/fat foods	High sugar/fat foods
6	Measure	20	Low sugar/fat foods+ Positive words	High sugar/fat foods+ Negative words
7	Measure	40	Low sugar/fat foods+ Positive words	High sugar/fat foods+ Negative words

3.11. IAT procedure

The present study included the use of two IATs performed; the first one was HS-LS and the second IAT replacing fat with sugar was presented immediately after the sugar version. The second IAT format was identical to the first. The test was explained to the participants that the experiment would include two computer-based tasks. To avoid any possible participants 'suspicion, no further information was given about the purpose of the test a planned degree of deception was used; the participants were told that the experiment was designed to measure the participant's cognitive functioning based on reaction time performance. The participants were required to categorize the displayed stimuli as quick and as accurately as possible, according to a concept or an attribute dimension, by pressing the corresponding key. Prior to the test day, to avoid any influence of being on an empty stomach or in a satiated state on the IAT results, the participants were instructed to consume a meal 2 hours prior to the measurement, before arriving for the test day. The test took place in a small and quiet lab.

Each participant completed and the test individually. The IAT began with a display and directions for the participants on how to do the test on the computer as it continued.

3.12. Explicit attitude test

The explicit attitude questionnaire (EAQ) was used to measure explicit attitude towards the fatty and sugary food (Courneya & Bobick, 2000). The original use for the questionnaire was to determine the instrumental and affective components of an individual's attitude towards exercise (Instrumental: useful-useless, wise-foolish, harmful-beneficial, good-bad; Affective: enjoyable-unenjoyable, interesting-boring, pleasant- unpleasant, relaxing-stressful). The questionnaire is based on the 7- point bipolar rating scale from 'extremely=1' over 'neither=4' to 'extremely=7'. The sum total will, therefore, range from between 8 and 56. Accordingly, the higher scores indicate more positive explicit attitudes towards the specific food (fatty or sugary food) and vice versa. In the current study, 4 EAQs including high fat, low fat, high sugar, and low sugar were administered, providing information about explicit attitudes towardseach specific food category. The test was carried out after the IAT was conducted. Types of food were rated by participants according to their attitude towards it using the given instrumental and affective components (Appendix 12).

Chapter 4: Effects of an 8-week Supervised Exercise Intervention on Appetite, Metabolic and Food Intake in Overweight/obese and Lean Sedentary Females

4.1. Introduction

The current obesity epidemic has been growing at such a rapid rate that the World Health Organization (WHO) has formally recognised it as a global epidemic, being particularly prevalent amongst women (WHO, 2015). Current statistics reveal that > 1.9 billion adults are overweight, and >600 million are reported clinically obese, as a result of prevalence in the UK increasing threefold since 1980 (World Health Organisation, 2015). The health implications of excess adiposity have been well documented, predisposing individuals to a greater risk of numerous co-morbidities such as; type II diabetes mellitus, hypertension, coronary heart disease, osteoarthritis, respiratory problems and cancers of breast, endometrium, prostate and bowel (Neary *et al*., 2004), with one or more conditions currently suffered by ~80% of obese adults (Must *et al*., 1999). As the prevalence of obesity continues to rise globally, effective strategies are needed to facilitate weight control, emphasized by the fact that even modest weight loss is capable to significantly reduce the risks of morbidity and mortality of overweight and obesity (Murphy and Bloom, 2004).

Exercise is repeatedly recommended as an effective means to tackle the obesity phenomena (Donnelly *et al*., 2009). However, as previously discussed, although an exercise program accompanied by *ad libitum* dietary control often results in weight loss (Ross *et al*., 2000; 2004), the magnitude of weight loss achieved is lower than the expected weight loss from a particular exercise program (Leon *et al*., 1979). Furthermore, individual variability is present in body weight changes following exercise training, highlighting the need for more individualised training programs (King *et al.*, 2007, 2008; Barwell *et al*., 2009). Possible

causes of less than expected weight outcomes are suggested to be: modified appetite, perceived reinforcement value of food and altered unstructured physical activity (Blundell *et al.,* 2003, King *et al.,* 2007, Church *et al.,* 2009). In consequence, the concept of compensators and non-compensators of negative energy balance has been established, although the mechanisms behind the individual variability are currently unknown (Finlayson *et al.,* 2009). Clearly, homeostatic and non-homeostatic (hedonic) mechanisms on various levels are involved but the amount of their contribution and what particular systems are responsible for the individuals' responses to exercise training remains difficult to examine and to predict. It is therefore crucial to determine the role of exercise in affecting energy intake and consequently body weight by the influence of homeostatic and hedonic aspects.

Adopting a sedentary lifestyle and consequently maintaining a poor cardiorespiratory fitness, is a major contributor to the increasing incidence of morbidity and mortality associated with cardiovascular disease (Durstine, 2001; Thompson, 2001), the development of type 2 diabetes (T2D) (Hielbronn, 2007; Tuomilehto, *et al.,* 2001), and obesity (Daniels *et al*., 2009). Research studies have identified a link between the cardiorespiratory fitness of an individual, and a tighter coupling between energy intake and energy expenditure, suggesting an increased sensitivity within the physiological mechanisms involved in appetite control in more exercised individuals (King *et al*., 1997). This hypothesis is demonstrated in the findings from Long *et al*. (2002), whereby, a better short-term appetite response to covert preload energy manipulation was present in active men, when compared to sedentary counterparts. The sedentary group were unable to adjust subsequent energy intake in response to either a high- or low- energy preload in *ad libitum* buffet meals 60 minutes following the exercise, but the active men were able to decrease subsequent energy intake to an almost exact (90%) amount.

The current body of literature regarding the effect of exercise on appetite control regulation is rather limited. The majority of research studies conducted are inclusive of lean, healthy individuals, regularly students of the university (King *et al., 1997; 2007,* Finlayson *et al*., 2009). Furthermore, the majority of studies conducting chronic exercise training in overweight and/or obese individuals suffer from many confounding variables, such as the control over participants' motivation to lose weight. Without adopting deceptive techniques to keep participants naïve to the aims of the weight loss study, there is increased bias in the results, skewed towards a false-positive from the participants' own desires to restrain food intake and achieve weight loss, thereby limiting our understanding of the mechanisms involved in appetite regulation.

The inability of long-term exercise to consistently yield significant weight loss has previously been attributed to the adoption of compensatory eating behaviour (Mayer, 1953) and changes in food selection and reward (Finlayson *et al*., 2009). Hopkins *et al*. (2014) found that fat mass and fat-free mass were positively associated with explicit liking for high fat foods, but both implicit liking and wanting were only associated with fat mass (r= 0.341, r= 0.414 respectively), suggesting that fat mass may predict food reward independently of fat free mass. Such findings are consistent in the literature, whereby separate roles for fat-free mass in satiation (Blundell *et al.,*2012) and hunger (Caudwell *et al*., 2013) and fat mass in hedonic eating behaviour traits (O'Neill, 2012) and neural activation to high energy foods (Luo *et al*., 2013) are seen. However, these findings are currently limited to the obese population and comparisons with lean individuals warrants investigation.

The increase in the susceptibility of obese individuals to overconsume may also be related to their tonic signals. Increased leptin and leptin resistance as a consequence of excess adiposity may reduce their sensitivity to short term appetite control (Flint *et al*., 2007). Indeed, Hopkins *et al*. (2014) show cross-sectional associations between fasting leptin and explicit liking and implicit wanting for high fat foods relative to low fat foods, and demonstrate negative relationship whereby a decline in fasting leptin is associated with an increase in explicit liking for high fat foods relative to low fat foods. Taken together, these findings suggest a dynamic role for fasting leptin as a regulatory signal of food reward during exercise-induced weight loss. In addition, substrate availability may influence eating behaviour, and given that whole-body carbohydrate sources are stored in relatively small quantities (400-800g) (Bjorntorp and Sjostrom, 1978), its depletion may induce a high priority signal for its restoration. In fact, decreases in carbohydrate availability have been linked to meal size and frequency (Finlayson *et al*., 2009), conversely, fatty acid oxidation has consistently failed to show an effect on eating behaviour (Langhans, 2008). This may have implications for obese individuals' post-exercise eating behaviour since obesity is characterized by an impaired ability to oxidize fat during rest and exercise, and a lower intensity-substrate crossover point when compared to lean individuals indicating an impairment of fat oxidation and a greater dependency on carbohydrate energy sources during exercise (Perez-Martin *et al*., 2001).

Considering this, the current study will aim to investigate the effects of an 8-week exercise training intervention between lean and obese populations on body composition, endocrine adaptations, hedonic responses and compensatory responses in females. To our knowledge, by deceiving participants on the study aims, we are the first study to avoid the influence of internal motivation to lose weight and expectations about outcomes, providing more control

over the potential bias towards weight loss in studies of this nature. The study hypotheses are as follows;

Hypotheses:

Weight/BMI changes

1. Overweight/obese and lean females would regulate their body weight successfully leading to no weight/BMI change after the training intervention

Appetite hormones

1. We hypothesize that there will be no change in fasting or postprandial ghrelin concentrations for both lean and obese groups following the 8-week intervention.
2. We hypothesize that there will be no change in fasting or postprandial insulin concentrations for both lean and obese groups following the 8-week intervention.
3. We hypothesize that there will be no change in fasting or postprandial leptin concentrations for both lean and obese groups following the 8-week intervention.
4. We hypothesize that a significant decrease in fasting and postprandial amylin concentrations will occur in the obese group, and either no change, or an increase in amylin concentrations in the lean group, following the 8-week intervention.
5. We hypothesize that a significant decrease in fasting and postprandial PYY concentrations will occur in the obese group, and either no change, or an increase in PYY concentrations in the lean group, following the 8-week intervention.

Individual variability

1. We hypothesize that the lack of weight loss in the groups will include a large degree of inter-individual variability.

Hedonic hypothesis (IAT and EAQ)

1. We also hypothesize that there would be higher implicit attitude scores towards high-fat and high-sugar foods in response to exercise in the obese group as compared to lean group at post-test.
2. It was also hypothesized that there would be higher explicit attitude scores towards low-fat and low-sugar foods in both groups.

Correlation between attitude, body composition and hormones

1. It is hypothesized that there exists a significant positive correlation between change in IAT and change in fat mass.
2. Whereas a significant negative correlation between fat intake and EAQ score for low-fat foods is predicted.
3. We hypothesize a significant positive correlation between ghrelin concentrations and implicit and explicit wanting and liking for high fat foods.

4.2. Methods

4.2.1. Ethical approval

Ethical approval for the research study was obtained by Bangor University, Faculty of Sport, Health and Exercise Sciences (SSHES) and the North Wales Research Ethics Committee – West (Ysbyty Gwynedd Hospital, Betsi Cadwaladr University Health Board– REC No 11/WA/0321 and 12/WA/0118).

4.2.2. Participant recruitment and subjects

The general required procedures for recruitment and inclusion criteria are explicated in Chapter 3.1. Subjects were classified lean (L) and overweight/obese (OV/OB) with BMI of 18 to 24.9 kg/m^2 and 25 to 39.9 kg/m^2 respectively. During the initial screening and prior to any testing, only participants who expressed no desire to lose weight via qualitative questionnaire were eligible to take part in the study. If participants were found to be taking part in any form of dieting (evident from the screening questionnaire) including the use of nutritional supplements (any supplement which may confound the objectives of the study, like diets aimed to lose weight, or supplementation to gain muscle mass like protein and creatine supplements), they were excluded. A total of 56 female participants were initially recruited for the study–34 individuals (OV/OB, n= 23; L, n= 11) completed the study, 22 (OV/OB, n= 15; L, n= 7) participants dropped out. Data from two individuals were removed from the study (OV/OB, n= 1; L, n= 1) due to beginning a restrained diet during the intervention. For baseline characteristics of groups see table 4.3.1. All participants who fully completed the study were provided with a pair of trainers up to the value of £100.

4.2.3. Study design

This study used a single blind, repeated measures design for conducting a longitudinal training intervention. The study investigated the effects of an 8-week supervised exercise intervention (circuit training exercise 50-95% VO_{2peak}, 3 times a week, 45-90 min) in *ad libitum* conditions on weight, appetite and eating behaviour in healthy, sedentary, lean and overweight/obese women. The study was completed in three separate trials, firstly, OV/OB participants were recruited in trial 1 (n= 12), however due to the high attrition rate of the study (68%), the trial was repeated and further OV/OB participants recruited in trial 2 (n = 11). The third trial was added to investigate the 8-week intervention in lean individuals only (n= 11), utilising the exact methodology from trials 1 & 2.

The study design had a unique feature in its recruitment, the participants were not recruited for weight-loss, given the importance of protecting the real aim of the study (weight, appetite regulation and eating behaviour), a degree of deception was unavoidable, because participants' awareness of the main research purpose might lead to consciously restrict or change their diet which in turn could affect appetite regulation and eating behaviour. Accordingly, during the recruitment, primary screening and throughout the study, the participants were informed the current study was investigating 8-weeks of supervised exercise that may improve their cardiovascular and cognitive function. However, after completion of study, they were thoroughly debriefed and informed about the true aim of the research.

All experiments were performed at pre (baseline) and post 8-week exercise intervention except explicit attitude (EAQ) which was measured only at post intervention to protect the

aim of the study and not to make the participants suspicious regarding real purpose of the study and any possibility of influencing on the participant's diet during the intervention. To analyse any potential changes in diet, participants were instructed to keep record of their diet three days a week (chosen at random) across the intervention, including pre and post intervention. The experiments consisted of anthropometrical measurements, and IAT (visit 1, 4), resting metabolic rate, fasting and postprandial blood sampling, test meal and DEXA scans (visit 2, 5), $\dot{V}O_{2Peak}$ (visit 3, 6) and EAQ (just post-test, visit 6) (Figure 4.2.3).

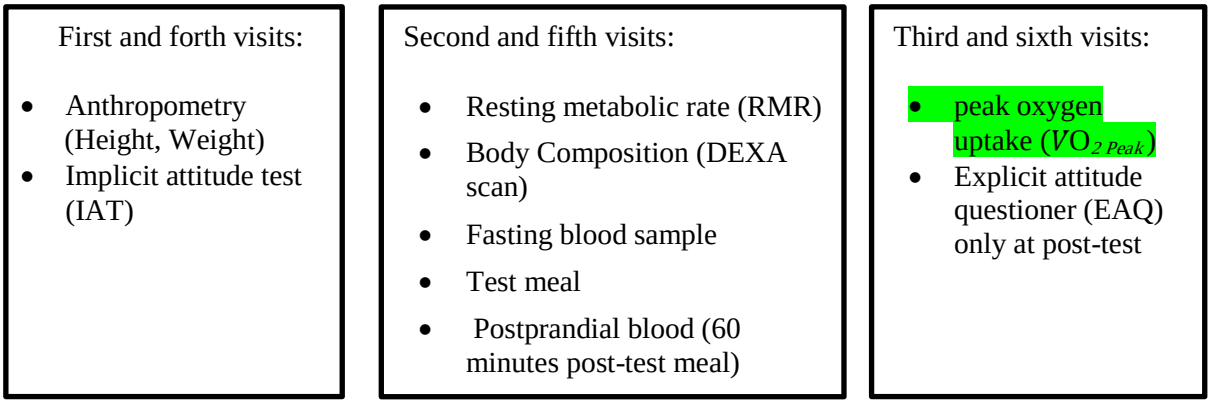

Figure 4.2.3: Protocol schematic.

4.2.4. Study intervention

The intervention consisted of 8 weeks of circuit-based exercise sessions on 3 days per week (Monday, Wednesday, Friday) commencing between 3-5pm. The circuit training protocols consisted of 12 to 16 exercise stations consisting of aerobic and resistance circuit-based exercise which when completed would result in a full body workout (See Appendix 13). All training sessions during the 8-week exercise intervention were supervised by at least 3 members of the research team.

High intensity group exercises were performed in three circuits, and the duration of each station in three circuits was 50, 40 and 30 seconds respectively, with 10 second rest periods between stations. They were given a 2-minute rest period between circuits. Moderate intensity exercises included 3 circuits, with 1-minute rest period per circuit, and no rest between stations, just 5 seconds of transition time between stations. The duration of sessions was dependent on the intensity of the exercise, ranging between 40–90 minutes, to achieve equal energy expenditure across groups. In order to manage training intensity, a telemetric monitoring system (Activio, Activio Sport System, Sweden) plus projector were implemented to display live heart rates to the participants for the duration of the session. This in turn encouraged the participants to remain in the correct zones, corresponding to $\dot{V}O_{2Peak}$. Accordingly, to control exercise intensity (heart rate) the participants were asked to maintain their target heart rate depending on which intensity they were (moderate or high) using the live heart rates displayed by projector via regulation their efforts during all sessions. Additionally, appropriate music of various tempos, helped the participants set the correct pace of movement. However, because many participants were unable to keep to their target HR and high variability in both groups, the data was collapsed into two training groups, (L, n=12, OV/OB, n=23) and exercise intensity (heart rate) was used as a covariate for the later statistical analysis.

To ensure that the exercising groups were matched in terms of energy expenditure during all sessions, the exercise-induced energy expenditure was monitored and regulated on a weekly basis (see energy expenditure and substrate utilization section). Consequently, all exercising groups completed the circuit training program with matched energy expenditure during exercise sessions.

4.2.5. Baseline measurements

Participants were asked to visit to the laboratory on three separate occasions. In the first visit, participant's anthropometric measurements were taken (Chapter 3.2) and implicit attitudes towards food (high fat-low fat and high sugar-low sugar) foods at both pre- and post-test were assessed using the Implicit Association Test (IAT) (See Chapter 3.11). During the second visit, resting metabolic rate (RMR) and respiratory exchange ratio (RER) were measured, following an overnight fasting (Chapter 3.4). Afterwards, body composition was measured using dual-energy x-ray absorptiometry (DXA) by a qualified individual (Chapter 3.2). Finally, fasting and postprandial blood samples were taken (Chapter 3.6). On the third visit, cardiorespiratory fitness was assessed (Chapter 3.3).

All measurements were conducted in the physiology and psychology laboratories in the School of Sport, Health & Exercise Sciences. Baseline measurements were conducted over two weeks prior to commencement of the 8-week exercise intervention with each participant being measured on three separate occasions. All measurements were repeated at least 72 hours following the final exercise session for post-testing. For all visits the ambient room temperature was 19°C.

4.2.6. Explicit attitude test

Explicit attitude towards four types of food (high fat, low fat, high sugar and low sugar) was assessed using the Explicit Attitude Questionnaire (EAQ) (See Chapter 3.12). The EAQ carried out only at post intervention to protect the aim of the study and not to make the participants suspicious regarding real purpose of the study and any possibility of influencing

on the participants 'diet during the intervention. It was administered after the IAT at post-test as a last measurement.

4.2.7. Heart rate dependent measures

Heart rate was measured and recorded at baseline (RMR, $\dot{V}O_{2peak}$), during all training sessions and post intervention for multiple purposes. To establish the proper intensity for each participant using the heat rate obtained during $\dot{V}O_{2max}$ assessment at baseline measurements. Heart rate reserve was calculated using heart rate recorded during RMR measurement and maximal heart rate throughout all training sessions. Additionally; Energy expenditure during all the sessions was calculated using the heart rate and wattage data from the baseline $\dot{V}O_{2peak}$ measurement at different stages of the test and comparing it to the HR recorded during the sessions using a telemetric system.

4.2.8. Blood sampling

Blood sampling was obtained as described in Chapter 3.6. Two blood samples (fasting and post-prandial) at baseline and after the 8-week intervention were collected. Fasting blood samples were taken on arrival at the laboratory, following an overnight fast (after measuring RMR. As for postprandial blood sample, it was obtained 60 minutes after consumption of the test meal (See below).

4.2.9. Test meal

A liquid test meal (See Chapter 3.5) was used to measure postprandial response of glucose and appetite-related hormones following the fasting blood sample. Consumption time was noted and samples of postprandial blood provided exactly 60 minutes later.

4.2.10. Blood parameters

Fasting and postprandial glucose and blood lipid profiles including total cholesterol, triglycerides and high-density lipoproteins using reflotron plus system were measured. In addition, low-density lipoproteins were calculated using the formula LDL= TC – HDL – (TG/2.17) (Friedewald, 1972). All appetite-related hormones including amylin, insulin, leptin, total ghrelin, acylated ghrelin, and total PYY were analysed after completion of post-testing using enzyme-linked immunosorbent assay (ELISA) kits. The Homeostasis Model Assessment (HOMA) (www.dtu.ox.ac.uk/homacalculator/) was conducted to estimate steady state beta cell function (%B), insulin resistance (IR) and insulin sensitivity (%S) by calculating fasting plasma insulin (μIU/mL) x fasting plasma glucose ($m.mol^{-1}$) (Wallace*et al*., 2004) (Chapter 3.7). All sample measurements were performed in duplicate. Any values out of the range were discounted from the final data analysis. The intra assay coefficient of variation for all appetite related hormones are as follows: amylin- 12%, insulin- 7%, leptin- 7%, total ghrelin- 4%, acylated ghrelin- 29% and PYY- 12%.

4.2.11. Energy intake, energy balance, and energy expenditure

Food intake records were obtained weekly using food record sheets. Participants were asked to maintain their current diets for the entire length of intervention. In order to justify our

request for participants to keep and record the food diary, as mentioned in the study design, the participants were informed that food records were needed because of potential effects of diet nutrients on CV performance and cognitive function.

The participants were provided a food record sheet and were instructed on how to complete (Appendix 14). The participants were required to record, the type, brand name, quantity, the location of eating (e.g. restaurants) and timing of their food intake and drink items for the whole week pre-testing period and during 8 weeks training intervention (3-day food diary randomly assigned days including two weekdays and one weekend day) throughout the 8-week duration of the exercise intervention) and a whole week following the completion of the study using food record sheets using standardised instructions (Gibson, 2005). To facilitate and standardize portion size estimates the recording; participants were given a set of measuring cups in order to promote accuracy and reliability of the food intake recording. The participants were asked to return filled food record sheet at the end of each week, rather than at the end of the study. Quantitative data was provided by diet diaries, noting the eating behaviour of individuals and assisted in assessing the alterations in food choice and that may have occurred over the time period of the program, in addition to energy intake, macronutrient and any possible changes resulting from the intervention. The diet diaries were monitored weekly whether participants started a diet which led to exclusion of 1 L and 1 OV/OB data set.

Energy intake and macronutrient composition were analysed by the United States Department of Agriculture, National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) and the Tesco Supermarket online database (http://www.tesco.com/groceries/) as these

provided nutritional composition information on a broad spectrum of ingredients and branded food goods, such as ready meals, that were consumed by participants.

DEXA data was used to calculate energy balance for the duration of the training intervention using the methods outlined by (Thomas, Schoeller *et al.*, 2010). (Eq.1). Energy intake (EI) was calculated (Eq.2), energy expenditure (EE) was also calculated (Eq. 3).

$$EB \approx cl\left(\frac{\Delta LM}{\Delta t}\right) + cf\ \left(\frac{\Delta FM}{\Delta t}\right)$$

Eq.1 LM is equal to 1 kilogramme L mass, FM to 1 kilogramme of fat mass, cl to 1100 kcal/kg and cf to 9300 kcal/kg, t is equal to time. FFM represents kilogrammes of fat-free mass, F to kilogrammes of fat mass and cl is equal to 1100kcal/kg and cf is equal to 9300 kcal/kg are the energy densities of FFM and F, respectively.

Energy expenditure during all the sessions was calculated using the heart rate, oxygen consumption and the power data from the baseline $\dot{V}O_{2\,peak}$ measurement at various test-stages (i.e., 25W, 50W, 70W, 90W, 110W etc.) as well as through the mean HR recorded during the sessions of the telemetric system. The obtained wattage was then used to estimate energy expenditure induced exercise using the linear relationship seen between wattage and energy expenditure from the baseline $\dot{V}O_{2peak}$ test. In order to obtain exercise-induced energy expenditure, total energy expenditure was corrected for RMR. Additionally, 20% of RMR for activities of daily living (ADL) (Roza & Shizgal, 1984) and 10% of RMR for diet induced thermogenesis (DIT) (Westerterp, 2004) were added. Values of energy intake (EI) were calculated (Eq. 2 and 3). The energy expenditure for all exercise session was added together to work out total calories burned during the 8 weeks' intervention.

$$EI = EB + EE$$

Eq.2 Energy intake (EI) energy balance (EB) and energy expenditure (EE).

$$EE = RMR + EEEx + ADL + DIT$$

Eq.3 Resting metabolic rate (RMR); activities of daily living (ADL); energy expenditure from exercise (EEEx); diet induced thermogenesis (DIT).

4.2.12. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics 20. Prior to commencing the statistical analysis, all data were assessed for relevant assumptions: normality, outliers, homogeneity of covariance. To determine whether data are normally distributed, the Shapiro-Wilk test was used. After testing the assumptions of ANOVA, fasting and postprandial insulin, aclayted ghrelin, and amylin (just postprandial) values did not display normal distribution; therefore, the original data was transformed using a logarithm 10 function to proceed) (See Table 4.3.6). As to checking for the probable outliers, we made use of the formula devised by Hoaglin and Iglewicz (1987). One-way ANOVA was used to compare baseline measurements, energy intake, energy expenditure during exercise sessions, total energy expenditure and EAQ between two groups (L and OV/OB). Data were analysed either by ANCOVA using exercise intensity (heart rate) during the 8weeks sessions as a covariate or by two-factor repeated measure ANOVA with one within factor (pre versus post intervention) and one between factor (L versus OV/OB) and appropriate post hoc analysis was used when needed. Pearson's correlations were used to analyse relationships between variables. Multi regression analyses using the enter method were conducted on variables of interest. All data are reported as means ± SD. Statistical significance was assumed at $P<$ 0.05, unless explicitly stated otherwise.

4.2.13 Power calculations

Our power calculations were performed retrospectively, an initial oversight in our design. However, past research had identified that a minimum of 8-9 participants were sufficient to detect significant changes in appetite >10% area under the curve (AUC) (Flint *et al.*, 2000) and appetite hormone parameters, such as acylated ghrelin (Broom *et al*., 2007).

Following the completion of the study, we conducted A priori and post-hoc power analysis with the program *G*Power* (Erdfelder, Faul, & Buchner, 1996) to find out whether our design in study 1 had sufficient power to detect a significant effect on our parameters.In the current study, the significant main effect for amylin was 0.58, classified as a "large effect" (i.e. > 0.4) (Cohen, 1977), as calculated from n^2= 0.257. The post-hoc power analysis output is shown below:

Analysis:	Post hoc: Compute achieved power	
Input:	Effect size f	= 0.5881284
	α err prob	= 0.05
	Total sample size	= 34
	Number of groups	= 2
	Number of measurements	= 2
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 1
Output:	Noncentrality parameter λ	= 47.0417220
	Critical F	= 4.1490974
	Numerator df	= 1.0000000
	Denominator df	= 32.0000000
	Power (1-β err prob)	= 0.9999986

Therefore, Study 1 registered a power of 0.99 with a sample size of 34 participants and 2 measurement time points, however we also conducted an A priori power calculation analysis to determine the minimum number of participants that were required with α= 0.05 to achieve a power of 0.95 with two groups. The output of the A Priori output is shown below:

Analysis:	A priori: Compute required sample size		
Input:	Effect size f	=	0.5881284
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
	Number of groups	=	2
	Number of measurements	=	2
	Corr among rep measures	=	0.5
	Nonsphericity correction ε	=	1
Output:	Noncentrality parameter λ	=	16.6029607
	Critical F	=	4.9646027
	Numerator df	=	1.0000000
	Denominator df	=	10.0000000
	Total sample size	=	12
	Actual power	=	0.9554278

Thus, our A priori power calculations show we require 12 subjects, 6 in each group to achieve a power effect of 0.95.

4.3. Results

This section reports the results of exercise training in L and OV/OB participants in a single blind experimental design. The study was conducted in three separate training trials comprised of three parts. The first and second parts were carried out with OV/OB participants and in the last part of the study only lean participants were recruited. All three trials were carried out over a period of three years, using the same methodology and procedures in the same time span of each year. 34 (OV/OB, n=23; L, n=11) out of 56 sedentary females completed their participation in the 8 week supervised exercise intervention (attrition rate ~39%). Training compliance was 79.17± 15.18 % across the training groups, with no difference between groups. The total energy expenditure from the exercise intervention was approximately matched (~3400 kcal) and there was no significant difference between groups' exercise energy expenditure (Table 4.3.4.1). Statistical significance of $P<0.05$ was applied for all statistical tests.

4.3.1. Baseline characteristics

The baseline body characteristics and blood parameters of the thirty-four participants (mean ± SD) are displayed in table 4.3.1. One-way ANOVA was performed in order to find any differences between group means (L group vs OV/OB group). It was found that the OV/OB group exhibited significantly higher values than their L counterparts for BMI, body weight, body fat, body fat percentage, RMR and lean mass than those of the L group, and lower relative VO_{2peak} (P<0.05).

Table 4.3.1. Anthropometric and metabolic parameters of participants at baseline.

Parameter (units)	L (n=11)	OV/OB (n=23)
Age (years)	24.6 ± 6.9	23.4 ± 5.7
BMI (kg m^{-2})	22.41 ± 2.14	30.27 ± 3.66†
Weight (kg)	63.71 ± 5.60	82.78 ± 11.88†
Fat mass (kg)	19.09 ± 3.54	32.42 ± 7.81†
Fat mass (%)	29.32 ± 4.72	38.74 ± 4.88†
Lean mass (kg)	43.32 ± 3.99	49.13 ± 5.44†
Lean mass (%)	67.46 ± 4.41	59.75 ± 4.74
Fasting glucose (mmol l^{-1})	4.51 ± 0.44	4.56 ± 0.42
Total cholesterol (mmol l^{-1})	3.59 ± 0.56	3.85 ± 0.83
HDL (mmol l^{-1})	1.68 ± 0.41	1.51 ± 0.47
LDL (mmol l^{-1})	1.75 ± 0.63	2.24 ± 0.71
VO_{2peak} (l min^{-1})	2.92 ± 0.56	2.64 ± 0.49
VO_{2peak} (l $min^{-1}kg^{-1}$)	45.89 ± 0.85	32.58 ± 6.27†
RMR (kcal d^{-1})	1361.35 ± 178.86	1619.15 ± 318.86†
RER	0.79 ± 0.07	0.77 ± 0.06

Data presented as mean ± SD. † represents significant differences (P < 0.05) between body types (OV/OB vs. L). L, lean; OV/OB, overweight and obese; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; VO_2peak, peak oxygen uptake; RMR, resting metabolic rate; and RER, respiratory exchange ratio.

4.3.2. Anthropometry

The mean values (*M*) and standard deviations (*SD*) for all anthropometric data and blood parameters for pre-test and post-test are shown in table 4.3.1. Two factor mixed-model ANOVA analysis revealed that both OV/OB and L groups resulted in no significant changes in body weight, BMI, RMR, RER, or $\dot{V}O_{2peak}$ from baseline. A significant change was reported in fat mass in L participants (F=7.95, η^2=0.21, P=0.008) whereby body fat % decreased significantly, whilst no change was reported in OV/OB individuals.

4.3.3. Metabolic and aerobic measures

Two factor mixed-model ANOVA analysis revealed that both OV/OB and L groups resulted in no significant changes in body weight, BMI, RMR, RER, or $\dot{V}O_{2peak}$ (P>0.05) from baseline (Table 4.3.3 and Figure 4.3.3).

Table 4.3.3. Physiological characteristics at baseline and post-intervention.

	L (n=11)		OV/OB (n=23)	
Parameter (units)	Pre	Post	Pre	Post
BMI (kg m^{-2})	22.41 ± 2.14	22.43 ± 2.12	30.27 ± 3.66†	30.43 ± 3.37
Weight (kg)	63.71 ± 5.60	63.79 ± 5.70	82.79 ± 11.89†	83.22 ± 12.06
Fat mass (kg)	19.09 ± 3.54	18.21 ± 3.88#	32.42 ± 7.81†	32.70 ± 7.79
Fat mass (%)	29.32 ± 4.72	28.16 ± 5.27#	38.74 ± 4.88†	38.90 ± 4.98
Lean mass (kg)	43.32 ± 3.99	43.93 ± 4.38	49.13 ± 5.44	49.28 ± 5.74
VO_{2peak} (l min^{-1})	2.92 ± 0.56	2.69 ± 0.59	2.64 ± 0.49)	2.69 ± 0.46
VO_{2peak} (l $min^{-1}kg^{-1}$)	45.89 ± 0.85	42.46 ± 8.38	32.58 ± 6.27†	33.19 ± 5.04
RMR (kcal d^{-1})	1361.35 ± 178.85	1477.93 ± 215.70	1619.15 ± 318.86†	1664.01 ± 303.71
RER	0.79 ± 0.07	0.82 ± 0.10	0.77 ± 0.06	0.80 ± 0.06

Data presented as mean ± SD. † indicates a significant difference (P < 0.05) between groups (OV/OB vs. L) at baseline; # indicates a significant interaction between groups following the 8-week intervention. L, lean; OV/OB, overweight and obese; BMI, body mass index; RMR, resting metabolic rate; VO_{2peak}, peak oxygen uptake; and RER, respiratory exchange ratio.

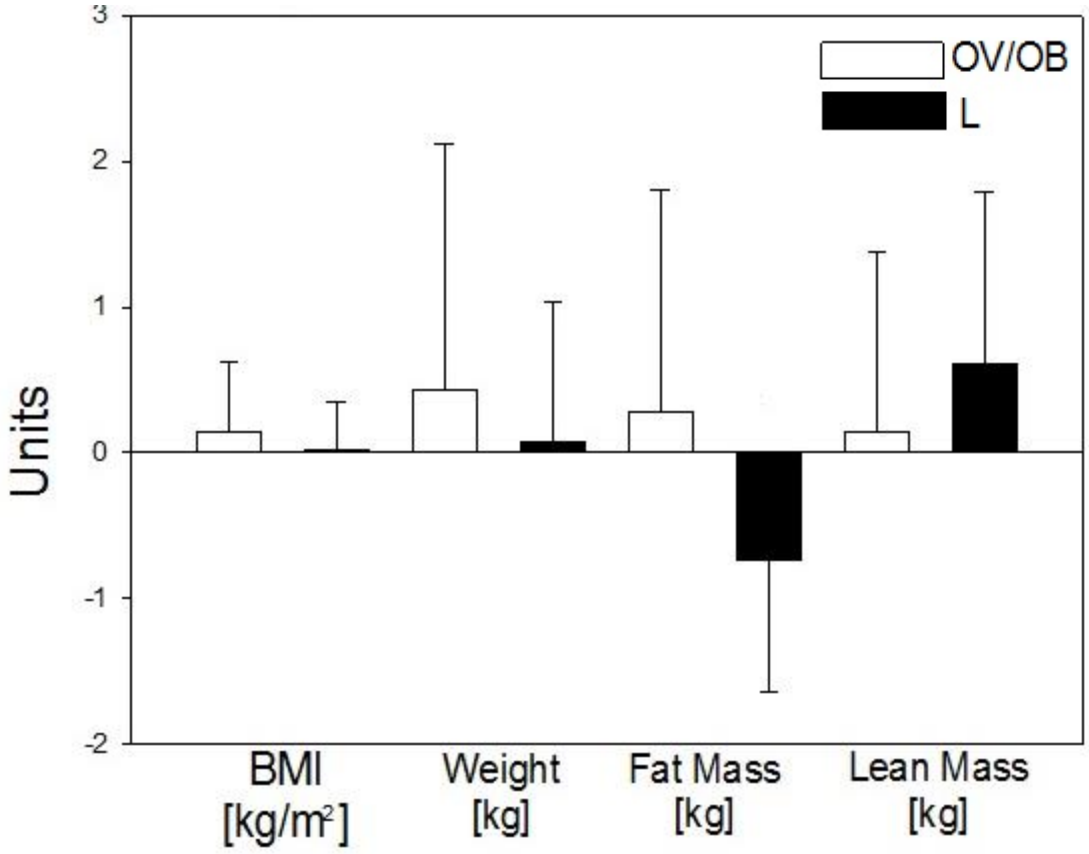

Figure 4.3.3. Participants characteristics (BMI, body mass index; weight; fat mass; and lean mass) at baseline and following 8-week intervention.

4.3.4. Energy intake, expenditure and balance

The weekly diet diaries, obtained from participants across the 8-week training intervention, were proposed to measure macronutrient intake, and total energy intake (kcal/d). Macronutrient intake was calculated based on diet diaries. However, unexpectedly primary analysis of the diet diaries revealed that there was no significant correlation between weight change, energy balance, macronutrient intake or total caloric intake, where RMR and exercise energy expenditure were used to calculate energy balance. Therefore, a degree of underreporting which has previously been documented in both genders, lean and obese subjects (Bandini *et al*., 1990) was hypothesised. Furthermore, due to large discrepancies

between the diet reports and calculated energy intake based on body composition and RMR measurements, the macronutrient intake displays a degree of underreporting particularly for the obese group and should only be seen as qualitative information of participants' diet. Taking all this into account, the percentage of macronutrient intake from diet records was still analysed, because they were the sole source of information regarding possible change in macronutrient choice over the time. However, the amount of macronutrient intake reported by participants is displayed to clarify underreporting of diet diaries (Table 4.3.4.2).

