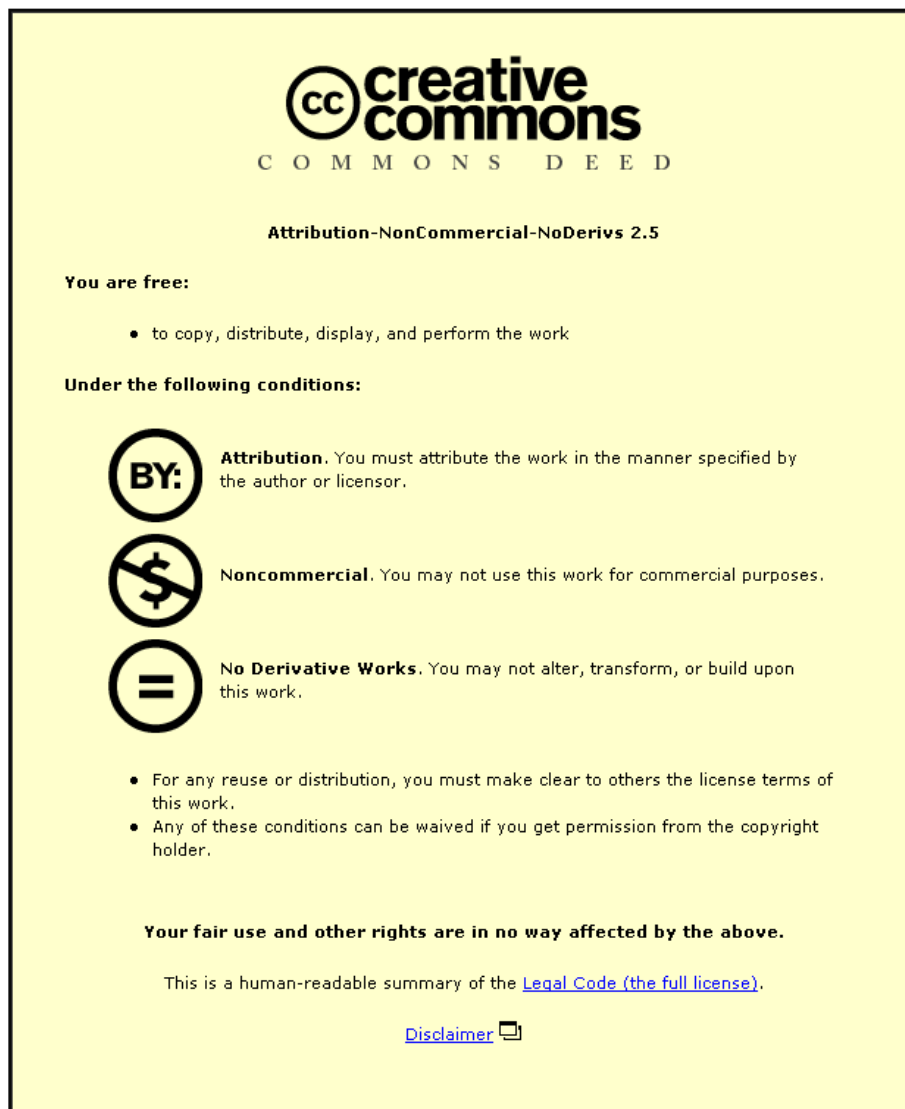




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
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**The acute effects of exercise on appetite  
perceptions, gut hormones and food intake in  
females**

**Nawal Alajmi**

A Doctoral Thesis

Submitted in partial fulfilment for the award of Doctor of Philosophy of  
Loughborough University

## Abstract

In recent years there has been growing interest in the role of gut hormones in regulating appetite, energy balance and weight control. Prominent among these hormones is the ‘hunger’ hormone ghrelin which is the only circulating hormone currently known to stimulate appetite. A variety of hormones are known to suppress appetite and notable among these is peptide YY (PYY). Both ghrelin and PYY exist in more than one form with acylated ghrelin and PYY<sub>3-36</sub> representing the biologically active forms of these hormones i.e. the form of each hormone with the most potent effects on appetite. Many studies have investigated ghrelin responses to exercise in male participants and some studies have also examined PYY responses. Far fewer studies have examined ghrelin and PYY responses in female participants and this was the primary purpose of the studies reported here.

This thesis comprises four main experimental chapters which collectively sought to clarify whether there is any evidence to support the hypothesis that appetite, gut hormone and food intake responses differ in female compared with male participants. A total of 123 participants took part in the studies reported in this thesis. The first of these studies was cross-sectional in nature and compared fasting appetite, plasma acylated ghrelin and dietary restraint questionnaire values (among other variables) in 34 males and 33 females. No significant differences were observed between sexes for any of these variables.

In the second study, appetite, plasma acylated ghrelin and *ad libitum* food intake responses to cycling exercise were examined in 13 female participants taking the oral contraceptive pill in both the luteal and follicular phases of the menstrual cycle. Although fasting hunger and prospective food consumption values were higher in the follicular than the luteal phase there was no difference in appetite, plasma acylated ghrelin and food intake responses to exercise between menstrual cycle phases.

