# ACUTE EFFECTS OF EXERCISE ON APPETITE, APPETITE REGULATORY HORMONES AND ENERGY INTAKE IN LEAN AND OVERWEIGHT MEN AND WOMEN

by

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## ABSTRACT

The acute effects of exercise on appetite, *ad libitum* energy intake and gut hormone responses have received much attention over the past two decades. The experiments in this thesis have contributed to this research by examining appetite, acylated ghrelin, peptide-YY (PYY), leptin and *ad libitum* energy intake responses to two consecutive days of moderate-high intensity running. To achieve this 15 individuals aged 21 (2) y, with a BMI of 23.0 (1.9) $kg \cdot m^{-2}$ were recruited. Additionally, appetite, acylated ghrelin, PYY, glucagon-like peptide-1 (GLP-1), and *ad libitum* energy intake responses to an acute bout of moderate intensity treadmill exercise were compared in lean and overweight/obese (ow/ob) males and females. Two separate cohorts of individuals were recruited; 22 lean individuals and 25 ow/ob individuals (aged 38 (15) and 45 (12) y, with a BMI of 22 (2) and 29 (3) $kg \cdot m^{-2}$, for lean and ow/ob individuals, respectively).

In Chapter 4, two consecutive days of 60 min treadmill running at 70% $\dot{V}O_2$ peak did not produce compensatory changes in appetite or energy intake over two days. There were no main effects of trial for acylated ghrelin or leptin. However a main effect of trial for PYY indicated higher concentrations on the exercise than control trial. A meta-analysis was completed in Chapter 5, suggesting further research in the effects of acute exercise on appetite regulatory hormones in individuals who are ow/ob was necessary. In Chapter 6, 60 min of treadmill exercise at 60% $\dot{V}O_2$ peak did not alter appetite sensations or energy intake in the 7 h after exercise in lean and ow/ob males and females. There were no main effects of sex, BMI or trial for acylated ghrelin; however, PYY and GLP-1 concentrations were higher in exercise than control trials.

This thesis has demonstrated that over two days, high volume exercise does not stimulate compensatory appetite regulatory changes, in lean healthy males. In the short term, lean and ow/ob males and females respond similarly to acute exercise, showing no alterations in appetite or food intake responses, whilst PYY and GLP-1 concentrations are higher in exercise than control trials.



**Key words:** exercise, appetite, energy intake, energy balance, compensations, acylated ghrelin, peptide-YY, glucagon-like peptide-1, hormones.

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## LIST OF ABBREVIATIONS

The following abbreviations are used throughout multiple chapter of this thesis. Where they appear in text, they have been defined in the first instance:

AgRP (agouti-related peptide)

ARC (arcuate nucleus)

BMI (body mass index)

DPP-IV (dipeptidyl peptidase IV)

EDTA (ethylenediaminetetra-acetic acid)

GHS-R (growth hormone secretagogue receptor)

GLP-1 (glucagon-like peptide-1)

GOAT (ghrelin O-acyltransferase)

HbA1c (haemoglobin A1c)

HOMA-IR (homeostatic model of assessment – estimated insulin resistance)

IRAS (integrated research application system)

NaOH (sodium hydroxide)

NEFA (non-essential fatty acids)

NPY (neuropeptide Y)

Ow/ob (overweight/obese)

PBS (potassium phosphate buffer)

PHMB (p-hydroxymercuribenzoic acid)

PFC (prospective food consumption)

POMC (pro-opiomelanocortin)

PYY (peptide-YY)

R&D (research and development)

REC (research ethics committee)

RPE (rating of perceived exhaustion)

REI (relative energy intake)

RER (non-protein respiratory exchange rate)

SSI (site-specific information)

TAG (triglycerides)

VAS (visual analogue scale)

$\dot{V}O_2$ (oxygen uptake)

# CHAPTER I

## Introduction

Obesity is defined as an abnormal or excessive accumulation of fat, which may impair health (WHO 2016). Body mass index (BMI), a product of weight in kilograms divided by height in metres squared, is commonly used to stratify body size. Specifically, those with a BMI equal to or greater than 25 $kg \cdot m^{-2}$ are classified as overweight, and those with a BMI more than or equal to 30 $kg \cdot m^{-2}$ are classified as obese. In 2014, it was estimated that there were more than 1.9 billion overweight adults worldwide, of whom 600 million were obese (WHO 2016). Overweight and obesity negatively impacts morbidity, disability and quality of life. In particular, increased BMI increases the risk of cardiovascular disease, type 2 diabetes, musculoskeletal disorders (i.e. osteoarthritis) and some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon (Bray 2004)).

Within the UK, the issue of overweight and obesity is just as pervasive. In 2014, approximately a quarter of adults were classified as obese, with estimations expecting this to rise to over 50% of the population by 2050 (HSCIC 2015). Obesity is thought to cost the UK's wider economy £47 billion a year, second only to the costs of smoking. Notably, there are direct costs to the NHS for treating overweight and obesity, costs to produce and supply obesity medication, provision of social care and the impact of obesity attributed sick days (Public Health England 2015).

Obesity is a result of sustained positive energy balance, whereby energy consumed outweighs that expended in metabolic processes and physical activity, over time. The soaring prevalence in overweight and obesity is a repercussion of recent population wide behavioural changes; for example, ease of access to energy dense foods, increased physical inactivity through the sedentary nature of work and transportation and increased urbanisation (WHO 2016).

Physiologically, energy balance and body weight are controlled by a plethora of gut, adipose and pancreatic derived substrates. These can be subdivided into those that regulate energy intake and energy metabolism on an acute or chronic basis (Murphy & Bloom 2006). Specifically, over the long-term, insulin and leptin suppress appetite and increase energy expenditure. Whilst, gut hormones including ghrelin, peptide-YY (PYY) and glucagon-like peptide-1 (GLP-1), regulate energy intake on a meal-to-meal basis. Ghrelin, is appropriately dubbed the 'hunger hormone', a unique peptide responsible for appetite stimulation and is

consequently thought to initiate a bout of eating. Conversely, PYY and GLP-1 are satiety hormones, concentrations rise in response to feeding, causing an individual to feel full and stop eating. These physiological mechanisms responsible for controlling appetite are dysfunctional in obese individuals, increasing susceptibility to over-eating and generation of a positive energy balance. Particularly, circulating concentrations of acylated ghrelin are not as effectively suppressed after meals and postprandial concentrations of PYY and GLP-1 are attenuated in comparison to lean individuals (Batterham et al. 2006; le Roux et al. 2006; Morpurgo et al. 2003). The feelings of reduced fullness and satisfaction associated with these hormone patterns, promotes food consumption and consequently the generation of a positive energy balance in this population.

These findings initiated research into drug treatments that could imitate the body's own regulatory signals and pathways (Field et al. 2009). To date, these have been largely unsuccessful due to efficacy and safety concerns. The only safe obesity drug recognised and prescribed in England, Orlistat, had a net cost of £15.3 million in 2014 (Baker & Bate 2016). Clearly, this outgoing cost is not sustainable, and as obesity is largely preventable, the identification of a more natural, effective and cheaper resolution to the overweight and obesity epidemic would be favourable. As a result, the government has begun to implement initiatives to encourage healthy food choices and regular physical activity. Current UK physical activity guidelines state individuals should aim to complete 150 min of moderate intensity exercise (Department of Health 2011). However, despite the government's best efforts, only 67% of men and 55% of women were reported to meet these guidelines in 2012 (Health Survey for England, 2012). The ACSM position stand furthers these guidelines, suggesting that 150 min of moderate intensity exercise is only enough to prevent weight gain (Donnelly et al. 2009). To help achieve modest weight loss, the position stand recommends that individuals complete 150-250 min of moderate intensity exercise per week. It is suggested that further increasing activity (>250 min per week) can result in clinically significant weight loss.

On the other hand, an energy deficit to help induce weight loss can also be achieved by energy restriction (i.e. dieting). "Fad" diets have risen in popularity concurrently with the overweight and obesity epidemic. In particular, low calorie, low carbohydrate, low fat and high protein diets have all received attention in recent years. Indeed, it has been reported that following such diets can lead to weight loss in the short term (Dansinger et al. 2005). However, it is often reported that dieting alone is unsuccessful in the long run, with weight

regain occurring in the majority of individuals (Lowe 2015). Conversely, others regard increased physical activity as the most valuable behaviour change to prevent and manage overweight and obesity. The associated health benefits of exercise (i.e. reduced risk of developing cardiovascular disease, type 2 diabetes, dementia and some cancers (King et al. 2008; Shaw et al. 2006)) has led to public health authorities advising the population to increase physical activity. Exercise training studies have shown that by increasing physical activity, weight loss can be achieved (Garrow & Summerbell 1995; Shaw et al. 2006; Wing 1999). However, in many cases the degree of weight loss achieved with exercise is less than predicted, perhaps a result of compensatory appetite responses (Church et al. 2009; King et al. 2008). Total weight loss is often used as an indicator of weight management; however, this can be misleading. For example, during an exercise training programme it might be expected for fat mass to be reduced and fat free mass to be increased. Consequently, total body weight may remain stable or even increase. However, in caloric restriction both fat and fat free mass could be reduced, showing a drop in total weight. In this example, using total weight as a measure of effectiveness would suggest that exercise training is less effective for weight management. Instead, body composition measures could be more informative as an indicator for weight management. It is likely that both diet and exercise need to be considered to accomplish and sustain successful weight loss and management. Public health authorities and the advertising industry should work hand in hand to help individuals make lifestyle changes towards 'healthier choices', particularly, choosing low sugar options and increasing physical activity.

The emphasis on increasing physical activity to help prevent weight gain and induce weight loss has sparked research into exploring the mechanisms controlling appetite, energy balance and exercise. To date, training studies lasting 4-12 months have found exercise to be unsuccessful for long-term weight loss (Hopkins et al. 2014; King et al. 2007; Wing 1999). This was explained by compensatory increases in appetite and food intake. On the contrary, compensatory responses are not seen after acute exercise, with no influence on energy intake (Schubert et al. 2013). It is possible that the compensatory responses of subjective appetite sensations and *ad libitum* energy intake to exercise occur over a longer duration than previously examined in acute laboratory studies i.e. over more than 24 h. However, extending this observation period up to 34 h, has still failed to show any alterations in *ad libitum* energy intake despite latent increases in hunger and motivation to eat after exercise (Beaulieu et al.

2015; Heden et al. 2013). Further research is required to extend upon these initial findings, to ascertain when compensation begins and to better understand the regulatory mechanisms.

With this is mind, the importance of exercise, appetite and weight management is not restricted to those who are overweight or obese. This also bears great significance to elite athletes seeking optimal body weight for performance. Matching an athlete's energy needs will not only help to maintain body weight required for competition, but also optimise training effects. Where energy intake is not adequate, muscle mass may be reduced, sensations of fatigue increased and as a result performance can suffer (Brouns 1992). It is therefore important to understand how the appetite regulatory system responds to an elite athlete's training regime, in particular repeated bouts of intense exercise, so that energy intake and body composition can be tightly regulated.

Subjective appetite perceptions, appetite regulatory hormone and *ad libitum* energy intake responses to acute exercise have mainly been examined in lean active males. Considering the implications that appetite responses to exercise have on the wider population, especially in individuals who are overweight and obese, research has begun to broaden to determine if the favourable effects of acute exercise on appetite translate into those who suffer from impaired appetite regulation. The findings in these initial studies have been divided, and further study is required to determine the efficacy of exercise in appetite regulation in this population.

Similarly, there is little work examining the responses of appetite perceptions, appetite regulatory hormone and *ad libitum* energy intake in females. This is likely due to the difficulties associated with controlling for the menstrual cycle. In particular, changes in the concentration of progesterone and estradiol could lead to females increasing their energy intake and/or energy expenditure during certain phases of the menstrual cycle. If sex-based differences in appetite regulatory responses to exercise were found, the prescription of physical activity and dietary based recommendations given by health authorities would need to be adapted to ensure the efficacy of weight loss and maintenance regimes.

Therefore, the aims of this thesis are threefold;

- To investigate the effect of a single bout of exercise on appetite regulatory hormone responses in lean vs. overweight/obese (ow/ob) males and females
- To investigate the effect of a single bout of exercise on subjective appetite perceptions and *ad libitum* energy intake responses in lean vs. ow/ob males and females

- To further examine where compensatory appetite regulatory hormone, subjective appetite perceptions and *ad libitum* energy intake responses begin after a bout of acute exercise.

It was hypothesised that in the hours after a bout of acute treadmill exercise, males and females who are lean and ow/ob would express similar appetite perceptions, energy intake and appetite regulatory responses. It was also hypothesised that two consecutive days of treadmill running, in lean healthy males, would generate a compensatory increase in appetite perceptions, energy intake and acylated ghrelin on the second day of exercise. Simultaneous compensatory decreases in circulating PYY and leptin would also be seen.

# CHAPTER II

# Literature Review

## 2.1. Introduction

This literature review will discuss the effects of acute exercise on subjective appetite perceptions, energy intake and appetite regulatory hormones in both lean and ow/ob individuals. The review will initially describe the components of energy balance and look at the gastrointestinal regulation of appetite and energy intake. Particular attention will be given to ghrelin, PYY, GLP-1 and leptin due to the significant attention they have received for their role in the hormonal regulation of appetite. Thereafter, the acute effects of exercise on subjective appetite perceptions, energy intake and appetite regulatory hormones in lean and ow/ob individuals will be examined.

## 2.2. Energy balance

Energy balance describes the relationship between energy intake and energy expenditure. The human body conforms to the first law of thermodynamics, which states that energy cannot be created or destroyed. When a positive imbalance exists between energy intake and utilisation, excess energy can be stored. On the other hand, when a negative imbalance exists between energy intake and utilisation, energy stores will be utilised. If either is sustained over time, this will lead to an alteration in body composition. To help recognise what is required to lose or maintain weight, it is important to understand the components of energy balance.

Energy intake describes the chemical energy consumed from food and beverages, specifically, carbohydrate, fat, protein and to a lesser extent alcohol (Hall et al. 2012). The energy densities of these macronutrients are 4 $kcal \cdot g^{-1}$ (17 $kJ \cdot g^{-1}$) for carbohydrate and protein, and 9 $kcal \cdot g^{-1}$ (38 $kJ \cdot g^{-1}$) for fat (Hall et al. 2012). This energy is used to fuel metabolic processes, but when consumed in excess of that required will be stored.

Rate of energy expenditure is highly variable, showing fluctuations across 24 h periods, and across a lifetime. Total energy expenditure is made up of resting energy expenditure (REE), dietary induced thermogenesis (DIT) and activity energy expenditure (AEE). REE is the largest component of energy expenditure, and accounts for approximately two-thirds of total energy expenditure (Hall et al. 2012). Specifically, this describes the energy required to keep the body functioning at rest, and includes processes such as breathing, control of body temperature and cell growth. There are huge variations in REE between individuals, dictated by body size and composition (Johnstone et al. 2005). On the other hand, DIT is the smallest contributor to total energy expenditure. This is the energy used to digest and absorb food and beverages. Again, DIT is varied between and within individuals. The macronutrient composition of ingested food and drink impacts the degree of energy required to digest and absorb these. Specifically, for isocaloric amounts of protein, carbohydrate and fat, protein utilises the most energy whilst fat utilises the least. Finally, AEE describes the energy required for physical activity, and is determined by body movement and body size. AEE is largely determined by an individuals want or wish to exercise, and consequently varies largely between individuals.

When an individual consumes more energy than that required for these obligatory processes and AEE, over time, obesity develops. Conversely, to induce weight loss, a negative energy balance must be maintained.

## 2.3. Gastrointestinal regulation of appetite and energy intake

### 2.3.1. Ghrelin

#### 2.3.1.1. Discovery

In 1999, Kojima and colleagues identified ghrelin as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). The name ghrelin is derived from 'ghre', the Proto-Indo-European root of the word 'grow', characterising its role in the release of growth hormone.

#### 2.3.1.2. Structure, production and regulation of secretion

Ghrelin is a 28 amino acid peptide, formed by the cleavage of preproghrelin, a larger precursor molecule (Kojima et al. 1999). Ghrelin is predominantly synthesised in the enteroendocrine X/A cells or "ghrelin cells" found in the mucosal layer of the stomach (Kojima & Kangawa 2005). These cells are also found within the intestine, but at lower concentrations to that in the stomach, with their presence continuing to decrease from the duodenum to the colon (Ariyasu et al. 2001; Kojima & Kangawa 2005; Peeters 2005).

There are two forms of ghrelin found within the circulation; acylated and desacylated. In its acylated form, the serine 3 residue is n-octanoylated. Here octanic acid is bound to the third amino acid residue (serine) via an ester bond (Figure 2.1 (Sato et al. 2012)). This process is catalysed by the enzyme ghrelin O-acyltransferase (GOAT), and allows ghrelin to cross the blood brain barrier and act on receptors in the brain (Yang et al. 2008). In particular, acylated ghrelin binds to and activates the growth hormone secretagogue receptor (GHSR-1a) stimulating hormone secretion, anabolic effects, gastric and cardiovascular function (Kojima & Kangawa 2005). The n-octanolyation at serine 3 is essential for ghrelin to bind to GHSR-1a and to activate this receptor (Kojima et al. 1999). For this reason, desacylated ghrelin was initially considered to be inactive. However, recent evidence suggests desacylated ghrelin can increase insulin sensitivity, improve glucose metabolism and inhibit lipid metabolism in humans (Delhanty et al. 2012). In some cases, desacylated ghrelin has been shown to antagonise the effects of acylated ghrelin (Broglio et al. 2004).

In human plasma, normal concentrations of acylated ghrelin are 34-67 $pg \cdot mL^{-1}$ and 337-506 $pg \cdot mL^{-1}$ for total ghrelin (acylated and desacylated ghrelin (Kojima & Kangawa 2005)). It is thought that ~90% of ghrelin in the circulation is desacylated because; there is a high

ghrelin to GOAT ratio within ghrelin secreting cells (Yang et al. 2008), and esterases present in the circulation breakdown the ester bond needed for acylation (Satou et al. 2010). The disproportional concentrations of acylated and desacylated ghrelin in the circulation could mean measuring total ghrelin may mask any effects caused by the acylated form.

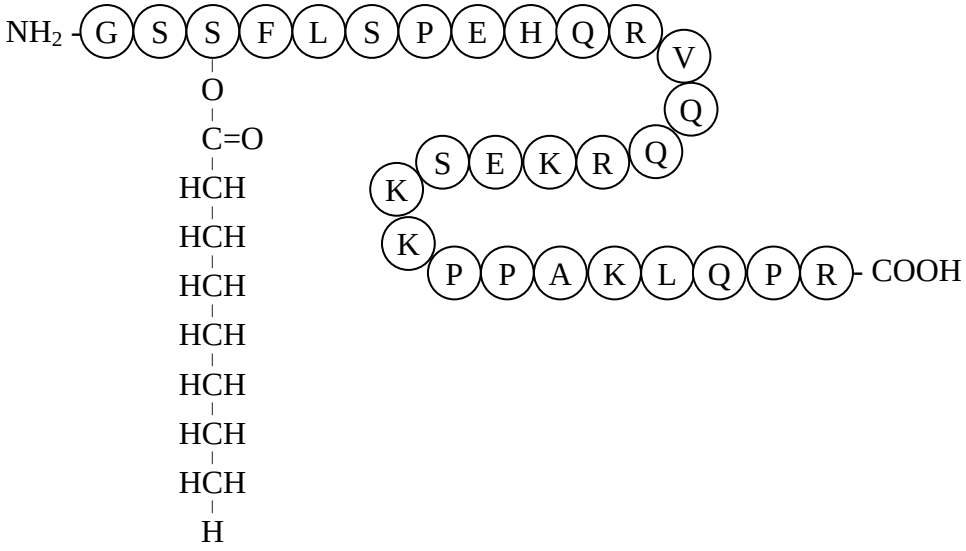


**Figure 2.1.** The structure of acylated ghrelin. Adapted from Sato et al. 2012.

Feeding is considered the most important factor in the regulation of ghrelin secretion. Plasma concentrations rise pre-prandially and fall after a meal (Cummings et al. 2001). This diurnal pattern and correlation with sensations of hunger has led to the suggestion that ghrelin plays a role in meal initiation (Cummings et al. 2001; Cummings et al. 2004).

The mechanisms behind the release of ghrelin in response to meals are unclear (Hosoda et al. 2006; Yin et al. 2009). Nutrient sensing appears to play a role, as the calorie and macronutrient content of a meal influences ghrelin secretion into the circulation (Callahan et al. 2004; Koliaki et al. 2010; Leidy & Williams 2006). Carbohydrates cause the highest degree of ghrelin suppression, with fats and protein being less effective (Erdmann et al. 2004; Monteleone et al. 2003). Despite this, protein is considered to be the most satiating macronutrient, as it generates a prolonged suppression of ghrelin in the postprandial state compared to balanced and high fat meals (Al Awar et al. 2005). Prolonging ghrelin suppression could increase time between meals by sustaining lower sensations of hunger and consequently reduce energy intake. Lipids appear to be the least effective macronutrient at postprandial ghrelin suppression, with consumption of high fat meals causing smaller

decreases in post-prandial ghrelin concentrations compared to high carbohydrate or high protein meals (Foster-Schubert et al. 2008; Monteleone et al. 2003; Tentolouris et al. 2004).

Several mechanisms have been proposed to explain why macronutrients differ in their effectiveness at suppressing ghrelin after a meal. Foster-Schubert and colleagues (2008) suggested that the rapid decreases seen in acylated ghrelin concentrations after high carbohydrate feeding could be caused by the fast gastric emptying rates associated with these meals. Furthermore, the shorter lived suppression of acylated ghrelin caused by carbohydrates could be due to the rapid breakdown and reabsorption of these molecules. Insulin may also contribute to the ghrelin response (Foster-Schubert et al. 2008; Yin et al. 2009). The pattern of ghrelin secretion is reciprocal to that of insulin, which is seen to be low before a meal and rises postprandially (Cummings et al. 2001). The poor ability for lipids to stimulate insulin secretion could explain the attenuated ghrelin profile in response to fat consumption (Foster-Schubert et al. 2008). The effects of gastric distension on circulating ghrelin concentrations have been dismissed. Comparing water ingestion to oral glucose administration showed that water alone could not alter ghrelin concentrations. This implies that mechanical distension of the stomach does not stimulate ghrelin release (Sato et al. 2012; Shiiya et al. 2002).

#### 2.3.1.3. Physiological functions and mechanisms of action

Ghrelin is commonly known for its unique characteristic as an orexigenic hormone, stimulating appetite when circulating concentrations are high (Druce et al. 2006; Wren 2001a). However, ghrelin has many other physiological functions that affect both the central and peripheral systems (Figure 2.2). In particular, ghrelin influences hormone secretion, anabolic reactions, gastric and cardiovascular function. Ghrelin is responsible for stimulating the release of growth hormone from the anterior pituitary by binding to GHRS-1a (Kojima et al. 1999). Ghrelin has also been shown to increase glucose concentrations and decrease insulin concentrations within the blood (Broglio 2001; Delhanty & van der Lely 2011). Anabolic effects include stimulating adiposity and increasing appetite (Wynne et al. 2005b).

Cardiovascular functions include increasing cardiac index and stroke volume, raising cardiac output, and decreasing mean and diastolic blood pressure (Hosoda et al. 2006; Nagaya et al. 2001). In addition, ghrelin alters gastric functions by increasing gastric acid secretion and stimulating gastric mobility and gastric emptying (Hosoda et al. 2006; Levin et al. 2006).

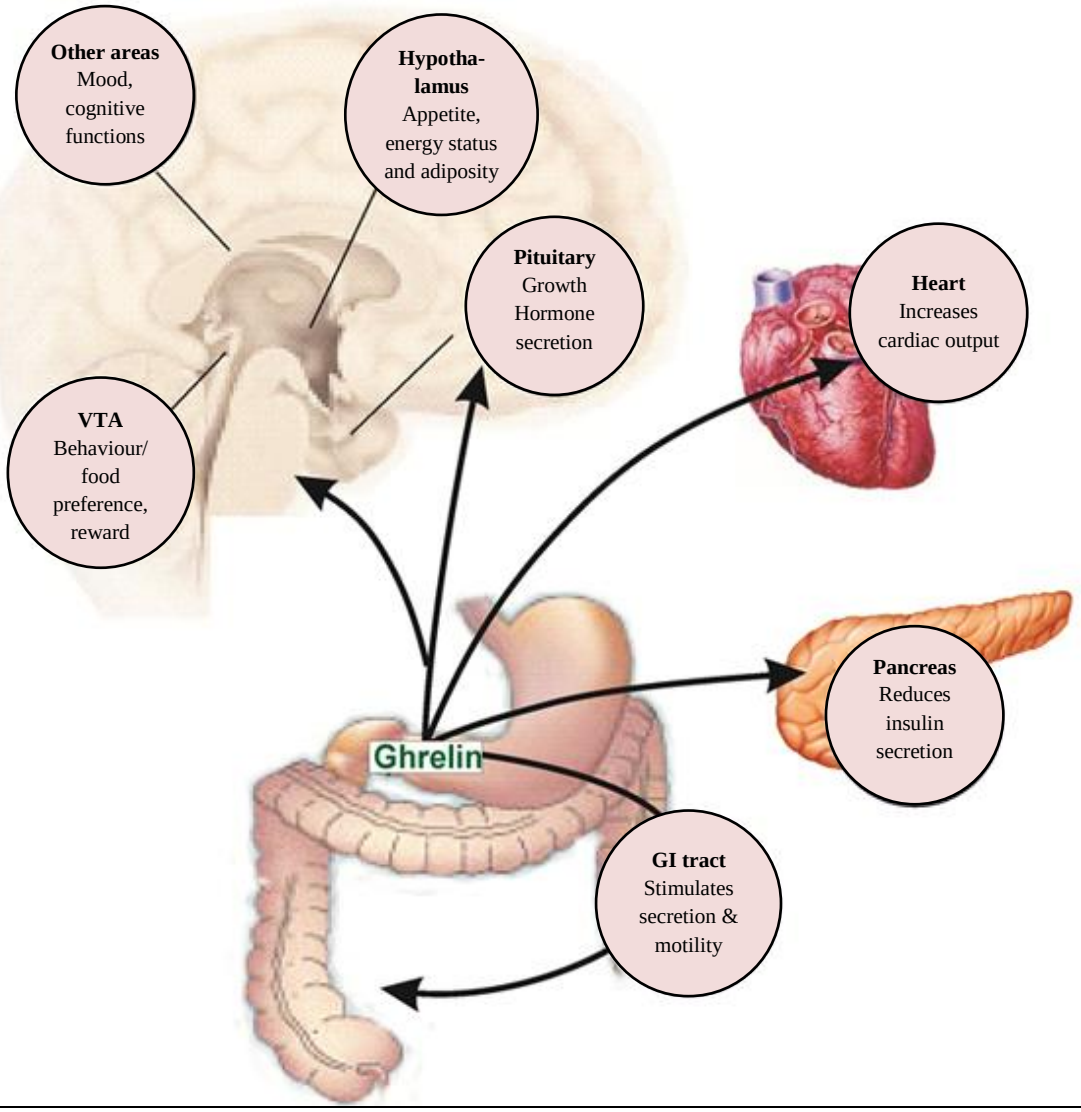


**Figure 2.2.** Physiological functions of ghrelin (adapted from Davenport et al. 2015). VTA, ventral tegmental area; GI tract, gastrointestinal tract.

Centrally, ghrelin is found within the hypothalamus (Kojima & Kangawa 2005). In particular ghrelin is found within the arcuate nucleus (ARC) (Kojima et al. 1999), a region of the brain responsible for appetite control. Ghrelin causes its orexigenic effects by stimulating neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons in the hypothalamus. This causes the release of NPY and AgRP which increases appetite (Sato et al. 2012).

#### 2.3.1.4. Acute energy regulation

Ghrelin infusion studies have been conducted in both rodents and humans in an attempt to understand its role in stimulating appetite. Intracerebroventricular and intraperitoneal infusion of ghrelin in rats caused dose dependent increases in food intake, weight gain and adiposity (Nakazato et al. 2001; Shintani et al. 2001; Tschöp et al. 2000; Wren et al. 2000; Wren et al.

2001b). Decreased fat utilisation was also observed, potentially contributing to the increase in adiposity (Tschöp et al. 2000). Similarly, ghrelin infusion in humans, sufficient to generate supra-physiological concentrations of circulating ghrelin, increased hunger and energy intake in a dose dependent manner (Akamizu et al. 2004; Druce et al. 2006; Huda et al. 2009; Neary et al. 2004; Wren 2001a; Wynne et al. 2005b). In normal weight males and females intravenous infusion of ghrelin (5 pmol·kg·min$^{-1}$, causing supra-physiological concentrations) for 75 min increased energy intake by 20% at an *ad libitum* buffet meal whereas obese individuals showed an increase of 70% (Druce et al. 2005). This suggests ghrelin is an appetite stimulator and ghrelin antagonists may be useful in therapeutic treatments for obesity.

#### 2.3.1.5. Chronic energy regulation

Ghrelin also seems to influence long-term body weight. In rats, repeated intracerebroventricular infusion of ghrelin causes hyperphagia and adiposity (Tschöp et al. 2000; Wren et al. 2001b). Whereas, ghrelin deficient rats show a slower rate of weight gain when fed a high fat diet compared to those administered ghrelin (Wortley et al. 2005). Similarly, GHS-R null mice are resistant to diet induced obesity (Zigman et al. 2005).

In humans, circulating ghrelin concentrations are negatively correlated with BMI (Shiiya et al. 2002). Obese individuals express lower mean total plasma ghrelin and acylated ghrelin concentrations compared to their lean counterparts (Huda et al. 2009; Marzullo et al. 2008; Shiiya et al. 2002; Tschöp et al. 2001; Vendrell et al. 2004), whilst those with anorexia nervosa and bulimia nervosa show significantly higher circulating ghrelin concentrations (Otto et al. 2001; Shiiya et al. 2002; Tanaka et al. 2002; Tolle et al. 2003). Together these findings support the notion of ghrelin playing a role in chronic energy regulation, however it cannot be assumed that ghrelin is a mechanism for weight gain. In fact, ghrelin may make weight loss and its maintenance more difficult. After both diet and exercise induced weight loss, overweight and obese individuals have shown increased ghrelin concentrations (Cummings et al. 2002; Foster-Schubert et al. 2005; Hansen et al. 2002; Leidy et al. 2004), a finding associated with increased hunger and energy intake.

#### 2.3.1.6. Differences between lean and obese individuals

Obese individuals express lower basal concentrations of circulating total and acylated ghrelin in comparison to their lean counterparts (le Roux et al. 2005; Marzullo et al. 2008; Shiiya et al. 2002; Tschöp et al. 2001). It is important to understand ghrelin's sensitivity to body size

as it could be a factor in the pathogenesis of obesity. It has been suggested that obese individuals exhibit lower basal concentrations of ghrelin as a compensatory response to prevent further weight gain (Koliaki et al. 2010). As obesity is associated with insulin resistance and hyperinsulinemia, it could be this that causes the reduced circulating concentrations of ghrelin in obese individuals (McLaughlin et al. 2004; Tschöp et al. 2001).

In obese individuals, the suppression of ghrelin after a meal is blunted (le Roux et al. 2005; Tentolouris et al. 2004). It is possible that this contributes to positive energy balance; failing to suppress appetite after a meal could consequently encourage further energy intake (Koliaki et al. 2010). Although expressing reduced circulating ghrelin concentrations, Druce and colleagues (2005) found obese individuals to remain sensitive to the effects of ghrelin. Both low (1 $pmol \cdot kg \cdot min^{-1}$) and high (5 $pmol \cdot kg \cdot min^{-1}$) dose ghrelin infusion caused increased energy intake in obese individuals. This implies that ghrelin receptor antagonists could be useful appetite reducing agents.

#### 2.3.1.7. Ghrelin as a remedy for obesity?

Surgery is an effective method for the treatment of obesity; however, it is very impractical to implement on a large scale. Consequently, circulating hormones identified in the regulation of appetite have been targeted for the development of anti-obesity treatments. There was doubt over the effectiveness of targeting ghrelin in the treatment of obesity as obese individuals already express lower circulating concentrations of ghrelin than lean individuals. However, as obese individuals remain sensitive to the effects of ghrelin (Druce et al. 2005), research into ghrelin as a therapy continues.

Several approaches have been used to target the ghrelin system to ameliorate obesity; antagonizing the ghrelin receptor, neutralizing circulating ghrelin and preventing production of ghrelin. It was hoped pre-prandial hunger could be suppressed by antagonizing ghrelin receptors (GHS-R1a) causing decreased circulating ghrelin concentrations. In obese rats, this treatment reduced body weight and improved glucose tolerance (Esler et al. 2007; Rudolph et al. 2007). However, no clinical trial has shown these to be successful as an obesity treatment in humans (Patterson et al. 2011). This would suggest that although ghrelin receptor antagonists are effective in rodent models, these have failed at later stages of testing/development.

By ‘neutralizing’ circulating ghrelin it was hoped the strength of the hunger signal could be reduced, restricting ghrelin from reaching and acting on receptors present in the brain (Patterson et al. 2011). In rats, this was achieved through an ‘anti-ghrelin’ vaccine, which initiated the development of antibodies against ghrelin (Zorrilla et al. 2006). Over 12 weeks of vaccination, the rats showed reduced weight gain, resulting from decreased feeding efficiency (body weight gained per unit energy intake). Again, when this vaccine was trialled in humans it was found to be ineffective (Cytos, 2006).

Ghrelin needs to be acylated at the serine 3 residue in order for it to bind to its receptor, GHS-R1a. GOAT is responsible for the acylation process (Gutierrez et al. 2008; Yang et al. 2008). In rats, GOAT inhibitors prevented acylated ghrelin production. When administered for a month, the rats showed reduced weight gain and fat mass in response to a high fat diet. Acute administration resulted in improved glucose tolerance and increased glucose stimulated insulin release. The rats used in this study were normal weight; consequently, these findings cannot be generalized to obese rats. The effectiveness of this treatment could also be limited by the already suppressed ghrelin concentrations observed in obese individuals.

To date, results in humans have been discouraging (Cytos, 2006; Patterson et al. 2011). However, if a successful GOAT inhibitor can be replicated in humans, it could prove useful for treatment of obesity and type 2 diabetes.

### 2.3.2. PYY

#### 2.3.2.1. Discovery

Peptide-YY was first isolated from upper small intestinal porcine tissues in 1980 (Tatemoto & Mutt 1980). PYY was given its name due to the presence of tyrosine (Y) residues at both ends of the molecule (Tatemoto 1982).

#### 2.3.2.2. Structure, production and regulation of secretion

Peptide-YY is a 36 amino acid peptide, found in two forms in the circulation; $PYY_{1-36}$ and $PYY_{3-36}$. $PYY_{3-36}$ is a truncated, 34 amino acid peptide produced from the cleavage of tyrosine and proline at the N terminal of $PYY_{1-36}$. This reaction is catalysed by the enzyme dipeptidyl peptidase IV (DPP-IV (Mentlein et al. 1993)). The majority of PYY found in the circulation is $PYY_{3-36}$ (Batterham et al. 2006), which is synthesised and released from endocrine L cells (Batterham et al. 2002; le Roux & Bloom 2005). L cells are present throughout the gut but are most abundant in the distal segments of the intestine, in particular the ilium and colon (Adrian et al. 1985). The surface of the L cell is partly exposed to the lumen of the intestine, allowing it to sense nutrients and other substances in the intestine. L cells are responsible for the secretion of both PYY and GLP-1, and circulating concentrations of these increase almost immediately after eating. The secretion of these hormones occurs before the nutrients will have reached the ilium, indicating that nutrient sensing by L cells is not the only mechanism to cause secretion of these hormones (le Roux & Bloom 2005).

The structure of PYY is altered when the N terminal is truncated. Consequently $PYY_{3-36}$ is only capable of binding to Y2 receptors whereas $PYY_{1-36}$ can bind to all forms of the Y receptor (Karra et al. 2009). Consequently, $PYY_{3-36}$ has a higher affinity for binding to Y2 receptors; this difference in receptor preference is thought to be necessary for modulating its function.

#### 2.3.2.3. Physiological functions and mechanisms of action

Peptide-YY acts as a satiety hormone; its release suppresses appetite after a meal and postpones the initiation of the next meal (Neary & Batterham 2009). In rodents, the infusion of PYY has been found to decrease food intake and body weight (Batterham et al. 2002; Challis et al. 2003; Chelikani et al. 2005; Martin et al. 2004; Pittner et al. 2004). Similarly, in humans a 90 min infusion of PYY (0.8 $pmol \cdot kg \cdot min^{-1}$ and 2 $nmol \cdot kg^{-1}$) caused reduced

hunger and food intake, further implicating its role as a satiety hormone (Batterham et al. 2002; Batterham et al. 2003).

As well as acting as a satiety hormone, PYY also influences energy expenditure and fuel partitioning. In rodents, chronic administration of $PYY_{3-36}$ resulted in a greater rate of fat oxidation (Adam & Westerterp-Plantenga 2004; van den Hoek et al. 2007). Similarly, in humans, peripheral administration of $PYY_{3-36}$, adequate to generate supra-physiological circulating concentrations of $PYY_{3-36}$, increased energy expenditure and fat oxidation (Sloth et al. 2007). Further to this, a negative correlation has been found between fasting PYY concentrations and 24 h respiratory quotient (Guo et al. 2006). Together these findings suggest that PYY could help regulate and induce weight loss by reducing food intake and increasing energy expenditure.

Peptide-$YY_{3-36}$ causes its satiety functions by acting on Y2 receptors in the ARC (Batterham et al. 2002). In particular, $PYY_{3-36}$ modulates neuronal activity within the hypothalamus, brainstem, mid brain and areas of the brain related with reward and processing (Batterham et al. 2007). PYY is also thought to promote satiety through the 'ileal brake reflex' (Figure 2.3), whereby proximal intestine and gastric motor activity are inhibited slowing large amounts of nutrients reaching the distal intestine (Hussain & Bloom 2013).

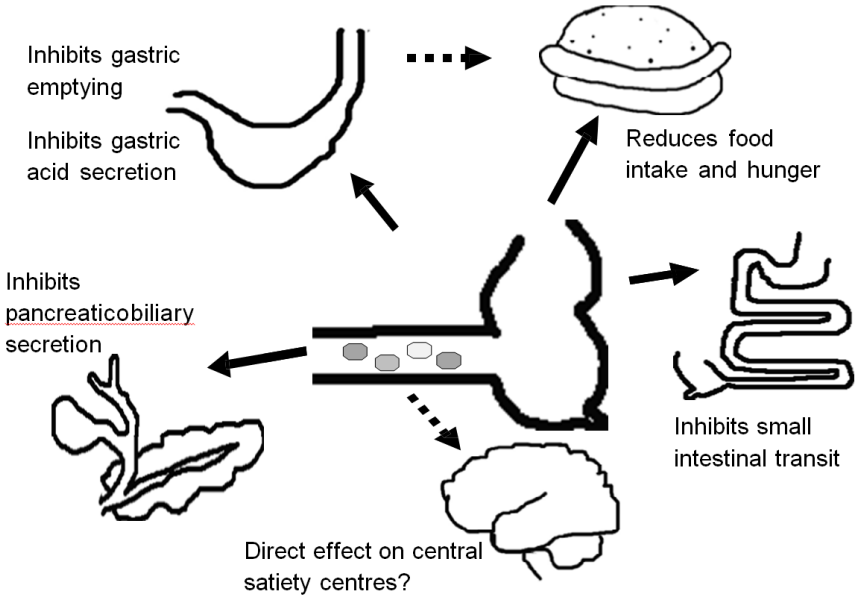


**Figure 2.3.** A diagram to show how the ileal brake mechanism influences hunger, food intake and the gastrointestinal tract. Adapted from Maljaars 2007.

Peripheral infusion of PYY has been shown to delay gastric emptying and inhibit secretions from the stomach, pancreas and gall bladder (Adrian et al. 1986). In addition to these effects on digestive function, PYY plays a role in vasoconstriction, with PYY infusion increasing both systolic and diastolic blood pressure (Adrian et al. 1986).

#### 2.3.2.4. Acute energy regulation

Peptide-YY concentrations are decreased pre-prandially and are shown to increase after feeding, suggesting it has a role in satiety signalling (Karra et al. 2009). After a meal, plasma concentrations of PYY increase within 15 min and peak at approximately 60 min, staying elevated for 6 h (Adrian et al. 1985; Batterham & Bloom 2003). The amount of PYY released from the gut, and the peak concentration after a meal is proportional to calorie intake and macronutrient content of the meal (Adrian et al. 1985). Specifically, in humans, high protein meals are associated with larger postprandial increases in PYY (Batterham et al. 2006), potentially distinguishing PYY's role in making protein a highly satiating macronutrient.

The anoeretic effects of PYY were confirmed using infusion studies to induce physiological (Batterham et al. 2002) and supra-physiological (Batterham et al. 2003; Degen et al. 2005) concentrations in humans. When normal weight males and females were infused with $PYY_{3-36}$ for 90 min (0.8 pmol·kg·min$^{-1}$) *ad libitum* energy intake was suppressed 2 h after the infusion and this was maintained for a total of 12 h (Batterham et al. 2002).

#### 2.3.2.5. Chronic energy regulation

In humans, there is a negative correlation between circulating concentrations of PYY and markers of adiposity, including BMI, body fat percentage and waist circumference (Batterham et al. 2003; Guo et al. 2006; le Roux et al. 2006). This suggests that PYY could play a role in the long-term regulation of energy balance, consequently influencing body weight.

In rodents, the administration of $PYY_{3-36}$ can attenuate weight gain (Batterham et al. 2002; Pittner et al. 2004). Furthermore, the absence of PYY in rodents can cause hyerphagia and obesity (Batterham et al. 2006). Here, PYY coding was deleted and daily cumulative food intake increased. This resulted in an increase of body fat and leptin levels. After weight gain, the PYY null mice were administered $PYY_{3-36}$. These mice were hypersensitive to the

anorexigenic effects of $PYY_{3-36}$, and obesity within these rats was reversed. The findings here suggest obesity was caused by a loss of PYY, implicating its role in chronic energy regulation.

It has also been suggested that PYY may beneficially affect metabolism by altering energy expenditure, thermogenesis and fuel partitioning (Boey et al. 2008; Guo et al. 2006; Sloth et al. 2007). Rodent studies have demonstrated that chronic administration of $PYY_{3-36}$ results in a favoured fat oxidation, and that those that overexpress PYY have increased basal temperatures (Adams et al. 2006; van den Hoek et al. 2007). Similarly, in humans it has been shown that the peripheral administration of $PYY_{3-36}$ leads to increased energy expenditure and fat oxidation, with circulating postprandial PYY concentrations being associated with postprandial energy expenditure and thermic effects of food (Doucet et al. 2008; Sloth et al. 2007). Together these findings suggest that PYY plays a role in the long-term regulation of body weight by reducing energy intake and by increasing energy expenditure.

#### 2.3.2.6. Differences between lean and obese individuals

Obese individuals exhibit lower fasting concentrations of PYY (Batterham et al. 2003; le Roux et al. 2006). Furthermore, fasting concentrations of PYY are found to correlate negatively with BMI; higher BMI's are associated with lower concentrations of PYY (Batterham et al. 2003).

Although normal weight and obese individuals express differing basal concentrations of PYY, similar decreases in energy intake and appetite following an infusion of PYY to generate supra-physiological concentrations (2 $nmol \cdot kg^{-2}$) have been observed (Batterham et al. 2003). This demonstrates that obese individuals are not resistant to the effects of PYY. However, despite consuming more calories than their lean counterparts, obese individuals expressed lower levels of PYY after an *ad libitum* meal (Batterham et al. 2003). This suggests that obese individuals are deficient in circulating concentrations of PYY and hence circulating PYY may be involved in the pathogenesis of obesity.

#### 2.3.2.7. PYY as a remedy for obesity?

Finding that obese individuals are sensitive to the effects of PYY, implicated its role as a potential remedy for obesity and weight management. Peptide hormones cannot be administered orally due to degradation within the stomach. Consequently, a PYY nasal spray was developed and administered 20 min before breakfast, lunch and dinner. Over 12 weeks,

obese individuals used either a placebo, 200 µg $PYY_{3-36}$ or 600 µg $PYY_{3-36}$ nasal sprays whilst consuming a hypocaloric diet and exercise regime (Gantz et al. 2007). The spray was not efficacious with 59% of individuals in the 600 µg $PYY_{3-36}$ nasal spray group dropping out of the study due to feelings of nausea and vomiting. It was suggested that these feelings were a result of sharp rises in plasma PYY levels caused by the nasal spray. For PYY to be used as an effective therapy, it will be necessary to deliver stable levels of the hormone that avoid sharp peaks (Troke et al. 2014). In particular, it has been suggested that manipulating the release of PYY through alterations in diet could be effective for treating obesity (Batterham et al. 2006).

### 2.3.3. GLP-1

#### 2.3.3.1. Discovery

The discovery of GLP-1 was initiated after the development of glucagon immunoassays (Unger et al. 1961). This highlighted the presence of glucagon like substances in the gastrointestinal tract after an oral glucose load (Unger et al. 1968), later determined as GLP-1 and GLP-2 (Bell et al. 1983).

#### 2.3.3.2. Structure, production, and regulation of secretion

Glucagon-like peptide-1 is cleaved from preproglucagon within the L cells of the intestinal mucosa (Donnelly 2012). GLP-1 is secreted into the circulation in two forms; GLP-$1_{1-37}$ and GLP-$1_{1-36}$ (Lockie 2013; Kieffer & Habener 1999; Neary & Batterham 2009). GLP-$1_{1-36}$ is the major form found in the blood (Neary & Batterham 2009), and is the form considered to be active. When in the circulation GLP-1 is rapidly broken down by DPP-IV into the inactive forms GLP-$1_{9-36}$ and GLP-$1_{9-37}$ (Lockie 2013). Due to the rapid break down of active GLP-1, most of the GLP-1 that leaves the gut is inactive, with only ~10-15% of the newly secreted GLP-1 being active in the circulation (Holst 2007). The secretion of GLP-1 is dictated by food consumption. During fasting, plasma concentrations of GLP-1 are very low, and these are seen to increase rapidly within 10 min of consuming a meal. The amount of GLP-1 released into the circulation is dependent on the size of the meal and the rate of gastric emptying (Kieffer & Habener 1999; Miholic et al. 1991; Vilsbøll et al. 2003). The release of GLP-1 is biphasic, with levels initially rising 10 min after food consumption, peaking after 30 min and remaining elevated for several hours (Orskov et al. 1996). However, the release of GLP-1 from L cells is not solely under the control of nutrients, hormones and neural inputs also play a role. This causes the biphasic pattern in GLP-1 release seen after a meal. The initial rise of GLP-1 in the 10 min after food intake is mediated both hormonally and neurally. Whereas the later secretion of GLP-1, seen 30 min after food consumption, is caused by direct nutrient contact with the L cells in the ileum (Kieffer & Habener 1999). The release of GLP-1 into the circulation stimulates insulin secretion and inhibits glucagon secretion (Field et al. 2009; Holst 2007; Lockie 2013). This allows for increased glucose uptake and glucose clearance, which is important for the regulation of glucose (Lockie 2013). Specifically, GLP-1 is classified as an incretin hormone (Holst 2007) a collection of molecules responsible for amplifying the secretion of insulin.

Fitting with GLP-1's incretin hormone role, carbohydrate ingestion stimulates GLP-1 secretion (Elliott et al. 1993; Herrmann-Rinke et al. 1995). However when glucose is systemically administered no rise in circulating GLP-1 is seen. This in tandem with the observation of increased GLP-1 after glucose infusion into the intestinal lumen suggests that the cells required to detect GLP-1 are distributed on the luminal side of the intestine (Kieffer & Habener 1999). As well as glucose, fat also appears to be partially responsible for the release of GLP-1 and other proglucagon derived peptides. Consuming either mixed fats or triglycerides (TAG) in humans, causes an increase in the secretion of GLP-1. The chain length and degree of saturation of the ingested fats influences GLP-1 secretion, with monounsaturated long chain fatty acids causing a greater secretion than short chain or medium chain polyunsaturated or saturated fatty acids (Kieffer & Habener 1999). When looking at the responses to amino acids and proteins, it has been found that GLP-1 secretion does not increase in humans. However, when mixed meals containing protein are consumed GLP-1 secretion increases (Kieffer & Habener 1999).

#### 2.3.3.3. Physiological functions and mechanisms of action

Glucagon-like peptide-1 has multiple physiological functions in the peripheral tissues (Figure 2.4), mediated through its own distinct receptor. These GLP-1 receptors are concentrated in areas closely related to appetite regulation, in particular on the beta cells of the pancreatic islets, the brain (in particular the hypothalamus), kidney, heart and GI tract (Holst 2007).

As very little active GLP-1 that leaves the L cells reaches the circulation, it has been proposed that GLP-1 may interact with afferent sensory nerve fibres as it crosses the capillary wall to induce its effects (Holst 2007). Specifically, sending impulses to the nucleus of the solitary tract, and onwards to the hypothalamus (Holst 2007).

Glucagon-like peptide-1 is suggested to be one of the most important incretin hormones (Vilsbøll & Holst, 2004). GLP-1 acts on pancreatic β cells to stimulate the release of insulin and inhibit the release of glucagon, in a glucose dependent manner (Field et al. 2009; Holst 2007). GLP-1 may also override the inhibition of insulin secretion by leptin (Kieffer & Habener 1999). This will ensure that an adequate insulin response occurs after a meal. Consequently, the release of GLP-1 from the gut is dependent on the quantity of glucose consumed, and will ensure that plasma glucose concentrations remain constant whatever the size of the glucose load (Holst 2007).

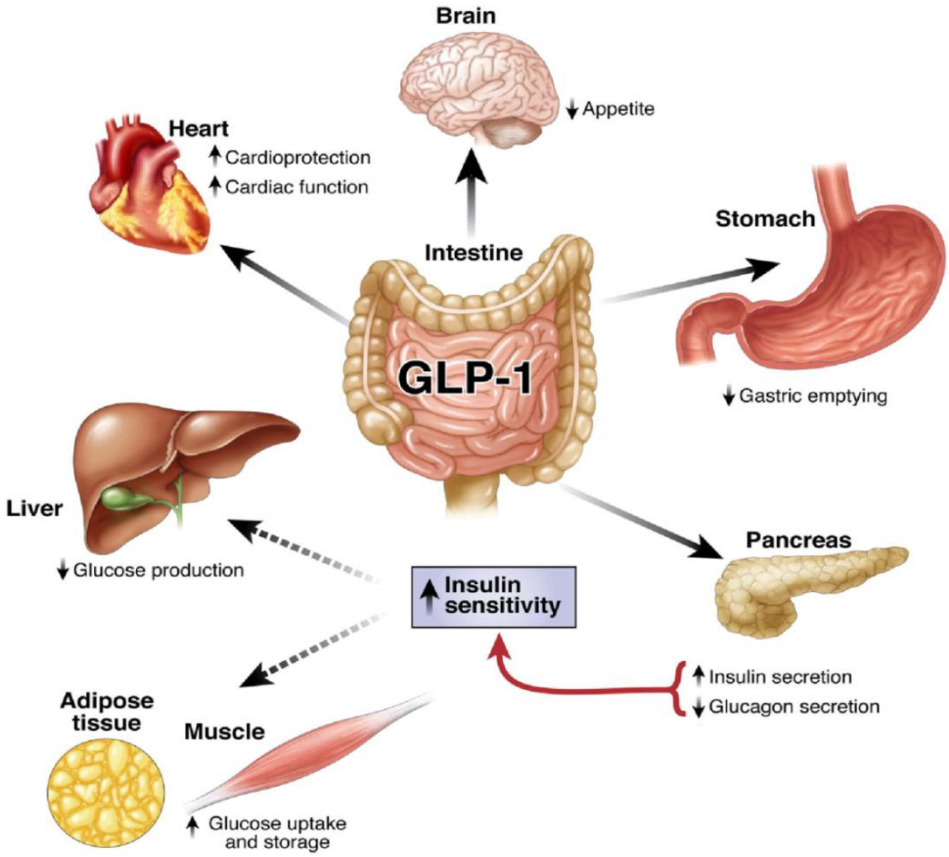


**Figure 2.4.** Physiological actions of GLP-1. Adapted from Baggio & Drucker (2007).

When those with type 2 diabetes are chronically infused with GLP-1, weight loss and improved glycaemic control are observed (Zander et al. 2002). This suggests that GLP-1 could be used in the treatment of type 2 diabetes; however, more investigation is required to determine its long term effects.

Glucagon-like peptide-1 inhibits gastric secretion and motility, gastric emptying and pancreatic secretions (Field et al. 2009; Holst 2007). This suggests that GLP-1, alongside PYY, is responsible for mediating the 'ileal brake' (Holst 2007). Specifically inhibiting upper gastrointestinal functions, due to the presence of unabsorbed nutrients in the ilium (Holst 2007), this effect is thought to be induced via afferent sensory neurons that are responsible for relaying impulses to the brain, in particular the hypothalamus (Holst 2007).

Glucagon-like peptide-1 has been shown to have satiating effects in humans, whereby energy intake at an *ad libitum* meal is reduced after acute intravenous infusion of GLP-1 (Flint et al. 1998). GLP-1 is suggested to mediate this effect by slowing gastric emptying and acting directly on the CNS (Field et al. 2009). Non-digestive effects include the inhibition of heart contractibility in the basal state and enhancing myocardial performance (Holst 2007; Kieffer

& Habener 1999). Additionally, GLP-1 has anabolic effects on the liver, skeletal muscle and fat cells, causing glucose uptake (Kieffer & Habener 1999).

#### 2.3.3.4. Acute energy regulation

After a meal, circulating concentrations of GLP-1 show a biphasic response. Levels rise within 10 min of the meal, and peak at about 30 min, remaining elevated for several hours (Orskov et al. 1996). The magnitude of this response is determined by the nutrients consumed (Holst 2013). This pattern of secretion mirrors that of satiety and for this reason the GLP-1 is thought to have an anoeretic effect.

In rats, the acute administration of GLP-1 by injection results in decreased food intake (Turron et al. 1996). Similarly, a dose dependent reduction in food intake with GLP-1 infusion has been shown in humans (Verdich et al. 2001). Together these further imply GLP-1 has an acute role in energy regulation.

The mechanism for satiety and food intake regulation is unclear. However, both central and peripheral mechanisms could play a role (McGrath et al. 2015). Firstly, GLP-1 may transmit nutritional signals via the vagus nerve in the gastrointestinal tract (McGrath et al. 2015). Secondly, the small amount of GLP-1 that is not broken down by DPP-IV could cross the blood brain barrier acting directly on GLP-1 receptors to induce satiation (Dailey & Moran 2013). Finally, feelings of satiation could be generated by decreased gastrointestinal motility (Holst 2007).

#### 2.3.3.5. Chronic energy regulation

After weight loss in humans, fasting concentrations of GLP-1 have been shown to decrease (Adam et al. 2005). It has been suggested that this could increase appetite and promote weight regain during and after dieting (Neary & Batterham 2009). However, after bariatric surgery, circulating concentrations and secretions of GLP-1 are elevated and this is believed to help reduce appetite and food intake and weight loss (Ashrafian & le Roux 2009). Together these imply GLP-1 plays a role in chronic energy balance and potentially contributes in the pathogenesis of obesity (Neary & Batterham 2009).

Similarly, by inducing supra-physiological circulating concentrations of GLP-1 through five days of prandial subcutaneous injections in obese individuals, calorie intake was reduced by 15% and 0.5 kg weight loss was seen (Näslund et al. 2004). This suggests that low

concentrations of GLP-1 could be responsible for obesity and restoration of these may increase satiety and cause weight loss.

#### 2.3.3.6. Differences between lean and obese individuals

With regards to fasting concentrations of GLP-1 in obese and normal weight individuals, mixed findings have been reported. Some show no difference (Adam & Westerterp-Plantenga 2004; Verdich et al. 2001), whereas others report lower concentrations in the obese (Chanoine et al. 2008; Ranganath et al. 1996). Discrepancies in these findings may have been caused by recruitment of obese individuals who are losing weight or have recently lost weight (Adam et al. 2005).

In response to a meal, obese individuals have shown attenuated postprandial GLP-1 secretion in comparison to lean individuals (Adam & Westerterp-Plantenga 2005; Ranganath et al. 1996; Verdich et al. 2001). However, again some studies have reported no difference (Fukase et al. 1993).

Irrespective of these differing reports between obese and lean individuals, it has been shown that both lean and obese individuals show a dose response relationship between GLP-1 infusion and energy intake with both populations showing equal sensitivity to GLP-1 (Verdich et al. 2001). This suggests that GLP-1 or its agonists could be useful in the treatment of obesity.

#### 2.3.3.7. GLP-1 as a remedy for obesity

The preservation of GLP-1's anoeretic effects in the obese sparked an interest in the potential GLP-1 may have in the treatment of obesity. However, a major barrier in the development of GLP-1 as a therapeutic drug is the short life of GLP-1 within the circulation. Two methods have been adopted to overcome this; production of a more stable GLP-1 analogue and inhibiting DPP-IV, to prevent GLP-1 breakdown.

Pharmaceutical companies have developed a number of long lasting GLP-1 agonists. Amylin pharmaceuticals developed 'exenatide', a product derived from exerdin-4, a potent GLP-1 analogue discovered in venomous lizards. Initially developed as a diabetes drug, it was also found to induce weight loss (Buse et al. 2004). When trialled in non-diabetic obese individuals nausea was observed, and this seemed to be the cause for observed weight loss. However, dose titration still resulted in weight loss whilst nausea was attenuated. Exenatide

is administered by injection, twice daily. To improve patient compliance a once weekly administration was trialled, with similar reductions in weight loss observed (Drucker et al. 2008). The final limitation to this drug is the high percentage of patients that develop antibodies to it, reducing its efficacy (Buse et al. 2004; Kendall et al. 2005). To help overcome the production of antibodies a GLP-1 analogue which has greater similarities to the human form is required. Novo Nordisk trialled 'litaglutide' for use in type 2 diabetic patients, which is 97% identical to GLP-$1_{1\text{-}37}$ (Knudsen et al. 2000). The drug also caused weight loss in this diabetic population (Marre et al. 2009). In a non-diabetic population, after 52 weeks of treatment more than 35% of participants achieved at least 10% weight loss (Novo Nordisk 2008). Only 10% of participants withdrew from the study, and the most common reason was for feelings of nausea. However, it was also reported that these sensations subsided with continual use of the drug.

Development of DPP-IV inhibiting drugs has been less successful. All initially licensed for type 2 diabetes treatment, and all administered orally, these have shown improvements in glycaemic control but not in weight loss (Green et al. 2006). Batterham & Neary (2005) suggest an alternative method would be to develop a drug whereby endogenous GLP-1 secretion is increased. Alternatively, to help reduce sensations of nausea, a combination drug could be developed where lower doses of two or more drugs are taken together to induce weight loss (Chaudhri et al. 2006).