Due to the poor quality of diet records, to obtain more accurate values of total energy expenditure (kcal/d), energy intake (kcal/d) and energy balance (kcal/d) for the duration of the 8 weeks training intervention, DEXA data was utilised (as previously described in the methods section). Where exercise induced energy expenditure; total energy expenditure was corrected using participants' RMR. Additionally, 20% of RMR for activities of daily living (ADL) (Roza & Shizgal, 1984) and 10% of RMR for diet induced thermogenesis (DIT) (Westerterp, 2004) were added. Values of energy intake (EI) were calculated according to EI = EB + EE; EE = RMR + EEEx + ADL + DIT. The energy expenditure for all exercise session was added together to work out total calories burned during the 8 weeks' intervention.

Using the newly calculated data, no significant difference between OV/OB and L groups for the exercise induced energy expenditure was seen (OV/OB, 3324.70±1060.38 kcal, L, 3194.21±1344.00 kcal, P=0.76). Furthermore, the exercise intervention was a similar intensity for both groups (OV/OB, 89.94±36.13 W, L, 78.84±20.06 w) (Table 4.3.4). A significant difference was observed between groups in both total energy expenditure (kcal/d)

and total energy intake (kcal/d), (*P*=0.024, *P*=0.009 respectively), both total energy expenditure (kcal/d) and total energy intake (kcal/d) were higher in OV/OB than their L counterparts. Furthermore, calculated energy balance over the 8 weeks training period was not significantly different between the OV/OB and L groups; however, a close trend towards significant difference (*P*=0.062) between groups was seen, where OV/OB calorie intake was higher with a positive energy balance while L females revealed a slightly negative (table 4.3.4.1). Percentage macronutrient intake of protein, carbohydrate, sugar and fat was calculated from the diet diaries of participants across the 8-week training intervention, and after analysis no significant differences in macronutrient composition were found between (Table 4.3.4.2).

Table 4.3.4.1. Energy balance parameters.

Parameter (units)	L (n=11)	OV/OB (n=23)
Training Energy Expenditure (kcal)	3194.21 ± 1344	3324.7 ± 1060.4
Training intensity (Watt)	78.84 ± 20.06	89.94 ± 36.13
Energy Intake (kcal day^{-1})	1661.32 ± 309.50 †	2078.49 ± 445.56
Energy balance (kcal $day^{-1)}$	-109.22 ± 138.37	49.23 ± 247.55

Data presented as mean ± SD. † denotes significant differences between groups (*P*<0.05). L, lean; and OV/OB, overweight and obese.

Table 4.3.4.2. Dietary analysis of macronutrient selection.

Macronutrients	L (n= 11)	OV/OB (n=19)
Protein (kcal/d)	302.09 ± 85.21	259.61 ± 59.20
Carbohydrate(kcal/d)	1020.03 ± 353.65	889.35 ± 264.87
Sugar (kcal/d)	326.92 ± 107.05	324.67 ± 159.84
Fat (kcal/d)	624.57 ± 193.51	533.31 ± 182.55
Protein (%)	15.74 ± 2.72	16.09 ± 2.93
Carbohydrate (%)	52.04 ± 4.85	53.23 ± 5.19
Sugar (%)	17.30 ± 5.16	19.74 ± 7.33
Fat (%)	32.22 ± 3.80	30.67 ± 5.54

Data presented as mean ± SD. L, lean; OV/OB, overweight and obese.

4.3.5. Blood parameters

Fasting insulin concentrations following the 8-week intervention indicated that there were no time main effect and interaction (body type x time) but a significant main effect of body type ($F=11.895$, $\eta^2=0.284$, $P=0.002$) was observed, with OV/OB group displaying higher concentrations than their lean counterparts.

There was a significant main effect of time in fasting glucose, with L participants significantly increased fasting glucose levels over the intervention ($F=9.345$, $\eta^2=0.232$, $P=0.005$). Analysis of postprandial glucose (one hour after the test meal) demonstrated that there was no change over the intervention for either of the groups over the 8-week intervention. However, there was a tendency for L participants to have a higher concentration of glucose compared to OV/OB group (Table 4.3.5).

To estimate insulin sensitivity (%S), resistance (IR) and beta cell function (%B) by using HOMA2 calculator, fasting values of glucose and insulin concentrations were used (Wallace, Levy & Matthews, 2004). At baseline, it was found that the OV/OB group exhibited significantly higher values for fasting insulin, %B, and IR than those of the L group, and lower %S than their L counterparts ($P<0.05$). After the 8 weeks' exercise training, there was no significant change in %S, %B and IR but a significant difference was seen between body types. Scores of both %B and IR were significantly greater in the OV/OB group, while the L group displayed a significantly greater %S (Table 4.3.5).

The analysis revealed that the 8-week exercise intervention did not significantly alter lipid profile; total cholesterol, HDL and LDL (Table 4.3.5). Only a small increase in LDL values at post-test in both L (9.71%) and OV/OB groups (11.16%) were detected ($P= 0.05$). Furthermore, a trend towards an interaction between time and body type was reported in HDL levels ($P=0.091$), HDL level increased in L group whereas OV/OB group showed a reduction over the 8 weeks intervention ($P=0.091$) (Table 4.3.5).

Table 4.3.5. Fasting and postprandial glycaemic and lipaemic parameters.

	L (n=11)		OV/OB (n=23)	
Parameters	Pre	Post	Pre	Post
F Glucose (mmol/l)	4.51 ± 0.44	4.79 ± 0.23*	4.57 ± 0.42	4.65 ± 0.28
PP Glucose (mmol/l)	4.88 ± 0.98	4.73 ± 1.12	4.55 ± 0.88	4.48 ± 0.80
F Insulin (mU/L)	4.16 ± 2.09	4.59 ± 0.61	7.52 ± 3.19†	7.93 ± 3.69†
PP Insulin (mU/L)	30.45 ± 17.50	37.91 ± 23.62	40.99 ± 19.43†	47.48 ± 19.45†
Beta Cell Function (%B)	74.37 ± 25.36	72.54 ± 27.10	111.34 ± 31.14†	109.91 ± 30.37†
Insulin Sensitivity (%S)	242.0 ± 129	211.49 ± 100.91	125.19 ± 55.43†	118.19 ± 48.85†
Insulin Resistance (IR)	0.53 ± 0.26	0.60 ± 0.33	0.95 ± 0.41†	1.02 ± 0.48 †
Tcho (mmol l^{-1})	3.59 ± 0.56	3.82 ± 0.76	3.85 ± 0.83	4.13 ± 0.68
HDL (mmol l^{-1})	1.68 ± 0.41	1.73 ± 0.48	1.51 ± 0.47	1.40 ± 0.33
LDL (mmol l^{-1})	1.75 ± 0.63	1.92 ± 0.70	2.24 ± 0.71	2.49 ± 0.64

Data presented as mean ± SD. * denotes a significant difference from pre to post intervention (P<0.05). † denotes a significant difference between the groups (P<0.05). L, lean; OV/OB, overweight/obese; F, fasting; PP, postprandial; Tcho, total cholesterol; HDL, high density lipoprotein; and LDL, low density lipoprotein.

4.3.6. Endocrine analysis

All appetite hormones except leptin (which was measured only in the fasting state) were measured in both a fasting and postprandial state using ELISA at baseline, and again after the 8-week intervention. All mean and *SD* values for fasting and postprandial appetite regulatory hormones are shown in table 4.3.6. At baseline, it was found that the OV/OB group exhibited significantly higher values than their L counterparts for leptin, insulin, amylin, and acylated ghrelin, as well as postprandial levels of insulin and acylated ghrelin (P<0.05). Fasting and postprandial levels of total ghrelin and PYY were not significantly different at baseline (Table 4.3.6).

Table 4.3.6. Fasting and postprandial plasma appetite-related hormone concentrations.

	L (n=11)		OV/OB (n=23)	
Parameters (units)	Pre	Post	Pre	Post
F Leptin (ng ml^{-1})	12.38±7.12	11.62±4.46	36.25±15.76 †	36.38±16.40 †
F Insulin (mU l^{-1})	4.16±2.09	4.59±2.61	7.52±3.19 †	7.93±3.69 †
	3.88(2.46-6.356)	*3.56(2.7-6.69)*	*7.19 (4.40-9.12)*	*6.78(5.44/10.13)*
PP Insulin (mU l^{-1})	30.45±17.50	37.91±23.62	40.99±19.43 †	47.48±19.45 †
	25.70(22.38-35.46)	*36.50(21.58-41.32)*	*36.97(32.88-44.31)*	*48.56(31.45-60.64)*
PP Insulin Change (mU l^{-1})	23.45±18.77	33.32±22.43	33.47±19.21	39.55±19.41
F Amylin (pmol l^{-1})	9.55±3.81	11.97±5.18	16.16±3.85 †	12.20±3.18 *
	17.81±5.60	21.18±6.76	20.42±3.64 †	17.55±3.96†, *
PP Amylin (pmol l^{-1})	*17.97(12.87-21.0)*	*20.07(14.78-27.03)*	*19.85(17.54-22.21)*	*16.88(14.79-19.38)*
PP Amylin Change (pmol l^{-1})	8.31±4.65	9.97±6.56	4.26±2.76†	5.35±4.45†
F Ghrelin (pg ml^{-1})	787.79±254.07	740.11±180.99	677.01±254.40	674.50±244.77
PP Ghrelin (pg ml^{-1})	563.14±198.47	479.53±119.45	452.30±205.99	491.49±216.42*
PP Ghrelin Change (pg ml^{-1})	-224.65±85.78	-260.56±94.25	-224.71±126.72	-183.00±93.74
F Acylated Ghrelin (pg ml^{-1})	23.38±13.89	19.73±6.70	46.90±30.90 †	50.50±24.56 †
	24.58(9.86-35.85)	*21.04(19.13-23.05)*	*38.98(23.20-55.64)*	*49.99(40.64-67.08)*
PP Acylated Ghrelin (pg ml^{-1})	17.28±8.35	17.42±9.26	38.52±21.11†	36.47±20.50 †
	13.17(12.28-23.34)	*18.31(9.0-26.20)*	*37.43(27.09-50.73)*	*33.44(21.32-51.41)*
PP Acylated Ghrelin Change (pg ml^{-1})	-3.40±10.71	2.04±3.22	-8.39±20.13	-14.03±17.52
F PYY (ng/ml)	130.59±63.88	152.65±58.26	158.67±54.03	158.84±70.73
PP PYY (ng/ml)	212.01±76.98	228.27±78.22	202.13±54.89	226.15±50.19
PP PYY Change (ng/ml)	81.42±33.24	75.62±37.84	43.45±51.97	67.32±50.38

Data are presented as means ± SD. † denotes a significant (P<0.05) difference between the groups, * denotes a significant interaction between groups within pre and post-test. L stands for lean, OV/OB for overweight/obese, F for fasting, PP for postprandial and PYY for peptide tyrosine-tyrosine. The figures shown in italic font represent M (Q_1-Q_3) which stands for median (quarter$_1$ & quarter$_3$).

4.3.7. Insulin

There were no significant changes in fasting or postprandial insulin concentrations following the 8 week intervention (P>0.05), no significant effects of time, or interactions were observed. However, a significant difference was measured between body types in both fasting (F=11.895, η^2=0.284, P=0.002) and postprandial states (one hour after a test meal) (F=4.533, η^2=0.128, P=0.041), with OV/OB participants displaying greater concentrations than their L counterparts (Table 4.3.6).

4.3.8. Leptin

Leptin was measured in the fasting state only due to the negligible effect of test meal consumption. There were no significant changes in fasting leptin concentrations following the 8 week intervention (P<0.05). Nevertheless, as expected, a significant effect of body type was detected at baseline and post intervention, with OV/OB exercising group displaying significantly higher amounts than their L counterparts (Table 4.3.6).

4.3.9. Amylin

There was no main effect of time for amylin concentrations however, more importantly a significant interaction between time and body type in both fasting and postprandial states was observed (F=16.137, η^2=0.358, P=0.001, F=10.033, η^2=0.257, P=0.004 respectively). In both fasting and postprandial amylin, baseline levels were significantly greater in the OV/OB individuals, and following the intervention amylin was also significantly reduced (P<0.05) in this group under fasting and postprandial conditions. In contrast, the L group do not display

this change, L participants actually increase post intervention, although this increase was not statistically significant (Table 4.3.6 and Figure 4.3.9).

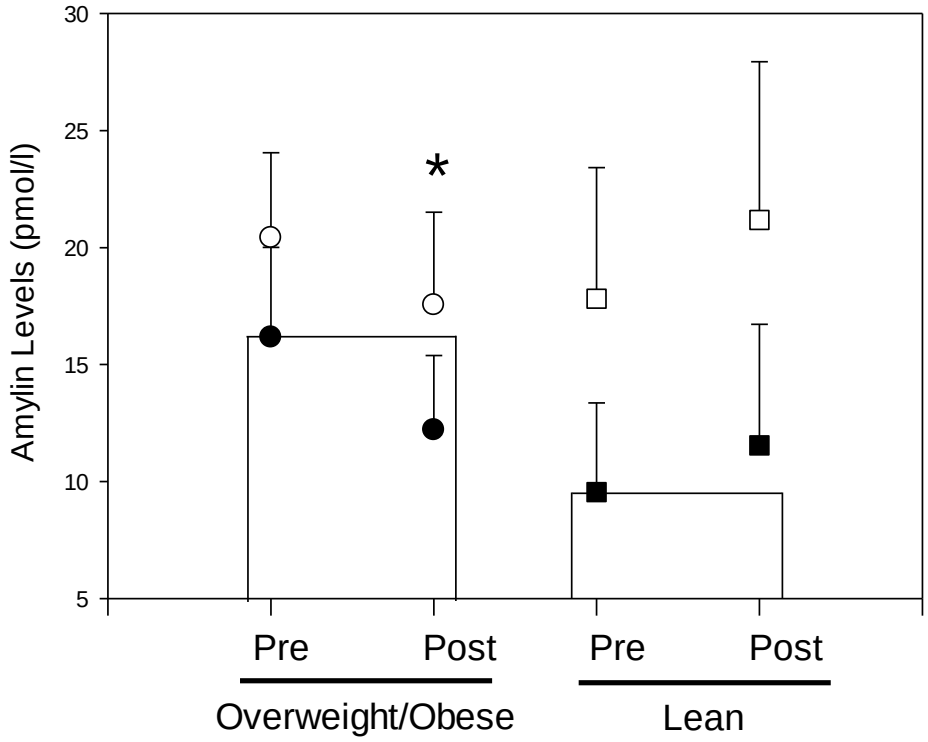

Figure 4.3.9. Plasma amylin concentrations (Fasting: ●, OV/OB; ■, L; postprandial: ○, OV/OB; □, L). * denotes a significant interaction between two groups from pre- to post-test (P<0.05).

4.3.10. Total ghrelin

The 8 week intervention did not induce significant effects of time, group or interaction in fasting plasma total ghrelin and the response to the test meal was unchanged in both groups. However, a significant interaction was observed between time and body type for total ghrelin postprandial concentrations (F=5.33, η^2=0.155, P=0.03), whereby the L group demonstrated a suppression in their total ghrelin response, and the OV/OB group displayed an increase which did not reach significance (Table 4.3.6).

4.3.11. Acylated ghrelin

There were no significant changes in fasting or postprandial acylated ghrelin concentrations following the 8-week intervention ($P< 0.05$). However, a significant difference was observed between groups. A trend towards significance was seen at baseline, for a higher fasting level of acylated ghrelin in OV/OB ($P= 0.084$), also a significant difference was seen post intervention, where OV/OB displayed higher levels of acylated ghrelin compared to L ($P= 0.006$). Regarding postprandial levels, OV/OB participants experienced significantly higher concentrations than their L counterparts at both baseline and post intervention ($P= 0.005$ and $P= 0.009$ respectively) (Table 4.3.6).

4.3.12. Total PYY

When analysed by ANCOVA, there were no significant changes in fasting or postprandial total PYY concentrations following the 8-week intervention ($P<0.05$), no effects of time, group or interactions were displayed. However, both groups did slightly increase at post-test when compared to baseline levels, but this difference was not significant.

4.3.13. Individual variability to BMI and fat loss

As previously mentioned, there was no significant change in BMI and weight within the whole group. However, there was wide variability in BMI in both L and OV/OB (-0.55 to +0.50, -0.98 to +2.77, respectively) and weight (−1.64 kg to +1.46 and −0.2.47 kg to +4.79, respectively) response to the exercise intervention in both groups (Figure 4.3.13). Normalisation of the standard variations of BMI changes on means of baseline BMI in both groups, show almost identical variability (OV/OB: 1.6%; L: 1.5%).

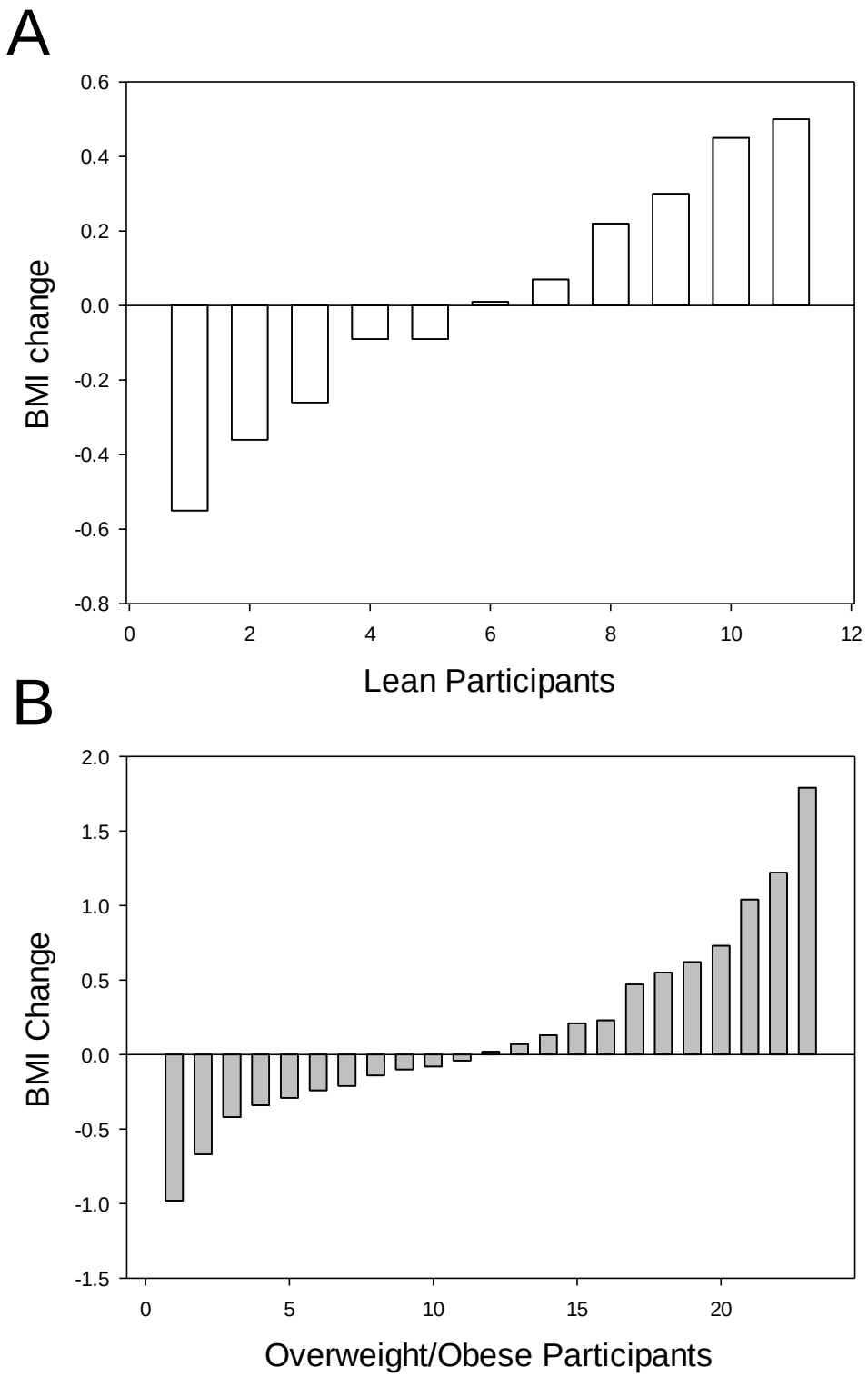

Figure 4.3.13. Individual BMI response to exercise in A) lean and B) overweight/obese individuals. BMI, body mass index.

Multi regression analysis (using enter method) was performed to predict the individuals' BMI changes induced by exercise training over 8 weeks, in both L and OV/OB groups. The appetite-related hormones, which were included as independent variables in the regression model, lead to a significant model for the OV/OB group. The post intervention levels (Table 4.3.13) of leptin (β=0.55) and postprandial levels of amylin (β=-0.36), predicted 45% of the variability of the BMI alterations (R^2=0.45, *P*= 0.003) in response to training in the OV/OB group. Other hormones did not play a significant role in the model, neither did they lead to a significant increase in prediction of the model. A single factor regression analysis was performed for L participants because of the small size of the group. The post intervention postprandial levels of PYY (β=-0.62) was the only significant hormonal predictor of change in BMI (R^2=0.39, *P*=0.041), explaining 39% of the variance of change in BMI in response to the intervention; other hormones did not show such significant results. For the group as a whole, leptin levels at post intervention were the best predictor of BMI change, with R^2=0.27, *P*=0.002 and β=0.52.

To summarize, BMI alterations in the OV/OB could be mainly explained by fasting leptin levels and postprandial levels of amylin (Table 4.3.13), while L participants' BMI change were partially predicted by postprandial PYY. Moreover, multivariate regression modelling indicated that energy expenditure from exercise was not significant in predicting fat change, because it was kept constant for all participants.

Table 4.3.13. Multi-regression analysis for prediction of fat mass changes in participants, and BMI changes in OV/OB group after 8 weeks' exercise training.

DV	IV	R^2	ΔR	p	B	SE B	β	p
Δ BMI N=23 OV/OB		0.45	0.45	0.003				
Constant					0.39	0.561		
	Amylin Postprandial post (mmol/l^{-1})				-0.058	0.028	-0.36	0.049
	Leptin post (ng ml^{-1})				0.021	0.007	0.55	0.004

Abbreviations: DV, dependent variable; IV, independent variable; SE, standard error.

4.3.14. Implicit attitudes

The study compared implicit attitudes towards four types of foods. These were high fat (HF), low fat (LF), high sugar (HS) and low sugar (LS), using the IAT (Greenwald et al., 1998), whereby it is possible to obtain scores of positive, negative or zero. Positive scores represent a positive implicit attitude while negative scores a negative attitude towards fatty/sugary food and a score of 0 indicates a neutral attitude.

Mean (*M*) scores and standard deviations (*SD*) for both IATs at pre- and post-test are given in table 4.3.14. Analysis of pre intervention levels of the two groups revealed no significant difference between L and OV/OB participants for either of the IATs (Table 4.3.14). Pre and post data of both IATs (HF-LF and HS-LS) were analysed, implicit attitude toward food was unaffected by the training intervention and no body type differences or interactions were seen

either (Table 4.3.14). This means that neither the L nor OV/OB participants significantly changed their implicit attitude toward HF-LF foods and HS-LS foods from baseline to post-intervention.

Table 4.3.14. Analysis of IATs (HF-LF and HS-LS) for both groups at pre-test and post-test.

	L (n=11)			OV/OB (n=22)		
	Pre-test	Post-test	Δ	Pre-test	Post-test	Δ
HF.LF	0.78 ± 0.60	0.55 ± 0.56	-0.23 ± 0.77	0.63 ± 0.59	0.49 ± 0.51	-0.13 ± 0.56
HS.LS	0.35 ± 0.77	0.22 ± 0.78	-0.13 ± 0.65	0.05 ± 0.65	0.23 ± 0.77	-0.02 ± 0.80

Data presented as mean ± SD. L, lean; OV/OB, overweight/obese; HF-LF, high fat-low fat; and HS-LS, high sugar-low sugar. *1 data set for IAT withdrawn from OV/OB group for poor English proficiency.*

4.3.15. Explicit attitudes

Explicit attitudes towards food (high fat, low fat, high sugar and low sugar) were assessed using the Explicit Attitude Questionnaire. This questionnaire consisted of 8 questions, each using a 7 points scale (1-7); the higher score indicating a higher explicit attitude towards the given construct and vice versa. Four questionnaires, one for each food type, were used to measure explicit attitudes towards the foods already mentioned. Mean explicit attitude scores for both L and OV/OB groups are displayed in table 4.3.15. Further analysis indicated that the explicit attitude scores towards low fat food was significantly different between the groups. The L participants' explicit towards low fat food attitude scores were significantly higher compared to OV/OB participants. Although there was no significant differences between the groups regarding the other food categorises (high fat, high sugar and low sugar) OV/OB participants' scores were slightly higher than L participants.

Table 4.3.15. Explicit attitude questionnaire (EAQ) results for four different categories.

	L (n=11)	OV/OB(n=22)
Parameters	Post	Post
Low Fat	5.67 ± 1.01	4.28 ± 1.46†
High Fat	3.80 ± 1.14	4.43 ± 1.26
Low Sugar	4.69 ± 0.81	4.41 ± 0.86
High Sugar	4.29 ± 0.59	4.57 ± 1.02

Data presented as mean ± SD. † represent significant difference between body types ($P < 0.05$). L, lean; OV/OB, overweight/obese. *1 data set for IAT withdrawn from OV/OB group for poor English proficiency.*

4.3.16. Associations between hormones, body characteristics and attitude measures

Pearson's correlations were used to investigate any potential relationships between both IATs and EAQ, and changes (post-test minus pre-test) in appetite-regulation hormones, BMI and weight. Table 4.3.16.1 shows the association between IAT/EAQ and appetite-related hormones in the OV/OB group. A significant negative correlation was found between changes in HF, LF, IAT and insulin change, from pre to post-test. For EAQ, there was a significant negative correlation between LF EAQ and fasting amylin changes. In addition, both fasting and postprandial total ghrelin in post-test had a positive significant correlation with HS EAQ. Additionally, a negative correlation was seen between LF EAQ and post fasting total ghrelin. Furthermore, a significant negative correlation was also reported between HF-LF IAT and postprandial insulin in pre-test. There was a negative moderately significant trend between changes in HF-LF IAT and leptin changes from pre to post-test. A significant negative correlation was observed between fasting amylin in pre-test and HS-LS IAT.

Table 4.3.16.1. Correlation between IAT/EAQ and appetite-related hormones in the OV/OB group.

OV/OB (n=22)								
	IAT				EAQ			
Parameters	Δ HF-LF		LF		LS		HS	
	r	p	r	p	r	p	r	p
Δ F Amylin					-0.506	0.016†	0.411	0.058
Δ F Insulin	-0.467	0.029 †						
Δ Leptin	-0.391	0.072						
F post total ghrelin			-0.440	0.041†			0.601	0.003†
PP post total ghrelin							0.519	0.013

Data is presented as means ± SD. † denotes significant correlation between IAT/EAQ and appetite-related hormones in the OV/OB group ($P<0.05$). IAT, implicit association test; EAQ, explicit attitude questionnaire; OV/OB, overweight/obese; F, fasting; PP, postprandial; HF-LF, high fat-low fat; LF, low fat; LS, low sugar; and HS, high sugar. *1 data set for IAT and EAQ withdrawn from OV/OB group for poor English proficiency.*

Additionally, when studying the L group (Table 4.3.16.2), significant correlations were found between the following relationships. There was a positive significant correlation between LF EAQ and fasting amylin in post-test (r=0.654, P=0.040); postprandial amylin in post-test was negatively associated with L.S EAQ (r=0.648, P=0.043). LF was positively correlated with both fasting PYY and fasting amylin in post-test (r=0.634, P=0.036 and r=0.654, P=0.040 respectively). LS and HS EAQs had negative and positive correlation respectively with changes in BMI and weight. Additionally there was a trend towards significant association between HF EAQ and changes in leptin. All other relations tested were found to be non-significant. No associations existed between IATs and appetite hormones, BMI and weight following the intervention.

Table 4.3.16.2. Correlation between EAQ and appetite-related hormones in L group.

L (n=11)								
EAQ								
Parameters	LF		HF		LS		HS	
	r	p	r	p	r	p	r	p
F PYY Post	0.634	0.036†						
PP Amylin Post					-0.648	0.043†		
F Amylin Post	0.654	0.040	-0.779	0.008†				
Δ Leptin			-0.599	0.067				
Δ BMI					-0.703	0.016†	0.672	0.024 †
Δ Weight					-0.671	0.024 †	0.645	0.032 †

Data is presented as means ± SD. † denotes significant correlation between IAT/EAQ and appetite-related hormones in the L group (P<0.05). IAT, implicit association test; EAQ, explicit attitude questionnaire; L, lean; F, fasting; PP, postprandial; HF-LF, high fat-low fat; LF, low fat; LS, low sugar; and HS, high sugar. *1 data set for IAT and EAQ withdrawn from OV/OB group for poor English proficiency.*

4.4. Discussion

4.4.1. Introduction

The aim of the current study was to investigate the effects of an 8-week exercise training intervention between lean and obese populations on body composition, endocrine adaptations, hedonic responses and compensatory responses in females. The lean and overweight/obese females who were naive to the aims and objectives of the trial and participants who expressed an interest to lose weight had been excluded from the study. To our knowledge, this is the first study which tried to achieve an *ad libitum* situation for participants facing negative energy balance via exercise whilst avoiding the influence of internal motivation to lose weight and expectations about outcomes.

4.4.2. Weight/BMI response to exercise

It was found that the intervention did not lead to significant weight loss/BMI change in both OV/OB and L groups (See Figure 4.3.3 and Table 4.3.3). This finding is consistent with our first hypothesis and we interpret this as indicative of intact weight regulation, even in overweight/obese individuals, if exposed to exercise training. This outcome, considering the mean weight changes, as well as individual responses to exercise, are in contrast to results which have been published before. King *et al*. (2007) utilised a design where individuals were recruited without concealing the study aims and not excluding individuals with motivation to lose weight, reporting a considerable weight loss with high variability among overweight/obese participants with outcomes skewed towards weight loss; outcomes which are in contrast to our study where gainers and losers are in balance in both OV/OB and L groups. This suggests that BMI/weight alterations in conventionally designed studies may rather reflect different restrained eating behaviour based on weight loss motivation and self-

control rather than the direct effect of exercise. However, our outcomes point clearly towards regulatory processes which may be different in mechanisms in both OV/OB and L groups but still functional on different levels of weight set point.

4.4.3. Individual variability in BMI changes and fat loss

The successful weight regulation in our groups, however, does not explain the large inter-individual variability in weight loss, ranging from considerable weight loss to weight gain (L: −1.64 kg to +1.46 and OV/OB: −0.2.47 kg to +4.79), an individual response also highlighted by King *et al*. (2007) leading to the concept of compensators and non-compensators. Often mechanisms are differentiated in homeostatic and hedonic regulation suggesting that humans are prone to be mainly driven by hedonic regulation (Berthoud, 2011). Otherwise it is known that both regulatory processes are heavily interlinked and difficult to separate; in particular appetite hormones are repeatedly shown to influence 'liking' and 'wanting' or reward perception as well as influencing energy intake and energy metabolism (Volkow, Wang *et al.*, 2011). We did not gather information about the individuals' motives for eating in our study but we did gather information about appetite hormones responses at fasting and postprandial levels to explain the variability of weight/BMI changes after the training intervention.

4.4.4. Hormonal response to exercise

Amylin was the only appetite hormone measured which revealed novel findings, with significant alterations after the training intervention at fasting and postprandial levels in the OV/OB group, while the L group revealed no changes (See Figure 4.3.9). Reports of alterations in amylin levels in response to exercise training are sparse; Izadpanah *et al.* (2012) and Roberts *et al*. (2013) reported a reduction of amylin in response to a combined diet

exercise intervention in obese children for 14 days but the investigation was aimed on markers of metabolic health and inflammation and was combined with diet induced weight loss. Additionally, acute responses to exercise bouts with a reduction of amylin after prolonged exercise bouts (Kraemer, Francois *et al*., 2011) and increase in higher intensity bouts (Kraemer, Acevedo *et al.*, 2002) were recently shown. Smith *et al*. (2007) previously demonstrated that amylin-analogue pramlintide administration produced weight lowering effects in obese individuals by a reduction in daily food intake, portion sizes, and binge-eating tendencies. Therefore, it would be logical to assume that a reduction in amylin concentrations would result in larger meal sizes, more between meal snacks, and therefore increased daily energy intake. However, in the current study, food intake did not significantly change across time, as assessed by 3-day diet diaries (See Table 4.3.4.1). The contradiction between hormonal adaptation and daily food intake observed may be explained by the limitations in obtaining accurate measurements of food intake during *ad libitum* studies. For instance, the introduction of dietary intake methodology can in itself alter dietary habits, at least in some individuals (Bingham, 1991; Stallone *et al*., 1997). Furthermore, self-reported diet diaries rely on the compliance of the individual to record everything consumed, whilst not changing typical eating habits (Elia *et al*., 2003). As such, it may be that our participants misreported the diet diaries, especially in the OV/OB group, since obese individuals are known to have under-reported food intake by as much as 30% (Lichtman *et al*., 1992). Thus, our novel finding, that amylin is reduced following exercise training in OV/OB individuals, may be a homeostatic defence mechanism to protect against a negative energy balance, which may explain why despite the increased energy expenditure yielded (~3324.66 ± 1060.38), we were unable to induce weight/fat loss following the 8-week intervention.

4.4.5. Prediction of BMI/weight changes

Having considered what cited previously, we made attempts to reveal whether the measured appetite hormone responses could explain the variability of BMI/weight changes of compensators and non-compensators. Indeed, multi regression analysis resulted in two prediction equations for BMI changes in OV/OB and L groups. With the contribution of leptin and postprandial amylin we could predict 45% of the variability of BMI changes in the OV/OB group (See Table 4.3.13), while other hormones did not contribute significantly to the model. Leptin is clearly known to be the most important tonic signal of energy stores and mainly sensed in the hypothalamus for the intrinsic drive to eat and consequently for the regulation of body weight and energy expenditure (Blundell& Gillett, 2001); therefore, the large contribution of leptin levels for OV/OB in the prediction model is not unexpected. Additionally, leptin's links towards perceptual response towards food was recently established identifying fasting leptin levels as a determinant of food reward showing also an influence of leptin on hedonic responses (Hopkins, Gibbons *et al*., 2014). However, the strong contribution of amylin for the model of the OV/OB group is remarkable. Amylin is known to play a role as a satiogenic signal, inhibitor of gastric emptying and possesses glucoregulatory functions; agonists are well established in supporting weight loss in obese (Smith, Blundell *et al*., 2007). Amylin expression in beta cells was recently shown to be controlled via carbohydrate-response-element-binding-protein (ChREBP) and thioredoxin-interacting-protein (TXNIP) responding to glucose availability directly (Jing, Westwell-Roper *et al.*, 2014). Interestingly, our data revealed a positive correlation between postprandial amylin levels and fasting glucose levels at post intervention in the OV/OB group which highlights a possible connection between glucose availability and amylin levels. Clearly, fasting glucose levels can be influenced via sugar/carbohydrate intake (Sartor, Jackson *et al.*, 2013) as well as exercise (Sartor, de Morree *et al.*, 2010). Moreover, amylin

and leptin are shown to share important functions in the hindbrain and hypothalamus. It is suggested that amylin enhances leptin signalling and lead to transient alteration of leptin responsiveness threshold (Trevaskis, Lei *et al.*, 2010). In consequence, in our study, decreased amylin levels (postprandial and fasting) could increase leptin responsiveness threshold in OV/OB participants and would have led to compensatory energy intake in response to exercise training. Our model predicts that participants who have a combination of high levels of leptin and low postprandial levels of amylin would gain weight during exercise training, and consequently would have overcompensated the exercise induced negative energy balance via energy intake. Regarding lean participants, with the limitation of numbers, we could establish that postprandial PYY explains a substantial proportion of the variability of BMI changes (39%) which was not seen in the OV/OB group. This finding is an agreement with the frequently reported importance of weight regulation via PYY in lean people (Manning, Batterham, 2014).

Regarding the need of body composition changes, in particular fat loss, our study shows in agreement with other studies (Bryner, Toffle *et al.*, 1997, Irving, Davis *et al*., 2008) that higher intensities are beneficial for fat loss but that energy intake is the dominant factor if energy expenditure is clamped. Our model would therefore predict that a combination of high intensity exercise with diet restriction would yield in the best reduction in fat mass.

4.4.6. Hedonic response to exercise

We hypothesised that there would be higher implicit attitude scores towards high-fat and high-sugar foods in response to exercise in the obese group as compared to lean group at post-test. However, neither the L nor OV/OB participants significantly changed their implicit

attitude toward high-fat, low-fat foods nor high-sugar, low-sugar foods from baseline to post-intervention (See Tables 4.3.14), although explicit attitude scores towards low-fat food was significantly different between the groups, with lean groups significantly higher than compared to the OV/OB group (See Table 4.3.16.2, and Table 4.3.16.1, respectively). This data suggests that the obese group had a higher liking and wanting for high-fat energy dense foods than their lean counterparts, however, this was not found to be the case, as there were no significant changes in explicit attitude scores for high-fat, high-sugar and low-sugar foods. Overall, this suggests that implicit attitude is not associated with weight loss in this study, and therefore does not seem to be important for the variance of weight gain and loss in either group.