In the third study, appetite, plasma acylated ghrelin, plasma PYY<sub>3-36</sub> and food intake responses to energy deficits created via diet and exercise were compared in 13 young, healthy female participants who completed three separate trials (control, exercise deficit and food deficit) in a random order. The findings revealed that, as with male participants, females experience compensatory appetite, gut hormone and food intake responses to

dietary induced energy deficits but not to exercise induced energy deficits (over the course of a nine hour observation period). The final study reported in this thesis compared appetite, plasma acylated ghrelin and *ad libitum* food intake responses to a one hour run in 10 male and 10 female participants. Suppressions of both hunger and plasma acylated ghrelin were noted during exercise but there was no significant difference in the responses of males and females during or after exercise.

Collectively, the studies reported here suggest: 1) that fasting appetite and plasma acylated ghrelin concentrations do not differ between male and female participants; 2) that appetite, ghrelin and food intake responses to cycling exercise do not differ according to the phase of the menstrual cycle in females; 3) that dietary restriction is more likely to elicit compensatory feeding responses than elevated exercise levels in females and 4) that males and females do not differ in their acute appetite, ghrelin and food intake responses to an acute bout of running exercise. Hence the studies reported here do not support the hypothesis that exercise will be less effective for controlling appetite and food intake in females than in males.

**Key words: exercise, appetite, food intake, ghrelin, peptide YY, gut hormones**

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## **Preface**

### **Conference communications and presentations**

Abstracts from the following studies have been peer reviewed and have been accepted for conference presentations as follows:

Alajmi NS, De Oliveira AR and Stensel DJ (2011) Acute effects of exercise on appetite, energy intake and plasma acylated ghrelin concentrations in males and females. *Journal of Sports Sciences*. 29 (Supplement 2): S108.

## List of Abbreviations

The abbreviations mentioned below are used throughout this thesis. They will be mentioned in full in the text and then abbreviated throughout the thesis.

- a-MSH (alpha-melanocyte stimulating hormone)
- AG (acylated ghrelin)
- AgRP (agouti related protein)
- ANOVA (analysis of variance)
- ARC (anorectic arcuate nucleus)
- AUC (area under the curve)
- BMI (body mass index)
- CART (cocaine and amphetamine regulated transcript)
- CCK (cholecystokinin)
- CNS (central nervous system)
- DBP (diastolic blood pressure)
- DPP-IV (dipeptidyl peptidase IV)
- EDTA (ethylenediamine tetra-acetic acid)
- EE (energy expenditure)
- EI (energy intake)
- FSH (follicular stimulating hormone)
- GABA (gamma-amino butyric acid)
- GH (growth hormone)
- GHS-R (growth hormone secretagogue receptor)
- GI (gastro-intestinal)
- GLP-1 (glucagon – like peptide-1)
- kcal (kilocalorie)
- kJ (kilojoules)
- LH (luteinizing hormone)
- NPY (neuropeptide Y)
- OCP (oral contraceptive pills)
- OXM (oxyntomodulin)



PFC (prospective food consumption)  
POMC (proopiomelanocortin)  
PP (pancreatic polypeptide)  
PYY (peptide tyrosine tyrosine)  
RMR (resting metabolic rate)  
RPE (rating of perceived exertion)  
RQ (respiratory quotient)  
SBP (systolic blood pressure)  
SD (standard deviation)  
SEM (standard error of the mean)  
SPA (spontaneous physical activity)  
SPSS (Statistical Package for Social Sciences)  
TAG (triacylglycerol)  
TEF (thermic effect of foods)  
TFEQ (three factor eating questionnaire)  
VAS (visual analogue scales)  
VO<sub>2</sub>max (maximum oxygen uptake)

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## 1 Introduction

Obesity has become a worldwide epidemic and its prevalence has increased by nearly double within developed and, increasingly, developing countries since 1980 according to the World Health Organisation (WHO, 2013). This has important consequences for health and for society. Obesity is a risk factor for several serious chronic diseases including diabetes mellitus, coronary heart disease and cancer (WHO 1998, Adams *et al.*, 2006; Tiriyaki-Sonmez *et al.*, 2013). The prevalence of co-morbidities associated with obesity (e.g. diabetes) continues to increase and experts have warned that the number of fatalities due to obesity may soon exceed those that are caused by smoking (Hennekens & Andreotti, 2013).

The WHO estimates that the prevalence of obesity has doubled since 1980, thus giving a figure of more than 1.4 billion adults aged 20 and above in 2008 being overweight. Amongst this figure it is estimated that 300 million women and 200 million men globally are obese. In terms of percentages it is calculated that worldwide 35% of adults aged 20 and over were overweight in 2008, and 11% were obese. Each year it is estimated that around 2.8 million adults die due to complications of obesity and being over weight. Moreover, 44% of the burden of ill health associated with obesity is made up from diabetes, 23% from ischaemic heart disease, and between 7% up to 41% from cancer (WHO, 2008 – 2013). The U.K. figures prominently in the list of nations where obesity is a major issue and it has been predicted that 60% of the U.K population will be obese by 2050 (Government Office for Science, 2007).