### 2.3.4. Leptin

#### 2.3.4.1. Discovery

Leptin was identified in 1994 following genetic cloning of the obese (ob) gene in mice (Zhang et al. 1994). The word leptin is derived from the Greek word ‘leptos’, meaning thin, this was chosen as the administration of this hormone to obese mice resulted in significant weight loss.

#### 2.3.4.2. Structure, production and regulation of secretion

Leptin is a 167-amino acid peptide with a 21 amino acid secretory signal sequence. The signal sequence is removed prior to release into the circulation, consequently leptin circulates in the blood as a 146 amino acid residue (Margetic et al. 2002; Zhang et al. 1994). Leptin is primarily produced by and secreted into the circulation from adipose tissue, the placenta, and the gastrointestinal tract (Margetic et al. 2002; Prolo et al. 1998).

Human plasma leptin concentrations express a circadian rhythm, which is affected by sleep patterns (Scheer et al. 2009). Plasma concentrations are found to peak between midnight and early morning, whilst lowest concentrations are seen in the early to mid-afternoon (Friedman & Halaas 1998; Kanabrocki et al. 2001). Obese individuals show higher circulating concentrations of leptin than their lean counterparts (Saad et al. 1998); rate of leptin production and circulating concentrations are related to fat mass with higher leptin concentrations when adipocyte stores are larger (Hamilton et al. 1995). Sex also influences the secretion of leptin (Frederich et al. 1995). Females express higher concentrations of circulating leptin compared to males, which is thought to be caused by reproductive hormones (Mantzoros & Moschos 1998). Feeding behaviour also appears to influence circulating concentrations of leptin, with both short and long term overfeeding increasing leptin expression in adipocytes and concentrations within the circulation (Kolaczynski et al. 1996; Levine et al. 1999).

Leptin binds to leptin receptors (ObRs) found in the brain, specifically the hypothalamus and cerebellum, stomach and placenta (Burguera et al. 2000; Henson et al. 1998; Sierra-Honigmann et al. 1998). Leptin acts on receptors in the brain to signal energy status. Leptin influences several other physiological functions, namely; wound healing, bone formation, angiogenesis, haematopoiesis, immune and inflammatory responses (Klok et al. 2007).

#### 2.3.4.3. Energy regulation

Leptin regulates energy homeostasis by acting on receptors in the hypothalamus. After secretion from adipose tissues, leptin crosses the blood brain barrier and binds to receptors, providing information on body energy stores (Sahu 2003). Leptin stimulates hypothalamic neurones resulting in anorexic effects on energy balance (Klok et al. 2007). In particular leptin induces weight loss by suppressing food intake and stimulating metabolic rate. Supporting this notion, a leptin deficient child born a normal weight, rapidly becomes obese due to overeating (Montague et al. 1997). Similarly, leptin treatment in leptin deficient patients sees decreased appetite, weight loss and increased physical activity (Licinio et al. 2004). Evidence on the effect of leptin on metabolic rate and energy expenditure is not as clear. Researchers show mixed findings of leptin concentrations and RMR, with some showing no correlation (Kennedy et al. 1997; Levine et al. 1999; Roberts et al. 1997), whilst others have found positive correlations (Jorgensen et al. 1998).

Leptin also appears to play a role in short term energy regulation, specifically in response to individual meals. Evidence to support this includes; leptin production in the stomach, insulin's ability to influence secretion of leptin, high fat meals lowering leptin concentrations and the possibility of leptin working in tandem with satiety peptides to reduce food intake (Attele et al. 2002; Dallongeville et al. 1998; Sobhani et al. 2002). More research is required to confirm this.

## 2.4. Exercise, appetite and energy intake in lean and obese individuals

### 2.4.1. Appetite assessment

Appetite is a subjective measurement assessed using indirect methods, such as, eating pattern and food intake questionnaires or by biomarkers i.e. appetite related hormones (Mattes et al. 2005). Initial appetite questionnaires, using visual analogue scales (VAS), showed a weak to moderate association between pre-meal hunger and energy intake at that meal (Hill & Blundell 1982). It was suggested that this was because only one aspect of 'hunger' was assessed, and that taking a multidimensional approach to hunger would be more appropriate. To fully understand a multidimensional model it is first important to clarify the different definitions of appetite. Appetite is a broad term covering food intake, selection, motivation and preference of food items (Blundell et al. 2010). Appetite can be broken down into a collection of feelings; hunger, satiation and satiety. Hunger is defined as the sensation that promotes the intake of food and is found to decrease after food intake (Mattes et al. 2005). Metabolic, sensory and cognitive signals all influence an individual's feeling of hunger (Blundell et al. 2010; Mattes & Friedman 1993). Hunger is objectively measured by rating sensations for the desire to eat. These are felt in various parts of the body including the stomach, limbs and head and are described as feelings of light-headedness, weakness or emptiness in the stomach (Blundell et al. 2010). Satiation is the sensation that determines how long we eat for and how much we consume (Blundell & Macdiarmid 1997). This feeling becomes stronger during a meal and contributes to an individual finishing a meal and beginning a phase of fasting (Mattes et al. 2005). Satiation, also termed intra-meal satiety or post-ingestive satiety, determines how long we fast between meals (Blundell et al. 2010; Mattes et al. 2005).

Measuring an individual's food intake is a common method used to assess hunger, based on the assumption that appetite and food intake are correlated (Mattes et al. 2005). However, there are several factors that can interfere with this relationship. Factors that could inhibit an individual eating enough include; lack of food availability and social constraints. Whereas factors such as boredom, availability of palatable food and emotional stress can all cause an individual to overeat (Mattes et al. 2005).

Visual analogue scales (VAS) and category scales are the most commonly used appetite questionnaires. VAS comprise of lines either 100 or 150 mm in length anchored with opposing phrases. The participant marks on the line how they feel, and this is measured using

a ruler by the investigator. Initially only one question ('how hungry are you right now?') was asked to determine hunger (Hill & Blundell 1982). However, to address the multidimensional nature of hunger it was suggested using a set of questions would be more appropriate. For example, 'how strong a desire to eat do you have now?', 'how much could you eat now?', 'how full are you now?', 'how strong a desire to eat something savoury/sweet do you have now' and 'how thirsty are you now?' (Mattes & Friedman 1993; Mattes et al. 2005). To ensure the multidimensional nature of appetite is assessed, questionnaires should incorporate a combination or all of the questions stated above (Mattes et al. 2005). Flint et al. (2000) measured the reproducibility of VAS measures in a group of 55 healthy normal weight male individuals. Participants ate a breakfast, *ad libitum* lunch meal and completed VAS over a period of four h. The trials were separated by one to three weeks. The scales were shown to be reproducible after identical meals, suggesting suitability to assess appetite in studies with a single meal. Additionally, the validity of VAS was determined by testing the correlation between hunger and energy intake at the *ad libitum* meal. Post-meal scores were found to have a stronger correlation with food intake at the meal than the pre-meal values and pre-post differences of appetite.

When deciding which VAS scales to use, it is necessary to consider their qualities; are the scales easily applied and can they be unambiguously interpreted by investigators and subjects? Do they demonstrate repeat-reliability? Do they show convergent validity with other similar scales? and are they suitable for relevant mathematical and statistical handling (Blundell et al. 2010)?

Category scales or line scales are a similar measure to VAS, but instead of being a continuous scale with anchors at each end, the line is divided into categories. Subjects tend to assume that each category is equally spaced; however, this is not always the case with scales not having ratio properties (Mattes et al. 2005).

Eating patterns is another method used to assess appetite. This is based on the assumption that there is a direct relationship between inter-meal fasts and hunger (Mattes et al. 2005). For this to be true the meal following an overnight sleep would be the largest. However, few individuals show hunger ratings to peak early in the morning. Portion sizes and meal times are also influenced by social norms (de Castro 1988). These drawbacks make this method of appetite assessment less reliable and valid.

Biomarkers of appetite, such as gut peptide hormones, are related to appetite ratings and food intake and can also be used as measures of appetite. In order for biomarkers to be relevant they need to meet a number of criteria (Mattes et al. 2005). Firstly, the marker must be clearly related to appetite physiology, and be sensitive to the changes seen in appetite. Secondly, it is necessary for the measurement of this biomarker to be reproducible under similar conditions. Measures of biomarkers are perhaps more useful for detecting potential mechanisms of behavioural patterns, rather than being direct measures of appetite themselves; this is partly due to the invasive nature for measuring these.

### 2.4.2. Food intake assessment

Both indirect and direct methods can be used to measure food intake. Indirect methods include diet history questionnaires and food diaries, whilst direct measures include *ad libitum* homogenous meals and buffets. There are three major concepts of food intake that need to be considered when determining the suitability of an assessment type; habitual intake, validity and precision (Livingstone & Black 2003). Habitual intake is the mean energy intake of an individual over a prolonged period of time, ideally measured over weeks or months. However this is hard to do accurately, as it varies so much from day to day for each individual (Livingstone & Black 2003). In order for food intake assessment to be valid, it needs to measure the true intake of an individual, i.e. it is accurate and complete for the measured days, and not influenced by taking part in the study (Livingstone & Black 2003). Finally, for a method to be precise it should provide the same results on repeated tests. However, this is difficult to achieve during food intake assessment as habitual patterns vary so widely (Livingstone & Black 2003). Each of the food intake assessment methods has strengths and limitations, summarised below.

Indirect methods of assessment include food diaries and diet recalls via questionnaire or interview. For a diet diary to be valid, it must be completed fully and accurately for all food and drink consumed on test days (Livingstone & Black 2003). Questionnaires allow assessments to be made whilst participants are ‘free living’, and theoretically the external validity of these measures is high (Blundell et al. 2010). However, in reality, the internal validity of these methods is limited by; a bias in habitual food intake, with a tendency for under-reporting of energy and misreporting of macronutrient content of the participant’s diet (Blundell et al. 2010; Livingstone & Black 2003).

Direct measures of food intake require *ad libitum* meals to be consumed within strict laboratory environments. Meal formats vary, with either a selection of buffet foods (hot or cold) or a homogenous meal presented to participants. The main function of an *ad libitum* meal is to measure satiation, i.e. the termination of an eating occasion (Blundell et al. 2010). Feelings of fullness and boredom of taste are two factors that contribute to the ending of a meal; a homogenous meal is most likely to be terminated due to boredom of taste whereas feelings of fullness are more likely to influence participant's cessation at a buffet meal (Blundell et al. 2010). Meals consumed within laboratory environments should have high internal validity and sensitivity as interventions and their outcomes can be controlled (Blundell et al. 2010). However, it is impossible to control for the participant's expectations and the novelty of the laboratory environment could influence behaviour (Blundell et al. 2010). Meal palatability, texture and energy density should be considered alongside the motivational state of the participant, environmental cues and cognitive factors as potential aspects that may influence energy intake (Blundell et al. 2010). *Ad libitum* buffet meals allow for participants to express their own individual selection pattern and have been shown to be reproducible for both macronutrient preferences and energy intake in lean healthy males (Arvaniti et al. 2000; Gregersen et al. 2008; Nair et al. 2009). This reproducibility still stands when assessing energy intake after exercise in lean males (Laan et al. 2010). However, this was not the case in obese women (Brown et al. 2012). Further research is required to confirm these findings, but this would suggest that it is also necessary to consider the group being examined when selecting methods to measure food intake.

To conclude, both direct and indirect methods of energy intake measurement have limitations. To control for these a combination of methods should be adopted (Blundell et al. 2010; Livingstone & Black 2003).

### 2.4.3. Exercise and appetite in lean individuals

In 1994, King and colleagues proposed and investigated the existence of 'exercise induced anorexia'; a condition where appetite is transiently suppressed after exercise. King et al. (1994) found that in lean healthy males appetite was temporarily suppressed during and after high intensity cycling (~70% $\dot{V}O_2$max). The suppression of appetite was found to last for 15 min after exercise, before returning to control values. In response to this finding, many more studies have further examined the effect of exercise on appetite. Most have supported the work of King et al. (1994) showing moderate to vigorous intensity exercise, using a range of

modes to suppress appetite (Bailey et al. 2015; Broom et al. 2007; Broom et al. 2009; Deighton et al. 2012; Deighton et al. 2013a; King & Blundell 1995; King et al. 2010a; King et al. 2010b; King et al. 2011a; Martins et al. 2007; Thompson et al. 1988; Tsofliou et al. 2003; Wasse et al. 2013; Westerterp-Plantenga et al. 1997). However, increases and no change in appetite during and after exercise have also been reported (Hubert et al. 1998; Imbeault et al. 1997; King et al. 1996; King et al. 1997; Lluch et al. 1998; Maraki et al. 2005; Martins et al. 2015; Melanson et al. 1999; Ueda et al. 2009b; Wasse et al. 2013).

Exercise induced anorexia is dependent on the intensity of the exercise bout performed. Low intensity exercise (30-35% $\dot{V}O_2$ max) has not been shown to induce a suppression of appetite, both during and after the bout of exercise (King et al. 1994; Thompson et al. 1988). Similarly, few studies have found suppression of appetite after moderate intensity exercise (50% $\dot{V}O_2$ max (Ueda et al. 2009b)). Instead, the majority of research shows that high intensity exercise is required to create exercise induced anorexia, implying a threshold of approximately 60% $\dot{V}O_2$max (Cheng et al. 2009; King & Blundell 1995; King et al. 1994; Kissileff et al. 1990; Martins et al. 2007; Thompson et al. 1988).

Several studies have directly compared the influence of exercise on appetite during fed and fasted conditions. All are in agreement; appetite is suppressed to a greater extent and for greater duration postprandially after exercise in the fed state vs. fasted state (Borer et al. 2005; Cheng et al. 2009; Deighton et al. 2012).

Little of the literature has directly compared appetite responses in males and females. However, after an acute bout of cycling at 70% $\dot{V}O_2$ max, lean healthy males and females showed no difference in appetite ratings (hunger, satisfaction, fullness (Hagobian et al. 2013)). Similarly, after an acute bout of treadmill running (70% $\dot{V}O_2$ max) males and females exhibited similar appetite responses (Alajmi et al. 2016). In both of these studies males and females were not matched for body fat percentage, but instead matched by physical activity level or age. This may have impacted findings as differences in body fat percentages can be a true difference of sex.

### 2.4.4. Exercise and appetite in obese individuals

The effects of exercise on appetite in obese individuals are unclear. Moderate intensity exercise has not been found to alter appetite perceptions, despite increasing PYY and GLP-1 (Ueda et al. 2009b; Unick et al. 2010). Ueda et al. (2009b) also observed a decrease in energy

intake and relative energy intake (REI) after exercise. This finding suggests that after a bout of exercise, it takes less food to satisfy this population. This could help to maintain the energy deficit created from exercise and hence aid weight loss. However, this study is limited by its short two hour observation period and low participant numbers. Moderate intensity exercise has also been shown to reduce appetite in this population (Tsofliou et al. 2003; Westerterp-Plantenga et al. 1997). In a group of obese females, moderate physical activity (20 min treadmill walking) reduced appetite in the 30 min after exercise (Tsofliou et al. 2003). Satiety and fullness were also shown to be higher after exercise in comparison to control conditions.

Preliminary research examining the effects of exercise intensity on appetite in overweight participants found no difference in appetite between trials (Sim et al. 2014). Healthy overweight young men completed a 30 min bout of; rest, continuous moderate exercise (60% $\dot{V}O_2$ peak), intermittent high intensity exercise (60 seconds at 100% $\dot{V}O_2$ peak: 240 seconds at 50% $\dot{V}O_2$ peak) and very high intermittent intensity exercise (15 seconds at 170% $\dot{V}O_2$peak: 60s at 32% $\dot{V}O_2$ peak). Unlike their lean counterparts who show that there is a clear intensity threshold for reducing appetite perceptions, no such observation has been seen in obese or overweight individuals. Further work within this population is necessary to help with the understanding of their appetite control systems.

### 2.4.5. Energy intake in response to acute exercise in lean individuals

Table 2.1 summarises studies looking at the effects of acute exercise on energy intake. The majority of studies have shown individuals do not compensate for the energy expended during exercise in the immediate hours after exercise through the alteration of energy intake. A recent meta-analysis quantified these findings, showing exercise to have a trivial effect on energy intake (~200 kJ, ES=0.14) and large effect on relative energy intake (~2017 kJ, ES=-1.35 (Schubert et al. 2013)). However, findings are not unanimous, with some studies showing an increase in energy intake after an acute bout of exercise (Erdmann et al. 2007; Laan et al. 2010; Larson-Meyer et al. 2012; Martins et al. 2007; Shorten et al. 2009). Schubert et al. (2013) suggested that despite discrepancies in energy intake reports, exercise remains effective at producing a short-term energy deficit.

**Table 2.1.** Acute exercise and energy intake in lean individuals.

| Dailey | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Balaguera-Cortes et al. 2011 | 10 (M) | 23.7 (2.0) | 45 min treadmill run @ 70% $\dot{V}O_2$ max | *Ad libitum* meal 30 min post-exercise | CON: | 5283 (1342) | CON: | 5027 (1531) |
| | | | | | EX: | 5516 (1558) | EX: | 2698 (1140) |
| | | | 45 min resistance exercise 3 sets of 12 reps or to failure, 8 exercises, 1 min between sets | | CON: | 5283 (1342) | CON: | 5027 (1531) |
| | | | | | EX: | 5441 (1503) | EX: | 4101 (830) |
| Deighton et al. 2012 | 12 (M) | 22.9 (2.1) | 60 min treadmill run @ 70% $\dot{V}O_2$ max | Standard breakfast 30 min post-exercise *Ad libitum* meals 4.5 and 8.5 h post-exercise | CON: | 13452 (2682) | CON: | 9774 (2694) |
| | | | | | EX: | 16652 (2385) | EX: | 6481 (2318)* |
| | | | | Standard breakfast 2.5 h pre-exercise *Ad libitum* meals 4.5 and 8.5 h post-exercise | CON: | 13452 (2682) | CON: | 9774 (2694) |
| | | | | | EX: | 12929 (2933) | EX: | 6017 (3050)* |
| Deighton et al. 2013a | 12 (M) | 24.2 (2.9) | 60 min cycling @ 65% $\dot{V}O_2$ max | Standardised breakfast 1.75 or 2.25 h pre-exercise *Ad libitum* meals 45 min and 4.25 h post-exercise Overnight food bags | CON: | 12941 (3113) | CON: | 12941 (3113) |
| | | | | | EX: | 13548 (3205) | EX: | 10908 (3238)* |
| | | | 30 min cycling including 6 x 30 second sprints | | CON: | 12941 (3113) | CON: | 12941 (3113) |
| | | | | | EX: | 12920 (2983) | EX: | 12326 (2987) |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Deighton et al. 2013b | 12 (M) | 23.7 (3.0) | 60 min cycling @ 60% $\dot{V}O_2$ max | Standardised meals 2 h pre-exercise and 45 min post-exercise *Ad libitum* meal 4 h post-exercise | CON: | 4759 (1268) | CON: | 4759 (1268) |
| | | | | | EX: | 4813 (1316) | EX: | 2362 (1224)* |
| | | | 10 x 4 min cycling bouts @ 85-80% $\dot{V}O_2$ max, 2 min rest | | CON: | 4759 (1268) | CON: | 4759 (1268) |
| | | | | | EX: | 4952 (1351) | EX: | 2523 (1402)* |
| Deighton et al. 2014 | 12 (M) | 23.8 (2.7) | 30 min cycling @ 65% $\dot{V}O_2$ max | Standardised meals 30 min and 3.5 h post-exercise *Ad libitum* meal 6.5 h post-exercise | CON: | 4376 (1634) | CON: | NM |
| | | | | | EX: | 4217 (1850) | EX: | NM |
| Erdmann et al. 2007 | 7 (5F:2M) | 21.4 (0.8) | 30 min cycling @ 50 W | *Ad libitum* meal 15 min post-exercise | CON: | 1721 (217) | CON: | NM |
| | | | | | EX: | 1825 (375) | EX: | NM |
| | | | 30 min cycling @ 100 W | | CON: | 1721 (217) | CON: | NM |
| | | | | | EX: | 1758 (262) | EX: | NM |
| | 7 (3F:4M) | 22.1 (NM) | 30 min cycling @ 50 W | | CON: | 2350 (135) | CON: | NM |
| | | | | | EX: | 1992 (412) | EX: | NM |
| | | | 60 min cycling @ 50 W | | CON: | 2350 (135) | CON: | NM |
| | | | | | EX: | 2386 (497) | EX: | NM |
| | | | 120 min cycling @ 50 W | | CON: | 2350 (135) | CON: | NM |
| | | | | | EX: | 3248 (511)* | EX: | NM |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| George & Morganstein 2003 | 12 (F) | 22.0 (1.0) | 60 min treadmill walk @ 60% HR max | Standard breakfast 90-150 min pre-exercise | CON: | 1846 (859) | CON: | NM |
| | | | | *Ad libitum* meal 30 min post-exercise | EX: | 1503 (536) | EX: | NM |
| Hubert et al. 1998 | 11 (F) | 21.5 (1.1) | 40 min cycling @ 70% $\dot{V}O_2$ max | Standardised breakfast immediately post-exercise (~270 kJ) | CON: | 3182 (783) | CON: | NM |
| | | | | *Ad libitum* lunch 3 h post-exercise | EX: | 2843 (1076) | EX: | NM |
| | | | | Standardised breakfast immediately post-exercise (~2100 kJ) | CON: | 2525 (821) | CON: | NM |
| | | | | *Ad libitum* lunch 3 h post-exercise | EX: | 2495 (900) | EX: | NM |
| Imbeault et al. 1997 | 11 (M) | 23.2 (2.3) | 2050 kJ EE treadmill walk @ 35% $\dot{V}O_2$ max | Standard breakfast 3.5 h pre-exercise | CON: | 6593 (NM) | CON: | 6593 (NM) |
| | | | | *Ad libitum* meal 15 min post-exercise | EX: | 7387 (NM) | EX: | 5719 (NM) |
| | | | 2050 kJ EE treadmill run @ 75% $\dot{V}O_2$ max | | CON: | 6593 (NM) | CON: | 6593 (NM) |
| | | | | | EX: | 6623 (NM) | EX: | 4796 (NM)* |
| Jokisch et al. 2012 | 10 (inactive M) | 23.0 (1.9) | 45 min cycling @ 65-75% HR max | *Ad libitum* meal 60 min post-exercise | CON: | 4497 (1968) | CON: | NM |
| | | | | | EX: | 3915 (929)* | EX: | NM |
| | 10 (active M) | 23.9 (1.5) | | | CON: | 4258 (1662) | CON: | NM |
| | | | | | EX: | 4643 (1629) | EX: | NM |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| King et al. 1994 | 12 (M) | 24.2 (NM) | ~60 min cycle @ 30% $\dot{V}O_2$ max | *Ad libitum* meal from 15 min post-exercise (participants instructed to eat when start to feel hungry) | CON: | 6443 (1305) | CON: | 6213 (1305) |
| | | | | | EX: | 6889 (1414) | EX: | 5397 (1439) |
| | | | ~30 min cycle @ 70% $\dot{V}O_2$ max | | CON: | 6443 (1305) | CON: | 6213 (1305) |
| | | | | | EX: | 6439 (1778) | EX: | 5017 (1816) |
| | 12 (M) | 23.2 (2.2) | ~30 min cycle @ 70% $\dot{V}O_2$ max | | CON: | 5841 (2251) | CON: | 5640 (2251) |
| | | | | | EX: | 6351 (2280) | EX: | 5113 (2289) |
| | | | ~60 min cycle @ 70% $\dot{V}O_2$ max | | CON: | 5841 (2251) | CON: | 5640 (2251) |
| | | | | | EX: | 5975 (2222) | EX: | 3720 (2142) |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| King & Blundell 1995 | 12 (M) | 22.7 (NM) | ~50 min cycle @ 70% $\dot{V}O_2$ max | Low-fat/high-carbohydrate *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 4782 (1397) | CON: | 4540 (1393) |
| | | | | | EX: | 5088 (1075) | EX: | 2502 (987) |
| | | | | Low-carbohydrate/high-fat *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 7439 (1987) | CON: | 7201 (1983) |
| | | | | | EX: | 8142 (2050) | EX: | 4753 (1987)* |
| | 12 (M) | 22.8 (NM) | ~50 min treadmill run @ 70% $\dot{V}O_2$ max | Low-fat/high-carbohydrate *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 4975 (1173) | CON: | 4745 (1167) |
| | | | | | EX: | 5314 (1305) | EX: | 2795 (1326)* |
| | | | | Low-carbohydrate/high-fat *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 8460 (2264) | CON: | 8460 (2264) |
| | | | | | EX: | 9042 (1770) | EX: | 6477 (1711)* |
| King et al. 1996 | 13 (F) | 21.9 (1.6) | ~50 min cycle @ 70% $\dot{V}O_2$ max | Low-fat/high-carbohydrate *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 2730 (549) | CON: | 2529 (646) |
| | | | | | EX: | 2998 (506) | EX: | 1537 (397)* |
| | | | | Low-carbohydrate/high-fat *ad libitum* meal available post-exercise or rest (participants instructed to eat when start to feel hungry) | CON: | 4484 (782) | CON: | 4283 (811) |
| | | | | | EX: | 4886 (996) | EX: | 3010 (844)* |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| King et al. 1997 | 8 (M) | 22.4 (1.8) | 50 min treadmill run @ 70% HR max | Free living choice for the rest of the trial day | CON: | 12154 (2453) | CON: | NM |
| | | | | | EX: | 12481 (2085) | EX: | NM |
| King et al. 2010a | 9 (M) | 23.6 (1.2) | 90 min treadmill running @ 70% $\dot{V}O_2$ max | *Ad libitum* meals 1, 4 and 8.5 h post-exercise Diet log completed overnight | CON: | 17191 (3432) | CON: | NM |
| | | | | | EX: | 17606 (4152) | EX: | NM |
| King et al. 2010b | 14 (M) | 23.4 (2.2) | 60 min self-paced "brisk walk" (45±7.5% $\dot{V}O_2$ max) | *Ad libitum* meals 30 min and 4 h post-exercise | CON: | 9212 (2200) | CON: | NM |
| | | | | | EX: | 9384 (2466) | EX: | NM |
| King et al. 2011a | 12 (M) | 22.8 (1.4) | 90 min treadmill run @ 70% $\dot{V}O_2$ max | Standardised meals 30 min and 3.25 h post-exercise *Ad libitum* meal 8 h post-exercise | CON: | 4004 (1479) | CON: | NM |
| | | | | | EX: | 4343 (2262) | EX: | NM |
| King et al. 2011b | 14 (M) | 23.2 (2.2) | 60 min intermittent swimming | Standardised breakfast 60 min pre-exercise *Ad libitum* meals 2 and 6.5 h post-exercise | CON: | 9161 (2690) | CON: | 9161 (2690) |
| | | | | | EX: | 9749 (3027) | EX: | 7828 (2896)* |
| Laan et al. 2010 | 19 (10F:9M) | 22.5 (1.8) | 35 min cycle @ 70% HRR | *Ad libitum* meal 30 min post-exercise | CON: | 3282 (373) | CON: | NM |
| | | | | | EX: | 3756 (402)* | EX: | NM |
| | | | 35 min resistance training 2 sets of 10 reps @ 70% 1-RM, 5 exercises | | CON: | 3282 (373) | CON: | NM |
| | | | | | EX: | 3868 (398)* | EX: | NM |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Larson-Meyer et al. 2012 | 9 (F) | 19.8 (1.0) | 60 min treadmill running @ 70% $\dot{V}O_2$ max | Standardised breakfast 90 min pre-exercise *Ad libitum* meal 2 h post-exercise | CON: | 2011 (529) | CON: | 1188 (505) |
| | | | | | EX: | 2034 (768) | EX: | -812 (862)* |
| | 10 (F) | 22.1 (3.4) | | | CON: | 2305 (680) | CON: | 1532 (769) |
| | | | | | EX: | 2612 (582)* | EX: | 531 (820)* |
| Martins et al. 2007 | 12 (6F:6M) | 22.0 (3.2) | 60 min cycling @ 65% HR max | Standardised breakfast 60 min pre-exercise *Ad libitum* lunch 60 min post-exercise | CON: | 3190 (1055) | CON: | 2366 (946) |
| | | | | | EX: | 3822 (1520)* | EX: | 1763 (1264)* |
| Melby et al. 2002 | 13 (F) | 21.6 (0.2) | 2160 kJ EE cycling @ 65% $\dot{V}O_2$ max (~75 min) | *Ad libitum* meal 90 min post-exercise | CON: | 3236 (1268) | CON: | 2495 (1177) |
| | | | | | EX: | 3266 (1223) | EX: | 331 (1117) |
| O'Donoghue et al. 2010 | 9 (M) | 22.4 (1.6) | 45 min running @ 75% $\dot{V}O_2$ max | *Ad libitum* meals 15 min, 5 and 10.5 h post-exercise | CON: | 19975 (5909) | CON: | NM |
| | | | | | EX: | 21145 (4507) | EX: | NM |
| Pomerleau et al. 2004 | 13 (F) | 22.2 (2.4) | 350 kcal treadmill walk @ 40% $\dot{V}O_2$ max | Standard breakfast 90 min pre-exercise *Ad libitum* meals 1 and 6.5 h post-exercise | CON: | 9567 (2495) | CON: | NM |
| | | | | | EX: | 10040 (1809) | EX: | NM |
| | | | 350 kcal treadmill walk @ 70% $\dot{V}O_2$ max | Snack bags 4.5 and 9 h post-exercise | CON: | 9567 (2495) | CON: | NM |
| | | | | | EX: | 10802 (2215) | EX: | NM |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Shorten et al. 2009 | 11 (M) | 24.1 (2.3) | 40 min treadmill run @ 70% $\dot{V}O_2$ max | *Ad libitum* meal 35 min post-exercise | CON: | 3744 (1566) | CON: | 3744 (1566) |
| | | | | | EX: | 5193 (1998)* | EX: | 2818 (1718) |
| Vatansever-Ozen et al. 2011 | 10 (M) | 22.0 (20.4) | 105 min treadmill run @ 59% $\dot{V}O_2$ max and 15 min @ 70% $\dot{V}O_2$ max | *Ad libitum* meal 60 min post-exercise | CON: | 8194 (2169) | CON: | 7210 (2177) |
| | | | | | EX: | 8587 (2889) | EX: | 3081 (2935)* |
| Wasse et al. 2012 | 10 (M) | 24.8 (2.4) | 60 min treadmill run @ 70% $\dot{V}O_2$ max | Standard meal 1 h post-exercise | CON: | 7535 (2112) | CON: | 7435 (2324) |
| | | | | *Ad libitum* meal 4.5 h post-exercise | EX: | 7909 (2599) | EX: | 4542 (2448) |

CON, control trial; EX, exercise trial; F, female; HR max, heart rate maximum; HRR, heart rate reserve; M, male; NM, data not measured; RM, repetition maximum; $\dot{V}O_2$ max, maximal oxygen uptake. *significantly different from control trial.

Relative energy intake takes into consideration the energy expended during exercise, and is calculated by subtracting exercise energy expenditure from energy intake in the exercise trial. REI in the exercise trial can be compared to the energy intake of the control condition to determine if a negative energy balance occurred as a result of the exercise bout. King et al. (1994) suggest that this could be a more meaningful analysis than simply comparing total energy intake between trials, and this is especially true when determining the efficiency of exercise to induce weight loss.

Direct examination of exercise intensity has revealed no significant effect on energy intake (Imbeault et al. 1997; Pomerleau et al. 2004). Imbeault et al. (1997) assessed energy intake 15 min after a bout of low intensity exercise (35% $\dot{V}O_2$ max), high intensity exercise (75% $\dot{V}O_2$ max), and rest. Energy expenditure for the differing intensities was matched and energy intake was assessed using an *ad libitum* buffet. Post-exercise energy intake did not significantly differ between the three conditions, but energy intake did tend to be lower after the high intensity exercise bout compared to the low intensity exercise bout. Schubert et al. (2013) also confirmed that exercise intensity had no effect on energy intake in their meta-analysis.

Exercise mode does not influence variation in energy intake (Schubert et al. 2013). Specifically, King & Blundell (1995) examined the effects of 50 min of cycling or running at 70% $\dot{V}O_2$ max on energy intake at an *ad libitum* meal immediately after exercise, and from food intake during the remainder of the trial day. No significant differences in energy intake between the two modes of exercise were found. Resistance exercise has shown similar absolute energy intake responses to aerobic exercise. However, due to the lower energy cost of resistance exercise, differences in REI are less pronounced after resistance exercise than aerobic exercise (Schubert et al. 2013). Laan et al. (2010) showed no difference in energy intake after a bout of resistance and a bout of aerobic exercise. Healthy men and women completed three trials in a randomised order; control, aerobic exercise and resistance exercise. Aerobic exercise was a 35 min cycle at 70% heart rate reserve. Resistance exercise consisted of two sets of 10 repetitions and a third set to voluntary fatigue at 70% 1-RM for chest press, leg press, seated leg extension, seated leg curl and seated row. Participants were presented with an *ad libitum* pasta meal 30 min after the exercise. Energy intake was higher after both the exercise sessions in comparison to the control session, but with no significant difference between these. When taking into consideration the energy expenditure of the exercise bouts, REI was lower after the aerobic bout vs. resistance and control conditions.

The size of the energy deficit generated by exercise does not seem to affect energy intake. In particular, King et al. (2010a) imposed a ~5000 kJ energy deficit in healthy males during a 90 min treadmill run. Energy intake in the 24 h after exercise did not differ significantly from control.

The majority of studies examining acute exercise and energy intake are limited by short observation periods; typically responses are limited to one meal. It is also important to consider the possibility that energy compensations could be made later on in the day, or on consecutive days. A few studies have continued to monitor energy intake for longer periods of time after an acute exercise bout (Deighton et al. 2012; King et al. 1997; King et al. 2010a; King et al. 2010b; O'Donoghue et al. 2010; Pomerleau et al. 2004). Some assessed energy intake multiple times on the day of exercise, via *ad libitum* buffets (Deighton et al. 2012; O'Donoghue et al. 2010; Pomerleau et al. 2004) others relied on food diaries to assess energy intake (King et al. 1997), whilst the remainder relied on a combination of these methods (King et al. 2010a; King et al. 2010b). Together these studies found that even by increasing the observation period, there was no compensation for the energy lost during exercise. Again, these studies are limited as those relying on *ad libitum* meals to assess energy intake still only looked at the response to exercise within 10.5 h of the bout ending (Deighton et al. 2012; O'Donoghue et al. 2010; Pomerleau et al. 2004). Similarly, the reliance on food diaries to assess food intake is unreliable. Therefore, it is still necessary for a robust study to be designed in which energy intake is accurately assessed for a longer period of time after the cessation of exercise to determine if the energy deficit is compensated for.

### 2.4.6. Energy intake in response to acute exercise in obese individuals

Fewer studies have examined the effect of exercise on energy intake in overweight and obese individuals (Table 2.2). Findings have been mixed, with some reporting no difference in absolute energy intake compared to control conditions (Martins et al. 2015; Tsofliou et al. 2003; Unick et al. 2010), with others report reductions in absolute energy intake after exercise (Sim et al. 2014; Ueda et al. 2009b). It is likely that these inconsistencies are due to differences in methodology. For example, a range of exercise intensities, modes and durations were utilised; 20 min brisk walk (Tsofliou et al. 2003), 40 min moderate walking (Unick et al. 2010), moderate intensity continuous 60 min cycling (Ueda et al. 2009b), and a range of moderate, high and very high intensity cycling sessions (Martins et al. 2015; Sim et al. 2014). In addition, studies differed in meal provision; with some providing homogenous

meals and others providing buffet style meals. These were provided at differing times after the exercise bouts, with many stopping observations immediately after the cessation of this meal. Consequently, the trial length of these studies was short, lasting no longer than 3.5 h. It is therefore unknown how this population respond to exercise later on in the day.

Despite these discrepancies, early findings within this population are similar to those within lean individuals. This would suggest that exercise is as useful at generating a negative energy balance, and exercise could be prescribed as a weight loss tool if negative energy balances are sustained. Further research is required within this population to gain a greater understanding of how exercise affects appetite later on in the day.

**Table 2.2.** Acute exercise and energy intake in overweight/obese individuals.

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Martins et al. 2015 | 12 (7F:5M) | 32.3 (2.7) | Cycling @ 85-90% HR max until 250 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling | Standard breakfast 1 h pre-exercise *Ad libitum* meal 3 h post-breakfast | CON: | 2013 (862) | CON: | 481 (806) |
| | | | | | EX: | 2213 (820) | EX: | -364 (728)* |
| | | | Cycling @ 70% HR max until 250 kcal expended: continuous cycle | | CON: | 2013 (862) | CON: | 481 (806) |
| | | | | | EX: | 1987 (757) | EX: | -590 (715)* |
| | | | Cycling @ 85-90% HR max until 125 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling | | CON: | 2013 (862) | CON: | 481 (806) |
| | | | | | EX: | 1941 (636) | EX: | -113 (594)* |
| Sim et al. 2014 | 17 (M) | 27.7 (1.6) | 30 min continuous cycling @ 60% $\dot{V}O_2$ peak | Standardised meal 5 min post-exercise *Ad libitum* meal 70 min post-exercise | CON: | 3199 (1642) | CON: | NM |
| | | | | | EX: | 2974 (1370) | EX: | NM |
| | | | 30 min cycling: alternating between 60s @ 100% $\dot{V}O_{2peak}$ and 240s @ 50% $\dot{V}O_2$ peak | | CON: | 3199 (1642) | CON: | NM |
| | | | | | EX: | 2602 (1086)* | EX: | NM |
| | | | 30 min cycling: alternating between 15s @ 170% $\dot{V}O_{2peak}$ and 60s @ 32% $\dot{V}O_2$ peak | | CON: | 3199 (1642) | CON: | NM |
| | | | | | EX: | 2488 (1202)* | EX: | NM |
| Tsofliou et al. 2003 | 10 (F) | 37.2 (6.5) | 20 min brisk walk | *Ad libitum* meal 1 h post-exercise | CON: | 3032 (NM) | CON: | NM |
| | | | | | EX: | 2860 (NM) | EX: | NM |

| Study | Participants | | Intervention | Meal(s) | Energy intake (kJ) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | Absolute energy intake | | Relative energy intake | |
| Ueda et al. 2009b | 7 (M) | 30.0 (3.1) | 60 min cycle @ 50% $\dot{V}O_2$ max | Standard breakfast<br>1 h pre-exercise<br>*Ad libitum* meal<br>1 h post-exercise | CON: | 3951 (737) | CON: | 2569 (364) |
| | | | | | EX: | 2769 (640)* | EX: | -387 (467)* |
| Unick et al. 2010 | 19 (F) | 32.5 (4.3) | Walk @ 70-75% age predicted HR max until 3.0 $kcal.kg^{-1}$ of body weight expended | *Ad libitum* meal<br>1 h post-exercise | CON: | 2296 (1200) | CON: | 2110 (1214) |
| | | | | | EX: | 2307 (1025) | EX: | 828 (1073)* |

CON, control trial; EX, exercise trial; F, female; HR max, heart rate maximum; M, male; NM, data not measured; $\dot{V}O_2$ max, maximal oxygen uptake; $\dot{V}O_2$ peak, peak oxygen uptake. Values are mean (SD). *significantly different from control trial.

### 2.4.7. Energy intake in response to acute exercise, differences between males and females

When comparing energy intake responses to exercise in males and females, no significant differences have been found for either total energy intake or REI (Hagobian et al. 2013; King et al. 1996; Laan et al. 2010); Schubert et al. (2013) reported no effect of sex on absolute energy intake. However the effect size was large and approached significance (ES=0.64, p=0.054). This suggests that the effects of exercise on energy intake are similar between males and females, showing exercise to be as effective at generating a negative energy balance on the day of exercise.

### 2.4.8. Exercise and macronutrient intake in lean individuals

An advantage for using *ad libitum* buffets for energy intake assessment is that these meals allow for participants to express their own selection pattern. Consequently the effect that exercise has on macronutrient intake can be measured. It is important to consider the composition of food selected as macronutrients differ in the amount of energy per gram of food. If participants were to favour high fat foods over proteins or carbohydrates, this could undo the energy deficit generated by exercise.

Although some studies have reported differences in macronutrient intake after exercise (Pomerleau et al. 2004; Shorten et al. 2009; Wasse et al. 2012), the majority of studies report there to be no change in macronutrient preference after exercise (Balaguera-Cortes et al. 2011, Deighton et al. 2012; Deighton et al 2013a; George & Morganstein 2003; Hubert et al. 1998; Imbeault et al. 1997; Jokisch et al. 2012; King et al. 1994; King et al. 1997; King et al. 2010a; King et al. 2010b; King et al. 2011a; King et al. 2011b; Larson-Meyer et al. 2012; Martins et al. 2007; O'Donoghue et al. 2010; Vatansever-Ozen et al. 2011).

It is likely that these differing results are caused by differences in protocol. Specifically, studies will have presented a different range of foods to participants, and this could have caused the favouring of certain foods.

### 2.4.9. Exercise and macronutrient intake in obese individuals

Of the few studies that have examined the effects of exercise on energy intake in obese individuals, only three examined macronutrient selection (George & Morganstein 2003; Martins et al. 2015; Tsofliou et al. 2013). These studies were in agreement, all showing no

effect of exercise on macronutrient choice at a buffet meal after exercise. Further research within this population is required to determine the true effects of exercise on macronutrient intake.

## 2.5. Effects of exercise on appetite regulating hormones in lean and obese individuals

### 2.5.1. Acute exercise and ghrelin

When looking at the effects of exercise on ghrelin concentrations results are divided. This is likely a result of differing study protocols; for example, exercise mode, intensity and form of ghrelin measured all vary. Table 2.3 summarises studies looking at the effects of aerobic exercise on ghrelin. A recent meta-analysis quantified the effects of acute exercise on acylated ghrelin (Schubert et al. 2014). It was found that exercise had a small suppressing effect on circulating concentrations of acylated ghrelin (ES = -0.20).

The concurrent suppression of appetite and acylated ghrelin concentrations during strenuous exercise sparked a plethora of research in this area. Of particular interest to researchers was the role of ghrelin in exercise induced anorexia and if ghrelin could play a role in the compensation of energy intake after exercise, to restore the energy deficit caused by exercise. These findings would have particular relevance in determining the efficacy of exercise for weight loss and management.

Ghrelin responses to exercise in obese and normal weight individuals have been found to differ (Heden et al. 2013). The morning after a bout of treadmill walking, obese individuals showed no change in fasting or postprandial acylated ghrelin concentrations vs. control. Normal weight individuals expressed reduced concentrations both pre- and post-prandially. Despite showing no changes in acylated ghrelin, obese individuals reported a standardised meal to be less filling. It was hypothesised that this could result in greater energy intake later in the day. However as energy intake was not measured in this study, this is only a speculation. Research into exercise and ghrelin in obese individuals is limited, and more is required to determine its effects, especially in relation to post exercise energy intake.

Mode of exercise appears to have little effect on ghrelin responses. When comparing running and cycling at the same relative intensity plasma acylated ghrelin concentrations were similarly suppressed vs. resting control (Wasse et al. 2013). However, energy expenditure was not matched between the two exercise trials, potentially confounding these findings. Further to this, Kawano et al. (2013) compared responses between rope skipping and cycling. Here the exercise bouts were matched for energy expenditure, showing acylated ghrelin to be similarly suppressed during and immediately after both exercise bouts.

Exercise intensity appears to be important when considering the response of ghrelin. When exercise intensity is low (i.e. treadmill walking), ghrelin concentrations remain unchanged to control conditions (King et al. 2010b; Ueda et al. 2009a; Unick et al. 2010). Whilst intense exercise (~70% $\dot{V}O_2$ max) is shown to decrease ghrelin concentrations (Broom et al. 2007; Broom et al. 2009; King et al. 2010a; King et al. 2011a; Wasse et al 2012).

Several studies have compared ghrelin responses to exercise in males and females (Alajmi et al. 2016; Burns et al. 2007; Hagobian et al. 2009; Hagobian et al. 2013). In lean individuals, no significant difference in total ghrelin response was seen between males and females after 1 h of treadmill running at 75% $\dot{V}O_2$ max (Burns et al. 2007). However, phase of menstrual cycle was not controlled for. Similarly, no sex differences in acylated ghrelin responses to moderate intensity exercise have been reported (Alajmi et al. 2016; Hagobian et al. 2013). Further research is required to confirm these findings.

**Table 2.3.** Effects of acute aerobic exercise on ghrelin.

| Study | Participants | | Intervention | Ghrelin form measured | Ghrelin response compared to control | |
|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | During exercise | After exercise |
| Broom et al. 2007 | 9 (M) | 22.2 (0.7) | 60 min treadmill running @ 72% $\dot{V}O_2$ max | Acylated | ↓ | ↔ |
| Broom et al. 2009 | 11 (M) | 23.1 (0.4) | 60 min treadmill running @ 70% $\dot{V}O_2$ max | Acylated | ↓ | ↔ |
| | | | 90 min resistance exercise; 3 sets of 12 reps @ 80% 12 RM max, 10 exercises | | ↓ | ↔ |
| Burns et al. 2007 | 18 (9F:9M) | 22.9 (0.6) | 60 min treadmill running @ 73.5% $\dot{V}O_2$ max | Total | ↔ | ↔ |
| Christ et al. 2006 | 11 (M) | 22.6 (0.5) | 180 min cycle @ 50% W max | Total | ↑ | NM |
| Dall et al. 2002 | 12 (M) | 23.6 (0.5) | 45 min cycle @ LT | Total | ↔ | ↔ |
| Deighton et al. 2013a | 12 (M) | 24.2 (2.9) | 60 min cycling @ 65% $\dot{V}O_2$ max | Acylated | ↔ | ↓ |
| | | | 30 min cycling including 6 x 30 s sprints | | ↓ | ↓ |
| Deighton et al. 2014 | 12 (M) | 23.8 (2.7) | 30 min cycling @ 65% $\dot{V}O_2$ max | Acylated | ↔ | ↔ |

| Study | Participants | | Intervention | Ghrelin form measured | Ghrelin response compared to control | |
|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | During exercise | After exercise |
| Erdmann et al. 2007 | 7 (5F:2M) | 21.4 (0.8) | 30 min cycle @ 50 W | Total | ↑ | ↑ |
| | | | 30 min cycle @ 100 W | Total | ↔ | ↔ |
| | 7 (3F:4M) | 22.1 (NM) | 30 min cycle @ 50 W | Total | ↔ | ↔ |
| | | | 60 min cycle @ 50 W | Total | ↔ | ↔ |
| | | | 120 min cycle @ 50 W | Total | ↔ | ↔ |
| Gholipour et al. 2011 | 9 (M) | 32.7 (0.8) | 36 min treadmill run: 10 min, 10 min, 5 min, 2 min @ 65% $\dot{V}O_2$ max, separated by 3 min @ 3 $km \cdot h^{-1}$ | Acylated | ↓ | ↓ |
| Hagobian et al. 2013 | 11 (M) | 26.0 (2.0) | Cycle @ 70% $\dot{V}O_2$ peak until 30% of total daily expenditure expended | Acylated | ↔ | ↔ |
| | 10 (F) | 24.0 (2.0) | | | ↔ | ↔ |
| Jurimae et al. 2007a | 9 (M) | 24.8 (NM) | Constant load sculling 5 bpm below anaerobic threshold | Total | ↔ | ↔ |
| | | | Constant load sculling 5 bpm above anaerobic threshold | Total | ↔ | ↔ |
| Jurimae et al. 2007b | 8 (M) | 97.4 (7.4) | 6000m maximal rowing ergometer test | Total | ↑ | ↔ |

| Study | Participants | | Intervention | Ghrelin form measured | Ghrelin response compared to control | |
|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | During exercise | After exercise |
| Kallio 2001 | 9 (2M:7F) | 22.8 (0.9) | 5 min warm up at ≥ 20 W<br>Workload increased by 20% $\dot{V}O_2$ max at 2 min intervals until 80% $\dot{V}O_2$ max was reached<br>Maintained for 12 min and followed by 10 min cooling period at 20% $\dot{V}O_2$ max | Total | ↔ | ↔ |
| Kelly et al. 2012 | 10 (M) | 23.9 (2.1) | 45 min treadmill run<br>@ 70% $\dot{V}O_2$ max | Total | ↔ | ↔ |
| King et al. 2010a | 9 (M) | 23.6 (0.4) | 90 min treadmill run<br>@ 70% $\dot{V}O_2$ max | Acylated | ↓ | ↔ |
| King et al. 2010b | 14 (M) | 23.4 (2.2) | 60 min self-paced "brisk walk"<br>(45% $\dot{V}O2$ max) | Acylated | ↔ | ↔ |
| King et al. 2011a | 12 (M) | 22.8 (1.4) | 90 min treadmill run<br>@ 70% $\dot{V}O_2$ max | Acylated | ↓ | ↔ |
| King et al. 2011b | 14 (M) | 23.2 (2.2) | 60 min<br>intermittent swimming | Acylated | ↓ | ↔ |
| Kraemer et al. 2004 | 6 (M) | NM | Intermittent treadmill exercise session:<br>10 min @ 60% $\dot{V}O_2$ max,<br>10 min @ 75% $\dot{V}O_2$ max,<br>5 min @ 90% $\dot{V}O_2$ max and<br>2 min @ 100% $\dot{V}O_2$ max.<br>Each stage was separated by 3.5 to 4 min of rest | Total | ↔ | ↔ |
| Martins et al. 2007 | 12 (6F:6M) | 22.0 (3.2) | 60 min cycle<br>@ 65% HR max | Total | ↔ | ↔ |

| Study | Participants | | Intervention | Ghrelin form measured | Ghrelin response compared to control | |
|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | During exercise | After exercise |
| Martins et al. 2015 | 12 (5F:7M) | 32.3 (2.7) | Cycling @ 85-90% HR max until 250 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling | Acylated | ↔ | ↓ |
| | | | Cycling @ 70% HR max until 250 kcal expended: continuous cycle | Acylated | ↔ | ↓ |
| | | | Cycling @ 85-90% HR max until 125 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling | Acylated | ↔ | ↔ |
| Marzullo et al. 2008 | 8 (M) | 22.1 (1.2) | Cycle at 60 rpm @ 20 W, increased by 20 W every 4 min until physical exhaustion | Acylated<br>Total | ↓<br>↔ | NM<br>NM |
| | 8 (M) | 33.7 (1.5) | On recovery subjects cycled for two more min effortlessly | Acylated<br>Total | ↓<br>↔ | NM<br>NM |
| Schmidt et al. 2004 | 8 (M) | 22.2 (0.5) | Treadmill exercise @ 50%, 70% and 90% of $\dot{V}O_2$ max | Total | ↔ | ↔ |
| Shorten et al. 2009 | 11 (M) | 24.1 (2.3) | 40 min treadmill run @ 70% $\dot{V}O_2$ max | Acylated | ↔ | ↔ |
| Sim et al. 2014 | 17 (M) | 27.7 (1.6) | 30 min continuous cycling @ 60% $\dot{V}O_2$ peak | Acylated | NM | ↓ |
| | | | 30 min cycling: alternating between 60s @ 100% $\dot{V}O_2$ peak and 240s @ 50% $\dot{V}O_2$ peak | | NM | ↔ |
| | | | 30 min cycling: alternating between 15s @ 170% $\dot{V}O_2$ peak and 60s @ 32% $\dot{V}O_2$ peak | | NM | ↔ |

| Study | Participants | | Intervention | Ghrelin form measured | Ghrelin response compared to control | |
|---|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^2$) | | | During exercise | After exercise |
| Tiryaki-Sonmez et al. 2013 | 9 (F) | 28.3 (1.8) | 60 min running @ 53% $\dot{V}O_2$ max | Acylated | ↓ | ↓ |
| Ueda et al. 2009b | 7 (M) | 22.4 (2.4) | 60 min cycle @ 50% $\dot{V}O_2$ max | Total | ↔ | ↔ |
| | 7 (M) | 30.0 (3.1) | 60 min cycle @ 50% $\dot{V}O_2$ max | | ↔ | ↔ |
| Unick et al. 2010 | 19 (F) | 32.5 (4.3) | 40 min walk @ 70-75% HR max | Acylated | ↔ | ↔ |
| Vatansever-Ozen et al. 2011 | 10 (M) | 22.0 (20.4) | 105 min treadmill run @ 59% $\dot{V}O_2$ max and 15 min @ 70% $\dot{V}O_2$ max | Acylated | ↓ | ↓ |
| Vestergaard et al. 2007 | 29 (8F:21M) | F 23.5 (0.2) M 22.4 (0.4) | Maximal exercise test according to their sport (either long distance cycling, sprint cycling or weight lifting) | Total | NM | ↓ |
| Wasse et al. 2012 | 10 (M) | 24.8 (2.4) | 60 min treadmill run @ 70% $\dot{V}O_2$ max | Acylated | ↔ | ↓ |
| Wasse et al. 2013 | 11 (M) | 23.4 (2.4) | 60 min treadmill run @ 70% $\dot{V}O_2$ max | Acylated | ↓ | ↓ |
| | | | 60 min cycle @ 70% $\dot{V}O_2$ max | | ↓ | ↓ |

BPM, beats per min; F, females; LT, lactate threshold; M, males; NM, not measured; $HR_{max,}$ heart rate maximum; $\dot{V}O_2$ max, maximal oxygen uptake; $\dot{V}O_2$ peak; W, watts; W max, watt max; ↓, decreased; ↑, increased; ↔, no change.

### 2.5.2. Acute exercise and PYY

After an acute bout of exercise, circulating concentrations of PYY have been found to be transiently increased (Broom et al. 2009; Deighton et al. 2013a; Deighton et al. 2013b; Deighton et al. 2014; King et al. 2011a; Martins et al. 2007; Shorten et al. 2009; Ueda et al. 2009a; Ueda et al. 2009b; Wasse et al. 2012). This suggests a role for PYY in exercise induced anorexia, as the reductions in PYY are synchronised with decreases in hunger. However concentrations have also been shown to be unaltered by acute exercise (Balaguera-Cortes et al. 2011; Holmstrup et al. 2013; Martins et al. 2015; Shorten et al. 2009; Sim et al. 2014). These discrepancies are also seen within overweight and obese individuals (Martins et al. 2015; Sim et al. 2014; Ueda et al 2009b). It is likely that the discrepancies in findings are a result of differences in study protocol; study population, form of PYY measured, exercise intensity and mode, and observation period all vary. Despite these discrepancies a recent meta-analysis (Schubert et al. 2014) quantified exercise to increase concentrations of PYY (ES = 0.24). In particular, circulating levels of PYY are seen to rise during a bout of exercise compared to control conditions (Broom et al. 2009; Deighton et al. 2013a; Deighton et al. 2013b; King et al. 2011a; Martins et al. 2007; Ueda et al. 2009a; Ueda et al. 2009b; Wasse et al. 2012) and remain elevated for several hours after (Broom et al. 2009; Deighton et al. 2013b; King et al. 2011a; Ueda et al. 2009a; Wasse et al. 2012). Further to this, PYY responses to a meal are heightened more so after an acute exercise bout vs. control, suggesting exercise enhances satiation (Broom et al. 2009; Cheng et al. 2009; King et al. 2011).

When examining the effects of exercise intensity on concentrations of PYY, the responses seem to be less clear cut than to those of ghrelin. Moderate intensity exercise (~50% $\dot{V}O_2$ max) both increases (Ueda et al. 2009a; Ueda et al. 2009b) and has no effect on concentrations of PYY (Cheng et al. 2009). Discrepancies are still seen at higher intensities (≥60% $\dot{V}O_2$ max (Balaguera-Cortes et al. 2011; Broom et al. 2009; Deighton et al. 2013a; Deighton et al. 2013b; King et al. 2011a; Martins et al. 2007; Shorten et al. 2009; Wasse et al. 2012)). It has instead been suggested that duration of exercise could impact concentrations of PYY, with changes only seen after 60 min of continuous exercise. A 45 min bout of treadmill running at 70% $\dot{V}O_2$ max caused no changes in PYY concentrations (Balaguera-Cortes et al. 2011), whereas a 60 min treadmill run at 70% $\dot{V}O_2$ max increased PYY concentrations (Wasse et al. 2012). However, this is unlikely to be a determinant, as Ueda and colleagues

(2009a) showed 30 min of cycling was enough to alter concentrations of PYY. Conflicting results could be a result of authors measuring different forms of PYY, with some measuring the active form ($PYY_{3-36}$) whilst others have measured the total form. Although it has been suggested that $PYY_{3-36}$ has more potent anorectic effects than total PYY (Chelikani et al. 2005), the responses of $PYY_{3-36}$ and total PYY are similar in response to high protein, high carbohydrate and high fat meals (Batterham et al. 2006). This suggests that measuring either form of PYY should form comparable findings.

To the authors knowledge no study has directly compared the effects of running and cycling on circulating concentrations of PYY. However, studies have compared the effects of resistance training to endurance training (Balaguera-Cortes et al. 2011; Broom et al. 2009). Again these studies found conflicting results. Broom and colleagues (2009) compared a bout of 60 min treadmill running at 70% $\dot{V}O_2$ max with a 90 min resistance training session. PYY concentrations increased during and after the run, but were unaffected by the resistance session. Perhaps strengthening the relationship between PYY and appetite, sensations of hunger were only found to decrease during the bout of endurance exercise. However, PYY concentrations were also observed to remain elevated for the rest of the trial day whereas sensations of hunger returned to control values. Balaguera-Cortes and colleagues (2011), compared a 45 min treadmill run at 70% $\dot{V}O_2$ max with a 90 min resistance training session and found no effect of either exercise session on concentrations of PYY. Further research is required to determine the true effects of different modes of exercise on circulating concentrations on PYY and how any changes impact sensations of appetite and energy intake.

### 2.5.3. Acute exercise and GLP-1

Few studies have examined the effects of acute exercise on GLP-1, with mixed findings. The majority of studies have reported increases of GLP-1 during exercise (Martins et al. 2007; Martins et al. 2015; Ueda et al. 2009a; Ueda et al 2009b), with concentrations remaining elevated compared to control conditions for the rest of the observation period (Martins et al. 2007; Martins et al. 2015; Ueda et al. 2009a; Ueda et al 2009b). However Unick et al. (2010) reported no change in GLP-1 concentrations during exercise, and that concentrations were reduced after exercise. Despite these discrepancies, a recent meta-analysis found GLP-1 to be increased by acute exercise (ES = 0.28 (Schubert et al. 2014)).