4.4.7. Correlation between hormones and attitude measures

In our findings, ghrelin was correlated to an increased wanting for high fat foods (See Table 4.3.16.1). This is an anticipated finding since higher ghrelin concentrations are associated with an increase in hunger, and therefore when people are hungrier it seems logical that they are more likely to desire high-fat, high-calorie, energy-dense foods to satiate their hunger. This finding supports the notion of intermittent snacking for weight loss, rather than feasting on a large meal in the evening, which would make an interesting future study in these populations. It is possible that by snacking throughout the day, ghrelin concentrations will be suppressed, and this will be reflected in the food choices made, with the scope of lower fat lower calorie consumption.

4.4.8. Conclusion

In conclusion, we have shown that under *ad libitum* condition, un-biased by internal and external motivation for weight loss, 8 weeks' exercise training did not result in weight loss in overweight/obese and lean females. We interpret this as evidence of eating behaviour that compensates for exercise energy expenditure based on an intact set point regulation. However, appetite hormones responses reveal that amylin decreased on fasting and postprandial levels only in overweight/obese, while postprandial PYY trended to increase in both groups. About half of the variance of the BMI changes after training in overweight/obese females can be predicted by postprandial amylin and leptin levels, pointing towards a new function of amylin in weight regulation during exercise training in overweight/obese females.

Chapter 5: Acute Appetite Responses to High and Moderate Intensity Exercise Training in Inactive, Lean and Overweight/obese Females

5.1. Introduction

Exercise seems to elicit compensatory eating behaviour when performed over a long period (>2-4 weeks), with only modest impact on weight loss as a result (Miller *et al*., 1997). However, following an acute bout of exercise, most studies show no impact on appetite (King *et al.,* 1996; Blundell *et al.,* 2003; Martins *et al.,* 2008) or subsequent energy intake (King *et al.,* 1996; King *et al.,* 1997; Imbeault *et al.,* 1997; Hubert *et al.,* 1998; Blundell and King 1999). In fact, acute strenuous bouts of exercise have been found to, paradoxically, suppress hunger in a phenomenon termed 'exercise-induced anorexia' (King & Blundell 1995). Furthermore, a temporary suppression of appetite reported in women is much more pronounced following intense exercise than moderate-low intensity exercise, often with no change following an acute bout of low intensity exercise (Bilski *et al*., 2009), suggesting an intensity threshold for exercise-induced anorexia. In this regard, recent studies have demonstrated intense exercise to be more effective than food intake restriction in creating an acute energy deficit without subsequent increases in appetite and energy intake (Hubert *et al.,* 1998; King *et al.,* 2011). This suggests that the compensatory coupling of energy intake to match exercise-induced energy expenditure requires a number of weeks to fully offset a negative energy balance, as observed in many studies (King *et al.,* 2008; Doucet *et al*., 2011).

Recent research highlights that acute exercise may have conflicting effects on appetite regulation in male and female participants. Females have been reported to increase food intake in response to exercise whereas most studies in males appear to suggest that increased energy expenditure is not undermined by an increase in energy intake in the short term

(Hagobian *et al.,* 2009; Pomerleau *et al.,* 2004). Hagobian and colleagues (2008) suggest that the compensatory intake following exercise interventions in women explains why females maintain body weight and body fat, and others have suggested that an alteration to a more high-calorie food preference (such as high fat foods) following exercise increases their predisposition to overconsumption. Currently, a sound understanding of how exercise influences appetite is lacking, therefore, it is important to identify the homeostatic and hedonic processes which could be mobilised by exercise to influence food intake, and therefore of maximising the effectiveness of exercise as a method of producing a negative energy balance for weight control. It is important to identify the intensity of exercise, since higher intensities (>60% VO_{2max}) utilise an increasingly greater proportion of energy derived from carbohydrate than fatty acids (Van Loon *et al.,* 2001), it may be that decreases in muscle glycogen stores will produce an effect on eating behaviour when compared to decreases in fatty acyl stores in adipose tissue. This link is supported by findings from Finlayson *et al*. (2009), who document an association between carbohydrate availability and increased meal size and frequency, supporting the role of muscle glycogen restoration as a high priority following intense exercise. As such, increasing exercise intensity in young women has shown to elicit an increase in energy intake, as reported by diet diaries (Stubbs *et al*., 2002), or during a subsequent *ad libitum* buffet test meal (Pomerleau *et al*., 2004) which was almost sufficient to totally compensate for the exercise-induced energy expenditure. In addition, individuals who were classified as high fat oxidizers based on exercise respiratory quotient (RQ) experienced significantly lower post-exercise energy intake than the high carbohydrate oxidizers ($P< 0.05$) (Almeras *et al*., 1995).

Implicit behaviours of ‘liking’ and ‘wanting’ reflect core processes that can operate without conscious awareness (Berridge and Robinson, 2003). Finlayson *et al*. (2007) emphasises that understanding how liking and wanting may influence behaviour towards eating since they

operate at implicit and explicit levels of conscious awareness. Similarly, Brug *et al*. (1995) highlight that an individual's attitude towards food could be an important factor contributing to obesity, controlled by two modes, implicit attitude, which can impact behaviour in an affective and instinctive way, irrespective of any consideration towards the advantages or disadvantages of that particular behaviour, and explicit attitude, which may guide behaviour through a deliberate and conscious analysis of the risk and reward of that behaviour (Craeynest *et al*., 2005).

The expression of habitual eating behaviour involves the co-ordination and interaction of both homeostatic and hedonic signals (Finlayson, King, & Blundell, 2008). Exploring this association, Gibson *et al*. (2010) demonstrate a stimulatory role for ghrelin within sites associated with reward-driven eating. Similarly, Batterham and colleagues (2007) documented that an infusion of typical postprandial PYY_{3-36} concentrations modulates neuronal activity in both the hypothalamus and regions of the brain specifically involved in reward processing, demonstrating the co-ordination of hedonic and homeostatic signalling n the acute regulation of food intake. The co-ordination of these signalling processes seem to be further modulated by nutritional state and body composition. As such, Hopkins *et al*., (2014) reported an association between an explicit liking for high-fat foods, whilst implicit wanting was only associated with fat-free mass, suggesting that fat mass may predict food reward independently of fat-free mass. Such findings are consistent in the literature, whereby separate roles for fat-free mass in satiation (Blundell *et al.,* 2012) and hunger (Caudwell *et al*., 2013) and fat mass in hedonic eating behaviour traits (O'Neill, 2012) and neural activation to high energy foods (Luo *et al*., 2013) are seen. However, these findings are currently limited to the obese population and comparisons with lean individuals warrants investigation.

Considering this, in the current study we attempt to capture the hedonic drive to eat, in addition to homeostatic parameters, therefore instead of using a general test for hunger such as the visual analogue scales, we will obtain dimensions of FCQ-T, and FCQ-S which assess features of food cravings that are stable across time, and temporary state-dependent cravings, respectively (Cepeda-Benito *et al*., 2001). This will aid us in our aim to identify whether there is a discrepancy between physiologic drives and hedonic drives in response to exercise that is not possible with a more general test for hunger seen in many other studies and to further explore whether divergent changes in appetite regulating hormone responses are associated with subjective feelings of appetite measured using FCQ-T and FCQ-S and implicit and explicit attitudes towards specific food preference (high-fat, low-fat, high-sugar, low-sugar). The study will assess the effects of two separate exercise bouts of high- and moderate-intensity conditions, of equivalent energy deficits, in obese and lean sedentary individuals on metabolic (blood glucose), lipaemic (blood lipid profile) and appetite-related hormone parameters (insulin, leptin, total ghrelin, acylated ghrelin, and amylin).

Hypotheses:

Appetite hormones

1. Acylated ghrelin concentrations will be significantly suppressed following an acute exercise bout of both moderate- and high-intensities for both lean and obese groups, with a greater suppression expected following high-intensity exercise;
2. No change in leptin or amylin concentrations for both lean and obese groups following either moderate or high intensity exercise;

Food cravings

1. The hunger dimension of FCQ-S will be suppressed in both populations immediately following exercise, and increase at 1 hour following the cessation of the protocol, but other dimensions will not follow suit in the overweight/obese when a suppression is expected in the lean group;
2. Both lean and obese individuals will demonstrate a heightened positive explicit attitude towards high-fat, high-sugar foods following the high intensity exercise bout compared to the moderate intensity bout but that the implicit attitude would not be affected because it would be an outcome of cognitive transfer of former experiences with the food into the subconscious;

Correlation between subjective hunger and appetite hormones

1. A significant positive correlation between changes in subjective hunger and acylated ghrelin concentrations will be present in our findings.

5.2. Methods

5.2.1. Ethical approval

The studies described in this chapter were approved by the ethics committee of the School of Sport, Health and Exercise Sciences, Bangor University.

5.2.2. Participant recruitment

The general procedures needed for recruitment and inclusion criteria are described in detail in Chapter 3.1.

5.3. Pilot Study

5.3.1. Subjects and design

Nine female participants meeting the inclusion criteria took part in this study, for which anthropometric data are shown in table 5.7.1. The study employed a randomised, cross-over design, whereby participants completed two trials; a high-intensity (90% $\dot{V}O_{2peak}$)- and moderate (50% $\dot{V}O_{2peak}$) exercise bout, each separated by 7 days apart and randomised in order. Participants were expected to visit the laboratory on 4 separate occasions, after meeting the inclusion criteria and agreeing to take part. During the first visit baseline measurements of all data markers were taken; anthropometric data, RMR, HR, fasting and postprandial blood sampling, IAT, EAQ, Food craving questionnaire-trait (FCQ-T, Appendix 15) and food craving questionnaire-state (FCQ-S, Appendix 16) responses to a test meal. During visit two, maximal oxygen consumption was assessed. Visits three and four were the main experimental trials, where high and moderate intensity exercise was randomised, and all other parts of the trials were identical. Trials began with IAT, EAQ and FCQ-S measurement, after which exercise followed.

5.3.2. Exercise protocol

All exercise was performed using an electronically braked cycle ergometer (Corival 400, Lode, Groningen, Netherlands) and participants were given a 5 minute warm up and cool down period. High intensity exercise consisted of 5, 4 minute intervals at 90% $VO_{2\ peak}$, with 2minutes rest between each interval; low intensity exercise consisted of 40 minutes' continuous exercise at 50% VO_{2peak}. Fluid intake was *ad libitum* during each trial and to monitor hydration pre exercise and post exercise body weight was taken to suitably rehydrate

participants (1.5L per 1kg weight loss), pre and post exercise hematocrit was also collected. Venous blood samples were taken immediately post and one-hour post exercise; IAT, EAQ and FCQ-S were also taken immediately post exercise. Prior to each trial participants were informed to keep to the same diet for 24 hours and to avoid any strenuous exercise, alcohol or caffeine. A flowchart of the current study procedures is also presented in figure 5.3.2.

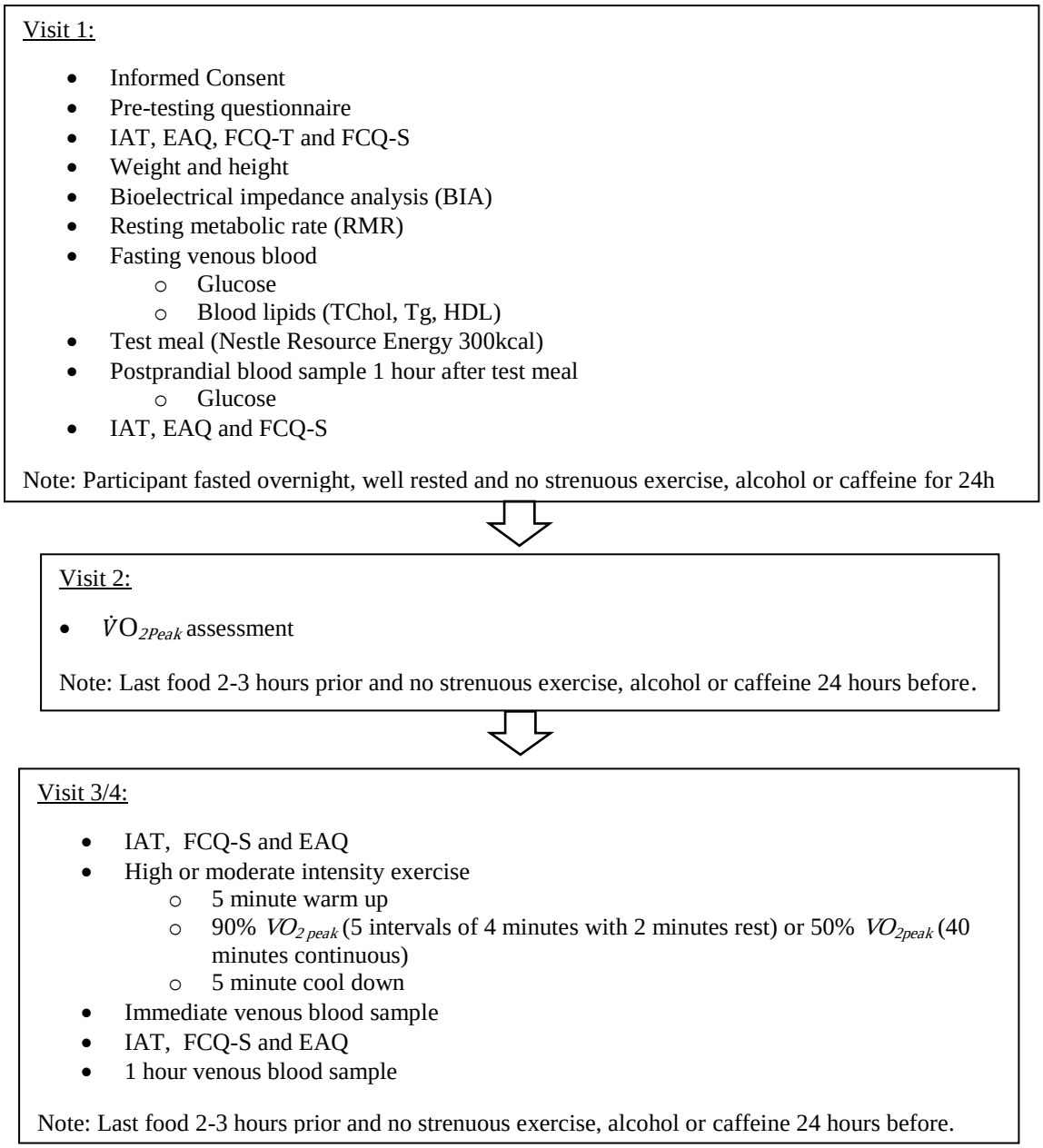

Figure 5.3.2. Protocol schematic of Pilot Study Experimental Procedure. IAT, implicit association test; EAQ, explicit attitude questionnaire; FCQ-T, food cravings questionnaire-Trait; FCQ-S, food cravings questionnaire-state; HDL, high density lipoprotein; LDL, low density lipoprotein; Tcho, total cholesterol; Tg, triglyceride; and VO_{2peak}, peak oxygen uptake.

5.4. Main Study

5.4.1. Subjects and design

19 female participants (Lean n=9; OV/OB n=10) meeting the inclusion criteria took part in this study, for which anthropometric data are shown in table 5.8.1. The main study largely resembled the pilot study and employed a randomised, counter-balanced, cross-over design, whereby participants completed two trials; a moderate- and high-intensity exercise bout. However, in some aspects, procedures and visits were altered in consideration of the findings from the first study.

Participants were once again required to visit the laboratory on 4 separate occasions, and as before, during visits one and two baseline measurements of all measures were obtained; visit one was used to collect anthropometric, RMR and fasting blood data; visit two was used to collect baseline behavioural data: IAT, EAQ, FCQ-T and FCQ-S in response to a test meal. EAQ and FCQ responses were taken pre meal, immediately post meal and 30 minutes' post meal; IAT was only taken immediately post meal. At the end of visit two maximal oxygen consumption was measured. Visits three and four were the main experimental exercise trials, where all procedures were replicated other than the chosen exercise. Exercise protocol was unchanged from the pilot study for both high (90% $\dot{V}O_{2peak}$) and moderate intensity (50% $\dot{V}O_{2peak}$) exercise. Before arriving at the laboratory participants were instructed to consume their last food within 2-3 hours of attending. Additionally, participants were asked to replicate their diet 24 hours before exercise and to avoid any strenuous exercise, alcohol or caffeine consumption. Prior to exercise, IAT, EAQ, and FCQ-S measurements were taken, venous blood samples, glucose levels, hematocrit and bodyweight were also obtained. All these measures were collected once more, immediately following exercise and one hour after

exercise (bodyweight and hematocrit were only measured immediately after exercise). Immediately after the one-hour post exercise measurement participants were given a test meal and straight after this EAQ and FCQ-S responses were collected. Finally, 30 minutes after the test meal was consumed IAT, EAQ and FCQ-S responses were obtained. A flowchart of all procedures during the main study are presented in figure 5.4.1.

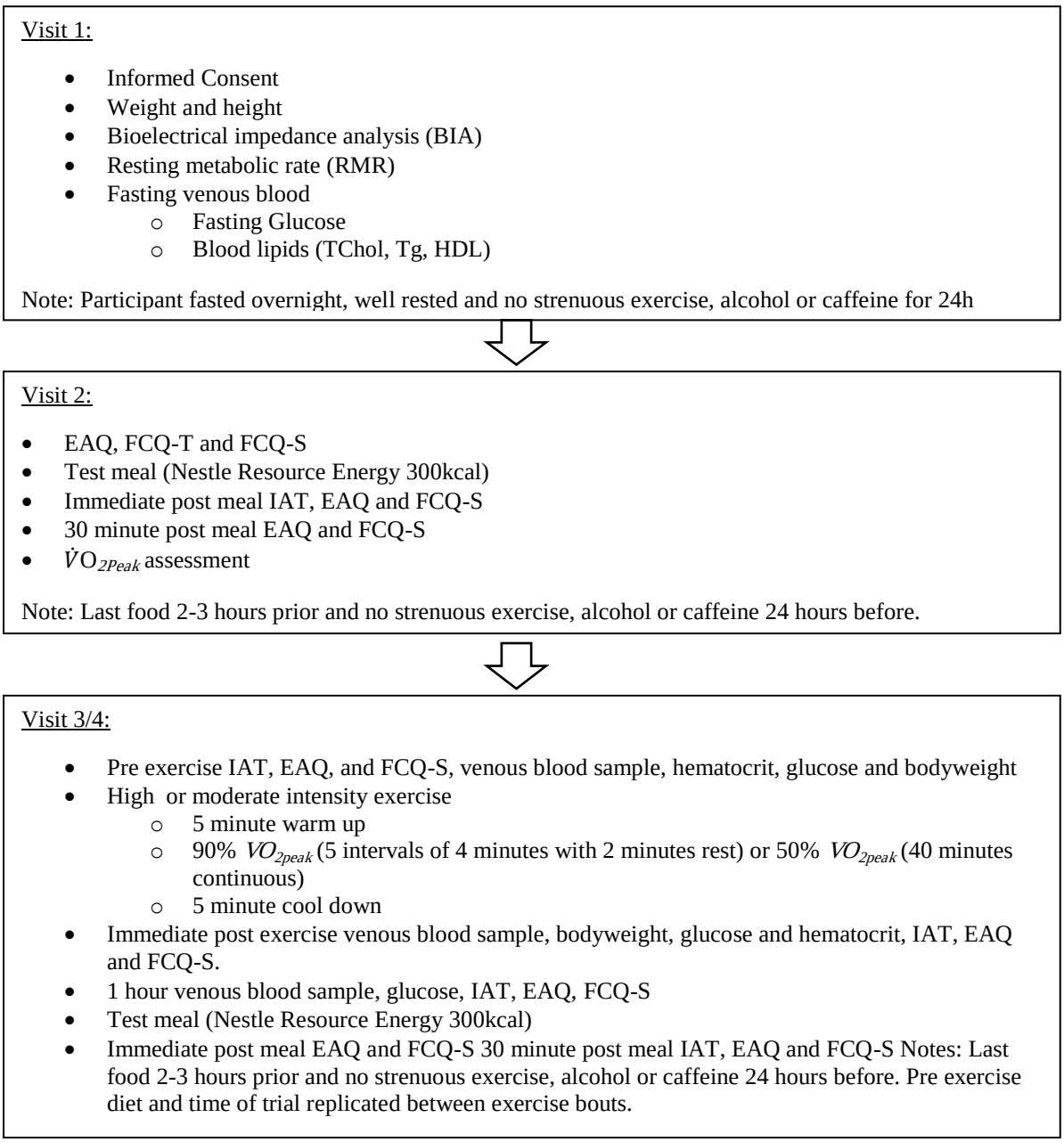

Figure 5.4.1. Protocol schematic of Main Study Experimental Procedure. IAT, implicit association test; EAQ, explicit attitude questionnaire; FCQ-T, food cravings questionnaire-Trait; FCQ-S, food cravings questionnaire-state; HDL, high density lipoprotein; LDL, low density lipoprotein; Tcho, total cholesterol; Tg, triglyceride; and $VO_{2peak,}$ peak oxygen uptake.

5.5. Pre-experimental procedures

Participants were asked to visit the laboratory. Participant's anthropometric measurements were taken as described in Chapter 3.2. Body mass index (BMI) was calculated by dividing the participants' weight in kilograms by the square of their height in metres ($kg.m^{-2}$). Resting metabolic rate (RMR) and respiratory exchange ratio (RER) were measured, following an overnight fasting (See Chapter 3.4). Cardiorespiratory fitness was assessed (See Chapter 3.3).

5.6. Experimental Procedures

5.6.1. Energy expenditure during exercise

For calculation of energy expenditure during both high and moderate intensity exercise trials, heart rate (HR) was monitored by telemetry throughout (Polar RS800CX - Polar Electro Oy, Kempele, Finland). Energy expenditure was then estimated from HR readings following previous recommendations by Keytel *et al*. (2005).

5.6.2. Test meal

A liquid test meal (See Chapter 3.5) was used to measure postprandial response of subjective appetite ratings (just in FCQ-S) following the fasting blood sample, one hour after a test meal, only in pilot study; also the same test was carried out in main study immediately post meal (1hour after exercise) and 30 min post meal (1 hour and 30 min after exercise). Participants were informed to consume the test meal as soon as possible and the exact time of consumption was recorded for subsequent measurements.

5.6.3. Blood sampling and analysis

Blood samples were obtained by a qualified phlebotomist as explained in Chapter 3.6. Fasting and postprandial glucose (pilot study only) and blood lipid profiles including total cholesterol, triglycerides and high-density lipoproteins using Reflotron plus system were measured. In addition, low-density lipoproteins were calculated using the formula LDL= TC – HDL – (TG/2.17) (Friedewald, 1972). The samples were then centrifuged for 10min at 3000rpm, 4^0C to separate plasma and frozen at -80°C until further analysis.

After all experimental trials were completed, frozen plasma samples were analysed to determine appetite-related hormone concentrations of amylin, total ghrelin, acylated ghrelin, leptin and insulin by enzyme-linked immunosorbent assay (ELISA). The homeostasis model assessment (HOMA-2) (www.dtu.ox.ac.uk/homacalculator/) was conducted to estimate steady state beta cell function (%B), insulin resistance (IR) and insulin sensitivity (%S) (Wallace *et al.,* 2004) (See Chapter 3.7). The intra assay coefficient of variation for all appetite related hormones for both pilot and main part of the study are as follows: amylin- 12%, 17%, insulin- 24%, 6%, leptin- 3%, 4%, total ghrelin- 4%, 3%, and acylated ghrelin- 25%, 20% respectively.

5.6.4. Implicit association task (IAT) and explicit attitude test (EAQ)

Implicit attitudes (high fat-low fat and high sugar-low sugar) and explicit attitude towards food (high fat, low fat, high sugar and low sugar) were assessed using the Implicit Association Test (IAT) (See Chapter 3.11) and Explicit Attitude Questionnaire (EAQ) respectively (See Chapter 3.11 and 3.12).

5.6.5. Subjective appetite ratings

Subjective appetite ratings were assessed by the food cravings questionnaire (FCQ) which has been shown to be a reliable and valid measure of food cravings (Cepeda-Benito *et al.,* 2001). There are two parts to the FCQ; the food cravings questionnaire-trait (FCQ-T) and the food cravings questionnaire-state (FCQ-S). The FCQ-T was developed to measure cravings in a general sense e.g. 'what would be true in a general' and it provides measures of 9 trait dimensions of food cravings. The 9 dimensions are:

- Positive reinforcement: relates to the anticipation that positive emotions will be achieved from eating.
- Negative reinforcement: relates to the anticipation of relief from negative feelings after eating.
- Cue dependent eating: relates to the extent to which food cues initiate eating.
- Pre occupation with food: relates to the extent to which thoughts are processed about consuming food.
- Feelings of hunger: relates to general feelings of fullness.
- Intentions to eat: relates to the extent to which plans are made to consume food.
- Lack of control: relates to the extent to which control is lost when eating begins.
- Negative effect: relates to the extent to which negative emotions effect food intake.
- Guilty feelings: relates to the extent of the guilt felt following giving in to cravings.

The FCQ-S was developed to measure food cravings at a particular moment in time e.g. 'right now, at this very moment' and it provides 5 state dimensions of food cravings. Additionally, in the present study participants were asked to complete the questionnaire in reference to sweet and savoury food. The 5 dimensions are:

- Intense desire to eat: refers to an urge to consume a specific kind of food,

- Positive reinforcement: refers to the extent to which positive emotions will be gained following eating,
- Negative reinforcement: refers to the extent to which consuming food will provide the relief of negative feelings,
- Feelings of hunger: refers to the feelings of hunger toward a specific type of food and
- Lack of control: refers to the extent to which control maybe lost when eating a specific type of food.

Participants were only required to complete the FCQ-T on one occasion at baseline. The FCQ-T asks participants to rate how frequently each statement "would be true for you in general" on a 6-point scale ranging from 1 (never) to 6 (always). The FCQ-S was completed by participants at varying time points, as outlined in the design section. Individuals were instructed to indicate the extent to which they agreed with each statement "right now, at this very moment" on a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree).

5.6.6. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics 20. Prior to commencing the statistical analysis, all data were assessed for relevant assumptions: normality, outliers, homogeneity of covariance. All variables measured in response to HI or MI exercise at multiple time points were analysed by mixed model ANOVA, with one or two between subject factors, intensity and body type (pilot study – intensity only, main study – intensity and body type); and one within subject factor, time. Where necessary, post hoc analyses were conducted by pairwise comparisons regarding effects of time and regarding between subject factors, independent *t*-tests were conducted. The relationships between variables were

investigated by using bivariate Spearman. All data are presented as means ± standard deviation, unless otherwise stated. To aid the understanding of results various tables and graphs are displayed. Where main effects of time have been reported independent of intensity, groups have been combined and where presented, means and standard deviations reflect this.

5.6.7. Power calculations

Following the completion of the study2 including the pilot and main studies, we conducted A priori and post-hoc power analysis with the program *G*Power* (Erdfelder, Faul, & Buchner, 1996). It was carried out based on previously obtained data (Taylor & Oliver, 2009) to find out whether our design in both pilot and main studies had sufficient power to detect a significant effect on our parameters, and that how many participants would be required. An A priori power calculation analysis was conducted to determine the minimum number of participants that were required with $\alpha = 0.05$ to achieve a power of 0.95.

As to the pilot study, relying on FCQ-S parameter, A priori analysis revealed that 22 participants would be required to guarantee the adequate power. Nevertheless, due to the considerable dropping out of the participants, the pilot study failed to meet this requirement, thus proved to be underpowered. Further, a post-hoc analysis of the pilot study revealed that in this research the effect size was below the satisfactory level because of the inadequate number of the participants.

Next, we conducted A priori and post-hoc power analysis on the main study with the same procedure for the pilot study. In the main study, the significant main effect for FCQ-S was 0.37, classified as a "large effect" (i.e. > 0.4) (Cohen, 1977), as calculated from n^2= 0.121. The post-hoc power analysis output is shown below:

Analysis:	Post hoc: Compute achieved power	
Input:	Effect size f	= 0.3710208
	α err prob	= 0.05
	Total sample size	= 19
	Number of groups	= 2
	Number of measurements	= 5
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 1
Output:	Noncentrality parameter λ	= 26.1547225
	Critical F	= 2.5066210
	Numerator df	= 4.0000000
	Denominator df	= 68.0000000
	Power (1-β err prob)	= 0.9875632

Therefore, the main study registered a power of 0.99 with a sample size of 19 participants and 5 measurement time points, however we also conducted an A priori power calculation analysis to determine the minimum number of participants that were required with α= 0.05 to achieve a power of 0.95 with two groups based on previously obtained data (Taylor & Oliver, 2009). The output of the A Priori output is shown below:

Analysis:	A priori: Compute required sample size	
Input:	Effect size f	= 0.4034733
	α err prob	= 0.05
	Power (1-β err prob)	= 0.95
	Number of groups	= 2
	Number of measurements	= 5
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 1
Output:	Noncentrality parameter λ	= 29.3023267
	Critical F	= 2.5153179
	Numerator df	= 4.0000000
	Denominator df	= 64.0000000
	Total sample size	= 18
	Actual power	= 0.9939404

Thus, our A priori power calculations show we require 18 subjects to achieve a power effect of 0.95.

5.7. Results of Pilot Study

5.7.1. Baseline characteristics and exercise bout responses

All participants completed both high (90% $\dot{V}O_{2peak}$) and moderate intensity (50% $\dot{V}O_{2peak}$) exercise bouts with average workloads of 129.60±29.46 W and 72±16.32 W, respectively. For full anthropometric, lipid, glucose and HOMA 2 profiles at baseline see table 5.7.1.

Table 5.7.1. Anthropometric and metabolic parameters at baseline.

Age (yrs)	23.5 ± 2.9
Height (m)	1.59 ± 0.06
Weight (kg)	55.50 ± 7.91
BMI ($kg \cdot m^{-2}$)	21.93 ± 2.16
Body Fat (%)	17.15 ± 0.05
RMR($kcal\ d^{-1}$)	1171.24 ± 379.22
RER	0.78 ± 0.08
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	40.00 ± 5.90
Tchol (mmol/l)	3.76 ± 0.44
HDL (mmol/l)	1.37 ± 0.38
LDL (mmol/l)	2.22 ± 0.51
Tg (mmol/l)	0.83 ± 0.08
F Glucose (mmol/l)	4.52 ± 0.30
P Glucose (mmol/l)	5.16 ± 0.84
Beta Cell Function (%B)	79.38 ± 9.92
Insulin Sensitivity (%S)	187.04 ± 26.84
Insulin Resistance (IR)	0.55 ± 0.08

Data presented as mean ± *SD* (n= 10). F, fasted; PP, postprandial (1-h post-meal); BMI, body mass index; RMR, resting metabolic rate; RER, respiratory exchange ratio; $\dot{V}O_{2peak}$, peak oxygen uptake; Tcho, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; and Tg, triglyceride.

5.7.2. Baseline appetite hormone levels

Firstly, appetite hormone levels were compared to see if there were any differences between fasting and postprandial levels of each hormone (Table 5.7.2). Leptin levels were only measured in fasting conditions as we did not expect to see any change. Paired samples *t*-tests revealed a significant postprandial increase in amounts of both insulin and amylin as expected. The test meal also induced a significant reduction in plasma total ghrelin, although no difference was seen in acylated ghrelin.

Table 5.7.2. Fasting and postprandial plasma appetite-related parameters.

	Fasting	Postprandial
Leptin (ngml-1)	14.08±7.71	
Insulin (mU l^{-1})	3.67±0.99	38.83±18.12*
Amylin (pmol l^{-1})	8.07±2.62	21.11±11.99*
Total Ghrelin (pg ml^{-1})	966.73±332.88	682.48±301.95*
Acylated Ghrelin (pg ml^{-1})	14.23±5.42	11.23±4.83

Data presented as mean ± SD, (n= 10). * represents significant difference between fasting and postprandial ($P<0.05$).

5.7.3. Exercise bout appetite hormone levels

Appetite hormone levels were analysed by mixed model ANOVA with repeated measures to outline any potential difference between immediate post exercise and one-hour post exercise levels, also any differences between HI and MI conditions, and finally any potential interactions between intensity and time (Means and SD for each hormone are presented in Table 5.7.3). Comparison of insulin levels post exercise and one-hour post exercise revealed a significant main effect of time (F=18.88, η^2 = 0.512, P= 0.01). Post hoc analysis showed this difference to be a decrease from immediate post exercise to one-hour post exercise following HI; this change was not observed after MI. Additionally, a significant interaction

between time and intensity was reported (F=6.31, η^2 = 0.260, P= 0.001) where insulin concentrations decreased in HI from immediate post exercise to the one hour later, no significant changes were observed in MI. Furthermore, no main effect of intensity was detected. Analysis of amylin concentrations by ANOVA showed no significant alterations over time or between intensities, also no significant interactions were observed. When leptin levels were analysed, a significant main effect of time was seen (F=6.98, η^2 = 0.279, P= 0.017), further paired samples t-tests showed a close trend towards a significant reduction from immediate post exercise to 1-hour post exercise in HI (P=0.051) whereas MI showed no change. Moreover, no main effect of intensity or interactions between time and intensity was observed. Total ghrelin concentrations, when analysed, demonstrated a trend towards a significant main effect of time (F= 4.17, η^2= 0.188, P= 0.056), when further investigated with follow up *t*-tests, this change was evident in MI where total ghrelin significantly increased 1-hour post exercise (P=0.04) but not in HI which remained unaltered. In addition, no main effects of intensity or interactions were observed for differences in total ghrelin. Finally, when acylated ghrelin levels were investigated a significant main effect of intensity was observed (F=6.55, η^2 = 0.522, P=0.043), independent samples *t*-tests revealed that immediate post exercise concentrations were significantly greater after MI compared to HI (P=0.031), 1-hour post exercise concentrations showed no difference. Further analysis of acylated ghrelin levels also illustrated a trend towards an interaction between time and intensity (F=5.16, η^2 = 0.462, P=0.063), where HI tended to increase in acylated ghrelin one-hour post exercise (P=0.076) and MI showed no significant shift.

Table 5.7.3. Immediate post exercise and 1-hour post exercise levels of appetite hormones.

	HI		MI	
	Post-Ex.	1hr Post-Ex.	Post-Ex.	1hr Post-Ex.
Leptin (ng ml^{-1})	13.25±5.11*	11.16±2.85*	13.25±6.01	12.26±7.44
Insulin (mU l^{-1})	9.50±3.28*	3.57±2.10*§	8.66±4.97	7.07±2.37
Amylin (pmol l^{-1})	9.24±4.13	8.27±4.41	10.71±2.13	10.17±2.12
Total Ghrelin (pg ml^{-1})	721.94±363.15	765.45±268.82	755.83±292.31	845.81±266.32
Acylated Ghrelin (pg ml^{-1})	11.40±4.10†	20.37±11.08‡	58.53±23.12†	40.99±50.32

Data presented as mean ± SD (n=10). ***** represents a significant difference between post exercise and 1-hour post exercise (Ex), (P<0.05), † represents a significant difference between high (HI) and moderate intensity (MI) bouts (P<0.05). **§** represents a significant interaction between intensities and training time (post exercise and 1-hour post exercise) (P<0.05).

5.7.4. Haematocrit

Haematocrit was measured pre and immediately after termination of exercise in both the high and moderate intensity exercise bouts (Figure 5.7.4) to provide a marker of hydration status (Shirreffs, 2000) and protect against the confounding factor of hypohydration on appetite and energy intake (Corney *et al*., 2015) and was also analysed by ANOVA. As a result, a significant main effect of time was observed in haematocrit (F=40.53, η^2=0.692, P=0.001), follow up tests showed a significant increase following HI (P=0.001), while no change was seen in haematocrit after MI. Additionally, a significant interaction was observed between time and intensity (F=18.65, η^2=0.509, P=0.001) although no main effect of intensity was observed.

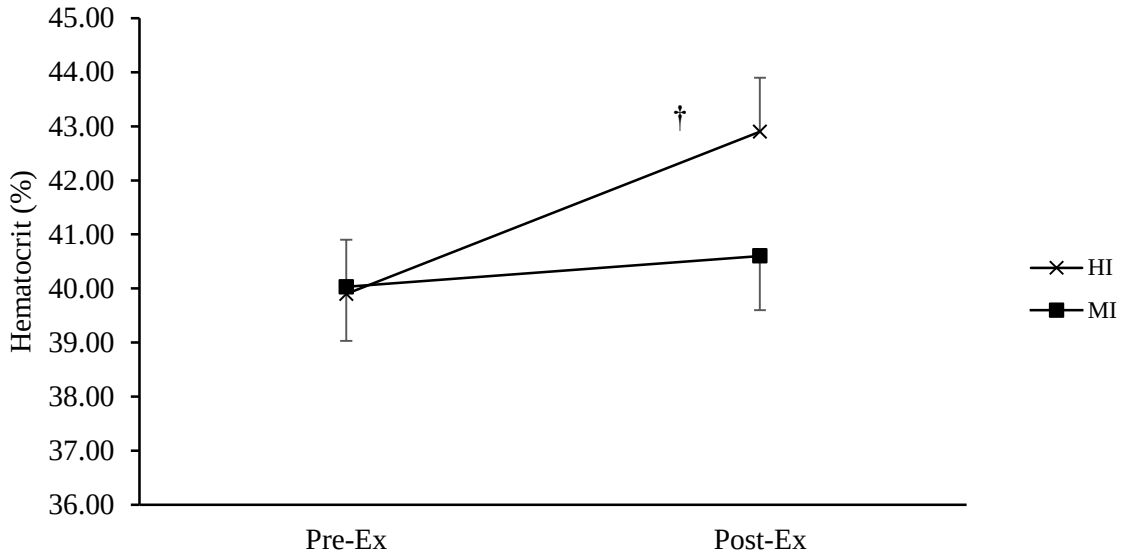

Figure 5.7.4. Haematocrit concentrations following high intensity (HI) and moderate intensity (MI) exercise (Ex). Data presented as mean ± SD. † indicates a significant difference from pre exercise and post exercise (P<0.05).