The growing prevalence of obesity worldwide has led to an urgent need to determine the factors that cause obesity in both developed and developing countries. In particular it is believed that developing countries lack the infrastructure to treat the chronic diseases associated with obesity adequately. It is common to blame the rise of marketed fast food and couch potato lifestyles for obesity, however, obesity is a complex issue and a great many factors are involved. One such issue is the role of genetics and whether the parental genetic material has any bearing on the BMI of the child. This is a theory supported by the work of Stunkard *et al.* (1986) in which a study of adopted children and their adoptive and biological parents found a strong relationship between the weight class of biological

parents and that of their children (who had been adopted many years earlier) but no relationship between the weight status of adoptive parents and that of the children they had adopted. A factor causing obesity is reasoned to be an energy imbalance between calories consumed and calories expended; thus a situation where energy intake exceeds energy expenditure over a prolonged period of time (Bray and Champagne, 2005). This may arise due to a high energy intake, a low energy expenditure or a combination of the two. As obesity prevalence continues to rise, effective strategies are needed to help prevent obesity and to facilitate weight loss (Kelly *et al.*, 2008).

Over the years a variety of diets and pharmacological weight loss agents (e.g. Adios/ Adios Max- herbal medicinal products) have been made available but the long term efficacy and safety of these diets and drugs and, hence their impact on overall health, has been questioned and many uncertainties remain. One such example is the Atkins diet. Although this diet has been shown to result in weight loss in the short term there are questions about the long-term safety and effectiveness of this diet (Astrup *et al.*, 2004). Physical activity is another strategy which is often recommended in the prevention and management of obesity and it has been very well studied in both animals and humans yet many questions remain over the long term effectiveness of exercise as a means of weight control (Donnelly *et al.*, 2003a, and Oscai *et al.*, 1971).

One major issue that is currently being debated is the extent to which men and women differ in their appetite, hormonal and food intake responses to exercise. It has yet to be determined conclusively that exercise and appetite differ between men and women. Some previous studies have suggested that women increase their food intake when carrying out bouts of exercise and this leads to maintenance of body weight rather than weight loss (Donnelly *et al.*, 2003a). Most studies in men, on the other hand, suggest that they do not increase their energy intake against the new higher energy expenditure at least in the short term (Hagobian *et al.*, 2009, Hickey *et al.*, 1997; Pomerleau *et al.*, 2004). However, this issue has not been extensively studied and there is insufficient evidence and a lack of scientific congruity to confirm the theory that males and females respond differently to exercise, therefore further investigation is required.

In the early nineties, the relationship between exercise and food intake was a subject area that was still unraveling. During this decade it became clear that there were a variety of hormonal factors that were influencing appetite and food intake. More was learnt about a variety of energy regulating hormones including ghrelin, peptide YY (PYY), glucagon – like peptide-1 (GLP-1), leptin and insulin. Aside from ghrelin all of these hormones exert appetite-suppressing effects.

Ghrelin is a unique gut hormone that stimulates an individual's appetite. Ghrelin is a 28-amino acid peptide that is modified by an acyl chain added to the serine at position 3. The hormone is expressed in peripheral tissues within the stomach, and acts as an autocrine/paracrine growth factor that also affects food intake (Kojima and Kangawa, 2005). Ghrelin, is believed to be the principal hormone dictating hunger. It is at its highest levels during fasting and at its lowest levels after meals (Mcgowan and Bloom, 2007; Hotta *et al.*, 2009).

Studies investigating the relationships between exercise, appetite, appetite regulating hormones and food intake have largely focused on male participants. This is because the relationship between exercise and appetite in women is more difficult to study due to confounding by the menstrual cycle. This is primarily due to changes in the concentration of menstrual cycle hormones particularly progesterone and estradiol. Changes in the concentration of these hormones may lead to females increasing their energy intake, their energy expenditure or both factors. Changes in the concentration of these hormones may also influence other factors which could influence food intake including mood, depression, irritability, breast tenderness and bloating (Endrikat *et al.*, 1997).

Although the studies highlighted above have made some headway in this field, there is a need to carry out further studies regarding the influence of physical activity on appetite and food intake, taking into consideration both male and females. Therefore, the work reported in this thesis sought to examine appetite, hormonal and food intake responses to exercise in males and females, but with specific focus on female participants.

The first study in this thesis (Chapter 4) examined relationships between physical activity, physical fitness, body composition, resting metabolic rate, appetite perceptions, dietary restraint and plasma acylated ghrelin in both men and women. Study two (Chapter 5) examined female participants and the effects of taking the OCP on appetite, acylated ghrelin and food intake responses to cycling exercise. Study three (Chapter 6) assessed appetite and hormonal (ghrelin and PYY) responses to exercise and food restriction in female participants. This study is a repeat of a previous study with male participants carried out by King *et al.* (2011a). The final study reported in this thesis (Chapter 7) examined the effects of exercise on appetite, energy intake and plasma acylated ghrelin concentrations in both males and females. This is one of the very few studies to directly compare appetite and hormonal responses to exercise in males and females. Collectively, the studies presented in this thesis begin to address the lack of information in the literature on appetite regulation during/after exercise in females.

The key hypotheses investigated in this thesis were as follows:

1. females would experience compensatory increases in appetite and energy intake in response to exercise,
2. females would experience compensatory increases in plasma acylated ghrelin in response to exercise,
3. females would experience compensatory decreases in PYY in response to exercise and;
4. females would experience a compensatory increase in food intake in the luteal phase compared with the follicular phase of the menstrual cycle.