Increases in GLP-1 concentrations during exercise coincided with decreases in hunger (Martins et al. 2007; Ueda et al 2009a), implying that GLP-1 may also play a role in exercise

induced anorexia. However, Martins et al. (2015) reported no difference in hunger during exercise despite seeing increases in GLP-1 concentrations. The association between GLP-1 and hunger is further weakened as GLP-1 concentrations have been shown to remain elevated later in the trial day, whilst hunger levels return to control values (Martins et al. 2007), remain suppressed after exercise (Ueda et al. 2009a) or are found to be similar to control values (Martins et al. 2015).

Due to the small number of studies looking at exercise and GLP-1, it is hard to make judgements on the effect that exercise intensity and mode, BMI and sex have on GLP-1 concentrations. In general it would appear that there is no effect of mode, with both cycling and running increasing GLP-1 concentrations (Martins et al. 2007; Martins et al. 2015; Ueda et al. 2009a; Ueda et al 2009b). Similarly, exercise intensity does not seem to influence GLP-1 responses, with both moderate and vigorous intensities causing increases in GLP-1 (Martins et al. 2007; Martins et al. 2015; Ueda et al. 2009a; Ueda et al 2009b). Further research is required to determine the effects of resistance exercise on GLP-1 responses. In addition, comparisons between sex, BMI and exercise intensity will help with the understanding of GLP-1effect on appetite and energy intake.

### 2.5.4. Acute exercise and leptin

The effects of exercise on leptin concentrations have received a lot of interest. As leptin levels have been shown to be up- and down- regulated through alterations in energy intake, focus has been placed on determining if manipulations in energy expenditure via exercise can influence leptin concentrations. After acute bouts of exercise, leptin concentrations have been shown to be reduced (Elias et al. 2000; Fisher et al. 1985; Kraemer et al. 1999) or remain unchanged (Racette et al. 1997; Torjman et al. 1999; Weltman et al. 2000) in the immediate hours after exercise. When leptin concentrations were controlled for haemoconcentration and circadian rhythm, exercise values no longer differed from control conditions. However, when observation time is expanded, leptin concentrations are reduced 48 h after exercise (Essig et al. 2000; Olive & Miller 2001; Yang et al. 2014). It should also be noted that these studies induced a large energy deficit; reductions in leptin could be reflections of alterations in energy balance rather than being caused by the exercise bout per-se (Borer et al. 2009; Hilton & Loucks 2000).

More recently, the effects of an acute bout of treadmill running (90 min at 70% $\dot{V}O_2$ max) on leptin concentrations in the subsequent 24 h was examined (King et al. 2015). In agreement

with previous findings, leptin concentrations expressed a delayed reduction. Specifically, a large energy expenditure of ~5020 kJ, caused a 33% reduction in fasting concentrations of leptin 20 h after exercise. This reduction was sustained throughout this second observation day (20-27 h post-exercise). Participants were required to follow strict dietary and physical activity control in the 27 h post-exercise; authors could therefore postulate that the reduced leptin concentrations were in response to the disturbance in energy balance.

The effects of resistance training on leptin concentrations have also been explored. Kanaley et al. (2001) found that in healthy individuals, lower and upper body resistance exercises (3-RM test on nine muscle groups) had no influence on 24 h leptin concentrations. However, when a large energy deficit (~850 kcal) was generated through resistance exercise (50 sets of four exercises), leptin concentrations were reduced 9, 12 and 13 h after exercise (Nindl et al. 2002). This finding supports previous work, showing that leptin concentrations are only reduced after greater energy expenditures, and subsequent disturbances in energy balance.

Further research is required to compare the effects of exercise and sex on leptin concentrations, and to compare responses of resistance and aerobic exercise. To help with the understanding of leptin's time course, longer observation periods and more frequent blood sampling are required, with stringent physical activity and diet controls. Together these will better explain the responses of leptin to exercise, help with designing the most effective exercise strategy for positive leptin concentration adaptations and aid weight management strategies.

## 2.6. Summary

The current literature suggests that in healthy lean individuals, acute bouts of exercise cause transient reductions in appetite during exercise, and that these rapidly return to control values after exercise. This is concordant with transient decreases in acylated ghrelin and increases in PYY and GLP-1. Energy intake after exercise generally remains unaltered, implying there is no compensatory response to acute exercise and that a negative energy balance is induced over the trial period. However, research is limited by short observation periods, lack of direct comparison between males and females, and overweight, obese and lean individuals. Furthermore, study protocols lack resemblance to 'real-life' scenarios, and consequently limit the extent to which findings can be applied to the general population. The role of appetite regulatory hormones in appetite and energy intake responses still remains unclear. This thesis therefore aims to examine the responses of appetite regulatory hormones, subjective appetite sensations and energy intake over longer periods and after repeated bouts of exercise. In addition comparisons will be made between lean and ow/ob individuals and between males and females.

# CHAPTER III

## A comparison of two methods of blood sample collection for determining plasma acylated ghrelin concentration in humans

### 3.1. Introduction

Acylated ghrelin is a unique orexigenic hormone found to stimulate appetite in humans when circulating concentrations are high (Cummings et al. 2001; Druce et al. 2006; Wren 2001a). Originally discovered in rats, ghrelin is a purified ligand made of 28 amino acids. Ghrelin is synthesised within the stomach and enters the circulation, with feeding being considered the most important factor for its release. Concentrations are seen to increase in short term fasting and decrease after feeding. Ghrelin is found in two forms in the circulation; acylated and desacylated. In the acylated form the serine 3 molecule is covalently linked to octanic acid via an ester bond (Sato 2012). An enzyme called GOAT is responsible for the conversion of desacylated ghrelin to acylated ghrelin (Yang 2008). With regards to appetite regulation, ghrelin is considered to be in its active form when it is acylated allowing ghrelin to cross the blood brain barrier and act on receptors in the brain (Kojima et al. 1999). However, the majority of ghrelin found within the circulation is desacylated, a form considered to be inactive, with respect to appetite regulation. Therefore when measuring total ghrelin concentrations, the effects of acylated ghrelin could be masked.

The ester bond formed by GOAT is both chemically and enzymatically unstable and the breakdown of this molecule can occur easily during storage and handling of these samples. In particular, the ester bond is easily cleaved and digested by a number of cellular proteases (Sato 2012). Consequently effective sample collection and processing protocols have been developed to allow for accurate determination of acylated ghrelin from plasma samples (Blatnik & Soderstrom 2011; Hosada et al. 2006; Hosada et al. 2012). Blatnik & Soderstrom (2011) pre-treated plasma with aprotinin, 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and a protease inhibitor cocktail. AEBSF and protease inhibitors yielded approximately three- and two-fold higher acylated ghrelin concentrations than aprotinin in fasting and fed samples, respectively. Furthermore, work by Hosoda and Colleagues (2006, 2012) identified that collecting blood samples with ethylenediaminetetra-acetic acid (EDTA) and aprotinin was most effective at stabilising acylated ghrelin concentrations in the plasma, compared with EDTA, heparin sodium and no pre-treatment.

Subsequent acidification of plasma samples to pH 4 using 1 M hydrochloric acid further preserved acylated ghrelin concentrations in these samples.

Within our laboratory, we currently use manufacture guidelines to preserve acylated ghrelin in plasma samples. Blood samples are collected with EDTA, p-hydroxymercuribenzoic acid (PHMB), potassium phosphate buffer (PBS), and sodium hydroxide (NaOH). Plasma is subsequently acidified and stored at -20°C until frozen and then transferred to -80°C for later analysis. This method requires plasma samples to be spun twice using a centrifuge, making this procedure time consuming. In addition, PHMB preparation requires a fume cupboard, as this chemical is toxic when inhaled. The additional resources required to follow this method could prevent or deter other research groups from measuring acylated ghrelin within human plasma. Furthermore, the cost associated with AEBSF, justifies identifying if the quicker, cheaper and safer method outlined by Hosoda and Colleagues (2006, 2012) is as effective at preserving acylated ghrelin concentrations and would facilitate research in this area. Therefore, the purpose of this study was to compare the use of aprotinin vs. PHMB for the determination of circulating acylated ghrelin in human plasma.

## 3.2. Methods

### 3.2.1. Participants

Loughborough University's Ethics Advisory Committee approved the following study and all participants provided written informed consent before taking part in this research experiment. Participants were recruited from Loughborough University by word of mouth and email advertisement.

Participants were provided with a participant information sheet, describing the purpose, protocol, demands and potential risks/harms of the study. After a verbal explanation of the study and discussion of any questions, participants completed an informed consent form (Appendix A) and a health screen questionnaire (Appendix B) before any experimental procedures began.

Inclusion criteria for participation were as follows: not taking any medication had no history of infectious disease within the previous month of the study or a history of cardiovascular or metabolic disease.

Nine healthy participants, five males and four females, volunteered to take part in this study. The physical characteristics of the participants were as follows: age 26 (3) y, BMI 22 (2) $kg \cdot m^{-2}$, body mass 68 (10) kg.

### 3.2.2. Preliminary visit

Prior to the main trial, participants visited the laboratory to provide anthropometric measures and to be familiarised with the laboratory environment. Specifically, height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany) and body mass was measured to the nearest 0.1 kg using a digital scale (Seca Ltd, Germany). For measurements of height and weight, participants wore light clothing and removed their shoes. BMI was subsequently calculated as weight in kilograms divided by squared height in metres.

### 3.2.3. Experimental protocol

The main trial lasted 1 h and began at 08:45 after an overnight fast of at least 10 h. The main trial commenced with the collection of a fasting venous blood sample. Participants were provided with a standardised breakfast meal at 09:00. This was consumed within 15 min. The trial ended approximately 30 min after the end of the test meal with the collection of a

postprandial venous blood sample. Participants rested between blood samples (sitting reading, working at a desk or watching television).

### 3.2.4. Standardised breakfast

The breakfast meal consisted of jam sandwiches (brown bread, margarine and strawberry jam), banana and orange juice. The macronutrient content of this meal was 72% carbohydrate, 10% protein and 18% fat. The breakfast meal provided 30% of the estimated daily energy needs for a healthy weight inactive individual (643 kcal for males, 578 kcal for females).

The energy needs for an inactive individual were calculated using the Mifflin equation and a physical activity factor of 1.4 (Mifflin et al. 1990). This equation was selected as it considers the participants age, height, body mass and sex allowing for a more accurate estimation. A 1.4 activity factor was selected to represent the sedentary nature of the experimental visit. The Mifflin equations for males and female are below:

Male resting energy expenditure = (10 x body mass) + (6.25 x height) – (5 x age) + 5;

Female resting energy expenditure = (10 x body mass) + (6.25 x height) – (5 x age) – 161.

Water was available *ad libitum* throughout the trial.

### 3.2.5. Blood sampling

Venous blood samples were taken by venepuncture of an antecubital vein. At each sampling point blood samples were collected into two pre-chilled 4.9 mL pre-chilled EDTA monovettes (Sarstedt, Leicester, UK) for the measurement of acylated ghrelin. One 4.9 mL EDTA monovette was treated with a 50 µL solution containing PHMB/PBS/NaOH (PHMB method). Immediately after the collection of a blood sample, this monovette was spun in a refrigerated centrifuge (Heraeus Labofuge 400R, Thermo Electron, Osterode, Germany) at 1165 x *g* for 10 min at 4°C. The plasma supernatant was immediately dispensed into a Polypropylene tube and 100 µL of 1 M hydrochloric acid was added per mL of plasma. Samples were then spun at 1165 x *g* for 5 min at 4°C prior to storage in 2 mL Eppendorf tubes at -20°C for later analysis. The second 4.9 mL EDTA monovette was treated with aprotinin (Nordic Pharma, Reading, UK) at a final concentration of 500 KIU per mL of blood (aprotinin method). This monovette was spun in a refrigerated centrifuge at 1165 x *g* for 10 min at 4°C. The plasma supernatant was immediately dispensed into an Eppendorf tube and

100 µL of 1 M hydrochloric acid was added per mL of plasma. The plasma was stored in the Eppendorf tube at -20°C for later analysis.

### 3.2.6. Biochemical analysis

Plasma acylated ghrelin concentrations were determined using a commercially available enzyme linked immunosorbent assay kit (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader to measure absorbance (Expert plus, ASYS Atlantis, Eugendorf, Austria). Precision of analysis was ensured by the quantification of an internal quality control on each assay plate. To eliminate inter-assay variation, samples from each participant were analysed on the same run. The within-batch coefficients of variation for the assays were 10.9%.

### 3.2.7. Statistics

Data were analysed using IBM SPSS statistics, version 21.0 for Windows. Data for the two preservation methods were compared using Student's paired t-tests. The Pearson product moment correlation coefficient was used to examine the relationship between the two preservation methods. A Bland-Altman analysis was used to assess the limits of agreement (LOA) between the two preservation methods, comparing the aprotinin method to the PHMB method. The 95% LOA were determined as the mean difference ± 1.96 standard deviations (s) of the mean difference. It is expected that the 95% LOA include 95% of the differences between the two measures. Statistical significance was accepted at 5%. Results are shown as mean (SD) in text and tables.

### 3.3. Results

The Pearson product moment correlation coefficient indicated that the two methods were moderately correlated at both the pre- and post-meal time points (see Table 3.1). However blood samples treated with aprotinin yielded significantly lower plasma acylated ghrelin concentrations that those treated with PHMB. The mean difference was 14% for the pre-meal values and 18% for the post-meal values.

**Table 3.1.** Acylated ghrelin ($pg \cdot mL^{-1}$) values at the pre- and post-meal time points assessed via the two methods, mean difference between the methods, t-test, correlation and LOA values for acylated ghrelin.

| | **PHMB** | **Aprotinin** | **Mean difference** | **T-test** | **Correlation** | **-1.96s** | **+1.96s** |
|---|---|---|---|---|---|---|---|
| Pre-meal | 184 (80) | 159 (66) | -25 (27) | P=0.024 | r=0.73, P=0.026 | -77 | 28 |
| Post-meal | 97 (55) | 80 (55) | -17 (9) | P<0.001 | r=0.75, P=0.021 | -35 | 1 |

Values are mean (SD). N=9. Mean difference, aprotinin minus PHMB; -1.96 and +1.96 show the lower and upper 95% LOA when using aprotinin.

Bland-Altman analysis suggests that for both pre- and post-meal concentrations of acylated ghrelin there was no relationship between the difference and mean of the two methods. Limits of agreement highlight a large variance when using the aprotinin method (See Figure 3.1 and 3.2).

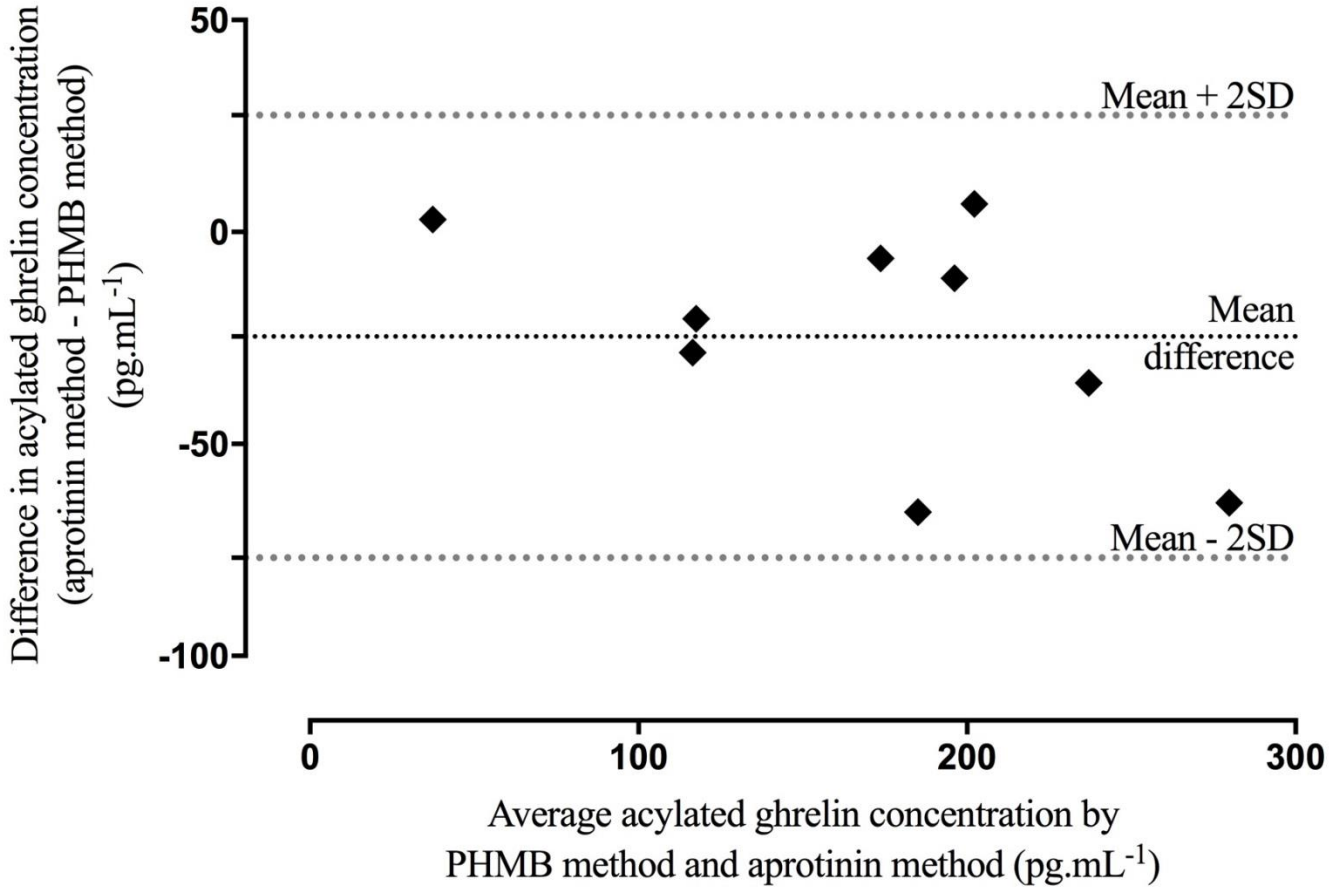


**Figure 3.1.** Difference against mean for acylated ghrelin data, pre-meal.

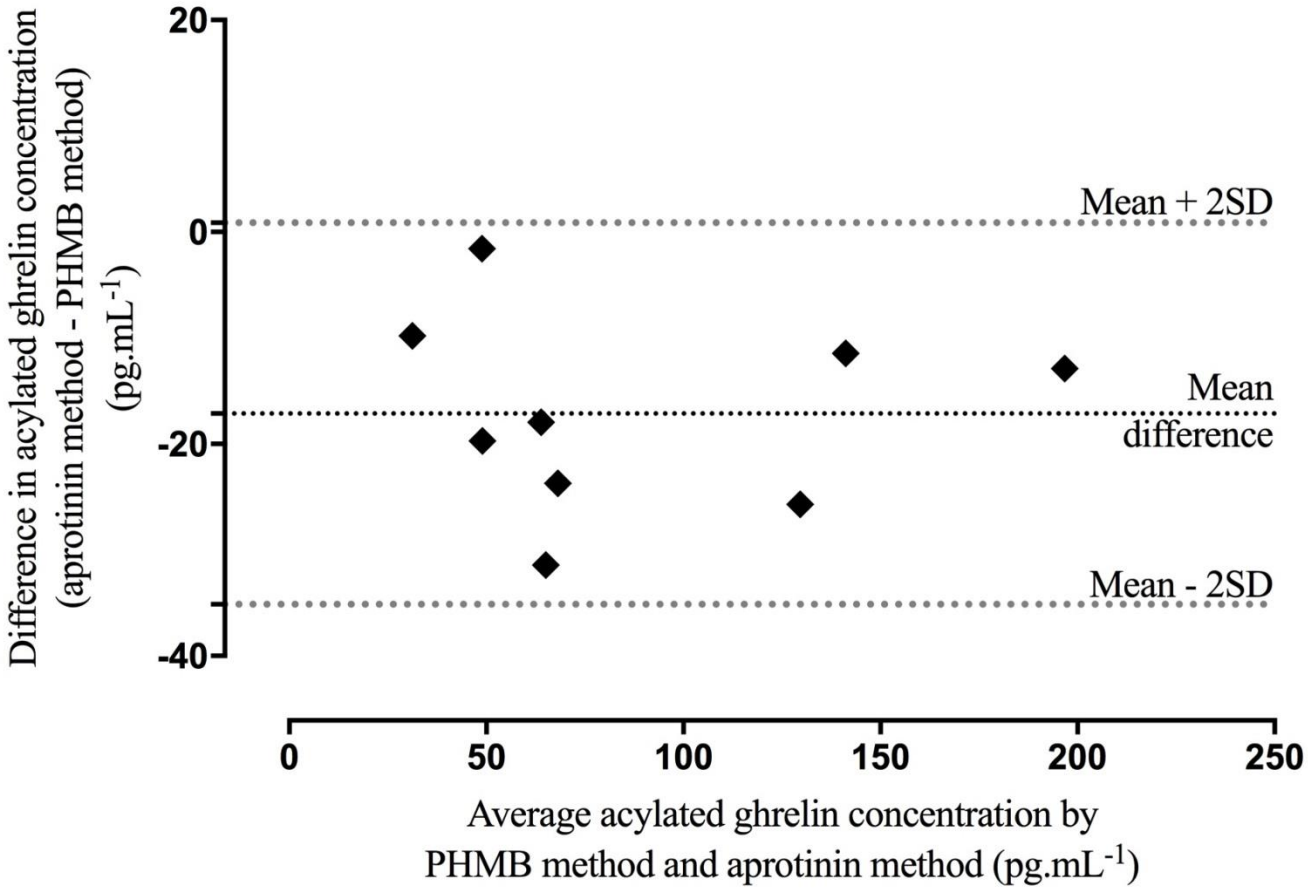


**Figure 3.2.** Difference against mean for acylated ghrelin data, post-meal.

### 3.4. Discussion

These findings suggest that the aprotinin method is less effective for preserving plasma acylated ghrelin than the PHMB method. The LOA values suggest that with a PHMB determined value of 184 pg·mL$^{-1}$ the aprotinin determined value could lie anywhere between 107 and 212 pg·mL$^{-1}$. Similarly, for a PHMB determined value of 97 pg·mL$^{-1}$ the aprotinin determined value could lie anywhere between 62 and 98 pg·mL$^{-1}$.

Despite aprotinin being less effective at preserving acylated ghrelin, this method was still able to detect a typical acylated ghrelin response post-feeding, and mirrored the pattern of change shown by the PHMB method. If time is limited or a fume cupboard is unavailable, these results suggest that it is still viable to use the aprotinin method to identify patterns of acylated ghrelin within human plasma.

However, for the studies within this thesis, blood collection tubes were pre-treated using the PHMB method for the determination of acylated ghrelin.

# CHAPTER IV

## Appetite, appetite hormone and energy intake responses to two consecutive days of running in healthy young men

### 4.1. Introduction

The interaction between exercise, appetite control and energy balance has direct relevance for the implementation of exercise as a therapeutic strategy for weight control. Over the last decade, many studies have investigated the impact of acute exercise, and exercise training, on appetite perceptions, food intake and energy balance (Hopkins et al. 2010). Currently, a disparity exists whereby on the one hand evidence suggests that exercise training (4-12 months) does not induce substantial weight loss due to compensatory responses i.e. increases in appetite and food intake (King et al. 2007; Hopkins et al. 2014; Wing 1999); while on the other hand acute laboratory-based studies consistently document that acute exercise has no influence *ad libitum* food intake in the hours after exercise (King et al. 2013; Wasse et al. 2012). Indeed, a recent meta-analysis concluded that acute exercise has a trivial effect on subsequent energy intake (Schubert et al. 2013). Studies have consistently shown a transient suppression of appetite after moderate to vigorous intensity acute exercise, lasting up to 15 minutes (Alajmi et al. 2016; Broom et al. 2007; Deighton et al. 2013a; King et al. 1994; King et al. 2011a). However, this is not seen to extend later into the trial day. It is possible that this inconsistency between outcomes is related to the rather loose coupling which exists between perturbations to energy balance induced by exercise and subsequent energy intake (Blundell & King 1998). Compensatory alterations in appetite and food intake in response to single bouts of exercise could possibly occur over a longer duration than that previously examined in acute laboratory studies i.e. over more than 24 h. To date, only two studies have looked at this proposition in detail under strict laboratory conditions. In one study, appetite responses before and after a test meal were examined on the day after a single bout of moderate-intensity exercise (Heden et al. 2013). The researchers reported that exercise on the previous evening reduced perceptions of fullness in obese volunteers, but had no influence in lean individuals. More recently, Beaulieu et al. (2015) examined appetite and *ad libitum* energy intake responses over 34 h with an acute bout of sprint interval exercise being performed at the beginning of the trial. The researchers observed a transient suppression of appetite, characteristic of exercise-induced anorexia (King et al. 1994), but then observed a latent increase in hunger and motivation to eat several hours after exercise leading to increased 34 h

area under the curve (AUC) values compared to control conditions. There was no effect of exercise on energy intake. However, it should be noted that in this study appetite perceptions and *ad libitum* energy intake may have been compromised by reports of nausea and vomiting during and after exercise (Seimon et al. 2010). Additional work is needed to extend these preliminary findings in order to identify the initial stage of compensation and to better understand the regulatory mechanisms.

Advancements in knowledge about the neuro-humoral regulation of appetite and energy homeostasis have catalysed research investigating the influence of exercise on hormones implicated in appetite control and energy balance (King et al. 2013; Schubert et al. 2014; Stensel 2010). Findings from this body of work were recently pooled in a meta-analysis which concluded that acute exercise has a small to moderate effect on appetite hormones, suppressing acylated ghrelin, and stimulating PYY, GLP-1 and pancreatic polypeptide (PP) (Schubert et al. 2014). These alterations are transient, and often coincide with appetite loss during and after moderate to high intensity exercise (however, there is often little effect on subsequent changes in food intake). Unfortunately, at present, there is a dearth of information regarding the more protracted effects of exercise on these important mediators of appetite control. As an exception, Heden et al. (2013) observed attenuated fasting and post-meal suppression of acylated ghrelin after a standardised breakfast meal in lean individuals on the morning after exercise the prior evening. This response is suggestive of a compensatory mechanism serving to defend energy balance i.e. less effective suppression of an appetite stimulating signal. No changes in acylated ghrelin were seen in obese individuals. Unfortunately in this study energy intake was not examined on the day after exercise; thus future research is required to identify if the highlighted changes in acylated ghrelin influence subsequent energy intake. It has been hypothesised that compensatory responses in appetite regulatory hormones and energy intake may be delayed, and future research is needed to investigate this by examining longer term responses to energy imbalance.

The present investigation sought to advance previous research by characterising the impact of exercise, performed on two consecutive mornings, on appetite, *ad libitum* food intake and key hormones implicated as important regulators of appetite and eating (acylated ghrelin, PYY, leptin and insulin). Specifically, by eliciting a marked perturbation to energy homeostasis by performing two prolonged bouts of moderate-high intensity treadmill running, we imposed a notable and unfamiliar stimulus (for the participants) through which to assess the impact of exercise in controlled conditions over a more prolonged period than typically implemented.

Based on previous findings, in response to this stimulus, it was hypothesised that appetite and energy intake would be unaltered on the first day of exercise, but that on the second day, compensatory increases in appetite, energy intake and acylated ghrelin would be seen. Conversely, compensatory decreases in circulating PYY and leptin may be apparent.

## 4.2. Methods

### 4.2.1. Participants

Loughborough University's Ethics Advisory Committee approved the following study and all participants provided written informed consent before taking part in this research experiment. Participants were recruited from Loughborough University by word of mouth, email and poster advertisement.

The inclusion criteria for participation were as follows;

- male
- aged 18-40 y
- non-smoker
- not taking medication
- weight stable for at least 3 months before the study (St Jeor et al. 1997)
- not dieting or exhibiting extreme eating habits
- no history of cardiovascular or metabolic disease
- tolerance for food items provided during the experiment
- sufficient ability to complete the exercise protocol

Fifteen healthy males (18-24 y) matching the inclusion criteria volunteered to participate in this study. Most participants recruited were students studying at Loughborough University, who were recreational games players, not accustomed to performing repeated, prolonged bouts of moderately-high intensity running. Table 4.1 describes the participant characteristics.

**Table 4.1.** Characteristics of the study participants.

| **Characteristic** | **Mean (SD)** |
|---|---|
| Age (y) | 21.1 (1.7) |
| BMI ($kg \cdot m^{-2}$) | 23.0 (1.9) |
| Body mass (kg) | 74.8 (6.8) |
| Waist circumference (cm) | 78.5 (5.2) |
| Treadmill peak oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$) | 57.9 (4.2) |

Values are mean (SD). N=15.

### 4.2.2. Preliminary trial

Prior to the main trials, participants visited the laboratory to undergo screening and preliminary anthropometric measures. Participants were also familiarised with the laboratory environment, test equipment and procedures before completing two preliminary exercise tests.

Participants were provided with a participant information sheet, describing the purpose, protocol and demands of the study (including any potential risks or harms). After a verbal explanation of the study and discussion of any questions participants completed an informed consent form (Appendix A) and a health screen questionnaire (Appendix B) before any experimental procedures began. Participants also completed questionnaires assessing physical activity levels (Appendix C) and a questionnaire assessing eating habits (Three-Factor Eating Questionnaire (TFEQ), Appendix D, Stunkard & Messick 1985) to screen participants who exhibited extreme dietary tendencies (including disinhibited and restrained eating). Participants scoring 11 or more for any factor on the TFEQ were determined to exhibit restrained or disinhibited eating tendencies, and consequently excluded from taking part. This score was selected, as it represents the mean suggested 'high' cut off for all three behaviours measured in this questionnaire (Stunkard & Messick 1985). Those who score either high or in the clinical range for these variables could confound appetite and energy intake data, justifying their exclusion from the study. A food preference questionnaire (Appendix E) was also completed to ensure participants enjoyed >80% of food items available.

Height and body mass were measured as described in Chapter 3. Waist circumference was determined as the narrowest part of the torso between the xiphoid process and the iliac crest (Ross et al. 2008). Measurements were taken in duplicate to the nearest 0.1 cm. If the measurements were within 1 cm of each other, the mean was calculated. If these measures differed by more than 1 cm, a third measurement was repeated, and so on (WHO, 2011).

Participants completed two preliminary exercise tests on a level motorised treadmill (Technogym Excite Med, Cesena, Italy). After familiarisation with the protocol and treadmill, participants completed a 16 min progressive submaximal test to determine the relationship between treadmill speed and oxygen consumption. The test was designed to exercise participants through a range of intensities from moderate to vigorous but was not exhaustive. The test was continuous in nature, subdivided into four, 4 min segments. The initial treadmill speed was set between 8 and 11 $km \cdot h^{-1}$ depending on the fitness level of each participant and was increased between 1 and 1.5 $km \cdot h^{-1}$ after the completion of each 4 min stage. Oxygen

consumption and carbon dioxide production were determined in the last min of each stage from expired air samples collected into Douglas bags (Plysu Protection Systems, Milton Keynes, UK) and analysed using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and paramagnetic oxygen/infra-red carbon dioxide analyser (Series 1400, Servomex, Crowborough, East Sussex, UK). Prior to sample analysis, the analysers were calibrated with certified reference gases. Expired air temperature was determined using a thermistor during evacuation (Edale, type 2984, Model C, Cambridge, UK). Expired air samples were corrected to standard temperature and pressure (dry). The Borg scale was used to obtain the participants rating of perceived exhaustion (RPE) at these time points (Borg 1973). This scale ranged from six (no exertion) to 20 (maximal exertion). Heart rate was measured throughout using short-range radio telemetry (Polar T31; Electro, Kempele, Finland).

After a 20 min rest participants completed a $\dot{V}O_2$ peak test. An incremental uphill treadmill protocol was used to test participants until volitional exhaustion. Run speed was constant and set at a speed corresponding to a heart rate of ~150 beats·min$^{-1}$. Treadmill gradient began at 3.5% and increased by 2.5% at 3 min intervals until exhaustion, which was reached within 9-12 min. Samples of expired air were collected for 1 min, 1.75 min into each stage i.e. 1:45-2:45, 4:45-5:45 etc. A final expired air sample was collected when participants indicated that they could continue for only one more minute. Heart rate and RPE were also collected at these time points.

To ensure attainment of $\dot{V}O_2$ peak participants were required to meet ≥2 of the following criteria:

- plateau in oxygen consumption
- heart rate within 10 beats.min$^{-1}$ of age predicted maximum
- non-protein respiratory exchange ratio (RER)≥1.1
- RPE≥19 (Cooke 2001)

The achieved $\dot{V}O_2$ peak of each participant was used in combination with individual running speed-oxygen uptake regression equations to determine a running speed corresponding to 70% $\dot{V}O_2$ peak. Participants started at this speed during main trials, with subtle adjustments made if necessary i.e. for cardiovascular drift.

### 4.2.3. Experimental protocol

Participants completed two, two-day experimental trials (exercise and control) in a randomised crossover design with each trial being separated by at least one-week (Figure 4.1). Each trial lasted 31 h and required participants to attend the laboratory for 7 h on day one and day two.

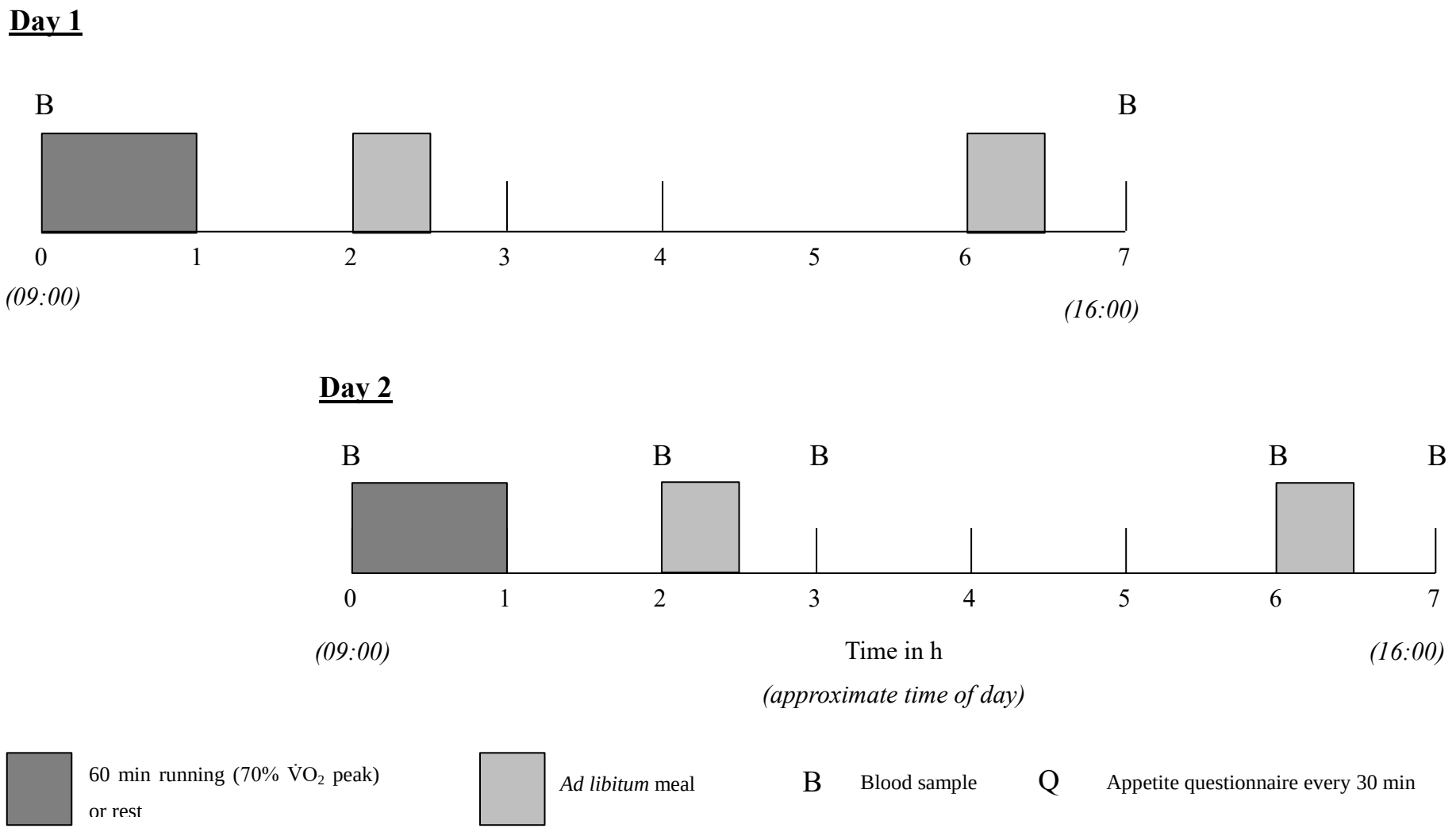


**Figure 4.1.** Schematic representation of the main trial protocol.

Each trial commenced at 09:00 after an overnight fast of at least 10 h. Before the first trial participants recorded their dietary intake for 48 h using a weighed food record. Participants were instructed to consume identical amounts of food and drink items in the 48 h before the second trial to ensure dietary standardisation. After 23:00 on the evening prior to each main trial participants refrained from eating, however water was available *ad libitum*. Alcohol, caffeine and structured physical activity were not permitted during this time. Participants were asked to confirm that they had adhered to the dietary and exercise standardisations before starting each experimental trial day.

Day one of the exercise trial commenced with a 60 min continuous treadmill run (Technogym Excite Med, Cesena, Italy) at a speed predicted to elicit 70% of $\dot{V}O_2$ peak. Expired air samples were collected at 15, 30, 45 and 60 min during exercise to estimate energy expenditure and to monitor the intensity with subtle adjustments being made to the treadmill speed if necessary. Heart rate and RPE were also assessed at these times. Energy expenditure during the exercise session was calculated from oxygen uptake and carbon dioxide production (Frayn 1983). These measurements were also used to calculate RER (carbon dioxide production divided by oxygen consumption).

After the run, participants rested in the laboratory for 6 h (sitting, reading, working at a desk or watching films). At the end of this time participants left the laboratory and were instructed to remain physically inactive in the time spent away from the laboratory in-between days one and two (<5000 steps; Tudor-Locke & Bassett 2004) and this was confirmed by a pedometer (Yamax DIGI-WALKER SW200, San Antonio, USA) which each participant wore. Participants returned the next morning at 09:00 (minimum 10 h overnight fast) to commence day two of the trial. Day two of main trials was identical to day one.

The procedures in the control trial were identical to the exercise trial except that no exercise was performed. The sole exception to this was that resting expired air samples were collected during the first hour of each control trial day to assess resting metabolic rate. These data subsequently permitted the calculation of net exercise energy expenditure i.e. gross exercise energy expenditure minus resting energy expenditure.

### 4.2.4. Appetite measures

During the laboratory phase of the main trials, appetite perceptions (hunger, fullness, satisfaction and prospective food consumption (PFC)) were assessed at baseline and at every 30 min thereafter using validated visual analogue scales (Appendix F, Flint et al. 2000). Each visual analogue scale was 100 mm in length, with opposing descriptors positioned at either end (e.g. 'I am not hungry at all' and 'I have never been more hungry'). Participants rated their appetite by making each scale with a vertical mark. Participants could not refer back to previous ratings when completing the scales. The scales were analysed by measuring the horizontal distance from the left hand side anchor of the scale to the point marked by the participant.

### 4.2.5. *Ad libitum* meals and overnight food bags

Energy and macronutrient intake were assessed at two *ad libitum* meals provided on both days of the exercise and control trials. Specifically, at 2 h a cold buffet meal was provided from which participants were free to consume food *ad libitum* for 30 min. Acceptability of the buffet items was ensured during the preliminary trial via the completion of a food preference questionnaire (Appendix E). The questionnaire required participants to rate preselected food items on a Likert scale ranging from one (dislike extremely) to ten (like extremely). Participants were excluded from the study if they rated >20% of the items at 4 or less. The items available are listed in Appendix G. The buffet foods were presented identically on each occasion and were in excess of expected consumption. Participants were instructed to eat until 'comfortably full' and informed that additional food was available if desired.

At 6 h *ad libitum* energy intake was assessed using a hot homogenous pasta meal. This method attempted to blind the participants from the amount of food they had eaten. The *ad libitum* meal was composed of white fusilli (Tesco Fusilli Pasta Twists), tomato sauce (Tesco Bolognese sauce), Cheddar cheese (Tesco British Mature White Cheddar) and olive oil (Tesco Extra Virgin Olive Oil). For each trial, 500 g of pasta was cooked in 2 L of unsalted water at 900 W for 11 min. The pasta was drained with the weight recorded 4 min after cessation of cooking. The cooked pasta was then thoroughly mixed with 156 g of cheese, and 375 g of pasta sauce, until homogenous in nature. The pasta was then covered and allowed to cool completely. Approximately 30 min before the *ad libitum* meal was due to be served; 50 g of olive oil was added and mixed into the pasta meal. The pasta was then reweighed and

divided equally between 5 smaller serving bowls. Immediately prior to serving the meal, a small bowl was reheated at 900 W for 1 min in the microwave. The bowl of pasta was allowed to cool for 90 s before weighing and presented to the participant. The next bowl was subsequently heated, cooled and replaced the partially consumed bowl. Each returned bowl was weighed. This process was continued until the participant felt 'comfortably full', with no time limit set. The macronutrient composition of the meal was balanced (52% carbohydrate, 14% protein and 34% fat), and designed to meet current UK dietary guidelines (Food Standards Agency, 2007).

Participants consumed meals in isolation so that social influence did not affect food selection or quantity eaten (except for the replacement of fresh, and retrieval of used pasta bowls). Leftovers were weighed and food consumption was determined as the weighed difference of items before and after each meal. The energy and macronutrient content of the items consumed were determined from manufacturers' values. Previously, authors have shown a high level of reproducibility when measuring energy intake by *ad libitum* buffets (Arvaniti et al. 2008; CV 8.2%) and hot homogenous meals (Gregersen et al. 2008; CV 14.5%). This suggests that these methods are reliable for assessing *ad libitum* energy intake in a laboratory environment.

For the time spent away from the laboratory in-between the visits on day one and day two participants were free to select, and consume, a selection of items presented at the cold buffet meal. Participants were permitted to consume these items after leaving the laboratory on day one and until 23:00. The food items available for selection are listed in Appendix H. Leftovers were returned the next day to determine actual consumption. Water was available *ad libitum* throughout trials.

### 4.2.6. Blood sampling

Venous blood samples were collected at 0 and 7 h on day one of each trial, and at 0, 2, 3, 6 and 7 h during day two. Both samples on day one, and the baseline sample on day two, were collected by venepuncture of an antecubital vein. The remaining blood samples on day two (2, 3, 6 and 7 h) were collected using a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) inserted into an antecubital vein. Patency of the cannula was maintained by flushing with 10 mL of non-heparinised saline (0.9 % (w/v) sodium chloride, Baxter Healthcare Ltd., Norfolk, UK) after each blood sample collection. To avoid dilution of the subsequent sample residual saline was drawn off immediately before each collection using a 2 mL syringe.

Venous blood samples were taken into two pre-chilled EDTA monovettes (4.9 mL and 9 mL) (Sarstedt, Leicester, UK) for the measurement of plasma total PYY, acylated ghrelin, leptin, glucose and insulin. To prevent the degradation of acylated ghrelin, blood samples collected into the 4.9 mL monovette were pre-treated and processed following manufacturer instructions (see Chapter 3). The remaining analytes were measured from the resulting plasma collected into the 9 mL monovette. After blood collection, the 9 mL monovette was immediately spun at 1165 x *g* for 10 min at 4°C in a refrigerated centrifuge (Heraeus Labofuge 400R, Thermo Electron, Osterode, Germany). The plasma supernatant was aliquoted into 2 mL Eppendorfs and stored at -20°C for future analysis.

At each sampling point duplicate 20 µL blood samples were collected into micropipettes for the determination of haemoglobin, and triplicate 20 µL blood samples were collected into heparinised micro haematocrit tubes for the determination of blood haematocrit concentration. These data were used to estimate plasma volume changes over time (Dill & Costill 1974). Haematocrit was determined using a microliter-haematocrit centrifuge (MIKRO, 20, Andreas Hettich GmbH and Co.KG, Tuttlingen, Germany). Haemoglobin was determined using cyanmethaemoglobin method with the aid of a spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, UK).

### 4.2.7. Biochemical analysis

Commercially available enzyme immunoassays were used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Billerica, USA), leptin (R&D Systems, Minneapolis, USA) and insulin (Mercodia, Uppsala, Sweden) with the aid of a plate reader to measure absorbance (Expert Plus, ASYS Atlantis, Eugendorf, Austria and Varioskan Flash Multiple Mode Reader, Vantaa, Finland). Plasma glucose concentrations were determined by enzymatic, colorimetric methods using a bench top analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France). Precision of analysis was ensured by quantification of an internal quality control exhibiting high and low values. To eliminate inter-assay variation, samples from each participant were analysed on the same run. The within-batch coefficients of variation for the assays were as follows: acylated ghrelin 10.9%, total PYY 7.7%, leptin 5.9% insulin 7.9% and glucose 0.6%.

### 4.2.8. Statistical analysis

Data were analysed using IBM SPSS statistics, version 21.0 for Windows. Appetite perceptions and appetite hormone total AUC calculations were performed using the trapezoidal method. Comparison between trials for exercise responses, energy and macronutrient intake, and day two total AUC appetite hormone data were compared using Student's paired t-tests. Two-way repeated measures ANOVA was used to examine differences between trials over time for appetite perceptions, energy and macronutrient intake and appetite hormones. Where significant trial and interaction effects were found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple comparisons (Atkinson 2002). The Pearson product moment correlation coefficient was used to examine relationships between variables. Corrections of plasma analyte values for changes in plasma volume did not alter the statistical significance of the results, therefore, for simplicity, the unadjusted values are presented. Statistical significance was accepted at 5%. Results are shown as mean (SD) in text and tables, and mean (SEM) in figures (for clarity).

## 4.3. Results

### 4.3.1. Exercise responses

The exercise responses for day one and day two are summarised in Table 4.2. There was no significant difference in oxygen consumption, exercise intensity, running speed, RPE, net energy expenditure, RER, carbohydrate or fat utilisation between the two days of exercise. Heart rate data was not available for two subjects, therefore analysis was completed on 13 subjects. Heart rate was found to be significantly higher on the first day of exercise (P=0.029). There was no difference between trials in pedometer-assessed physical activity (Control 4337 (1683); Exercise 4524 (2112) counts P=0.991, r=0.84) demonstrating that participants remained equally inactive outside of formal exercise periods during main trials (<5000 steps; Tudor-Locke & Bassett 2004).

**Table 4.2.** Exercise responses during the 60 min treadmill run on day one and day two.

| Response | Day one | Day two | P |
|---|---|---|---|
| **$VO_2$ ($mL.kg^{-1}.min^{-1}$)** | 40.6 (3.1) | 40.5 (3.5) | 0.87 |
| **$VO_2$ ($L.min^{-1}$)** | 3.0 (0.2) | 3.0 (0.2) | 0.77 |
| **Exercise intensity (% of $\dot{V}O_2$peak)** | 70.1 (2.5) | 70.0 (3.2) | 0.85 |
| **Running speed ($km \cdot h^{-1}$)** | 11.9 (1.0) | 11.9 (1.0) | - |
| **RPE (6 – 20)** | 15 (2) | 15 (2) | 0.62 |
| **Heart rate ($beats.min^{-1}$)** | 175 (11) | 171 (13) | 0.03 |
| **Net energy expenditure (kJ [kcal])** | 3779 (327) [903 (78)] | 3787 (327) [905 (78)] | 0.85 |
| **RER** | 0.95 (0.04) | 0.93 (0.03) | 0.17 |
| **Carbohydrate utilisation (%)** | 83.5 (11.5) | 82.1 (10.0) | 0.56 |
| **Fat utilisation (%)** | 16.5 (11.5) | 17.9 (10.0) | 0.56 |

Values are mean (SD). N=15, except for heart rate where N=13.

### 4.3.2. Resting expired air samples

Expired air samples were not available for one subject, therefore analysis was completed on 14 subjects. The resting expired air values for day one and day two are summarised in Table 4.3. There was no significant difference in oxygen consumption or energy expenditure between the two resting days. RER and carbohydrate utilisation were found to be

significantly higher on the second day of rest (P≤0.003). Fat utilisation was found to be significantly higher on the first day of rest (P=0.003).

**Table 4.3.** Resting expired air values on day one and day two.

| Response | Day one | Day two | P |
|---|---|---|---|
| **$VO_2$ ($mL.kg^{-1}.min^{-1}$)** | 2.6 (0.5) | 2.8 (0.6) | 0.64 |
| **$VO_2$ ($L.min^{-1}$)** | 0.20 (0.04) | 0.21 (0.04) | 0.34 |
| **Energy expenditure (kJ [kcal])** | 252.1 (50.5) | 272.8 (55.8) | 0.13 |
| | [60.3 (12.1)] | [65.2 (13.3)] | |
| **RER** | 0.85 (0.06) | 0.91 (0.04) | <0.01 |
| **Carbohydrate utilisation (%)** | 53.21 (18.97) | 74.36 (13.05) | <0.01 |
| **Fat utilisation (%)** | 46.79 (18.97) | 25.64 (13.05) | <0.01 |

Values are mean (SD). N=14.

### 4.3.3. Appetite

Fasting appetite perceptions (hunger, satisfaction, fullness and PFC) did not differ between trials at baseline on either day of each trial (all P>0.061). Two-way ANOVA revealed significant main effects of time (all P<0.001) and interaction effects (all P<0.001) for each appetite perception (Figure 4.2). Significant trial effects were also reported for hunger (P=0.046) and PFC (P=0.045), indicating suppressed levels in the exercise trial across days one and two. Compared with control, post-hoc analysis revealed suppressed hunger ratings at 1.5 h on day one (P=0.029) and at 0.5 (P=0.029) and 1 h (P<0.001) on day two of the exercise trial. Fullness ratings were significantly elevated at 1 h on day one of the exercise trial (P=0.030), whilst PFC was lower at 0.5 (P=0.029) and 1 h (P<0.001) on day two of the exercise trial.

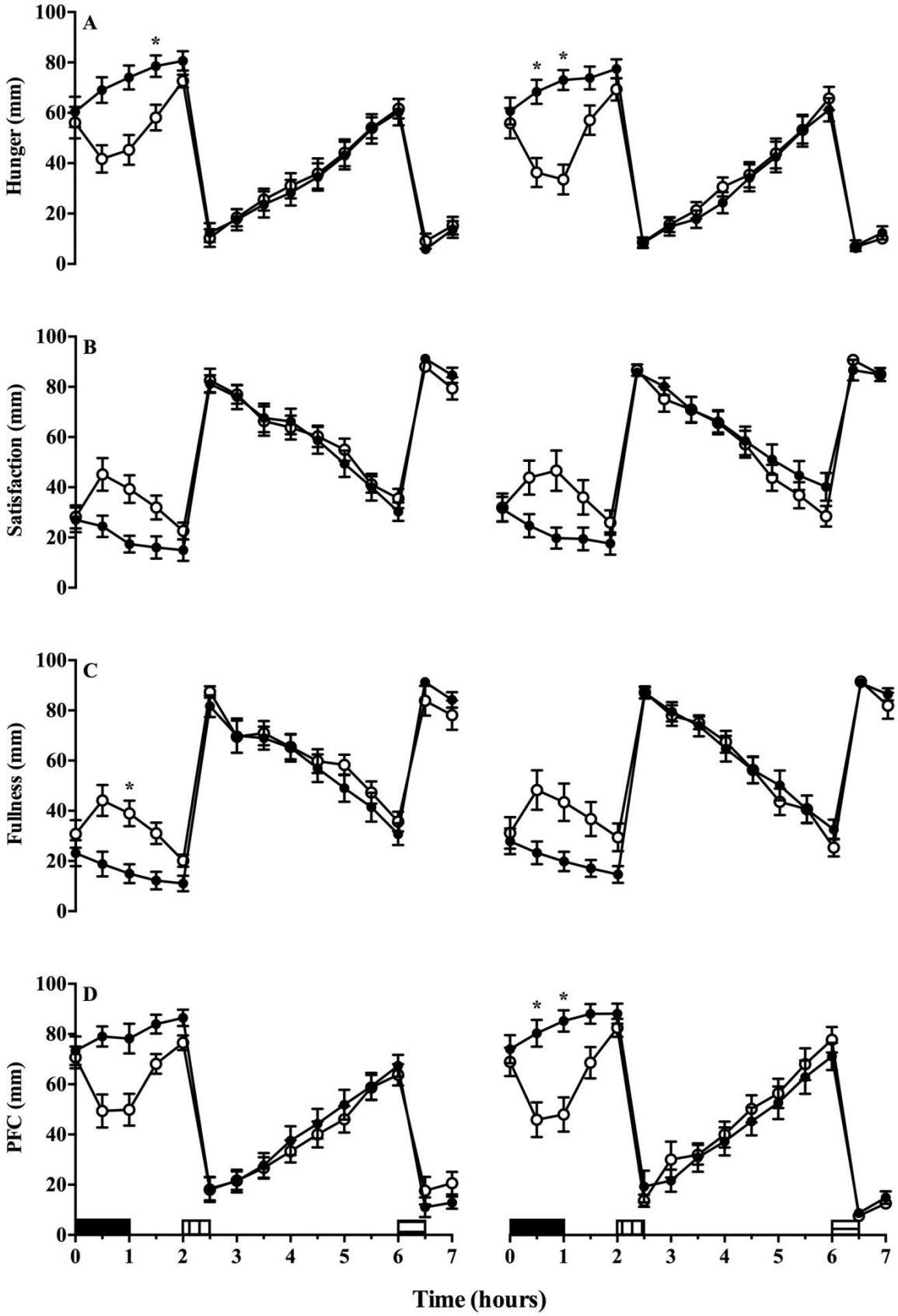


**Figure 4.2.** Perceptions of hunger (A), satisfaction (B), fullness (C) and prospective food consumption (D) in control (●) and exercise (○) trials. Values are mean (SEM), N=15. Black rectangles indicate exercise, vertically shaded rectangles indicate cold buffet meals, and horizontally shaded rectangles indicate hot meals. *Significant difference between control and exercise ($P<0.03$).

Analysis of the appetite total AUC (calculated separately for day one and two) using two-way ANOVA (day vs. trial) confirmed significant trial effects for hunger (P=0.042), fullness (P=0.050), and PFC (P=0.036) but did not show any differences in appetite between trial days one and two (Table 4.4).

**Table 4.4.** Total area under the curve values for appetite perceptions in the control and exercise trials.

| | **Day One (7 h)** | **Day Two (7 h)** | **Total Trial (14 h)** |
|---|---|---|---|
| Hunger | | | |
| Control | 309 (96) | 301 (83) | 610 (173) |
| Exercise | 272 (79) | 259 (80) | 531 (148)* |
| Satisfaction | | | |
| Control | 345 (80) | 361 (73) | 705 (143) |
| Exercise | 381 (75) | 382 (80) | 763 (141) |
| Fullness | | | |
| Control | 333 (85) | 353 (74) | 685 (152) |
| Exercise | 384 (64) | 389 (61) | 772 (102)* |
| PFC | | | |
| Control | 355 (86) | 332 (83) | 687 (161) |
| Exercise | 308 (80) | 299 (74) | 606 (140)* |

Values are mean (SD). N=15. *significant difference between trials.

### 4.3.4. Energy and macronutrient intake

Table 4.5 and 4.6 show the energy and macronutrient intake data from the exercise and control trials. No significant main effects were apparent for energy intake (all P>0.137). There was no effect of trial order on total energy intake (P=0.77). For macronutrient intake, no significant main effects were found for carbohydrate or fat intake (all P>0.261), however a main effect of time was observed for protein intake (P<0.001) which was reduced during the evening period between days 1 and 2 on both the control and exercise trials.

**Table 4.5.** *Ad libitum* energy intake during the control and exercise trials.

| Day | Meal | Exercise (kJ) | Control (kJ) | *P* |
|---|---|---|---|---|
| 1 | Cold Buffet (2 h) | 5950 (1855) | 6053 (2363) | 0.84 |
| 1 | Hot Meal (6 h) | 5777 (1407) | 6052 (902) | 0.43 |
| 1 | Overnight | 5085 (1087) | 5163 (1214) | 0.80 |
| 2 | Cold Buffet (2 h) | 5432 (1231) | 5839 (1587) | 0.39 |
| 2 | Hot Meal (6 h) | 6288 (1738) | 6109 (821) | 0.60 |
| | Total Trial | 28532 (3899) | 29217 (4006) | 0.45 |

Values are mean (SD). N=15. Food consumption outside of the lab on day 1 (overnight) was between 16:30 and 22:30.

**Table 4.6.** Macronutrient intake in the control and exercise trials.

| Day | | Fat (g) [%] | Carbohydrate (g) [%] | Protein (g) [%] |
|---|---|---|---|---|
| | Control Trial | | | |
| 1 | Cold Buffet (2 h) | 51 (35) | 203 (62) | 45 (17) |
| | | [29 (10)] | [58 (11)] | [13 (4)] |
| 1 | Hot Meal (6 h) | 54 (8) | 187 (28) | 51 (8) |
| | | [34 (0)] | [52 (0)] | [14 (0)] |
| 1 | Overnight Food bag | 47 (14) | 188 (46) | 14 (3) |
| | | [34 (5)] | [61 (6)] | [5 (0)] |
| 2 | Cold Buffet (2 h) | 47 (24) | 201 (55) | 43 (16) |
| | | [29 (11)] | [59 (12)] | [13 (5)] |
| 2 | Hot Meal (6 h) | 54 (7) | 189 (24) | 52 (7) |
| | | [34 (0)] | [52 (0)] | [14 (0)] |
| | Total Trial | 252 (59) | 968 (133) | 205 (29) |
| | | [32 (4)] | [56 (4)] | [12 (2)] |
| | Exercise Trial | | | |
| 1 | Cold Buffet (2 h) | 51 (28) | 195 (48) | 46 (19) |
| | | [30 (11)] | [57 (11)] | [13 (4)] |
| 1 | Hot Meal (6 h) | 54 (13) | 188 (44) | 52 (12) |
| | | [34 (0)] | [52 (0)] | [14 (0)] |
| 1 | Overnight Food bag | 47 (12) | 184 (43) | 14 (3) |
| | | [35 (5)] | [60 (5)] | [5 (0)] |
| 2 | Cold Buffet (2 h) | 48 (24) | 175 (24) | 43 (16) |
| | | [31 (11)] | [56 (12)] | [13 (3)] |
| 2 | Hot Meal (6 h) | 58 (13) | 201 (45) | 54 (13) |
| | | [34 (0)] | [52 (0)] | [14 (0)] |
| | Total Trial | 258 (45) | 942 (111) | 208 (41) |
| | | [33 (4)] | [55 (4)] | [12 (1)] |

Values are mean (SD). N=15. Food consumption outside of the lab on day 1 (overnight) was between 16:30 and 22:30.