5.7.5. FCQ-S and attitude response to test meal

The FCQ-S was used to measure participants' food cravings in a fasted state and one hour after a test meal, this was separated into five dimensions; intense desire to eat, positive reinforcement, negative reinforcement, lack of control and feelings of hunger, with a score for each. In addition, both a sweet and savoury version of the FCQ-S was completed, giving a separate score for each dimension. As well as the FCQ-S, both explicit and implicit attitudes towards food were measured in a fasted and postprandial state. The EAQ was administered to measure explicit attitudes to 4 types of food; high fat/low fat/high sugar/low sugar and the IAT was employed to analyse implicit attitudes to both high fat/low fat and high sugar/low sugar. For all craving and attitude measures pre and post-test meal scores were assessed for statistical differences by paired samples *t*-tests, these revealed no significant change in all FCQ-S dimensions from pre to post meal for both sweet and savoury foods (P>0.05). Regarding the results of the EAQ, a significant change in attitude was only observed towards foods low in fat (fasting, 5.16±0.89 and postprandial, 4.73±0.81, t=2.91, P=0.009), attitudes

to high fat, low sugar and high sugar were unaltered. Interestingly, the high fat/low fat IAT also saw a significant change following the test meal (fasting, 0.71±0.55 and postprandial, 0.32±0.5, t=3.48, P=0.002), whereas, the high sugar/low sugar IAT demonstrated no alteration (for complete tables of all data see Appendix 17).

5.7.6. FCQ-S and attitude response to exercise

For both HI and MI exercise bouts, FCQ-S, EAQ and IAT were all used, as with the test meal, to analyse cravings, explicit and implicit attitudes towards food, respectively. Participants completed each of these before and immediately after exercise. Any differences in response to exercise trials was analysed by repeated measures ANOVA. Analysis of FCQ-S results illustrated alterations in positive reinforcement towards savoury food only (F=7.25, η^2=0.287, P=0.015). Follow up tests clearly demonstrated a significant decrease following the HI trial (Pre Ex., 13±4.62 and post Ex., 9.3±4.76, P=0.039), compared to the MI trial which observed no change (Figure 5.7.6). Positive reinforcement towards sweet foods also revealed a significant main effect of time (F=9.21, η^2=0.338, P=0.007). Follow up tests reported only a trend towards a decrease after HI exercise (Pre Ex., 13±4.0 and post Ex., 9.8±5.65, P=0.068), however, the MI trial significantly decreased post exercise (Pre Ex., 13.4±4.9 and post Ex., 11.1±4.15, P=0.039). All other dimensions remained unchanged from pre to post exercise, no differences were expressed between responses to HI, and MI exercise trials either. Furthermore, no significant alterations were observed in scores for any of the EAQ or IAT versions (for complete tables of all data see Appendix 17).

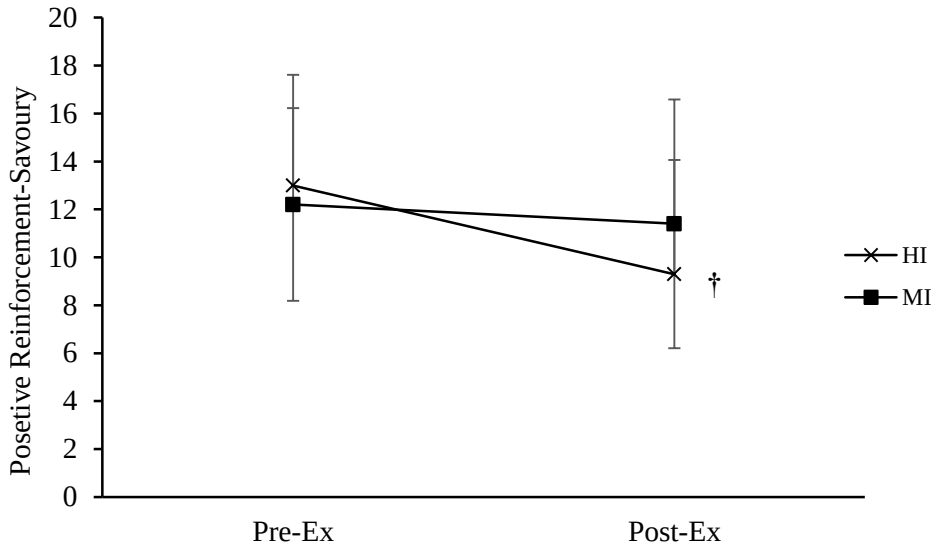

Figure 5.7.6. Positive reinforcement-savoury following high intensity (HI) and moderate intensity (MI) exercise (Ex). Data presented as mean ± SD. † indicates a significant difference from pre to post exercise ($P < 0.05$).

5.7.7. FCQ-T

The FCQ-T was administered to participants in a fasted state at the beginning of the study to characterise individuals' cravings towards food in a stable condition regardless of time and/or scenario. Table 5.7.7 shows the mean score for each dimension of the FCQ-T ± standard deviation.

Table 5.7.7. Food Cravings Questionnaire-Trait analysis.

Positive Reinforcement	18.50 ± 6.51
Negative Reinforcement	8.40 ± 2.44
Intentions to Eat	12.20 ± 4.56
Cue Dependent Eating	14.30 ± 5.98
Preoccupation with Food	18.90 ± 5.43
Feelings of Hunger	15.60 ± 2.56
Lack of Control	18.30 ± 8.76
Negative Effect	14.40 ± 6.59
Guilty Feelings	12.70 ± 5.07
Sum	133.30 ± 36.05

Data presented as mean ± SD, (n= 10).

5.7.8. Relationships between FCQ-T, appetite hormones and body composition

Potential relationships between scores for each dimension of the FCQ-T and the fasting appetite hormones were investigated by multiple Pearson's correlations between trait food craving scores and fasting hormones. The primary purpose of this was to assess whether or not the measured hormones had any relationship with the control of hunger and/or specific food cravings. Table 5.7.9 shows the correlation between each dimension of the FCQ-T and each hormone, *r* and *p* values are only reported where $P<0.1$. As table 5.7.9 illustrates, insulin was positively correlated with guilty feelings and cue dependent eating items; amylin was negatively correlated with both lack of control and guilty feelings; leptin had a positive correlation with positive reinforcement; and lastly, acylated ghrelin was negatively correlated with feelings of hunger, and positive reinforcement. BMI and weight both positively correlate with six items; intentions to eat, cue dependent eating, preoccupation with food, lack of

control, negative effect and guilty feelings. Finally, they were both positively associated with the sum of all items. In summary, body composition shows the greatest associations with food cravings, where the higher the participants' BMI or weight, the higher their cravings of food are. Amongst the appetite hormones, acylated ghrelin shows the most correlations with food cravings.

5.7.9. Relationships between FCQ-S, appetite hormones and body composition

As with the FCQ-T, multiple Pearson's correlations were performed with the FCQ-S to discover any potential relationships between the food craving scores and the fasting hormone levels. Correlations were performed between each hormone and each dimension of the FCQ-S for both sweet and savoury food categories. Of all the dimensions of the FCQ-S, negative reinforcement is correlated with the most appetite hormones. Although, when comparing sweet and savoury food, negative reinforcement of savoury food is correlated with all five hormones measured, of which total ghrelin has the strongest correlation (r=0.791). Additionally, feelings of hunger and intense desire to eat correlate with multiple hormones suggesting they have a strong influence on appetite regulation; however, positive reinforcement and lack of control show few. Regarding the appetite hormones, total ghrelin, insulin and amylin correlate with the most items of the FCQ-S, opposed to leptin, which has no significant correlation with any of the items. Moreover, to investigate any further relationships between FCQ-S scores and appetite hormones, immediate post exercise measures of both sets of data were analysed by correlations (See Table 5.7.9). A Pearson's correlation was performed between each item, both sweet and savoury, and each hormone. However, no significant relationships were observed between the many factors, only a single trend was seen between total ghrelin and intense desire to eat sweet food. This indicates that

food cravings and appetite regulating hormones are more closely linked in fasting conditions than immediately following exercise.

Table 5.7.9. Correlation between fasting FCQ-S items and fasting appetite hormones.

n=9	Insulin	Leptin	Amylin	Total Ghrelin	Acylated ghrelin
Intense desire to eat_SW	*r*= -0.654 *p*= 0.002				
Positive reinforcement_SA					*r*= 0.534 *p*= 0.022
Positive reinforcement_SW	*r*= -0.746 *p*= 0.001				
Negative reinforcement_SA	*r*= -0.589 *p*= 0.006		*r*= 0.452 *p*= 0.046	*r*= 0.791 *p*= 0.001	
Negative reinforcement_SW	*r*= -0.692 *p*= 0.001		*r*= 0.515 *p*= 0.020	*r*= 0.636 *p*= 0.003	
Feelings of hunger_SA			*r*= 0.489 *p*= 0.029	*r*= 0.770 *p*= 0.001	
Feelings of hunger_SW			*r*= 0.597 *p*= 0.005	*r*= 0.742 *p*= 0.001	

Correlation coefficients (*r*) and significance values (*p*) are given only when *P*<0.1. FCQ-S, food craving questionnaire-state; SW, sweet; and SA, savoury.

5.8. Results of Main Study

5.8.1. Baseline characteristics and exercise bout responses

All lean participants completed both high and moderate intensity exercise bouts with average workloads of 143.0±21.58 W and 79.44±11.99 W, respectively. OV/OB completed both high and moderate intensity exercise bouts with average workloads of 162.0±25.12 W and 90.0±13.95 W, respectively. For full overview of baseline characteristics of each group see table 5.8.1. All baseline characteristics were compared between groups by independent *t*-tests and significant differences are highlighted in table 5.8.1.

Table 5.8.1. Anthropometric and metabolic parameters at baseline.

Parameter (units)	L (n=9)	OV/OB (n=10)
Age (years)	21.6 ± 2.7	23.7 ± 5.2
Height	1.67 ± 0.42	1.68 ± 0.05
BMI (kg m^{-2})	20.94 ± 2.84	29.51 ± 4.31*****
Weight (kg)	58.77 ± 9.74	83.53 ± 12.78*****
Body Fat (%)	17.77 ± 10.05	32.83 ± 8.40*****
Fasting glucose (mmol l^{-1})	4.69 ± 0.35	4.46 ± 0.65
Beta Cell Function (%B)	72.99 ± 14.71	100.16 ± 39.42*****
Insulin Sensitivity (%S)	196.24 ± 50.37	152.72 ± 31.88*****
Insulin Resistance (IR)	0.55 ± 0.20	0.69 ± 0.19*****
Tcho (mmol l^{-1})	3.88 ± 1.21	3.88 ± 0.77
HDL (mmol l^{-1})	1.50 ± 0.63	1.26 ± 0.12
LDL (mmol l^{-1})	2.38 ± 1.35	2.56 ± 0.72
VO_{2peak} (l min-1kg^{-1})	44.69 ± 8.80	35.06 ± 5.24*****
RMR (kcal d^{-1})	1360.11 ± 351.22	1589.25 ± 249.62*****
RER	0.82 ± 0.01	0.75 ± 0.41*****

Data presented as mean ± SD. * denotes a significant difference between groups (P<0.05). L, lean; OV/OB, overweight/obese; BMI, body mass index; Tcho, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VO_2peak, peak oxygen uptake; RMR, resting metabolic rate; and RER, respiratory exchange ratio.

5.8.2. Baseline appetite hormone levels

To look for any baseline differences in levels of fasting appetite hormones, independent samples *t*-tests were conducted between groups. As demonstrated in table 5.8.2, OV/OB participants displayed significantly greater leptin and insulin levels than the L group, as well as significantly lower circulating total ghrelin concentrations. In addition, OV/OB individuals showed a trend towards significantly higher amylin than L individuals when fasted.

Table 5.8.2. Fasting and postprandial plasma appetite-related parameters.

	L (n=9)	OV/OB (n=10)
Leptin (ng/ml)	12.84 ± 6.29	34.53 ± 18.17*****
Insulin (mU/L)	4.08 ± 1.20	5.48 ± 1.53*****
Amylin (pmol l^{-1})	9.94 ± 5.45	11.82 ± 7.38
Total Ghrelin (pg/ml)	596.94 ± 179.22	409.06 ± 165.0*****
Acylated Ghrelin (pg/ml)	27.09 ± 14.58	27.56 ± 15.13

Data presented as mean ± SD. * denotes a significant difference between groups ($P<0.05$). L, lean; and OV/OB, overweight/obese.

5.8.3. Exercise energy expenditure

In the pilot study, energy expenditure was calculated during exercise trials using HR telemetry to make sure it matched between trials. Intensities were compared by independent *t*-test and no significant difference was observed (MI, 420.26±155.24 kcal and HI, 371.33±80.74 kcal). Once again, in the main study, to see if energy expenditure was matched, means were compared by independent *t*-test and no significant difference was detected, confirming energy expenditure was matched between high and moderate intensity exercise. Additionally, L and OV/OB group means were compared in this study by independent *t*-test

and this was not matched, energy expenditure was found to be significantly higher in the L participants (Table 5.8.3, P=0.03).

Table 5.8.3. Energy expenditure during high- and moderate-intensity exercise trials for L and OV/OB individuals.

	Energy Expenditure
MI (50% $\dot{V}O_{2peak}$)	377.23 ± 59.90 kcal
HI (90% $\dot{V}O_{2peak}$)	342.12 ± 66.29 kcal
L	383.46 ± 68.12 kcal †
OV/OB	338.28 ± 54.86 kcal †

Data presented as mean ± SD. † indicates a significant difference between body types (P < 0.05). L, lean; OV/OB, overweight/obese; HI, high intensity; and MI, moderate intensity.

5.8.4. Exercise bout appetite hormone levels

After testing the assumptions of ANOVA, insulin, leptin and amylin values did not display normal distribution therefore original data was transformed using a logarithm 10 function to proceed (Table 5.8.4). Analysis of insulin concentrations by mixed model ANOVA with repeated measures (group x condition x time) revealed significant main effects for time (F=23.54, η^2=0.457, P=0.001). Pairwise comparisons showed in both groups participants had significantly lower insulin one-hour post exercise compared to pre exercise and immediately post exercise (P<0.05). A trend was also observed for a main effect of intensity (F=3.53, η^2=0.112, P=0.071), follow up independent samples t-tests revealed only a difference between HI and MI at one hour after exercise (P=0.040, Figure 5.8.4.1). No main effect of group or significant interactions was seen.

Table 5.8.4. Appetite-related hormone concentrations at pre-exercise, post-exercise and one hour post-exercise.

	L (n=9)			OV/OB (n=10)		
Parameters (units)	Pre Ex	Post Ex	Post Ex+1H	Pre Ex	Post Ex	Post Ex+1H
Leptin (ng ml^{-1})	10.94 ± 4.92 *11.08(6.04-14.77)*	10.90 ± 5.36 *11.28(5.29-15.64)*	10.95± 5.96 *10.60(5.15-14.84)*	28.66 ± 14.48 *24.04(17.28-39.28)*	25.32 ± 13.44 *20.61(15.42-32.40)*	27.14 ± 13.86 *21.17(16.84-35.04)*
Insulin (mU l^{-1})	8.77 ± 6.06 *6.89(4.67-13.23)*	6.46 ± 3.68 *6.39(3.34-9.31)*	3.02 ± 1.83 *2.71(1.88-3.23)*	9.12 ± 6.78 *7.69(5.75-10.25)*	8.88 ± 4.81 *7.08(4.90-13.29)*	4.48 ± 2.63 *4.14(3.22-4.68)*
Amylin (pmol l^{-1})	17.37 ± 12.60 *11.39(8.53-27.56)*	12.53 ± 7.89 *8.71(47.12-17.44)*	12.23 ± 10.39 *7.74(5.86-15.75)*	14.31 ± 10.20 *11.64(6.03-16.83)*	12.70 ± 9.26 *8.66(6.02-18.62)*	11.71 ± 8.11 *8.10(5.60-19.66)*

Data presented as mean ± SD. The figures shown in italic font represent M (Q_1-Q_3) which stands for median (quarter$_1$ & quarter$_3$).

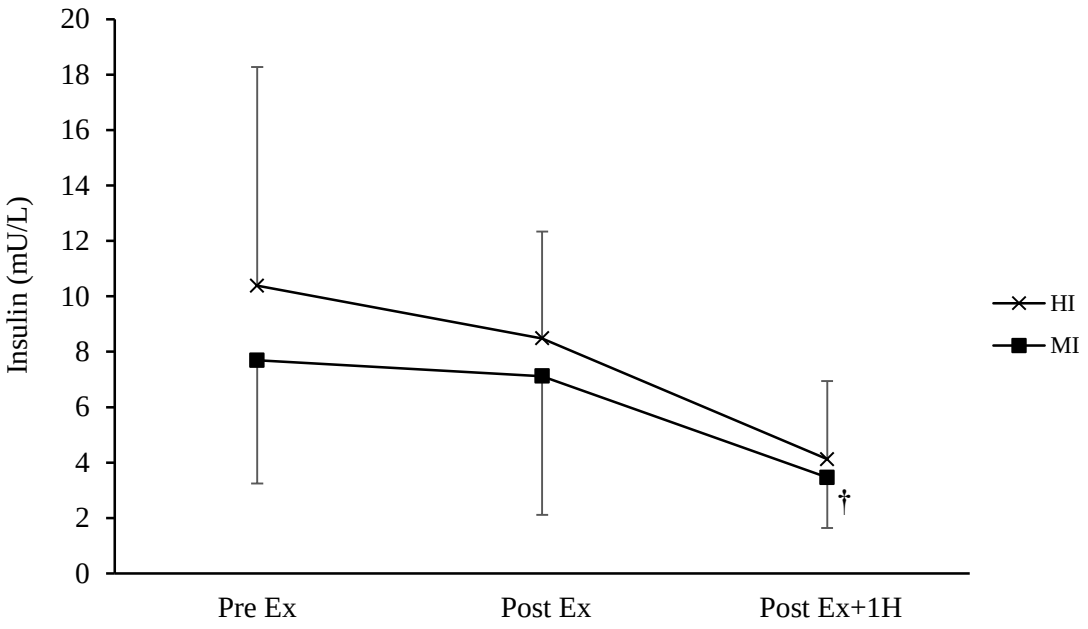

Figure 5.8.4.1. Plasma insulin alteration following high (HI) and moderate (MI) intensity exercise. Data are presented as mean ± SD. † indicates a significant reduction from pre- to one hour-post exercise ($P < 0.05$).

Analysis of leptin levels by ANOVA revealed a significant main effect for time (F=3.68, η^2=0.098, P=0.030) where a decrease in post exercise levels were observed although this difference was not present after 1-hour post exercise (Figure 5.8.4.2). Post hoc analysis revealed a significant difference between OV/OB participants but this difference was not present in the lean group. Additionally, a significant main effect for group (F=30.05, η^2=0.469, P=0.001) was also observed where OV/OB participants showed greater leptin levels across all time points. Moreover, no significant main effect for intensity was observed although there was a significant trend for interaction between time, intensity and group (F=2.60, η^2=0.071, P=0.082). Further analysis of this interaction shows the OV/OB group have the greatest change in leptin levels over time during the moderate intensity bout where a reduction in leptin from pre to post exercise is expressed (P=0.074), unlike the L group who

show the greatest change over time during the high intensity but this change was not significant.

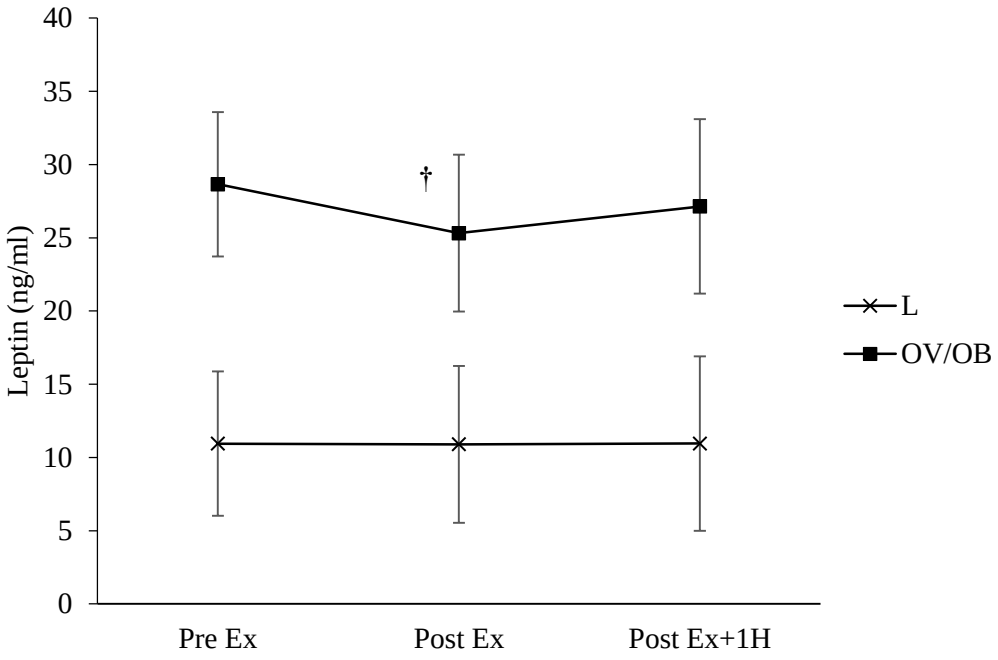

Figure 5.8.4.2. Plasma leptin alteration following high (HI) and moderate (MI) intensity exercise. Data presented as mean ± SD. † indicates a significant reduction from pre- to post-exercise (Ex) ($P < 0.05$). L, lean; and OV/OB, overweight/obese.

The results from the mixed model ANOVA for amylin showed a significant main effect for time (F=4.03, η^2=0.134, P=0.024), where a significant reduction was observed between pre exercise levels and one-hour post exercise samples, this difference was only seen in the OV/OB participants (P=0.041, Figure 5.8.4.3) and not in the L group. No main effects were observed for group and intensity; however, a significant interaction was seen between time, intensity and group (F=5.82, η^2=0.454, P=0.014), upon closer inspection the OV/OB group show the greatest response to HI where amylin reduced from pre to one-hour post exercise by as much as 46% (P=0.010, Figure 5.8.4.4).

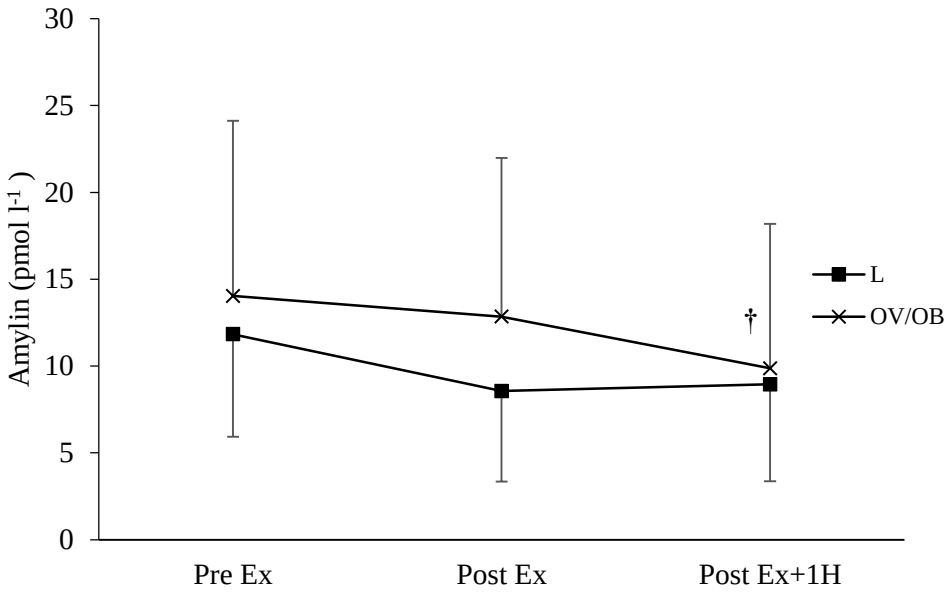

Figure 5.8.4.3. Plasma amylin alteration following exercise regardless of intensity for lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † indicates a significant reduction from pre exercise to 1h post exercise in OV/OB group ($P < 0.05$).

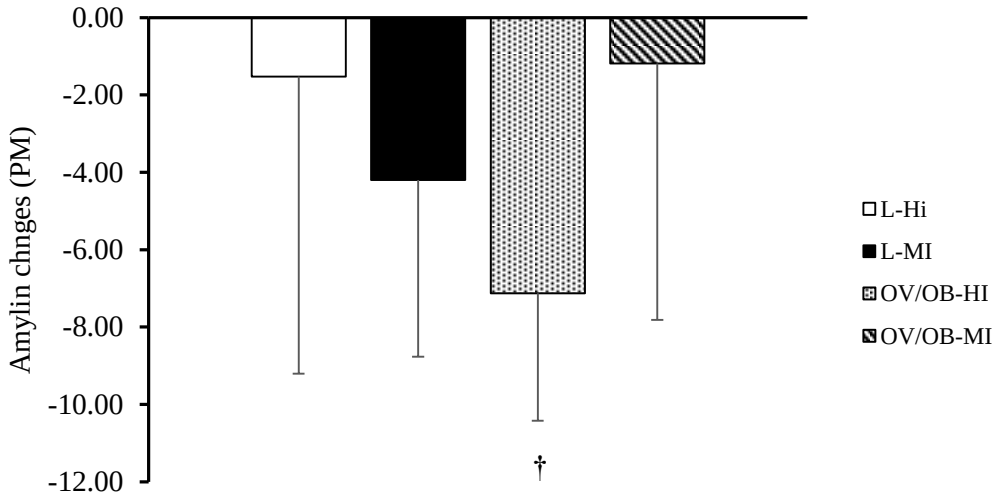

Figure 5.8.4.4. Plasma amylin alteration following high and moderate intensity exercise in L and OV/OB females. Data presented as mean ± SD. † indicates a time, intensity and group interaction ($P < 0.05$). L-HI, lean-high intensity; L-MI, lean-moderate intensity; OV/OB-HI, overweight/obese- high intensity; and OV/OB-MI, overweight/obese moderate intensity.

RM ANOVA for total ghrelin levels displayed a significant main effect for time (F=20.25, η^2=0.380, P=0.001). As shown in figure 5.8.4.5, there was an initial decrease in total ghrelin

post exercise but following a further hour levels were increased back towards pre exercise levels, this pattern was observed in both the L and OV/OB groups (P<0.05). Moreover, analysis revealed a trend towards a significant main effect for group (F=4.06, η^2=0.110, P=0.052); this was displayed between groups immediately post exercise and one-hour post exercise where L participants had greater total ghrelin level at both time points (P<0.05). Finally, no main effect for intensity was reported and only a trend towards a significant interaction between all three variables was seen (F=2.49, η^2=0.070, P=0.090). When analysing this interaction further, in the L group, only the MI exercise bout effected total ghrelin levels post exercise, whereas in the OV/OB group, both HI and MI exercise have the same impact on total ghrelin concentrations (P<0.05).

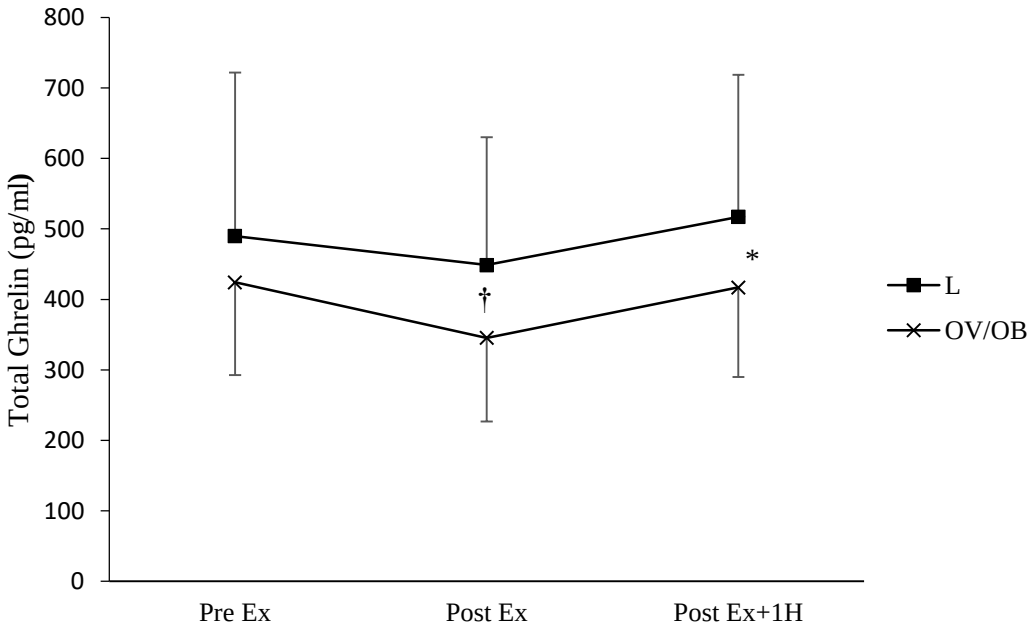

Figure 5.8.4.5. Plasma total ghrelin alteration following exercise regardless of intensity in lean (L) (■) and overweight/obese (OV/OB) (×) females. Data presented as mean ± SD. † denotes a significant reduction from pre-exercise to post-exercise. * denotes a significant increase from post-exercise to 1h post exercise (P < 0.05).

No significant time or group effects were seen in levels of acylated ghrelin, however a significant effect of intensity was observed (F=4.68, η^2=0.175, P=0.042, Figure 5.8.4.6). This

was evident pre exercise and one-hour post exercise but not immediately post exercise, the moderate intensity bout displayed a greater acylated ghrelin level than the high intensity bout (P<0.1). Furthermore, no interactions were seen between group and intensity although a trend was seen for an interaction between group and time (F=284, η^2=0.114, P=0.086, Figure 5.8.4.7). Follow up tests revealed a significant increase in L individuals from post exercise to one-hour post (P=0.030) and a movement in the other direction for OV/OB individuals although this was not significant.

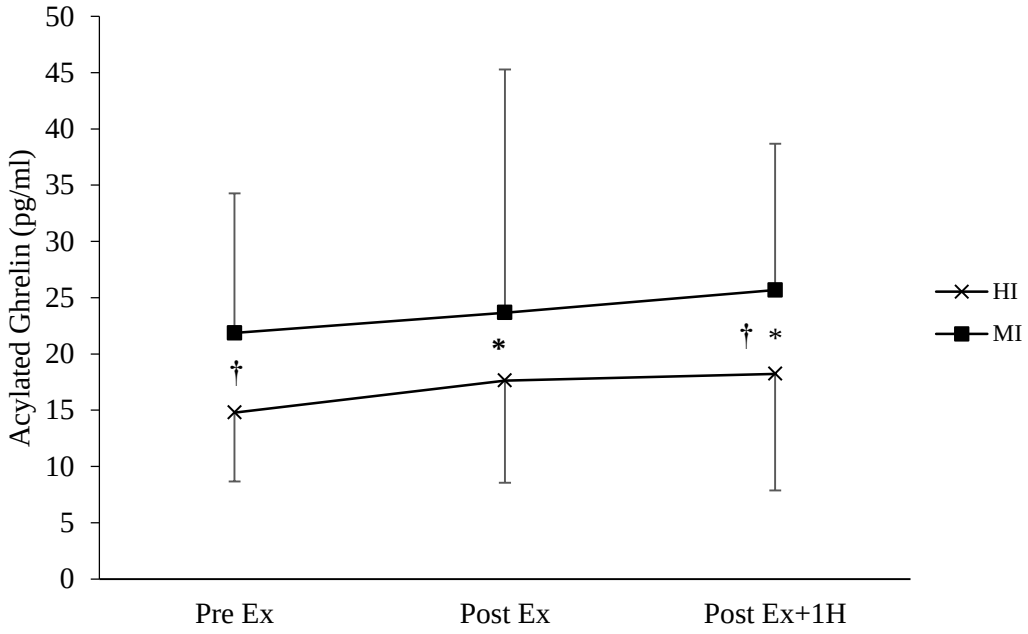

Figure 5.8.4.6. Plasma acylated ghrelin alteration following high intensity (HI) (×) and moderate intensity (MI) (■) exercise. Data presented as mean ± SD. * denotes a significant difference from pre-exercise to 1h-post-exercise. † denotes a significant difference from post-exercise to 1h-post-exercise (P<0.05).

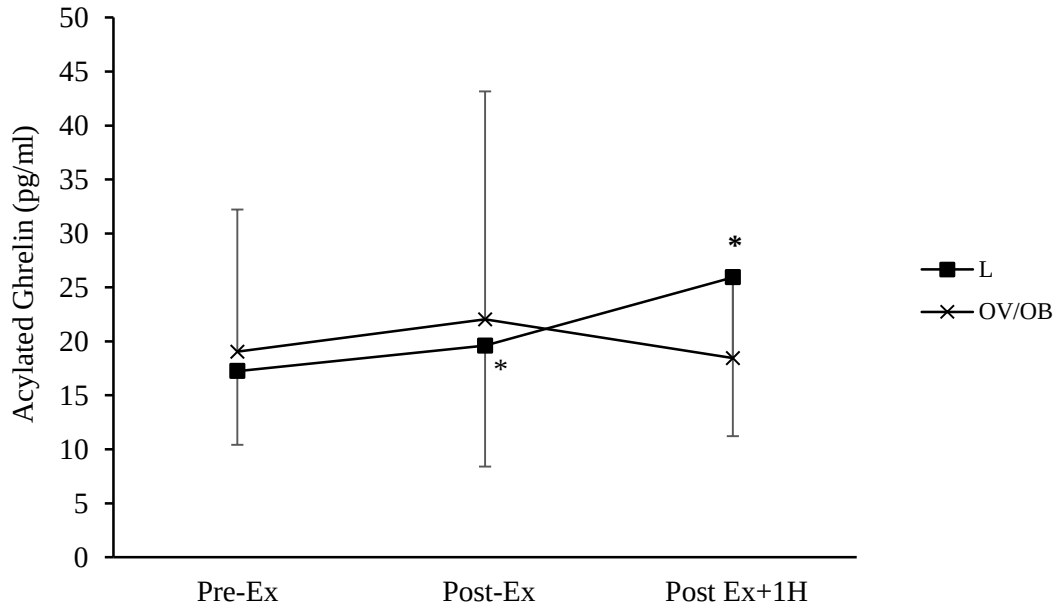

Figure 5.8.4.7. Plasma acylated ghrelin alteration in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. * denotes a significant difference from post-exercise to 1h-post-exercise ($P < 0.05$).

5.8.5. Haematocrit and glucose

Haematocrit concentration was measured pre and immediately after both HI and MI exercise bouts in L and OV/OB females. Mixed model ANOVA showed a significant main effect of time (F=11.74, η^2=0.268, P=0.002). Follow up tests indicated a significant increase in L group from pre to post-exercise (P=0.021). Additionally, a significant interaction was observed between exercise intensities from pre to post-exercise (F=7.09, η^2=0.181, P=0.012). Further analysis displayed only a significant increase after HI exercise (Table 5.8.5, P=0.002). Haematocrit concentration was unaltered, following the MI trial. No main effect of intensity or group was observed.

Glucose was also measured before and immediately after each exercise trial, with the addition of a final measurement one hour after exercise. Mixed model ANOVA was used to

analyse any differences or interactions between time points, intensities and groups. A significant main effect of time was witnessed (F=6.25, η^2=0.155, P=0.003) between glucose levels although upon further inspection this change was only evident in the L group (L-pre Ex 4.34 ± 0.62, post Ex 4.82 ± 0.77, 1hr Post Ex 4.33 ± 0.29 vs. OV/OB-pre 4.45 ± 0.42, post Ex 4.60 ± 0.68, 1hr Post Ex 4.38 ± 0.43). Here glucose concentrations decreased from post exercise to one-hour post exercise (P=0.022), and additionally, a trend towards an increase from pre to post exercise was observed (P=0.062). No significant main effects of intensity and group or interactions were reported.

Table 5.8.5. Glycaemic and haematocrit concentrations.