Over the last few decades the prevalence of overweight and obesity has become a major crisis around the globe with significant health and economic concerns for individuals and for society. Efforts to reverse this trend have involved diet, exercise, drugs and surgery but despite these efforts the prevalence of overweight and obesity remains high as does the prevalence of the co-morbidities associated with obesity. Exercise is still seen as an important weight loss strategy, however, for those with severe obesity it is often not practical and surgery has proven to be a far more effective solution to weight loss (Christou

*et al.*, 2004). Understanding the relationship between the different types of exercise, appetite and food choices is extremely important in helping to counter rising obesity rates and in introducing successful weight loss programs for both women and men.

## 2 Literature review

### 2.1 Appetite

#### 2.1.1 Definition of appetite

The most common definition of appetite found in the literature is “the desire to eat”. Rolfes and Whitney (1996) argue that the desire to eat relates to a psychological drive and define appetite as “the psychological desire to eat or an interest in food” (Rolfes and Whitney, 1996, p. 279). Appetite and hunger are commonly used in nutrition to reflect the initiating of eating although Rolfes and Whitney (1996) state that the two terms do not always match. Hunger is based on a physiological drive whereas appetite reflects the psychological; that is, it is a learned response to food. In contrast, satiety is defined as a feeling of fullness while satiation is “the process that leads to the termination of eating” (Benelam, 2009).

Appetite, hunger and satiety are influenced by signals that control people's eating behaviour with each signal resulting from stimuli produced by hormonal and nervous input. Both hunger and satiety are controlled by several areas within the hypothalamus. This is due to the signals sent from the gastrointestinal tract to the regulatory centres of appetite within the central nervous system (CNS) (Gardiner *et al.*, 2008). These areas are the hunger centres in the lateral hypothalamus and the satiety centres in the ventromedial hypothalamus (Benelam, 2009; Bender, 2002).

Hormonal and neural signals are believed to have important roles in the short-term regulation of appetite (Murphy and Bloom, 2006), inducing immediate food intake and controlling the size and frequency of meals. When the stomach feels empty, it releases hormones which in turn send signals to hunger centres asking for the initiation of eating. On the other hand, when the stomach feels full, it releases other hormones which are responsible for sending signals to the satiety centres to terminate eating. In addition to short-term regulation, there is long-term control of food intake and energy expenditure which regulates the balance between energy needs and energy intake for several days (Bender, 2002).

There are several methods for measuring appetite. The most common method used is the Visual Analogue Scale (VAS) which provides ratings for hunger, satisfaction, fullness and prospective food consumption (PFC). Prospective food consumption can be defined as the quantity of food that participants think they can eat (Poortvliet *et al.*, 2007) and offers quantitative data about a person's satiety (Kristensen *et al.*, 2002). These quantitative data, deriving from a scale varying between two extremes such as "I have never been more hungry" to "I am not hungry at all" can be analysed using statistical software; participants express their feeling by placing a cross or line on the scale. Quantification of the measurements is carried out by measuring the distance from the left end of the line to the mark (Flint *et al.*, 2000).

Measuring food intake is a common method of assessing subjects' satiety. However, the eating patterns of subjects may be influenced by factors other than internal appetite signals. Eating patterns can be monitored directly by keeping a record of the subjects' food consumption. The recorded eating pattern can then be analysed using appropriate nutritional analysis software to calculate energy and nutrient intakes (Benelam, 2009). Monitoring food intake relies on the study participants self reporting dietary intake and these measurements are exposed to bias. Participants may not report accurately or may miss recording some of their energy intake. It is also important to stress the lack of control over the participants eating environment that may influence energy intake (Black *et al.*, 1993; Goldberg and Black, 1998).

### **2.1.2 Control of appetite**

Appetite is influenced by a variety of physiological, social and psychological factors. Physiological factors include age, bodyweight, sex, smoking, alcohol consumption, hormones, physical activity, cold and heat. Appetite decreases with age for a variety of reasons such as a weakening of olfactory sensitivity and gustation (the ability to detect the flavour of food) (Murphy *et al.*, 1991; Kaneda *et al.*, 2000); increased gastrointestinal satiation signals (e.g. cholecystokinin: CCK); a decline in the opioid modulation of feeding which occurs mainly in older females (Chapman *et al.*, 2002); rises in leptin levels which occur mainly in older males due to a decline in testosterone leading to decreased neuropeptide Y (NPY) and decreased food intake (Morley, 2001). This decline in

testosterone is correlated with a decline in body mass and strength in older males (Morley *et al.*, 1997). Differences in body mass also elicit differences in energy requirements. For example, obese subjects expend more energy at rest than lean subjects and this may result in a greater food intake, together with a delay in satiety (Benelam, 2009).

Males and females regulate adiposity-relevant parameters differently; in females, plasma leptin correlates positively with body fat but, on the other hand, plasma insulin correlates positively with body fat in males (Casabiell *et al.*, 1998, Björntorp, 1997a; 1997b, Clegg *et al.*, 2003). This is because insulin and leptin reflect different fat stores which are differentially distributed in males and females (Clegg *et al.*, 2003). Leptin is secreted from subcutaneous fat than from visceral fat (subcutaneous fat is generally higher in females while visceral fat is generally higher in males). This leads to the circulating leptin correlating better with total subcutaneous fat than with total body fat (Casabiell *et al.*, 1998, Björntorp, 1997a; 1997b).