### 4.3.5. Relative energy intake

Relative energy intake was calculated for days one and two as energy intake minus the net energy expenditure elicited by exercise (exercise day one: 13302 (2450) kJ; exercise day two: 8090 (1342) kJ; control day one: 17268 (2775) kJ; control day two: 11948 (1692) kJ). Significant trial ($P<0.001$) and time ($P<0.001$) effects were found, indicating lower REI across days one and two on the exercise trial and also reflecting the fewer number of meals consumed on day two compared with day one (three vs. two) on both trials.

### 4.3.6. Plasma total PYY, leptin, acylated ghrelin, triglyceride, glucose and insulin concentrations

Due to problems with blood sampling data for plasma acylated ghrelin, total PYY and leptin are presented for 14 (rather than 15) participants while data for plasma insulin are presented for 13 (rather than 15) participants. Figure 4.3 shows the circulating appetite hormone responses on days one and two of the control and exercise trials.

Fasting acylated ghrelin concentrations did not differ between the exercise and control trials at baseline on day one or day two (control day one: 128 (113); exercise day one: 144 (169); control day two: 127 (121); exercise day two: 117 (143) $pg \cdot mL^{-1}$ (P for trial, time and interaction all >0.05)) (Figure 4.3A). Across the whole trial, two-way repeated measures ANOVA did not show any significant main effects for plasma acylated ghrelin (all $P>0.05$).

Fasting plasma total PYY concentrations did not differ between trials at baseline on day one or day two of main trials (control day one: 84 (40); exercise day one: 95 (63); control day two: 87(37); exercise day two: 104 (50) $pg \cdot mL^{-1}$ (P for trial, time and interaction all >0.05)) (Figure 4.3B). Across the whole trial, two-way repeated measured ANOVA revealed a significant main effect of trial ($P=0.029$) indicating higher total PYY concentrations on the exercise trial. There was also a significant main effect of time ($P<0.001$) for total PYY.

Fasting plasma leptin concentrations did not differ between trials at baseline on day one or day two of the exercise and control trials (control day one: 1543 (1042); exercise day one: 1738 (1716); control day two: 1860 (2044); exercise day two: 1710 (1733) $pg \cdot mL^{-1}$, $P>0.333$) (Figure 4.3C). Across trial days, two-way repeated measures ANOVA revealed a significant main effect of time ($P<0.001$) but no trial or interaction effects ($P>0.05$). Fasting plasma insulin concentrations were found to differ across time ($P=0.0015$), however there were no trial or interaction effects (both $P>0.05$) (control day one: 21 (5); exercise day one: 25 (10);

control day two: 30 (10); exercise day two: 29 (13) $pg \cdot mL^{-1}$) (Figure 4.3D). Across trial days, two-way repeated measures ANOVA revealed a significant trial ($P=0.025$), time ($P<0.001$) and interaction effect ($P=0.019$) for plasma insulin concentrations. Post-hoc analysis showed insulin levels to be significantly lower for the exercise condition in comparison to the control condition at 2 h on day two ($P<0.001$).

Total area under the curve calculations for day two of each trial revealed no significant differences for acylated ghrelin (control: 833 (1090); exercise: 909 (1337) $pg \cdot mL \cdot h^{-1}$) and leptin (control: 10376 (10652); exercise: 9783 (9244) $pg \cdot mL \cdot h^{-1}$) (both $P>0.050$). For insulin, values were significantly lower during the exercise trial (control: 1446 (785); exercise: 852 (316) $pg \cdot mL \cdot h^{-1}$, $P=0.050$), whilst total PYY values were significantly higher (control: 720 (253); exercise: 883 (374) $pg \cdot mL \cdot h^{-1}$, $P=0.010$).

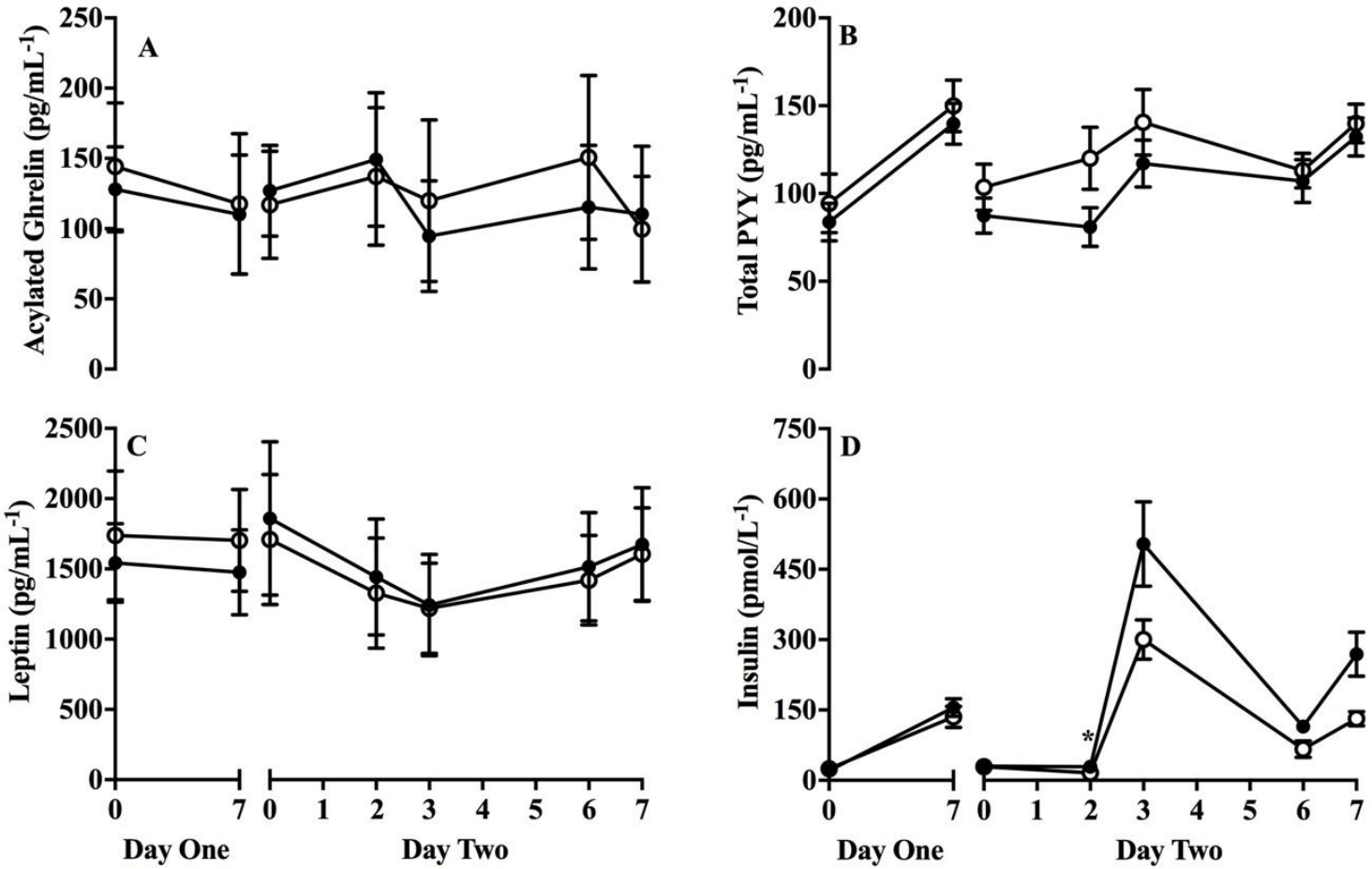


**Figure 4.3.** Plasma concentrations of acylated ghrelin, N=14 (A), total PYY, N=14 (B), leptin, N=14 (C), and insulin, N=13 (D) in control (●) and exercise (○) trials. Values are mean (SEM). *Significant difference between control and exercise. NB: meals were consumed at 2 and 6 h on days 1 and 2.

### 4.3.7. Correlations

Correlation analyses were performed for all major outcomes (total AUC hormone data and appetite ratings, energy intake and subject characteristics); however no statistically significant outcomes were found (data not shown).

### 4.4. Discussion

The consensus from the research literature suggests that exercise does not stimulate appetite on the day it is performed, even when responses have been examined for several hours post-exercise. Conversely, daily exercise undertaken over four to five consecutive days has been shown to increase hunger, motivation to eat or reduce satiation (Mackelvie et al. 2007; Hagobian et al. 2009). The present study sought to explore whether initial alterations in appetite could be detected after two bouts of exercise performed on consecutive days across a period of 30 h after the first bout of exercise. Besides witnessing a transient exercise-induced suppression of appetite on each day, we observed no subsequent changes in any of the subjective appetite measurements assessed. Thus, repeated bouts of exercise on two days did not impact hunger, satiation or satiety. These findings indicate that over this duration, exercise and its associated energy expenditure, are not compensated for.

Previously, King et al. (1997) examined appetite responses across two days with two bouts of exercise being performed on the first day of the exercise trial (combined energy expenditure 4983 (1004) kJ). Similar to the current investigation, these researchers observed no alterations in appetite. It should however be noted that in this particular study appetite assessments were made in participants' free living environment; meaning that various extraneous factors may have confounded the results. More recently, Heden et al. (2013) also observed no changes in fasting or postprandial ratings of hunger and fullness in response to a test meal consumed on the morning after moderate-intensity exercise completed on the previous evening. In contrast to these findings, Beaulieu et al. (2015) recently published data showing a greater motivation to eat on the day after a single bout of sprint interval training (supra-maximal exercise) in a small group of trained men (N=8). Taken collectively, the available data suggests that acute exercise, even when repeated on two days, does not automatically stimulate appetite. Very high intensity exercise may be an exception; however additional studies with a larger group of more diverse participants are needed to state this with any certainty.

An additional aim of the present investigation was to characterise the extended impact of exercise on energy and macronutrient intake. Consistent with the lack of alteration in appetite, we also observed no significant changes in energy or macronutrient intake assessed from several *ad libitum* meals consumed across days one and two. Previous studies have shown that over an extended period (one to two weeks) individuals may begin to partially increase

the amount of food consumed in an attempt to counter the heightened energy expended during exercise (Stubbs et al. 2002a; Stubbs et al. 2002b; Whybrow et al. 2008). The precise point at which this response is initiated is not known, however it is clear that such compensation does not occur immediately on the actual day that exercise is performed (Schubert et al. 2013). When considering responses on the day after exercise, King et al. (1997) previously reported a lack of change in energy intake. Unfortunately, in this study the implementation of diaries to assess energy intake, and the aforementioned lack of study control, limits the strength of these findings. The present study sought to provide a more rigorous analysis by utilising laboratory based assessments of energy and macronutrient intake. The present results are consistent with recent findings reported by Beaulieu et al. (2015) who observed no changes in energy intake within the 36 h after sprint interval training. Together, these data indicate that the initial stages of energy intake compensation are not seen on the day after exercise, even when a successive bout is performed on the second day. It is likely that compensatory responses will begin to appear after additional days of exercise with a higher level of energy expenditure and/or negative energy balance.

Appetite and food intake are subject to regulation by a complex network of neural-hormonal mediators which act acutely (meal to meal) and chronically (over days/weeks) to facilitate energy homeostasis (Neary & Batterham 2009; Perry & Wang 2012). Notable within the short-term regulation of appetite and energy intake are acylated ghrelin and PYY which work antagonistically to coordinate meal initiation and termination (De Vriese et al. 2010; Manning & Batterham 2014). Specifically, acylated ghrelin remains unique as the only known hormonal stimulant of appetite, with circulating levels increasing prior to meal initiation and subsequently decreasing postprandially (Cummings et al. 2004). Conversely, PYY is a key mediator of satiation and satiety, with circulating levels increasing in response to nutrient intake and remaining elevated between meals (Le Roux et al. 2005). Acylated ghrelin and PYY are also implicated in the chronic regulation of energy homeostasis with each being sensitive to deviations in energy balance/stores. Specifically, negative energy balance and weight loss increase circulating levels of acylated ghrelin (King et al. 2011; Leidy et al. 2007) and reduced those of PYY (Hill et al. 2011; King et al. 2011). These responses serve to promote the restoration of energy balance and limit perturbations in body weight. The present study sought to characterise the latent impact of exercise on acylated ghrelin and PYY. Specifically, we wanted to determine how exercise, and the associated high level of energy expenditure, would impact circulating levels of these hormones over an

extended duration. It was anticipated that fasting and/or postprandial levels of acylated ghrelin on day two would be elevated, whilst those of PYY would be reduced as mechanisms to preserve energy homeostasis. In contrast, we saw no changes in acylated ghrelin whilst PYY was transiently elevated on day two after exercise. The present findings therefore contrast recent data reported by Heden et al. (2013) who observed attenuated fasting and postprandial suppression of acylated ghrelin at a test meal consumed on the morning after exercise completed on the previous evening. The discrepancy in findings is likely related to key differences between protocols such as the time between exercise and blood sampling and the additional bout of exercise performed in the present study. This inconsistency in outcome identifies a need to further scrutinise the protracted impact of exercise on acylated ghrelin.

In the present investigation, we saw no change in fasting levels of PYY on day two; however, a transient increase in circulating PYY levels was apparent after the second bout of exercise i.e. on day two. This investigation is the first to assess the impact of exercise on the fasting level of PYY on the day after exercise and therefore the lack of change despite a high level of energy expenditure is a novel observation. The short-term increase in PYY after exercise on day two is consistent with previous research, which has described a transient increase in PYY in response to moderate-high intensity exercise (Broom et al. 2009; Martins et al. 2007; Ueda et al. 2009). It is beyond the scope of the present thesis to speculate on the mechanisms mediating this response; it is clear however that this acute effect is maintained with repeated bouts of exercise on consecutive days. Nonetheless, there was no observation of any compensatory down-regulation of PYY in response to exercise-related energy expenditure across the time-frame examined. Furthermore, as shown in previous studies, this exercise-induced change in PYY does not appear to have any influence on appetite or energy intake. PYY has many physiological functions in the body e.g. gastrointestinal transit, substrate metabolism, thermogenesis; and it is possible that the change witnessed with exercise is unrelated to appetite control and food intake. Conversely, it is also possible that the response is relevant but is insufficient to overcome ingrained habits and behaviour relating to food intake so that visible effects on these outcomes may only be seen over a greater period of time or with a larger stimulus.

Episodic signals regulating appetite and energy intake function synergistically with additional chronic hormonal mediators which are primarily sensitive to perturbations in energy balance and bodily energy stores. Within this system, leptin and insulin are well defined; being positively associated with energy availability and responding dynamically to perturbations in

energy balance (Gautron & Elmquist 2011; Woods et al. 2006). Previous research has shown that leptin is sensitive to negative energy balance induced by exercise (≥3340 kJ) with compensatory decreases in circulating levels being detectable after a latency period of 12-24 h (Essig et al. 2000; Olive & Miller 2001). In the present investigation, despite a net energy expenditure of ~3765 kJ on day one, we did not detect any change in fasting leptin on the following morning; nor did we see any changes after the second bout of exercise on day two. It is possible that the second bout of exercise on day two may have masked any latent changes which may have otherwise occurred. Furthermore, despite exercise on day one inducing a relative energy deficit of 4234 kJ, the somewhat high amount of energy intake across each trial on day one (control 17268 (2775); exercise 16812 (2899) kJ) may have been sufficient to block any exercise-related decrease in leptin.

In addition to its central role in regulating glycaemia, insulin is also a tonic signal regulating energy balance either by acting directly within the hypothalamus, or by modulating the brain's sensitivity to episodic appetite signals (McMin et al. 2000; Woods et al. 2006). Chronically, circulating levels of insulin are positively related to adiposity and insulin resistance within key metabolic tissues. An increase in insulin sensitivity is a well-characterised effect of acute exercise that endures for up to 72 h after each individual bout (Hawley & Lessard 2008). In the present study, our participants were young and healthy, with low basal insulin levels. It is therefore perhaps unsurprising that fasting concentrations on day two were not lowered further by prior exercise. On day two we did however observe reduced levels of insulin in response to the morning meal; a response which is consistent with an enhancement of insulin sensitivity induced by exercise. Although important for glycaemic control, this transient response is unlikely to have any influence on energy balance.

This study has some notable limitations. By estimating participants' energy requirements (Mifflin et al. 1990) it appears that participants overate during main trials. This was likely due to the wide selection of foods available at *ad libitum* meals and an associated 'banquet effect'. Consequently, the sensitivity to detect changes in energy intake after consecutive days of exercise may have been reduced. Furthermore, such overconsumption may have also dampened the ability to detect changes in appetite hormones in response to exercise. Another limitation of this study is that performance of a second bout of exercise may have masked any changes in study outcomes on day 2; however, this is unlikely given other recent findings of ours (King et al. 2015). Finally, this study focused solely on homeostatic factors contributing to the regulation of appetite and food intake. Recent investigations have highlighted the

importance of hedonic factors contributing to these outcomes (Crabtree et al. 2014; Evero et al. 2012).

In summary, this investigation sought to characterise the impact of two bouts of moderate to high intensity exercise, performed on consecutive mornings, on appetite perceptions, food intake and circulating levels of key appetite regulatory hormones. This study found that besides inducing a transient inhibition of appetite, exercise did not exert any other influence on subjective appetite perceptions and did not alter *ad libitum* energy or macronutrient intake on day one or two. Furthermore, exercise had no impact upon circulating levels of leptin or acylated ghrelin, but did augment levels of total PYY and reduce those of insulin, on day two. These findings support previous data in lean individuals, indicating that acute exercise does not induce compensatory appetite or food intake responses within the short-term (King et al. 1994; King et al. 2013; Wasse et al. 2012). This study has demonstrated that this lack of response persists on the day after exercise.

# CHAPTER V

## Acute exercise and appetite-regulating hormones in overweight and obese individuals: A meta-analysis

### 5.1. Introduction

'Exercise induced anorexia' was a term coined in 1994 by King and colleagues, to describe the condition where appetite is suppressed after acute exercise. King et al. (1994) showed that appetite was temporarily suppressed during and after high intensity exercise in lean healthy males. Subsequent researchers confirmed these earlier findings (Broom et al. 2007; Broom et al. 2009; King et al. 2010a; King et al. 2010b; King et al. 2011). At rest, feelings of hunger are mediated by gut hormones such as acylated ghrelin, PYY and GLP-1 (Cummings et al. 2001; Harrold et al. 2012; Karra et al. 2009). It has been hypothesised that 'exercise induced anorexia' is mediated by altered concentrations of these hormones. For example, acylated ghrelin (an appetite stimulating hormone) has been found to be suppressed after vigorous exercise (Broom et al. 2009; King et al. 2011; Ueda et al. 2009b). In contrast, circulating concentrations of PYY and GLP-1 (satiety hormones), have been shown to increase after exercise in healthy lean adults (Broom et al. 2009; King et al. 2011; Ueda et al. 2009b). Researchers have also examined the effects that exercise has on energy intake after exercise. The majority of studies indicate that individuals do not compensate for the energy expended during exercise in the immediate hours after exercise (Schubert et al. 2013). Therefore, these individuals are in an energy deficit, and if maintained over time this could result in weight loss.

Most studies on exercise and appetite regulation involve crossover designs and relatively small sample sizes. Meta-analyses can be useful to quantify the effects of an intervention with greater precision from a pooled estimate. A standardised mean difference (SMD) is often reported. This is a summary statistic, and is used when studies measure the same outcome but report it in a variety of ways (i.e. different units of concentration or time). The SMD standardises the results of the studies so that they can be combined. In particular, the SMD is calculated as follows:

$$\text{SMD} = \frac{\text{(Difference in mean outcome between groups)}}{\text{(Standard deviation of outcome among participants)}}$$

Therefore, regardless of how outcomes are reported in each study, the SMD expresses the size of the invention effect relative to the variability observed in that study. Findings can then be pooled and analysed using meta-analysis software.

Recently, the effects of acute exercise on appetite regulatory hormones were examined in lean, ow/ob individuals (Schubert et al. 2014). It was concluded that an acute bout of exercise suppresses acylated ghrelin (SMD: 0.20) and increases PYY (SMD: 0.24), GLP-1 (SMD: 0.28) and PP (SMD: 0.50). Of the 25 studies included in this review, only two involved ow/ob individuals. Clearly, there are fewer studies on ow/ob individuals and no previous systematic review and meta-analysis have been undertaken on this population. Such a review would capture new studies involving ow/ob individuals, and clarify if they respond in a similar way to their lean counterparts. This in turn could enhance understanding of the role exercise plays in weight maintenance and control. Therefore, we aimed to synthesise this evidence from studies investigating acute exercise bouts and circulating concentrations of acylated ghrelin, total PYY and total GLP-1, measured in ow/ob participants.

## 5.2. Methods

### 5.2.1. Data source

A systematic review of peer-reviewed studies was undertaken comparing concentrations of appetite regulatory hormones, quantified as time averaged area under the curve. The review was registered with the PROSPERO database (CRD42014006265).

The literature search was conducted by an information specialist using commonly-used research databases (Applied Social Sciences Index and Abstracts (ASSIA), Campbell Collaboration, Centre for Review and Dissemination, Database of Promoting Health Effectiveness Reviews (DoPHER), Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews, Cochrane Methodology Register, Database of Abstracts of Reviews or Effects (DARE), EMBASE, NHS Economic Evaluation Database (NHS EED), PROSPERO, PubMed, PsycINFO MEDLINE (Ovid), Sports Discus, SCOPUS, Web of Knowledge and CINAHL). These databases were searched in January 2014 with an update search in June 2014 and October 2016. Keyword searches were performed for 'exercise', 'physical activity', 'energy expenditure', 'energy intake', 'appetite', 'hunger', 'food intake', 'ghrelin', 'acylated ghrelin', 'total ghrelin', 'acyl ghrelin', 'peptide YY', 'PYY', 'peptide $YY_{3-36}$', '$PYY_{3-36}$', 'total PYY', 'glucagon like peptide-1', 'GLP-1', 'active GLP-1', 'GLP-1(7-36)', 'GLP-1(9-36)', 'obese', 'overweight', 'meta-analysis' and 'appetite hormones'. Details of the search strategy are provided in Appendix I.

### 5.2.2. Inclusion criteria

For inclusion, studies were required to meet the following criteria:

- study participants were;
    - overweight or obese
    - had no history of diabetes, gastrointestinal, inflammatory, metabolic, cardiovascular or psychological disease
    - non-smokers
    - had no abnormal eating tendencies
- studies were;
    - not limited by the duration or observation period post-exercise
    - not limited by exercise intensity, duration or modality

    - required to have a control condition (completed by the same participants who completed the exercise condition)
    - required to use identical exercise and control conditions (minus the exercise bout)
    - measure acylated ghrelin, total PYY or total GLP-1 plasma concentrations
- investigators were not blinded (due to the nature of the study)
- published in;
    - peer-reviewed journals
    - conference proceedings
    - theses
    - dissertations

A broad range of sources for study inclusion where chosen to minimize the risk of small study effects, which can occur if only published studies are included.

### 5.2.3. Exclusion criteria

Studies were excluded if:

- did not measure acylated ghrelin, total PYY or total GLP-1 responses to an exercise bout
- did not measure responses in ow/ob individuals
- did not include a control trial

### 5.2.4. Study selection

Potential studies were identified by examining the abstracts and full-text copies were obtained if they met the initial criteria of evaluating appetite hormone changes in response to an acute exercise bout. This process was checked independently by an expert in the field. Notes of the two researchers were compared, and a mutual consensus reached. In the original literature search conducted in January 2014, five studies met the inclusion criteria. Two updated searches conducted in June 2014 and October 2016, identified one further study. Together six studies met the inclusion criteria for the current meta-analysis (Figure 5.1).

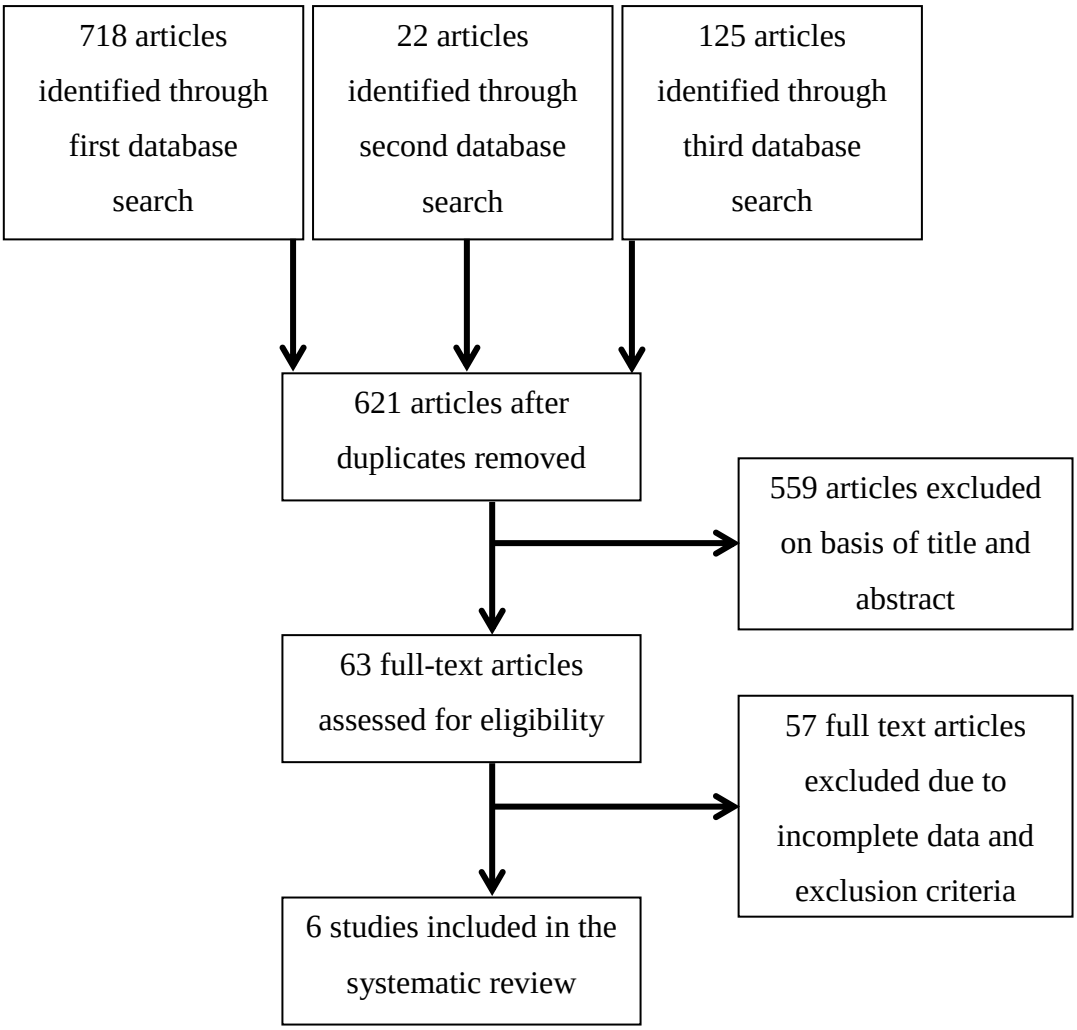


**Figure 5.1.** Flowchart of study selection.

### 5.2.5. Data synthesis

Included studies were assessed for quality and validity, and independently checked by another expert in the field, using established criteria (Physiotherapy Evidence Database [PEDro], http://www.pedro.org.au/english/downloads/pedro%20scale/). Data on the study methods, sample size, participant characteristics, blood analytical methods, exercise intervention information, and hormone ($pmol \cdot L^{-1}\ h^{-1}$, $pg \cdot mL^{-1}\ h^{-1}$, $\mu U \cdot mL^{-1}\ h^{-1}$) and appetite AUC data were extracted for both control and exercise conditions into a computerised spreadsheet. Data entry was checked by one other expert in the field and discrepancies discussed and checked again. If standard error of the mean (SEM) was reported, these were converted to standard deviations (Deeks et al. 2011).

### 5.2.6. Meta-analysis procedures

Comprehensive Meta-Analysis software (Version 2.2.064; Biostat, Englewood, NJ, USA), was used to conduct a random effects (DerSimonian-Laird inverse variance approach) meta-analysis of the mean difference in acylated ghrelin, total PYY and total GLP-1 during control and exercise trials (Deeks et al. 2011). The inputted data included sample sizes, AUCs for the control and exercise conditions (with their respective standard deviations) and an imputed correlation coefficient to take into account the fact that all studies were crossover in nature. These correlation coefficients were estimated from prior reliability studies in our laboratory and were as follows; acylated ghrelin; r=0.93, total PYY: r=0.71 and GLP-1: r=0.94. The software calculated the pooled standardised difference in means to determine the effect size (Higgins et al. 2011). All data are presented as means (95% confidence interval).

We interpreted SMD values of <0.2 as trivial, 0.2-0.3 as small, 0.4-0.8 as moderate and >0.8 as large. A negative effect size indicates that exercise was associated with decreased hormone concentrations, while a positive effect size indicates that hormone concentrations increased with exercise (Hopkins et al. 2009). Heterogeneity was explored using a Q-test, I-square statistic and the tau-squared statistic. A Q statistic was deemed significant when $P<0.05$, and an I-square statistic >50% was deemed to be indicative of substantial heterogeneity.

#### 5.2.6.1. Meta-regression analyses for BMI of acylated ghrelin time-averaged AUC between exercise and control conditions

BMI was used as a moderator in a meta-regression analysis (methods-of-moments model), to determine if BMI could explain the variation in effect size values seen between studies for acylated ghrelin concentrations (Higgins et al. 2011). Mean BMIs were pooled from studies collected in the current review together with those reported for lean individuals in a recent review by Schubert et al. (2014). Mean BMI was included as a moderating variable, based on the hypothesis that a negative association exists between study mean BMI and acylated ghrelin for a pooled sample of studies across a full range of BMIs. This analysis was only performed for acylated ghrelin as there were not enough studies (N<3) reporting data for total PYY or total GLP-1 to obtain sufficiently-precise and meaningful estimations of meta-regression slope

#### 5.2.6.2. Exploration of small study effects

Small study effects were explored with a funnel plot of standard difference in means vs associated standard errors (Sterne et al. 2011) and by quantifying Egger's linear regression intercept. A large and statistically significant Egger's statistic indicates the presence of a small study effect. This analysis was only performed for acylated ghrelin as there were not enough studies reporting data for a precise exploration of total PYY and total GLP-1.

## 5.3. Results

### 5.3.1. Overview

Six studies involving a total of 73 participants met the inclusion criteria for the meta-analysis. All of these had been published (or accepted for publication) in peer-reviewed scientific journals. The experimental trials in each study lasted between 2 and 3 h, with exercise conducted in a fasted condition or following a standardised breakfast. Three studies included standardised meals before the exercise bout (Martins et al. 2014; Ueda et al. 2009; Unick et al. 2010) and four studies included *ad libitum* meals after the exercise bout (Martins et al. 2014; Sim et al. 2014; Ueda et al. 2009b; Unick et al. 2010). Blood samples were collected at regular intervals throughout all trials.

The included studies are summarised in Table 5.1. The majority of studies recruited participants of the same sex. One study recruited both males and females (Martins et al. 2015). Two of the studies involved more than one exercise intensity trial (Martins et al. 2015; Sim et al. 2014), and are reported in the analysis as "multiple trials". Accounting for these, the total number of trials is 10, each including one control and one exercise condition. Six studies (10 trials) reported acylated ghrelin AUC data, two studies (four trials) reported total PYY AUC data and two studies (four trials) reported total GLP-1 AUC data. Of the 10 trials, one used treadmill walking as the mode of exercise, two used treadmill running and seven used a cycle ergometer. The mean PEDro score for the six studies was 6±0, rating all studies to have "good" methodological quality.

### 5.3.2. Participant demographics and exercise intervention characteristics

Of the 73 participants included in the meta-analysis, 57 were male (78%) and 16 were female (22%). Mean BMI values of the 73 participants ranged from 27.7 to 32.7 $kg \cdot m^{-2}$ (mean 30.6 $kg \cdot m^{-2}$). Four studies used exercise which was aerobic in nature (Sim et al. 2014; Tiryaki-Sonmez et al. 2013; Ueda et al. 2009b; Unick et al. 2010), and two compared aerobic exercise with two variations of high intensity exercise (Martins et al. 2015; Sim et al. 2014). The exercise interventions lasted between nine and 60 min (mean 34 min), and exercise intensity was set between 50 and 65% $VO_2$ peak (mean 58% $\dot{V}O_2$ peak, N=6) or 72.5 and 87.5% $HR_{max}$ (mean 79.4% $HR_{max}$, N=4). Between 7 and 19 participants took part in each study (mean 12 participants) (see Table 5.1 for summaries of study protocols).

**Table 5.1.** Hormone time averaged area under the curve (AUC) data for the six studies included in the meta-analysis.

| Study | Participants | | Intervention | Hormone AUC time (h) | Hormone AUC (pg·mL$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Acylated Ghrelin | | Total PYY | | Total GLP-1 | |
| | N | BMI (kg·m$^{-2}$) | | | | | | | | |
| Gholipour et al. 2011 | 9 (M) | 32.7 (0.8) | 36 min treadmill run: 10 min, 10 min, 5 min, 2 min at 65% $\dot{V}O_2$ max, separated by 3 min at 3 km·h$^{-1}$ | 2.85 | CON: | 3512 (654) | CON: | NM | CON: | NM |
| | | | | | EX: | 1935 (302)* | EX: | NM | EX: | NM |
| Martins et al. 2015 | 12 (7F:5M) | 32.3 (2.7) | Cycling @ 85-90% $HR_{max}$ until 250 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling (mean duration, 18 ± 3 min) | 3.00 | CON: | 3921 (1318) | CON: | NM | CON: | 4181 (1262) |
| | | | | | EX: | 3315 (1219)* | EX: | NM | EX: | 4272 (969) |
| | | | Cycling @ 70% $HR_{max}$ until 250 kcal expended: continuous cycle (mean duration, 27 ± 6 min) | | CON: | 3921 (1318) | CON: | NM | CON: | 4181 (1262) |
| | | | | | EX: | 3296 (1058)* | EX: | NM | EX: | 4638 (1305) |
| | | | Cycling @ 85-90% $HR_{max}$ until 125 kcal expended: 8 s all out sprinting separated by 12 s easy pedalling (mean duration, 9 ± 2 min) | | CON: | 3921 (1318) | CON: | NM | CON: | 4181 (1262) |
| | | | | | EX: | 3532 (1393) | EX: | NM | EX: | 4072 (1156) |
| Sim et al. 2014 | 17 (M) | 27.7 (1.6) | 30 min continuous cycling @ 60% $\dot{V}O_{2peak}$ | 1.58 | CON: | 70 (37) | CON: | 85 (43) | CON: | NM |
| | | | | | EX: | 69 (30) | EX: | 87 (37) | EX: | NM |
| | | | 30 min cycling: alternating between 60s @ 100% $\dot{V}O_{2peak}$ and 240s @ 50% $\dot{V}O_{2peak}$ | | CON: | 70 (37) | CON: | 85 (43) | CON: | NM |
| | | | | | EX: | 62 (28) | EX: | 83 (40) | EX: | NM |
| | | | 30 min cycling: alternating between 15s @ 170% $\dot{V}O_{2peak}$ and 60s @ 32% $\dot{V}O_{2peak}$ | | CON: | 70 (37) | CON: | 85 (43) | CON: | NM |
| | | | | | EX: | 56 (26) | EX: | 88 (34) | EX: | NM |

| Study | Participants | | Intervention | Hormone AUC time (h) | Hormone AUC ($pg \cdot mL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Acylated Ghrelin | | Total PYY | | Total GLP-1 | |
| | N | BMI ($kg \cdot m^{-2}$) | | | | | | | | |
| Tiryaki-Sonmez et al. 2013 | 9 (F) | 28.3 (1.8) | 60 min running @ 53% $\dot{V}O_{2max}$ | 2.00 | CON: | 51 (8) | CON: | NM | CON: | NM |
| | | | | | EX: | 47 (5) | EX: | NM | EX: | NM |
| Ueda et al. 2009b | 7 (M) | 30.0 (3.1) | 60 min cycle @ 50% $\dot{V}O_{2max}$ | 2.00 | CON: | 15779 (10046) | CON: | 393 (50)* | CON: | NM |
| | | | | | EX: | 16641 (10725) | EX: | 425 (46)* | EX: | NM |
| Unick et al. 2010 | 19 (F) | 32.5 (4.3) | Walk @ 70-75% age predicted $HR_{max}$ until 3.0 kcal/kg of body weight expended (mean energy expenditure, 354 ± 72 kcal; mean duration 42 ± 8 min) | 2.00 | CON: | 6527 (2646) | CON: | NM | CON: | 211400 (51600) |
| | | | | | EX: | 6361 (3339) | EX: | NM | EX: | 201200 (49400)* |

CON, control trial; EX, exercise trial; F, female; $HR_{max}$, maximum heart rate; M, male; NA, AUC data not available; NM, not measured. *Significantly different from control ($P<0.05$). Values are mean (SD).

### 5.3.3. Meta-analysis

Individual study statistics and results for both trials including ow/ob subjects and lean subjects are summarised in the appendix (See Appendix J, K, L).

### 5.3.4. Effect size and moderator variable for acylated ghrelin time-averaged AUC analysis

In ow/ob individuals there was a statistically significant moderate suppression in mean acylated ghrelin time-averaged AUC concentrations in exercise trials compared with resting trials (pooled effect size -0.34, 95% confidence interval -0.533 to -0.146; N=10; P=0.001; Figure 5.2). Heterogeneity was found to be high between these studies ($I^2$=87.7%; Q=73.4, $T^2$=0.084, $d_f$=9). For this reason a random effects model was chosen to conduct the meta-analyses (Ades et al. 2005).

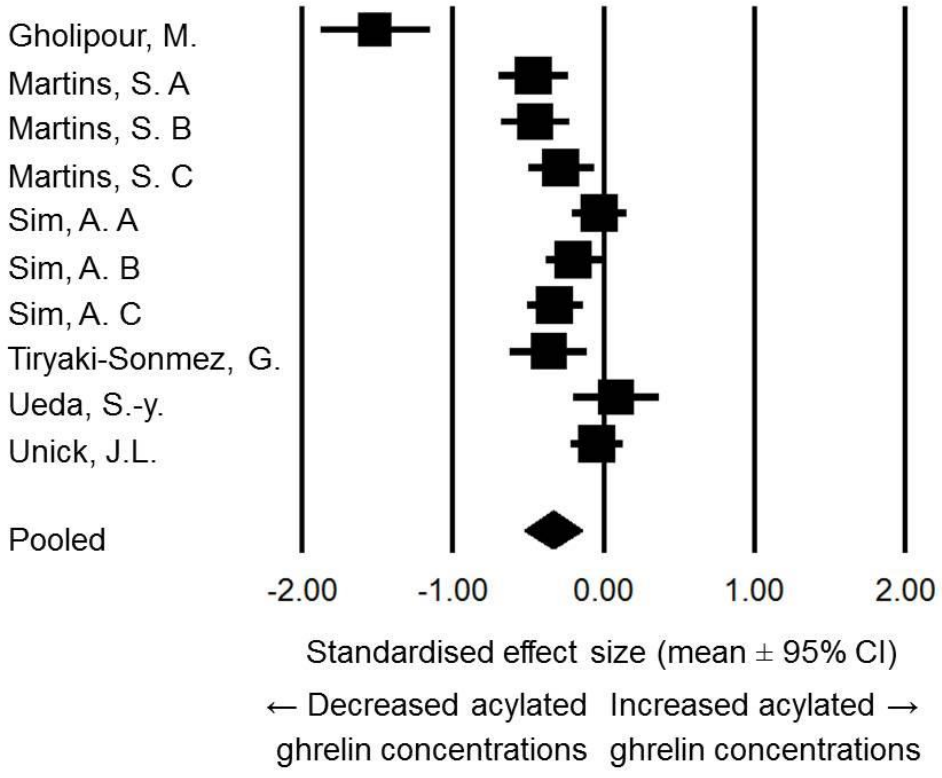


**Figure 5.2.** Forest plot of effect sizes (means±95% confidence intervals [CIs]) for studies evaluating acylated ghrelin time-averaged AUC values in response to exercise in ow/ob individuals.

Sensitivity analysis showed that the study by Gholipour et al. (2011) increased the effect size of exercise on mean acylated ghrelin time-averaged AUC concentrations. The removal of this study decreased the pooled effect size to -0.23 (95% confidence interval -0.35 to -0.11, P<0.001).

When data from lean individuals were included into the meta-analysis, with that of the previously-reported ow/ob individuals the pooled standardised effect size of exercise on acylated ghrelin time-averaged AUC data was reduced (pooled effect size -0.215, 95% confidence interval -0.324 to -0.105; N=33; P<0.001).

Using BMI as a moderator in a meta-regression model, a higher mean BMI was associated with a greater exercise induced suppression of acylated ghrelin time-averaged AUC concentration. The slope of regression for BMI was shallow, but significantly negative (95% confidence interval -0.073 to -0.010; P=0.044; Figure 5.3). The standardised reduction in acylated ghrelin for exercise vs. control conditions was found to be 0.037 units more marked for every 1 $kg \cdot m^{-2}$ increase seen in BMI. Including baseline acylated ghrelin concentrations and BMI into a multiple meta-regression model had little effect on the results; the slope of regression became slightly more negative so that the standardised reduction in acylated ghrelin for exercise vs. control was 0.040 units more marked for every 1 $kg \cdot m^{-2}$ increase in BMI (95% confidence interval -0.080 to -0.010; P=0.020). Sensitivity analysis showed that the study by Gholipour et al. (2011) strengthened the relationship between BMI exercise induced suppression of acylated ghrelin time-averaged AUC concentration. The removal of this study decreased the slope of regression, making it non-significant (95% confidence interval -0.052 to 0.014; P=0.25). However, no methodological reason was identified for the relatively high effect size in this particular study.

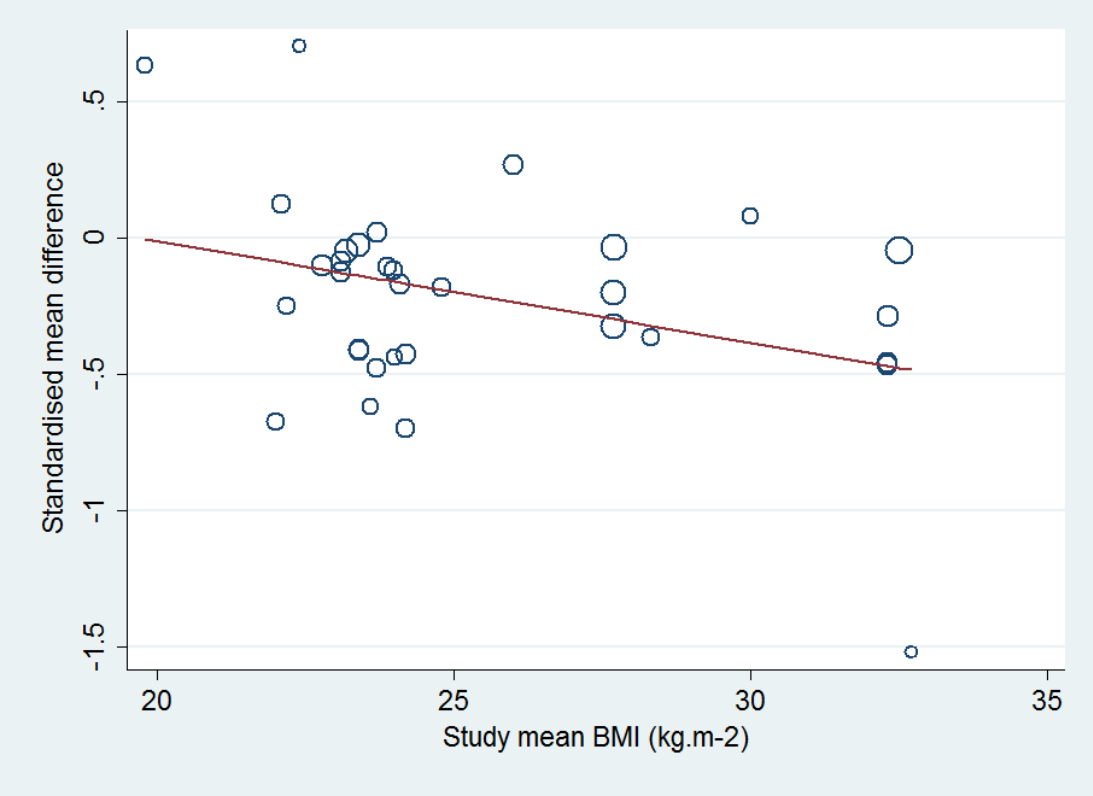


**Figure 5.3.** Univariable meta-regression for study mean BMI vs. the SMD for acylated ghrelin. A negative correlation was observed which persisted even when baseline (control) mean ghrelin concentration was added as a covariate. The area of each circle is proportional to that study's weight in the analysis.

Inspection of the funnel plot (Figure 5.4) and Egger's regression intercept revealed that there was little evidence of small study effects (intercept=-3.647, 95% confidence interval -9.080 to 1.785, P=0.264).

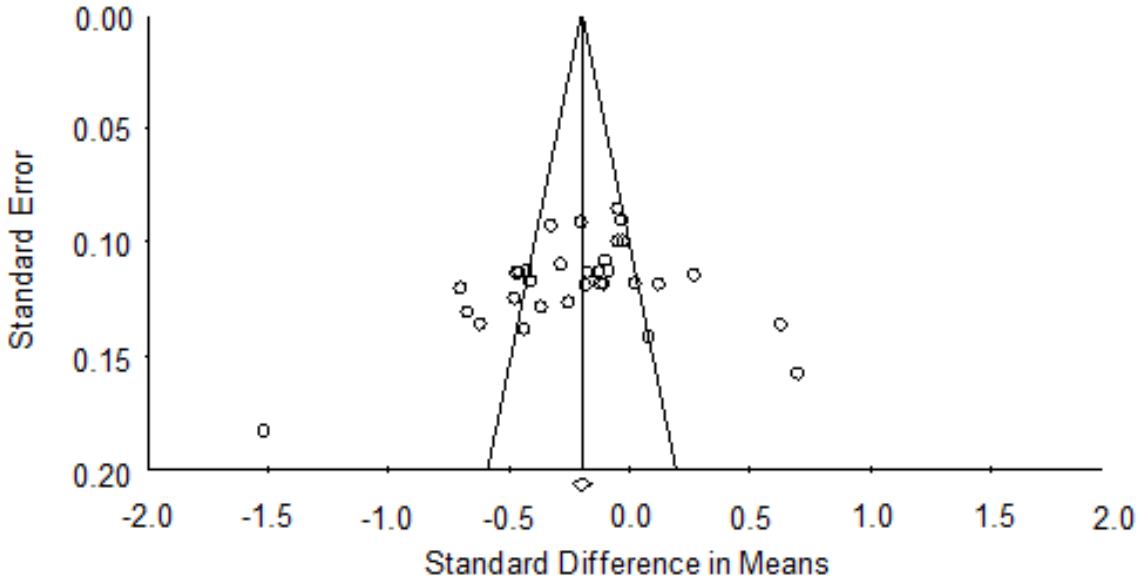


**Figure 5.4.** Funnel plot of standard error by standard difference in means for studies evaluating the influence of acute exercise on acylated ghrelin time-averaged AUC values in ow/ob individuals.

### 5.3.5. Effect size for total PYY time-averaged AUC analysis

In ow/ob individuals there was a trivial mean effect of exercise on total PYY (pooled effect size 0.099, 95% confidence interval -0.133 to 0.311; N=4; Figure 5.5), and this was not significantly different from zero (P=0.404). Heterogeneity was found to be low between these studies ($I^2$=24.17%; Q=3.96, $T^2$=0.014, $d_f$=3).

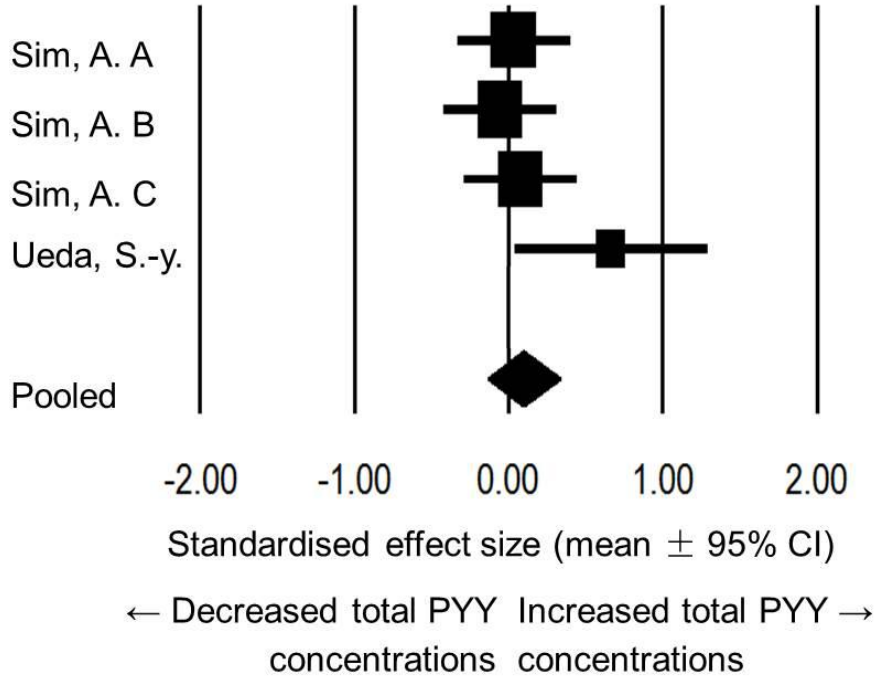


**Figure 5.5.** Forest plot of effect sizes (means±95% confidence intervals [CIs]) for studies evaluating the influence of acute exercise on total PYY time-averaged AUC values in ow/ob individuals.

### 5.3.6. Effect size and moderator variable for total GLP-1 time-averaged AUC analysis

In ow/ob individuals there was a trivial mean effect of exercise on GLP-1 (Pooled effect size -0.026, 95% confidence interval -0.184 to 0.133; N=4; Figure 5.6), and this was not significantly different from zero (P=0.749). Heterogeneity was found to be high between these studies ($I^2$=65.7%; Q=8.74, $T^2$=0.017, $d_f$=3).

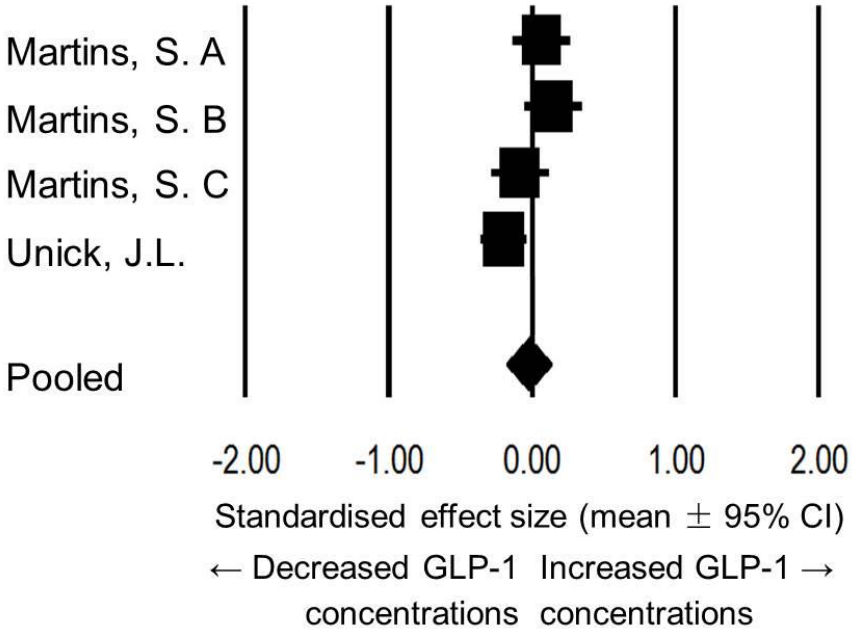


**Figure 5.6.** Forest plot of effect sizes (means±95 % confidence intervals [CIs]) for studies evaluating the influence of acute exercise on total GLP-1 time-averaged AUC values in ow/ob individuals.

## 5.4. Discussion

Understanding the responses of appetite regulatory hormones to exercise and consequently the effect they may have on energy intake and appetite could enhance understanding of the role of exercise in weight control. The purpose of this review was to examine the concentration changes of acylated ghrelin, total PYY and total GLP-1 after acute exercise in ow/ob individuals. We found acylated ghrelin to be moderately suppressed by acute exercise, whilst there were trivial effects of exercise on total PYY and total GLP-1. Ghrelin is an appetite stimulating hormone and our results suggest that exercise in ow/ob individuals alter acylated ghrelin in a direction that would be associated with decreased hunger and energy intake. We can only speculate the effects ghrelin has on appetite and food intake as not all studies included these measures in their protocol. Future research should examine energy intake in addition to appetite and appetite regulatory hormone responses to clarify this assumption.

The results of the current review appear to mirror those of lean individuals. In a recent review, lean individuals showed a small reduction in acylated ghrelin after exercise, whilst total PYY and total GLP-1 showed small increases (Schubert et al. 2014). Our findings suggest that ow/ob individuals show broadly similar appetite hormone responses to exercise in lean individuals, in such a direction that could alter energy intake and achieve weight loss if sustained over prolonged periods of time. Again, this can only be speculated as the studies included in both the review by Schubert et al. (2014) and the current review were acute in nature.

The present meta-regression demonstrated greater exercise-induced suppression of acylated ghrelin as BMI increased. Although ow/ob individuals have shown a moderate suppression of acylated ghrelin after exercise, this suppression becomes more prominent as BMI increases from 27.7 to 32.7 $kg \cdot m^{-2}$. This finding differed from that of Schubert et al. (2014), where BMI was shown to have no influence on appetite regulatory hormones. Schubert et al. (2014) included 23 studies examining responses of lean individuals and two studies with ow/ob individuals. The inclusion of six, rather than two, studies with overweight/obese individuals in the present meta-regression may explain the differences found between the two reviews.

The current review found ow/ob individuals to express a moderate reduction in acylated ghrelin during exercising conditions. Large variations in fasting and postprandial ghrelin concentrations between individuals make it difficult to establish the clinical relevance that

exercise has on this hormone. In lean individuals circulating concentrations of acylated ghrelin in the range of 40-67 $pg \cdot mL^{-1}$ may be expected in fasting conditions (Sato et al. 2012), with obese individuals expressing lower concentrations (Tschöp 2001; Shiiya et al. 2002). We attempted to control for differences in baseline levels of acylated ghrelin between studies by accounting for fasting values observed in resting trials. This had negligible influence on the relationship between increased BMI and acylated ghrelin.

The current review found three studies that examined the acute effects of exercise on appetite regulatory hormones in males, two studies in females and one including both males and females. Due to the limited number of studies in this review, no conclusions can be drawn upon the effect of exercise and sex on appetite regulatory hormones. It has been hypothesised that exercise could influence acylated ghrelin differently in obese males and females. In one study, after four days of consecutive exercise females experienced an increase in acylated ghrelin, whereas males showed no change (Hagobian et al. 2009). This suggests that females may be prone to increasing energy intake after exercise training. However, after acute exercise lean individuals have shown no sex difference in responses of $PYY_{3-36}$ or acylated ghrelin (Hagobian et al. 2013). Similar relative energy intakes were observed in males and females, suggesting that acute exercise is equally effective for both sexes. Future research is required to understand and compare the responses of males and females.

The current review has several limitations. First, only six studies were identified as relevant following our literature searches. We recognise that meta-analyses are not immune from statistical power-related issues, and that the pooling of data from such a small number of studies may still provide relatively low statistical precision (wide confidence interval for pooled effect). For example, despite finding substantial heterogeneity amongst studies in which ghrelin and GLP-1 were measured, precise analyses such as one for the presence of outliers could not be performed due to the small number of studies. We restricted our search to acute exercise trials, further reviews should examine the effects of repeated bouts of exercise and exercise training on appetite regulatory hormones in ow/ob individuals although at present the literature on this aspect is very limited. The longest trial length in the current review was 3 h, future research should examine what happens to this population later on in the exercising day. Despite trials only lasting 2-3 h, protocols differed widely between studies, potentially confounding appetite hormone time-averaged AUC estimations. Specifically, the timing of exercise and meal provision within the study protocol. Time-averaged AUC calculations were made over the duration of each trial, irrespective of when exercise occurred.

The inclusion of rest periods prior to exercise into time-averaged AUC calculations could potentially underestimate the effect of exercise on hormone responses. Additionally, studies differed by exercising participants in both fasted and fed states. Further still, studies varied in meal provision (standardised, *ad libitum* or no meal) after exercise. Together these could further cofound the study of hormonal responses to exercise. We meta-regressed the SMD in acylated ghrelin vs. study mean BMI. Such study-level explorations of potential moderators of effect size should be interpreted with caution as within-study relationships can sometimes disagree with between-study relationships (Petkova et al. 2013). The mean BMI of the participants in this review was 30.6 $kg \cdot m^{-2}$ (i.e. borderline obese), therefore we cannot generalise the findings of this review to those who fall higher into the obese or severely obese category. Despite our best efforts, we cannot guarantee that we captured all the relevant studies for this review. Finally, we cannot directly link the effects of exercise on appetite regulatory hormones to weight loss and management due to the acute nature of the studies, and the lack of consistency in studies including energy intake and appetite ratings.

An evidence synthesis of the six studies on ow/ob individuals indicated that a moderate reduction in acylated ghrelin occurs after acute exercise. Only trivial effects of exercise were quantified for total PYY and GLP-1.

# CHAPTER VI

## The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women (INTAKE)

### 6.1. Introduction

The findings reported in Chapter 4 of this thesis suggest that that there is no compensation in subjective appetite ratings, appetite regulatory hormones or *ad libitum* energy intake after two consecutive days of exercise. Like many other studies to date, this study was completed in lean healthy individuals (Schubert et al. 2013; Schubert et al. 2014). This limitation of the current literature is highlighted in Chapter 5, which found only six studies to examine the effects of exercise on appetite regulatory hormones in ow/ob individuals, at the time of review. In addition, few have compared the responses of lean and ow/ob individuals (Holmstrup et al. 2013; Martins et al. 2015; Sim et al. 2014; Ueda et al. 2009b) or males and females (Alajmi et al. 2016; Hagobian et al. 2009). It is therefore unknown as to whether the effects of exercise on appetite regulation seen in lean healthy males translate into females, or to those who are overweight or obese. Thus, a direct comparison of subjective appetite, appetite regulatory hormones and *ad libitum* energy intake responses in lean and ow/ob, males and females would be needed to confirm this.