	HI	MI
Pre-Ex glucose (mmol l-1)	4.45 ± 0.42	4.69 ± 0.35
Post-Ex glucose (mmol l-1)	4.60 ± 0.68	4.69 ± 0.35
1hr Post-Ex glucose (mmol l-1)	4.38 ± 0.44	4.33 ± 0.29
Pre-Ex haematocrit	42.46 ± 2.94*	41.91 ± 2.34
Post-Ex haematocrit	44.28 ± 3.01*****	42.14 ± 2.33

Data presented as mean ± SD (n= 19). * denotes a significant difference from pre to post-Ex (P < 0.05). HI, high intensity; MI, moderate intensity; and Ex, exercise.

5.8.6. FCQ-T

Firstly, FCQ-T scores were compared between L and OV/OB groups to see if there were any underlying differences in food cravings irrespective of an exercise or food challenge. For all but one item of the FCQ-T there was no significant differences between groups, only a significant difference was seen between the scores for negative effect, where OV/OB participants' scores were significantly higher than their L counterparts (Table 5.8.6.1,

P=0.010). However, because participants of the pilot study also completed the FCQ-T under fasting conditions, subjects from this group were added to the sample of the main study and analysis was performed once more. In this case, when compared participants had significantly different scores for 6 items including, negative reinforcement, cue dependent eating, feelings of hunger, lack of control, negative effect and the sum of scores (Table 5.8.6.2, *P*<0.05).

Table 5.8.6.1. Food Craving Questionnaire-Trait analysis for main intervention study.

	L (n=9)	OV/OB (n=10)
Positive Reinforcement	18.22 ± 7.45	20.10 ± 7.17
Negative Reinforcement	11.33 ± 3.79	12.50 ± 4.66
Intentions to Eat	13.89 ± 3.64	13.80 ± 3.83
Cue Dependent Eating	19.78 ± 3.90	20.50 ± 5.64
Preoccupation with Food	24.56 ± 7.75	24.90 ± 10.72
Feelings of Hunger	17.89 ± 5.12	20.20 ± 5.80
Lack of Control	21.78 ± 7.82	25.70 ± 10.95
Negative Effect	14.33 ± 5.25	18.60 ± 4.41*****
Guilty Feelings	12.78 ± 5.22	14.60 ± 2.91
Sum	154.56 ± 36.46	170.90 ± 47.40

Data presented as mean ± SD. * denotes a significant difference between groups (*P* < 0.05). L, lean; and OV/OB, overweight/obese.

Table 5.8.6.2. Food Craving Questionnaire-Trait analysis for pilot study and main intervention study.

	L (n=19)	OV/OB (n=10)
Positive Reinforcement	18.37 ± 6.88	20.10 ± 7.17
Negative Reinforcement	9.79 ± 3.44	12.50 ± 4.66*
Intentions to Eat	13.00 ± 4.18	13.80 ± 3.83
Cue Dependent Eating	16.89 ± 5.75	20.50 ± 5.64*
Preoccupation with Food	21.57 ± 7.14	24.90 ± 10.72
Feelings of Hunger	16.89 ± 4.09	20.20 ± 5.80*
Lack of Control	19.95 ± 8.40	25.70 ± 10.95*
Negative Effect	14.37 ± 5.91	18.60 ± 4.41*
Guilty Feelings	12.74 ± 5.07	14.60 ± 2.91
Sum	143.37 ± 37.33	170.90 ± 47.40*

Data presented as mean ± SD. * indicates a significant difference between groups (P <0.05). L, lean; and OV/OB, overweight/obese.

5.8.7. FCQ-S and attitude response to test meal

The FCQ-S was administered to participants in a fasted state prior to test meal, immediately after and 30 minutes after (See Appendix 17 for complete tables of all data). As with the pilot study, a sweet and savoury version of the FCQ-S was completed at each time point and answers were computed to give scores for the same five dimensions. Intense desire to eat savoury food revealed a significant main effect for time (F=5.09, η^2=0.130, P=0.009), where a decrease from pre to post meal in the L participants was seen (P=0.039) and a significant decrease from immediate post to post 30 min was observed in the OV/OB (P=0.001). A significant interaction between time and group was also reported (F=3.772, η^2=0.100, P=0.028), where L participants desire to eat savoury food decreased significantly and OV/OB

tended to increase their desire to eat savoury food (See Figure 5.8.7.1). No other main effects or interactions were observed for intense desire to eat savoury food and when the same analysis was performed for intense desire to eat sweet food, no main effects or interactions were seen at all.

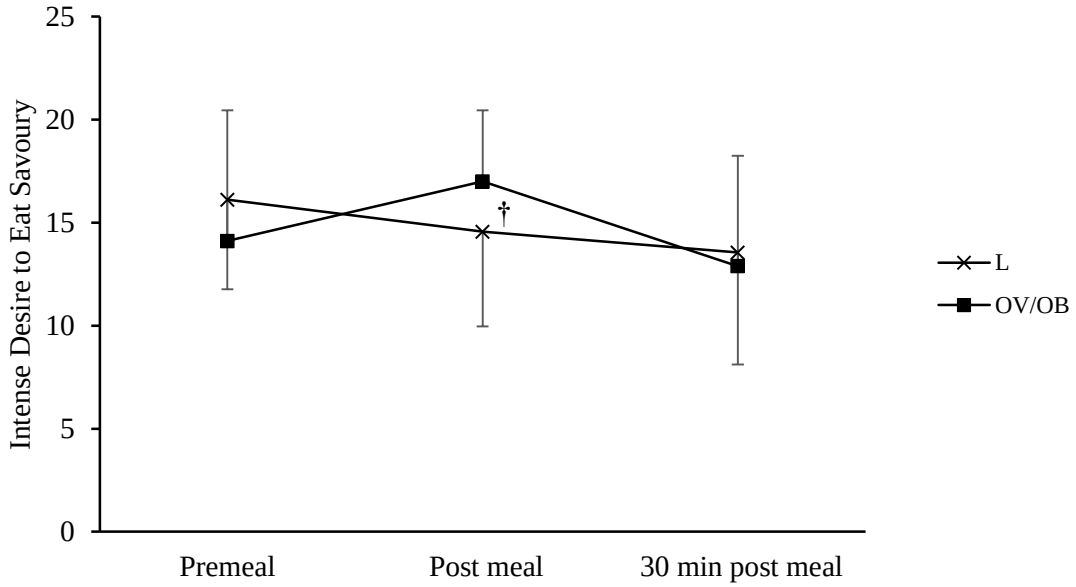

Figure 5.8.7.1. Food Craving Questionnaire analysis: Intense desire to eat savoury foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant interaction between groups from pre- to post-meal ($P < 0.05$).

Positive reinforcement towards savoury food displayed no significant alterations over time or between groups, no interactions were displayed either. However, positive reinforcement towards sweet food did demonstrate a significant interaction between time and group (F=4.82, η^2=0.138, P=0.011) although no further interactions or main effects were observed. A more in depth view of the interaction between time and group revealed a trend towards an increase in L participants from pre to post meal (P=0.091) and a decrease in OV/OB participants although this movement was not significant (Figure 5.8.7.2).

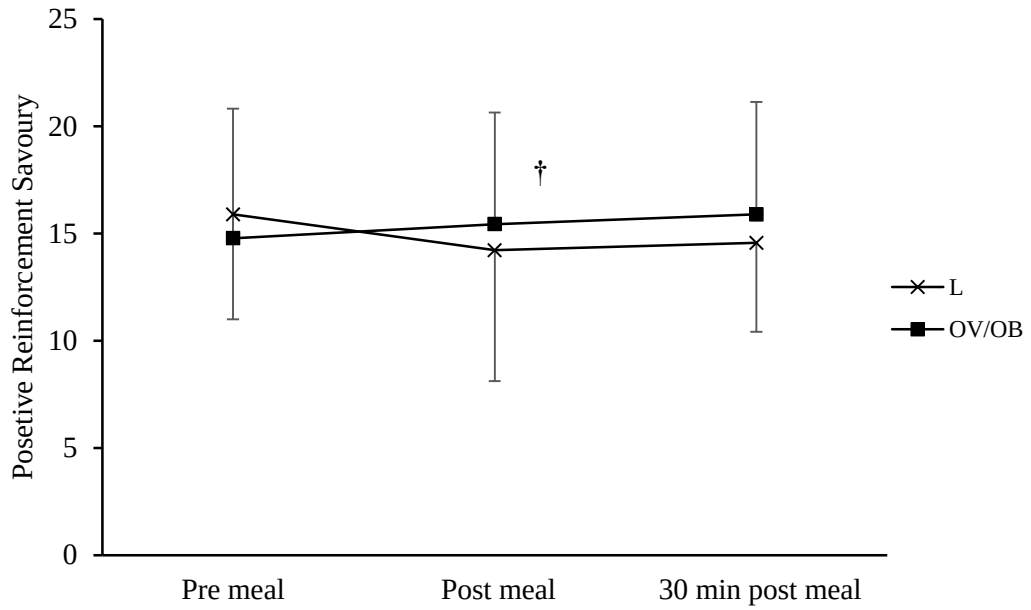

Figure 5.8.7.2. Food Craving Questionnaire analysis: Positive reinforcement for savoury foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant interaction between groups from pre to post meal ($P < 0.05$).

When negative reinforcement towards savoury food was analysed a significant effect of time was the only significant observation (F=3.96, η^2=0.104, P=0.024) and this was only evident in the OV/OB individuals from immediately after the test meal to 30 minutes later (P=0.009). No other effect of group or interactions were reported and when analysing negative reinforcement towards sweet foods no significant alterations were detected (Figure 5.8.7.3).

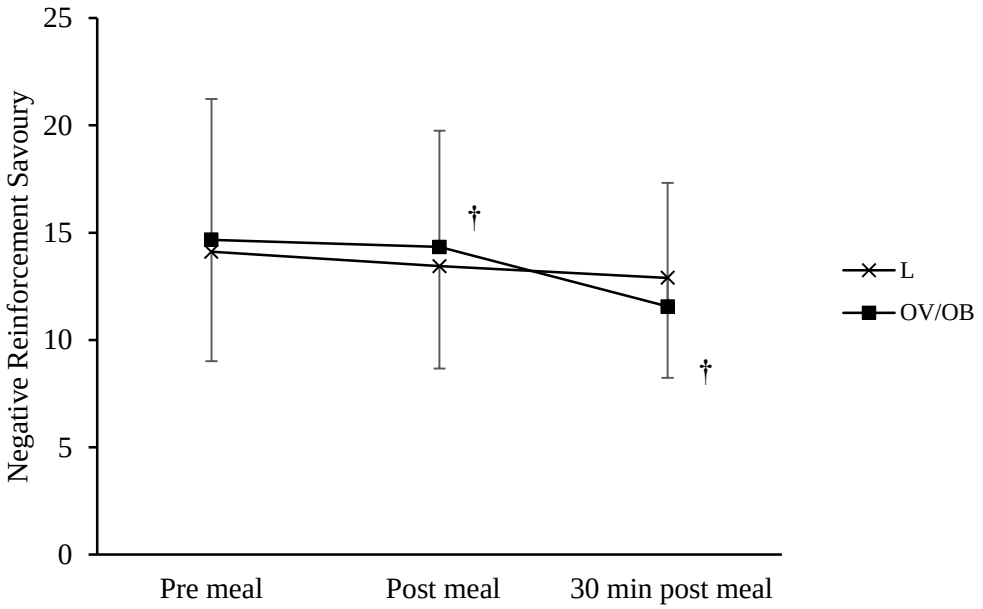

Figure 5.8.7.3. Food Craving Questionnaire analysis: Negative reinforcement for savoury foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant difference from post-meal to 30 min-post-meal in OV/OB ($P < 0.05$).

Much like negative reinforcement, lack of control towards savoury food only displayed a significant main effect over time (F=3.66, η^2=0.097, P=0.031). However, this time the L group demonstrated a significant reduction from pre to 30 min post meal (P=0.041, Figure 5.8.7.4), interestingly no difference was seen immediately after the test meal and on the contrary, the OV/OB group scores remained unaltered. Lack of control towards sweet food did not display any significant changes in response to the test meal.

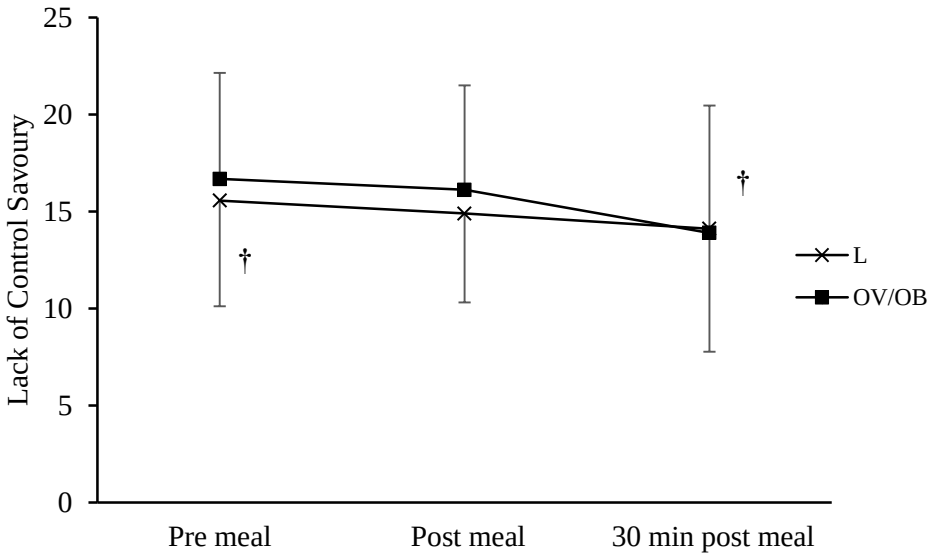

Figure 5.8.7.4. Food Craving Questionnaire analysis: Lack of control for savoury foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant reduction from pre-meal to 30 min-post-meal in L ($P < 0.05$).

Feelings of hunger was the last dimension of the FCQ-S to be analysed in response to a test meal and regarding feelings of hunger towards savoury food a significant main effect of time (F=7.72, η^2=0.185, P=0.001, Figure 5.8.7.5) and interaction between time and group were witnessed (F=4.22, η^2=0.110, P=0.019). After follow up tests, the main effect of time was evident for L individuals between pre to post-test meal, where feelings of hunger decreased (P=0.001) and OV/OB individual's feelings tended to increase post meal although this change was not significant (P>0.1). When analysing feelings of hunger towards sweet food, no significant main effects of group or time were reported but a significant interaction between these two variables was seen (F=8.24, η^2=0.205, P=0.001). This interaction took place between pre and post-test meal, where L participants feelings tended to decrease and OV/OB participants increased significantly (P=0.017, Figure 5.8.7.6).

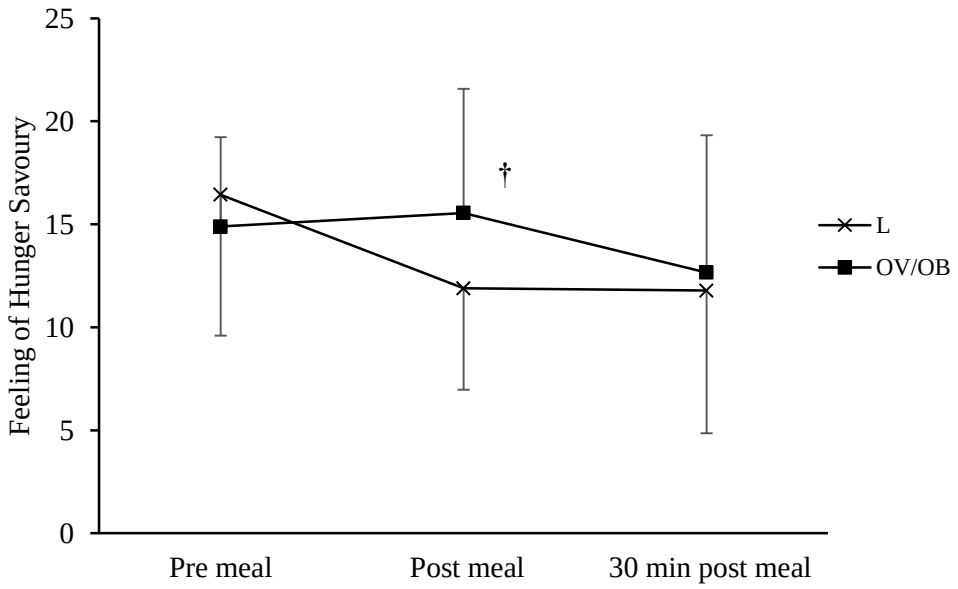

Figure 5.8.7.5. Food Craving Questionnaire analysis: Feeling of hunger towards savoury foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant interaction between groups from pre to post meal ($P < 0.05$).

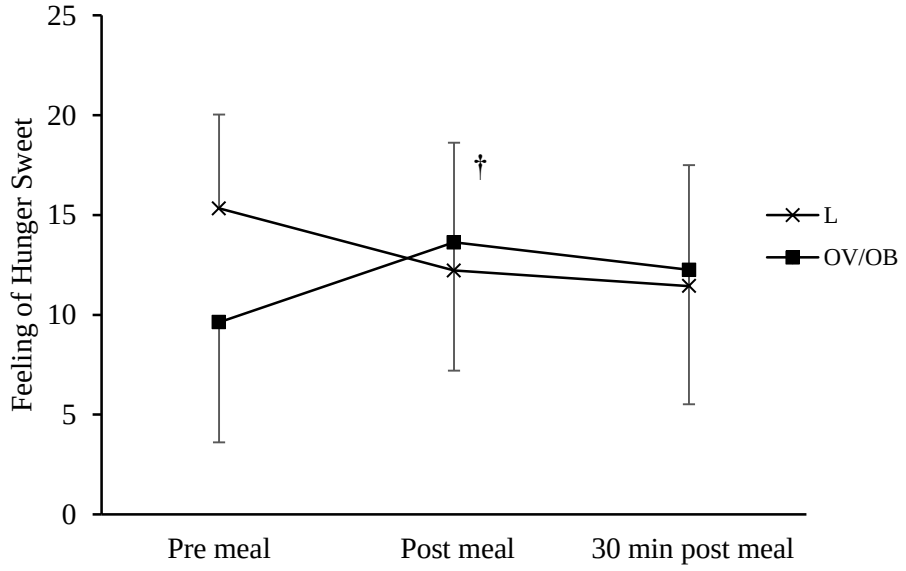

Figure 5.8.7.6. Food Craving Questionnaire analysis: Feeling of hunger towards sweet foods in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant interaction between groups from pre- to post-meal ($P < 0.05$).

Implicit attitudes towards sweet food and fatty foods were assessed during the same test meal scenario but these were only measured once immediately after consumption of the meal, therefore results can only be compared between groups. Independent samples *t*-tests showed only a significant trend between L and OV/OB participants scores for the HF/LF IAT (L 0.38±0.37 vs. OV/OB 0.60±0.42, P=0.094) and no significant differences between scores of the HS/LS IAT (L 0.27±0.46 vs. OV/OB -0.01±0.59).

Explicit attitudes towards sweet and fatty foods were monitored in response to a test meal as per FCQ-S, pre meal, post meal and 30 min post meal. Explicit attitudes to foods low in sugar displayed no main effects of time or group but a significant interaction was observed (F=5.08, η^2=0.124, P=0.009). This was illustrated immediately after the test meal where the L group scores reduced although not significantly, opposed to the OV/OB group which displayed a trend towards a significant increase (P=0.079). Explicit attitudes towards foods high in sugar did display a significant main effect of time (F=15.03, η^2=0.307, P=0.001), this was shown in both groups L and OV/OB where a reduction expressed from post meal to 30 minutes post meal (P=0.007 and P=0.001, respectively). A trend for an interaction between group and time factors was also evident from the repeated measures ANOVA (F=2.68, η^2=0.073, P=0.076), from pre to post meal L individuals tended to increase and OV/OB tended to do the opposite. Finally, when explicit attitudes to foods high and low in fat were assessed no significant main effects or interactions were observed.

5.8.8. FCQ-S and attitude response to exercise

FCQ-S was administered to participants at 5 time points during each exercise trial (For complete tables of all data see Appendix 17); pre exercise, immediately post exercise, 1-hour

post exercise, immediately post meal (1 hour after exercise) and 30 min post meal (1 hour and 30 min after exercise). A mixed model ANOVA was performed for each dimension in response to sweet and savoury food. Intense desire to eat savoury food was the first dimension analysed, where a significant interaction was observed between time and group (F=2.66, η^2=0.072, P=0.036), a significant trend for a 3-way interaction between time, group and intensity was also displayed (F=2.35, η^2=0.065, P=0.057, Figure 5.8.8.1). Although, neither group saw any significant changes in cravings over time, between 1-hour post exercise and immediately post meal, OV/OB increased their desire to eat savoury food compared to L which showed no change. On the contrary, intense desire to eat sweet food did not display any significant interactions or effects of group and intensity but a significant main effect of time was reported (F=2.65, η^2=0.072, P=0.036). Further analysis of this main effect showed the greatest change in cravings one hour after exercise and following the test meal, L participants tended to increase immediately after the test meal (P=0.086) and OV/OB participants increased 30 min later after the test meal (P=0.005, Figure 5.8.8.2).

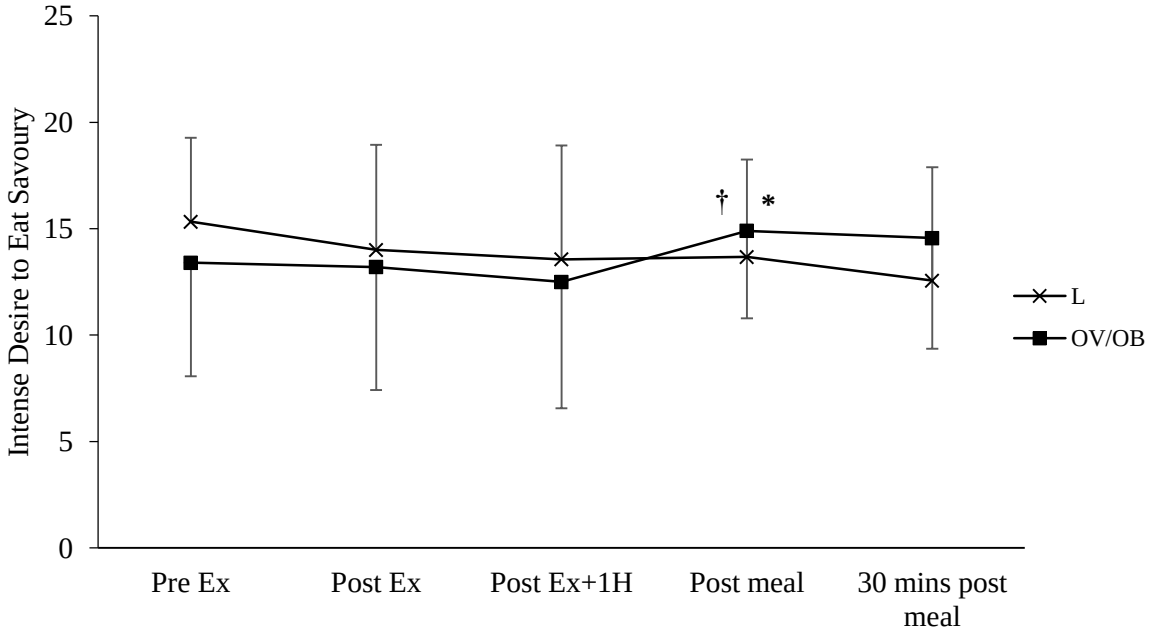

Figure 5.8.8.1. Food Craving Questionnaire analysis: Intense desire to eat savoury foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a trend toward significant interaction between groups from post to 1-hour post exercise. * denotes a significant interaction between the groups from 1-h-post-exercise and post-meal (P < 0.05).

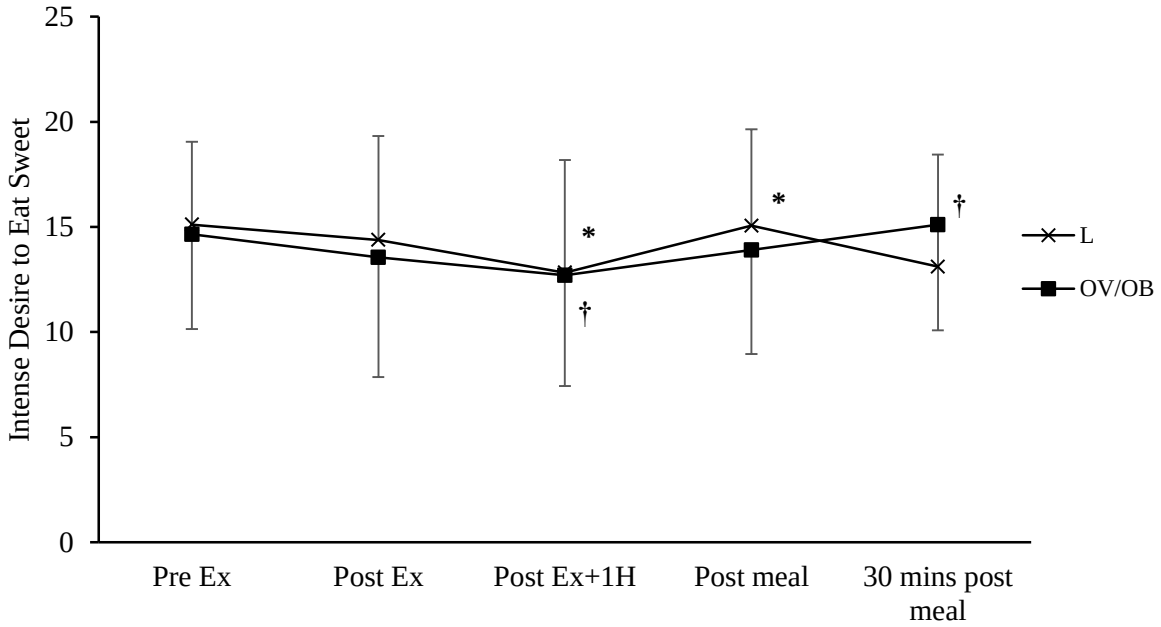

Figure 5.8.8.2. Food Craving Questionnaire analysis: Intense desire to eat sweet foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant difference from 1h post exercise to 30 min post meal in OV/OB. ***** denotes a trend toward significant difference from 1h post exercise to post meal in L (P = 0.063).

When scores of positive reinforcement towards savoury food were compared by repeated measures ANOVA no significant main effects or interactions were reported. However, a significant main effect for time was observed for positive reinforcement towards sweet food (F=2.98, η^2=0.076, P=0.021). Follow up tests revealed only a significant increase in the OV/OB groups positive reinforcement, this was observed from one-hour post exercise to 30 min after test meal (P=0.025, Figure 5.8.8.3). A trend was also seen for a decrease from pre exercise to one-hour post exercise in the OV/OB group (P=0.063). No further main effects or interactions were seen.

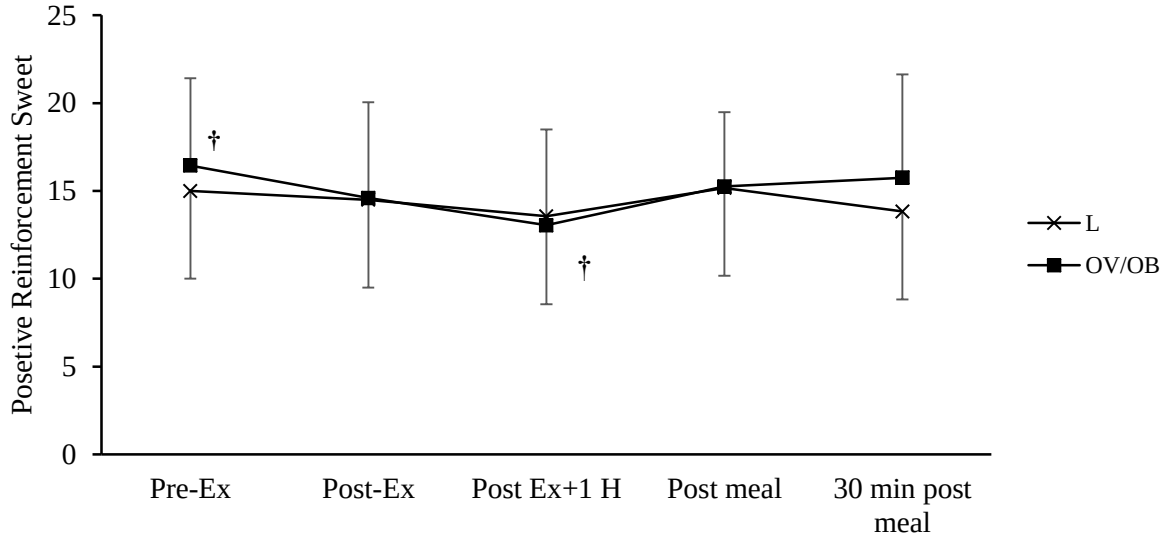

Figure 5.8.8.3. Food Craving Questionnaire analysis: Positive reinforcement towards sweet foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant difference from pre exercise to 1h post exercise in OV/OB ($P < 0.05$).

Negative reinforcement towards savoury food displayed both a significant main effect for time (F=2.51, η^2=0.069, P=0.045) and an interaction between time and group (F=2.68, η^2=0.073, P=0.034, Figure 5.8.8.4) but no other effects or interactions were observed. The main effect of time was investigated further to reveal only changes in OV/OB participants and in particular OV/OB showed greater negative reinforcement both immediately and 30 min after test meal compared to post exercise ratings (P<0.1). Analysis of negative reinforcement regarding sweet food found a significant main effect of time (F=4.01, η^2=0.105, P=0.004) as well as an interaction trend between time and group factors (F=2.39, η^2=0.066, P=0.054). Post hoc analysis using pairwise comparisons showed time differences only evident in the OV/OB group, more specifically an initial decrease from pre exercise to one hour post exercise(P=0.017), followed by an increase 30 min after the test meal (P=0.018). Further inspection of the interaction shows a difference in how the groups responded to the test meal after exercise, L display a decrease in negative reinforcement and OV/OB increase in negative reinforcement (Figure 5.8.8.5).

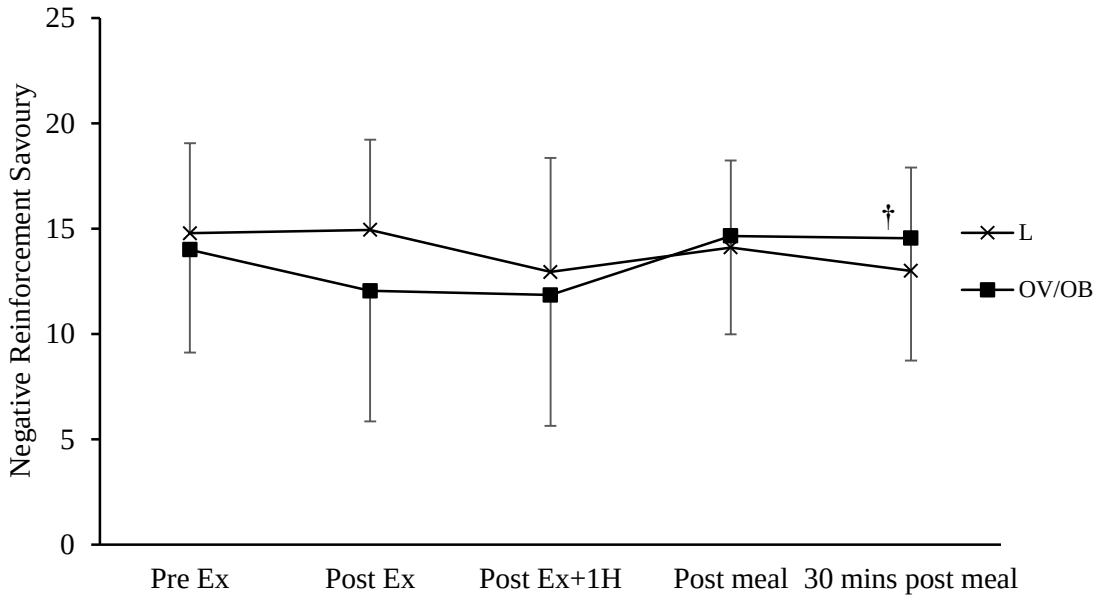

Figure 5.8.8.4. Food Craving Questionnaire analysis: Negative reinforcement towards savoury foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant interaction between groups from post meal to 30 min post meal ($P < 0.05$).

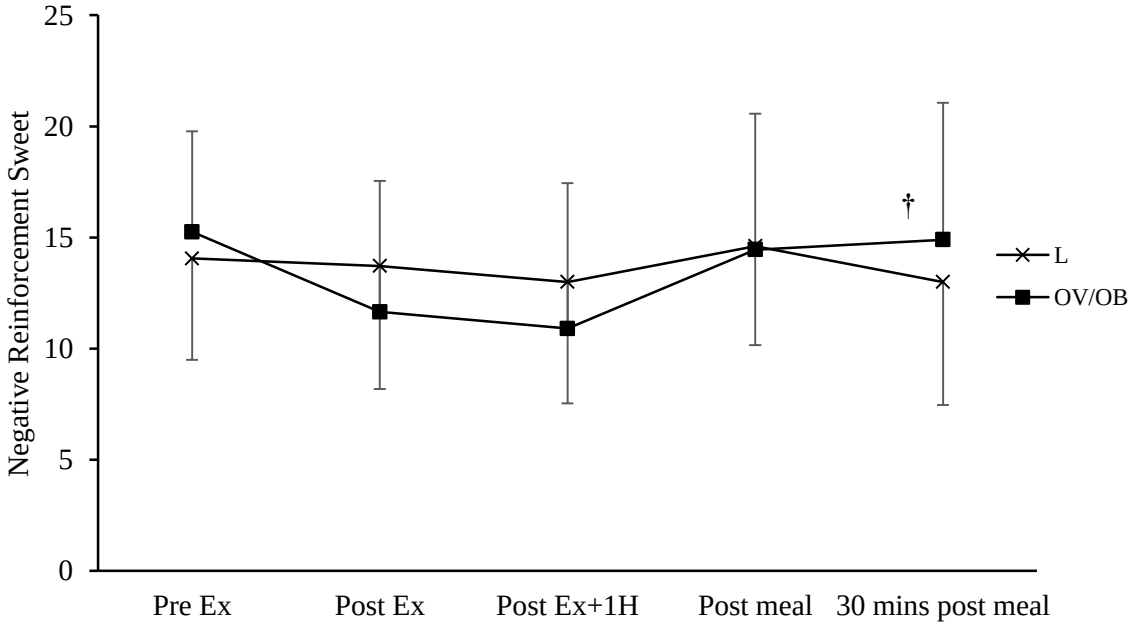

Figure 5.8.8.5. Food Craving Questionnaire analysis: Negative reinforcement towards sweet foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a trend toward significant interaction between groups from post meal to 30 min post meal ($P < 0.05$).

Lastly, feelings of hunger associated with savoury food did show some change in response to exercise, both a significant main effect of time (F=4.69, η^2=0.121, P=0.003) and interaction trend between time and group were reported (F=2.18, η^2=0.060, P=0.091). Once again, the OV/OB group display the greatest changes in craving scores; L participants' feelings of hunger remain unaltered by either exercise trial. Pairwise comparisons in the OV/OB group show a significant decrease in feelings from pre exercise to one-hour post exercise (P=0.022), then from one-hour post exercise to immediately after the meal (P=0.005) and 30 minutes after (P=0.074) feelings increase significantly greater and greater still, though only a trend (Figure 5.8.8.6). Although no significant difference of time was seen in the L individuals, a small decrease from post meal to 30 min post meal is evident, this is a movement in the opposing direction to OV/OB individuals' feelings who experience a close to significant drop during the same time points. This suggests OV/OB and L participants respond very differently to a test meal after exercise regardless of intensity. In response to feelings of hunger associated with sweet food a similar pattern to savoury food is seen, a significant main effect of time (F=5.35, η^2=0.136, P=0.002), although no significant interaction was observed. In the OV/OB group pairwise comparisons show a significant drop in feelings from pre to one-hour post exercise (P=0.007, Figure 5.8.8.7), feelings did then increase after the test meal and 30 min later but this was not significant. L participants' feelings did not significantly alter over time.

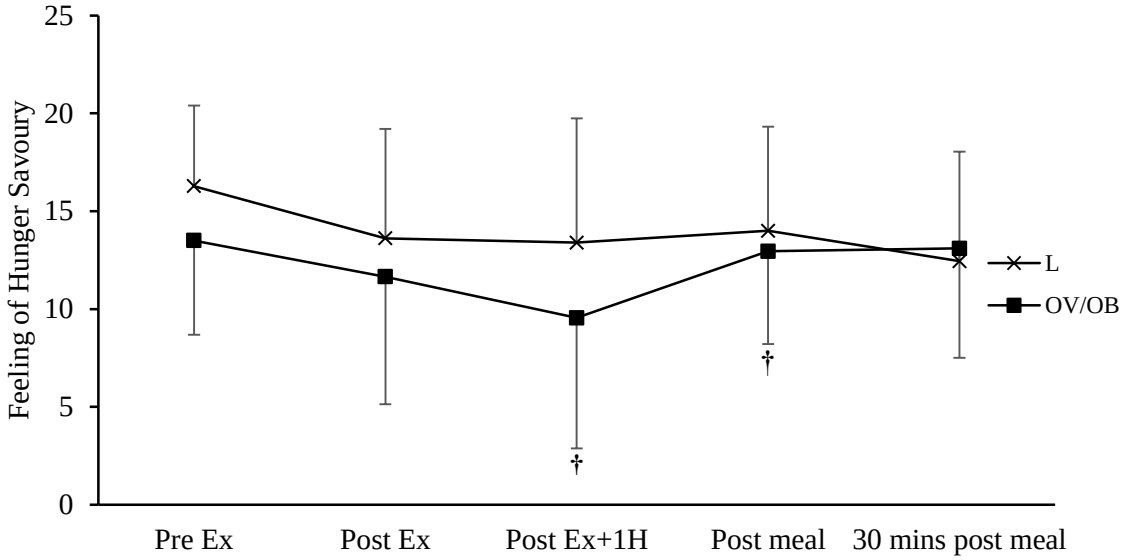

Figure 5.8.8.6. Food Craving Questionnaire analysis: Feelings of hunger for savoury foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant difference from pre Ex to 1h post exercise and1h post exercise to post meal in OV/OB (P<0.05).