Previous research has indicated that tobacco smoking has an acute influence on hunger and food intake in both males and females. A survey carried out by Jarry and co-workers (1998) highlighted that female dieters report gaining more weight after they stop smoking than non-dieters do. Shimada *et al.* (1998) found mice that lacked melanin-concentrating hormone (MCH), due to the administration of nicotine, exhibited lower body weights and the enhanced leanness associated with marked hypophagia, compared with control animals (Shimada *et al.*, 1998). Furthermore, Jo *et al.* (2002) concluded that smokers were leaner and cessation of smoking without nicotine replacement therapy typically led to significant and sustained hyperphagia and weight gain. On the other hand, findings from a study by Perkins and colleagues (1994) indicated that there is no difference in total caloric intake or in food taste selection in response to smoking versus non-smoking in a study that consisted of male and female participants.

Caton *et al.*, (2004, 2005) concluded that high doses of alcohol have a modest influence on food intake, as it increases appetite and plays a role in delaying the development of satiety (Caton *et al.*, 2004). Some alcoholic beverages that are categorised as aperitifs (or appetizers) are also considered to be appetite stimulators (Molina *et al.*, 2007). Yeomans and colleagues (1999) reported that alcohol failed to have any effect on the pleasantness or

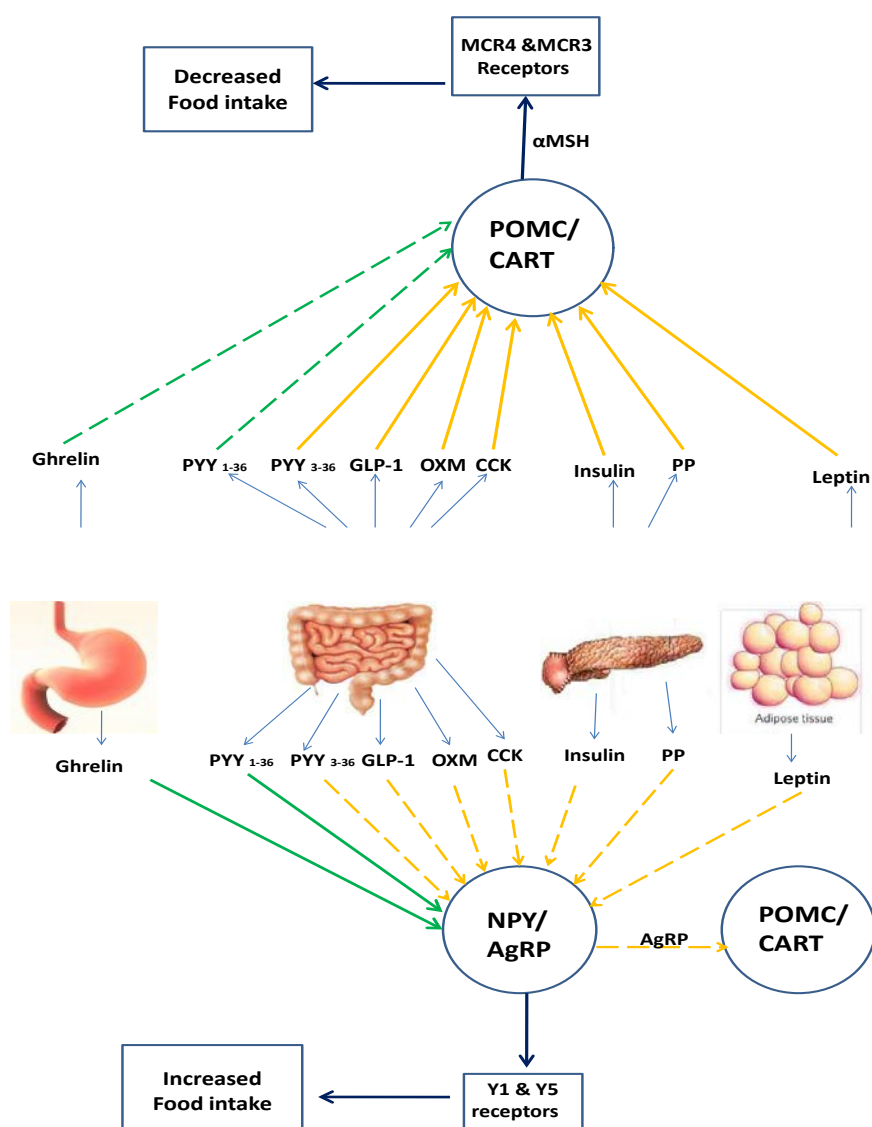


the taste of food items while several research studies indicate that, when alcohol is consumed before or with a meal, it does not reduce energy intake but contributes to stimulate appetite (Westerberp-Plantenga and Verwegen, 1999; Yeomans and Phillips, 2002). However, it is suggested that, under controlled conditions, alcohol can stimulate satiety and reduce energy intake in a similar way to other macronutrients (Raben *et al.*, 1995; Benelam, 2009). The reason for this is the disruption of post-ingestive satiety signals after alcohol consumption (Yeomans *et al.*, 2003).

Hormones play a significant role in controlling short- and long-term appetite. Such hormones are of two types: orexigenic (appetite stimulating) and anorexigenic (appetite suppressing) (Table 2.1). Anorexigenic hormones are also known as satiety peptides and function by sending signals to the satiety centres in the brain; these hormones inhibit food intake by stopping the eating process and/or prolonging satiety. Receptors in the GI tract detect nutrients and signal satiety after each meal (Arora, 2006). These peptides interact with the appetite centre in the hypothalamus and brainstem to relay information about the nutritional state of the subject (McGowan and Bloom, 2007) (Figure 2.1).