Energy balance is the difference between energy consumed and energy expended. A sustained positive energy balance (whereby energy consumed outweighs that expended) results in weight gain and can cause overweight and obesity. The occurrence of a positive energy balance can be partially explained by dysfunctional appetite control. A number of gut hormones are responsible for appetite control, including acylated ghrelin, an appetite stimulating hormone, and appetite supressing hormones such as PYY and GLP-1. Typically, acylated ghrelin is suppressed after a meal, and PYY and GLP-1 increased, signalling meal cessation. The suppression of acylated ghrelin after a meal is attenuated in obese individuals (Morpurgo et al. 2003; Tentolouris et al. 2004) whilst PYY and GLP-1 responses are blunted after a meal (Adam & Westerterp-Plantenga 2005; Batterham et al. 2006; le Roux et al. 2005; le Roux et al. 2006; Ranganath et al. 1996; Verdich et al. 2001). Together these reduced feelings of fullness and satisfaction, promote food consumption and positive energy balance.

After acute vigorous intensity exercise, lean individuals show transiently suppressed appetite. This is associated with suppressed circulating concentrations of ghrelin and increased levels

of PYY and GLP-1 (Broom et al. 2009; King et al. 1994; King et al. 2010a; King et al. 2011a; Martins et al. 2007). In addition to the negative energy balance generated from exercise, the associated hormone changes suggest appetite control could be altered in a fashion that could sustain this deficit.

There are few studies examining the effects of acute exercise on subjective appetite perceptions, appetite regulatory hormones and *ad libitum* energy intake in ow/ob individuals. After exercise, acylated ghrelin has been shown to be decreased (Martins et al. 2015; Sim et al. 2014; Tiryaki-Sonmez et al. 2013) and unaltered (Ueda et al. 2009a; Unick et al. 2010), compared to control conditions. Similar discrepancies have also been found for other major appetite regulatory hormones, including PYY and GLP-1 (Martins et al. 2015; Sim et al. 2014; Tiryaki-Sonmez et al. 2013; Ueda et al. 2009a; Unick et al. 2010). Despite studies in this population finding contradictory responses for appetite regulatory hormones, all seem to agree that appetite perceptions do not differ during exercise and control conditions and show REI to be reduced. To date, research in ow/ob mirrors that in lean individuals with the mechanism responsible for transient appetite suppression yet to be confirmed. Presently, it is difficult to draw any conclusions from these studies. Specifically, data is limited by the small number of participants in each study, the short observation periods after exercise and differing study protocols, including exercise duration and intensity. Further research is required to determine if the effects of exercise on appetite control seen in lean participants translate into the overweight and obese.

Research on subjective appetite perceptions, appetite regulatory hormone and *ad libitum* energy intake responses to acute exercise has predominantly been conducted in males. Many have neglected responses in females, or failed to directly assess sex-based differences. A study by Hagobian and colleagues (2009) showed that after four consecutive days of acute exercise, designed to induce an energy deficit, a compensatory increase in circulating concentrations of acylated ghrelin was observed in females but not males, whilst hunger ratings remained unaltered in males and females. More recently, both males and females showed equal suppressions in appetite and circulating acylated ghrelin concentrations with no change in *ad libitum* energy intake after acute exercise (Alajmi et al. 2016). It is likely that these conflicting findings result from differing protocols, specifically, trial length and exercise intensity. Data on other key appetite regulatory hormones, such as, GLP-1 and total PYY within females is lacking. Further research is required to determine if males and females respond similarly to a bout of acute exercise.

The present investigation sought to advance on previous literature by comparing the effects of a single bout of aerobic exercise on subjective appetite perceptions, *ad libitum* energy intake and circulating concentrations of appetite regulating hormones (acylated ghrelin, desacylated ghrelin, total PYY, total GLP-1) as well as insulin, glucose, NEFA and TAG in lean and ow/ob males and females. Specifically, subjective appetite perceptions were examined during one hour of moderate intensity continuous exercise (60% V̇O2 peak) and for seven hours after. Plasma concentrations of appetite regulatory hormones were measured in responses to exercise, two standardised meals and an *ad libitum* buffet meal. The effects of exercise on energy intake were established at an *ad libitum* meal provided six hours after the cessation of exercise. Based on previous findings, in response to exercise, it was hypothesised that subjective appetite and *ad libitum* energy intake would be unaltered in lean individuals despite seeing transient increases in circulating concentrations of PYY and suppressed circulating concentrations of acylated ghrelin. Furthermore, it was hypothesised that females and ow/ob individuals would express similar appetite perceptions, energy intake and appetite regulatory responses to males and individuals who are lean, respectively.

## 6.2. Methods

### 6.2.1. Ethics procedures

Study design took several months, with input from experts in the field. Due to the nature of the project, it was necessary to seek NHS ethical approval. The ethics approval process was comprised of several stages (Figure 6.1).

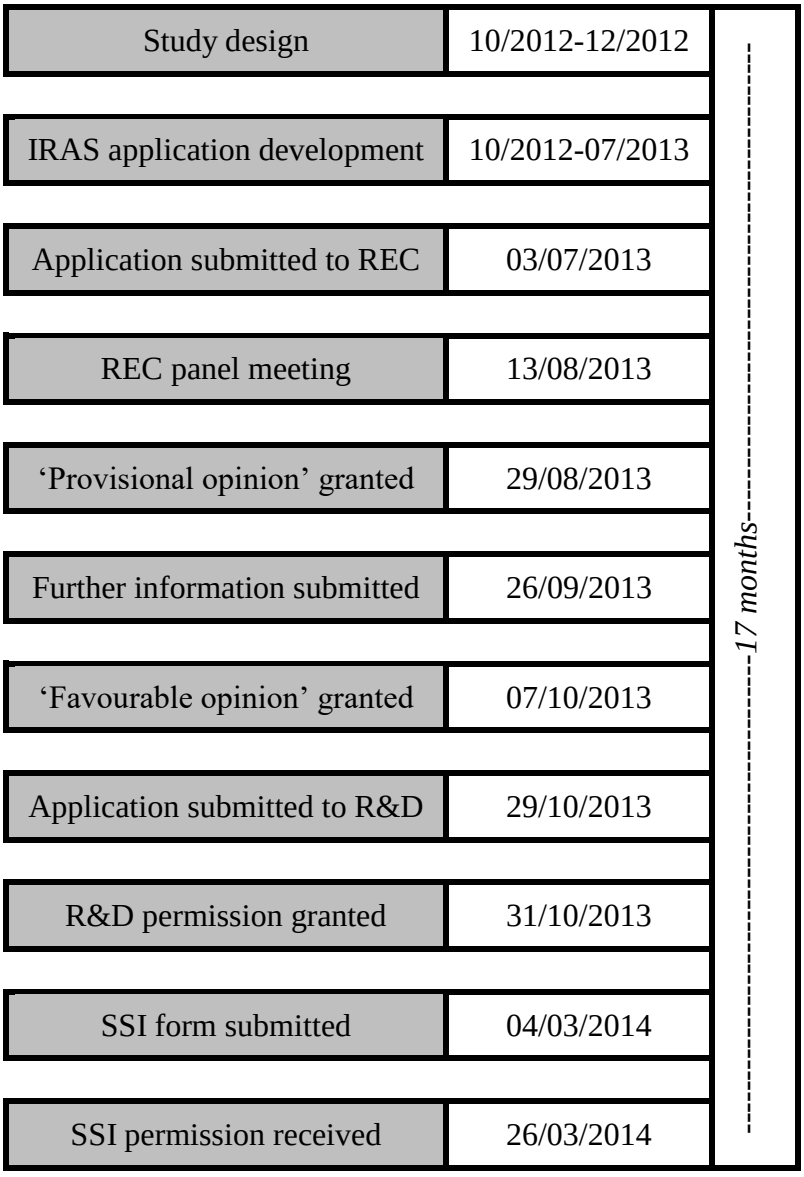


**Figure 6.1.** Schematic representation of the NHS ethics procedure. IRAS, integrated research application system; REC, research ethics committee; R&D, research and development; SSI, site-specific information.

The Integrated Research Application System (IRAS) was used to collate information required to apply for NHS/health and social care (HSC) Research & Development (R&D) and NHS/HSC Research Ethics Committee (REC) approval and to complete the Site Specific Information (SSI) form. Completion of the IRAS form also required submission of supporting study documentation, including;

- Study protocol document (Appendix M)
- Participant information sheet (Appendix N)
- Consent form (Appendix O)
- Study invitation letters (Appendix P)
- Trial reminder letters (Appendix Q)
- Recruitment posters (Appendix R)
- Screening questionnaires (Appendix S)

Upon completion, the IRAS application form and supporting documentation were submitted to REC for review. Part of the review process required research team members to attend a panel review meeting. Here the study protocol and supporting documentation were scrutinised by committee members. Subsequent email communication confirmed the outcomes of the meeting, requesting information for those areas needing further clarification and outlining any required document changes. After the submission of additional information, REC granted a 'favourable opinion'. Both R&D and SSI applications were then submitted. After acceptance of these, the study had full approval to begin.

During the study, it was necessary to make amendments to the original ethics documentation (Figure 6.2). Substantial amendments refer to those that made alterations to the study protocol, influenced safety of the participants or conduct of the study. Both REC and R&D were required to review these changes, and grant their approval. Non-substantial amendments refer to those where additional members of staff were added to the research team, minor changes were made to the study documentation (i.e. contact information), and study deadlines extended. Only R&D were required to grant approval for these changes.

We adhered to Good Clinical Practice guidelines issued by the International Conference on Harmonisation, and to the Declaration of Helsinki. Independent local ethics committees approved the protocol, amendments, and informed consent documents. Participants provided written consent before study procedures began.

| | | |
|---|---|---|
| NSA # 1<br>(Change to contact details and staff) | Submitted: 14/04/2014<br>Approved: 17/04/2014 | *3 days* |
| NSA # 2<br>(Change staff) | Submitted: 05/06/2014<br>Approved: 12/06/2014 | *7 days* |
| NSA # 3<br>(Change staff) | Submitted: 30/06/2014<br>Approved: 01/07/2014 | *1 day* |
| NSA # 4<br>(Change staff) | Submitted: 26/01/2015<br>Approved: 10/02/2015 | *15 days* |
| NSA # 5<br>(Change staff) | Submitted: 09/02/2015<br>Approved: 10/02/2015 | *1 day* |
| NSA # 6<br>(Change staff) | Submitted: 14/04/2015<br>Approved: 17/04/2015 | *3 days* |
| NSA # 7<br>(Study extension) | Submitted: 13/01/2016<br>Approved: 18/01/2016 | *5 days* |
| SA # 1<br>(Change to screening procedure and inclusion of extra blood samples) | Submitted: 24/04/2014<br>REC Approved: 06/05/2014<br>R&D Approved: 07/05/2014<br>Sponsor Approved: 13/05/2014 | *19 days* |
| SA # 2<br>(Measurement of HbA1c at screening, change in investigators) | Submitted: 03/07/2014<br>REC Approved: 25/07/2014<br>R&D Approved: 11/08/2014<br>Sponsor Approved: 14/08/2014 | *42 days* |
| SA # 3<br>(Change to inclusion criteria, study posters (Appendix T) and recruitment methods) | Submitted: 02/10/2014<br>REC Approved: 14/10/2014<br>R&D Approved: 24/10/2014<br>Sponsor Approved: 30/10/2014 | *28 days* |

**Figure 6.2.** Schematic representation of NHS amendments made. NSA, non-substantial amendment; SA, substantial amendment; REC, research ethics committee; R&D, research and development; HbA1c, haemoglobin A1c.

### 6.2.2. Recruitment

A complex and thorough approach was adopted to recruit the necessary participants for this study (Figure 6.3). The original protocol document submitted for ethical approval allowed for recruitment within the local community. Specifically the protocol document permitted distribution of study posters and leaflets in public places and local companies. Posters and leaflets were required to contain a brief overview of the study, key inclusion and exclusion criteria and further contact information. Individuals who felt eligible to participate contacted the research team via email or telephone, at which time they provided contact details (telephone number or email address) so that a participant information sheet, and if appropriate, a study invitation letter could be provided. In particular, posters were distributed around Loughborough University campus and Loughborough town centre (shops, restaurants, local businesses, supermarkets, town hall, community centres, religious centres and leisure centres). E-mails were sent to Loughborough University departments, local clubs and societies. The internet and social media was another useful outlet for study advertisement; local clubs and societies mentioned the study in newsletters, Loughborough University included the study in 'Twitter' posts, news articles were posted on the Loughborough University and NIHR homepages. In addition to these 'passive' methods, a recruitment stand was also present at two NIHR participant and patient involvement (PPI) days, a series of public lecture events, local businesses and supermarkets (targeting both staff and customers), and local wellbeing fairs. A live interview on a local BBC radio channel was also broadcast.

Original ethics documentation also allowed for contact with individuals who had previously consented to take part in future studies investigating diabetes from NHS study databases. Study invitation letters, reply slips and participant information sheets were sent to postal addresses. There was only one NHS study database within the department, where study inclusion criteria matched those of the current study. Eighty individuals were contacted from the 'STAND' (REC ref: 10/H0403/13) study, among the reply slips only two individuals were interested in taking part in the study. Only one matched the inclusion criteria, who later withdrew from the study after screening.

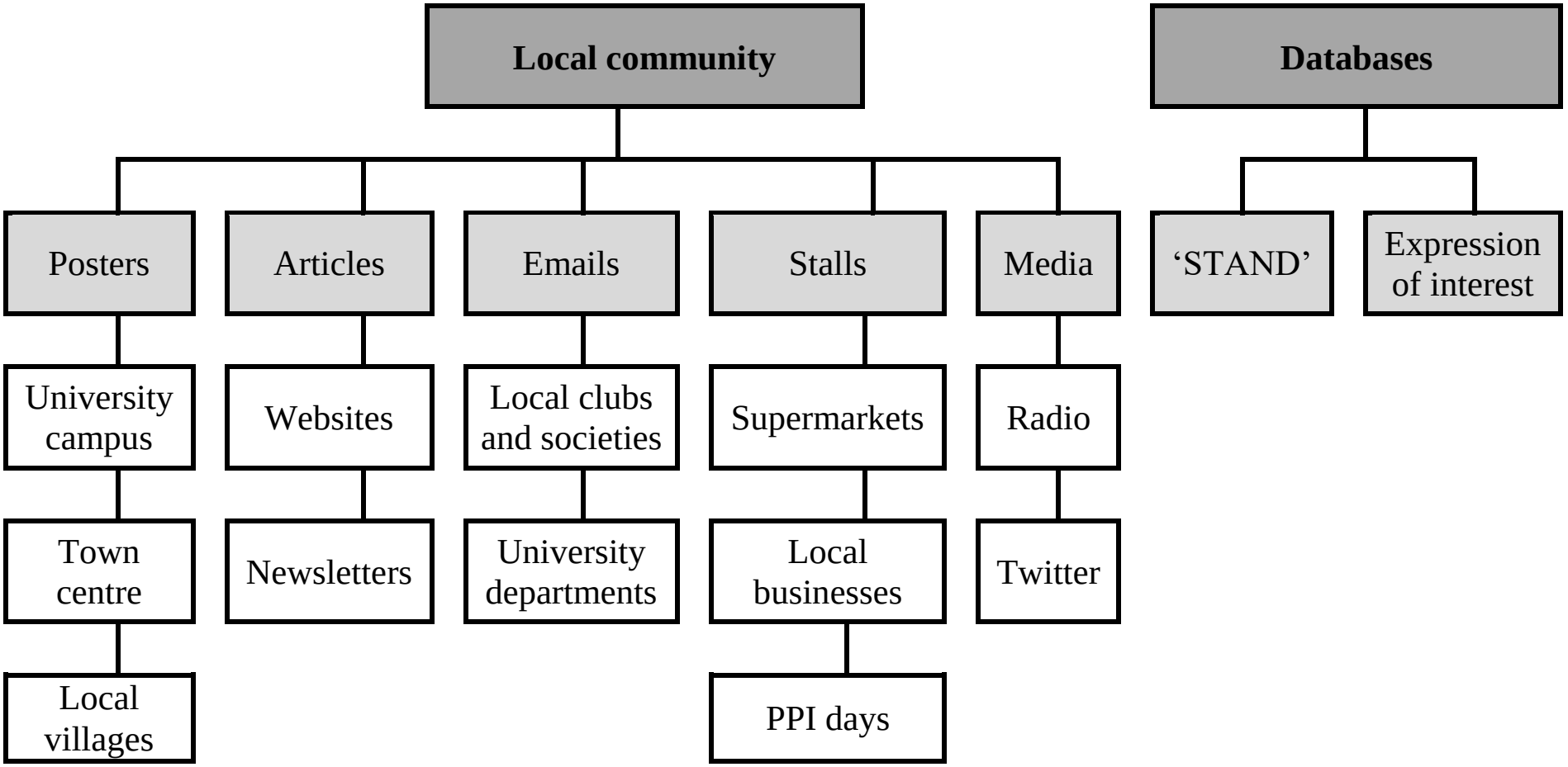


**Figure 6.3.** Schematic representation of the recruitment strategy. PPI, patient and public involvement.

### 6.2.3. Participants

Initial inclusion criteria for participation were as follows:

- 18-50 y
- non-smoker
- BMI of 18.5-24.9 or 30.0-39.9 $kg \cdot m^{-2}$
- inactive or moderately active (category 1 or category 2 on International Physical Activity Questionnaire, IPAQ)
- non-diabetic (fasting whole blood capillary glucose <7.0 $mmol.L^{-1}$) and no history of diabetes (HbA1c <6.5%)
- blood pressure <160/100 mmHg
- weight stable for at least 3 months before the study
- not dieting or exhibiting extreme eating habits
- not currently suffering from gastrointestinal, inflammatory, metabolic, cardiovascular or psychological diseases and no history of these conditions
- breakfast eaters
- not night shift workers
- no recent history of infectious disease
- not taking medication
- tolerance of food items provided during the experiment
- if female:
    - premenopausal
    - not pregnant

Later, due to slow recruitment, a substantial amendment was made to the inclusion criteria (Figure 6.2). Specifically, the criteria were widened, raising the age range to 18-65 y, the obese group was broadened to include those who were overweight (i.e. anyone with a BMI of 25.0 to 39.9), and postmenopausal females and non-breakfast eaters could be included.

Sixty participants matching the inclusion criteria provided formal written consent to take part in the study. Thirteen withdrew from the study; therefore, reported data is for 47 participants. Reasons for withdrawal included;

- cannulation issues (N=3)
- injury or illness (N=2)
- lost contact (N=3)
- moved away from the area (N=1)
- did not pass the screening visit (N=4).

Table 6.1 describes the participant characteristics. Of the 22 females, 12 were pre-menopausal (lean, 6; overweight, 6) and 10 post-menopausal (lean, 5; overweight, 5). Three females reported to be using contraceptives (lean, 2; overweight, 3).

**Table 6.1.** Characteristics of the study participants (N=47).

| | Lean Females (N=11)[a] | Ow/ob Females (N=11)[b] | Lean Males (N=11)[c] | Ow/ob Males (N=14)[d] | Effect Size | |
|---|---|---|---|---|---|---|
| | | | | | Sex | BMI |
| **Age (years)** | 38.6 (15.9) | 45.5 (13.2) | 36.4 (15.1) | 44.6 (12.1) | 0.08 | 0.05 |
| **Height (cm)** | 162.1 (4.0) | 164.9 (7.2) | 174.4 (5.2) | 176.8 (6.0) | 2.13 | 0.40 |
| **Weight (kg)**[*†] | 57.5 (4.9) | 78.5 (13.6) | 69.8 (5.0) | 92.9 (12.1) | 0.99 | 1.96 |
| **BMI ($kg \cdot m^{-2}$)**[*] | 21.9 (1.6) | 28.7 (2.8) | 23.0 (1.4) | 29.6 (3.0) | 0.35 | 2.93 |
| **Waist circumference (cm)**[*] | 69.7 (4.1) | 85.4 (7.3) | 81.6 (5.3) | 96.9 (7.9) | 1.25 | 1.88 |
| **Body fat (%)**[*†] | 24.9 (4.2) | 38.1 (3.5) | 16.9 (3.6) | 26.6 (4.2) | 1.25 | 1.66 |
| **Treadmill peak $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)**[*†] | 39.2 (9.9) | 29.7 (6.0) | 48.1 (13.0) | 38.6 (9.0) | 0.79 | 0.84 |
| **Treadmill peak $\dot{V}O_2$ ($L \cdot min^{-1}$)**[†] | 2.2 (0.6) | 2.3 (0.5) | 3.5 (0.7) | 3.5 (0.7) | 2.01 | 0.18 |
| **Fasting plasma glucose ($mmol \cdot L^{-1}$)** | 5.0 (0.7) | 4.7 (1.1) | 4.5 (0.9) | 4.7 (0.8) | 0.29 | 0.03 |
| **Fasting plasma total cholesterol ($mmol \cdot L^{-1}$)**[*] | 3.9 (1.1) | 4.4 (1.6) | 3.9 (1.1) | 5.1 (1.2) | 0.33 | 0.71 |
| **Fasting plasma HDL-cholesterol ($mmol \cdot L^{-1}$)**[*†] | 2.5 (0.2) | 1.8 (0.6) | 1.5 (0.5) | 1.2 (0.4) | 1.49 | 0.94 |
| **Fasting plasma LDL-cholesterol ($mmol \cdot L^{-1}$)**[*†] | 1.2 (1.0) | 2.3 (1.5) | 2.1 (1.1) | 3.5 (1.1) | 0.85 | 1.05 |
| **Fasting plasma triglyceride ($mmol \cdot L^{-1}$)** | 1.3 (1.0) | 1.6 (1.0) | 1.0 (0.7) | 1.7 (1.1) | 0.08 | 0.56 |
| **HbA1c (%)** | 5.3 (0.2) | 5.5 (0.3) | 5.5 (0.2) | 5.3 (0.3) | 0.11 | 0.07 |
| **Fasting leptin ($pg \cdot mL^{-1}$)**[*†] | 13752 (10380) | 23328 (9755) | 2795 (930) | 9063 (3796) | 1.47 | 0.74 |
| **HOMA-IR** | 1.0 (0.6) | 1.4 (1.1) | 1.1 (0.4) | 1.5 (1.0) | 0.12 | 0.51 |
| **Physical activity ($METS \cdot min^{-1} \cdot week^{-1}$)** | 1777 (1016) | 1367 (1000) | 1668 (965) | 1537 (1776) | 0.04 | 0.13 |

*Significantly different between lean and ow/ob ($P \leq 0.03$). [†]Significantly different between males and females ($P < 0.01$). [a]HbA1c values only available for N=9. [b]Body fat and HbA1c values are only available for N=10. [c]Body fat values are only available for N=10 and HbA1c for N=5. [d]Body fat values are only available for N=13 and HbA1c for N=10. Physical activity values were determined using the International Physical Activity Questionnaire. Values are mean (SD).

### 6.2.4. Preliminary testing

Prior to main trials participants visited the laboratory for two preliminary trials; (i) participant screening and (ii) exercise familiarisation.

#### 6.2.4.1. Participant screening

Before the start of any study procedures participants provided formal written consent. Participants underwent screening and preliminary anthropometric measures. After familiarisation with the laboratory environment, test equipment and test procedures, participants also completed two preliminary exercise tests.

Participants were provided with a participant information sheet (Appendix M), describing the purpose, protocol, demands of the study and any potential risks or harms of taking part. Participants were allowed at least 48 h to read over the participant information sheet. After further verbal explanation of the study and discussion of any questions, participants completed an informed consent form (Appendix N). Written informed consent was obtained by means of participant dated signature and dated signature of the designated researcher who presented and obtained informed consent. The informed consent process was undertaken in privacy. The researcher taking consent was adequately trained, following completion of a Good Clinical Practise and Consent for Research training course.

Experimental procedures began with the completion of a set of questionnaires to help determine participant's suitability to take part in the study. Specifically, participants completed questionnaires (Appendix S) to assess;

- health status (health screen questionnaire)
- physical activity readiness (PAR-Q)
- food preference and breakfast pattern questionnaire
- dietary habits (TEFQ)

The health status questionnaire screened for regular medication use and recent history of infectious disease. The PAR-Q is a validated questionnaire, used to determine if physical activity could pose a problem or hazard to participants. Tolerance to food items available at the standardised and buffet meals was assessed using the food preference questionnaire, those expressing a dislike for >20% of the food items available were excluded from the study.

The TFEQ screened participants who exhibited extreme dietary tendencies (including disinhibited and restrained eating). Participants scoring 11 or more for any factor on the TFEQ were considered to exhibit restrained or disinhibited eating tendencies, and consequently questionnaires were further scrutinised before determining suitability to take part in the study.

Height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany) and body mass was measured to the nearest 0.1 kg using a segmental body composition analyser (Tanita, Illinois, US). Body fat percentage was measured using bioelectrical impedance (Tanita, Illinois, US). For measurements of height, body mass and body fat percentage participants wore light clothing and removed their shoes and socks. BMI was subsequently calculated as body mass in kilograms divided by squared height in metres. Waist circumference was determined as the narrowest part of the torso between the xiphoid process and the iliac crest (Ross et al. 2008). A manual sphygmomanometer and stethoscope was used to measure resting blood pressure. An appropriately sized blood pressure cuff was selected and positioned over the brachial artery. Measurements were taken in triplicate after sitting for at least five min and the mean of these were used.

A fasting blood sample was collected to confirm that participants were non-diabetic (fasting glucose concentrations <7 $mmol.L^{-1}$, HbA1c <6.5%), and for the measurement of fasting total cholesterol, high density lipid-cholesterol, low density lipid-cholesterol and triglyceride concentrations. To measure glucose, cholesterol and triglyceride a blood sample was collected from the participant's finger using a lancet (Accu-Check Lancets, Roche, Basel, Switzerland). Samples were immediately dispensed onto test strips and analysed using hand held monitors (Accutrend Plus, Roche, Basel, Switzerland and CardioChek, PTS diagnostics, Indianapolis, USA). For the measurement of HbA1c, a venous blood sample was collected by venepuncture of an antecubital vein.

All participants underwent a 12 lead resting echocardiogram (ECG), performed by a suitably qualified member of the research team. The results from the resting ECG determined whether the participant was suitable to continue in the study, or if further ECG examination was required under exercising conditions in a clinical setting by trained staff.

Finally, a suitably trained member of staff performed a physical examination of the participant to assess the nervous, pulmonary and circulatory systems for abnormalities. A

verbal detailed medical history was also obtained, and this subsequently determined the suitability of the participant to continue in the study.

Provided it was determined safe for participants to exercise, participants completed two preliminary exercise tests on a level motorised treadmill (Technogym Excite Med, Cesena, Italy). After familiarisation with the protocol and treadmill, participants completed a 16 min progressive submaximal test, as described in Chapter 4. The initial treadmill speed was set between 4.0 and 8.0 $km \cdot h^{-1}$ depending on the fitness level of each participant and was increased between 0.5 and 1.0 $km \cdot h^{-1}$ after the completion of each 4 min stage. Oxygen consumption and carbon dioxide production were determined from continuous breath-by-breath analysis (MetaMax 3B, Cortex, Biophysik, Leipzig, Germany). Prior to sample analysis, the analyser was calibrated with certified reference gases. The Borg scale was used to obtain the participants RPE at the end of each 4 min stage (Borg 1973). This scale ranged from six (no exertion) to 20 (maximal exertion). Heart rate was measured throughout using short-range radio telemetry (Polar T31; Electro, Kempele, Finland).

After a period of rest, dictated by the participant, participants completed a $\dot{V}O_2$ peak test. An incremental uphill treadmill protocol was used to test participants' until volitional exhaustion. Run speed was constant and set at a speed corresponding to a heart rate of ~150 $beats \cdot min^{-1}$. Treadmill gradient began at 1% and increased by 1% at 1 min intervals until exhaustion, which was reached within 5 to 18 min. Samples of expired air and heart rate were collected throughout. Measures of RPE were collected at the end of each 1 min stage.

To ensure attainment of $\dot{V}O_2$ peak participants were required to meet ≥2 of the following criteria:

- plateau in oxygen consumption
- heart rate within 10 $beats \cdot min^{-1}$ of age predicted maximum
- RER≥1.1
- RPE≥19 (Cooke 2001)

The achieved $\dot{V}O_2$ peak of each participant was used in combination with individual walking/running speed-oxygen uptake regression equations to determine a walking/running speed corresponding to 60% $\dot{V}O_2$ peak. Participants started at this speed during familiarisation trials, with subtle adjustments made if necessary i.e. for cardiovascular drift.

#### 6.2.4.2. Exercise familiarisation

Participants visited the laboratory on a second occasion for an exercise familiarisation trial, at least 7 days after the screening visit. Participants performed 60 min of continuous treadmill exercise on a level motorised treadmill (Technogym Excite Med, Cesena, Italy) at a speed predicted to elicit 60% $\dot{V}O_2$ peak. Work rate was measured throughout (MetaMax 3B, Cortex, Biophysik, Leipzig, Germany) to monitor the intensity of the treadmill exercise, with adjustments made to the treadmill speed if necessary. Heart rate was also measured throughout (Polar T31; Electro, Kempele, Finland). Ratings of perceived exhaustion were collected at 15, 30, 45 and 60 min.

To ensure that participants adhered to the strict dietary and physical activity standardisation before each main trial, participants were given verbal and written instructions at the familiarisation visit. Specifically, participants were reminded to refrain from alcohol, caffeine, and taking part in any structured form of physical activity in the 48 h before each main trial. An explanation was provided for how to record and subsequently replicate dietary intake before each main trial using a weighed food diary. Before the first trial participants recorded their dietary intake for 48 h and were instructed to consume identical amounts of food and drink items, at identical times, in the 48 h before the second trial. Participants were provided with a food record diary and set of food weighing scales, if required, to assist with this. Participants were provided with food to consume the eve before each main trial, along with verbal and written instructions on how to prepare this meal. This meal was the final meal consumed before each trial and was consumed before 21:00, water was available *ad libitum* after this time. Participants were instructed to note the time this meal was consumed on the eve of the first trial, and this was to be strictly matched on the eve of the second trial. Participants were asked to confirm that they had adhered to the dietary and exercise standardisations before starting each experimental trial day.

### 6.2.5. Experimental protocol

Participants completed two main trials (control and exercise) in a crossover design with each trial being separated by at least 7 days in males, and by 28 days in females to control for the effects of the menstrual cycle (Figure 6.4).Each trial lasted 8 h and commenced at 09:00 after an overnight fast of at least 12 h.

The exercise trial commenced with 60 min continuous treadmill exercise (Technogym Excite Med, Cesena, Italy). The speed of the treadmill was set to that determined in the exercise familiarisation trial to elicit 60% $\dot{V}O_2$ peak. Heart rate and expired air samples were collected by breath-by-breath analysis (MetaMax 3B, Cortex, Biophysik, Leipzig, Germany) throughout the treadmill session. Ratings of perceived exertion were assessed at 15, 30, 45 and 60 min during the exercise. Energy expenditure during the exercise session was calculated from oxygen uptake and carbon dioxide production (Frayn, 1983). These measurements were also used to calculate RER (carbon dioxide production divided by oxygen consumption). After the run participants rested in the laboratory for 7 h (sitting, reading, working at a desk or watching films). Blood samples and appetite questionnaires were completed periodically. Set meals were provided at 1.5 h and 4 h, and a buffet meal at 7 h.

The procedures in the control trial were identical to the exercise trial except that no exercise was performed. The sole exception to this was that resting expired air samples were collected during the first hour to assess resting metabolic rate. These data subsequently permitted the calculation of net exercise energy expenditure i.e. gross exercise energy expenditure during exercise minus resting energy expenditure.

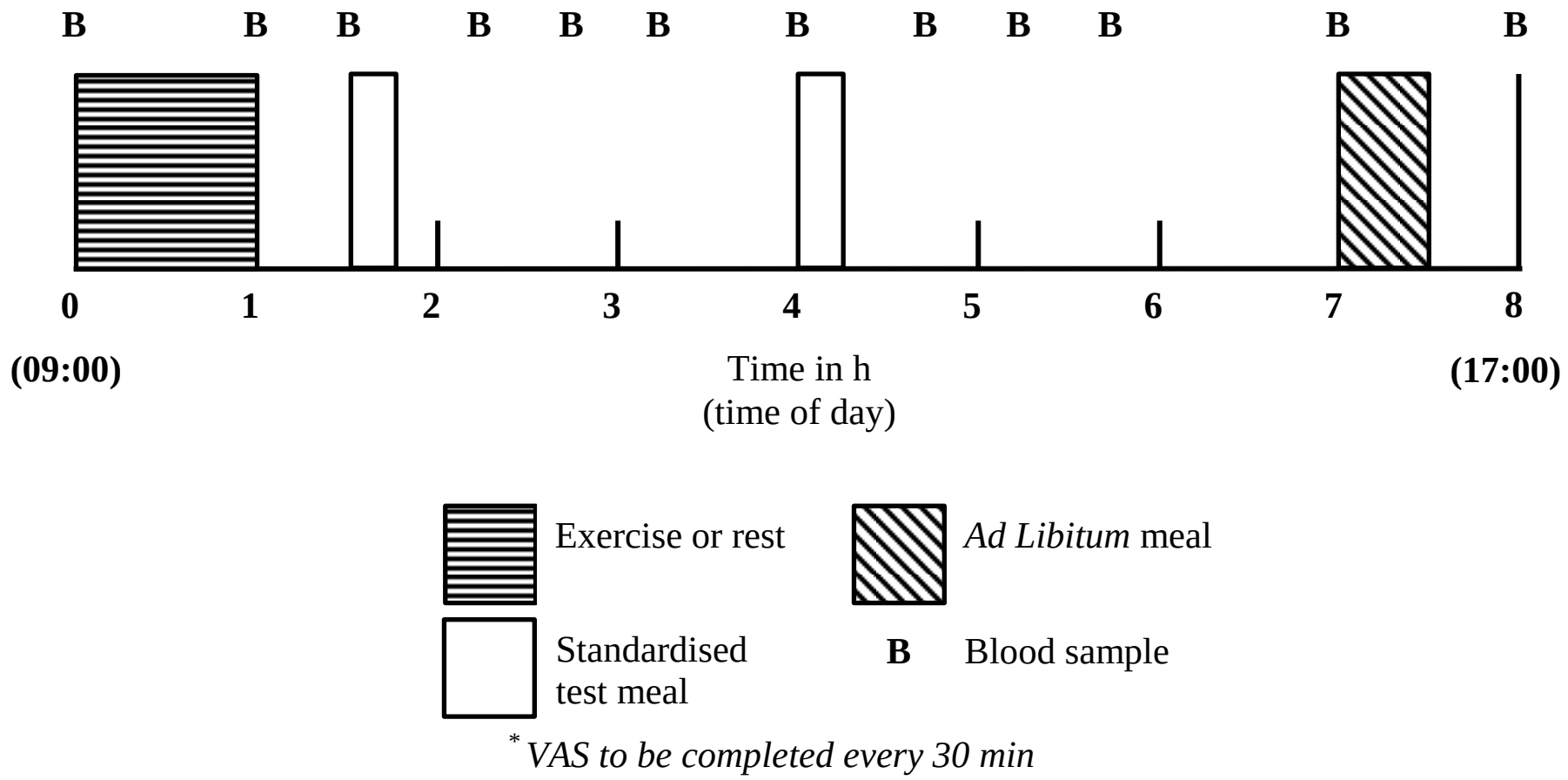


**Figure 6.4.** Schematic representation of the main trial protocol. VAS, visual analogue scale.

### 6.2.6. Appetite measures

During each trial appetite perceptions (hunger, satisfaction, fullness and PFC) were assessed at baseline and at every 30 min thereafter, as described in Chapter 4.

### 6.2.7. Food provision, standardised test meals and *ad libitum* buffet meal

To assist with dietary standardisation, participants were provided with a food package to consume on the eve of each main trial. Food packages contained white fusilli (Tesco fusilli pasta twists), tomato sauce (Dolmio Express tomato and basil sauce) and chocolate biscuits (Kit Kat milk chocolate biscuit). The calorie content of this meal was 750 kcal for males and 674 kcal for females, and the macronutrient content was 71% carbohydrate, 11% protein and 18% fat. The meal was designed to provide ~35% of the estimated daily energy needs for a healthy weight, inactive individual. The energy needs for an inactive individual were calculated using the Mifflin equation and a physical activity factor of 1.4 (Mifflin et al. 1990). This equation was selected as it considers the participants age, height, body mass and sex allowing for a more accurate estimation. A 1.4 activity factor was selected to represent the sedentary nature of the experimental visit. The Mifflin equations for males and females are below:

Male resting energy expenditure = (10 x body mass) + (6.25 x height) – (5 x age) + 5;

Female resting energy expenditure = (10 x body mass) + (6.25 x height) – (5 x age) – 161.

Written and verbal instructions on how to prepare the meal were given at the exercise familiarisation visit (as described in chapter 6.2.4.2.). Participants were instructed to consume the meal by 21:00 on the eve of each main trial, and that water could be consumed *ad libitum* thereafter.

At 1.5 h (10:30) participants were provided with a standardised breakfast, which consisted of a jam sandwich (brown bread (Tesco wholemeal medium bread), margarine (Tesco butter me up original spread), and strawberry jam (Tesco strawberry jam)), banana and orange juice (Tesco pure orange juice smooth). The calorie content of this meal was 643 kcal for males and 578 kcal for females, and the macronutrient content was 72% carbohydrate, 10% protein and 18% fat. The meal was designed to provide ~30% of the estimated daily energy needs for a healthy weight, inactive individual. Participants were instructed to eat all of the meal within 15 min.

At 4 h (13:00) a standardised lunch meal consisting of either a tuna (Tesco tuna chunks pole and line in spring water) and mayonnaise (Tesco mayonnaise) or cheese (Tesco lighter mature cheese) white bread (Tesco white medium bread) sandwich (depending on preference, determined at the screening visit), with salted crisps (Tesco ready salted crisps), golden delicious apple and a chocolate muffin (Tesco chocolate muffins). The calorie content of this meal was 750 kcal for males and 674 kcal for females, and the macronutrient content of this meal was 43% carbohydrate, 32% protein, 25% fat. Participants were instructed to eat all of the meal within 15 min.

Energy and macronutrient intake were assessed at an *ad libitum* meal provided at 7 h (16:00) on each trial day. Acceptability of the buffet items was ensured during the preliminary trial via the completion of a food preference questionnaire (Appendix S). The questionnaire required participants to rate preselected food items on a Likert scale ranging from one (dislike extremely) to ten (like extremely). Participants were excluded from the study if they rated >20% of the items at 4 or less. The items available are listed in Appendix U. The buffet foods were presented identically on each occasion and were in excess of expected consumption. Participants were allowed up to 30 min to consume the buffet foods but instructed to eat until 'comfortably full' and informed that additional food was available if desired. Participants consumed meals in isolation so that social influence did not affect eating speed, food selection or quantity eaten. Participants were not overtly aware that their food intake was being measured. After the *ad libitum* meal leftovers were weighed and food consumption was determined as the weighed difference of items before and after the meal. The energy and macronutrient content of the items consumed were determined from manufacturers' values.

### 6.2.8. Blood sampling

Upon arrival to the laboratory, participants rested in a semi-supine position and a cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein. Blood samples were collected at: baseline, 1, 1.5, 2.25, 2.75, 3.25, 4, 4.75, 5.25, 5.75, 7 and 8 h. Samples from the cannula were taken as described in Chapter 4.

Venous blood samples were taken into two pre-chilled EDTA monovettes (4.9 and 9 mL) (Sarstedt, Leicester, UK) for the determination of plasma acylated and desacylated ghrelin, total PYY, total GLP-1, insulin, glucose, TAG and non-essential fatty acids (NEFA). To prevent the degradation of acylated ghrelin, blood samples collected into the 4.9 mL

monovette were pre-treated and processed following manufacturer instructions (see Chapter 3). The remaining analytes were measured from the resulting plasma collected into the 9 mL monovette as described in Chapter 4. The plasma supernatant was aliquoted into 2 mL Eppendorfs and stored at -20°C, then transferred to -80°C for future analysis. Changes in plasma volume were estimated, as described in Chapter 4.

### 6.2.9. Biochemical analysis

Commercially available enzyme immunoassays were used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), desacylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Billerica, USA), total GLP-1 (Millipore, Billerica, USA) and insulin (Mercodia, Uppsala, Sweden) with the aid of a plate reader to measure absorbance (Varioskan Flash Multiple Mode Reader, Vantaa, Finland). Plasma glucose, TAG and NEFA concentrations were determined by enzymatic, colorimetric methods using a bench top analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France). Plasma concentrations of total ghrelin were calculated by adding plasma acylated ghrelin and desacylated ghrelin concentrations. Precision of analysis was ensured by quantification of an internal quality control exhibiting high and low values. To eliminate inter-assay variation, samples from each participant were analysed on the same run. The within-batch coefficients of variation for the assays were as follows: acylated ghrelin 5.2%, desacylated ghrelin 4.8%, total PYY 6.1%, insulin 4.5%, total GLP-1 10.2%, glucose 0.7%, TAG 1.5%, and NEFA 2.4%.

### 6.2.10. Statistical analysis

Data were analysed using IBM SPSS statistics, version 21.0. for Windows. Appetite perception (hunger, satisfaction, fullness and PFC) and appetite hormone (acylated ghrelin, desacylated ghrelin, total ghrelin, total PYY, total GLP-1, insulin, TAG, glucose, NEFA) total AUC calculations were performed using the trapezoidal method. For the purpose of hormone total AUC calculations, missing sample points were inputted by multiplying the preceding sample point by the mean change across these two time points. i.e.:

$$x = (x-1) * \frac{\overline{x}}{\overline{x-1}}$$

where $x$ is the missing sample point, $(x-1)$ is the preceding sample, $\overline{x}$ is the group mean value of the hormone at the same time point as missing sample $x$, and $\overline{x-1}$ is the group

mean value of the hormone at the preceding time point to the missing sample. Given the behavioural and unstandardized nature of the *ad libitum* buffet meal at 7 h, AUC calculations were completed for the whole 8 h trial period, and the first 7 h of each trial. However, shortening the AUC period did not alter the statistical significance of the results; therefore, for simplicity, only the 8 h values are presented.

Two-factor linear mixed models were used to examine participant characteristics and exercise responses with fixed (sex and BMI) and random (participant) factors. Three-factor linear mixed models were used to examine baseline measurements, appetite perception total AUC and appetite hormone total AUC data with fixed (sex, BMI and trial) and random (participant) factors. Four-factor linear mixed models were used to examine appetite perceptions and appetite hormones across trials with fixed (sex, BMI, trial and time) and random (participant) factors. Where significant trial vs. time interaction effects were found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple comparisons (Atkinson 2002). Data were also plotted on graphs for clarity. Where significant trial vs. BMI and trial vs. sex interactions were found, data were graphically represented and written descriptions made. The Pearson product moment correlation coefficient was used to examine relationships between waist circumference and key outcomes (i.e. absolute energy intake, hunger total AUC, and acylated ghrelin, desacylated ghrelin, PYY and GLP-1 total AUC). Corrections of plasma analyte values for changes in plasma volume did not alter the statistical significance of the results; therefore, for simplicity, the unadjusted values are presented. Statistical significance was accepted at 5%. Absolute standardised effect sizes (ES) were calculated to supplement important findings. The differences between mean values (control vs. exercise, lean vs. overweight, or females vs. males) were divided by the pooled standard deviation. An ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (Cohen 1988). Results are shown as mean (SD) in text and tables, and mean (SEM) in figures (for clarity).

### 6.2.11. Power calculations

The power calculation for this study was based on its primary outcome; in particular, the effect of acute exercise on circulating concentrations of appetite regulatory hormones. Power calculations were determined by obtaining effect sizes from previous research within our laboratory (King et al. 2011). This study examined differences in circulating concentrations of acylated ghrelin and $PYY_{3-36}$ between an exercise and control trial (total trial AUC). This

previous data revealed effect sizes of 0.6. Using this data, and assuming an alpha of 0.05 and acceptable power of 0.8, the present study requires a sample size of 64 participants (16 lean females, 16 obese females, 16 lean males and 16 obese males).

However, it must be noted that the stated sample size was calculated using the original ethics documentation. It is likely that by broadening the participant inclusion criteria, i.e. wider age and BMI range, the sample size required to detect a meaningful result would have increased. However, there are no borderline statistics reported in this chapter. This would suggest there are no cases of type 2 error. To address the issue of under-powering, effect sizes are reported alongside significance values.

### 6.3. Results

#### 6.3.1. Exercise responses

The exercise responses are summarised in Table 6.2. Participants completed the 60 min bout of treadmill exercise at 6.7 (1.2) km·h$^{-1}$. This elicited a mean oxygen consumption equivalent to 59 (4) % of $\dot{V}O_2$ peak and a net energy expenditure of 1851 (601) kJ. The RER was 0.88 (0.06), which reflected proportional contribution to energy provision of 62% (22%) carbohydrate and 38% (22%) fat. Heart rate and RPE were 135 (25) beats·min$^{-1}$ and 12 (2), respectively.

**Table 6.2.** Exercise responses (N=47).

| | Lean Females (N=11)[a] | Ow/ob Females (N=11)[b] | Lean Males (N=11)[c] | Ow/ob Males (N=14)[d] | Effect Size | |
|---|---|---|---|---|---|---|
| | | | | | Sex | BMI |
| **Treadmill speed (km·h$^{-1}$)*†** | 6.6 (1.6) | 5.8 (0.7) | 7.6 (1.0) | 6.8 (0.8) | 0.81 | 0.67 |
| **Mean heart rate (beats·min$^{-1}$)** | 136 (31) | 118 (14) | 141 (29) | 142 (21) | 0.55 | 0.24 |
| **Mean relative $\dot{V}O_2$ (mL·kg·min$^{-1}$)*†** | 24 (6) | 17 (3) | 28 (6) | 23 (5) | 0.77 | 1.00 |
| **Mean absolute $\dot{V}O_2$ (L·min$^{-1}$)†** | 1.4 (0.4) | 1.3 (0.3) | 2.0 (0.4) | 2.1 (0.4) | 1.87 | 0.06 |
| **Exercise intensity (% of $\dot{V}O_2$ peak)*** | 61 (3) | 57 (4) | 59 (3) | 58 (3) | 0.07 | 0.66 |
| **RPE (6-20)** | 11 (3) | 12 (2) | 12 (1) | 12 (1) | 0.40 | 0.17 |
| **Net energy expenditure (kJ)†** | 1503 (449) | 1350 (333) | 2211 (507) | 2265 (481) | 1.89 | 0.02 |
| **Mean RER** | 0.86 (0.06) | 0.86 (0.06) | 0.89 (0.07) | 0.89 (0.07) | 0.54 | 0.02 |
| **Carbohydrate utilisation (%)** | 56.5 (21.5) | 55.2 (21.9) | 66.5 (22.6) | 66.9 (21.8) | 0.51 | 0.01 |
| **Fat utilisation (%)** | 43.5 (21.5) | 44.8 (21.9) | 33.5 (22.6) | 33.1 (21.8) | 0.51 | 0.01 |

*Significantly different between lean and ow/ob (P<0.01). †Significantly different between males and females (P<0.01).[a]Mean treadmill oxygen uptake, exercise intensity, net energy expenditure mean RER, carbohydrate and fat utilisation only available for N=9. [b]Mean heart rate only available for N=10.[c]Mean heart rate, mean treadmill oxygen uptake, exercise intensity, net energy expenditure mean RER, carbohydrate and fat utilisation only available for N=9. [d]Net energy expenditure only available for N=13. Net energy expenditure was calculated as exercise energy expenditure minus energy expenditure at rest. Values are mean (SD).

Treadmill speed, mean absolute $\dot{V}O_2$, mean relative $\dot{V}O_2$, and net energy expenditure were significantly higher for males than females (treadmill speed, 7.2 (1.0) vs. 6.2 (1.3) $km \cdot h^{-1}$; mean absolute $\dot{V}O_2$, 2.0 (0.4) vs. 1.4 (0.3) $L \cdot min^{-1}$; mean relative $\dot{V}O_2$, 25 (6) vs. 20 (6) $mL \cdot kg \cdot min^{-1}$; net energy expenditure, 2243 (481) vs. 1419 (386) kJ for males and females, respectively; all $P \leq 0.01$). Treadmill speed, mean relative $\dot{V}O_2$ and exercise intensity were significantly higher for lean than ow/ob individuals (treadmill speed, 7.1 (1.4) vs. 6.3 (0.9) $km \cdot h^{-1}$; mean relative $\dot{V}O_2$, 26 (7) vs. 20 (5) $mL \cdot kg \cdot min^{-1}$; exercise intensity (% of $\dot{V}O_2$ peak), 60 (3) vs. 58 (4) for lean and ow/ob, respectively; all $P \leq 0.01$).

### 6.3.2. Resting expired air samples

The resting expired air values are summarised in Table 6.3. There were no significant differences in mean RER, carbohydrate or fat utilisation between sex or BMI group. Mean relative $\dot{V}O_2$ was significantly higher for lean than ow/ob individuals (3.72 (0.62) vs. 3.18 (0.57) $mL \cdot kg \cdot min^{-1}$ for lean and ow/ob, respectively; $P<0.01$). Mean absolute $\dot{V}O_2$ and energy expenditure were significantly higher for males than females (mean absolute $\dot{V}O_2$, 0.28 (0.05) vs. 0.23 (0.05) $L \cdot min^{-1}$; energy expenditure, 5.93 (0.93) vs. 4.74 (1.04) $kJ \cdot min^{-1}$ for males and females, respectively; $P \leq 0.01$).

**Table 6.3.** Resting expired air values. (N=46).

| | Lean Females (N=11) | Ow/ob Females (N=11) | Lean Males (N=11) | Ow/ob Males (N=13) | Effect Size | |
|---|---|---|---|---|---|---|
| | | | | | Sex | BMI |
| **Mean relative $\dot{V}O_2$ ($mL \cdot kg \cdot min^{-1}$)*** | 3.69 (0.62) | 3.03 (0.49) | 3.75 (0.65) | 3.31 (0.62) | 0.23 | 0.45 |
| **Mean absolute $\dot{V}O_2$ ($L \cdot min^{-1}$)†** | 0.22 (0.06) | 0.24 (0.05) | 0.27 (0.05) | 0.30 (0.04) | 1.04 | 0.90 |
| **Energy expenditure ($kJ \cdot min^{-1}$)†** | 4.58 (1.16) | 4.90 (0.93) | 5.62 (0.89) | 6.18 (0.92) | 1.20 | 0.02 |
| **Mean RER** | 0.85 (0.08) | 0.82 (0.05) | 0.87 (0.07) | 0.85 (0.05) | 0.35 | 0.37 |
| **Carbohydrate utilisation (%)** | 51.9 (28.4) | 43.1 (17.9) | 59.4 (24.1) | 52.4 17.9) | 0.36 | 0.33 |
| **Fat utilisation (%)** | 48.1 (28.4) | 56.9 (17.9) | 40.7 (24.1) | 47.6 (17.9) | 0.36 | 0.33 |

*Significantly different between lean and ow/ob ($P<0.01$). †Significantly different between males and females ($P<0.01$). Values are mean (SD).

### 6.3.3. Appetite responses

Fasting appetite perceptions (hunger, fullness, satisfaction and PFC) did not differ between sex, BMI group or trial at baseline (all P≥0.10, Table 6.4). Despite this, baseline hunger was seen to be lower in the exercise than control trial for lean females and ow/ob males. Prior knowledge of the trial participants would be completing could have influenced baseline hunger ratings.

**Table 6.4.** Baseline appetite perceptions in the control and exercise trials (N=47).

| Fasting appetite perception (mm) | Trial | Lean Females (N=11) | Ow/ob Females (N=11) | Lean Males (N=11) | Ow/ob Males (N=14) |
|---|---|---|---|---|---|
| **Hunger** | Control | 47 | 49 | 52 | 37 |
| | | (26) | (24) | (28) | (27) |
| | Exercise | 34 | 48 | 52 | 26 |
| | | (29) | (24) | (25) | (22) |
| **Satisfaction** | Control | 19 | 37 | 30 | 30 |
| | | (11) | (16) | (19) | (23) |
| | Exercise | 29 | 24 | 38 | 40 |
| | | (28) | (10) | (29) | (22) |
| **Fullness** | Control | 16 | 26 | 25 | 27 |
| | | (13) | (18) | (18) | (19) |
| | Exercise | 26 | 23 | 28 | 35 |
| | | (29) | (13) | (21) | (24) |
| **PFC** | Control | 66 | 63 | 63 | 62 |
| | | (24) | (20) | (22) | (29) |
| | Exercise | 49 | 61 | 65 | 50 |
| | | (31) | (11) | (20) | (25) |

Values are mean (SD).

Across trials, four-factor linear mixed modelling (sex vs. BMI vs. trial vs. time) revealed a significant main effect of trial for hunger and PFC (both P≤0.01; Figure 6.5), indicating suppressed ratings in the exercise trial for all participants. A significant trial vs. time interaction effect was found for hunger (P≤0.01), satisfaction (P=0.04) and PFC (P≤0.01). Compared with control, *post-hoc* analysis revealed suppressed hunger ratings at 0.5 (P=0.02) and 1.0 h (P=0.04) of the exercise trial. Similarly, *post-hoc* analysis revealed suppressed PFC perceptions at 0.5 (P<0.01) and 1.0 h (P=0.01). A significant trial vs. sex interaction was found for PFC, males showed a greater suppression of PFC than females during the exercise trial (11 vs. 2%, P=0.04; Appendix V). Significant trial vs. BMI interactions were found for

satisfaction (P=0.04; Appendix V) and PFC (P<0.01; Appendix V). For satisfaction, lean and ow/ob responded in opposite directions, with ow/ob being less satisfied in the exercise compared to control trial and lean individuals showing an increase in satisfaction in the exercise trial. For PFC, lean individuals showed a greater suppression of PFC than ow/ob during the exercise trial (12 vs. 1%, for lean and ow/ob, respectively).

Analysis of the appetite total AUC using three-factor linear mixed modelling (sex vs. BMI vs. trial) did not show any differences in appetite perceptions (Table 6.5).

**Table 6.5.** Total area under the curve (AUC) values (0-8 h) for hunger, satisfaction, fullness and prospective food consumption (PFC) during control and exercise trials (N=47).

| AUC (0-8h) | Trial | Lean Females (N=11) | Ow/ob Females (N=11) | Lean Males (N=11) | Ow/ob Males (N=14) |
|---|---|---|---|---|---|
| **Hunger** | **Control** | 217 (97) | 261 (117) | 312 (127) | 226 (86) |
| | **Exercise** | 170 (90) | 237 (117) | 288 (146) | 221 (85) |
| **Satisfaction** | **Control** | 438 (98) | 452 (133) | 401 (69) | 442 (93) |
| | **Exercise** | 461 (96) | 446 (112) | 416 (132) | 440 (96) |
| **Fullness** | **Control** | 434 (94) | 431 (118) | 375 (76) | 441 (99) |
| | **Exercise** | 446 (115) | 427 (95) | 393 (105) | 431 (85) |
| **PFC** | **Control** | 304 (111) | 318 (120) | 365 (105) | 311 (98) |
| | **Exercise** | 242 (88) | 310 (115) | 342 (125) | 317 (90) |

Values are mean (SD).

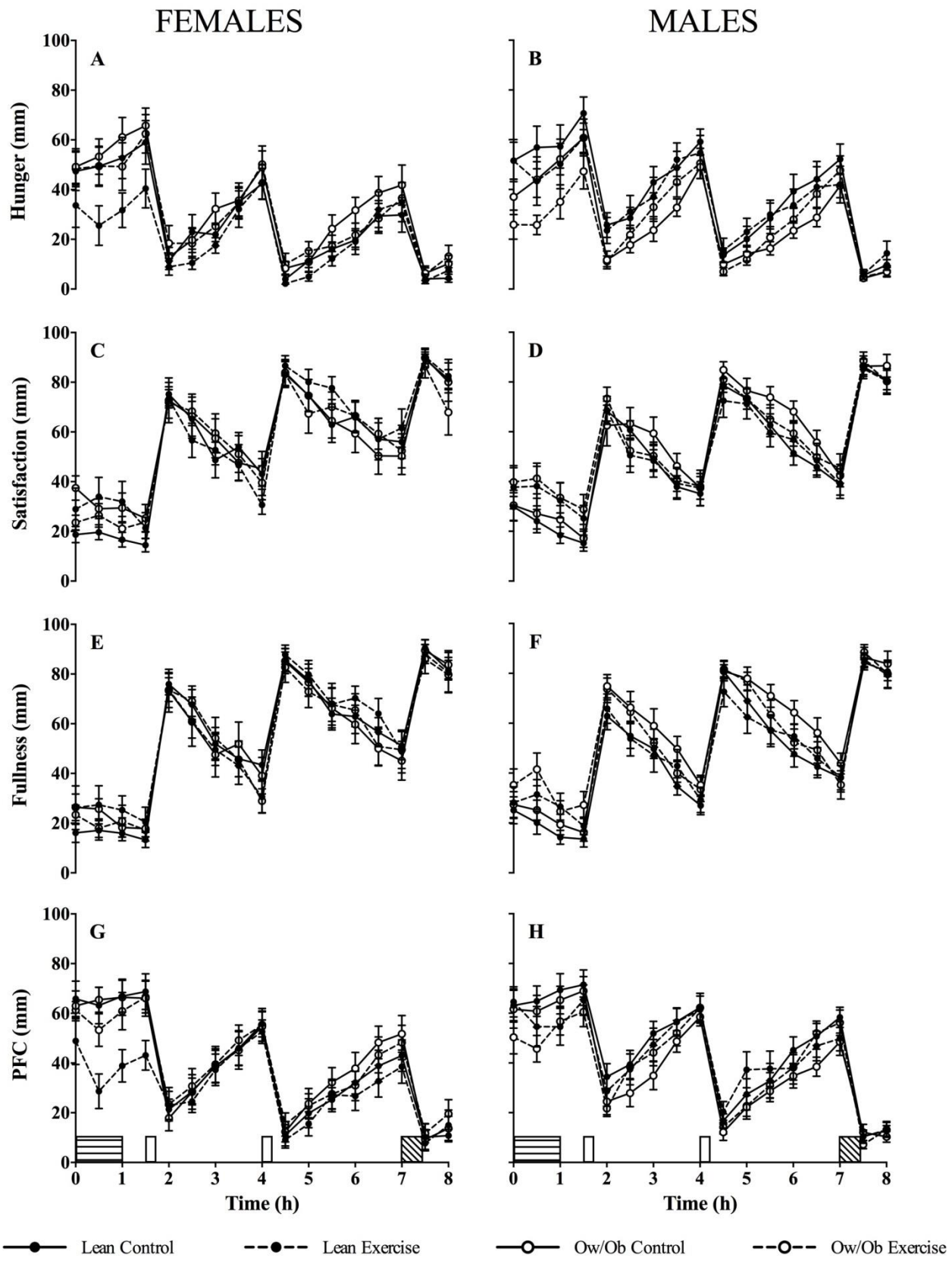


**Figure 6.5.** Perceptions of hunger in females (A) and males (B), satisfaction in females (C) and males (D), fullness in females (E) and males (F) and prospective food consumption in females (G) and males (H) in lean (●) and overweight/obese (○), during control (——) and exercise (- - -) trials. Values are mean ± SEM, N=47. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.4. Energy and macronutrient intake

Table 6.5 shows the energy and macronutrient intake data from the control and exercise trials. Three-factor linear mixed modelling (sex vs. BMI vs. trial) identified a main effect of sex for absolute energy intake (kJ), carbohydrate (g), protein (g) and fat (g) intake (all $P<0.01$). Males consumed more kilocalories and more grams of carbohydrate, protein and fat than females. No main effect of trial, BMI or interaction effects were found (all $P\geq0.18$). There was no effect of trial order on *ad libitum* energy intake ($P=0.12$). Three-factor linear mixed modelling revealed a significant main effect of trial for *ad libitum* water intake (1071 (406) vs. 1366 (480) g for control and exercise, respectively; $P<0.01$).