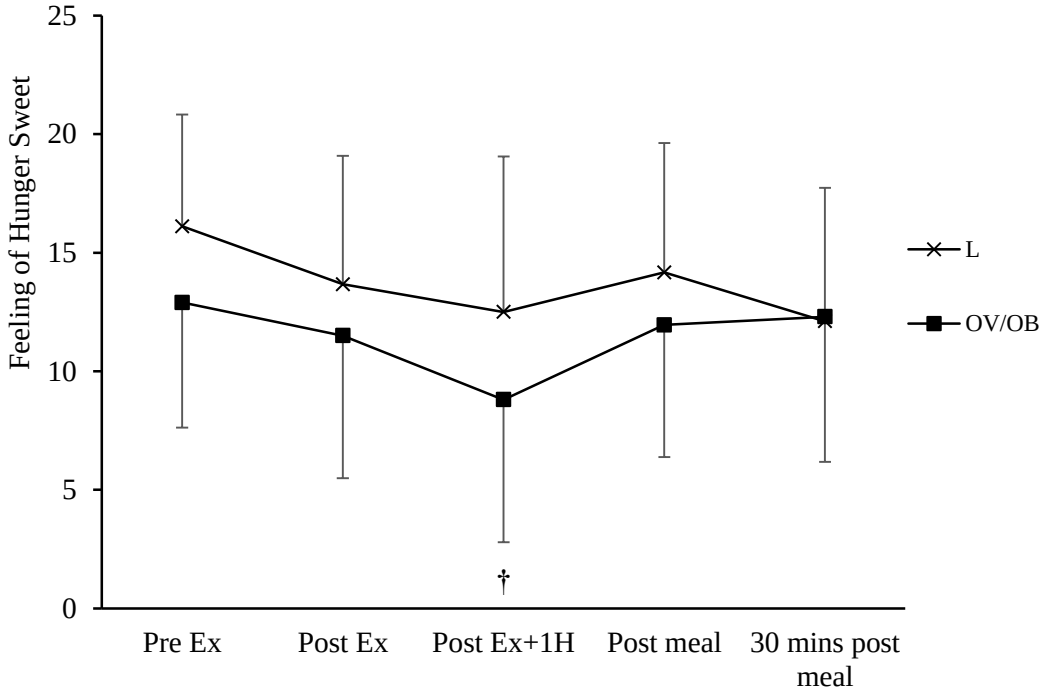

Figure 5.8.8.7. Food Craving Questionnaire analysis: Feelings of hunger towards sweet foods following exercise in lean (L) and overweight/obese (OV/OB) females. Data presented as mean ± SD. † denotes a significant difference from pre Ex to 1h post exercise in OV/OB (P < 0.05).

All versions of the EAQ (high fat, low fat, high sugar, low sugar) were administered with the FCQ-S at all five time points during both the HI and MI exercise trials. Mixed model ANOVA was performed on the data obtained from each EAQ and no significant differences were observed for any of the food categories. Implicit attitudes towards both fat and sugar where measured by the IAT at three time points during the exercise trials (pre exercise, immediately after exercise and immediately after test meal). Data from each IAT were explored again by ANOVA and no significant differences were observed from the HS/LS IAT, however, the HF/LF IAT did show a significant trend towards a main effect for time (F=2.61, η^2=0.073, P=0.081). Looking in more detail, both groups show a decrease across each trial, from pre exercise to post exercise and post meal.

5.8.9. Relationships between FCQ-T, appetite hormones and body composition

FCQ-T scores were correlated with fasting appetite hormone levels. Table 5.8.9.1 shows the correlation between each item of the FCQ-T and each fasting appetite hormone for the OV/OB group. Interestingly, for the L individuals fasting amylin is correlated with all food craving dimensions, insulin and total ghrelin also show some correlation but leptin and acylated ghrelin show no correlation. This is in staunch contrast to the OV/OB individuals where fasting leptin shows the greatest correlation with food craving items and amylin is correlated with very few dimensions. Furthermore, as L participants in both the pilot study and the main study provided FCQ-T scores and appetite hormone levels, this data was combined to give a larger group (n=18) and table 5.8.9.2 shows the correlations for this group. With the combination of studies, amylin is still highlighted as the greatest indicator of food craving scores and leptin the least, additionally insulin is also correlated with more items suggesting it too is strongly correlated with food cravings in the fasted state.

Table 5.8.9.1. Correlation between FCQ-T, fasting appetite hormones, BMI and weight in OV/OB participants in main intervention study.

n=10	Insulin	Leptin	Amylin	Total Ghrelin	Acylated ghrelin
Positive Reinforcement		r= -0.459 p= 0.042			
Negative Reinforcement		r= -0.568 p= 0.009			
Intentions to Eat				r= -0.566 p= 0.009	
Cue Dependent Eating		r= -0.759 p= 0.001			
Preoccupation with Food		r= -0.637 p= 0.003	r= 0.453 p= 0.045		
Feelings of Hunger	r= 0.463 p= 0.040			r= -0.580 p= 0.007	
Lack of Control		r= -0.655 p= 0.002			
Guilty Feelings		r= -0.433 p= 0.050	r= -0.465 p= 0.039		r= -0.660 p= 0.020
Sum		r= -0.608 p= 0.001			

FCQ-T, food craving questionnaire-trait; BMI, body mass index; and OV/OB, overweight /obese.

Table 5.8.9.2. Correlation between FCQ-T, fasting appetite hormones, BMI and weight for L participants in pilot and main intervention study.

n=18	Insulin	Amylin	Total Ghrelin	BMI	Weight
Positive Reinforcement		r= -0.470 p= 0.007			
Intentions to Eat	r= 0.424 p= 0.009	r= -0.355 p= 0.046			r= 0.523 p= 0.001
Cue Dependent Eating	r= 0.530 p= 0.001		r= -0.524 p= 0.001		r= -0.525 p= 0.001
Preoccupation with Food		r= -0.438 p= 0.012			
Feelings of Hunger		r= -0.376 p= 0.034		r= -0.324 p= 0.047	
Lack of Control	r= 0.481 p= 0.003	r= -0.481 p= 0.005	r= -0.359 p= 0.027	r= 0.352 p= 0.030	r= 0.489 p= 0.002
Negative Effect	r= 0.364 p= 0.027	r= 0.418 p= 0.017			r= 0.321 p= 0.049
Guilty Feelings		r= -0.536 p= 0.002			r= 0.413 p= 0.010
Sum	r= 0.391 p= 0.017	r= -0.525 p= 0.002			r= 0.417 p= 0.009

FCQ-T, food craving questionnaire-trait; BMI, body mass index; and L, lean.

5.8.10. Relationships between FCQ-S, appetite hormones and body composition

To better understand how food cravings and hormone levels interact in response to exercise a Pearson's correlation was performed between each dimension of the FCQ-S, including savoury and sweet foods, and each appetite hormone; fasting, pre exercise, post exercise and 1-hour post exercise. Correlations were performed separately on L and OV/OB groups. Table 5.8.10.1 shows correlations between fasting hormones and fasting FCQ-S dimension in both lean and OV/OB females. The L group; insulin, total ghrelin and amylin each correlates with one item and leptin shows no significant correlations. In the OV/OB group, only amylin was

significantly correlated with three craving items, regardless of food category. The other hormones show no significant relationships.

Table 5.8.10.1. Correlation between fasting FCQ-S items and appetite hormones at fasting in lean and overweight/obese females.

	L (*n*=9)			OV/OB (*n*=10)
	Insulin	Amylin	Total Ghrelin	Amylin
Intense desire to eat_SA			r= -0.474 p= 0.047	
Intense desire to eat_SW				r= -0.543 p= 0.013
Lack of control_SA	r= 0.616 p= 0.007			r= 0.513 p= 0.021
Feelings of hunger_SA		r= -0.658 p= 0.020		r= -0.446 p= 0.049

FCQ-S, food craving questionnaire-state; OV/OB, overweight/obese; L, lean; SW, sweet; and SA, savoury.

Correlation analysis of pre exercise hormone levels with FCQ-s domains scores revealed only few significant correlations detectable for the lean group; only acylated ghrelin was correlated with two FCQ-s domains (Table 5.8.10.2), while the remaining hormones revealed no significant correlations with cravings. In contrast, amylin was significantly correlated with several craving dimensions at pre exercise in the OV/OB group (Table 5.8.10.3); in particular with 'positive reinforcement' and 'lack of control' regardless of food category. Furthermore, insulin, leptin and total ghrelin correlated each with one craving dimension.

Immediate after exercise, no significant relationships were observed between hormones and FCQ-S of the L group, hence no correlation data is presented. However, OV/OB individuals post exercise hormone levels are still correlated with FCQ-S items (Table 5.8.10.3). More specifically, unlike fasting conditions, amylin shows little correlation and leptin is correlated with three items. Moreover, total ghrelin is significantly negatively correlated with feelings of hungers towards sweet food. Finally, analysing 1-hour post exercise relationships in the L group (Table 5.8.10.2) demonstrates the only significantly negative correlation of amylin with feelings of hunger towards sweet food. Similarly, the OV/OB group (Table 5.8.10.3) exhibits few correlations in 1 hour after exercise; however, upon this occasion only total ghrelin is significantly correlated with FCQ-S dimensions; negative reinforcement towards sweet food and feelings of hunger towards both sweet and savoury.

Table 5.8.10.2. Correlation between pre exercise, 1hr post exercise FCQ-S items and appetite hormones in lean females.

n=9	Acylated Ghrelin-Pre-Ex	Amylin-1h-Post-Ex
Negative reinforcement_SW	r= -0.573, p= 0.051	
Feelings of hunger_SW	r= -0.581, p= 0.047	r= -0.55, p= 0.041

FCQ-S, food craving questionnaire-state; Ex, exercise; SW, sweet; and SA, savoury.

Table 5.8.10.3. Correlation between pre exercise, post exercise,1hr post exercise FCQ-S items and appetite hormones in overweight/obese females.

n=10	Insulin-Pre-Ex	Leptin-Pre-Ex	Leptin-Post-Ex	Amylin-Pre-Ex	Total Ghrelin-Pre-Ex	Total Ghrelin-Post-Ex	Total Ghrelin-1h-Post-Ex
Intense desire to eat_SW			r= 0.427 p= 0.036				
Positive reinforcement _SA				r= -0.559 p= 0.010			
Positive reinforcement _SW		r= 0.468 p= 0.037	r= 0.543 p= 0.013	r= -0.707 p= 0.001			
Negative reinforcement _SW	r= 0.545 p= 0.019				r= -0.580 p= 0.007		r= -0.567 p= 0.009
Lack of control_SA				r= -0.696 p= 0.001			
Lack of control_SW			r= 0.585 p= 0.007	r= -0.774 p= 0.001			
Feelings of hunger_SA				r= -0.445 p= 0.049			r= -0.652 p= 0.010
Feelings of hunger_SW				r= -0.564 p= 0.010		r= -0.546 p= 0.013	r= -0.638 p= 0.002

FCQ-S, food craving questionnaire-state; Ex, exercise; SW, sweet; and SA, savoury.

5.9. Discussion

5.9.1. Introduction

To the best of our knowledge, this is the first study aiming to evaluate the effects of acute exercise bouts of moderate and high intensity of equivalent energy deficits on food craving dimensions, implicit and explicit attitude towards food, and on selected appetite hormones (insulin, leptin, ghrelin, acylated ghrelin, amylin), in lean and overweight/obese sedentary females.

We hypothesized that: i) acylated ghrelin concentrations will be significantly suppressed following an acute exercise bout of both moderate- and high-intensities for both lean and obese groups, with a greater suppression expected following high-intensity exercise; ii) no change in leptin or amylin concentrations for both lean and obese groups following either moderate or high intensity exercise; iii) the hunger dimension of FCQ-S will be suppressed in both populations immediately following exercise, and increase at 1 hour following the cessation of the protocol, but other dimensions will not follow suit in the overweight/obese when a suppression is expected in the lean group; iv) both lean and obese individuals will demonstrate a heightened positive explicit attitude towards high-fat, high-sugar foods following the high intensity exercise bout compared to the moderate intensity bout but that the implicit attitude would not be affected because it would be an outcome of cognitive transfer of former experiences with the food into the subconscious; v) a significant positive correlation between changes in subjective hunger and acylated ghrelin concentrations will be present in our findings.

5.9.2. Subjective appetite response (FCQ-T and FCQ-S) response to exercise

Our data documents that lean and OV/OB individuals respond differently in their subjective food craving scores to exercise. We predicted that, consistent with the exercise-induced anorexia phenomenon reported in previous studies (Broom *et al*., 2007; 2009), individuals' subjective feelings of hunger to eat both sweet and savoury foods would be suppressed following both moderate- and high-intensity exercise bouts in both lean and OV/OB females. Conversely, our findings report that only OV/OB individuals' feelings of hunger to both sweet and savoury foods were suppressed at post- and 1h post moderate- and high-intensity exercise when compared to baseline with no significant change reported in the lean group (See Figures 5.8.8.6 and 5.8.8.7). Therefore, our data demonstrates exercise-induced anorexia following exercise in OV/OB individuals compared to lean individuals, as has been reported in similar studies in obese participants (Schneider *et al*., 2009), although that the suppression does not happen to all craving dimensions is an important point because in reality the person will be driven to eating by all dimensions combined. Furthermore, our study discovered that this transient suppression of appetite is not maintained following the ingestion of a test meal 1h following the cessation of exercise.

Our data demonstrates a marked difference between lean and OV/OB individuals' subjective food craving scores in the postprandial state. Irrespective of whether exercise was performed prior to the test meal there were increases in 'feelings of hunger towards' both sweet and savoury foods when compared to pre-meal values (pre-meal to post-meal; 1h post exercise to post-meal) in the OV/OB group (See Figures 5.8.7.5; 5.8.7.6; 5.8.8.6; and 5.8.8.7). Whereas either a decrease, or no change, was reported in 'feelings of hunger towards' sweet and savoury foods in the lean group following the consumption of the test meal on either the

fasting test-meal challenge trial or the exercise trial, respectively. In summary, it appears that when presented with a test-meal OV/OB individuals demonstrate an increase in hunger, not evident in our lean participants, independent of whether hunger was transiently suppressed following moderate- and high-intensity exercise. It is not known in the current study whether the increase in hunger following the test-meal would have translated into a greater energy intake at an *ad libitum* test-meal and previous research have provided inconsistent findings. George and Morganstein (2003) compared obese and lean individuals exercising at a moderate intensity (60% HR_{max}), reporting that obese individuals consumed significantly more calories following exercise than lean, however, Schneider *et al.* (2009) also investigated the response to moderate-intensity exercise, but reported that obese individuals did not consume more calories following exercise than after a sedentary activity session. The results from the current study are suggestive that the OV/OB individuals would have consumed significantly greater kcal in an *ad libitum* test-meal than the lean individuals, even despite the initial transient suppression of hunger at 1h post-exercise.

5.9.3. Subjective appetite (FCQ-S) response to the test meal

The above findings are paradoxical considering the ingestion of a 300kcal test meal, and suggestive that hunger can be initiated by smell, taste, and reward sensations in OV/OB individuals. Previous research has already demonstrated how even in the presence of homeostatic signals that indicate an individual is satiated, feeding can still be initiated by the taste and/or olfactory sensation of foods (Van Vugt, 2010). The present study suggests that this response also applies to the state of exercise-induced anorexia. It would be logical to expect that participants would alter their responses for their ‘intense desire to eat’, ‘lack of control’, and ‘positive/negative reinforcement’ to reflect the added drive to eat stimulated by

olfactory sensations, and as such, significant increases in individuals' 'intense desire to eat' for savoury and sweet foods were reported immediately following the meal and 30mins after the test meal, respectively (See Figures 5.8.8.1 and 5.8.8.2). Although no significant changes were documented in FCQ-S categories 'lack of control' and/or 'positive/negative reinforcement' towards sweet and savoury foods (See Figures 5.8.8.3, 5.8.8.5 and 5.8.8.6). Studies in recent years using brain imaging suggest that gut peptides such as ghrelin can influence areas in the brain associated with hedonic and reward-driven hunger, suggesting that food intake can be regulated by reward-driven processes even when an individual is satiated (Gibson *et al.*, 2010). Although a major limitation of our study is that we were unable to obtain blood samples at the postprandial time-points, we were able to document the homeostatic effects of appetite hormones up to 1h post moderate- and high-intensity exercise.

5.9.4. Hormonal Response to Exercise

5.9.4.1. Total ghrelin

Although we expected to find no change in concentrations of total ghrelin following either moderate- or high-intensity exercise bout, as is often reported in the literature (Dall *et al*., 2002; Burns *et al.,* 2007), our data demonstrate an initial decrease in total ghrelin concentrations post exercise which return to baseline values 1h following the cessation of the protocols in both lean and OV/OB participants (See Figure 5.8.4.5). A similar finding was reported by Vestegaard and colleagues (2008), with an acute suppression in total ghrelin concentrations 1h post exercise, who emphasized that the result should be treated with caution, since the observed total ghrelin changes could, in part, be attributable to haemoconcentration, which may also explain the finding in the current study since a significant increase in haemoconcentration was reported for the high-intensity (42.49±3.02

vs. 44.35±3.09, *P*=0.002), whilst the moderate-intensity group remained unchanged (41.91±2.34 vs. 42.14±2.33, *P* > 0.05) (See Table 5.8.5).

5.9.4.2. Acylated ghrelin

A main aim of the current study was to determine whether a transient suppression of appetite could be explained by a concomitant suppression in acylated ghrelin concentrations, as documented by Broom and colleagues (2007) where acylated ghrelin and subjective ratings of hunger were both reduced in response to high-intensity exercise and, importantly, both outcomes were positively correlated. As such, we predicted that acylated ghrelin concentrations will be significantly suppressed following an acute exercise bout of both moderate- and high-intensities for both lean and obese groups, with a greater suppression expected following high-intensity exercise. Our data does not support this hypothesis, whereby no changes were observed immediately following the protocol for acylated ghrelin concentrations, although at 1h post exercise the high intensity group did demonstrate a greater suppression when compared to the moderate-intensity group, which is consistent with our hypothesis (See Figures 5.8.4.6 and 5.8.4.7). Recent research suggests that acylated ghrelin concentrations are suppressed following acute exercise for up to 3h (Broom *et al*., 2007; 2009) in both lean and obese individuals (Marzullo *et al*., 2008). That our exercise protocols were unable to suppress acylated ghrelin concentrations may be down to threefold inconsistencies in the protocols utilised between the current study and previous research. Firstly, although Marzullo *et al*. (2008) reported a suppression in acylated ghrelin in lean and obese individuals, they utilised a graded exercise test to exhaustion with blood samples obtained at ‘peak exercise’, it is possible that our high-intensity protocol was not of a sufficient intensity to elicit a suppression of acylated ghrelin concentrations. Moreover,

although Broom *et al*. (2007; 2009) employed an intensity of 70% $\dot{V}O_{2max}$, the population studied were of healthy, physically active lifestyles, with a $\dot{V}O_{2max}$ of 63.3±2.0 ml·kg^{-1}·min^{-1} and 62.1±1.8 ml·kg^{-1}·min^{-1}, respectively. In the current study, our participants were of sedentary activity level, and hence were unlikely to provide a true $\dot{V}O_{2max}$ in a protocol to exhaustion, and instead only provided a $\dot{V}O_{2peak}$ of 44.69±8.80 ml·kg^{-1}·min^{-1} and 35.06±5.24 ml·kg^{-1}·min^{-1}, for L and OV/OB respectively. Furthermore, during the high-intensity exercise bout in the present study, both lean and overweight individuals struggled to maintain the 90% $\dot{V}O_{2peak}$ workload required, and adjustments to percentage workload had to be made so that the exercise bout could be completed. Lastly, the duration of high-intensity exercise, a major factor in exercise induced anorexia, was significantly shorter in our study (28 minutes vs. 60 minutes), which would translate to a much lower exercise induced energy deficit than in the studies by Broom and colleagues (2007; 2009). Therefore, our study was unable to support a positive correlation between subjective hunger and acylated ghrelin concentrations, suggesting an intensity and/or energy expenditure threshold was not met by our protocol, although the intermittent protocol designed was necessary to provide time and intensity dimensions similar to what is achievable at maximal effort by our sedentary population, therefore we were limited in the intensity we could meet.

5.9.4.3. Leptin

It is widely accepted that an acute bout of exercise has no short-term effect on circulating leptin concentrations (Kraemer *et al*., 2002), therefore we expected leptin values to remain unchanged following both moderate- and high-intensity exercise protocols in both populations, however, our observations demonstrate an immediate decrease in leptin which was not present at 1h post exercise in the OV/OB participants when compared to the lean

group (See Figure 5.8.4.2). Our finding may have been due to alterations in haematocrit, a decrease in the plasma volume resulting in an increase in the concentration of red blood cells in the blood (See Table 5.8.5), as demonstrated previously (Kraemer *et al*., 2001; Fisher *et al*., 2001). Such differences between the groups were identified for haematocrit values, with a highly significant increase in lean participants immediately post-exercise (P= 0.02) and an almost, but non-significant increase in the OV/OB group. Although unfortunately since haematocrit measurements were performed at pre- and post-exercise only, we cannot document 1h post-exercise haematocrit values, and therefore can only speculate that haematocrit levels were returned to baseline levels at 1h post exercise. Alternatively, reported changes of leptin values during exercise were have been suggested to be a result of an elevated production of non-esterified fatty acids (NEFA) during the exercise (Elias *et al*., 2000), as shown previously to be inversely correlated with leptin levels (Duclos *et al*., 1999), which was not measured in the current study.

5.9.4.4 Amylin

Current, but limited, research on the effects of exercise on amylin concentrations led us to predict that amylin would remain unchanged following moderate- and high-intensity exercise protocols. Although our observations show that while no change was shown in the lean individuals, OV/OB individuals demonstrate a significant reduction 1h post exercise, with the greatest response evident following the high-intensity protocol where amylin was reduced from pre- to 1h post-exercise by 46 % (See Figures 5.8.4.3 and 5.8.4.4). Although previous research has demonstrated that amylin concentrations are lowered by an exercise intensity that meets a threshold of around 90% $\dot{V}O_{2max}$ (Kraemer *et al*., 2002), we were conservative in our hypothesis since our population studied were of sedentary activity level, and hence were

unlikely to provide a true $\dot{V}O_{2max}$ in a protocol to exhaustion. However, it is paradoxical that in the current study high-intensity exercise elicited a greater decrease in amylin concentrations than the moderate-intensity exercise in OV/OB individuals, although Kraemer *et al*. (2002) have previously suggested that their reported changes in amylin once exercise intensity reached 90% $\dot{V}O_{2max}$ during exercise may be influenced by elevated blood glucose levels. Our results show an elevated glucose response were present only in the lean population, which may explain the lowered amylin concentrations in the OV/OB group, and since a positive relationship exists between the elevation of blood glucose during high-intensity exercise and greater fitness levels of the population studied (Kjaer *et al*., 1986), the inconsistencies between our findings and those of Kraemer and colleagues (2002) may, ultimately, be a result of the differences in cardiorespiratory fitness between populations.

5.9.4.5. Insulin

The insulin response to exercise in lean males is fairly well established in the literature, however, in females, both lean and sedentary, similar understanding is currently lacking. Kreisman and colleagues (2000) investigated insulin concentrations before and after moderate intensity (60% $\dot{V}O_{2max}$) in 7 trained males, documenting a suppression of insulin levels following exercise. In contrast, a more intense bout of exercise (85% $\dot{V}O_{2max}$) does not elicit the same response, with no change observed in plasma insulin concentrations. Whereas when intensity is increased further to approaching maximal effort, plasma insulin concentrations were significantly augmented in fit males, which remained elevated for up to 20 minutes following the cessation of exercise (Marliss *et al*., 1992). Since insulin also plays a role in appetite control in addition to glucose homeostasis, it was difficult to predict the response in our sedentary population, and therefore no hypothesis was made on this outcome.

We observed no differences reported between the moderate- and high-intensity exercise bouts, both groups had significantly lower insulin one-hour post-exercise compared to pre-exercise and immediately post exercise (See Figure 5.8.4.1). This finding is conflicting to the previous research, high-intensity exercise did not elicit an augmented insulin response, however, the populations tested present a key difference in the studies referenced. As explained above, our participants were of sedentary activity level, and hence were unlikely to provide a true $\dot{V}O_{2max}$ in a protocol to exhaustion, and instead only provided a $\dot{V}O_{2peak}$, whereas Marliss *et al*. (1992) recruited fit, young lean male subjects who are much more able to provide a true $\dot{V}O_{2max}$ with far greater absolute values than our participants could achieve. Our research is however, in support of Kreisman *et al.* (2000), where insulin is suppressed following moderate intensity exercise, further indicating that our exercise protocol was not of sufficient intensity to elicit an augmented insulin response. This theory is supported by our glucose findings, where no changes were reported between moderate- and high-intensity groups (See Table 5.8.5). During exercise of a low-moderate intensity, glycaemia remains in tight control whilst insulin secretion is inhibited by β-cell α-adrenergic receptor activation (Marliss and Vranic, 2002). In contrast, glycaemic values during exercise intensities approaching maximal effort glucose is utilised faster in muscle glycogen stores than hepatic glucose production, with an increase in insulin concentrations for 40-60min for the replenishment of energy stores (Marliss *et al*., 1991; Marliss *et al*., 1992). Therefore, as exercise was not of a sufficient intensity to stimulate a rise in glycaemia in the present study, there were no concomitant rises documented for plasma insulin concentrations.

5.9.5. Correlation between cravings for food and appetite regulating-hormones

Although we were limited in our ability to document correlations between appetite control hormones and food craving at postprandial time-points, we were able to investigate correlations at fasting and following moderate- and high-intensity exercise for up to 1h. In the fasted state amylin appeared to correlate very highly with fasting FCQ-T items in lean (all items), but not OV/OB individuals (2 items). In contrast, leptin correlated very highly with fasting FCQ-T items in OV/OB individuals (7 items) (See Table 5.8.9.1) but not in lean individuals (0 items) (See Table 5.8.9.2). Since fasting levels of leptin were significantly greater in OV/OB individuals compared to their lean counterparts, these contrasting findings may suggest that eating behaviour may be modulated differently in OV/OB individual's dependent on their level of fasting leptin concentrations. Our observations of FCQ-S responses provided a major outcome in our study, where amylin, again, was very highly correlated (See Tables 5.8.10.1). There was an association between hormones and cravings at some, but not all time points, suggesting that exercise caused a disruption in the association of hormones with the perception of craving. Importantly, the disruption observed was greater in lean than OV/OB individuals, and reappears in OV/OB individuals after 1 h only (See Table 5.8.10.3). This is a novel finding not seen in any other literature demonstrating a complete disruption between the association between dimensions of FCQ-S and appetite controlling hormones in lean, but only a partial disruption in OV/OB females.

The findings from this study may have important implications for eating behaviour and weight loss in overweight and obese females. Our finding that OV/OB females increase their feelings of hunger upon entering a postprandial state, despite the transient suppression of appetite (See Figures 5.8.8.6 and 5.8.8.7), suggest that exercise-induced anorexia may not

translate into a reduction in appetite in these individuals. As discussed, olfactory processes may override the suppressed state achieved via exercise, suggesting OV/OB females may be more susceptible to overcompensating at a later, *ad libitum*, meal than lean individuals, a study which could be investigated in future by our research team. Therefore, the current study suggests that future exercise programmes should emphasize diet manipulation and self-control, in addition to exercise adherence, to achieve weight loss which may help restore body compositional, hormonal, and food cravings parameters in overweight individuals following exercise to match their lean counterparts, and could lead to maintained weight if regular exercise is performed, in absence of dietary control in the future. However, although we conclude that exercise does not necessarily impact weight loss in overweight/obese females, the role of exercise in improving health outcomes should not be understated, and therefore the prescription of exercise is warranted in any sedentary individual.

5.9.6. Conclusion

In conclusion, this investigation has shown that an acute bout of moderate- and high-intensity exercise resulted in a suppression of appetite in OV/OB individuals which was not evident in their lean counterparts. The study also demonstrates that OV/OB individuals have a greater drive to eat in a postprandial state following the ingestion of a 300kcal test-meal. Importantly, the exercise-induced anorexia state did not reverse this pattern, whereby the transient suppression of appetite caused by moderate- and high-intensity exercise was offset by the stimulus of food, possibly mediated by olfactory mechanisms as demonstrated previously in the literature when an individual is full. Although this response could not be fully explained by either food craving items, or appetite hormone parameters, the current study nonetheless

highlights the importance of dietary restriction in addition to exercise in OV/OB individuals for providing an energy deficit post-exercise and therefore, potential long-term weight loss.

Chapter 6: General Discussion

6.1. Introduction

In recent years there has been a rapid growth in our understanding of the physiological regulation of energy homeostasis. Specifically, our knowledge of how gut hormones play a role in the regulation of energy balance has been developed substantially. Since exercise is known to be an important determinant of energy balance, much recent research has sought to characterise the effects of exercise on gut hormones. These novel studies contribute to an already established body of research which has examined the effects of exercise on appetite, hormonal and energy intake parameters. The purpose of this chapter is to collectively discuss the findings presented within the experimental chapters of this thesis.

A main aim of the studies presented within this thesis was to characterise the effect of exercise on appetite. Although aware of the significant body of research conducted previously on this topic, the studies presented within this thesis have endeavoured to extend knowledge within the literature by examining the effects of moderate- and high- exercise intensities over the long-term (8 week) and short-term (acute bout) in lean and overweight and obese females.

Although exercise is a recommended and widely accepted means of preventing weight gain and achieving weight loss and maintenance, its effect in the absence of energy restriction seems to be modest at best (Shaw *et al*., 2006). The ability of exercise to create and maintain a negative energy balance relies not only directly on its ability to increase energy expenditure but also indirectly on its potential to modulate energy intake and appetite (King *et al*., 1997; Martins *et al*., 2008). It was demonstrated in long term studies that lower than expected

weight loss in response to exercise interventions, which can be explained, in part, by changes in behaviour towards compensatory eating (King *et al*., 2008). However, this behaviour modification seen in long-term studies is not evident following acute exercise bouts, several studies have shown that exercise suppresses appetite when performed at moderate-high intensities, an effect which has been termed 'exercise-induced anorexia' (King *et al*., 1994). However, many of these studies have only focused on young, lean, healthy and physically active individuals (King *et al*., 1994; Broom *et al*., 2007), or less frequently, only obese individuals (Martins *et al*., 2014). The investigations within this thesis sought to examine the effects of the same long-term and short-term protocols on both lean and obese females to uncover whether differences existed in these populations. The focus on female population in this thesis reflects the well-known differences in appetite response towards energy deficit and other appetite related stimuli between genders.

The findings within this thesis confirm that lean and obese females respond differently to exercise in both the long- and short-term, contributing novel findings, which could be of major importance for the current body of literature. In the first investigation, we ensured lean and overweight/obese females were naive to the aims and objectives of the trial. We have shown that under *ad libitum* condition, un-biased by internal and external motivation for weight loss, 8 weeks' exercise training did not result in weight loss in overweight/obese and lean females (See Figure 4.3.13 and Table 4.3.4.2). We interpret this as evidence of eating behaviour that compensates for exercise energy expenditure based on an intact set point regulation. In contrast, King *et al*. (2007) demonstrated elected not to conceal study aims, nor set exclusion criteria for those individuals with motivation to lose weight, and hence, report considerable weight loss with high variability among overweight/obese participants with outcomes skewed towards weight loss. Since these results are in stark contrast to our

findings, this suggests that the success of exercise interventions on weight loss outcomes may reflect restrained eating behaviour based on weight loss motivation and self-control rather than a direct effect of exercise. This finding shows the importance of participant recruitment that minimizes external confounding factors within the same population.

6.2. Chronic study

In recent years, our knowledge of how gut hormones play a role in the regulation of energy balance has been developed substantially, however, further investigation on how exercising at different intensities affects individuals from lean and overweight/obese populations is still needed. In study 1, following an 8-week exercise training program amylin decreased on fasting and postprandial levels in overweight/obese, while no change was evident in their lean counterparts (See Table 4.3.6 and Figure 4.3.9). Furthermore, about half of the variance of the BMI changes after training in overweight/obese females could be predicted by postprandial amylin and leptin levels, pointing towards a new function of amylin in weight regulation during exercise training in overweight/obese females, while other hormones did not contribute significantly to the model (See Table 4.3.6). The strong contribution of amylin for the model of the OV/OB group is remarkable, and agonists are well established in supporting weight loss in obese (Smith *et al*., 2007). Since amylin is suggested to enhance leptin signalling and lead to transient alteration of a leptin responsiveness threshold (Trevaskis *et al.,* 2010), the decreased amylin levels documented in study 1 (postprandial and fasting) could increase leptin responsiveness threshold in OV/OB participants and would have led to compensatory energy intake in response to exercise training. Our model predicts that participants who have a combination of high levels of leptin and low postprandial levels

of amylin would gain weight during exercise training, and consequently would have overcompensated the exercise induced negative energy balance via energy intake.

6.3. Bout study

Furthermore, study 2 has shown that an acute bout of moderate- and high-intensity exercise resulted in a suppression of appetite in OV/OB individuals, not seen in their lean counterparts in our studies (See Figures 5.8.8.6 and 5.8.8.7). This study utilised FCQ-S measures for the first time in this type of experiment capturing a greater desire to eat by OV/OB individuals in the postprandial state independent of whether exercise was performed prior to the ingestion of the 300kcal test-meal producing a transient exercise-induced suppression of appetite (See Figures 5.8.7.5, 5.8.7.6, 5.8.8.6 and 5.8.8.7). It is suggested that the increased drive to eat following the test-meal in OV/OB participants may be due to the gustatory-olfactory mechanisms, whereby the sight, smell and taste of food can stimulate appetite even in the presence of homeostatic signals that indicate an individual is satiated (Van Vugt, 2010). In addition, our observations of FCQ-S responses discovered an association between hormones and cravings at some, but not all time points, suggesting that exercise caused a disruption in the association of hormones with the perception of craving, with greater disruption seen in the lean individuals. This is a novel finding not seen in any other literature demonstrating a complete disruption following exercise between the association between dimensions of FCQ-S and appetite controlling hormones in lean, but only a partial disruption in OV/OB females.

Following a short-term exercise bout, a significant reduction post-exercise was observed, with the greatest response evident following the high-intensity protocol where amylin was reduced from pre- to 1h post-exercise by 46% (See Figure 5.8.4.3). However, it should be

noted that since amylin concentrations are influenced by elevations in glucose levels, and concentrations of blood glucose increased only in lean individuals, that the lower amylin concentrations may be resultant of altered glycaemic response to exercise between the populations. Leptin responses were as expected following both studies, whereby in an absence of weight loss, leptin concentrations were unchanged following a chronic exercise program. Changes were reported following the acute bout, whereby an immediate decrease in leptin which was observed in the OV/OB participants when compared to the lean group, however, since a highly significant increase in haematocrit were reported in the lean group, but not seen in the OV/OB group, the changes observed may have been due to haemoconcentration (See Table 5.8.5), as reported in previous studies (Kraemer *et al*., 2002). Similarly, insulin concentrations remained similar following the long-term exercise intervention, as was expected without significant weight loss (See Table 4.3.6). In the short-term no differences were reported between the moderate- and high-intensity exercise bouts, both groups had significantly lower insulin one-hour post-exercise compared to pre-exercise and immediately post exercise (See Table 5.7.2 and Figure 5.8.4.1). Investigations into the hunger hormone, ghrelin, demonstrated that in the long term, both total ghrelin and acylated ghrelin concentrations were increased following 8 weeks of an exercise program in OV/OB individuals, but not in lean where an increase and no change were reported for total- and active- form of ghrelin, respectively. A short-term exercise bout however saw an initial decrease in total ghrelin concentrations post exercise which return to baseline values 1h following the cessation of the protocols in both lean and OV/OB participants, which is suggestive of exercise induced anorexia, however, similar to leptin, may be due to changes in haemoconcentration (See Table 5.8.5). The acylated form of ghrelin demonstrates a suppression following high-intensity exercise when compared to the moderate-intensity group, although no change was reported for main effect of time (See Figure 5.8.4.6). It may

be that our exercise intensity was not of a high enough intensity due to limitations in our sedentary participants' cardiovascular fitness.