**Table 2.1 Gut hormones and their actions, (adapted from Benelam, 2009; Chaudhri, *et al.*, 2006)**

<b>Hormones</b>	<b>Primary sites of synthesis</b>	<b>Effect on appetite</b>	<b>Mechanism</b>	<b>Additional effects</b>
Ghrelin	A-cells of gastric fundus; small and large intestine; hypothalamic nuclei	↑Hunger (Orexigenic)	Via ghrelin receptors in the brain	Long-term effect on energy balance. increases food intake promotes gastric motility promotes PP release
Cholecystokinin (CCK)	I-cells of duodenum, jejunum; widespread CNS expression	↑Satiety (Anorexigenic)	Via the vagus nerve	Delays gastric emptying. Stimulates pancreatic enzyme secretion. Stimulates gall bladder contraction.
Glucagon-like peptide-1 (GLP-1)	L-cells of distal small and large intestine; immunoreactivity in hypothalamus, dorsovagal complex, pituitary	↑Satiety (Anorexigenic)	Via GLP-1R in brain	Incretin (stimulates insulin production) Slows gastric emptying
Oxyntomodulin (OXM)	L-cells of distal small and large intestine; immunoreactivity in hypothalamus, pituitary	↑Satiety (Anorexigenic)	Via GLP-1R in brain Via reductions in ghrelin	Slows gastric emptying
Peptide YY (3-36) (PYY <sub>3-36</sub> )	L-cells of distal small and large intestine; immunoreactivity in hypothalamus	↑Satiety (Anorexigenic)	Via Y <sub>2</sub> receptors	Slows gastric emptying and intestinal transport, reduces gastric secretions
Pancreatic polypeptide (PP)	pancreatic islets of Langerhans; some reports of expression in hypothalamus, pineal gland, pituitary	↑Satiety (Anorexigenic)	Via Y <sub>5</sub> receptors in brain via vagus nerve	Relaxation of gallbladder, equivocal effect on gastric emptying



**Figure 2.1 Homeostatic regulation of food intake**

Figure 2.1 show the hormones that regulate food intake at the level of the arcuate nucleus. It shows the hormones source of secretion. Ghrelin enters the arcuate nucleus via the vagus nerve. The arrows represent two factors. The first is the inhibition of firing of proopiomelanocortin (POMC) /cocaine and amphetamine regulated transcript (CART) neurons. This is represented by green dashed lines. The second is the firing of the orexigenic neurons through NPY and agouti related protein (AgRP). This is represented by the solid green lines. Leptin, insulin and PYY directly diffuse into the arcuate nucleus. They exert indirect influence on food intake regulation, by suppressing expression of NPY and AgRP, which are orexigenic. This leads to an anorexigenic signal which is represented by the yellow lines.

Insulin and leptin are responsible for long-term appetite control which is associated with body-fat stores. Insulin is critical for regulating energy balance; it controls food intake and energy expenditure and regulates the storage of absorbed nutrients. Deficiency of insulin leads to hyperphagia while its increase leads to satiety. Leptin, a hormone produced in adipocytes in proportion to fat mass, plays an indispensable role in preserving energy homeostasis. Leptin acts as a feedback signal to the hypothalamus, informing the brain of the adipose energy reserves; this leads to an increase in food intake when the body's fat stores are depleted (Badman and Flier, 2005; Minor *et al.*, 2009; Korner and Leibel, 2003).

Leptin is synthesised mainly by white adipose tissue in proportion to metabolic activity (Arora, 2006; Friedman and Halaas, 1998). The hormone is also produced in certain foetal organs, brown adipose tissue, the stomach, ovarian follicles, the heart, mammary glands, and the placenta (Trayhurn *et al.*, 1999; Trayhurn and Beattie, 2001). Leptin is a single peptide which consists of 146 amino acids (Zhang *et al.*, 1994); it is known as the “satiety hormone” (Benatti and Lancha Junior, 2007). In the arched nucleus of the hypothalamic regions, leptin has an action in the neurons which produce neuropeptides and primary neurotransmitters. It acts in two ways: directly by stimulating anorexigenic neurons such as proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) and indirectly by inhibiting orexigenic neurons such as NPY and agouti related protein (AgRP); thus, the main role of leptin is to control food intake and appetite (Benatti and Lancha Junior, 2007; Negrão and Licinio, 2000). The other vital functions of leptin are to regulate adipose tissue mass and body weight by sending an afferent feedback signal to the CNS (Houseknecht *et al.*, 1998).

Insulin is a hormone that is produced in the islets of Langerhans, the endocrine cells of the pancreas (Benatti and Lancha Junior, 2007). Insulin concentration increases after meals or after other positive energetic balance conditions; on the other hand, it decreases during fasting and after negative energetic balance conditions (Benoit *et al.*, 2004). Pancreatic  $\beta$  cells secrete insulin in response to any increase in the glucose circulating in the body which leads to rapid secretion of insulin after a meal (Polonsky *et al.*, 1988). Benatti and Lancha Junior (2007) suggested that insulin is a catabolic hormone which plays an important role in the central regulation of adiposity and energy intake. Insulin receptors are widely expressed on neurons in the hypothalamus, particularly in the arcuate nucleus. Thus, insulin

acts as a satiety signal by stimulating the anorexigenic POMC/CART neurons and by inhibiting the orexigenic NPY/ AgRP/ gamma-amino butyric acid (GABA) neurons (Arora, 2006).