### 6.3.5. Relative energy intake

Relative energy intake was calculated as *ad libitum* energy intake minus the net energy expenditure elicited by exercise (Table 6.2). A significant trial effect ($P<0.01$) was found, indicating lower REI for all participants in the exercise trial. A significant effect of sex ($P<0.01$) shows that males had a higher REI than females.

**Table 6.6.** Absolute *ad libitum* energy intake, relative *ad libitum* energy intake and macronutrient intake at the buffet meal (7 h) during control and exercise trials (N=47).

| | | Trial | Lean Females (N=11)[a] | Ow/ob Females (N=11) | Lean Males (N=11)[a] | Ow/ob Males (N=14)[b] | Effect Size | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Sex | BMI | Trial |
| **Absolute energy intake** | **(kJ)**[†] | **Control** | 2497 (1158) | 2830 (1378) | 3869 (1295) | 3964 (1578) | 1.15 | 0.26 | 0.01 |
| | | **Exercise** | 2149 (998) | 2525 (1575) | 3976 (1858) | 4340 (1566) | | | |
| **REI** | **(kJ)**[†#] | **Control** | 2497 (1158) | 2830 (1378) | 3869 (1295) | 3964 (1578) | 0.87 | 0.18 | 1.26 |
| | | **Exercise** | 467 (1110) | 1155 (1450) | 1975 (1911) | 2020 (1673) | | | |
| **FAT** | **g**[†] | **Control** | 31 (17) | 34 (17) | 40 (15) | 45 (27) | 0.73 | 0.29 | 0.06 |
| | | **Exercise** | 26 (16) | 29 (17) | 42 (21) | 48 (28) | | | |
| | **%** | **Control** | 41 (12) | 45 (11) | 40 (9) | 40 (12) | 0.23 | 0.14 | 0.05 |
| | | **Exercise** | 40 (8) | 45 (9) | 41 (10) | 39 (10) | | | |
| **CHO** | **g**[†] | **Control** | 63 (30) | 70 (39) | 114 (50) | 99 (37) | 1.11 | 0.10 | 0.06 |
| | | **Exercise** | 61 (24) | 69 (49) | 107 (68) | 115 (37) | | | |
| | **%** | **Control** | 43 (9) | 42 (15) | 48 (11) | 43 (10) | 0.09 | 0.18 | 0.16 |
| | | **Exercise** | 49 (8) | 43 (16) | 44 (14) | 47 (11) | | | |
| **PRO** | **g**[†] | **Control** | 20 (10) | 23 (14) | 26 (11) | 36 (13) | 1.14 | 0.26 | 0.07 |
| | | **Exercise** | 18 (14) | 18 (12) | 36 (27) | 38 (20) | | | |
| | **%** | **Control** | 15 (9) | 13 (6) | 12 (5) | 16 (6) | 0.15 | 0.01 | 0.07 |
| | | **Exercise** | 14 (11) | 12 (9) | 15 (11) | 14 (5) | | | |

[†]Significantly different between males and females (P<0.01). [#]Significantly different between control and exercise trials (P<0.01). [a]Exercise energy intake only available for N=9. [b]Exercise energy intake only available for N=13. Values are mean (SD).

### 6.3.6. Blood analyses

Due to problems with cannulation, blood sampling data for fasting and across trial plasma acylated, desacylated and total ghrelin, insulin, TAG, glucose and NEFA are presented for 43 (rather than 47) participants. Data for fasting and across trial plasma total PYY are presented for 42 participants, whilst data for fasting and across trial plasma total GLP-1 are presented for 40 participants. Of the participant blood sets presented 3% of samples could not be collected for acylated and desacylated ghrelin, total PYY, insulin, TAG, glucose and NEFA. Total ghrelin and total GLP-1 are missing 4% of sample points.

### 6.3.6.1. Acylated ghrelin concentrations (N=43)

There were no main effects of trial, BMI or interaction effects for baseline acylated ghrelin concentrations (all P≥0.38; Figure 6.6). A significant main effect of sex showed females to have higher circulating baseline concentrations than males (143 (91) vs. 87 (79) pg·mL$^{-1}$ for females and males, respectively; P<0.01).

Across the whole trial, a four-factor mixed model showed no significant main effects of sex, BMI or trial (all P≥0.13). A significant trial vs. sex interaction highlights that females expressed 8% higher circulating acylated ghrelin concentrations in the exercise than control trial, whilst males showed 5% lower concentrations (P=0.01; Appendix W).

Total area under the curve calculations revealed no significant differences (Figure 6.7 and Table 6.7).

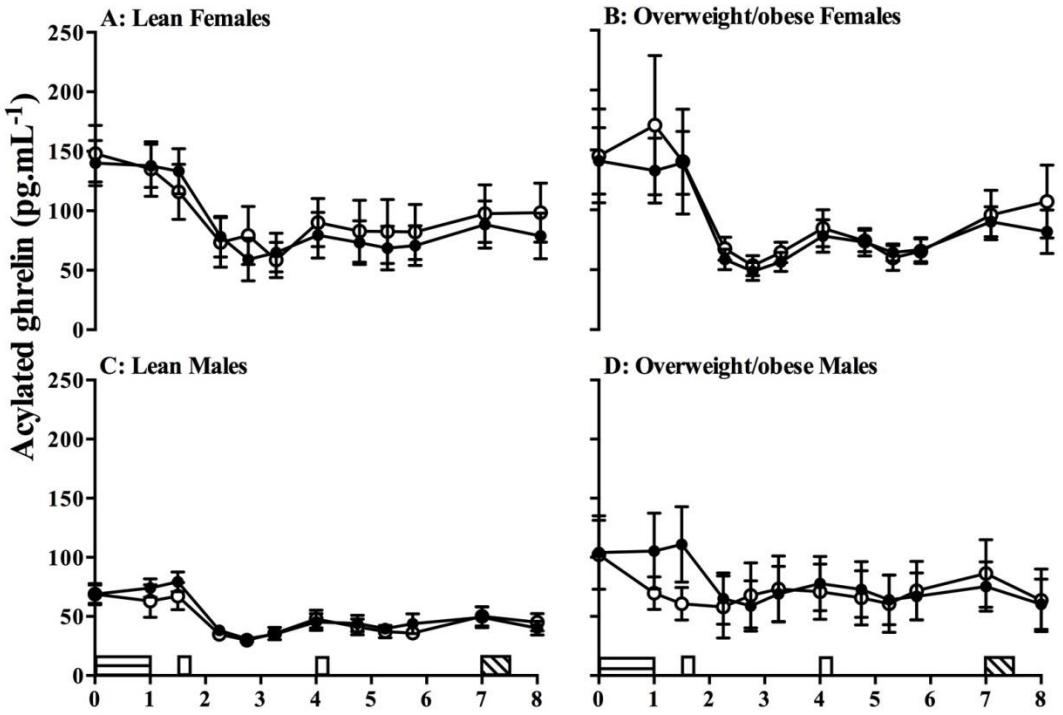


**Figure 6.6.** Plasma concentrations of acylated ghrelin in: (A) lean females, N=10, (B) overweight/obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

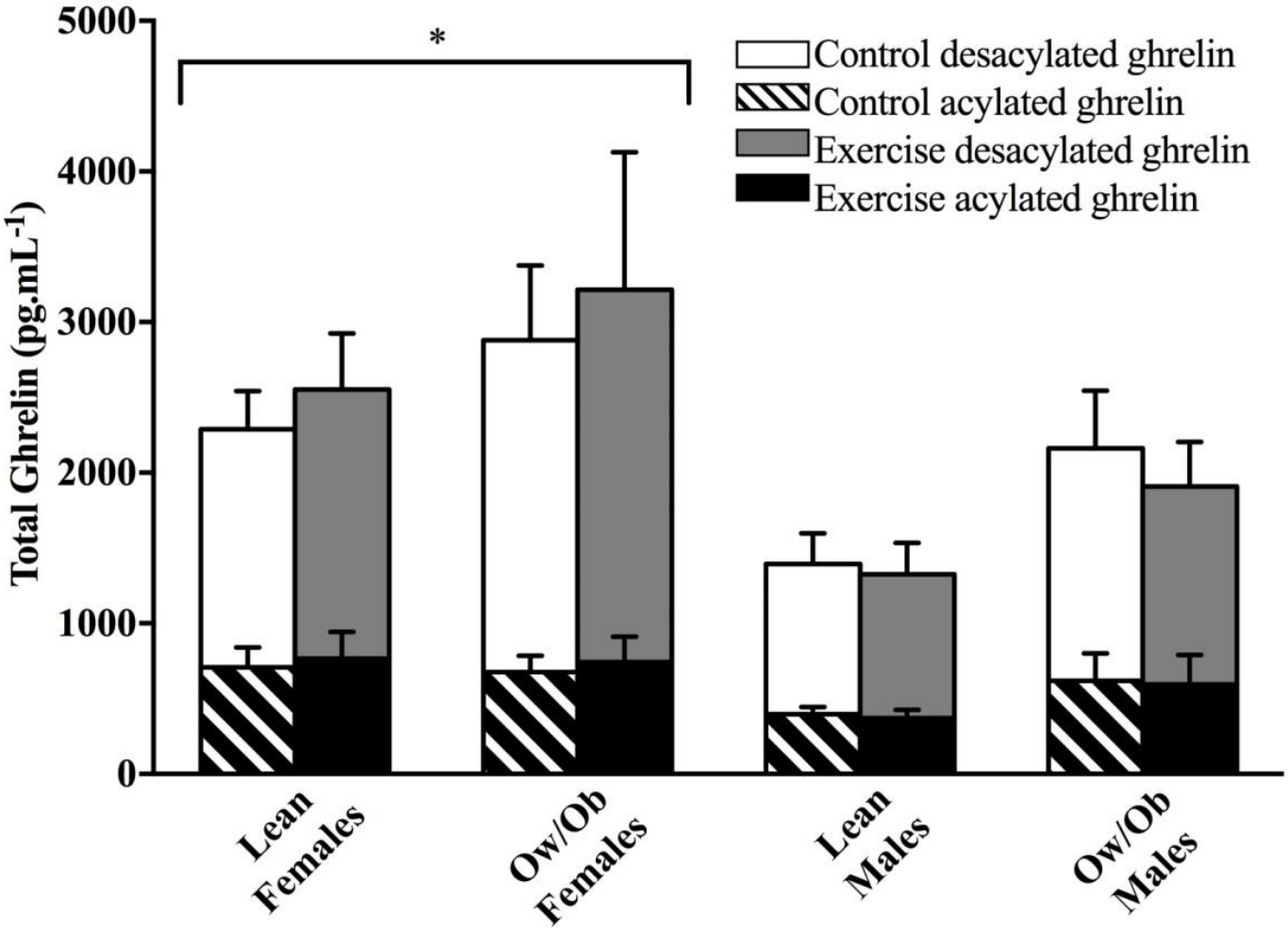


**Figure 6.7.** Area under the curve (AUC) concentrations of total ghrelin, acylated ghrelin and desacylated ghrelin in lean females, N=10, overweight/obese females, N=11, lean males, N=10, and overweight/obese males, N=12 in control and exercise trials. *Significantly different AUC concentrations of total ghrelin (P=0.05) between males and females. Values are mean ± SEM.

**Table 6.7.** Total area under the curve (AUC) values (0-8 h) for acylated ghrelin, desacylated ghrelin, total ghrelin, total peptide-YY (PYY), total glucagon-like peptide-1 (GLP-1), insulin, triglycerides (TAG), glucose, and non-essential fatty acids (NEFA) during control and exercise trials (N=43).

| AUC (0-8h) | Trial | Lean Females (N=10)[a] | Ow/ob Females (N=11)[b] | Lean Males (N=10) | Ow/ob Males (N=12) | Effect Size | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Sex | BMI | Trial |
| **Acylated ghrelin ($pg \cdot mL^{-1} \cdot h^{-1}$)** | **Control** | 708 (416) | 676 (363) | 396 (150) | 619 (634) | 0.23 | 0.10 | 0.03 |
| | **Exercise** | 766 (558) | 742 (561) | 372 (169) | 594 (681) | | | |
| **Desacylated ghrelin ($pg \cdot mL^{-1} \cdot h^{-1}$)** | **Control** | 1581 (800) | 2204 (1648) | 1000 (636) | 1544 (1324) | 0.27 | 0.18 | 0.02 |
| | **Exercise** | 1787 (1177) | 2473 (3029) | 955 (660) | 1316 (1019) | | | |
| **Total ghrelin ($pg \cdot mL^{-1} \cdot h^{-1}$)$^{\dagger}$** | **Control** | 2289 (1110) | 2880 (1975) | 1396 (752) | 2163 (1763) | 0.28 | 0.17 | 0.03 |
| | **Exercise** | 2553 (1645) | 3215 (3571) | 1327 (794) | 1910 (1454) | | | |
| **Total PYY ($pg \cdot mL^{-1} \cdot h^{-1}$)** | **Control** | 947 (497) | 985 (588) | 1041 (302) | 775 (570) | 0.08 | 0.13 | 0.06 |
| | **Exercise** | 1034 (548) | 1034 (563) | 1080 (393) | 761 (625) | | | |
| **Total GLP-1 ($pM \cdot h^{-1}$)$^{\#}$** | **Control** | 154 (99) | 178 (81) | 186 (74) | 178 (57) | 0.12 | 0.11 | 0.28 |
| | **Exercise** | 179 (122) | 219 (126) | 215 (97) | 244 (152) | | | |
| **Insulin ($pmol \cdot L^{-1} \cdot h^{-1}$)$^{\#}$** | **Control** | 1433 (962) | 1762 (884) | 1164 (451) | 1425 (549) | 0.21 | 0.27 | 0.22 |
| | **Exercise** | 1063 (388) | 1655 (457) | 1066 (464) | 1240 (531) | | | |
| **TAG ($mmol \cdot L^{-1} \cdot h^{-1}$)*$^{\dagger}$** | **Control** | 9.29 (2.90) | 12.26 (4.36) | 11.71 (6.27) | 19.07 (8.31) | 0.43 | 0.47 | 0.02 |
| | **Exercise** | 8.96 (2.07) | 12.57 (4.07) | 12.27 (5.79) | 19.39 (7.05) | | | |
| **Glucose ($mmol \cdot L^{-1} \cdot h^{-1}$)*** | **Control** | 50.12 (4.98) | 55.76 (9.75) | 50.54 (5.19) | 51.02 (4.04) | 0.15 | 0.31 | 0.01 |
| | **Exercise** | 49.95 (3.61) | 54.96 (5.03) | 50.37 (2.61) | 52.41 (4.30) | | | |
| **NEFA ($mmol \cdot L^{-1} \cdot h^{-1}$)$^{\#}$** | **Control** | 1.78 (0.71) | 1.99 (0.64) | 1.53 (0.82) | 1.84 (0.43) | 0.12 | 0.13 | 1.04 |
| | **Exercise** | 3.09 (1.11) | 2.98 (0.81) | 2.64 (0.86) | 3.12 (0.97) | | | |

*Significantly different between lean and ow/ob ($P \leq 0.01$). $^{\dagger}$Significantly different between males and females ($P \leq 0.05$). $^{\#}$Significantly different between control and exercise trials ($P \leq 0.05$). [a]N=8 for total GLP-1. [b]N=10 for total PYY and total GLP-1. Values are mean (SD).

### 6.3.6.2. Desacylated ghrelin concentrations (N=43)

There were no main effects of trial or BMI or interaction effects for baseline desacylated ghrelin concentrations (all P≥0.09; Figure 6.8). A significant main effect of sex showed females to have higher circulating baseline concentrations than males (414 (499) vs. 221 (198) $pg \cdot mL^{-1}$ for females and males, respectively; P=0.02).

Across the whole trial, a four-factor mixed model showed no significant main effects of sex, BMI or trial (all P≥0.07). A significant trial vs. sex interaction highlights that females expressed 12% higher circulating desacylated ghrelin concentrations in the exercise than control trial, whilst males showed 11% lower concentrations (P=0.02; Appendix W).

Total area under the curve calculations revealed no significant differences (Figure 6.7 and Table 6.7).

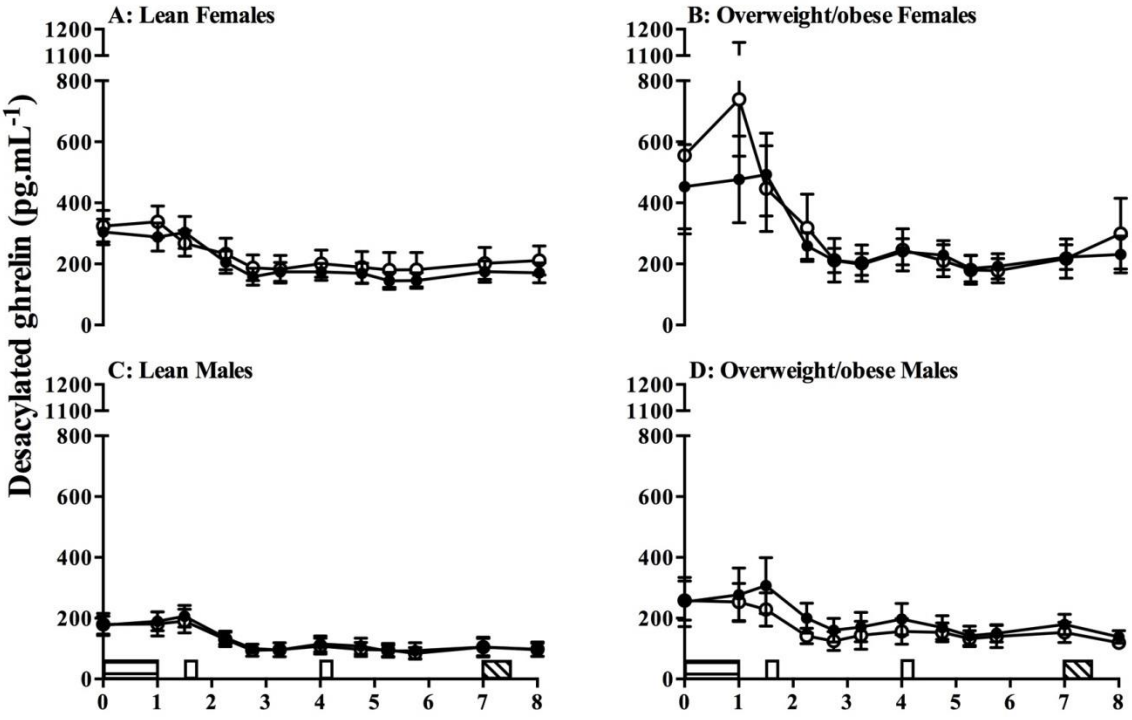


**Figure 6.8.** Plasma concentrations of desacylated ghrelin in: (A) lean females, N=10, (B) overweight/obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.3. Total ghrelin concentrations (N=43)

There were no main effects of trial or BMI or interaction effects for baseline total ghrelin concentrations (all P≥0.14; Figure 6.9). A significant main effect of sex showed females to have higher circulating baseline concentrations than males (557 (577) vs. 308 (259) $pg \cdot mL^{-1}$ for females and males, respectively, P=0.01).

Across the whole trial, a four-factor mixed model showed no significant main effects of sex, BMI or trial (all P>0.06). A significant trial vs. sex interaction found that females expressed 12% higher circulating total ghrelin concentrations in the exercise than control trial, whilst males showed 7% lower concentrations (P=0.02; Appendix W).

Total area under the curve calculations revealed a significant main effect of sex with females expressing higher total AUC concentrations than males (2749 (2247) vs. 1730 (1308) $pg \cdot mL^{-1}$, for females and males, respectively; P=0.05; Table 6.7 and Figure 6.7).

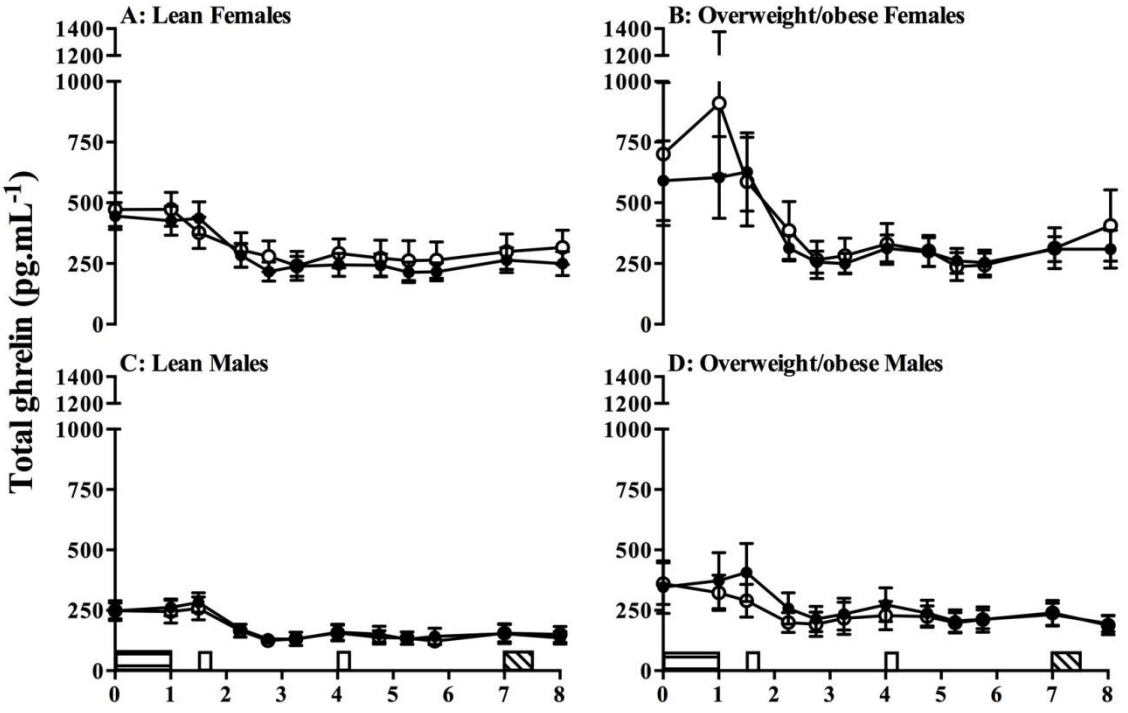


**Figure 6.9.** Plasma concentrations of total ghrelin in: (A) lean females, N=10, (B) overweight/obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.4. Total peptide-YY concentrations (N=42)

Total PYY concentrations did not significantly differ between trial or group at baseline (all P≥0.25; Figure 6.10).

Across the whole trial, a four-factor mixed model showed a significant main effect of trial with circulating concentrations significantly higher in the exercise than control trial (119 (75) vs. 113 (68) $pg \cdot mL^{-1}$, for exercise and control, respectively; P<0.01; Appendix X).

Total area under the curve calculations revealed concentrations to be higher in the exercise than control trial (967 (539) vs. 929 (500) $pg \cdot mL^{-1} \cdot h^{-1}$, for exercise and control, respectively; P=0.051; Table 6.7).

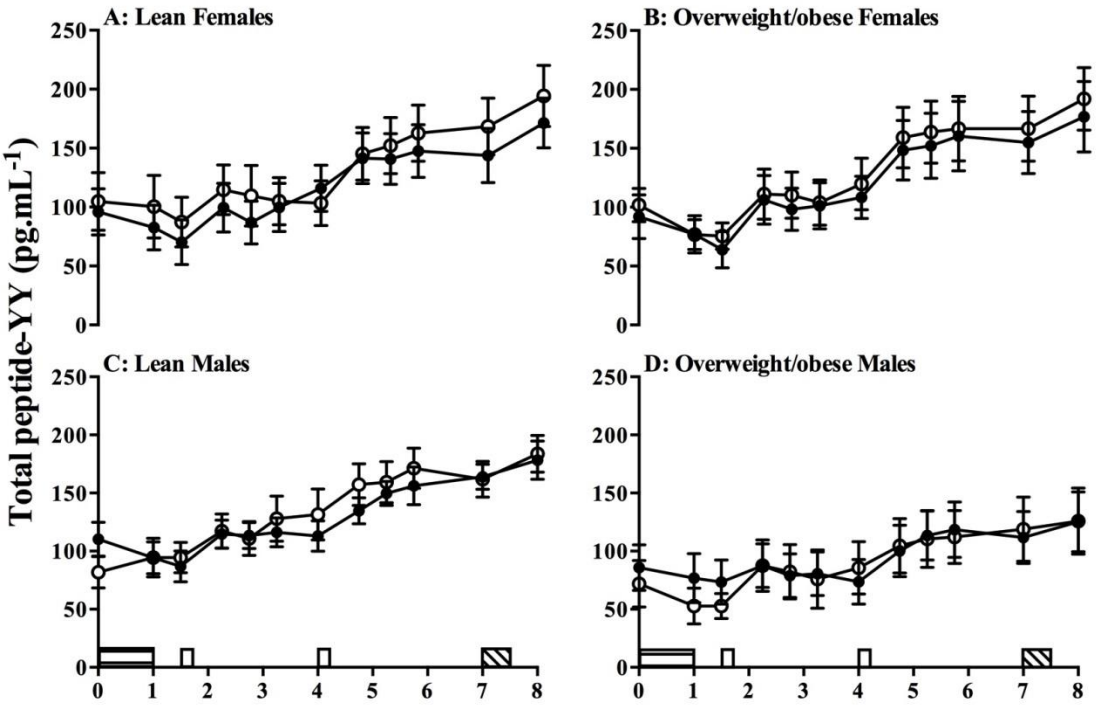


**Figure 6.10.** Plasma concentrations of total peptide-YY in: (A) lean females, N=10, (B) overweight/obese females, N=10, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (O) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.5. Total glucagon-like peptide-1 concentrations (N=40)

Total GLP-1 concentrations did not significantly differ between trial or group at baseline (all P≥0.15; Figure 6.11).

Across the whole trial, a four-factor mixed model showed a significant main effect of trial, with concentrations higher in the exercise than control trial (27 (20) vs. 22 (12) $pM.L^{-1}$, for exercise and control, respectively; P<0.01; Appendix X). A significant trial vs. time interaction was also found (P<0.01). *Post-hoc* analysis showed total GLP-1 concentrations to be significantly elevated during exercise trials at 2.25 h (P<0.01) and 2.75 h (P=0.04), compared to control.

Total area under the curve calculations revealed a significant effect of trial, with total AUC concentrations higher in the exercise than control trial (217 (128) vs. 175 (79) $pM \cdot mL^{-1} \cdot h^{-1}$, for exercise and control, respectively; P=0.01; Table 6.7).

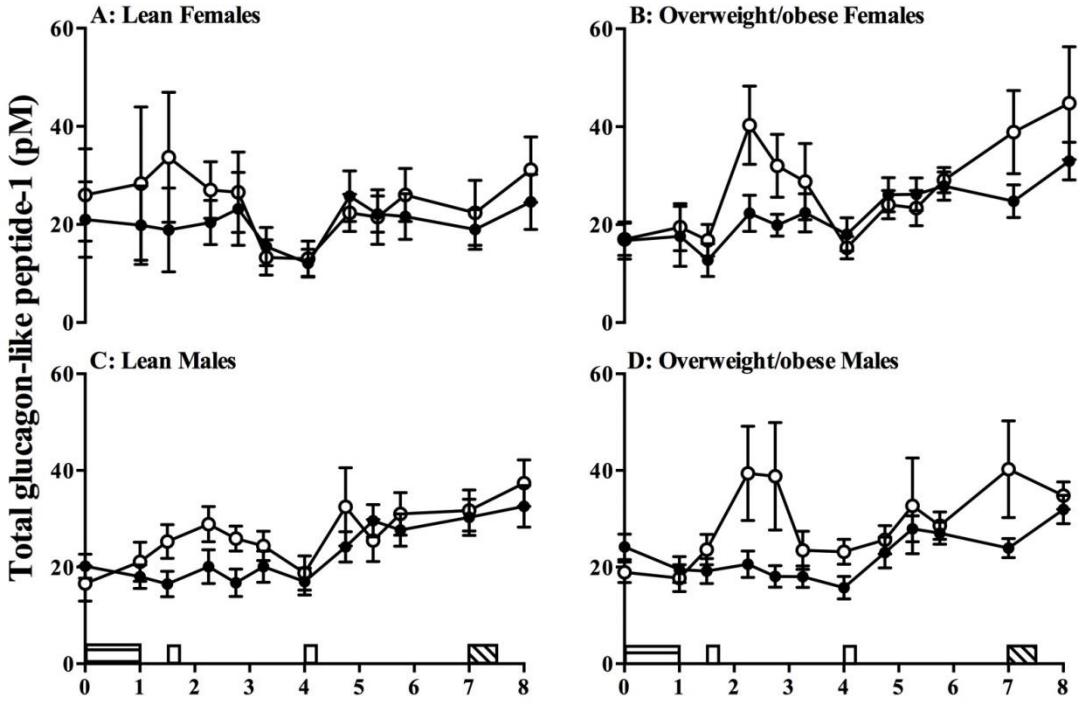


**Figure 6.11.** Plasma concentrations of total glucagon-like peptide-1 in: (A) lean females, N=8, (B) overweight/obese females, N=10, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.6. Insulin concentrations (N=43)

Insulin concentrations did not significantly differ across trial or group at baseline (all P≥0.13; Figure 6.12).

Across the whole trial, a four-factor mixed model showed a significant main effect of BMI with concentrations higher in ow/ob than lean individuals (198 (166) vs. 150 (135) pmol.$L^{-1}$, for ow/ob and lean, respectively; P=0.03). A significant main effect of trial showed concentrations of insulin to be higher in the control than exercise trial (186 (169) vs. 165 (137) pmol.$L^{-1}$, for control and exercise, respectively; P<0.01; Appendix X).

Total area under the curve calculations revealed a significant main effect of trial showed that total AUC values were higher in the control than exercise trial (1452 (743) vs. 1264 (511) pmol.$L^{-1}$·$h^{-1}$, for control and exercise, respectively; P=0.03). Concentrations were found to be higher in ow/ob than lean individuals (1520 (846) vs. 1181 (906) pmol.$L^{-1}$·$h^{-1}$, for lean and ow/ob, respectively; P=0.051; Table 6.7).

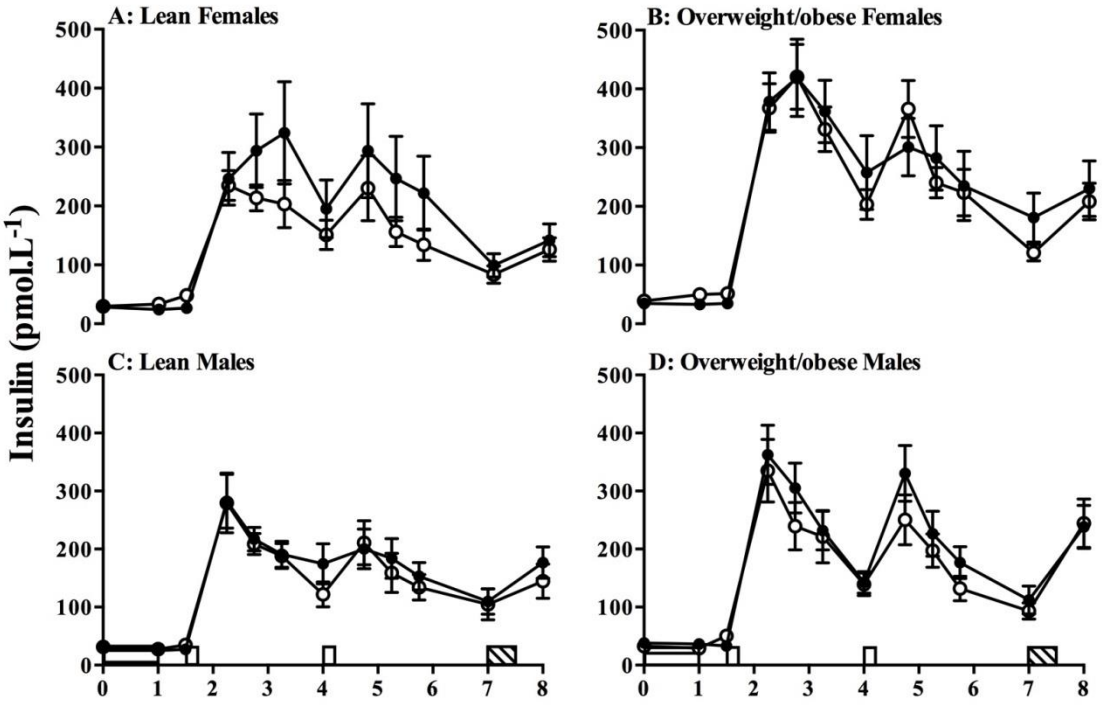


**Figure 6.12.** Plasma concentrations of insulin in: (A) lean females, N=10, (B) overweight/ obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.7. Triglyceride concentrations (N=43)

There were no main effects of trial or interaction effects for baseline TAG concentrations (all $P \geq 0.62$; Figure 6.13). A significant main effect of sex showed males to have higher circulating baseline concentrations than females (1.5 (0.8) vs. 1.1 (0.5) $mmol.L^{-1}$ for males and females, respectively; P=0.01). A significant main effect of BMI showed ow/ob to have higher circulating baseline concentrations than lean individuals (1.5 (0.7) vs. 1.0 (0.4) $mmol.L^{-1}$ for ow/ob and lean, respectively; P<0.01).

Across the whole trial, a four-factor mixed model showed a significant main effect of sex, with males expressing higher concentrations of TAG than females (1.9 (1.2) vs. 1.3 (0.6) $mmol.L^{-1}$ for males and females, respectively; P=0.01). A significant main effect of BMI showed ow/ob to express higher concentrations of TAG than lean individuals (1.9 (1.1) vs. 1.3 (0.7) $mmol.L^{-1}$, for ow/ob and lean, respectively; P<0.01).

Total area under the curve calculations revealed a significant main effect of sex, with males having higher total AUC concentrations than females (16.0 (7.7) vs. 10.9 (3.7) $mmol.L^{-1} \cdot h^{-1}$, for males and females, respectively; P<0.01; Table 6.7). A significant main effect of BMI showed that ow/ob had higher total AUC concentrations than lean individuals (16.0 (7.1) vs. 10.6 (4.8) $mmol.L^{-1} \cdot h^{-1}$, for ow/ob and lean, respectively; P<0.01).

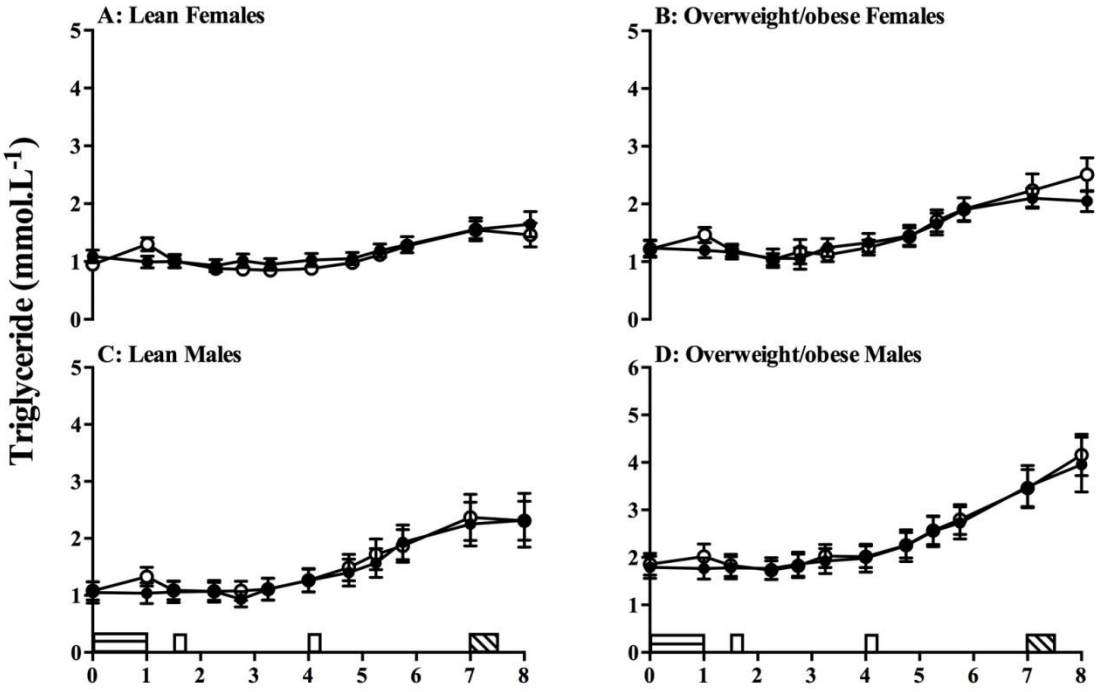


**Figure 6.13.** Plasma concentrations of triglyceride in: (A) lean females, N=10, (B) overweight/obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (O) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.6.8. Glucose concentrations (N=43)

There were no main effects of trial or sex or interaction effects for baseline glucose concentrations (all P≥0.44; Figure 6.14). A significant main effect of BMI showed ow/ob to have higher circulating baseline concentrations than lean individuals (6.0 (0.6) vs. 5.6 (0.3) $mmol.L^{-1}$ for ow/ob and lean, respectively; P<0.01).

Across the whole trial, a four-factor mixed model showed a significant main effect of BMI with ow/ob expressing higher concentrations of insulin than lean individuals (6.8 (1.4) vs. 6.3 (1.2) $mmol.L^{-1}$, for ow/ob and lean, respectively; P=0.02).

Total area under the curve calculations revealed a significant main effect of BMI, with ow/ob showing higher total AUC concentrations than lean individuals (50.2 (4.2) vs. 53.5 (6.2) $mmol.L^{-1} \cdot h^{-1}$, for lean and ow/ob, respectively; P=0.03).

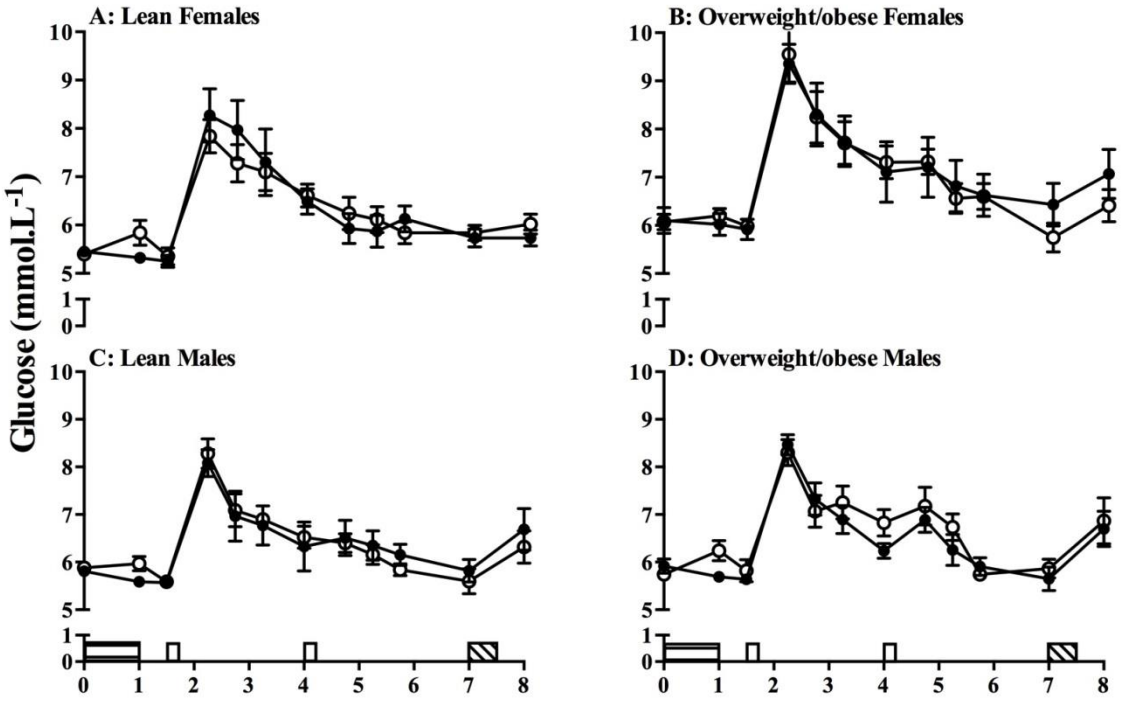


**Figure 6.14.** Plasma concentrations of glucose in: (A) lean females, N=10, (B) overweight/ obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

#### 6.3.6.9. NEFA concentrations (N=43)

There were no main effects of trial or BMI or interaction effects for baseline NEFA concentrations (all P≥0.18; Figure 6.15). A significant main effect of sex showed females to have higher circulating baseline concentrations than males (0.7 (0.3) vs. 0.5 (0.2) $mmol.L^{-1}$ for females and males, respectively; P<0.01).

Across the whole trial, a four-factor mixed model showed a significant main effect of trial with concentrations of NEFA higher in the exercise than control trial (0.3 (0.4) vs. 0.2 (0.2) $mmol.L^{-1}$, for exercise and control, respectively; P<0.01; Appendix X). A significant trial vs. time interaction was also found (P<0.01). *Post-hoc* analysis showed NEFA concentrations to be significantly elevated during exercise trials at 1.0 and 1.5 h (both P≤0.01), compared to control.

Total area under the curve calculations revealed a significant main effect of trial, with total AUC concentrations higher in the exercise than control trial (3.0 (0.9) vs.1.8 (0.8) $mmol.L^{-1}\cdot h^{-1}$, for exercise and control trials, respectively; P<0.01).

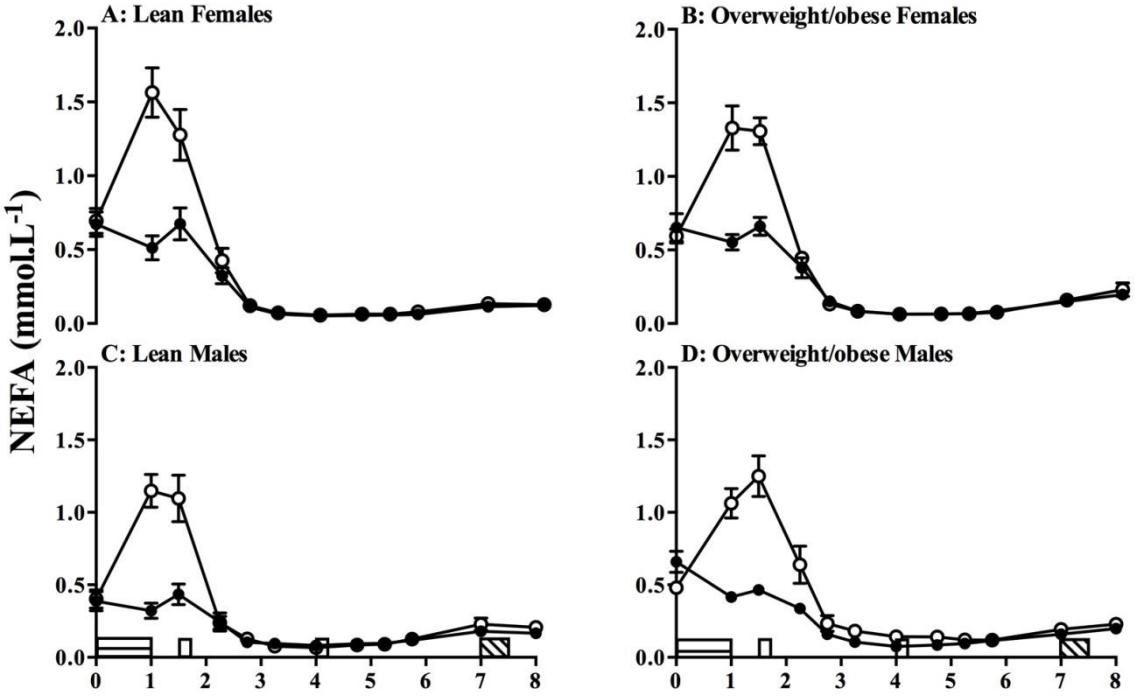


**Figure 6.15.** Plasma concentrations of NEFA in: (A) lean females, N=10, (B) overweight/ obese females, N=11, (C) lean males, N=10, and (D) overweight/obese males, N=12, in control (●) and exercise (○) trials. Values are mean ± SEM. Horizontally shaded rectangles indicate exercise, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.

### 6.3.7. Correlations

A positive, but weak, relationship was found between waist circumference and *ad libitum* energy intake for both control and exercise trials (control: r= 0.32, P=0.03, exercise: r=0.39, P<0.01). There was no correlation between waist circumference and acylated ghrelin total AUC, desacylated ghrelin total AUC, GLP-1 total AUC, PYY total AUC or hunger total AUC.

## 6.4. Discussion

The purpose of this investigation was to compare the effects of a single bout of aerobic exercise on subjective appetite perceptions, circulating concentrations of appetite regulatory hormones and *ad libitum* energy intake in lean and ow/ob males and females. The primary findings are that 1) exercise induced a transient suppression of subjective appetite in all participant groups, 2) concentrations of PYY and GLP-1 were higher in the exercise than control trial, 3) mean subjective appetite, acylated ghrelin, and *ad libitum* energy intake did not differ between control and exercise trial days, for all participant groups. Together, the findings of the current study suggest that the beneficial effects of exercise on appetite control are seen equally in males and females, and in lean and ow/ob individuals, i.e. acute exercise does not induce compensatory increases in subjective appetite in the 7 h after exercise or *ad libitum* energy intake at a buffet meal 6 h after exercise. Together this allowed for the maintenance of an energy deficit. If this can be sustained over longer periods of time, moderate intensity exercise could be useful for weight loss and management.

Subjective ratings of hunger and PFC were suppressed for all participant groups during and for immediately after exercise. This suppression of subjective appetite during exercise is consistent with previous reports of exercise induced anorexia during exercise ≥60% $\dot{V}O_2$ max (Blundell et al. 2003; King et al. 1994). However, few have compared responses of males and females (Alajmi et al. 2016; Hagobian et al. 2013), or ow/ob and lean individuals (Ueda et al. 2009b). In the present study, we found appetite responses to be similar among all participants, irrespective of sex or BMI group. The mechanisms that cause appetite suppression during exercise remain unclear; however, recent evidence suggests that exercise-induced changes in appetite regulatory hormones such as acylated ghrelin, PYY, GLP-1 and PP may play a role (Broom et al. 2009; Hazell et al. 2016; Schubert et al. 2013; Ueda et al. 2009a). The transient suppression of appetite after exercise seen in the present study, occurred in the absence of any simultaneous changes in appetite regulatory hormones. This questions the role of appetite regulatory hormones on subjective appetite perceptions, and suggests other mechanisms of appetite control may be more influential.

In accordance with previous research, it was expected for circulating concentrations of acylated ghrelin to be transiently suppressed after exercise, in lean individuals. Contrary to this, acylated ghrelin concentrations were found to be similar between control and exercise trials, for all participant groups. It has been suggested that the redistribution of blood from the

splanchnic region to active skeletal muscle during exercise is important for suppressing ghrelin (Hazell et al. 2016). As blood flow redistribution is dependent on exercise intensity, it is possible that the exercise intensity used in the current study was not great enough to elicit these expected changes in acylated ghrelin. In concert with the current findings, other low intensity exercise, including treadmill walking and cycling at 50% of $\dot{V}O_2$ max, has failed to alter concentrations of acylated ghrelin in comparison to control trials (King et al. 2010b; Ueda et al. 2009a; Unick et al. 2010). However, intense exercise (~70% $\dot{V}O_2$ max) has been shown to suppress acylated ghrelin concentrations (Broom et al. 2007; Broom et al. 2009; King et al. 2010a; King et al. 2011a; Wasse et al 2012).

Although there was no main effect of gender for acylated ghrelin, female participants expressed higher circulating concentrations of acylated ghrelin across both trials compared with males. This is in agreement with previous research (Alajmi et al. 2016; Burns et al. 2007; Hagobian et al. 2013). A significant trial by sex interaction found females to have 8% higher mean concentrations of acylated ghrelin in exercise than control trials, and males to have 5% lower concentrations in the exercise trials. These differences were predominantly driven by the response of ow/ob males and females during and 30 min after exercise. In particular, females showed increased circulating concentrations of acylated ghrelin immediately after the exercise bout. Males showed lower concentrations immediately and 30 min after exercise. This divergent response supports previous suggestions that females exhibit compensatory responses in appetite regulatory hormones to energy deficits to preserve energy balance and reproductive function (Hagobian and Braun 2010). However, despite these differences, concentrations were not significantly different between males and females at any time point, and total AUC values did not significantly differ between males and females, questioning the importance of this interaction.

In the current study, both acylated and desacylated ghrelin were measured. This consequently allowed for the calculation of total ghrelin concentrations (acylated ghrelin plus desacylated ghrelin concentrations). Acylated and desacylated ghrelin responded similarly in all participant groups, across both trials (control, $r=0.65$, $P<0.01$; exercise, $r=0.69$, $P<0.01$). Acylation is considered necessary to allow ghrelin to cause its appetite regulatory effects (Neary et al. 2006). Previously, the measurement of total ghrelin has been shown to be unreliable in detecting changes seen in acylated ghrelin (Hosoda et al. 2006). The findings in the present study do not support this, and provide some evidence to suggest that the

measurement of desacylated ghrelin could summarise changes in concentrations of ghrelin. The measurement of acylated ghrelin is time consuming, and uses potentially toxic chemicals. If further research supports the findings of the current study, future studies could use quicker and safer methods of determining ghrelin responses (i.e. measuring desacylated ghrelin from EDTA plasma).

Across trial days total PYY concentrations were found to be higher in the exercise than control trial, for all participant groups. To date, the majority of studies have found transient increases in circulating concentrations of PYY after exercise (Broom et al. 2009; Deighton et al. 2013a; Deighton et al. 2013b; Deighton et al. 2014; King et al. 2011a; Martins et al. 2007; Shorten et al. 2009; Ueda et al. 2009a; Wasse et al. 2012). However, some have found no change (Balaguera-Cortes et al. 2011; Shorten et al. 2009). Similar discrepancies are also reported when comparing the responses of lean and ow/ob individuals (Holmstrup et al. 2013; Martins et al. 2015; Sim et al. 2014; Ueda et al. 2009b). It is likely that the conflicting findings are a result of variances in study protocol and form of PYY measured. It has been suggested that $PYY_{3-36}$ has more potent anorectic effects than total PYY (Chelikani et al. 2005). However, after high protein, high carbohydrate and high fat meals, $PYY_{3-36}$ and total PYY have been shown to respond similarly. This suggests that measuring either form of PYY is valid, and that discrepancies in research to date may be ruled by other variables. Regardless of these conflicting findings, a recent review found exercise to increase concentrations of PYY (Schubert et al. 2014). The findings of the current study are in concert with these.

Mean concentrations of total GLP-1 were found to be higher in the exercise than control trial. In particular, this trial effect appeared to be driven by significantly elevated GLP-1 concentrations immediately and 30 min after the standard breakfast meal in the exercise compared to the control trial, for all participant groups. Beyond this time point, all participant groups expressed similar GLP-1 responses in exercise and control trials. To the author's knowledge, this is the first study to examine sex differences in GLP-1 responses to exercise. Data from the current study suggests males and females exhibit similar GLP-1 responses to exercise. When comparing responses of lean and ow/ob individuals, previous studies have also shown mixed findings. One possible reason for these discrepancies is the influence that weight loss has on circulating GLP-1 concentrations. In particular, after a period of weight loss (6 weeks), fasting GLP-1 concentrations have been shown to decrease and the release of GLP-1 is negated after feeding (Adam et al. 2005). Further to this, after 6 weeks of weight loss, concentrations of GLP-1 were shown to return to baseline after a 3 month weight-

maintenance period (Adam et al. 2006). These findings suggest that the GLP-1 response to an acute bout of exercise was similar in lean ow/ob males and females, up to 7 h after moderate intensity acute exercise.

Insulin is regarded as a tonic regulator of appetite which generates anorectic effects by supressing activity of NPY and AgRP neurones, stimulating POMC neurones in the ARC of the hypothalamus and altering the brain's sensitivity to episodic appetite signals (McMinn et al. 2000; Murphy and Bloom 2004; Woods et al. 2006; Wynne et al. 2005a). Chronically, circulating concentrations of insulin are positively related to adiposity and insulin resistance in key metabolic tissues (Woods and Seeley 1998). Insulin resistance was crudely assessed in the current study using HOMA1-IR (Matthews et al. 1985), which suggested that none of the participants were insulin resistant (HOMA1-IR>2.7, Geloneze et al. 2009). However, individuals who were ow/ob did have higher insulin levels than those who were lean, across trial days. Therefore it could be expected for ow/ob individuals to report lower appetite sensations than their lean counterparts, however this was not seen. It is well established that acute exercise can increase insulin sensitivity by increasing glucose uptake into muscle cells, and this is seen for up to 72 h after exercise (Hawley and Lessard 2008). In the current study insulin AUC was reduced with exercise, whilst glucose AUC remained similar between control and exercise trials, for all participants. This implies that the moderate bout of exercise was sufficient to enhance insulin sensitivity in lean, overweight and obese individuals. However, it is also important to consider the role of GLP-1, an incretin hormone that stimulates insulin secretion (Field et al. 2009; Holst 2007; Lockie 2013).The current study found GLP-1 AUC to be increased in the exercise vs. control trial, for all participants. The between-trial difference for insulin was in the opposing direction, observing reduced concentrations with exercise. Therefore, it appears that the moderate exercise bout did not influence the insulinotropic actions of GLP-1 in this study.

Despite finding a transient suppression of hunger, elevated concentrations of total PYY and total GLP-1 during exercise, there were no differences in absolute energy or macronutrient intake at the *ad libitum* meal provided 6 h after the exercise or control period. This supports previous evidence that exercise does not cause compensatory increases in energy intake in the hours after acute exercise (Schubert et al. 2013). Furthermore, this study found that lean and ow/ob males and females responded similarly, with no significant differences in absolute energy or macronutrient intake between exercise and control trials. When taking into consideration the energy requirements of the exercise bout, it was found that REI was

significantly reduced in all participant groups. This suggests that lean and ow/ob males and females do not exhibit compensatory food intake behaviour in the 6 h after exercise to recover the exercise induced energy deficit. In the present study, participants were only provided with the opportunity to compensate for the energy deficit 6 h after the exercise bout. Further research is required to determine if this deficit can be maintained over a longer period of time, and to help ascertain the effectiveness of exercise in weight loss and weight maintenance.

Given the nature of the current study, all of the measured hormones and substrates showed significant effects of time (all $P<0.01$). However, only GLP-1 and NEFA, expressed significant time vs. trial interactions. *Post-hoc* analysis revealed the significant differences to be in close temporal proximity to the exercise bout. Specifically, circulating concentrations of NEFA were elevated at 1.0 and 1.5 h in the exercise trial, whilst circulating concentrations of GLP-1 were elevated at 2.25 and 2.75 h, for all participants. NEFA and GLP-1 concentrations were similar at all other time points. The transient increase in NEFA concentrations seen during and 30 min after exercise were expected. This represents the mobilisation of fat for the working muscles to use as fuel, during exercise (Frayn, 2010). The heightened response of total GLP-1 to feeding after exercise, would suggest a beneficial effect of exercise on appetite regulation, i.e. exercise enhances satiation after feeding. This could be especially useful to those who express dysfunctional appetite mechanisms. However, these changes in GLP-1 were not concordant with alterations in appetite.

Although there were no differences in subjective appetite ratings, appetite regulatory hormones, or *ad libitum* energy intake responses to acute exercise between lean and ow/ob males and females, baseline measures were found to differ between groups. Specifically, females expressed higher baseline values of acylated, desacylated and total ghrelin and NEFA, and lower baseline concentrations of TAG, than males. Lean individuals showed lower baseline concentrations of TAG and glucose, than ow/ob individuals. As fasting concentrations of appetite regulatory hormones differ between groups, this highlights the importance of examining relative change in appetite regulatory hormones rather than absolute concentrations. The observation of similar ghrelin, total PYY and GLP-1 responses to exercise between groups, may explain why similar subjective appetite and energy intake responses were seen.

This study is limited by the selected group cut-off criteria. In particular, relying on BMI and waist circumference measures to classify individuals as 'lean' or 'overweight/obese'. Body mass index does not take into account body shape and body fat percentage when grouping participants. This may confound the comparisons between lean and ow/ob individuals, and future studies should aim to use a more vigorous set of criteria to determine body size and subsequently classify individuals, for example, by using measures of skinfold thickness or DEXA scans. Another limitation of the current study was the prescription of regular feeding bouts to participants. Test meals were provided at 1.5 and 4 h, and an *ad libitum* meal provided at 7 h. These eating times were often inconsistent with the self-reported eating habits of the participants, with participants often still feeling full at the start of the *ad libitum* meal. This could have impacted the amount of food eaten at the *ad libitum* meal, and reduced the sensitivity of the meal to detect changes in energy intake. In addition to this, participants were not familiarised with the *ad libitum* meal, potentially resulting in a 'banquet effect', in which participants over eat in response to the wide-selection of foods available. In an attempt to control for this, a cross over design was adopted with participants randomly assigned to either the control (N=23) or exercise trial (N=24) first. Furthermore, there was found to be no effect of trial order on *ad libitum* energy intake (P=0.12). Future studies using this method of assessment should present buffet meals to participants in preliminary visits to help prevent the 'banquet effect' from occurring and to improve the validity of this measure. The primary aim of this study was to determine the physiological, i.e. hormonal and subjective appetite, response to exercise. As a result, this study provided participants with standard and buffet meals at strict time intervals. This limits the study's ability to evaluate the compensatory eating behaviour of participants. Future studies should incorporate 'real world' influences and less rigid experimental control to gain a greater insight into the compensatory responses to feeding. Finally, circulating concentrations of the hormones measured in this study are related to body size. Given that we grouped individuals who were overweight and obese, the responses seen by individuals who were overweight could have diluted the responses of those seen in individuals who were obese. It is therefore possible that the current study is underpowered to detect significant differences between the four groups. In an attempt to control for the effects of the menstrual cycle on appetite regulatory hormones and subjective appetite sensations, main trials were separated by 28 days in females. However, females rarely exhibit cycles lasting 28 days, and participants may have been tested in different phases of the menstrual cycle. Future studies should control for the between- and inter-subject variations in menstrual cycle length. Total ghrelin concentrations were indirectly

measured in this study by summing concentrations of acylated and desacylated ghrelin. Samples used to determine acylated ghrelin concentrations were pre-treated to optimize its preservation, minimising its breakdown to desacylated ghrelin during storage. However, samples used to determine desacylated ghrelin were not pre-treated. Consequently, total ghrelin values may have been overestimated, as concentrations of desacylated ghrelin could have been higher in EDTA-plasma than in pre-treated plasma.