6.4. Limitations of the study

There have been considerable limitations in this study. One of the limitations of our study, which was common in both studies, was the small sample size due to the dropout of a number of participants at baseline measurement and during the studies. As a result, the sample size was relatively small which culminated in low power of many analyses.

Exercise is shown to influence appetite regulating hormones affecting acute and chronic appetite (Martins *et al*., 2008). There is accordingly a tie between exercise, appetite and food intake, which is not known in great detail. Clearly, the influence of exercise, with or without weight loss, on appetite hormones will not be completely detectable if fasting hormone levels are measured alone. While fasting levels are certainly a valuable indicator for metabolic status, e.g. in connection with glucose measurements, for calculation of HOMA, and many studies show specific differences in several appetite hormone in fasting levels between lean and overweight/obese (Bowen *et al.*, 2007), this gives no information about responses to nutrient intake. Clearly, for investigating the reasons for weight loss in connection with exercise it is imperative that appetite hormones are also measured at postprandial state due to the fact that their changes after food intake are part of the satiation process and therefore possibly involved in the control of caloric compensation of energy deficit through exercise. In consequence, test meals or *ad libitum* buffet meals are a possible method to investigate postprandial hormone levels (Brennan *et al*., 2012). In our study, we have had an interest to investigate differences in postprandial hormone responses to a meal after exercise training in

overweight/obese and lean female. Therefore, a standardised test meal with defined macronutrient composition was essential for the study because hormonal responses depend largely on nutrient composition, texture, and amount of intake (Tieken *et al*., 2007). Clearly, freely selected meals (buffet) would have not provided standardisation of the former factors due to variability in food choice; otherwise, a set meal often has problems with general acceptance and consequently variance in intake as well. Moreover, based on the repeated measure design of our study, it would not be helpful having two separate buffet-test meals due to the low chance of consumption of the same caloric value and composition by the same participants; a huge variation in energy intake has been reported in these designs (Stensel, 2010). Standardized nutrient intake was therefore an option; glucose tolerance tests use a similar approach for investigation of insulin response (Maffeis *et al*., 2010) but this limits the response to glucose, while obviously gastrointestinal hormones are released or suppressed via proteins and lipids intake as well, not only via carbohydrates (Karhunen *et al*., 2010).In several studies, investigating the response of appetite hormones to the main macronutrient modalities, it is obvious that hormonal responses are specific and vary in people depending on gender, age, BMI (Karhunen *et al*., 2010). Moreover, texture of meals affects the release of hormones from the GI, while liquid meals lead to more rapid release than solid with same nutrient composition (Tieken *et al*., 2007). Consequently, we decided to use an industrial product with defined nutrient composition and good palatability, which is liquid and contains all macronutrient factors usually expected in westernized balanced meals in the recommended composition (Brennan *et al*., 2012). Moreover, this test meal product is generally used in clinical context for patients who have problems with solid food intake (Resource® Energy, Nestle Healthcare Nutrition); therefore, less problems with acceptance and tolerance of this product were expected. In summary, the choice of using the liquid test meal with standardized composition gave us the opportunity to investigate appetite hormones

response to the full spectrum of macronutrients in a reliable and repeatable manner. This promised to enable us to provide a paradigm for investigating postprandial responses with smallest possible variability based on the test meal itself and possible food preferences of participants.

Regarding the 8-week study, one limitation was that we were unable to measure the energy expended by our participants over the 8-week period directly. We suggest that the energy deficit caused by the exercise sessions was negated by an increase in energy intake via compensatory eating behaviour, however, it is possible that the level of exercise and physical activity (stair climbing etc) was decreased due to the participants feeling fatigue, or a sense of reward resulting in a further sedentary lifestyle. This confounding factor was minimised in our study since both OV/OB and L groups were sedentary individuals however, so it is unlikely that physical changes to lifestyle would have significantly undermined the exercise training sessions.

Another limitation was self-reported diet diaries that were dependent upon the compliance of the individual to record everything consumed with a high level of accuracy, whilst not changing typical eating habits (Elia *et al*., 2003). As such, it may be that our participants misreported information within their diet diaries, as highlighted by Lictman *et al*. (1992) who reported under-reported food intake by as much as 30% in obese individuals when compared to control individuals.

A further limitation to the present study is not measuring excess post-exercise oxygen consumption (EPOC). This phenomenon is an increase in oxygen uptake that occurs after

exercise in the recovery period. EPOC can contribute to energy balance disturbance, which in turn affects body composition. EPOC is affected by both duration and intensity of exercise. EPOC may last from several hours after low or moderate intensity exercise session (Gaesser and Brooks, 1984) up to 24 hours following a vigorous exercise (Borsheim and Bahr 2003).

As for the bout study, there were many limitations of this study in addition to those already discussed, such as the failure of participants to i) provide a true $\dot{V}O_{2max}$; and ii) adequately perform the exercise intensity required of them, as well as the absence of blood samples at postprandial time-points, both on the test-meal challenge trial and exercise trials. It was thought that collecting blood samples over 5 time-points in a short period would have been too stressful on the participants, and subsequently, may have impacted on our success during participant recruitment. However, it may have been a better option to perform blood collection via cannulation methodology, a relatively routine procedure allowing for serial blood sampling whilst ensuring minimal discomfort for the patients, which would have allowed the collection multiple blood samples to further document time-course changes in glycaemia and appetite control hormones over a prolonged period of time. Another limitation of the study was identified in our inclusion criteria, with participants selected based on their BMI score, opposed to body fat %. Although BMI is a good predictor of body fat, especially in sedentary individuals with relatively low lean mass, it would have been more appropriate to have collected information regarding body composition prior to the recruitment of participants to reduce this confounding factor. Also, although all participants were informed of the necessity to repeat their evening-breakfast meals on the day before and morning of the moderate- and high-intensity exercise trials, it would have been helpful for all meals to have been provided, and consumed, within our laboratory so that accurate measurements could

have been made on macronutrient composition and overall quantity of the meal to ensure a standardised baseline state for all participants was achieved.

6.5. Future investigations

Our studies produce some very exciting findings that should be investigated further in the future. We have discovered a new role for amylin in weight regulation during exercise training in overweight/obese females. Similar research should be conducted on male participants to identify whether the changes in amylin are gender specific, and may explain why undertaking an exercise training program under *ad libitum* conditions is often reported as less effective in females than their male counterparts (Donnelly *et al*., 2003, Potteiger *et al*., 2003). One theory put forward by David Stensel (2010) to explain these differences in gender may be the necessity for women to maintain sufficient body fat stores for reproductive success.

Amylin was shown to involve in the regulation of food intake and body weight. Pramlintide is a human amylin analogue to be used in conjunction with insulin to treat type 1 or 2 diabetes. Several studies (Aronne et al. 2007; Smith et al. 2007; Smith et al. 2008) indicated that pramlintide can also affect weight loss in overweight or obese patients with and without diabetes. To do more studies, concentration on treatment of obesity with a combination of exercise and pramlintide, owing to the role of pramlintide in weight loss, would prove beneficial. Design would be a placebo controlled trial where one group receives exercise plus placebo and one group pramlintide with exercise; additionally it would be ideal to look into having a group without treatment and one with pramlintide alone. All groups should be randomised and at least single blind.

Moreover, investigating the reduction of amylin to find whether it is down to the reduction of glucose availability where exercise training in individuals with same caloric intake but with varied carbohydrate composition (high versus low carbohydrate diet) is undergone. With the help of a few related hormone measurements such as insulin, CCK, GLP-1and PYY, these changes associated with the glucose availability can be readily detected.

Further studies could be investigating the impact of 'intention to lose weight' where participants receive the same exercise but will be recruited differently–one group with uncovered objectives taking people with interest in weight loss and one group recruited with hidden objectives and people with interest in weight loss excluded. It sounds ideal to have two control groups who are not doing any exercise. Obviously measuring some questionnaire based psychological parameters as an addition seems necessary (eating restraint etc).

A future investigation that is warranted following on from this thesis would be to answer the question of whether changes we have reported in appetite hormones is translated into altered eating behaviour in an *ad libitum* test meal. This would provide an opportunity to assess food intake directly, rather than relying on self-report diet diaries, and the timing of the test meal could be manipulated (1h post, 2h post, 3h post) as to provide information pertaining to the duration of 'exercise induced anorexia', with the possibility of a test meal the next morning following an overnight fast, to see if overcompensation occurs the next day in high- or moderate intensity, or control (rest) trials.

6.6. Conclusion

In conclusion, it is clear that the clinical utility of exercise alone as a mediator of weight loss is unsupported, however the role of exercise in improving health outcomes should not be understated, and therefore the prescription of exercise is warranted in any sedentary individual. Our investigations in both lean and overweight/obese individuals suggests that exercise should be used in conjunction with dietary restriction and self-control to maximise the chance of successful weight loss programs. Studies that have reported success in overweight/obese individuals may be confounded by the participants' desire to lose weight during the study, exhibiting increased self-control in their eating behaviour alongside the exercise intervention. However, more studies are needed in future to assess the effects of exercise in lean and overweight/obese individuals over a more prolonged period both in the long-term (>8 weeks) and in an acute bout (>1h post-exercise), with a focus on investigating whether individuals' diets are manipulated in the long-term without the limitation of self-report diet diaries. Furthermore, whether the increased drive in hunger at meal times, despite the transient suppression of appetite in overweight/obese individuals, translates to an increased energy intake during an *ad libitum* test meal.

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Appendices

Appendix 1

Poster of the Study

Ydych chi'n fenyw rhwng 18 a 40 mlwydd oed? Ddim yn gwneud ymarfer corff?

Pam ddim cymeryd rhan yn ein arbrawf dros yr Hydref?

Manteision o gymeryd rhan yw:

- **hyfforddiant fitrwydd am ddim (3 gwaith yr wythnos am 8 wythnos).**
- **Par o dreiners newydd gwerth hyd at £100.**
- **Gwella eich iechyd**
- **Asesiad fitrwydd manwl efo offer technegol ora ar y farchnad (gwerth hyd at £250)**

Are you female? Aged 18-40? Currently Inactive? Want to improve your health and fitness levels?

Why not participate in our study this autumn?

What are the benefits of participating in our study?

- **8 weeks FREE fitness training (3 times a week).**
- **FREE pair of trainers up to the value of £100.**
- **Improved overall health and well being.**
- **State of the art fitness assessment, (up to the value of £250).**

If you are interested or would like more information please contact:

Fardin Fatahi: pepc09@bangor.ac.uk Tel: 01248388254

Appendix 2

Poster of bout study

Are you female?

Aged 18-40?

Currently inactive?

Why not participate in our study?

What's in it for you:

- Earn £40 reward for taking part in our study.
- Free fitness assessment and health counselling worth £250.
- Only 4 visits to complete study

If you are interested and would like further information please contact Dr Hans-Peter Kubis & Fardin Fatahi, School of Sport, Health and Exercise Sciences, Bangor University:

Email: pepc09@bangor.ac.uk

Tel:01248 388254

Appendix 3

Participant Information Sheet

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Participant Information sheet

Study Title: Effects of exercise intensity on substrate utilisation and cognitive function.

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and take time to decide whether you wish to take part, or not.

Please contact the researchers if anything is unclear or you would like more information.

Matthew Jackson pepa2f@bangor.ac.uk Tel: 01248388254

Fardin Fatahi pepc09@bangor.ac.uk Tel: 01248388254

What is the background of the study?

The main aim of this study is to investigate the effects of exercise intensity on substrate utilisation in inactive women. Specifically, we will be analysing how exercise performed at low and high intensities can differ in its effects on this factor. Substrate utilisation is the composition of fuel we use, both when exercising and at rest, this fuel can be derived from either carbohydrate, protein or fat and the amount of each can alter based on;- energy requirements, energy balance, dietary composition, body composition and aerobic capacity. It is also known that regular exercise is beneficial for a number of health related outcomes, including; - improved cardiovascular fitness, reduction in markers of lifestyle diseases such as blood pressure, blood lipid profile, insulin sensitivity and enhanced cognitive performance. The effects of exercise on substrate utilisation may be one of the underlying mechanisms responsible for these beneficial outcomes. An 8 week exercise intervention will be used to test these assumptions. A further aim of the study is to investigate the influence of different exercise intensities on cognitive function e.g. reaction times in a task combining pictures and words in a computer based test.

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 free to withdraw at any time and without giving a reason. This will not affect the marks you receive, the outcome of your period of study or your standing with your supervisor, other staff members or with the university.

What will happen to me if I take part?

Summary

You will be asked to keep a diet diary for two weeks before the study begins, in which you will need to write down precisely and accurately what you have had to eat and drink each day. After this the first bout of testing will take place;- these tests will include a dual-energy x-ray absorptiometry (DEXA) scan to assess your body composition, a VO2 max test to measure your aerobic/endurance fitness, analysis of resting metabolic rate, blood sampling before and after a test meal, and a cognitive function task. The tests will be completed over three visits to the lab. Each of these tests are discussed in greater depth below. Once the preliminary testing has been carried out the exercise intervention begins. You will be asked to attend exercise sessions 3 times a week for an hour or so on each occasion. These exercise classes will be conducted in a group format, and exercise will be done in a circuit training fashion. The exercise programme will last for 8 weeks in total, during this time you will also need to fill out a diet diary for two days of each week. Once the 8 week training period is finished, a second bout of testing will take place, in exactly the same format as the first with all the same tests. You will then be asked to fill out a diet diary once again as before.

Diet Diary

We will give you a Food Record Sheet (FRS) where you should describe with accuracy and precision your daily food and beverage intake. You will be asked to keep a record of your diet for 7 days pre-intervention, 7 days post-intervention, and 2 days per week during the intervention. The 7 days pre and post-intervention will be randomly chosen over a period of two weeks and the 2 days of each week during the intervention will also be randomised. How to describe foods and drinks will be fully explained in the Food Record Sheet. The analysis of your food composition is necessary to exclude influence of certain foodstuff on measures we take. It is fine to eat whatever you wish, but be sure to note everything that you eat on your food record sheet.

Cognitive Function Task

You will be asked to undergo a computerised test of the speed with which you respond to pictures of different objects. This will allow us to assess your cognitive function and whether it is affected by exercise. The test will last for approximately 20 minutes.

DEXA Scan

A whole body low radiation (DEXA) scan will be performed to assess how much bone, fat and muscle mass you have. For this procedure you need to wear shorts, socks, underwear (no bra) and t-shirt. The scan will last 15 minutes. This procedure is painless, and it is not more than lying down on a bed. Although there are small risks involved in radiation exposure, the amount of exposure during a DEXA scan is minimal and is lower than the amount of background radiation that we are exposed to during the course of two weeks outside in North Wales.

VO2 Max Assessment

VO2 max is a measure of your aerobic capacity and it is achieved with an incremental exercise test. You will begin by cycling at a fixed speed and a low resistance, and with every minute of the test the resistance will be increased slightly so the exercise becomes harder. The resistance will be increased until you have reached maximal exertion and can carry on no longer. During the test will be monitoring your heart rate, oxygen uptake and carbon dioxide production, The latter two will be

measured with a breath by breath gas analyser, which collects the air you breath in and out. This is necessary so we can gain an accurate measure of your cardiovascular fitness. The test will normally last no longer than 30 minutes.

Resting Metabolic rate

You will be asked to arrive at the laboratory in an overnight fasted state and then to lie on a bed for about 30 minutes. As with the VO2 max test we will measure your oxygen uptake and carbon dioxide production with a breath by breath gas analyser. This test is necessary to find out more about your metabolism.

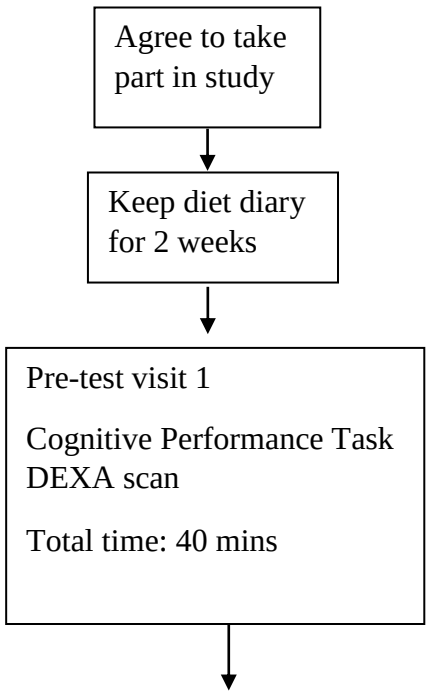
Blood Sampling and Test Meal

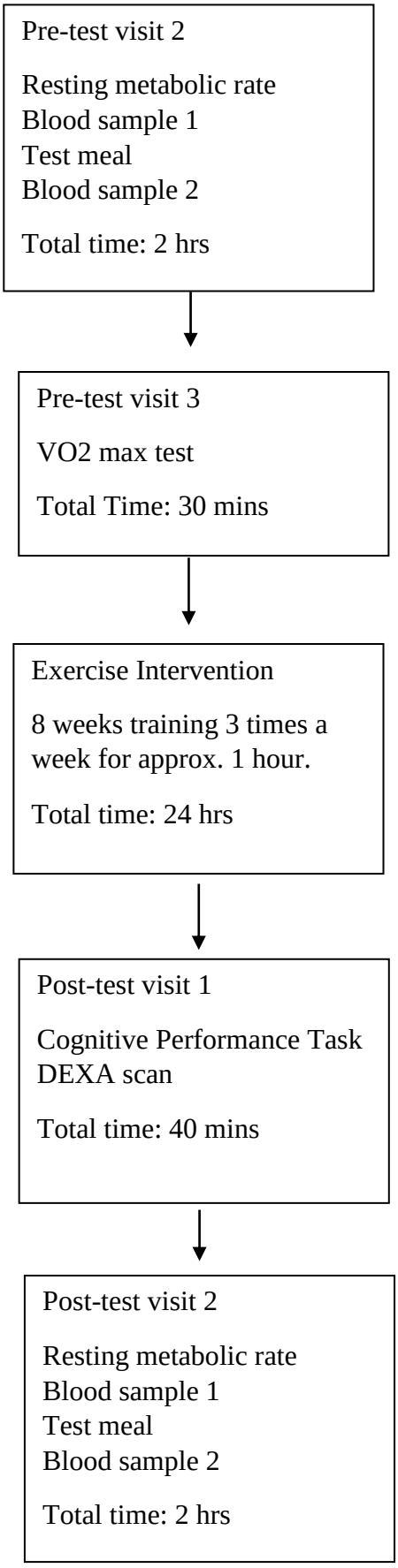
We will take two blood samples (i.e. pre and post-meal) from a vein of your forearm, on two separate occasions (i.e. pre and post exercise intervention). A small amount (8 ml) of blood will be collected (0.2% of the total blood volume). We will use sterile needles to draw the blood samples. It is very important that you have fasted overnight. Afterwards, we will give you a test meal which will be in the form a milk shake. Then one hour after the test meal we will take the second blood sample. The blood samples will be use to measure hormone levels in your blood, which will give us important information about how exercise alters the way foodstuff is consumed in your body.

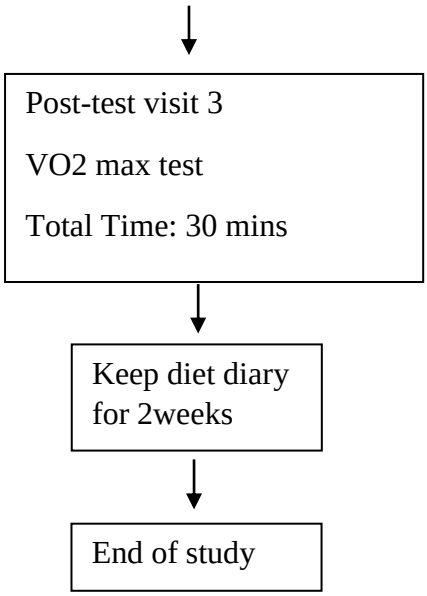
Exercise Intervention

Once you have completed all the baseline measures, we will begin the exercise intervention. As stated above, the exercise programme will last for 8 weeks and you will be asked to attend 3 exercise sessions per week, each exercise session will last for an hour or so and participants will train as a group. There will be 2 exercise groups in this study, a high intensity exercise group and a low intensity exercise group. Each participant will be randomly allocated to one of these groups for the entire duration of the study. Exercise sessions will be in a circuit training format, this is where a series of exercise stations are completed one after another until a whole circuit is completed. During exercise sessions you will also be required to where chest belts so that we can monitor your heart rate. Finally, exercise sessions will take place at Normal Site Sports Hall, you will need to make your own way there.

Timeline




Pre-test visit 2
Resting metabolic rate
Blood sample 1
Test meal
Blood sample 2
Total time: 2 hrs
Pre-test visit 3
VO2 max test
Total Time: 30 mins
Exercise Intervention
8 weeks training 3 times a week for approx. 1 hour.
Total time: 24 hrs
Post-test visit 1
Cognitive Performance Task
DEXA scan
Total time: 40 mins
Post-test visit 2
Resting metabolic rate
Blood sample 1
Test meal
Blood sample 2
Total time: 2 hrs



What are the possible disadvantages and risks of taking part?

You may experience some discomfort during blood sampling. The blood sampling might be stressful for people who do not like needles or seeing blood. You may also find the VO2 max testing somewhat uncomfortable as you are required to exercise up to maximal exertion. There is a relatively large time commitment for this study, pre-testing and post-testing will last for just over 3 hours each and you will need to exercise for approximately 3 hours per week for 8 weeks.

What are the possible benefits of taking part?

By taking part in this study you have the opportunity to join in a comprehensive 8 week exercise intervention with a team of sport scientists who are passionate about the benefits of exercise to health and who have many years of combined experience in fitness training. You have a chance of reducing the possible risks of lifestyle related diseases associated with low physical activity and improving your overall health. Other benefits of exercise include improved cardiovascular fitness and metabolism, and possibly cognitive performance. Your participation in this study will also provide us with valuable insights into how the human body and mind adapts to exercise and will add to our understanding of how exercise positively influences health.

Confidentiality

All information which is collected about you during the course of the research will be kept strictly confidential. Any data which is to leave the school, for possible publications or reports, will have your name and any other personal information removed so that you cannot be recognised from it. It will not be possible to identify you in any of these reports or publications.

Who is organising or funding the research?

This study has been organised by Matthew Jackson (PhD student), Fardin Fatahi (PhD student), Dr Hans-Peter Kubis (Senior lecturer) and Dr David Markland (Senior lecturer) from the School of Sport, Health and Exercise Sciences (SSHES) of Bangor University.

Who has reviewed the study?

This study has been reviewed by the SSHES Ethics Committee.

Feedback and conduct of research

SSHES is always keen to hear the views of research participants about their experience. If you would like to feedback, please ask your researcher to provide you with Form 6 – Participant Feedback Form – from the Ethics Guidelines Handbook. Completion of this form is optional. The completed form should be returned to Dr Andrew Lemmey, Chair, SSHES Ethics Committee, SSHES, Bangor University, Bangor LL57 2PZ. All information will be treated in a strictly confidential manner.

Any questions?

Please ask us if you have any questions. Here are the contact details of all investigators in this study:

Matthew Jackson (PhD student)	pepa2f@bangor.ac.uk	Tel: 01248 38 8254
Fardin Fatahi (PhD student)	pepc09@bangor.ac.uk	Tel: 01248 38 8254
Dr Hans-Peter Kubis (Senior Lecturer)	pes203@bangor.ac.uk	Tel: 01248 38 8261
Dr David Markland (Senior Lecturer)	d.a.markland@bangor.ac.uk	Tel: 01248 38 3487

Appendix 4

Participant information sheet-part1

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Participant Information sheet

Study Title: Acute effects of altering exercise intensity on metabolism and cognitive function.

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and take time to decide whether you wish to take part, or not.

Please contact the researchers if anything is unclear or you would like more information.

Matthew Jackson pepa2f@bangor.ac.uk Tel: 01248388254

Fardin Fatahi pepc09@bangor.ac.uk Tel: 01248388254

What is the background of the study?

The main aim of this study is to investigate the effects of exercise intensity on metabolism in inactive women. A further aim of the study is to investigate the influence of different exercise intensities on cognitive function e.g. reaction times in a task combining pictures and words in a computer based test. It is known that regular exercise is beneficial for cardiovascular fitness and enhances cognitive performance. The effects of exercise on metabolism may be one of the underlying mechanisms responsible for these beneficial outcomes. One high and one low intensity exercise bout will be used to test these assumptions.

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 free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

Summary

After agreeing to take part in the study and once informed consent has been received you will be invited to the School of Sport, Health and Exercise Sciences (SSHES) to complete the first batch of testing which will include; Bioelectrical Impedance Analysis (BIA) to assess your body composition, a VO2 max test to measure your aerobic/endurance fitness, analysis of resting metabolic rate (RMR), blood sampling and a cognitive function task. Each of these tests are discussed in greater depth below. Once the preliminary testing has been carried you will be asked to attend 2 exercise bouts sessions. You will be required to complete one low intensity session and one high intensity session, the order of these will be randomised. These exercise sessions will be conducted in a group format, and performed on an exercise bike. After both sessions you will be asked to complete a cognitive performance task and we will also be collecting blood samples. Following the exercise sessions you will be asked back to the department for one final visit, during this session you will be presented with a test meal and as with exercise sessions you will need to complete the cognitive task and provide blood samples.

Cognitive Function Task

You will be asked to undergo a computerised test of the speed with which you respond to pictures of different objects. This will allow us to assess your cognitive function and whether it is affected by exercise. The test will last for approximately 20 minutes.

Bioelectrical Impedance Analysis

A BIA scan will be performed to assess how much fat and muscle mass you have. For this procedure you need to wear shorts, socks, underwear and t-shirt. This procedure is painless and will only last a couple of minutes, you will be asked to lie down whilst electrodes are placed on your wrists and ankles.

VO2 Max Assessment

VO2 max is a measure of your aerobic capacity and it is achieved with an incremental exercise test. You will begin by cycling at a fixed speed and a low resistance, and with every minute of the test the resistance will be increased slightly so the exercise becomes harder. The resistance will be increased until you have reached maximal exertion and can carry on no longer. During the test will be monitoring your heart rate, oxygen uptake and carbon dioxide production, The latter two will be measured with a breath by breath gas analyser, which collects the air you breath in and out. This is necessary so we can gain an accurate measure of your cardiovascular fitness. The test will normally last no longer than 30 minutes.

Resting Metabolic rate

You will be asked to arrive at the laboratory in an overnight fasted state and then to lie on a bed for 30 minutes. As with the VO2 max test we will measure your oxygen uptake and

carbon dioxide production with a breath by breath gas analyser. This test is necessary to find out more about your metabolism.

Blood Sampling

All blood samples will be taken from a vein of your forearm, only a small amount (8 ml) of blood will be collected (0.2% of the total blood volume) each time. Sterile needles will be used to draw the blood samples. The blood samples will be use to measure hormone levels in your blood, which will give us important information about how exercise alters the way foodstuff is consumed in your body.

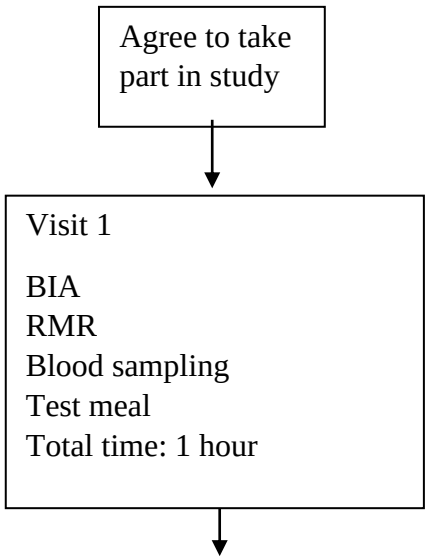
Exercise Bouts

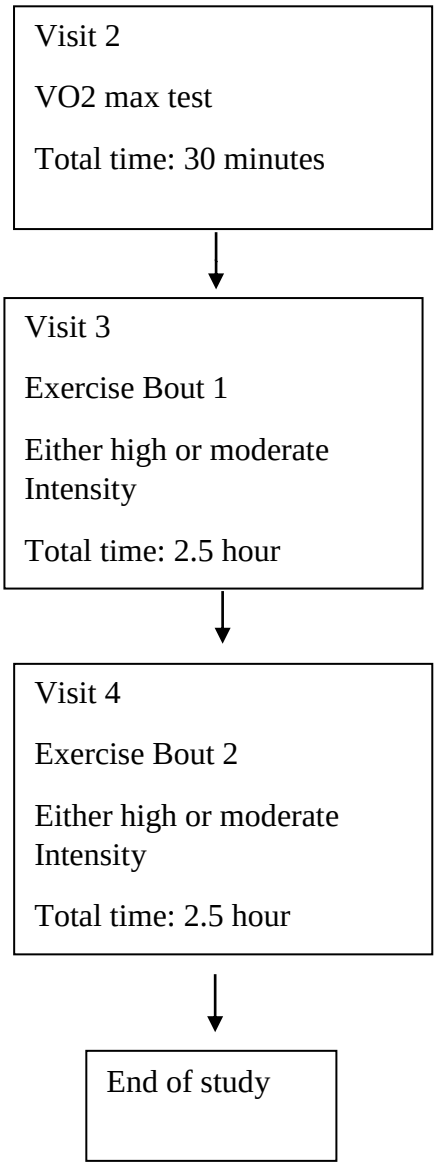
Once you have completed all the baseline measures, we will begin the exercise bouts. Each exercise session will last for an hour or so and participants will train as a group. There will be a high intensity exercise bout, and a low intensity exercise bout. Each participant will be randomly allocated to decide which order they will complete the bouts. Exercise bouts will be carried out on exercise bikes and during exercise sessions you will also be required to where chest belts so that we can monitor your heart rate. After exercise a blood sample will be collected immediately and another after one hour. You will also be asked to perform the cognitive function task.

Test Meal

It is very important that arrive in an overnight fasted state. You will need to be well rested and asked to avoid any alcohol or caffeine consumption the day before, as well as any intense exercise. Upon arrival you will be given a test meal, this will be liquid based and much like a milk shake. One hour after the test meal a blood sample will be taken and as with the exercise bouts, the cognitive function task will need to be completed post test meal.

Timeline





What are the possible disadvantages and risks of taking part?

You may experience some discomfort during blood sampling. The blood sampling might be stressful for people who do not like needles or seeing blood. You may also find the VO2 max testing somewhat uncomfortable as you are required to exercise up to maximal exertion.

What are the possible benefits of taking part?

Your participation in this study will provide us with valuable insights into how the human body and mind adapts to exercise and will add to our understanding of how exercise positively influences health. In addition to the above, upon completion of the study you will be entered into a prize draw for an Apple IPod Touch worth £170.

Confidentiality

All information which is collected about you during the course of the research will be kept strictly confidential. Any data which is to leave the school, for possible publications or reports, will have your name and any other personal information removed so that you cannot be recognised from it. It will not be possible to identify you in any of these reports or publications.

Who is organising or funding the research?

This study has been organised by Matthew Jackson (PhD student), Fardin Fatahi (PhD student), Dr Hans-Peter Kubis (Senior lecturer) and Dr David Markland (Senior lecturer) from the School of Sport, Health and Exercise Sciences (SSHES) of Bangor University.

Who has reviewed the study?

This study has been reviewed and approved by the SSHES Ethics Committee.

Feedback and conduct of research

SSHES is always keen to hear the views of research participants about their experience. If you would like to give feedback, please ask your researcher to provide you with Form 6 – Participant Feedback Form – from the Ethics Guidelines Handbook. Completion of this form is optional and can be done anonymously. The completed form should be returned to Dr Andrew Lemmey, Chair, SSHES Ethics Committee, SSHES, Bangor University, Bangor LL57 2PZ. All information will be treated in a strictly confidential manner.

Complaints

If during the course of this study you feel the need to complain about how the research has been carried out, for any reason, then please contact the Head of School, Dr Tim Woodman, SSHES, Bangor University, Bangor LL57 2PZ.

Any questions?

Please ask us if you have any questions. Here are the contact details of all investigators in this study:

Matthew Jackson (PhD student) pepa2f@bangor.ac.uk Tel: 01248 388254
Fardin Fatahi (PhD student) pepc09@bangor.ac.uk Tel: 01248 388254
Dr Hans-Peter Kubis (Senior Lecturer) pes203@bangor.ac.ukTel: 01248 388261
Dr David Markland (Senior Lecturer) d.a.markland@bangor.ac.ukTel: 01248 383487

Appendix 5

Participant information sheet-part 2

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Participant Information sheet

Study Title: Investigation of energy balance regulation with focus on the impact of metabolism on perception of food and cognition

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and take time to decide whether you wish to take part, or not.

Please contact the researchers if anything is unclear or you would like more information.

Fardin Fatahi pepc09@bangor.ac.uk Tel: 01248388254
Matthew Jackson pepa2f@bangor.ac.uk Tel: 01248 388254

What is the background of the study?

The main aim of this study is to investigate the effects of bouts of exercise with different intensities on metabolism in inactive women. A further aim of the study is to investigate how food is perceived and recognised in response to different exercise trials. Exercise has plenty of positive effects on cardiovascular system but it is not known how it interacts with perceptual and cognitive systems regulating the energy balance. The effects of exercise on metabolism may be one of the underlying mechanisms responsible. A baseline aerobic fitness test and one trial high and one trial low intensity exercise bout will be used in our investigation.

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 free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

Summary

After agreeing to take part in the study and once informed consent has been received you will be invited to the School of Sport, Health and Exercise Sciences (SSHES) to complete several tests which will include; Bioelectrical Impedance Analysis (BIA) to assess your body composition, analysis of resting metabolic rate (RMR), a VO2 max test to measure your aerobic/endurance fitness and fasting venous blood sampling. Additional blood samples will be taken before and after two exercise trials. Moreover, you will be asked to fill out questionnaires regarding your food preference and appetite, as well as preforming a cognitive task for food recognition and test meals. Tests will be spread across four visits to our laboratory and each test is described in greater depth below. Once the preliminary testing has been carried out you will be asked to attend two exercise bout sessions. Exercise bouts will be completed one week after pretesting and there will also be one week between bouts. You will be required to complete one low intensity exercise bout and one high intensity exercise bout, the order of these will be randomised. All exercise will be performed on an exercise bike. Before and after both bouts you will be asked to complete questionnaires and a cognitive performance task, we will also be collecting blood samples before and after exercise. Following this you will be given a test meal after both exercise bouts and you will need to complete the cognitive performance task once more. Finally, during the 2 week period around the exercise bouts we will ask participants to record their diet on 3 days per week; these will be chosen at random. Participants will also need to record their diet on the 2 days of exercise bouts.

Cognitive Function Task

You will be asked to undergo a computerised test of the speed with which you respond to pictures of different objects. This will allow us to assess your cognitive function and whether it is affected by exercise. The test will last for approximately ten minutes. Following the test you will also be asked to complete two short questionnaires related to the computerised test. Participants will need to complete the cognitive function task and associated questions on 4 occasions during each exercise bout session; before, immediately after, one hour after exercise and thirty minutes after test meal. The task will also be completed one last time after the test meal without prior exercise.

Bioelectrical Impedance Analysis

A BIA scan will be performed to assess your fat mass and percentage body fat. For this procedure you will need to remove your socks and shoes and stand on what appear to be a set of weighing scales. The base of the machine has two electrodes which you will stand on and two further electrodes which you will need to hold in both hands. The procedure is painless and will only last a couple of minutes. Participant's height and weight will also be recorded on this occasion.

VO2 Max Assessment

VO2 max is a measure of your aerobic capacity and it is achieved with an incremental exercise test. You will begin by cycling at a fixed speed and with a low resistance, and with every minute of the test the resistance will be increased slightly so the exercise becomes harder. The resistance will be increased until you have reached maximal exertion and can carry on no longer. During the test will be monitoring your heart rate, oxygen uptake and carbon dioxide production, The latter two will be measured with a breath by breath gas analyser, which collects the air you breath in and out. This is necessary so we can gain an accurate measure of your cardiovascular fitness. The test will normally last no longer than 30 minutes.

Resting Metabolic rate

Participants will be asked to arrive at the laboratory in an overnight fasted state and then to lie on a bed for 30 minutes. It is vitally important that you arrive in an overnight fasted state to gain a true measure of your RMR. You will also need to be well rested and asked to avoid any alcohol or caffeine consumption the day before, as well as any intense exercise. As with the VO2 max test we will measure your oxygen uptake and carbon dioxide production with a breath by breath gas analyser. This test is necessary to find out more about your metabolism. During this test you will also be required to wear a heart rate monitor and electrodes will be attached to your wrists and ankles to monitor the electrical activity of your heart (ECG), this will allow us to check you have no heart complications before starting exercise testing.

Intravenous Blood Sampling

Intravenous blood will be collected from a vein of your forearm 7 times over 3 visits to our lab, these will be: Fasted after RMR measurement; before, immediately after and one hour after high intensity exercise; and before, immediately after and one after low intensity exercise. Only a small amount (8 ml) of blood will be collected each time. Sterile needles will be used to draw blood samples. The blood samples will be used to measure hormone levels in your blood, which will give us important information about how exercise influences the regulation of energy balance.