Gut hormones constitute the most important physiological factor in short-term appetite control. In response to satiety and hunger, a variety of hormones are secreted from the gut. These include: ghrelin, peptide YY (PYY), pancreatic polypeptide (PP), cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM), which are peptides released from the gastro-intestinal (GI) tract. These influence instant food intake and control the size and frequency of meals (McGowan and Bloom, 2007; Hameed *et al.*, 2009; Gardiner *et al.*, 2008; Murphy and Bloom, 2006).

Cholecystokinin is derived from 115 amino acid precursors and selective cleavage. Selective cleavage gives rise to a number of bioactive forms of CCK and CCK was the first appetite regulating gut peptide to be discovered (Chaudhri *et al.*, 2006). CCK is considered as a satiety hormone; it is produced by the mucosal enteroendocrine cells of the duodenum and jejunum, and is secreted in reaction to the ingestion of a rich meal of protein and fat (Badman and Flier, 2005). The hormone acts as an integrator of upper gastrointestinal tract functions by balancing the intake of food that needs to be digested with the capacity for digestion. Therefore, the hormone slows the delivery of food from the stomach by inhibiting gastric emptying and food intake; on the other hand, it stimulates pancreatic enzyme secretion and gall bladder contraction thereby increasing digestive capacity (Dockray, 2009; Hameed *et al.*, 2009).

It has also been demonstrated that endogenous CCK has an important role in the control of meal size; several studies have uncovered the pathways by which CCK mediates these effects (Konturek *et al.*, 2003). CCK inhibits food intake by suppressing NPY in the hypothalamus, therefore opposing the effect of ghrelin (Moran and Kinzig, 2004). CCK also provides a satiety message to the brain by stimulating the vagus nerve (by increasing the discharge of neurotransmitters in the brain) to affect meal termination. Vagal afferent neurons (going from the gut to the brain) carry receptors for CCK (CCK1-R) (Hameed *et al.*, 2009). Moreover, CCK induces the release of leptin from the stomach which may enhance the short-term satiety signal (Emond *et al.*, 1999).

Oxyntomodulin (OXM) is a 37-amino acid peptide. It is rapidly released after food ingestion from the L cells of the distal small intestine and the central nervous system (CNS) in proportion to the calorie intake of a meal (Nogueiras *et al.*, 2006). According to Dakin and colleagues (2001; 2004), in studies with rats, OXM is considered to be an effective food intake inhibitor when administered intraperitoneally or intracerebroventricularly. Therefore, OXM promotes satiety.

Oxyntomodulin has an anorexigenic effect on food intake which may contribute to the normal postprandial suppression of plasma ghrelin concentration. According to studies of humans (Cohen *et al.*, 2003) and of rats (Dakin *et al.*, 2004), all results show a fall in ghrelin concentration after preprandial administration of OXM. In addition, the incubation of hypothalamic explants with OXM has been shown to cause a significant increase in the release of the anorectic arcuate nucleus (ARC) peptide alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) (Dakin *et al.*, 2004). This is co-secreted with glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY<sub>3-36</sub>) after the ingestion of a meal; its release is proportional to the ingested food, leading to satiety (Stanley *et al.*, 2004; Gagliardino, 2005). Physiologically, OXM reduces gastric motility and gastric acid secretion (Dakin *et al.*, 2004; Nogueiras *et al.*, 2006) and this leads to fullness and satiety.

Pancreatic polypeptide (PP) is released from and synthesised by the endocrine pancreas (Murphy and Bloom, 2006); it is also produced in small quantities in the rectum and colon (Adrian *et al.*, 1976). The hormone has high affinity for binding to two receptors: Y4 and Y5 (Murphy and Bloom, 2004). Pancreatic polypeptide is released after a meal, leading to a reduction in appetite (Murphy and Bloom, 2004). Batterham *et al.* (2003) showed that the anorexigenic effects of PP occur as it reduces energy intake by 22% when infused two hours before a buffet meal. Infusion of PP was also observed to reduce energy intake by 25% over a 24-hour period in this study. Benelam (2009) states that PP messages to the brain occur by way of the Y receptors' family.

Glucagon-like peptide-1 is a 30 amino acid peptide released in response to food intake (Herrmann *et al.*, 1995). Glucagon-like peptide-1 is a product of the proglucagon gene, expressed in the intestine, brain and pancreas (Benelam, 2009). Experimental investigations have indicated that the infusion of GLP-1 reduces food intake, increases fullness ratings

and decreases ratings of hunger in both obese subjects and those of normal weight (Flint *et al.*, 2000; Naslund *et al.*, 1999). Receptors for GLP-1 can be found in those areas of the brain involved in appetite; it is thought that GLP-1 mediates its effects on satiety by acting directly upon these areas (Arora, 2006). Glucagon-like peptide-1 also slows gastric emptying and modulates gastric acid secretion, contributing to the 'ileal brake' mechanism of the upper gastrointestinal tract; it also has an inhibitory effect on hunger and food intake in man. It has a combination of effects that controls the transit of food from the stomach into the intestines (Schmidt *et al.*, 2003).