In conclusion, this study has shown that an acute bout of treadmill exercise supresses appetite during and immediately after exercise in lean and ow/ob males and females. Circulating concentrations of PYY and GLP-1 are increased across the exercise trial day in comparison to the control trial. Despite this, energy intake remains unchanged, in all participant groups. These findings provide novel information regarding subjective appetite perception, appetite regulatory hormone and *ad libitum* energy intake by directly comparing responses in lean and ow/ob, males and females.

# CHAPTER VII

# General Discussion

## 7.1. Introduction

Amid present concerns over the prevailing overweight and obesity epidemic there has been an explosion of research into the effects of exercise on appetite regulation and energy intake.

The studies within this thesis were designed with the intention to add to this field of study by; (i) further characterising when compensatory subjective appetite, appetite regulatory hormone (acylated ghrelin, total PYY and leptin) and *ad libitum* energy intake responses start after exercise, and to (ii) compare subjective appetite, appetite regulatory hormone (acylated ghrelin, total PYY and total GLP-1) and *ad libitum* energy intake responses to acute aerobic exercise in lean and ow/ob males and females.

The purpose of this chapter is to consider the main outcomes of the experimental chapters presented in this thesis and to consolidate these findings. Table 7.1 summarises the protocols and variables measured in each experimental chapter.

**Table 7.1.** Summary of the study protocols presented within the experimental chapters of this thesis.

| Chapter | Participants | | Trials (time, h) | Exercise duration and intensity | Measurements |
|---|---|---|---|---|---|
| | N | BMI ($kg \cdot m^{-2}$) | | | |
| **4** | 15 (M) | 23.0 (1.9) | CON (31h) | 60 min of treadmill running at 70.1 (2.5)% (Day 1) and 70.0 (3.2)% (Day 2) $\dot{V}O_2$ peak on two consecutive mornings | Appetite<br>Energy/macronutrient intake<br>Acylated ghrelin<br>Total PYY<br>Leptin<br>Insulin, glucose, TAG |
| | | | EX (31h) | | |
| **6** | 11 (F) | 21.9 (1.6) | CON (8h) | 60 min of treadmill exercise at 58.8 (3.6)% $\dot{V}O_2$ peak | Appetite<br>Energy/macronutrient intake<br>Acylated ghrelin<br>Desacylated ghrelin<br>Total ghrelin<br>Total PYY<br>Total GLP-1<br>Leptin<br>Insulin, glucose, TAG and NEFA |
| | 11 (F) | 28.7 (2.8) | | | |
| | 11 (M) | 23.0 (1.4) | EX (8h) | | |
| | 14 (M) | 29.6 (3.0) | | | |

CON, control; EX, exercise; F, female; M, male; N, participant number. Values are mean (SD).

## 7.2. Appetite

A large body of evidence supports the existence of 'exercise induced anorexia', a condition where appetite is transiently suppressed after exercise (King et al. 1994). Work in Chapter 4 further expands on this notion by showing that on two consecutive days of acute moderately-vigorous aerobic exercise, appetite is suppressed during and up to 30 min after each exercise bout. In Chapter 6 lean and ow/ob, males and females expressed an exercise induced suppression of subjective appetite during and immediately after acute moderate intensity aerobic exercise. The research in this thesis confirms that the suppression of subjective appetite is short-lived, returning to values reported after a period of rest within 30 min of an exercise bout.

Previous research has shown that subjective appetite ratings do not exhibit compensatory responses in the hours after exercise (i.e. do not increase later in the trial day). The findings in Chapter 4 and 6 are consistent with these, showing that subjective appetite responses are similar to control conditions in the 7-30 h after both moderate and moderately-vigorous intensity exercise. Chapter 6 extends on these findings by showing that lean and ow/ob, males and females respond similarly in the hours after exercise. This suggests that the beneficial effects of exercise on appetite control (i.e. no compensatory increase in subjective appetite) seen in lean participants translate into ow/ob individuals.

It has been suggested that compensatory subjective appetite responses may be delayed, occurring after the observation time in the laboratory (~24 h). However, recent evidence has found that even on the day after acute aerobic exercise, subjective appetite responses after exercise did not significantly differ from control conditions (Beaulieu et al. 2015; King et al. 2015). Findings from Chapter 4 are in agreement with these, and supplement this to show subjective appetite responses can withstand the effects of moderately-vigorous aerobic exercise performed on two consecutive days. Further research should determine if this response is maintained after additional days of exercise, as beneficial effects of exercise on subjective appetite control could support the role of exercise in weight control. It is likely that such a study would be designed so that participants visit the laboratory on consecutive days, being allowed to return home overnight. It would be difficult to have full control over what the participants did outside of the laboratory, therefore making it difficult to objectively assess subjective appetite responses over long durations.

Few studies have compared subjective appetite responses in males and females (Alajmi et al. 2016; Hagobian et al. 2013). Both of these studies found no difference in subjective appetite responses between males and females after a bout of moderate intensity exercise (~70% $\dot{V}O_2$ max). The findings in Chapter 6 support these findings, and extend upon them by showing that appetite responds similarly, irrespective of sex, at moderate intensity exercise (~60% $\dot{V}O_2$ peak).

To date subjective appetite responses to acute exercise in ow/ob individuals have been inconsistent. Reductions in appetite during and for 30 min after exercise (Tsofliou et al. 2003; Westerterp-Plantenga et al. 1997), and no difference in appetite (Ueda et al. 2009b; Unick et al. 2010) have been reported in comparison to control conditions. To the authors' knowledge, no previous studies have made direct comparisons between individuals who are lean and ow/ob. In Chapter 6 both lean individuals and individuals who are ow/ob express suppressed sensations of appetite during and immediately after acute exercise. Additionally, no correlation was observed between waist circumference and hunger AUC. Therefore the findings in Chapter 6 add to the current literature, showing individuals who are lean and ow/ob to have similar appetite responses to moderate intensity treadmill exercise.

### 7.3. Energy intake

Currently, the majority of research is in agreement that exercise does not influence energy intake in the immediate hours after exercise (12-14 h, Schubert et al. 2013). The findings in Chapter 4 and 6 support this, showing absolute energy intake to be similar during control and exercise trials. It has been suggested that compensatory increases in *ad libitum* energy intake may occur outside this 12-14 h window post-exercise. However, when observation times have been increased to longer than 24 h, *ad libitum* energy intake during exercise trials remains indifferent to those of control trials (Beaulieu et al. 2015; King et al. 2015). Chapter 4 expands on this, to show that two consecutive days of moderately-vigorous exercise, fails to provoke a difference in absolute energy intake between trials. The inability for exercise to influence energy intake supports the subjective appetite response seen in Chapters 4 and 6.

It has been suggested that females, compared to males, could exhibit more potent compensatory responses to exercise induced energy deficits in an attempt to preserve energy balance and reproductive function (Hagobian and Braun 2010). However, in a recent meta-analysis, Schubert et al. (2013) showed males and females to exhibit similar *ad libitum* energy intake responses to exercise. Specifically, both males and females display no change

in *ad libitum* energy intake after an acute bout of exercise (Alajmi et al. 2016; Schubert et al. 2013). The findings in Chapter 6 confirm this and suggest that if an exercise induced energy deficit can be sustained over long periods of time, exercise could be equally effective for weight loss and maintenance in both males and females.

Across studies, the findings for a*d libitum* energy intake responses of ow/ob individuals to acute exercise are inconsistent. Some have found ow/ob individuals to express no difference (Martins et al. 2015; Tsofliou et al. 2003; Unick et al. 2010) and others report reductions in absolute energy intake after exercise compared to rest (Sim et al. 2014; Ueda et al. 2009b). Discrepancies are likely due to differences in study protocol, for example, exercise intensity, mode and duration. In addition, trial length of these studies was short (up to 3.5 h) restricting assumptions about how ow/ob individuals respond later in the trial day. In particular, *ad libitum* energy intake could only be assessed approximately 1.5 h after exercise. The findings in Chapter 6 broaden the findings of the current literature by extending this observation to 6 h after exercise and by directly comparing the response of ow/ob individuals to that of lean individuals. The findings from this thesis, along with previous research, would suggest that exercise is equally effective at generating a negative energy balance in lean and ow/ob individuals. The failure for acute exercise to increase *ad libitum* energy intake 6 h after exercise supports a role of exercise in weight management.

In both Chapter 4 and 6 of this thesis, participants were not familiarised with the buffet meals presented to them in main trials. To maintain the internal validity of buffet meals to assess energy intake it is important to keep all factors, other than the intervention, constant. By omitting buffet meal familiarisation, two factors will have been inadvertently changed at the buffet meals, i.e. the intervention and an unusual feeding environment on the first exposure vs. familiar feeding environment on subsequent exposures. This could have compromised internal validity, providing participants the potential to over-eat during the first exposure to these meals, in response to the wide-selection of foods available. However, no trial order effect was found for *ad libitum* energy intake in both Chapter 4 and 6 ($P=0.77$ and $P=0.12$, respectively). Future research must consider this limitation, and incorporate familiarisation to buffet meals, to ensure reliable and sensitive food intake assessment.

### 7.4. Acylated ghrelin

Circulating concentrations of acylated ghrelin are shown to be transiently suppressed during and immediately after exercise (Broom et al. 2007; Broom et al. 2009; Deighton et al. 2013a;

King et al. 2010a; King et al. 2011a; Wasse et al. 2012), concurrent with suppressions in subjective ratings of appetite. In view of ghrelin's unique ability to stimulate hunger, studies have tried to determine whether exercise induced anorexia is regulated by suppressions in ghrelin. However, few have identified significant correlations between appetite and acylated ghrelin concentrations (Broom et al. 2007; Broom et al. 2009). Chapter 4 also failed to correlate subjective appetite and acylated ghrelin concentrations; subjective appetite was suppressed immediately after exercise despite acylated ghrelin concentrations being similar to those in the control trial. Similarly, in Chapter 6 a suppression in subjective appetite was seen despite no differences in acylated ghrelin concentration immediately after exercise.

Data from this thesis questions the importance of acylated ghrelin in appetite regulation; transient suppressions in appetite did not coincide with suppressions in circulating concentrations of acylated ghrelin. Appetite control is complex, a plethora of tonic and acute hormones contribute to changes in appetite, amongst many other factors (i.e. behavioural, environmental and psychological). It is likely that the control of appetite after exercise is not solely regulated by one hormone, but instead the interaction of several hormones and other psychological, behavioural and environmental factors.

Numerous studies, including Chapters 4 and 6 of this thesis, have shown that in the hours after exercise acylated ghrelin concentrations are no different to those seen in control conditions i.e. there are no delayed rises to attempt to compensate for the energy expended during the exercise bout. Chapter 4 extended upon this pool of evidence by lengthening the exercise period over two days, again showing no delayed compensatory increase in acylated ghrelin. It is possible that the second day of exercise blocked the influences of the first exercise bout on circulating concentrations of acylated ghrelin as King et al. (2015) found acylated ghrelin concentrations to be reduced 24-27 h after exercise. This finding was unexpected and implies an improved appetite response to feeding the day after exercise. Further research is required to confirm the responses of acylated ghrelin >12-14 h after exercise.

To date, several studies have compared ghrelin responses in males and females. Initial findings suggested that males and females respond differently (Hagobian et al. 2009), however more recently studies suggest that there is no difference in acylated ghrelin response to exercise between males and females (Alajmi et al. 2016; Burns et al. 2007; Hagobian et al. 2009; Hagobian et al. 2013). The findings of Chapter 6 confirm these.

Research on responses of circulating ghrelin concentrations to exercise in ow/ob individuals are limited, with very few studies comparing these responses to those of lean individuals (Ueda et al. 2009b). Chapter 6 is one of the first studies to compare acylated ghrelin responses in lean and ow/ob individuals. These suggest that lean and ow/ob individuals respond similarly to a bout of acute treadmill exercise i.e. no suppression after exercise, and no compensatory increase later in the trial day. This supports the findings of Ueda et al. (2009b), who also showed no difference in ghrelin response to exercise (60 min cycling at 50% $\dot{V}O_2$ max) between lean and ow/ob individuals. Furthermore, Chapter 6 found there to be no correlation between waist circumference and acylated ghrelin AUC. The data presented in this thesis show that ow/ob individuals do not exhibit compensatory increases in acylated ghrelin, responding in a similar fashion to exercise as their lean counterparts, therefore supporting a role for exercise in the generation of an energy deficit.

### 7.5. Peptide YY

Studies examining the responses of circulating concentrations of PYY to acute bouts of exercise have shown mixed findings. Transient increases (and concordant decreases in hunger; Broom et al. 2009; Deighton et al. 2013a; Deighton et al. 2013b; Deighton et al. 2014; King et al. 2011a; Martins et al. 2007; Shorten et al. 2009; Ueda et al. 2009a; Ueda et al. 2009b; Wasse et al. 2012) after exercise, and no difference compared to control (Balaguera-Cortes et al. 2011; Holmstrup et al. 2013; Martins et al. 2015; Shorten et al. 2009; Sim et al. 2014) have been found. When expanding these comparisons to the whole intervention period, circulating concentrations of PYY are higher during exercise compared to control conditions (Cheng et al. 2009; King et al. 2011a). Both Chapters 4 and 6 confirm these reports, and further this to show that these increased PYY concentrations during exercise trials are concordant in males, females, lean and ow/ob individuals. Although not significantly different, ow/ob tended to express lower fasting concentrations of total PYY in Chapter 6 (87 (60) and 98 (56) $pg \cdot mL^{-1}$, for ow/ob and lean individuals, respectively). This is in concert with previous research, showing obese individuals to exhibit lower fasting concentrations of PYY (Batterham et al. 2003; le Roux et al. 2006). To date, there is little research comparing the responses of males vs. females and lean vs. ow/ob, making the findings of Chapter 6 novel.

## 7.6. GLP-1

Few have measured the responses of circulating concentrations of GLP-1 to acute exercise (Larson-Meyer et al. 2012; Martins et al. 2007; Martins et al. 2015; Ueda et al. 2009a; Ueda et al 2009b; Unick et al. 2010). A recent meta-analysis compiled these studies to show that GLP-1 concentrations are elevated on exercise compared to resting days (Schubert et al. 2014). Findings from Chapter 6 confirm those of the recent review, showing GLP-1 concentrations to be higher in the 7 h after a bout of moderate treadmill exercise than rest. Individuals were shown to respond similarly, irrespective of sex or BMI group. Furthermore, no correlation was found between waist circumference and GLP-1 AUC concentrations. This novel finding adds to the literature, and suggests that GLP-1 concentrations are altered in a way that will increase satiety after exercise. This in hand with the absence of exercise induced changes in *ad libitum* energy intake 6 h after a bout of acute exercise, suggests that satiety is maintained after exercise and consequently prevents a compensatory increase in energy intake. This could be of particular benefit to those who are using exercise as a weight loss or maintenance tool, but further work is required to confirm these findings.

## 7.7. Leptin

Circulating concentrations of leptin remain unaltered in the immediate hours (0-4 h) after acute exercise (Racette et al. 1997; Torjman et al. 1999; Weltman et al. 2000). However, in the 24-48 h after acute exercise circulating concentrations of leptin are seen to be reduced (Essig et al. 2000; King et al. 2015; Olive & Miller 2001; Yang et al. 2014). Contrary to this, in Chapter 4, leptin concentrations were unaltered by two consecutive days of exercise. The large energy requirements of each hour treadmill run (~3800 kJ) generated a significant difference in REI between control and exercise trials (control 29217 (4006); exercise 21394 (3031) kJ). Previously, the delayed reduction of leptin concentrations after acute exercise has been attributed to the generation of a negative energy balance and low energy availability (Borer et al. 2009; Hilton and Louks 2000). Perhaps, the high-energy intake seen on day one of the exercise trial (16812 (2899) kJ) could explain why leptin concentrations were not seen to be lower on day two of the exercise trial, compared to control. It is possible that the energy consumed was sufficient to block any exercise-related decreases in leptin. Owing to the importance of exercise-induced disturbances in energy balance for weight loss and maintenance, it is important to further examine the response of leptin to exercise induced energy deficits. Energy intake should be strictly controlled, so that the energy availability is

representative of a typical diet. This will aid in the design of effective exercise strategies to generate weight loss.

## 7.8. Limitations

The experimental chapters presented in this thesis contain several common limitations. Firstly all participants were relatively young (18-60 y). Considering the ageing population and large percentage of the UK population living outside the healthy life expectancy, it is becoming increasingly important to determine the effectiveness of exercise on appetite control and weight management in individuals who are elderly. Although Chapter 6 added to the current literature by comparing responses of lean and ow/ob individuals, participants were all otherwise healthy i.e. no history or currently suffering from diabetes, cardiovascular disease, high blood pressure etc. These are all conditions associated with increases in BMI, and therefore it would be useful to understand how individuals suffering from these conditions respond to exercise. Again, the findings from Chapter 6 are limited to those who are lean, overweight or obese. Future studies should examine appetite related responses to exercise in morbidly obese individuals. Chapter 6 is also restricted by the relatively short observation period (8 h). This also impacts the validity of the *ad libitum* meal provided during Chapter 6. This meal was provided at 7 h (~16:00), participants may have refrained from eating until 'comfortably full' knowing that they would be free to leave the laboratory one hour later, and subsequently be free to eat what they wish and not be confined to that available at the buffet.

## 7.9. Future directions

Further research is required to compare the responses of lean and ow/ob individuals to longer observation periods i.e. days after exercise and repeated bouts of exercise. This will help to determine if the energy deficit generated by single or repeated bouts of exercise can be sustained over time and ultimately aid in weight management. However, this will be difficult to measure objectively. In particular, it will be hard to control participant behaviour outside of the laboratory. Adopting a life-like study design, instead of time structured protocols (i.e. fixed meal times), will allow for the true determination of appetite compensation. Similarly, it is important to take a more holistic approach to study design, by incorporating genetic, psychological and environmental measures. For example, blood samples could be analysed to determine if participants express the FTO gene, measures of mood and anxiety could be collected periodically throughout trial days. Finally, with regard to the control of appetite

regulation, future research should look to extend upon the findings of this thesis by incorporating the measurement of active GLP-1 and $PYY_{3-36}$, cholecystokinin and PP.

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# APPENDICES

## APPENDIX A


**The comparison of two methods of blood sample collection for determining plasma acylated ghrelin concentration in humans**

**INFORMED CONSENT FORM**

**(to be completed after Participant Information Sheet has been read)**

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Approvals (Human Participants) Sub-Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others.

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

**APPENDIX B**

Loughborough University

Name/Number .....…….

**Health Screen Questionnaire for Study Volunteers**

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

If you have a blood-borne virus, or think that you may have one, please do not take part in this research.

**Please complete this brief questionnaire to confirm your fitness to participate:**

**1. At present,** do you have any health problem for which you are:

| | | | | | | |
|---|---|---|---|---|---|---|
| (a) | on medication, prescribed or otherwise ..... | Yes | | No | | |
| (b) | attending your general practitioner ..... | Yes | | No | | |
| (c) | on a hospital waiting list ..... | Yes | | No | | |

**2**. **In the past two years,** have you had any illness which required you to:

| | | | | | | |
|---|---|---|---|---|---|---|
| (a) | consult your GP..... | Yes | | No | | |
| (b) | attend a hospital outpatient department ..... | Yes | | No | | |
| (c) | be admitted to hospital ..... | Yes | | No | | |

**3. Have you ever** had any of the following:

| | | | | | | |
|---|---|---|---|---|---|---|
| (a) | Convulsions/epilepsy ..... | Yes | | No | | |
| (b) | Asthma ..... | Yes | | No | | |
| (c) | Eczema ..... | Yes | | No | | |
| (d) | Diabetes ..... | Yes | | No | | |
| (e) | A blood disorder ..... | Yes | | No | | |
| (f) | Head injury ..... | Yes | | No | | |
| (g) | Digestive problems ..... | Yes | | No | | |
| (h) | Heart problems ..... | Yes | | No | | |

| | | | | | |
|---|---|---|---|---|---|
| (i) | Problems with bones or joints ..... | Yes | | No | |
| (j) | Disturbance of balance/coordination ..... | Yes | | No | |
| (k) | Numbness in hands or feet ..... | Yes | | No | |
| (l) | Disturbance of vision ..... | Yes | | No | |
| (m) | Ear / hearing problems ..... | Yes | | No | |
| (n) | Thyroid problems ..... | Yes | | No | |
| (o) | Kidney or liver problems ..... | Yes | | No | |
| (p) | Allergy to nuts ..... | Yes | | No | |

**4**. **Has any,** otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ..... Yes ☐ No ☐

**If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)**

.....
.....
.....

**5. Allergy Information**

| | | | | | |
|---|---|---|---|---|---|
| (a) | are you allergic to any food products? | Yes | | No | |
| (b) | are you allergic to any medicines? | Yes | | No | |
| (c) | are you allergic to plasters? | Yes | | No | |

**If YES to any of the above, please provide additional information on the allergy**

……..
……...

**6. Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.**

Name:

……...

Telephone Number:

……...

Work ☐ Home ☐ Mobile ☐

Relationship to Participant:

…….....

**7. Are you currently involved in any other research studies at the University or elsewhere?**

Yes ☐ No ☐

If yes, please provide details of the study

……
……
……

## APPENDIX C

## INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don’t know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don’t know/Not sure

**This is the end of the questionnaire, thank you for participating.**

## APPENDIX D

## Three Factor Eating Questionnaire

**Part 1: please answer true/false**

1. **When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal**

   True □ False □

2. **I usually eat too much at social occasions, like parties and picnics**

   True □ False □

3. **I am usually so hungry that I eat more than three times a day**

   True □ False □

4. **When I have eaten my quota of calories, I am usually good about not eating any more**

   True □ False □

5. **Dieting is too hard for me because I just get too hungry**

   True □ False □

6. **I deliberately take small helpings as a means of controlling my weight**

   True □ False □

7. **Sometimes things just taste so good that I keep on eating even when I am no longer hungry**

   True □ False □

8. **Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat**

   True □ False □

9. **When I am anxious, I find myself eating**

   True □ False □

10. **Life is too short to worry about dieting**

    True □ False □

**11. Since my weight goes up and down, I have been on weight reducing diets more than once**

True □ False □

**12. I often feel so hungry that I just have to eat something**

True □ False □

**13. When I am with someone who is overeating, I usually overeat too**

True □ False □

**14. I have a pretty good idea of the number of calories in common food**

True □ False □

**15. Sometimes when I start eating, I just can't seem to stop**

True □ False □

**16. It is not difficult for me to leave something on my plate**

True □ False □

**17. At certain times of the day, I get hungry because I have gotten used to eating then**

True □ False □

**18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it**

True □ False □

**19. Being with someone who is eating often makes me hungry enough to eat also**

True □ False □

**20. When I feel blue, I often overeat**

True □ False □

**21. I enjoy eating too much to spoil it by counting calories or watching my weight**

True □ False □

**22. When I see a real delicacy I often get so hungry that I have to eat it right away**

True □ False □

**23. I often stop eating when I am not really full as a conscious means of limiting what I eat**

True □ False □

**24. I get so hungry that my stomach often feels like a bottomless pit**

True □ False □

**25. My weight has hardly changed at all in the last ten years**

True □ False □

**26. I am always hungry so it is hard for me to stop eating before I finish all the food on my plate**

True □ False □

**27. When I feel lonely, I console myself by eating**

True □ False □

**28. I consciously hold back at meals in order not to gain weight**

True □ False □

**29. I sometimes get very hungry late in the evening or at night**

True □ False □

**30. I eat anything I want, anytime I want**

True □ False □

**31. Without even thinking about it, I take a long time to eat**

True □ False □

**32. I count calories as a conscious means of controlling my weight**

True □ False □

**33. I do not eat some foods because they make me fat**

True □ False □

**34. I am always hungry enough to eat at any time**

True □ False □

**35. I pay a great deal of attention to changes in my figure**

True □ False □

**36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods**

True □ False □

**Part 2:**

**37. How often are you dieting in a conscious effort to control your weight?**

1 (rarely) 2(sometimes) 3(Usually) 4(always)

**38. Would a weight fluctuation of 5 lbs affect the way you live your life?**

1(not at all) 2(slightly) 3(moderately) 4(very much)

**39. How often do you feel hungry?**

1(only at meal times) 2(sometimes between meals) 3(often between meals) 4(almost always)

**40. Do your feelings of guilt about overeating help you to control your food intake?**

1(never) 2(rarely) 3(often) 4(always)

**41. How difficult would it be for you to stop eating half way through dinner and not eat again for four hours?**

1(easy) 2(slightly difficult) 3( moderately difficult) 4(very difficult)

**42. How conscious are you of what you are eating?**

1(not at all) 2(slightly) 3(moderately) 4(extremely)

**43. How frequently do you avoid 'stocking up' on tempting foods?**

1 (almost never) 2(seldom) 3(usually) 4(almost always)

**44. How likely are you to shop for low calorie foods?**

1(unlikely) 2(slightly unlikely) 3(moderately likely) 4(very likely)

**45. Do you eat sensibly in front of others and splurge alone?**

1(never) 2(rarely) 3(often) 4(always)

**46. How likely are you to consciously eat slowly in order to cut down on how much you eat?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**47. How frequently do you skip desert because you are no longer hungry?**

1(almost never) 2(seldom) 3(at least once a week) 4(almost every day)

**48. How likely are you to consciously eat less than you want?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**49. Do you go on eating binges though you are not hungry?**

1(never) 2(rarely) 3(sometimes) 4(at least once a week)

**50. On a scale of 0-5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?**

0

Eat whatever you want, whenever you want it

1

Usually eat whatever you want, whenever you want it

2

Often eat whatever you want, whenever you want it

3

Often limit food intake, but often 'give in'

4

Usually limit food intake, rarely 'give in'

5

Constantly limiting food intake, never 'give in'

**51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of a number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'**

1(not like me) 2(little like me) 3(pretty good description of me) 4( describes me perfectly)

## APPENDIX E

## Food Preferences

Please circle the number which best describes your liking of the following foods:

Coco-Pops

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cornflakes

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Rice Krispies

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Semi-Skimmed Milk

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Nutri-grain Bars

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Brown Bread

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tuna

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cheese

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ham

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mayonnaise

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Salted Crisps

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Apple

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mini-Rolls

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Muffins

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Chip Muffins

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Chip Cookies

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mars Bars

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Pasta

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tomato Sauce

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

## APPENDIX F

**Subject Number:** **Trial:** **Date:**

Visual Analogue Scale

**Time:**

**Please indicate how hungry you are now by circling a relevant number**

Not Hungry Fairly Hungry Hungry Very Hungry

**0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15**

**How hungry do you feel?**

I am not hungry at all — I have never been more hungry

**How satisfied do you**

I am completely empty — I cannot eat another bite

**How full do you feel?**

Not at all full — Totally full

**How much do you think you can eat?**

Nothing at all — A lot

**How nauseous do you feel?**

Not at all — Very nauseous

| Temperature (°C) | Humidity (%) |
|---|---|
| | |

## APPENDIX G

Semi-skimmed milk

Coco Pops

Cornflakes

Rice Krispies

White bread

Brown bread

Margarine

Mayonnaise

Ham

Tuna

Crisps

Chocolate rolls

Mars bars

Cereal bars

Cookies

Chocolate chip muffins

Triple chocolate chip muffins

Apples

Oranges

Bananas

## APPENDIX H

Crisps

Chocolate rolls

Mars bars

Cereal bars

Cookies

Chocolate chip muffins

Triple chocolate chip muffins

Apples

Oranges

Bananas

## APPENDIX I

Chapter V search strategy.

**Keywords**

| *Study Population* | *Intervention* |
|---|---|
| Obese | Exercise; Physical exercise; Aerobic exercise |
| Overweight | Physical activity |
| | Energy expenditure |
| | Energy intake; Caloric intake |
| | Food intake |
| | Appetite |
| | Hunger |
| | Appetite hormones; Appetite regulating hormones |
| | Ghrelin; Acylated ghrelin; Total ghrelin; Acyl ghrelin |
| | Peptide YY; PYY; Peptide YY 3-36; PYY3-36; PYY (3-36); Total PYY |
| | Glucagon like peptide 1; Glucagon-like peptide-1; GLP-1; total GLP-1; active GLP-1; GLP-1 (7-36); GLP-1 (9-36) |

I. Obese or overweight
II. Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure
III. Energy intake or caloric intake or food intake
IV. Appetite regulatory hormones or appetite hormones
V. Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY
VI. Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin
VII. Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36)
VIII. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure (I and II)
IX. Obese or overweight and Energy intake or caloric intake or food intake (I and III)
X. Obese or overweight and Appetite regulatory hormones or appetite hormones (I and IV)
XI. Obese or overweight and Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY (I and V)
XII. Obese or overweight and Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin (I and VI)

XIII. Obese or overweight and Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36) (I and VII)

XIV. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Energy intake or caloric intake or food intake (I and II and III)

XV. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Appetite regulatory hormones or appetite hormones (I and II and IV)

XVI. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY (I and II and V)

XVII. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin (I and II and VI)

XVIII. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36) (I and II and VII)

XIX. Obese or overweight (RCT filter)

XX. Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure (RCT filter)

XXI. Energy intake or caloric intake or food intake (RCT filter)

XXII. Appetite regulatory hormones or appetite hormones (RCT filter)

XXIII. Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY (RCT filter)

XXIV. Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin (RCT filter)

XXV. Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36) (RCT filter)

XXVI. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure (I and II) (RCT filter)

XXVII. Obese or overweight and Energy intake or caloric intake or food intake (I and III) (RCT filter)

XXVIII. Obese or overweight and Appetite regulatory hormones or appetite hormones (I and IV) (RCT filter)

XXIX. Obese or overweight and Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY (I and V) (RCT filter)

XXX. Obese or overweight and Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin (I and VI) (RCT filter)

XXXI. Obese or overweight and Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36) (I and VII) (RCT filter)

XXXII. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Energy intake or caloric intake or food intake (I and II and III) (RCT filter)

XXXIII. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Appetite regulatory hormones or appetite hormones (I and II and IV) (RCT filter)

XXXIV. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Peptide YY or PYY or Peptide YY 3-36 or PYY3-36 or PYY (3-36) or Total PYY (I and II and V) (RCT filter)

XXXV. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Ghrelin or Acylated ghrelin or Total ghrelin or Acyl ghrelin (I and II and VI) (RCT filter)

XXXVI. Obese or overweight and Exercise or physical exercise or aerobic exercise or physical activity or energy expenditure and Glucagon like peptide 1 or Glucagon-like peptide-1 or GLP-1 or total GLP-1 or active GLP-1 or GLP-1 (7-36) or GLP-1 (9-36) (I and II and VII) (RCT filter)

## APPENDIX J

Individual study characteristics for studies evaluating acylated ghrelin.

| Study | Category | Standard difference in means | Standard error | Variance | Lower 95% confidence interval | Upper 95% confidence interval | Z-value | p-value | Weight (%) |
|---|---|---|---|---|---|---|---|---|---|
| Gholipour, M. | ow/ob | -1.517 | 0.183 | 0.033 | -1.876 | -1.159 | -8.295 | 0.000 | 8.303 |
| Martins, S. A | ow/ob | -0.468 | 0.114 | 0.013 | -0.691 | -0.245 | -4.115 | 0.000 | 10.064 |
| Martins, S. B | ow/ob | -0.456 | 0.113 | 0.013 | -0.678 | -0.234 | -4.019 | 0.000 | 10.070 |
| Martins, S. C | ow/ob | -0.284 | 0.110 | 0.012 | -0.500 | -0.069 | -2.582 | 0.010 | 10.148 |
| Sim, A. A | ow/ob | -0.032 | 0.091 | 0.008 | -0.210 | 0.146 | -0.353 | 0.724 | 10.578 |
| Sim, A. B | ow/ob | -0.201 | 0.092 | 0.008 | -0.381 | -0.021 | -2.193 | 0.028 | 10.559 |
| Sim, A. C | ow/ob | -0.325 | 0.093 | 0.009 | -0.507 | -0.142 | -3.490 | 0.000 | 10.529 |
| Tiryaki-Sonmez, G. | ow/ob | -0.367 | 0.129 | 0.017 | -0.619 | -0.114 | -2.847 | 0.004 | 9.697 |
| Ueda, S.-y. | ow/ob | 0.082 | 0.142 | 0.020 | -0.196 | 0.359 | 0.577 | 0.564 | 9.374 |
| Unick, J.L. | ow/ob | -0.047 | 0.086 | 0.007 | -0.216 | 0.121 | -0.552 | 0.581 | 10.678 |
| Mean | | -0.340 | 0.099 | 0.010 | -0.533 | -0.146 | -3.443 | 0.001 | 100 |
| Broom et al. 2007 | Lean | -0.253 | 0.127 | 0.016 | -0.501 | -0.004 | -1.994 | 0.046 | 4.290 |
| Broom et al. 2009 | Lean | -0.085 | 0.113 | 0.013 | -0.307 | 0.136 | -0.753 | 0.451 | 4.427 |
| Broom et al. 2009-1 | Lean | -0.125 | 0.113 | 0.013 | -0.347 | 0.097 | -1.104 | 0.269 | 4.424 |
| Shorten et al. 2009 | Lean | -0.172 | 0.114 | 0.013 | -0.395 | 0.051 | -1.512 | 0.131 | 4.421 |
| Ueda et al. 2009a | Lean | 0.701 | 0.158 | 0.025 | 0.391 | 1.010 | 4.439 | 0.000 | 3.960 |
| King et al. 2010b | Lean | -0.620 | 0.136 | 0.019 | -0.887 | -0.353 | -4.553 | 0.000 | 4.192 |
| King et al. 2010a | Lean | -0.023 | 0.100 | 0.010 | -0.219 | 0.173 | -0.233 | 0.816 | 4.549 |
| Balaguera-Cortes et al. 2011 | Lean | 0.023 | 0.118 | 0.014 | -0.209 | 0.255 | 0.197 | 0.844 | 4.374 |
| Balaguera-Cortes et al. 2011-1 | Lean | -0.476 | 0.125 | 0.016 | -0.721 | -0.231 | -3.813 | 0.000 | 4.309 |
| King et al. 2011a | Lean | -0.100 | 0.108 | 0.012 | -0.312 | 0.112 | -0.924 | 0.355 | 4.472 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| King et al. 2011b | Lean | -0.047 | 0.100 | 0.010 | -0.243 | 0.149 | -0.468 | 0.640 | 4.549 |
| Vatansever-Ozen, 2011 | Lean | -0.672 | 0.131 | 0.017 | -0.929 | -0.415 | -5.129 | 0.000 | 4.246 |
| Becker et al. 2012 | Lean | -0.437 | 0.138 | 0.019 | -0.708 | -0.165 | -3.155 | 0.002 | 4.168 |
| Kelly et al. 2012 | Lean | -0.106 | 0.119 | 0.014 | -0.339 | 0.126 | -0.896 | 0.370 | 4.371 |
| Larson Meyer et al. 2012 | Lean | 0.631 | 0.137 | 0.019 | 0.363 | 0.899 | 4.621 | 0.000 | 4.187 |
| Larson Meyer et al. 2012a | Lean | 0.123 | 0.119 | 0.014 | -0.110 | 0.356 | 1.036 | 0.300 | 4.370 |
| Wasse et al. 2012 | Lean | -0.181 | 0.119 | 0.014 | -0.415 | 0.053 | -1.515 | 0.130 | 4.365 |
| Wasse et al. 2013-1 | Lean | -0.407 | 0.117 | 0.014 | -0.637 | -0.176 | -3.463 | 0.001 | 4.384 |
| Wasse et al. 2013 | Lean | -0.412 | 0.117 | 0.014 | -0.642 | -0.181 | -3.502 | 0.000 | 4.383 |
| Deighton et al. 2013 | Lean | -0.427 | 0.113 | 0.013 | -0.648 | -0.206 | -3.785 | 0.000 | 4.428 |
| Deighton et al. 2013-1 | Lean | -0.700 | 0.121 | 0.015 | -0.936 | -0.464 | -5.808 | 0.000 | 4.353 |
| Hagobian et al. 2013 | Lean | 0.270 | 0.115 | 0.013 | 0.044 | 0.495 | 2.347 | 0.019 | 4.409 |
| Hagobian et al. 2013-1 | Lean | -0.119 | 0.119 | 0.014 | -0.352 | 0.113 | -1.004 | 0.315 | 4.370 |
| Mean | | -0.159 | 0.067 | 0.005 | -0.291 | -0.027 | -2.358 | 0.018 | 100 |

## APPENDIX K

Individual study characteristics for studies evaluating total PYY.

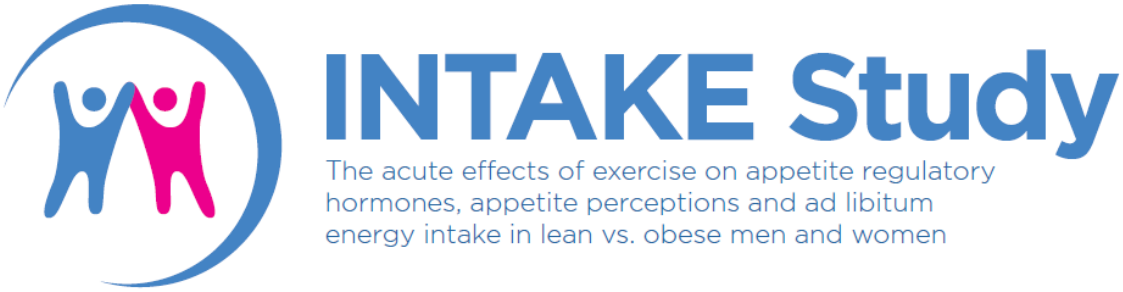
| Study | Category | Standard difference in means | Standard error | Variance | Lower 95% confidence interval | Upper 95% confidence interval | Z-value | p-value | Weight (%) |
|---|---|---|---|---|---|---|---|---|---|
| Sim, A. A | ow/ob | 0.034 | 0.185 | 0.034 | -0.328 | 0.396 | 0.186 | 0.853 | 29.296 |
| Sim, A. B | ow/ob | -0.052 | 0.185 | 0.034 | -0.414 | 0.310 | -0.281 | 0.779 | 29.280 |
| Sim, A. C | ow/ob | 0.078 | 0.185 | 0.034 | -0.285 | 0.441 | 0.422 | 0.673 | 29.245 |
| Ueda, S.-y. | ow/ob | 0.666 | 0.318 | 0.101 | 0.042 | 1.289 | 2.092 | 0.036 | 12.179 |
| Mean | | 0.099 | 0.118 | 0.014 | -0.133 | 0.331 | 0.835 | 0.404 | 100 |
| Martins et al. 2007 | Lean | 0.056 | 0.311 | 0.097 | -0.553 | 0.666 | 0.181 | 0.856 | 4.857 |
| Broom et al. 2009a | Lean | 0.647 | 0.253 | 0.064 | 0.152 | 1.142 | 2.564 | 0.010 | 7.373 |
| Broom et al. 2009b | Lean | -0.086 | 0.230 | 0.053 | -0.537 | 0.365 | -0.375 | 0.707 | 8.885 |
| Shorten et al. 2009 | Lean | 0.165 | 0.231 | 0.053 | -0.288 | 0.619 | 0.716 | 0.474 | 8.798 |
| Ueda et al. 2009a | Lean | 0.465 | 0.303 | 0.092 | -0.129 | 1.058 | 1.533 | 0.125 | 5.122 |
| Balaguera-Cortes et al. 2011a | Lean | 0.085 | 0.241 | 0.058 | -0.388 | 0.558 | 0.354 | 0.723 | 8.078 |
| Balaguera-Cortes et al. 2011b | Lean | -0.133 | 0.242 | 0.059 | -0.607 | 0.341 | -0.549 | 0.583 | 8.037 |
| Kelly et al. 2012 | Lean | 0.153 | 0.242 | 0.059 | -0.321 | 0.628 | 0.633 | 0.527 | 8.013 |
| Larson Meyer et al. 2012a | Lean | 0.023 | 0.254 | 0.064 | -0.474 | 0.521 | 0.093 | 0.926 | 7.295 |
| Larson Meyer et al. 2012b | Lean | 0.553 | 0.259 | 0.067 | 0.046 | 1.059 | 2.137 | 0.033 | 7.034 |
| Wasse et al. 2013 | Lean | 0.236 | 0.244 | 0.060 | -0.242 | 0.715 | 0.968 | 0.333 | 7.888 |
| Deighton et al. 2012a | Lean | 0.337 | 0.226 | 0.051 | -0.106 | 0.780 | 1.493 | 0.135 | 9.205 |
| Deighton et al. 2012b | Lean | 0.259 | 0.223 | 0.050 | -0.179 | 0.697 | 1.158 | 0.247 | 9.414 |
| Mean | | 0.204 | 0.069 | 0.005 | 0.070 | 0.339 | 2.979 | 0.003 | 100 |

## APPENDIX L

Individual study characteristics for studies evaluating total GLP-1.

| Study | Category | Standard difference in means | Standard error | Variance | Lower 95% confidence interval | Upper 95% confidence interval | Z-value | p-value | Weight (%) |
|---|---|---|---|---|---|---|---|---|---|
| Martins, S. A | ow/ob | 0.066 | 0.100 | 0.010 | -0.131 | 0.262 | 0.655 | 0.512 | 24.108 |
| Martins, S. B | ow/ob | 0.146 | 0.101 | 0.010 | -0.051 | 0.343 | 1.450 | 0.147 | 24.033 |
| Martins, S. C | ow/ob | -0.087 | 0.100 | 0.010 | -0.284 | 0.109 | -0.872 | 0.383 | 24.093 |
| Unick, J.L. | ow/ob | -0.200 | 0.080 | 0.006 | -0.358 | -0.043 | -2.497 | 0.013 | 27.765 |
| Mean | | | | | | | | | 100 |
| Martins et al. 2007 | Lean | 0.628 | 0.155 | 0.024 | 0.325 | 0.932 | 4.060 | 0.000 | 13.908 |
| Ueda et al. 2009a | Lean | 0.381 | 0.136 | 0.018 | 0.116 | 0.647 | 2.813 | 0.005 | 15.608 |
| Ueda et al. 2009b | Lean | 0.518 | 0.117 | 0.014 | 0.290 | 0.747 | 4.442 | 0.000 | 17.441 |
| Ueda et al. 2009b-1 | Lean | 0.518 | 0.117 | 0.014 | 0.290 | 0.747 | 4.443 | 0.000 | 17.441 |
| Larson Meyer et al. 2012 | Lean | 0.155 | 0.116 | 0.013 | -0.072 | 0.383 | 1.336 | 0.182 | 17.492 |
| Larson Meyer et al. 2012a | Lean | 0.136 | 0.110 | 0.012 | -0.079 | 0.352 | 1.239 | 0.215 | 18.109 |
| Mean | | | | | | | | | 100 |

**APPENDIX M**




## Study Protocol

### Full title

The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women.

### Short title

Acute exercise & appetite regulation in lean vs. obese men and women (INTAKE).

### Ethics ref:

### Chief Investigator

Professor Melanie Davies – Professor in Diabetes Medicine and Honorary Consultant Physician (University Hospitals of Leicester and University of Leicester)

### Investigators

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Dr David Stensel - Reader in Exercise Metabolism (Loughborough University)

Dr James King – Senior Research Associate (Leicester-Loughborough Diet, Lifestyle & Physical Activity BRU)

Professor Myra Nimmo – Professor in Exercise Physiology (Loughborough University) and Leicester-Loughborough Diet, Lifestyle & Physical Activity BRU theme lead

Andrew Jackson - Grade 7 Biochemist (Loughborough University)

Dr David Webb – Clinical Senior Lecturer in Diabetes Medicine (University of Leicester)

**Research sponsor**

University of Leicester

**Funder**

The NIHR Leicester-Loughborough Biomedical Research Unit in Diet, Lifestyle & Physical Activity

**Confidentiality statement**

This document contains confidential information that must not be disclosed to anyone other than the sponsor, investigator team, NHS trust, regulatory authorities, or members of the Research Ethics Committee.

**Study Summary**

Energy balance and body weight control are mediated by the interplay between energy intake (food and drink) and energy expenditure through metabolic processes and physical activity. Evidence has shown that exercise can favourably influence body weight regulation, most notably by augmenting daily energy expenditure. Additionally, recent research has shown that exercise may also facilitate healthy body weight control by improving appetite regulation. Specifically, it has been demonstrated that exercise can improve the ability to sense the energy/nutrient content of meals and this is associated with an improved capacity to match energy intake to energy requirement. One of the key mechanisms by which exercise mediates this effect appears to be related to favourable changes in circulating concentrations of several gut hormones which are known to be critical regulators of appetite control and energy balance.

The physiological regulation of appetite and energy homeostasis is orchestrated by several neuro-endocrine mediators that are released from the pancreas/gastrointestinal tract and which travel to the brain and exert their influence by targeting receptors on key appetite regulatory neuronal populations. Importantly, we now know that this regulatory system is dysfunctional in obese individuals with the most notable characteristics being an attenuated meal related increase in appetite inhibiting signals and a reduced post-meal suppression of appetite stimulating signals. The proposed investigation seeks to determine whether the beneficial effects of exercise on appetite control observed in lean participants translates into the obese. Specifically, the proposed investigation will examine the acute effects of a single bout of exercise on subjective perceptions of appetite, energy intake and appetite regulatory hormones (ghrelin, peptide $YY_{3-36}$ and GLP-1). Comparisons will be made between lean and overweight/obese men and women.

To accomplish this we will recruit 16 overweight/obese and 16 normal weight men and 16 overweight/obese and 16 normal weight women, aged 18-65 years (64 participants in total). All participants will be inactive and non-diabetic. Each participant will complete one screening visit, one exercise familiarisation session and two, 8 hour main experimental trials (control and exercise). The order of main trials will be counterbalanced. In the control trial

individuals will be required to rest for 8 hours and 12 blood samples will be drawn from an intravenous cannula during the trial. A standardised breakfast, lunch and a buffet evening meal will be provided during trials. Subjective appetite ratings will be determined at 30 minute intervals throughout. Identical procedures will be completed in the exercise trial except that during the first trial hour participants will complete 60 minutes of moderate intensity treadmill walking/running (60% of maximum oxygen uptake).

This research will determine the acute impact of exercise on appetite and its key physiological regulators. Critically, this investigation will compare the short-term effects of exercise on appetite control in lean vs. obese men and women and in doing so will enhance knowledge regarding the interaction between obesity, appetite regulation, energy homeostasis and exercise.

**Background and rationale**

Globally the prevalence of obesity has reached epidemic levels and continues to escalate. Current World Health Organisation (WHO) estimates indicate that 1.5 billion adults are overweight, of which 500 million individuals also qualify as obese (WHO, 2012). Furthermore, levels of obesity in children and adolescents are now significant and this may drive up rates of adult obesity in the future. The adverse health and economic consequences associated with being overweight make these statistics a significant cause for concern. Consequently there is an urgent need to identify effective preventative and/or management strategies. This feat relies upon possessing a comprehensive understanding of the critical mechanisms and influences governing energy homeostasis.

Energy balance and body weight are controlled physiologically by a network of gut, adipose and pancreatic derived hormones which regulate energy intake and energy metabolism homeostatically on both an acute and chronic basis (Murphy & Bloom, 2006). Specifically, on a meal-to-meal basis appetite and food intake are mediated by several circulating gut peptides (e.g. ghrelin, peptide YY, GLP-1) which rhythmically control perceptions of pre-meal hunger and post-meal satiation and satiety. Furthermore, within the long-term, appetite and energy homeostasis are also influenced by insulin and leptin, two potent long-term signals which suppress appetite and increase energy expenditure. Collectively these signals serve to prevent large perturbations in energy balance and therefore help to maintain body weight/composition within a healthy/ ‘evolutionally fit’ range.

Obesity is the result of chronic positive energy balance whereby over-time the energy consumed as food and drink surpasses that expended through metabolic processes and physical activity. Research has shown that the physiological appetite control system is dysfunctional in obese individuals and this predisposes to overconsumption (Batterham et al., 2006; Cummings et al., 2002; le Roux et al., 2006). In particular, in recent years it has been identified that circulating levels of ghrelin, an appetite stimulating hormone, are not effectively suppressed after meals in obese individuals (Morpurgo et al., 2003). Moreover, it has also been demonstrated that postprandial levels of PYY and GLP-1, two important appetite inhibiting signals and mediators of satiation and satiety, are significantly attenuated

in comparison with responses in lean individuals (Batterham et al., 2006; le Roux et al., 2006). In concert, these derangements result in impaired feelings of fullness and satisfaction and promote increased food consumption which ultimately perpetuates the obese state. This knowledge has fuelled an explosion of research and development activity into a subset of obesity pharmacotherapy with the aim of mimicking the body's own appetite regulatory signals/pathways (Field, Chaudhri, & Bloom, 2009). Unfortunately, to date the development of drug treatments within this sphere have been unsuccessful due to a combination of lack of efficacy and safety concerns. The identification of more natural solutions to this problem is therefore welcomed.

Over half a century ago it was first recognised that being active is important for individuals to successfully match energy intake to energy requirements and therefore maintain a healthy body weight (Mayer, Roy, & Mitra, 1956). More recently it has been shown that sedentary individuals, when compared with their active counterparts, suffer from an impaired appetite regulatory system which manifests as an inability to sense and respond to the energy content of ingested foods (Long, Hart, & Morgan, 2002). This notion has been confirmed in a six week exercise intervention in previously sedentary men and women (Martins, Morgan, Bloom, & Robertson, 2007). These researchers observed that exercise training led to an improved ability to detect the energy content of ingested foods (preloads) and to subsequently respond by altering energy intake at following meals. These findings indicate that exercise improves the sensitivity of the appetite control system. Preliminary data suggests that the mechanism responsible for these changes is at least partly due to alterations in the circulating concentrations of appetite regulatory hormones (acylated ghrelin, PYY and GLP-1) (Martins, Robertson, & Morgan, 2010). As yet it is unknown whether such benefits translate into obese populations who demonstrate an impaired appetite control system.

In healthy volunteers several studies have examined the acute impact of single bouts of exercise on circulating appetite regulatory hormones (Broom, Batterham, King, & Stensel, 2009; King, Miyashita, Wasse, & Stensel, 2010; King et al., 2011; King, Burley, & Blundell, 1994; Martins et al., 2007; Ueda et al., 2009). The consensus from this research suggests that acute exercise transiently suppresses appetite and that this is associated with suppressed circulating concentrations of ghrelin and increased levels of PYY and GLP-1. To date, very little of this research has been conducted in those who are overweight or obese. One study has shown that high intensity exercise suppressed ghrelin in obese men to the same extent as in lean individuals however ghrelin assessments in this study were limited to merely during and immediately after exercise (Marzullo et al., 2008). Another investigation showed that circulating levels of PYY and GLP-1 increase during exercise with levels of GLP-1, but not PYY, remaining higher for 60 minutes afterwards (Ueda et al., 2009). Both of these studies are limited by the brief duration of assessment and most importantly the failure to determine post-exercise effects on prandial hormone changes.

The proposed investigation seeks to compare the effects of a single bout of aerobic exercise on appetite perceptions, *ad libitum* energy intake and circulating levels of key appetite regulatory hormones in lean vs. overweight/obese men and women. Subjective appetite perceptions will be examined during exercise and for seven hours after. Circulating levels of

acylated ghrelin, PYY and GLP-1 will be measured in response to exercise and two standardised meals during seven hours after exercise. The effects of exercise on food intake will be ascertained by examining energy intake at an *ad libitum* meal provided six hours after exercise.

This research will extend knowledge about the impact of exercise on appetite regulation and energy balance. In particular, this study will determine whether previously reported favourable effects translate into real benefits in a population who suffer from impaired appetite regulation.

## Objectives

**Primary objective:** to investigate the effect of a single bout of exercise on appetite regulatory hormones (i.e. acylated ghrelin, $PYY_{3-36}$, GLP-1) in lean vs. overweight/obese men and women.

**Secondary objective:** to investigate the effect of a single bout of exercise on subjective appetite perceptions and *ad libitum* energy intake in lean vs. overweight/obese men and women.

## Trial design

This study is a randomised crossover intervention which will require participants to visit the laboratory on four occasions. Participants will be recruited and pre-assessed according to the 'Risk Definition and Standard Operating Procedures for Exercise Testing' document (Version 2, 28/05/2013) and will be tested at either Leicester Diabetes Centre or Loughborough University. The four visits will include:

1. Preliminary visit - ~90 minute visit
2. Exercise familiarisation - ~90 minute visit
3. Main experimental trial 1 - ~8.5 h visit
4. Main experimental trial 2 - ~8.5 h visit

Each participant will complete two main experimental trials (exercise and control) in a counterbalanced order. Each main experimental trial will be separated by at least one-week.

## Participant Flow

### Study visit 1 – Leicester Diabetes Centre or Loughborough University

#### *Informed consent*

Informed consent will be obtained before any study related procedures commence and only after participants have had sufficient time to thoroughly read the participant information sheet (always > 48 h). At this point participants will be reminded of the importance to comply with dietary and physical activity standardisation. Informed consent will be obtained by a designated researcher involved in this study who has undergone consent training and who holds an up to date Good Clinical Practice Certificate. Written informed consent will be

obtained by means of participant dated signature and dated signature of the individual who presented and obtained informed consent. A copy of the signed informed consent form will be given to the participants along with a copy of the participant information sheet. The original signed form will be retained in the study site file. The informed consent process will be undertaken in privacy.

***Study questionnaires***

Participants will also be required to complete a set of questionnaires at their first visit. Questionnaires will assess participants' health status (non-validated questionnaire), habitual physical activity level (IPAQ, validated questionnaire), physical activity readiness questionnaire (PAR-Q, validated questionnaire), food preference and breakfast pattern questionnaire (in house – non-validated questionnaire), and dietary habits (Three-Factor Eating Questionnaire, validated questionnaire). Participants will also be asked about their medical history and consequently a risk SCORE assessment will be calculated. Results from these will determine if the participant will need to complete the subsequent exercise test with an ECG. Details of this can be found in the 'Risk Definition and Standard Operating Procedures for Exercise Testing' (Version 2 , 28/05/2013).

***Anthropometric Measures and Physical Exam***

Participants' height, body weight, blood pressure and waist circumference will be measured using standard techniques. A suitably qualified member of the research team will complete a physical exam on the participant.

***Fasting blood sample***

A fasting venous blood sample to confirm that participants are non-diabetic (fasting glucose concentrations < 7 mmol/L, HbA1c <6.5%) will be taken. A cholesterol measurement will also be taken.

***Resting ECG***

A suitably qualified member of the research team will perform a 12 lead resting ECG.

The results from the resting ECG will determine whether the participant is suitable to continue in the study.

***Submaximal-incremental treadmill walking/running test***

Participants will complete a submaximal-incremental walking/running test on a motorised treadmill. The test will last for 16 minutes and will consist of four, four minute stages. Initial treadmill speed will be set according to the fitness of the participant. Treadmill speed will be increased every four minutes by 0.5-1.5 km/h (depending on the individual). The treadmill gradient will remain at zero throughout the test. Expired air samples will be collected continuously throughout the test using an online breath-by-breath gas analysis system. Heart rate will be monitored throughout and ratings of perceived exertion (RPE) will be measured

at the end of each stage using the Borg scale (Borg, 1973). This test will be used to determine the relationship between walking/running speed and oxygen consumption.

***Maximum oxygen uptake test***

Participants' maximum oxygen uptake will be determined using an incremental treadmill protocol whereby participants continue to exercise until volitional exhaustion. During this test the speed of the treadmill will remain constant throughout with this being determined by the fitness level of the participant. The test will begin with the treadmill gradient set at 1% and this will be increased by one per cent at intervals of one minute. The duration of this test will be approximately 12 minutes. After the test participants will relax for five minutes before a verification procedure is undertaken. For this procedure, participants will be asked to exercise at the gradient attained one stage prior to the last stage completed in the maximum oxygen uptake test. The test will then continue as per the maximum oxygen uptake test with the purpose being to verify that participants obtained a true maximum oxygen uptake value (Midgley & Carroll, 2009; Pettitt, Clark, Ebner, Sedgeman, & Murray, 2012). Expired air samples will be collected continuously throughout the test using an online breath-by-breath gas analysis system. Heart rate, expired air samples and RPE will be taken throughout.

The information gathered from the submaximal-incremental treadmill walking/running test and the maximum oxygen uptake test will allow us to calculate the treadmill speed necessary to elicit the desired exercise intensity (60% of maximum oxygen uptake) during the main experimental (exercise) trial.

Following the exercise tests, participants will be provided with refreshments to break the overnight fast required for the fasting blood sample.

The information gathered from the pre-assessment will determine if the participant matches all the inclusion criteria, and confirm if they can continue in the study.

**Study visit 2 – Leicester Diabetes Centre or Loughborough University**

***Exercise Familiarisation***

In order to ensure that participants can complete the bout of exercise required during the exercise main trial, and to familiarise participants with the associated procedures, each participant will complete 60 minutes of walking/running on a treadmill at the speed calculated to elicit 60% of their maximum aerobic capacity. During this procedure we will familiarise participants with the expired gas analysis collection procedures and ratings of perceived exertion assessments.

***Dietary and physical activity standardisation***

In order to ensure that strict dietary and physical activity standardisation occurs in the 48 h before each main experimental trial a member of the research team will talk participants through all of the procedures for recording and subsequently replicating their diet using a weighed food record. To assist with this they will then be provided with a set of weighing

scales and food record diary. Participants will also be provided with food to consume the night before each main trial. This will be a food package containing dried pasta shapes, tomato pasta sauce, and chocolate biscuits. The calorie content of this meal will be standardised (approximately 750 kcal for males and 674 kcal for females). Instructions on how to prepare the meal will be provided.

**Study visit 3 and 4 (main experiment trials) – Leicester Diabetes Centre or Loughborough University**

Visits 3 and 4 will be for participants' main experimental trials (exercise and control) which will each last for 8 h (~9am – 5pm). Appendix one provides a schematic illustration of the main trial protocol. Prior to each main trial participants will refrain from completing any structured form of physical activity or from consuming alcohol or caffeine. Participants will also standardise their dietary intake during this period using a weighed food record. Female participants with regular menstrual cycles will be tested during days one to eight of the follicular phase. All participants will complete both trials on the same day of the week, and will not have been overseas for one week prior to the trial days.