Fingertip Blood Sampling

Fingertip blood samples will be collected 4 times over 2 visits to our lab, these will be; before and after the high intensity exercise bout and before and after the low intensity exercise bout. On each occasion a small needle will pierce the skin and blood will be drawn into a small glass cylinder by massaging the fingertip. Only a very small amount of blood will be taken and this will be used to analyse hematocrit. Hematocrit is the percentage of red blood cells in the blood and this will be used to measure hydration status of participants.

Exercise Bouts

Once all the baseline measures have been completed, we will begin the exercise bouts. Each exercise trial will last for approximately 3 hours but you will only be expected to exercise for up to 45 minutes. During the high intensity exercise bout you will have to perform 5 times 4

minute high intensity intervals with a 2 minute rest between each interval and during the low intensity exercise bout you will be asked to complete 40 minutes of continuous low intensity exercise. Before both of the exercise bouts participants will begin with a 5 minute warm up. Each participant will be randomly allocated to decide which order they will complete the bouts. Both exercise bouts will be carried out on the same exercise bike and during exercise sessions you will also be required to where chest belts so that we can monitor your heart rate. Before, immediately after and one hour after both exercise sessions an intravenous blood sample will be collected. Fingertip blood samples will be taken and participant's bodyweight will be recorded before and after both exercise bouts. Finally, you will also be asked to perform the cognitive function task before and after both exercise bouts.

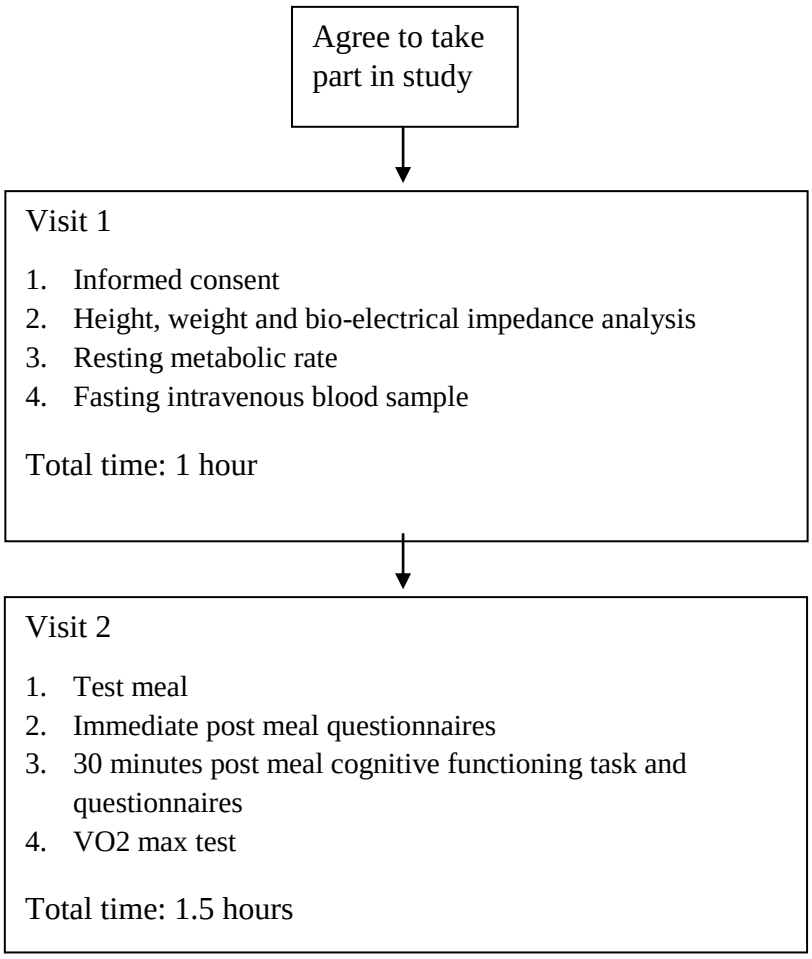
Test Meal

Participants will be asked to consume a test meal one hour after both exercise bouts and on a separate occasion at the same time of day without prior exercise. The test meal is a vanilla flavoured drink and much like a milkshake. The cognitive function task and associated questionnaires will be completed thirty minutes after the test meal.

Diet Record

You will be asked to record all food and drink that you consume on the day of both the high and low intensity exercise bouts and on 3 days per week during the 2 week period around the exercise bouts; these will be chosen at random. It is important that you record with as much detail and accuracy as possible. You will be asked to keep the same diet before you arrive at the lab on the day of both bouts. Diet records will be supplied.

Timeline



Visit 3 - Exercise Bout 1

1. Pre exercise cognitive functioning task and questionnaires
2. Bodyweight, venous and fingertip blood sample
3. 5 minute warm up
4. High intensity or moderate intensity exercise bout
5. Bodyweight, venous and fingertip blood sample
6. Immediate post exercise cognitive functioning task and questionnaires
7. One hour post exercise cognitive functioning task and questionnaires
8. One hour intravenous blood sample
9. Test meal
10. Immediate post meal questionnaires
11. 30 minutes post meal cognitive functioning task and questionnaires

Total time: 3 hours

Visit 4 - Exercise Bout 2

1. Pre exercise cognitive functioning task and questionnaires
2. Bodyweight, venous and fingertip blood sample
3. 5 minute warm up
4. High intensity or moderate intensity exercise bout
5. Bodyweight, venous and fingertip blood sample
6. Immediate post exercise cognitive functioning task and questionnaires
7. One hour post exercise cognitive functioning task and questionnaires
8. One hour intravenous blood sample
9. Test meal
10. Immediate post meal questionnaires
11. 30 minutes post meal cognitive functioning task and questionnaires

Total time: 3 hours

End of study

What are the possible disadvantages and risks of taking part?

You may experience some discomfort during blood sampling. The blood sampling might be stressful for people who do not like needles or seeing blood. You may also find the VO2 max testing somewhat uncomfortable as you are required to exercise to maximal exertion.

What are the possible benefits of taking part?

Your participation in this study will provide us with valuable insights into how the human body and mind adapts to exercise and will add to our understanding of how exercise positively influences health. In addition to the above, upon completion of the study you will be paid £40 as a reward for taking part in our study.

Confidentiality

All information which is collected about you during the course of the research will be kept strictly confidential. Any data which is to leave the school, for possible publications or reports, will have your name and any other personal information removed so that you cannot be recognised from it. It will not be possible to identify you in any of these reports or publications.

Who is organising or funding the research?

This study has been organised by Matthew Jackson (PhD student), Fardin Fatahi (PhD student), Dr Hans-Peter Kubis (Senior Lecturer) and Dr David Markland (Senior Lecturer) from the School of Sport, Health and Exercise Sciences (SSHES) of Bangor University.

Who has reviewed the study?

This study has been reviewed and approved by the SSHES Ethics Committee.

Feedback and conduct of research

SSHES is always keen to hear the views of research participants about their experience. If you would like to give feedback, please ask your researcher to provide you with Form 6 – Participant Feedback Form – from the Ethics Guidelines Handbook. Completion of this form is optional and can be done anonymously. The completed form should be returned to Prof. Andrew Lemmey, Chair, SSHES Ethics Committee, SSHES, Bangor University, Bangor LL57 2PZ. All information will be treated in a strictly confidential manner.

Complaints

If during the course of this study you feel the need to complain about how the research has been carried out, for any reason, then please contact the Head of School, Dr Tim Woodman, SSHES, Bangor University, Bangor LL57 2PZ.

Any questions?

Please ask us if you have any questions. Here are the contact details of all investigators in this study:

Fardin Fatahi (PhD student) pepc09@bangor.ac.uk Tel: 01248 388254
Matthew Jackson (PhD student) pepa2f@bangor.ac.uk Tel: 01248 388254
Dr Hans-Peter Kubis (Senior Lecturer) pes203@bangor.ac.uk Tel: 01248 388261
Dr David Markland (Senior Lecturer) d.a.markland@bangor.ac.uk Tel: 01248 383487

Appendix 6

Informed Consent Form

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Informed Consent to Participate in a Research Experiment

1	Title of project	An investigation into the effects of exercise intensity on substrate utilisation and cognitive function.
2	Name and e-mail address(es) of all researcher(s)	Matthew Jackson – pepa2f@bangor.ac.uk Fardin Fatahi – pepc09@bangor.ac.uk Charlotte Jelleyman – peu662@bangor.ac.uk Sayali Phatak – pepc11@bangor.ac.uk Carwyn Lewis – pepc25@bangor.ac.uk Dr. Hans-Peter Kubis - pes203@bangor.ac.uk Dr. David Markland - d.a.markland@bangor.ac.uk

Please tick boxes

1. I confirm that I have read and understand the Information Sheet dated ……. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor, other staff members of with the School. ☐

4. I understand that I may register any complaint I might have about this experiment with the Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

5. I agree to take part in the above study. ☐

Name of Participant …….

Signature ……. Date ……..

Name of Person taking consent…….

Signature ……. Date ……..

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE

Appendix 7

Informed consent

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Informed Consent to Participate in a Research Experiment

1	Title of project	Investigation of energy balance regulation with focus on the impact of metabolism on perception of food and cognition
2	Name and e-mail address(es) of all researcher(s)	Matthew Jackson – pepa2f@bangor.ac.uk Fardin Fatahi – pepc09@bangor.ac.uk Dr. Hans-Peter Kubis - pes203@bangor.ac.uk Dr. David Markland - d.a.markland@bangor.ac.uk

Please tick boxes

1. I confirm that I have read and understand the Information Sheet dated 31/10/2012 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐
3. I understand that I may register any complaint I might have about this experiment with the Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐
4. I agree to take part in the above study. ☐

Name of Participant …….

Signature …….....Date ……

Name of Person taking consent……Signature ……

Date …….

Appendix 8

Physiology Informed Consent and Medical questionnaire

Bangor University

SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of Participant ……..……..

Age ……

Are you in good health? YES ☐ NO ☐

If no, please explain

How would you describe your present level of activity?

Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once per week		> 5 times per week	

Have you suffered from a serious illness or accident? YES ☐ NO ☐

If yes, please give particulars:

Do you suffer from allergies? YES ☐ NO ☐

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis					

Are you currently taking medication? YES ☐ NO ☐

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? YES ☐ NO ☐

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?

YES ☐ NO ☐

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;

- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……20……

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature *(participant)* …….. Date ……..

Print name ……

Signature *(experimenter)* ……. Date ……..

Print name ……

Appendix 9

Health screening and physical activity questionnaire

School of Sport, Health & Exercise Sciences
Bangor University
George Building
Holyhead Road
Bangor
Gwynedd
LL57 2PZ

Pre-screening Questionnaire

Name of Participant: ……..…… Date of Birth: .....

Height: …….. Email:……...

Weight: ..... Contact No:……

Nationality:……..

HEALTH:

1. Are you in good health? YES ☐ NO ☐

   If no, please explain:

2. Have you suffered from a serious illness or accident? YES ☐ NO ☐

   If yes, please provide details:

3. Do you suffer or have you ever suffered from:

	YES	NO
Asthma		
Bronchitis		
Diabetes		
Epilepsy		
Heart Condition		
High Blood Pressure		
Bone or Joint Problem		

4. Are you currently taking medication? YES ☐ NO ☐

   If yes, please provide details:

5. Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? YES ☐ NO ☐

   If yes, please provide details:

6. Do you have any allergies? YES ☐ NO ☐

   If yes, please provide details:

7. Do you smoke? YES ☐ NO ☐

8. Menstruation:
   a. Please indicate your current menstrual status:

      ☐ No menstruation

      ☐ Irregular menstruation

      ☐ Regular menstruation

   b. How many days since your last menstruation? .....

c. Do you currently take an oral contraceptive? YES NO

☐ ☐

PHYSICAL ACTIVITY:

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< once per month		2 times per week	
once per month		3-4 times per week	
2 times per month		> 5 times per week	
1 times per week			

NUTRITION:

1. How would you describe your current diet:

   Westernised ☐

   Traditional ☐

2. Are you currently:

   Vegetarian YES NO

   ☐ ☐

   Vegan YES NO

   ☐ ☐

3. Are you currently attempting to lose weight? YES NO

   ☐ ☐

Have you, or are you presently taking part in any other laboratory experiment?

	YES	NO
PLEASE READ THE FOLLOWING CAREFULLY	☐	☐

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature ……. Date ……..
Participant

Signature ……. Date ……..
Experimenter

Appendix 10

Implicit Association Task - Images


ENERGY
orange

Appendix 11

Positive and Negative Words used in both IATs

Implicit attitude task	
Positive words	Negative words
Lovely	Horrible
Glorious	Awful
Superb	Nasty
Wonderful	Painful
Beautiful	Humiliate
Joyful	Tragic
Pleasure	Terrible
Marvellous	Agony

Appendix 12

Explicit Attitude Questionnaires

EXPLICIT ATTITUDE QUESTIONNAIRE

NAME _____ **NUMBER** _____

On the scales below, please circle the number that best describes your feelings about low fat food. Be sure to circle just ONE number for each pair of words.

Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about low fat food.

For me, eating low fat foodis …

	extremely	quite	slightly	neither	slightly	quite	extremely	
Enjoyable	1	2	3	4	5	6	7	**Unenjoyable**
Harmful	1	2	3	4	5	6	7	**Beneficial**
Useful	1	2	3	4	5	6	7	**Useless**
Boring	1	2	3	4	5	6	7	**Interesting**
Pleasant	1	2	3	4	5	6	7	**Unpleasant**
Stressful	1	2	3	4	5	6	7	**Relaxing**
Wise	1	2	3	4	5	6	7	**Foolish**
Bad	1	2	3	4	5	6	7	**Good**

Thank you very much for completing this questionnaire

EXPLICIT ATTITUDE QUESTIONNAIRE

NAME _____ **NUMBER** _____

On the scales below, please circle the number that best describes your feelings about high fat food. Be sure to circle just ONE number for each pair of words.

Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about high fat food.

For me, eating high fat food is …

	extremely	quite	slightly	neither	slightly	quite	extremely	
Enjoyable	1	2	3	4	5	6	7	**Unenjoyable**
Harmful	1	2	3	4	5	6	7	**Beneficial**
Useful	1	2	3	4	5	6	7	**Useless**
Boring	1	2	3	4	5	6	7	**Interesting**
Pleasant	1	2	3	4	5	6	7	**Unpleasant**
Stressful	1	2	3	4	5	6	7	**Relaxing**
Wise	1	2	3	4	5	6	7	**Foolish**
Bad	1	2	3	4	5	6	7	**Good**

Thank you very much for completing this questionnaire

EXPLICIT ATTITUDE QUESTIONNAIRE

NAME _____ **NUMBER** _____

On the scales below, please circle the number that best describes your feelings about low sugar food. Be sure to circle just ONE number for each pair of words.

Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about low sugar food.

For me, eating low sugar food is …

	extremely	quite	slightly	neither	slightly	quite	extremely	
Enjoyable	1	2	3	4	5	6	7	**Unenjoyable**
Harmful	1	2	3	4	5	6	7	**Beneficial**
Useful	1	2	3	4	5	6	7	**Useless**
Boring	1	2	3	4	5	6	7	**Interesting**
Pleasant	1	2	3	4	5	6	7	**Unpleasant**
Stressful	1	2	3	4	5	6	7	**Relaxing**
Wise	1	2	3	4	5	6	7	**Foolish**
Bad	1	2	3	4	5	6	7	**Good**

Thank you very much for completing this questionnaire

EXPLICIT ATTITUDE QUESTIONNAIRE

NAME _____ **NUMBER** _____

On the scales below, please circle the number that best describes your feelings about high sugar food. Be sure to circle just ONE number for each pair of words.

Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about high sugar food.

For me, eating high sugar food is …

	extremely	quite	slightly	neither	slightly	quite	extremely	
Enjoyable	1	2	3	4	5	6	7	**Unenjoyable**
Harmful	1	2	3	4	5	6	7	**Beneficial**
Useful	1	2	3	4	5	6	7	**Useless**
Boring	1	2	3	4	5	6	7	**Interesting**
Pleasant	1	2	3	4	5	6	7	**Unpleasant**
Stressful	1	2	3	4	5	6	7	**Relaxing**
Wise	1	2	3	4	5	6	7	**Foolish**
Bad	1	2	3	4	5	6	7	**Good**

Thank you very much for completing this questionnaire

Appendix 13

Circuit training - workout

Circuit Training - Workout 1	
HI	**LI**
General Warm-up (5 minutes): Moving in a circle around the gym • Walking • Jogging • High knees • Heel kicks • Side stepping • Skipping • Arm swings • Side twists • Hip rotations Circuit: 10 stations 1. Press ups 2. Tricep dips 3. Dumbbell punching 4. Step ups 5. Squats 6. Lunges 7. Star jumps 8. Dorsal raises 9. Crunches 10. Russian twists Circuit 1: 45 seconds exercise/15 seconds rest Circuit 2: 30 seconds exercise/15 seconds rest 3 minutes rest between circuits Cool down 5 minutes: Moving in a circle around the gym • Jog • Walk • Stretching	General Warm-up (10 minutes): Moving in a circle around the gym • Walking • High knees • Heel kicks • Arm swings • Side twists • Hip rotations • Swimming Circuit: 12 stations 1. Skaters 2. Squat with bicep curl 3. oodchopper 4. Multi-planar lunge 5. Te warrior 6. Plies 7. Supermans 8. Tread the needle 9. Russian twists 10. Bridges 11. Heel touches 12. Single leg raises 4 circuits, 1 minute per station, everyone does each exercise together. Cool down 10 minutes: Moving in a circle around the gym • Walk • Mobilisation • Stretching

Circuit Training-Workout 2	
HI	**LI**
General Warm-up (5 minutes): Moving in a circle around the gym • Walking • Jogging • High knees • Heel kicks • Side stepping • Arm swings • Side twists • Hip rotations Circuit: 10 stations Participants move from base to base doing one exercise from one base and then moving to the next. Base 1 – Split squats, Mountain climber, Star jumps, Astride jumps Base 2 – Press ups, Tricep dips, curl to press, shoulder raises Base 3 – Bicycle kicks, leg raises, supermans, reverse ab curl Circuit 1: 45 seconds exercise/15 seconds rest Circuit 2: 30 seconds exercise/15 seconds rest Circuit 3: 30 seconds exercise/no rest 2 minutes rest between circuits Cool down 5 minutes: Moving in a circle around the gym • Jog • Mobilisation • Walk • Stretching	General Warm-up (10 minutes): Moving in a circle around the gym • Walking • High knees • Heel kicks • Arm swings • Side twists • Hip rotations • Swimming Circuit: 10 stations Squat with calf raise Lunges with twist Dorsal raises Knee ups Upward dog/downward dog Single leg drops Toe touches Single leg anterior reach Side bridges Windscreen wipers 4 Circuits of 1 minute per exercise/15 second rest 2 minutes rest between circuits Cool down 10 minutes: Moving in a circle around the gym • Walk • Mobilisation • Stretching

Appendix 14

Food Record Sheet

The purpose of the 3 days food record is to provide a quantitative assessment of food intake over 3 days. To assess portion sizes household measures or weighing scales should be used. Accuracy and precision are of course necessary. *The information that you provide will be held confidential by the researcher*.

How to describe foods and drinks

Use a separate line for each food or food type when recording, and separate out components of composite dishes such as sandwiches, salad, or casseroles. For all foods, include the following details:

1. **Method of cooking** (e.g. roasted, stewed, fried, boiled, steamed)
2. **Kind of food** (e.g. raw or cooked; peeled or unpeeled; white or whole wheat bread; fresh, canned frozen, dried, whole or skim milk)
3. **Brand names** of all processed foods wherever applicable (e.g. Kraft Macaroni and Cheese, Campbell's soup, Kellogg's corn flakes)
4. **Include all condiments** (e.g. pickles, sauces, catsup, mustard)
5. **Provide as much label information as possible** and the brad name of any unusual or special foods consumed (e.g. sodium-reduced soup)
6. If a **recipe** is used to make composite products such as casseroles, baked goods, sauces, etc., record, on the back of the food record sheet, the complete recipe, giving the measured amount of each ingredient, the total number of serving for dish, and the amount of the dish eaten.
7. **Record the amounts** of all **food and beverages** in the form they are consumed (from Nutritional Assessment by R S Gibson, 1993)

Example:

Place Eaten	Time	**Description of Food or Drink**	Brand Name	Amount
Home	12.30	Beef hamburger		3 oz
" "	12.30	Fried tomato		2 tbs.
" "	12.30	Spaghetti and tomato sauce	Campbell	¾ cup
" "	12.30	Tea		1 cup
" "	12.30	Whole Milk	Tesco	2 tbs.

Thank you

Please write your Name here:_____

Day 1 – Tuesday

Place Eaten	Time	**Description of Food or Drink**	Brand Name	Amount

Appendix 15

Food craving questionnaire-trait (FCQ-T)

NAME: _____ DATE: _____

FCQ-T

How frequently each statement "would be true for you in general" using a 6-point scale that ranged from 1 (*never*) to 6 (*always*);

	1	2	3	4	5	6
Being with someone who is eating often makes me hungry						
When I crave something, I know I won't be able to stop eating once I start						
If I eat what I am craving, I often lose control and eat too much						
I hate it when I give in to cravings						
Food cravings invariably make me think of ways to get what I want to eat						
I feel like I have food on my mind all the time						
I often feel guilty for craving certain foods						
I find myself preoccupied with food						
I eat to feel better						
Sometimes, eating makes things seem just perfect						
Thinking about my favourite foods makes my mouth water						
I crave foods when my stomach is empty						
I feel as if my body asks me for certain foods						
I get so hungry that my stomach seems like a bottomless pit						
Eating what I crave makes me feel better						
When I satisfy a craving I feel less depressed						
When I eat what I am craving I feel guilty about myself						
Whenever I have cravings, I find myself making plans to eat						

Eating calms me down						
I crave foods when I feel bored, angry, or sad						
I feel less anxious after I eat						
If I get what I am craving I cannot stop myself from eating it						
When I crave certain foods, I usually try to eat them as soon as I can						
When I eat what I crave I feel great						
I have no will power to resist my food crave						
Once I start eating, I have trouble stopping						
I can't stop thinking about eating no matter how hard I try						
I spend a lot of time thinking about whatever it is I will eat next						
lf I give in to a food craving, all control is lost						
When I'm stressed out, I crave food						
I daydream about food						
Whenever I have a food craving, I keep on thinking about eating until I actually eat the food						
If I am craving something, thoughts of eating it consume me						
My emotions often make me want to eat						
Whenever I go to a buffet I end up eating more than what I needed						
It is hard for me to resist the temptation to eat appetizing foods that are in my reach						
When I am with someone who is overeating, I usually overeat too						
When I eat food, I feel comforted						
I crave foods when I'm upset						

Appendix 16

Food craving questionnaire-state (FCQ-S)

FCQ-S

Indicate the extent to which you agreed with each statement "right now, at this very moment" using a 7-point scale that ranged from 1 (*strongly agree*) to 7 (*strongly disagree*).

	1	2	3	4	5	6	7
I have an intense desire to eat something tasty							
I'm craving savoury food (e.g. pizza)							
I have an urge for savoury food							
Eating savoury food would make things just perfect							
If I were to eat what I'm craving, I am sure my mood would improve							
Eating savoury food would feel wonderful							
If I ate something, I wouldn't feel so sluggish and lethargic							
Satisfying my craving would make me feel less grouchy and irritable							
I would feel more alert if I could satisfy my craving							
If I had savoury food, I could not stop eating it							
My desire to eat savoury food seems overpowering							
I know I'm going to keep on thinking about savoury food until I actually have it							
I am hungry							
If I ate right now, my stomach wouldn't feel as empty							
I feel weak because of not eating							

Indicate the extent to which you agreed with each statement "right now, at this very moment" using a 7-point scale that ranged from 1 (*strongly agree*) to 7 (*strongly disagree*).

	1	2	3	4	5	6	7
I have an intense desire to eat something tasty							
I'm craving sweet food (e.g. chocolate bar)							
I have an urge for sweet foods							
Eating sweet foods would make things just perfect							
If I were to eat what I'm craving, I am sure my mood would improve							
Eating sweet foods would feel wonderful							
If I ate something, I wouldn't feel so sluggish and lethargic							
Satisfying my craving would make me feel less grouchy and irritable							
I would feel more alert if I could satisfy my craving							
If I had sweet food, I could not stop eating it							
My desire to eat sweet foods seems overpowering							
I know I'm going to keep on thinking about sweet foods until I actually have it							
I am hungry							
If I ate right now, my stomach wouldn't feel as empty							
I feel weak because of not eating							

Appendix 17

Acute FCQ-S, IAT, and EAQ responses to MI, HI and meal challenge (Chapter 5)

Table A17.1. Pilot - FCQ-S response to test meal

	Pre-meal	Post-meal
Intense desire to eat-Sa	12.40±4.19	13.20±4.05
Intense desire to eat-Sw	11.90±5.17	12.70±6.00
Positive reinforcement-Sa	12.40±5.45	12.30±4.70
Positive reinforcement-Sw	12.30±4.83	13.60±5.13
Negative reinforcement-Sa	12.90±2.92	10.10±4.99
Negative reinforcement-Sw	11.40±4.80	11.40±5.47
Lack of control-Sa	13.50±7.57	15.10±4.60
Lack of control-Sw	12.60±5.66	15.60±4.45
Feelings of Hunger-Sa	11.00±5.19	10.10±4.99
Feelings of Hunger-Sw	11.00±5.25	10.20±5.09

Values are means ± SD

Table A17.2. Pilot - FCQ-S response to exercise

	HI		MI	
	Pre Ex	Post Ex	Pre Ex	Post Ex
Intense desire to eat-Sa	13.00±4.64	7.60±5.38	11.90±4.80	9.90±5.51
Intense desire to eat-Sw	12.20±5.29	8.80±5.77	12.20±5.01	11.40±4.50
Positive reinforcement-Sa	13.00±4.62	9.30±4.76	13.00±4.00	11.40±5.19
Positive reinforcement-Sw	13.00±4.00	9.80±5.65	13.40±4.90	11.10±4.15
Negative reinforcement-Sa	13.10±4.53	9.00±4.94	12.40±4.84	11.50±5.29
Negative reinforcement-Sw	13.10±5.00	8.70±5.21	12.90±5.92	11.80±5.47
Lack of control-Sa	16.00±3.71	10.40±6.89	16.00±3.97	14.20±4.08
Lack of control-Sw	14.40±6.03	12.80±7.02	14.70±6.04	13.60±6.35
Feelings of Hunger-Sa	12.30±4.57	7.00±4.74	12.00±5.19	10.50±5.78
Feelings of Hunger-Sw	11.70±4.72	7.50±5.06	11.90±5.82	10.50±5.86

Values are means ± SD

Table A17.3. Pilot - IAT/EAQ non exercise response to meal

	Pre Meal	Post Meal
IAT HF/LF	0.32±0.50	0.37±0.52
IAT HS/LS	0.71±0.55	0.46±0.51
EAQ LF	5.16±0.87	4.73±0.79
EAQ HF	3.59±0.73	3.73±0.64
EAQ LS	4.61±0.94	4.38±0.67
EAQ HS	4.22±0.88	3.88±0.55

Values are means ± SD

Table A17.4. Pilot - IAT/EAQ response to exercise

	Pre Meal	Post Meal
IAT HF/LF	0.10±0.57	0.16±0.52
IAT HS/LS	0.19±0.41	0.11±0.39
EAQ LF	4.75±0.67	4.69±0.74
EAQ HF	3.71±0.79	3.69±0.80
EAQ LS	4.47±0.78	4.46±0.72
EAQ HS	3.65±0.93	4.03±1.66

Values are means ± SD

Table A17.5. Main trial - FCQ-S response to HI- L

	Pre Ex	Post Ex	Post Ex 1H	Post Meal	30 min Post meal
Intense desire to eat_SA	14.44±3.91	13.44±6.04	14.89±4.94	13.78±3.80	12.33±5.57
Intense desire to eat_SW	14.55±4.00	15.00±5.55	14.44±5.34	16.00±4.03	13.22±6.48
Positive reinforcement_SA	14.56±4.28	14.56±5.70	15.33±4.36	14.89±3.79	13.56±5.25
Positive reinforcement_SW	15.33±4.69	15.89±5.97	15.00±4.58	16.44±3.32	14.4±6.33
Negative reinforcement_SA	14.89±4.57	14.33±4.03	13.44±4.72	15.00±3.67	13.33±4.77
Negative reinforcement_SW	14.56±4.48	13.67±6.04	13.89±4.31	15.89±3.69	13.22±5.60
Lack of control_SA	15.33±5.32	13.67±4.80	14.67±3.91	15.44±4.67	14.22±5.17
Lack of control_SW	14.89±4.68	14.78±4.94	15.33±5.27	16.22±5.02	14.67±6.86
Feelings of hunger_SA	16.00±4.39	13.00±5.74	14.56±6.06	14.44±5.50	12.22±6.32
Feelings of hunger_SW	15.33±4.64	13.89±6.01	13.11±6.66	14.44±5.53	11.78±6.26

Values are means ± SD

Table A17.6. Main trial - FCQ-S response to MI- L

	Pre Ex	Post Ex	Post Ex 1H	Post Meal	30 min Post meal
Intense desire to eat_SA	16.22±3.99	14.56±3.84	12.22±5.70	13.56±5.48	12.78±5.43
Intense desire to eat_SW	15.67±4.87	13.78±4.41	11.22±4.68	14.11±4.26	13.00±5.10
Positive reinforcement_SA	14.67±5.10	15.22±4.32	13.22±6.87	14.00±5.10	13.22±5.56
Positive reinforcement_SW	14.67±5.50	13.11±4.78	12.11±6.11	13.89±4.83	13.22±5.73
Negative reinforcement_SA	15.56±4.67	12.44±6.29	13.22±4.58	12.67±5.32	14.55±5.53
Negative reinforcement_SW	13.56±4.85	13.78±5.36	12.11±6.57	13.33±4.97	12.78±5.38
Lack of control_SA	14.56±5.53	14.44±4.13	14.33±5.50	14.67±5.10	13.56±5.68
Lack of control_SW	14.33±6.76	13.00±5.15	12.44±6.31	13.44±5.66	13.33±6.52
Feelings of hunger_SA	16.56±4.07	14.22±5.70	12.22±6.78	13.56±5.41	12.67±5.15
Feelings of hunger_SW	15.89±5.06	13.44±5.10	11.89±6.81	13.88±5.71	12.44±5.29

Values are means ± SD

Table A17.7. Main trial - FCQ-S response to HI- OV/OB

	Pre Ex	Post Ex	Post Ex 1H	Post Meal	30 min Post meal
Intense desire to eat_SA	14.70±4.97	14.40±5.58	12.10±5.90	15.00±4.32	14.80±5.92
Intense desire to eat_SW	15.40±4.62	14.20±5.63	13.00±5.61	14.80±4.02	16.00±5.10
Positive reinforcement_SA	15.80±4.73	15.60±4.53	11.80±6.05	15.70±4.16	15.50±5.74
Positive reinforcement_SW	16.80±4.21	14.30±5.83	12.90±6.56	16.30±4.64	16.30±5.25
Negative reinforcement_SA	14.10±5.20	12.60±6.52	11.50±5.50	14.50±4.02	14.80±6.20
Negative reinforcement_SW	15.10±4.43	12.20±5.79	11.20±6.73	15.40±5.15	15.40±6.29
Lack of control_SA	16.90±4.38	16.40±5.61	14.90±5.90	17.70±3.89	17.10±4.89
Lack of control_SW	17.60±3.34	15.30±6.41	15.60±5.93	18.20±4.13	17.20±5.07
Feelings of hunger_SA	14.20±4.57	12.60±7.21	8.50±5.38	12.40±4.62	13.20±6.18
Feelings of hunger_SW	13.20±5.57	13.10±6.35	8.50±5.80	12.50±4.99	13.10±6.51

Values are means ± SD

Table A17.8. Main trial - FCQ-S response to MI- OV/OB

	Pre Ex	Post Ex	Post Ex 1H	Post Meal	30 min Post meal
Intense desire to eat_SA	12.10±5.61	12.00±6.02	12.90±6.28	14.80±4.13	14.30±4.67
Intense desire to eat_SW	13.90±4.51	12.90±5.97	12.40±5.17	13.00±5.81	14.20±5.03
Positive reinforcement_SA	14.90±5.99	13.30±7.20	14.60±6.79	15.70±4.57	16.30±4.47
Positive reinforcement_SW	16.10±5.82	14.90±6.51	13.20±6.60	14.20±7.00	15.20±5.39
Negative reinforcement_SA	13.90±4.82	11.50±6.17	12.20±7.13	14.80±5.41	14.30±5.74
Negative reinforcement_SW	15.40±4.86	11.10±6.24	10.60±6.70	13.50±7.11	14.40±6.32
Lack of control_SA	16.10±5.92	14.80±5.92	15.50±15.00	16.50±4.03	16.50±4.06
Lack of control_SW	16.40±5.56	15.60±5.76	15.50±5.91	14.90±6.98	16.00±5.33
Feelings of hunger_SA	12.80±5.18	10.70±5.96	10.60±6.04	13.50±5.04	13.00±5.29
Feelings of hunger_SW	12.60±5.25	9.90±5.50	9.10±6.51	11.40±6.31	11.50±5.95

Values are means ± SD

Table A17.9. Main trial - IAT/EAQ non exercise response to meal- L

Pre Meal	Post Meal	30 min Post meal	
IAT HF/LF		0.38±0.37	
IAT HS/LS		0.27±0.46	
EAQ LF	5.41±0.82	5.16±0.93	5.06±0.98
EAQHF	3.24±1.18	3.24±0.90	3.21±1.02
EAQLS	5.01±0.88	4.76±1.07	4.86±1.12
EAQHS	3.42±0.97	4.00±1.11	3.18±1.05

Values are means ± SD

Table A17.10. Main trial - IAT/EAQ non exercise response to meal- OV/OB

	Pre Meal	Post Meal	30 min Post meal
IAT HF/LF		0.60±0.42	
IAT HS/LS		-0.01±0.59	
EAQ LF	5.26±0.61	5.24±0.71	5.27±1.13
EAQ HF	3.35±0.74	3.30±0.82	3.22±1.15
EAQ LS	4.34±0.64	4.67±0.69	4.78±1.10
EAQ HS	3.63±0.93	3.77±0.75	3.14±0.82

Values are means ± SD

Table A17.11. Main trial - IAT/EAQ response to exercise- L

	Pre Ex	Post Ex	Post Ex+1H	Post Meal	30 min Post meal
High Intensity					
IAT HF/LF	0.19±0.59	0.22±0.43	0.04±0.26	-0.16±0.25	
IAT HS/LS	-0.03±0.46	-0.99±0.50	-0.01±0.32	-0.15±0.40	
EAQ LF	5.39±0.94	5.27±0.98	5.04±1.14	5.49±1.05	5.71±1.01
EAQ HF	3.25±1.52	3.47±0.88	3.47±1.10	3.49±1.37	3.38±0.94
EAQ LS	5.17±0.90	4.95±1.27	4.84±1.24	5.07±1.14	5.01±1.31
EAQ HS	3.61±1.31	3.67±1.28	3.09±1.61	3.51±1.32	3.42±1.32
Moderate Intensity					
IAT HF/LF	0.18±0.35	0.01±0.21	0.03±0.32	-0.01±0.27	
IAT HS/LS	0.77±0.41	0.21±0.16	-0.11±0.34	-0.10±0.45	
EAQ HF	5.32±0.81	5.27±1.12	5.04±1.02	5.20±1.27	5.39±0.89
EAQ LF	3.16±0.94	3.45±1.01	3.46±0.95	3.45±1.34	3.49±1.13
EAQ HS	4.98±1.02	4.78±0.88	5.07±1.04	5.14±1.04	5.11±0.89
EAQ LS	3.27±0.86	3.55±0.74	3.43±0.95	3.60±1.30	3.47±1.301

Values are means ± SD

Table A17.12. Main trial - IAT/EAQ response to exercise- OV/OB

	Pre Ex	Post Ex	Post Ex+1H	Post Meal	30 min Post meal
High Intensity					
IAT HF/LF	0.23±0.45	0.24±0.47	0.01±0.45	0.02±0.37	
IAT HS/LS	0.00±0.29	-0.07±0.44	-0.01±0.45	0.04±0.45	
EAQ HF	5.49±0.64	5.49±0.52	5.18±0.66	5.40±0.50	5.32±0.59
EAQ LF	3.40±0.84	3.29±0.85	3.59±0.70	3.40±0.90	3.23±0.79
EAQ HS	4.80±0.74	5.09±0.70	4.76±0.65	5.12±0.42	5.13±0.63
EAQ LS	3.70±0.80	3.51±0.77	3.72±0.64	3.32±0.82	3.40±0.83
Moderate Intensity					
IAT HF/LF	0.23±0.44	0.19±0.39	0.09±0.59	0.23±0.34	
IAT HS/LS	0.04±0.50	0.48±0.54	-0.04±0.45	0.15±0.36	
EAQ HF	5.42±0.61	5.48±0.62	5.34±0.66	5.34±0.72	5.35±0.1
EAQ LF	3.12±0.83	3.33±1.05	3.24±0.95	3.24±0.82	3.47±0.83
EAQ HS	4.80±0.80	5.08±0.57	5.04 ±0.72	5.05±0.59	5.03±0.74
EAQ LS	3.21±0.68	3.34±1.10	3.46±0.97	3.39±0.77	3.63±0.87

Values are means ± SD