On the control trial participants will arrive at the laboratory at ~8:30am having not eaten since 9:00pm the prior evening. An intravenous cannula will then be inserted into an antecubital vein to allow repeated blood sampling to occur over the duration of the trial. Participants will then rest for ~20 minutes before a baseline blood sample is taken (~9:00am, t = 0). Participants will continue to rest in a participant waiting room for the subsequent 8 h, the only exception being that during the first trial hour measurements of resting metabolic rate will be performed. During the trial participants will be provided with a standardised breakfast (t = 1.5 h, energy = 643 kcal for males, 578 kcal for females) and lunch (t = 4 h, energy = 750 kcal for males, 674 kcal for females). Breakfast will consist of jam sandwiches, fruit and fruit juice. The macronutrient content of this meal will be 72% carbohydrate, 10% protein and 18% fat. Lunch will consist of either a tuna and mayonnaise or cheese sandwich, with salted crisps, apple and a chocolate muffin. The macronutrient content of this meal will be 43% carbohydrate, 32% protein, 25% fat. Seven hours into the trial participants will be provided with the opportunity to eat *ad libitum* from a selection of cold buffet foods over 30 minutes and food intake will be monitored (See Appendix 2 for a list of foods available to the participant). The type and amount of food provided at this meal will be identical between main trials and participants will consume this meal within isolation. Food intake at this meal will be determined by weighing the amount of food initially provided and that remaining at the end of the meal. Manufacturer values will be used to translate this information into actual energy intake data.

At 30 minute intervals throughout trials participants will complete a set of appetite rating scales to assess subjective perceptions of hunger, fullness, satisfaction and prospective food consumption. Scales for meal palatability will also be included at 2 h, 4.5 h and 7.5 h.

Across each main trial visit 12 blood samples will be withdrawn from the cannula. A total of 208 mL will be collected across the duration of each trial. Blood will be collected into pre-

cooled monovettes and syringes containing necessary preservatives to ensure sample viability for hormone/metabolite biochemical analysis. After each blood withdrawal cannulas will be flushed with saline in order to maintain patency. Once blood samples have been collected they will immediately be spun in a refrigerated centrifuge (4$^{o}$C) and the plasma will be obtained and aliquoted into eppendorf tubes. These samples will then be frozen (initially at -20 $^{o}$C but then transferred to -80 $^{o}$C) until required for analysis.

Identical procedures will be completed in the exercise trial with the exception that during the first trial hour participants will complete 60 minutes of moderate intensity (60% of maximum oxygen uptake) walking/running. During exercise heart rate will be monitored continuously throughout and ratings of perceived exertion will be ascertained. Expired air samples will also be obtained periodically during exercise to monitor the intensity (4 x 1 minute air samples). Adjustments to the treadmill speed will be made if necessary.

***Biochemical analysis***

Biochemical analysis of plasma samples will take place at Loughborough University. Consequently, samples collected at the Leicester Diabetes Centre (UHL) will be transported to the analytical laboratories at Loughborough via a commercial courier. Samples will be analysed for hormones known to be important in the neuro-endocrine control of appetite and energy balance, as well as a selection of hepatokines. Specifically, acylated and non-acylated ghrelin (hunger stimulating) and GLP-1 (appetite inhibiting) will be measured using commercially available ELISA kits. $PYY_{3-36}$ (appetite inhibiting) will be measured using radio-immunoassay. Plasma insulin (ELISA) and glucose (colorimetry) will also be measured.

***Statistical analysis***

The statistical aspects of this study have been reviewed by a statistician within our BRU. The statistical analysis for this study will be undertaken by members of the research team with support being provided by our BRU statistician, Danielle Morris and Laura Gray.

The power calculation for this study is based on our primary outcome which relates to changes in circulating levels of appetite hormones in response to acute exercise. Data collected from the present study will be analysed using factorial analysis of variance with two important comparisons – BMI (lean vs. overweight/obese) and exercise (or control). To perform our power calculation we first obtained effect sizes from previous research of ours (King et al., 2011) which examined differences in circulating levels of gut hormones (acylated ghrelin and PYY3-36) between an exercise and a control trial (total trial AUC). This previous data revealed effect sizes of 0.6. Using this data, and assuming an alpha of 0.05 and acceptable power of 0.8, the present investigation will require us to recruit 32 males (16 lean, 16 overweight/obese) and 32 females (16 lean, 16 overweight/obese).

## Trial participants

For this study we will recruit 64 participants to the groups identified below. All participants will be inactive or moderately active (defined by IPAQ) and between the age of 18 – 50 years.

- N = 16 Males – lean (BMI 18.5 – 24.9 kg·m$^2$ and waist circumference < 102 cm)
- N = 16 Males – overweight/obese (BMI 25.0 – 39.9 kg·m$^2$ and waist circumference ≥ 102cm)
- N = 16 Female – lean (BMI 18.5 – 24.9 kg·m$^2$ and waist circumference < 88 cm)
- N = 16 Female – overweight/obese (BMI 25.0 – 39.9 kg·m$^2$ and waist circumference ≥ 88cm)

All participants will meet the following inclusion/exclusion criteria.

### *Key inclusion criteria*

- 18 - 65 years of age
- Inactive to moderately inactive (Category 1 and Category 2 on IPAQ)
- Non-diabetic (fasting whole blood capillary glucose < 7.0 mmol/L) and no history of diabetes (HbA1c <6.5%)
- Blood pressure < 160/100 mmHg
- Weight stable (weight change less than 2kg in last 3 months)
- If female:
    - Not pregnant

### *Exclusion criteria*

- Less than 18 or more than 65 years of age
- Diabetic (fasting whole blood capillary glucose ≥ 7.0 mmol/L, HbA1c ≥6.5%)
- Smokers
- Currently following any special diets e.g. low carbohydrate
- Recent history of infectious disease
- Presence of gastrointestinal, inflammatory, metabolic, cardiovascular or psychological disease
- Presence of a blood borne virus
- Abnormal psychological eating tendencies (as determined by the three-factor eating questionnaire)
- Highly active (Category 3 on IPAQ)
- Shift workers
- If female:
    - Pregnant

## Recruitment Strategy

There will be two opportunities at which potential participants may be identified and recruited into the study as follows:

1) **Advertisement within the local community**. Study posters and leaflets will be displayed in public places and sent to local companies and health care practises. Information on these will include a brief overview of the study, key inclusion/exclusion criteria and further contact information. After reading these publications, if individuals feel they are eligible to participate and would like to know more about the study they will be asked to express their interest by telephoning/emailing the research team.

When contacting the research team, potential participants will be asked to provide contact details (email/postal address and telephone number) so that they can be sent a formal study invitation letter and participant information sheet. A pre-paid envelope addressed to the research team will also be sent to prospective participants. These individuals will be asked to complete, tear off, and send back a reply slip to the research team, indicating whether they would like to take part in the study.

2) **Recruitment from NHS participant databases.** Individuals, who have previously consented to be contacted for future diabetes studies within the department, will be contacted to take part in INTAKE. Only researchers working on the relevant studies will have access to participants' information. These researchers will inform potential participants about the study (via telephone/email/mail) and if desired will be sent a study invitation letter and participant information sheet. The participant information sheet will ask participants to contact the research team (telephone/email/mail) if they are interested in taking part and to return the reply slip in a pre-paid envelope that will also be sent to the participants.

## Trial Management

### *Definition of end of trial*

The end of trial will be when the last participant has completed the study.

### *Discontinuation/withdrawal of study participants*

Each participant has the right to withdraw from the study at any time without giving a reason. The research team may also discontinue a participant if they consider necessary e.g. adverse event, consent withdrawal, incomplete data collection.

### *Source data, data handling and record keeping*

Email correspondence between study investigators and patients, patient reply slips, and notes made during telephone correspondence will be considered as source data in this study. Data collected at participant study visits will be recorded directly onto a case report form (CRF) and this information will be uploaded onto an electronic database. On all study specific documents, other than the signed consent form, the participant will be referred to by their study ID number, and not by name. Source data will be stored securely in a lockable filing

cabinets at the Leicester Diabetes Centre/Loughborough University. Only members of the research team will have access to these documents. Once the study has been completed data will be archived in accordance with local R&D procedures.

***Report writing/publication***

Results from this investigation will be published within internal reports necessary to fulfil the requirements of the funding body. Results will also be presented at international conferences and published in peer-reviewed academic journals. The chief investigator and lead researchers will determine the most appropriate place to publish findings depending on the outcomes of the study. Manuscripts will be written by the lead researchers and refined by all members of the research team. Authorship will be determined by the chief investigator.

**Definitions and reporting of serious adverse events**

We will follow the University of Leicester guidelines for managing and reporting a SAE or SUSAR, which follow those outlined in Good Clinical Practice guidance.

The following standard definitions will be used in the INTAKE Study.

**Adverse event (or adverse experience):** any untoward medical occurrence in a trial subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

**Serious adverse event (SAE) or serious adverse reaction (SDR) is:** any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

(a) results in death

(b) is life-threatening

(c) requires hospitalisation or prolongation of existing hospitalisation

(d) results in persistent or significant disability or incapacity

(e) consists of a congenital anomaly or birth defect

Additionally we will also define an event as serious if it is an important and significant medical event that may not be immediately life threatening or resulting in death or hospitalisation but, based upon appropriate medical judgement may jeopardise the patient or may require intervention to prevent one or more outcomes listed above.

Adverse events which do not fall into these categories are defined as non-serious.

**A Suspected Unexpected serious adverse reaction:** an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in the summary of product characteristics.

### Reporting of SAE and timelines

All SAEs will be reported internally to the R+D office and the sponsor (University of Leicester) using appropriate reporting forms, within 24 hours of the study team becoming aware of the event. The immediate report may be made orally or in writing and shall be followed by a detailed written report of the event. Additional information can be provided if requested to the sponsor, main Research Ethics Committee (REC), or R+D (e.g. in the event of a death). The principal investigator is responsible for the review and sign off the SAE, or in their absence, another member of the team (in order to avoid a delay).

The sponsor and/or R+D personnel will ensure that all relevant information about a SUSAR which occurs during the course of the ACUTE Study and is fatal or life-threatening is reported as soon as possible to the main REC not later than seven calendar days after they were first aware of the reaction. Any additional relevant information will be sent within eight days of the report. The sponsor and/ or R+D will ensure that a SUSAR which is not fatal or life-threatening is reported to the main REC no later than 15 calendar days after they were first aware of the reaction.

The investigator site file will contain documentation for:

- SAE, SAR and SUSAR reports
- Evidence of submission of SAEs to the sponsor within 24 hours of the team becoming aware of an event
- Evidence of timely SUSAR submission to the main REC.

## Ethics

### Declaration of Helsinki

The Investigators will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

### ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

**Approvals**

The protocol, informed consent form, study invitation letter, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee and host institution(s) for written approval. The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

**Participant Confidentiality**

The trial staff will ensure that the participants' anonymity is maintained at all times. The participants will be identified only by participant ID number on participant result sheets and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

**Finance**

The NIHR Biomedical Research Unit at University Hospitals of Leicester and Loughborough University is funded by the National Institute for Health Research and this investigation is being carried out as part of our BRU.

The total costs for this study are displayed below.

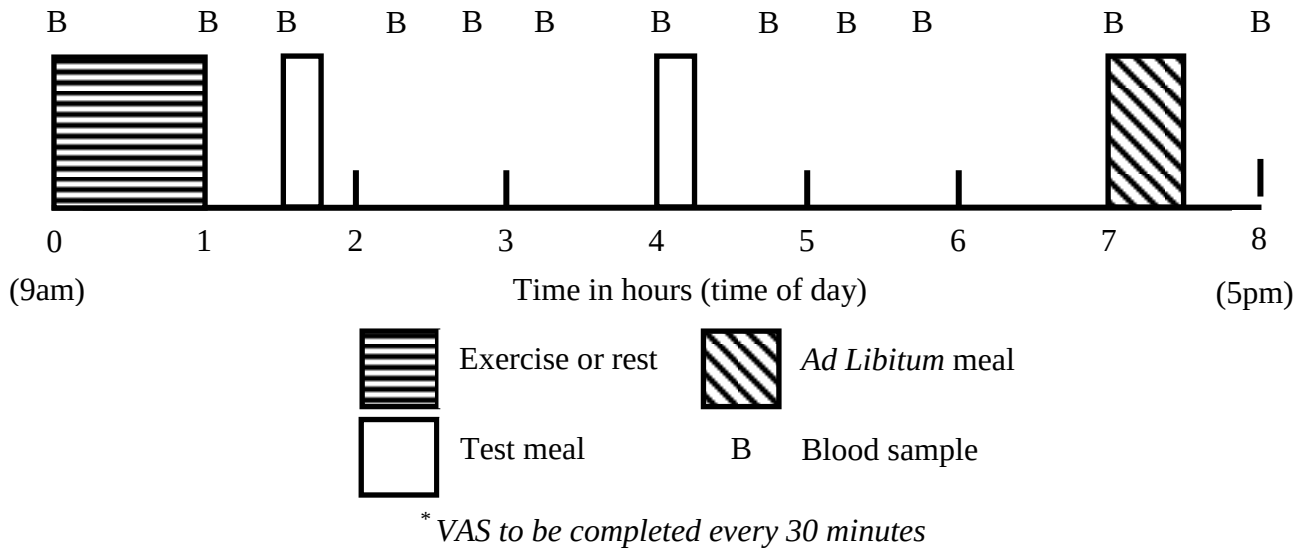
| | |
|---|---|
| **Assay costs** (acylated ghrelin, PYY3-36, GLP-1) | £24,171 |
| **Consumables** (blood collection equipment and blood tubes) | £2,007 |
| **Sample transportation** | £500 |
| **Study food costs** | £1600 |
| **Participant transport costs** | £3840 |
| **Total** | £32,118 |

**Insurance**

NHS insurance/indemnity scheme will apply to this trial to meet the potential legal liability of investigators arising from harm to participants in the conduct of the research.

**Appendices**

Appendix 1: Trial Visit Summary




* *VAS to be completed every 30 minutes*

Appendix 2: Buffet food items

White bread

Brown bread

Cheddar cheese

Ham

Tuna

Salted crisps

Mayonnaise

Margarine

Apple

Orange

Banana

Chocolate rolls (Mini-rolls)

Muffins (chocolate, chocolate chip)

Cookies

Cereal bars (Nutrigrain)

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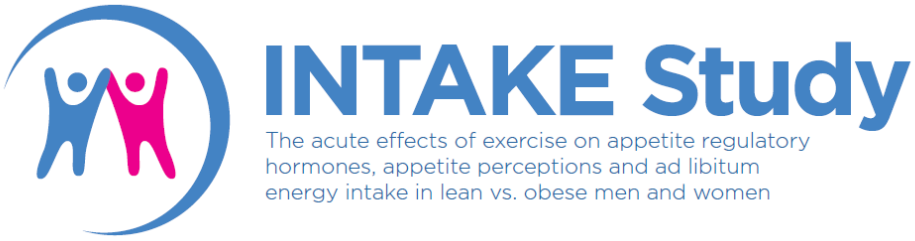
**APPENDIX N**

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**Does an acute bout of exercise alter appetite hormones, appetite perceptions and energy intake?**

**Chief Investigator:** Professor Melanie Davies
**Investigators:** Ms Jessica Douglas, Dr David Stensel, Dr James King, Professor Myra Nimmo, Mr Andrew Jackson, Dr David Webb

*This work is being carried out as part of a research project supported by the School of Sport, Exercise and Health Sciences, Loughborough University and the NIHR Leicester-Loughborough Biomedical Research Unit in Diet, Lifestyle & Physical Activity. Additionally this work will contribute to the partial fulfilment of Jessica Douglas' PhD.*

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. If anything is not clear or you would like more information, please do not hesitate to contact us. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of your consent form and this leaflet.

**What is the purpose of the study?**

Energy balance is the difference between the energy we take in, and the energy we use over the course of a day. When the energy we take in is more than the energy we use up, it results in weight gain. To lose weight we can either increase the amount of exercise we do or decrease the amount of food we eat. It has been suggested that there is an interaction between the amount of exercise we do and what we eat. One off exercise sessions in healthy weight individuals have previously been shown to alter how hungry we feel and how much we eat. As exercise is often prescribed as a method for weight management it is important to know in what way it affects what we eat, and if this will lead to weight loss.

The purpose of this study is to see how a single exercise session influences energy balance in both lean and non-lean individuals.

**What does the study involve?**

*Preliminary Visit*

Before you formally enter the study, you will be asked to attend either Loughborough University or the Leicester Diabetes Centre, in the morning, for a screening visit to ensure that you are eligible to participate. We will ask you not to eat or drink anything, other than water for 10 hours before this visit. This is in preparation for your visit and does not mean you have to take part. During this screening visit, which will take approximately 90 minutes, we will (i) ask you to sign a consent form following an explanation of the study, (ii) ask you to complete a set of questionnaires regarding your health status and history, physical activity habits and ability to exercise, food preference and breakfast patterns, and dietary habits (iii) take measurements of height, weight, waist circumference and blood pressure (iv) complete a physical examination (v) take a blood sample from your arm to measure sugar and cholesterol levels (vi) complete a resting and/or exercising electrocardiogram (vii) complete two walking/running exercise tests to determine your fitness. The results of your blood test, resting and/or exercising electrocardiogram, and information collected from the questionnaires during this screening visit will help to determine if you are suitable to take part in the study.

*Familiarisation Visit*

If you are suitable to participate, you will then be invited to return for a 'familiarisation visit' at either Loughborough University or the Leicester Diabetes Centre. Here you will be familiarised with the main trial procedures. This will include a 60 minute walk/run on a treadmill at moderate intensity. You will also be familiarised with expired air collection procedures and perceived exertion assessment.

**During this visit you will also be shown how to record and replicate your diet for the 48 hours before the main trial visits using a weighed food diary. You will also be provided with food and instructions on how to cook a meal to eat the night before each main trial.**

*Main Trial Visits*

Following the preliminary visits you will be invited back to either Loughborough University or the Leicester Diabetes Centre to complete two main experimental trials. These will include an exercise trial and control trial. **Each visit will require a time commitment of 8.5 h**.

For the exercise trial you will report to a lab at 8.30 am after consuming your standardised evening meal at 9.00 pm and having fasted overnight and **not consumed breakfast**. At 9.00 am you will perform 60 minutes of continuous walking/running at moderate intensity. You will be provided with a breakfast meal at 10:30 am and lunch at 1.00 pm. A free choice cold buffet meal will be provided at 4.00 pm. For the remainder of the trial you will rest within the laboratory (reading, watching TV/DVDs, playing computer games, working at a computer etc.). Over the course of the day we will ask you to complete some questionnaires about your

appetite. We will also collect twelve blood samples from a cannula positioned in the arm (approximately 41 teaspoons / 208mls of blood in total).
In the control trial procedures will be identical to the above trials but there will be no exercise.

*Summary*
In total the time commitment for this study will be;
*Preliminary Visit* ~1.5 hours

Exercise Familiarisation ~1.5 hours

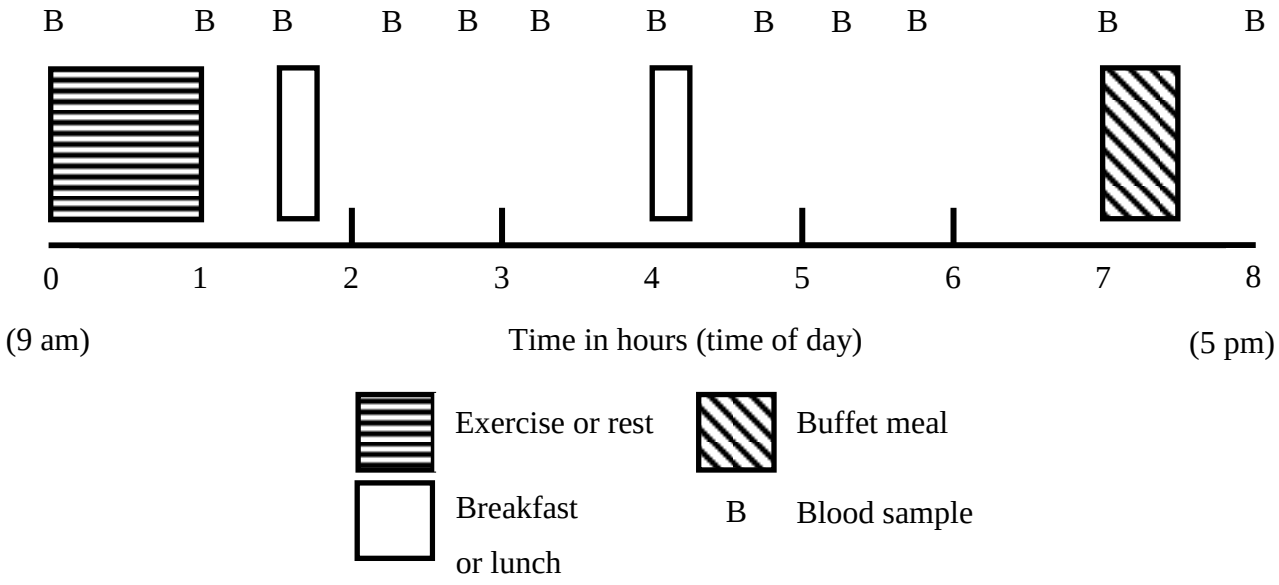
Main Experimental Trial # 1 ~8.5 hours

Main Experimental Trial # 2 ~8.5 hours

Throughout the study you should carry on your everyday life as normal. However, we will ask you not to take part in any other research studies, or give a blood donation over this time. The preliminary visits and experimental trials will be arranged at a time to suit you.

You will also be asked to fast 10 hours the night before the 'Preliminary' visit, and from 9 pm the night before each main trial. You will also be asked to refrain from strenuous physical activity, alcohol and caffeine for the 48 hours before the main experimental trials.

*Trial Visit summary*




[*] *Appetite questionnaires to be completed every 30 minutes*

**What will happen to the samples that are collected from me?**
The samples collected from you will be analysed for a number of hormones involved in the regulation of energy balance. These samples will be kept frozen in anonymised form until the analysis is completed. After this point your samples will either be discarded or will be stored frozen for potential use in future research if you consent to this additional aspect. This option will be provided on the study informed consent form.

**Why have I been asked to take part?**
You may be suitable to take part in the study if you are between the age of 18 and 65; you have a body mass index between 18.5 – 24.9 or 25.0 – 39.9 $kg/m^2$, are either not active or moderately active and can commit to attending the laboratory for all the study visits.

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

The data that we collect during the course of this study will be kept for 10 years. Our records of your personal information e.g. contact details, will be deleted 3-6 months after the study.

**What are the side effects of any treatment or procedures received when taking part?**
Research studies can involve some risks, not all of which may be currently known. The insertion of a cannula can be uncomfortable. Blood sampling also carries a small risk of causing inflammation of the vein, tenderness of the surrounding area and bruising, however the likelihood of these occurring are minimal.

The exercise bouts are performed at a moderate intensity and your heart rate will be monitored throughout. As you are healthy there is unlikely to be any side effects. All study procedures are carried out by experienced staff within our research group. At all times staff (doctors, other suitably trained healthcare practitioner, or member of research staff) fully trained in basic first aid and cardiac life support will be on hand.

If you do suffer any other symptoms or side-effects outside of the lab you should report them to Jessica Douglas immediately (contact details below), or another member of the research team.

**What are the exclusion criteria for this study?**
Unfortunately you would not be suitable to take part if you are very active, have any medical problems including diabetes or have a blood borne virus (e.g. AIDs, Hepatitis B), take certain regular medication, smoke or could be pregnant (pregnancy tests can be provided if there is a possibility of this).

**What are the possible disadvantages and risks of taking part?**
Healthy volunteers have been chosen for this study. However, if during the screening or study visits we find any abnormal results or a medical condition of which you were unaware, we will discuss the findings with you and all the relevant information will be made available to you. Contact with your GP will be made with your permission. We will be measuring your blood glucose levels during the study and it is possible that we could discover that you have diabetes. In the event that this occurs, a medical doctor who specialises in diabetes will be available to discuss this condition with you, if you so wish. Be advised, that future insurance status e.g. for life insurance or private medical insurance, could be affected by these diagnoses.

**Confidentiality:**
All data will be dealt with under the strictest of guidelines and according to the Data Protection Act (1998). All of your data will be anonymised and will only be discussed amongst the lead investigators.

**Will I get any payment and/or expenses?**
You will be reimbursed for any travel costs incurred by you throughout the study. Please keep any receipts.

**Further Information:**
If you require any further information or you would like to discuss the study further please contact:

**Jessica Douglas – J.Douglas@lboro.ac.uk 01509 226352**

*The University has a policy relating to Research Misconduct and Whistle Blowing which is available online at:*
*http://www.lboro.ac.uk/admin/committees/ethical/Whistleblowing(2).html*

**Who has reviewed this study?**
Top protect your safety, rights, well-being and dignity, all research involving patients is looked at by an independent group of people, called a Research Ethics Committee. This study has been reviewed by East Midlands – Nottingham 1 National Research Ethics committee in accordance with local regulations.

**What if I am harmed by the study?**
It is very unlikely that you would be harmed by taking part in this type of research study. However, if you wish to complain or have any concerns about the way you have been approached or treated in connection with the study, you should ask to speak to Professor Melanie Davies (01162 586481) who will do their best to answer your questions. If you remain unhappy and wish to address your concerns or complaints on a formal basis, you should contact Patient Information & Liaison Service at pils.complaints.compliments@uhl-tr.nhs.uk, The Firs, c/o Glenfield Hospital, Groby Road, Leicester, LE 3 9QP. Freephone: 0808 1788337.

**APPENDIX O**

# INTAKE Study

The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women

## Consent Form

Participant ID: _____

**Please read this form carefully before initialling each box and signing at the end.**

By initialling the box at the end of each statement:

Please initial box

- I confirm that I have read and understood the Participant Information Sheet (Version 4 - 15/09/2014) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
- I understand that following the consent procedure I may be excluded from the study if I do not meet the inclusion criteria. ☐
- I understand that my participation is voluntary and I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐
- I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the research team, from regulatory authorities, the sponsor or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Yes No

- I agree to being contacted with details of future research into diabetes and for my details to be stored on a NHS computer for this purpose. ☐ ☐

Yes No

- I consent for my samples to be transported to Loughborough University in anonymised form for biochemical analysis. ☐ ☐

Yes No

- I consent for my identifiable details i.e. name, address, email, telephone number to be stored on secure computers at Loughborough University/UHL during the course of this research. ☐ ☐

- I consent for my blood samples to be used in future research and understand that if they are used for this purpose my samples will be fully anonymised.

Yes ☐ No ☐

- I agree to my GP being informed of my results.

Yes ☐ No ☐

- I agree to take part in the above study.

Yes ☐ No ☐

**Participant Name: _____ Date: DD/MM/YYYY Signature: _____**

**Name of person**

**taking consent: _____ Date: DD/MM/YYYY Signature: _____**

## APPENDIX P


Leicester-Loughborough Diet, Physical Activity
& Lifestyle Biomedical Research Unit
Loughborough University,
Ashby Road, Loughborough, LE11 3TU
Tel : +44 (0) 1509 226445
Email: J.Douglas@lboro.ac.uk
Web: http://www.ll.dlpa.bru.nihr.ac.uk/

Dear FIRST NAME,

**Re:** Invitation to participate in the INTAKE study.

I am enclosing an information sheet giving details of the INTAKE study. Please take the time to read through this as it explains what the study involves.

If you would like to participate in the study, please complete and sign the enclosed reply slip and return it in the pre-paid envelope provided. The study team will then contact you directly to arrange a preliminary visit.

If you feel that you would like any further information before completing the documentation, please contact the team on the email address or phone number provided above.

Yours Sincerely

Jessica Douglas

## APPENDIX Q


Leicester-Loughborough Diet, Physical Activity
& Lifestyle Biomedical Research Unit
Loughborough University,
Ashby Road, Loughborough, LE11 3TU
Tel : +44 (0) 1509 ……
Fax : +44 (0) 1509 ……
Email: …@lboro.ac.uk
Web: http://www.ll.dlpa.bru.nihr.ac.uk/

[Insert name]
[Insert 1st line address]
[Insert 2nd line address]
[Insert town]
[Insert postcode]
[Insert date]

Dear [Insert Name],

**Re:** The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women (INTAKE).

Thank you for agreeing to take part in our study.

Please find below details for your preliminary appointment. If for any reason you cannot keep the appointment (or if you would like to change the date or time) please contact us on the number above.

**Appointment Date:** [Insert day, date]
**Time:** [Insert time]
**Location:** [Clyde Williams Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU / Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, LE5 4PW] (delete as appropriate/insert relevant map)

Please find enclosed a map.

Your appointment will take approximately 90 minutes.

You will need to fast from the night before, so we would ask that no food be eaten after 11.00 pm the night before the preliminary appointment and that only water is consumed before your visit. This is in preparation for your first visit and does not mean that you have to take part.

Drinks and biscuits will be provided for you after the appointment. Could we please ask you to bring along a list of any current medication that you are taking.

Yours faithfully

Study Co-Ordinator

Leicester-Loughborough Diet, Physical Activity
& Lifestyle Biomedical Research Unit
Loughborough University,
Ashby Road, Loughborough, LE11 3TU
Tel : +44 (0) 1509 ……
Fax : +44 (0) 1509 ……
Email: …@lboro.ac.uk
Web: http://www.ll.dlpa.bru.nihr.ac.uk/

INTAKE Study
The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women

[Insert name]
[Insert 1st line address]
[Insert 2nd line address]
[Insert town]
[Insert postcode]
[Insert date]

Dear [Insert Name],

**Re:** The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women (INTAKE).

Thank you for taking part in our study.

Please find below details for your familiarisation appointment. If for any reason you cannot keep the appointment (or if you would like to change the date or time) please contact us on the number above.

**Appointment Date:** [Insert day, date]
**Time:** [Insert time]
**Location:** [Clyde Williams Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU / Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, LE5 4PW] (delete as appropriate/insert relevant map)

Please find enclosed a map.

Your appointment will take approximately 90 minutes.

Drinks and biscuits will be provided for you after the appointment. Please bring with you a set of clothes comfortable to exercise in. Showers will be available for you to use following the trial, so please bring a towel if you would like to shower before leaving.

Yours faithfully

Study Co-Ordinator

Leicester-Loughborough Diet, Physical Activity
& Lifestyle Biomedical Research Unit
Loughborough University,
Ashby Road, Loughborough, LE11 3TU
Tel : +44 (0) 1509 ……
Fax : +44 (0) 1509 ……
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INTAKE Study
The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women

[Insert name]
[Insert 1st line address]
[Insert 2nd line address]
[Insert town]
[Insert postcode]
[Insert date]

Dear [Insert Name],

**Re:** The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women (INTAKE).

Thank you for taking part in our study.

Please find below details for your resting trial appointment. If for any reason you cannot keep the appointment (or if you would like to change the date or time) please contact us on the number above.

**Appointment Date:** [Insert day, date]
**Time:** [Insert time]
**Location:** [Clyde Williams Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU / Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, LE5 4PW] (delete as appropriate/insert relevant map)

Please find enclosed a map.

Your appointment will take approximately 8.5 hours.

You will need to fast the night before this visit, so we would ask that you eat the provided meal before 9.00 pm and no other food be consumed after this time. Only water should be drunk before your visit.

Could we please ask you to record your diet and physical activity levels for 48 hours before this visit. This is to ensure that you can replicate your diet for 48 hours before both main trials. Your evening meal will have been provided to you at the familiarisation appointment. Please refrain from alcohol, caffeine and strenuous physical activity in the 48 hours before this visit. These points are very important in order to prevent variables influencing the study findings and these will be discussed in depth at the familiarisation trial.

If you live within one mile of the laboratory you should walk in slowly on the morning of the appointment. Please do not run or cycle. If you live more than one mile from the laboratory then you should drive in. If you do not have access to a car please tell us and we will arrange for you to be collected.

We ask that you bring with you, your completed food diary [and weighing scales] (delete as appropriate).

Yours faithfully

Study Co-Ordinator


Leicester-Loughborough Diet, Physical Activity
& Lifestyle Biomedical Research Unit
Loughborough University,
Ashby Road, Loughborough, LE11 3TU
Tel : +44 (0) 1509 ……
Fax : +44 (0) 1509 ……
Email: …@lboro.ac.uk
Web: http://www.ll.dlpa.bru.nihr.ac.uk/

[Insert name]
[Insert 1st line address]
[Insert 2nd line address]
[Insert town]
[Insert postcode]
[Insert date]

Dear [Insert Name],

**Re:** The acute effects of exercise on appetite regulatory hormones, appetite perceptions and *ad libitum* energy intake in lean vs. obese men and women.

Thank you for taking part in our study.

Please find below details for your exercise trial appointment. If for any reason you cannot keep the appointment (or if you would like to change the date or time) please contact us on the number above.

**Appointment Date:** [Insert day, date]
**Time:** [Insert time]
**Location:** [Clyde Williams Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU / Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, LE5 4PW] (delete as appropriate)

Your appointment will take approximately 8.5 hours.

You will need to fast the night before this visit, so we would ask that you eat the provided meal before 9.00 pm and no other food be consumed after this time. Only water should be drunk before your visit.

Could we please ask you to record your diet and physical activity levels for 48 hours before this visit. This is to ensure that you can replicate your diet for 48 hours before both main trials. Your evening meal will have been provided to you at the familiarisation appointment. Please refrain from alcohol, caffeine and strenuous physical activity in the 48 hours before this visit. These points are very important in order to prevent variables influencing the study findings and these will be discussed in depth at the familiarisation appointment.

If you live within one mile of the laboratory you should walk in slowly on the morning of the appointment. Please do not run or cycle. If you live more than one mile from the laboratory then you should drive in. If you do not have access to a car please tell us and we will arrange for you to be collected.

We ask that you bring with you, your completed food diary [and weighing scales] (delete as appropriate), a set of clothes comfortable to exercise in and a towel as showers are available for you to use following the exercise.

Yours faithfully

Study Co-Ordinator

**APPENDIX R**

Leicester-Loughborough
Diet, Lifestyle and Physical Activity
Biomedical Research Unit

NHS
National Institute for
Health Research

# Would you like to take part in a study to help establish effective weight loss and maintenance strategies?

## INTAKE Study

The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women

**To participate in our study you must be:**

- Aged 18 – 50 years
- Moderately active or inactive
- Have a BMI between 18.5-24.9 or 30-39.9
- Have a blood pressure less than 160/100

IF YOU ARE UNSURE OF THESE PLEASE GET IN TOUCH AND WE CAN CHECK

- Weight stable and not following a special diet
- Non-smoker
- Not currently taking anti-inflammatory medications
- Have no history of gastrointestinal/inflammatory/psychological/cardiovascular/metabolic disease such as diabetes
- If female:
  - Premenopausal
  - Not pregnant

If you meet the criteria and would like to know more about this study please don't hesitate to get in touch with us

**Study Administrator (A.Stanley@lboro.ac.uk)**

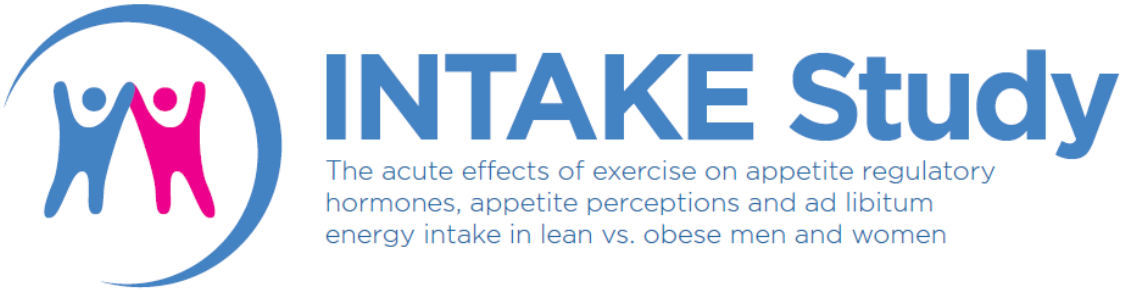
**Ms Jessica Douglas (J.Douglas@lboro.ac.uk) Tel: 01509 226351**

Leicester Diabetes Centre
Committed to Growing International Research, Education & Innovation

Loughborough University

INTAKE Advertisement – Version 2 – 14/04/14

**APPENDIX S**




## Health Screen Questionnaire for Study Volunteers

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to ensure study data collected is valid.

If you have a blood borne virus, or think that you may have one, please do not take part in this research.

**Please complete this brief questionnaire to confirm your fitness to participate:**

**1. At present,** do you have any health problem for which you are:

| | | | | | |
|---|---|---|---|---|---|
| (a) | on medication, prescribed or otherwise | Yes | | No | |
| (b) | attending your general practitioner | Yes | | No | |
| (c) | on a hospital waiting list | Yes | | No | |

**2. In the past two years,** have you had any illness which required you to:

| | | | | | |
|---|---|---|---|---|---|
| (a) | consult your GP..... | Yes | | No | |
| (b) | attend a hospital outpatient department ..... | Yes | | No | |
| (c) | be admitted to hospital ..... | Yes | | No | |

**3. Have you ever** had any of the following:

| | | | | | |
|---|---|---|---|---|---|
| (a) | Convulsions/epilepsy | Yes | | No | |
| (b) | Asthma | Yes | | No | |
| (c) | Eczema | Yes | | No | |
| (d) | Diabetes | Yes | | No | |
| (e) | A blood disorder | Yes | | No | |
| (f) | Head injury | Yes | | No | |
| (g) | Digestive problems | Yes | | No | |
| (h) | Heart problems | Yes | | No | |
| (i) | Problems with bones or joints | Yes | | No | |
| (j) | Disturbance of balance/coordination | Yes | | No | |
| (k) | Numbness in hands or feet | Yes | | No | |
| (l) | Disturbance of vision | Yes | | No | |
| (m) | Ear / hearing problems | Yes | | No | |
| (n) | Thyroid problems | Yes | | No | |
| (o) | Kidney or liver problems | Yes | | No | |
| (p) | Allergy to nuts | Yes | | No | |

**4**. Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ..... Yes ☐ No ☐

**If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.)**

.....

.....

.....

**5. Allergy Information**

| | | | | | |
|---|---|---|---|---|---|
| (a) | are you allergic to any food products? | Yes | | No | |
| (b) | are you allergic to any medicines? | Yes | | No | |
| (c) | are you allergic to plasters? | Yes | | No | |

**If YES to any of the above, please provide additional information on the allergy**

……

……

**6. Additional questions for female participants**

| | | | | | | |
|---|---|---|---|---|---|---|
| (a) | are your periods normal/regular? ..... | Yes | ☐ | No | ☐ |
| (b) | are you on "the pill"? ..... | Yes | ☐ | No | ☐ |
| (c) | could you be pregnant? ..... | Yes | ☐ | No | ☐ |
| (d) | are you taking hormone replacement therapy (HRT)? | Yes | ☐ | No | ☐ |

**7. Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.**

Name:……..…

Telephone Number:……

Work ☐ Home ☐ Mobile ☐

Relationship to Participant:…….

**8. Are you currently involved in any other research studies at the University or elsewhere?**

Yes ☐ No ☐

If yes, please provide details of the study

……

……

……

……


## Physical Activity Readiness Questionnaire (PAR-Q)

For most people, physical activity should not pose any problems or hazard. PAR-Q has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them.

Common sense is your best guide in answering these few questions. Please read carefully and check **YES** or **NO** opposite the question if it applies to you. If yes, please explain.

| QUESTION | YES | NO |
|---|---|---|
| 1. Has your doctor ever said you have heart trouble? If yes, please state: | | |
| 2. Do you frequently have pains in your heart and chest? If yes, please state: | | |
| 3. Do you often feel fain or have spells of severe dizziness? If yes, please state: | | |
| 4. Has a doctor ever said your blood pressure was too high? If yes, please state: | | |
| 5. Has your doctor ever told you that you have a bone or joint problem(s), such as arthritis that has been aggravated by exercise, or might be made worse with exercise? If yes, please state: | | |
| 6. Is there a good physical reason, not mentioned here, why you should not follow an activity program even if you wanted to? If yes, please state: | | |

| | | |
|---|---|---|
| 7. Are you or have you been pregnant in the last 6 months? | | |
| 8. Do you suffer from any problems of the lower back, i.e., chronic pain, or numbness?<br>If yes, please state: | | |
| 9. Are you currently taking any medications? If Yes, please specify. | | |
| 10. Do you currently have a disability or a communicable disease? If yes, please state: | | |

If you answered NO to all questions above, it gives a general indication that you may participate in physical and aerobic fitness activities. The fact that you answered NO to the above questions, is no guarantee that you will have a normal response to exercise. If you answered Yes to any of the above questions, then you may need written permission from a physician before participating in physical and aerobic fitness activities.

_____ _____ _____

Print Name Signature Date


## Food Preferences and Breakfast Pattern Questionnaire

### Food Preferences

Please circle the number which best describes your liking of the following foods:

Wholemeal Bread

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Strawberry Jam

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange Juice

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tuna

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mayonnaise

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cheddar Cheese

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ready Salted Crisps

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Apple

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Muffin

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ham

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mini-Rolls

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Double Chocolate Muffins

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Chip Muffins

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Chip Cookies

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Nutrigrain Cereal Bars

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Pasta

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tomato Pasta Sauce

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

KitKat

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

**Breakfast Patterns**

Please answer the following questions regarding your breakfast eating habits:

| | Yes | No |
|---|---|---|
| Do you normally consume breakfast? | ☐ | ☐ |

What do you normally consume for breakfast?

_____

_____

_____

_____

_____


## Three Factor Eating Questionnaire

**Part 1 - please answer true/false:**

1. **When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal**

   True □ False □

2. **I usually eat too much at social occasions, like parties and picnics**

   True □ False □

3. **I am usually so hungry that I eat more than three times a day**

   True □ False □

4. **When I have eaten my quota of calories, I am usually good about not eating any more**

   True □ False □

5. **Dieting is too hard for me because I just get too hungry**

   True □ False □

6. **I deliberately take small helpings as a means of controlling my weight**

   True □ False □

7. **Sometimes things just taste so good that I keep on eating even when I am no longer hungry**

   True □ False □

8. **Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat**

True □ False □

9. **When I am anxious, I find myself eating**

True □ False □

10. **Life is too short to worry about dieting**

True □ False □

11. **Since my weight goes up and down, I have been on weight reducing diets more than once**

True □ False □

12. **I often feel so hungry that I just have to eat something**

True □ False □

13. **When I am with someone who is overeating, I usually overeat too**

True □ False □

14. **I have a pretty good idea of the number of calories in common food**

True □ False □

15. **Sometimes when I start eating, I just can't seem to stop**

True □ False □

16. **It is not difficult for me to leave something on my plate**

True □ False □

17. **At certain times of the day, I get hungry because I have gotten used to eating then**

True □ False □

18. **While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it**

True □ False □

**19. Being with someone who is eating often makes me hungry enough to eat also**

True □ False □

**20. When I feel blue, I often overeat**

True □ False □

**21. I enjoy eating too much to spoil it by counting calories or watching my weight**

True □ False □

**22. When I see a real delicacy I often get so hungry that I have to eat it right away**

True □ False □

**23. I often stop eating when I am not really full as a conscious means of limiting what I eat**

True □ False □

**24. I get so hungry that my stomach often feels like a bottomless pit**

True □ False □

**25. My weight has hardly changed at all in the last ten years**

True □ False □

**26. I am always hungry so it is hard for me to stop eating before I finish all the food on my plate**

True □ False □

**27. When I feel lonely, I console myself by eating**

True □ False □

**28. I consciously hold back at meals in order not to gain weight**

True □ False □

**29. I sometimes get very hungry late in the evening or at night**

True □ False □

**30. I eat anything I want, anytime I want**

True □ False □

**31. Without even thinking about it, I take a long time to eat**

True □ False □

**32. I count calories as a conscious means of controlling my weight**

True □ False □

**33. I do not eat some foods because they make me fat**

True □ False □

**34. I am always hungry enough to eat at any time**

True □ False □

**35. I pay a great deal of attention to changes in my figure**

True □ False □

**36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods**

True □ False □

**Part 2 - Please circle the appropriate answer:**

**37. How often are you dieting in a conscious effort to control your weight?**

1 (rarely) 2(sometimes) 3(usually) 4(always)

**38. Would a weight fluctuation of 5 lbs affect the way you live your life?**

1(not at all) 2(slightly) 3(moderately) 4(very much)

**39. How often do you feel hungry?**

1(only at meal times) 2(sometimes between meals) 3(often between meals) 4(almost always)

**40. Do your feelings of guilt about overeating help you to control your food intake?**

1(never) 2(rarely) 3(often) 4(always)

**41. How difficult would it be for you to stop eating half way through dinner and not eat again for four hours?**

1(easy) 2(slightly difficult) 3(moderately difficult) 4(very difficult)

**42. How conscious are you of what you are eating?**

1(not at all) 2(slightly) 3(moderately) 4(extremely)

**43. How frequently do you avoid 'stocking up' on tempting foods?**

1 (almost never) 2(seldom) 3(usually) 4(almost always)

**44. How likely are you to shop for low calorie foods?**

1(unlikely) 2(slightly unlikely) 3(moderately likely) 4(very likely)

**45. Do you eat sensibly in front of others and splurge alone?**

1(never) 2(rarely) 3(often) 4(always)

**46. How likely are you to consciously eat slowly in order to cut down on how much you eat?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**47. How frequently do you skip desert because you are no longer hungry?**

1(almost never) 2(seldom) 3(at least once a week) 4(almost every day)

**48. How likely are you to consciously eat less than you want?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**49. Do you go on eating binges though you are not hungry?**

1(never) 2(rarely) 3(sometimes) 4(at least once a week)

**50. On a scale of 0-5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?**

0

Eat whatever you want, whenever you want it

1

Usually eat whatever you want, whenever you want it

2

Often eat whatever you want, whenever you want it

3

Often limit food intake, but often 'give in'

4

Usually limit food intake, rarely 'give in'

5

Constantly limiting food intake, never 'give in'

**51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of a number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'**

1(not like me) 2(little like me) 3(pretty good description of me)

4(describes me perfectly)

**APPENDIX T**

Leicester-Loughborough
Diet, Lifestyle and Physical Activity
Biomedical Research Unit

NHS
National Institute for
Health Research

# Looking for a healthier future?

## Why not take part in our study?

We are looking into the relationship between the amount of exercise we do and how hungry it makes us feel.

If you are aged 18-65 contact us for more information on how you can take part!


**Jessica Douglas**
PhD Student
Email: J.Douglas@lboro.ac.uk
Tel: 01509 .....

.....
Research Administrator
Email: .....
Tel: .....


Leicester Diabetes Centre
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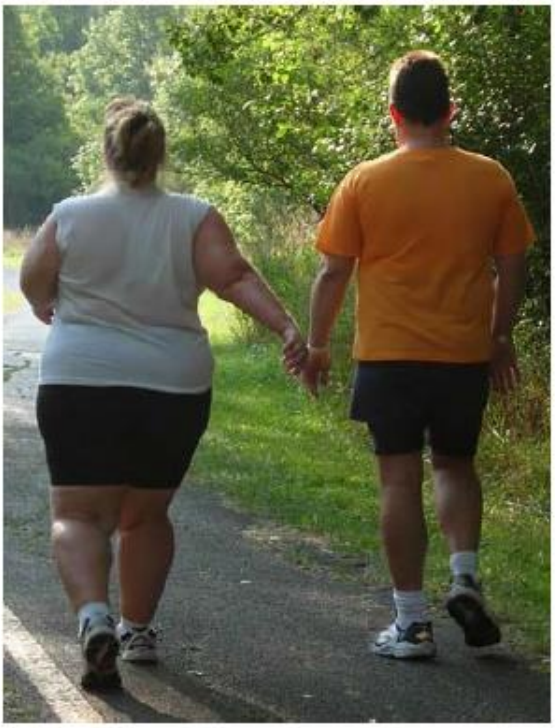
# Looking for a healthier future?



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## APPENDIX U

White bread

Brown bread

Cheddar cheese

Ham

Tuna

Salted crisps

Mayonnaise

Margarine

Apple

Banana

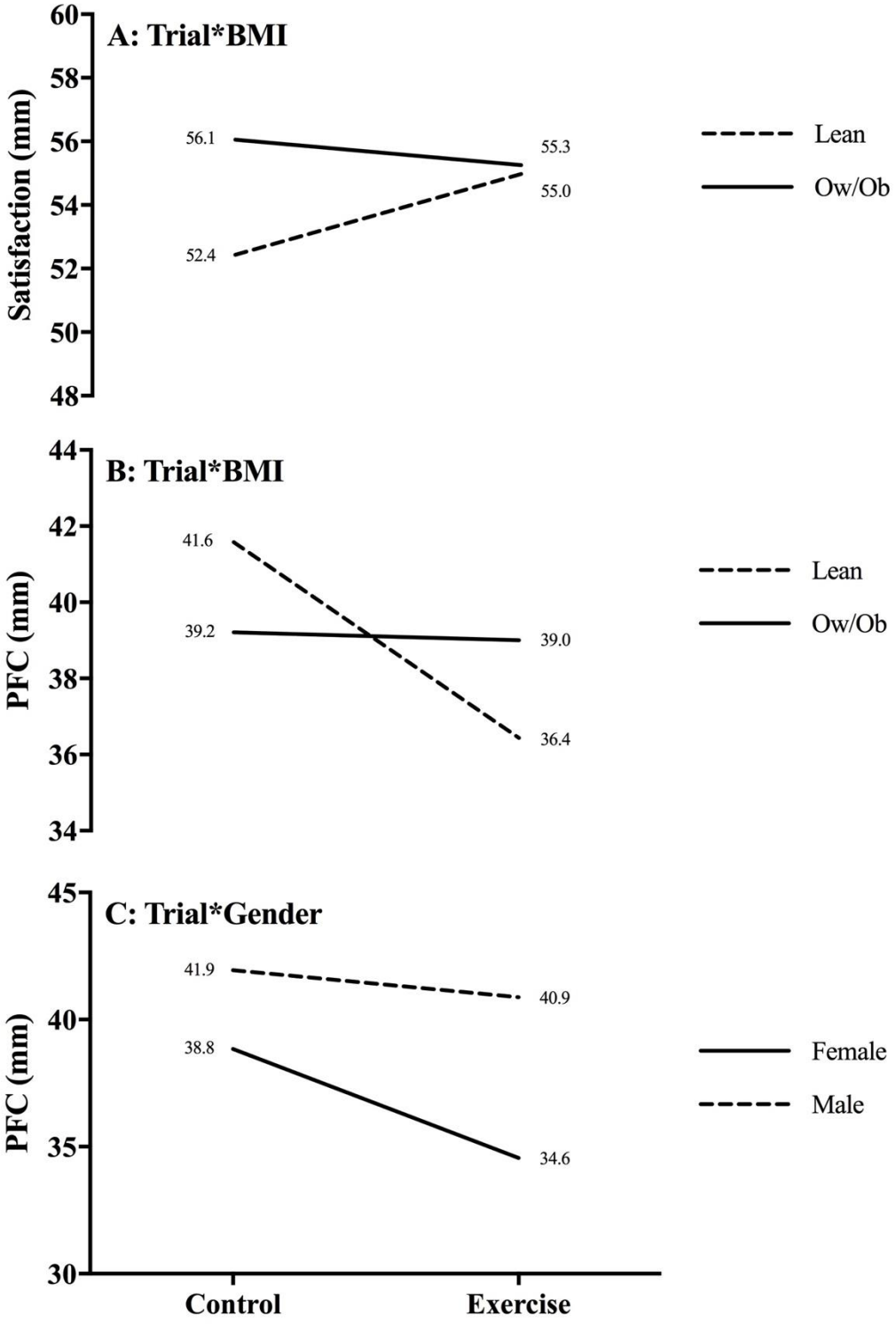
Chocolate rolls (Mini-rolls)

Muffins (chocolate, chocolate chip)

Cookies

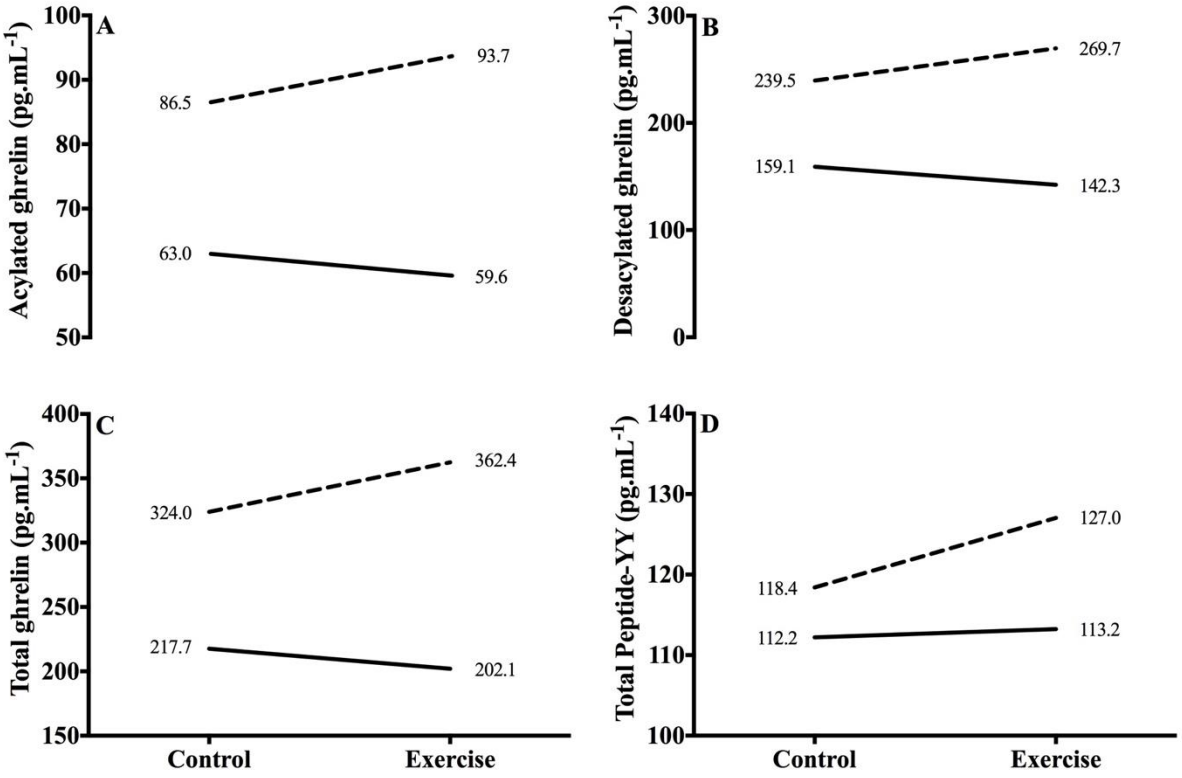
Cereal bars (Nutrigrain)

## APPENDIX V



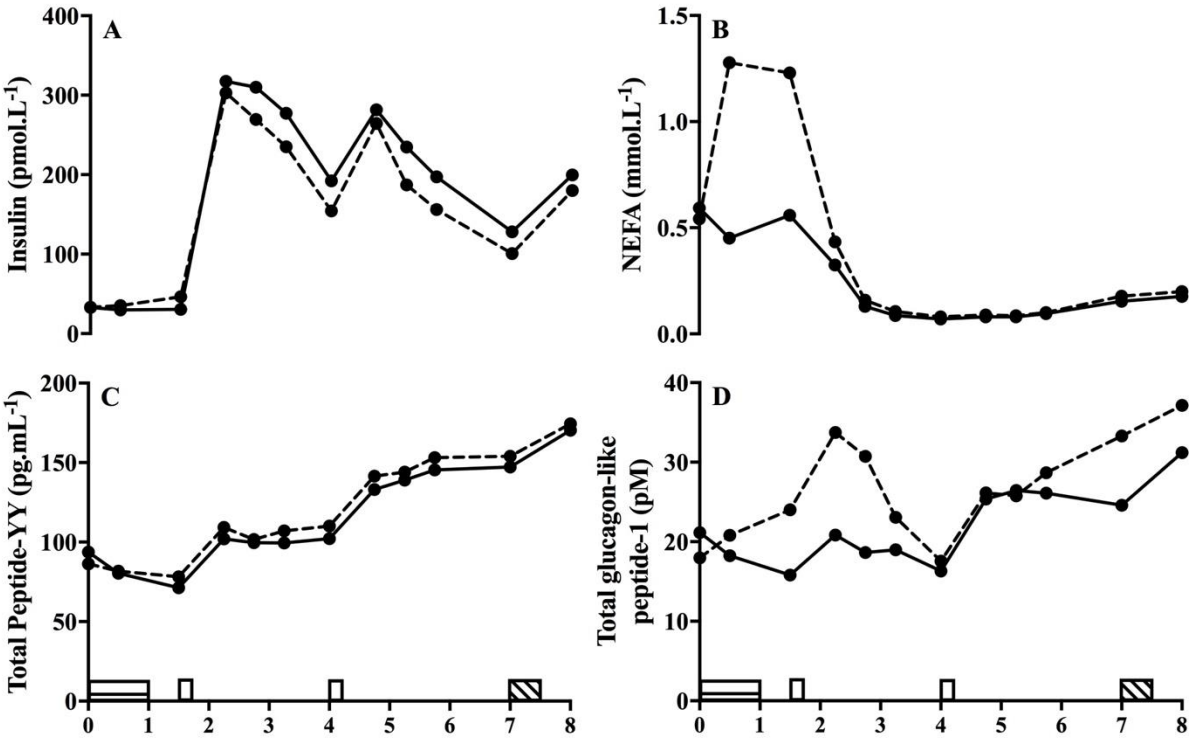

**Appendix V.** Graphical representation of: (A) significant trial*BMI interaction for satisfaction, (B) significant trial*BMI interaction for prospective food consumption, and (C) significant trial*gender interaction for prospective food consumption. PFC; prospective food consumption. Values are means, N=47.

## APPENDIX W




**Appendix W.** Graphical representation of trial*gender interactions for: (A) acylated ghrelin, N=43, (B) desacylated ghrelin, N=43, (C) total ghrelin, N=43, and (D) total peptide-YY, N=42. Solid lines (—) represent males, and dashed lines represent females (- - -).Values are means.

## APPENDIX X




**Appendix X.** Graphical representation of main effect of trial for: (A) insulin, N=43, (B) NEFA, N=43, (C) total peptide-YY, N=42, and (D) total glucagon-like peptide-1, N=40. Solid lines (—) represent control trials, and dashed lines represent exercise trials (- - -).Values are means. Horizontally shaded rectangles indicate exericse, white shaded rectangles indicate standardised meals, and diagonally shaded rectangles indicate *ad libitum* meal.