Ghrelin is a 28-amino acid peptide which is released from the stomach and is modified by an acyl side chain, added to the amino acid serine at position 3. This acylation is essential for binding to the growth hormone secretagogue receptor (GHS-R) and for applying its effect to food intake (Kojima and Kangawa, 2005). Ghrelin is the only orexigenic 'hunger hormone' which circulates in the blood. Circulating plasma levels of ghrelin rise before meals and fall upon eating. This supports the hypothesis that ghrelin plays a physiological role in meal initiation in humans (Cummings *et al.*, 2001; McGowan and Bloom, 2007) by increasing the feeling of hunger (Wren *et al.*, 2001). The main site of ghrelin's activity in the central nervous system (CNS) is in the hypothalamic ARC. The ARC is targeted by appetite-stimulating peptides NPY/AgRP, and by an appetite-suppressing hormone, leptin (Morton *et al.*, 2006; Rolfes and Whitney, 1996). Ghrelin is released from neurons in the brain containing ghrelin by way of the orexigenic message of ghrelin (from the stomach) via the vagus nerve. Ghrelin sends afferent fibers onto NPY/AgRP neurons to stimulate production and secretion of NPY and AgRP peptides. On the other hand, these peptides act as inhibitors to the firing of POMC/CART neurons (Arora, 2006; Cowley *et al.*, 2003; Kojima and Kangawa, 2005).

Peptide tyrosin tyrosin (PYY) is a gastrointestinal peptide which is crucial in the regulation of food intake and energy homeostasis. It is a 36-amino-acid peptide which is secreted by intestinal L cells in the ileum and colon (Ellacott *et al.*, 2006; Ueno *et al.*, 2008). Peptide YY is released into the circulation in two forms: PYY<sub>1-36</sub> and PYY<sub>3-36</sub>, depending on the affinity of receptors (Adrian *et al.*, 1985). PYY<sub>3-36</sub>, the major form in the circulation, is created by the cleavage of the N-terminal Tyr-Pro amino residues of PYY<sub>1-36</sub> by the enzyme dipeptidyl peptidase IV (DPP-IV) (Gardiner *et al.*, 2008; Ueno *et al.*, 2008).

Peripheral PYY<sub>3-36</sub> acts as a satiety signal to the hypothalamic arcuate nucleus, probably via the vagal afferent nerve, after binding to Y2 receptors (Ueno *et al.*, 2008). This leads to a direct anorexigenic effect (by stimulating POMC) and an indirect orexogenic effect (by inhibiting NPY neurons) (Arora, 2006).

The pleasure of eating relates to factors such as the texture, taste and odour of food; these can suppress or stimulate appetite (Rolfes and Whitney, 1996). The environment also plays an important role in controlling appetite. Factors such as the time of day, the place, the season, and an assessment of future food supply, may influence food intake. For example, people tend to prefer hot food in winter when it is cold. Physical activity is also identified in the literature as a physiological factor that influences appetite and food intake; post-meal exercise influences the suppressive effects of meal consumption on appetite (Cheng *et al.*, 2009). King and team (1994; 1995) and Westerterp-Plantenga and colleagues (1997) suggest that perceived hunger in men and women is reduced or that eating is delayed immediately after a bout of exercise (King *et al.*, 1994; King and Blundell, 1995; Westerterp-Plantenga *et al.*, 1997). The next section introduces and discusses the influence of exercise on appetite and food intake.

## **2.2 Exercise, appetite and food intake**

Over the past few decades there has been an increased emphasis on exercise. One reason for this may be the relationship between the exercise and food intake. Food intake and the consumption of energy play an important role in regulating body weight. In humans, the process of regulating the balance of energy intake and energy expenditure constitutes a complex physiological system. This system consists of afferent signals, integrated by peripheral nerves and brain centres (Bilski *et al.*, 2009; Stanley *et al.*, 2005). Exercise may have acute (short term) and chronic (long term) affects on appetite and these are reviewed below.



### 2.2.1 Effect of acute exercise

It is a general belief that exercise causes an automatic increase in hunger and a drive to eat and the findings from studies such as that carried out by Pomerleau *et al.* (2004) on a sample of moderately active women observed an increase in subsequent energy intake after an acute bout of exercise and Hagobian *et al.*, (2009) with sedentary, overweight/obese male and female participants found the same. Verger *et al.* (1992; 1994) employed a sample of physically active (not athletes) participants and reached the same verdict. However, these results were only found in a minority of the studies in this field; the majority of studies have shown that hunger and energy intake (EI) are not increased by acute exercise (Thompson *et al.*, 1988; King *et al.*, 1996; King and Blundell, 1995; Westerterp-Plantenga *et al.*, 1997; Lluch *et al.*, 1998; Blundell and King, 1999) regardless of the exercise intensity (King *et al.*, 1994; Kissileff *et al.*, 1990). These observations are supported by similar studies which show that vigorous exercise significantly reduces hunger causing a phenomenon known as "exercise-induced anorexia" (King and Blundell, 1995; King *et al.*, 1994).

There are methodological differences between many of these studies. For example, several different modes of exercise have been examined including running (King *et al.*, 2010a; Deighton *et al.*, 2012), walking (Pomelaue *et al.*, 2004; King *et al.*, 2010b), swimming (King *et al.*, 2011b; Verger *et al.*, 1992), cycling (King & Blundell, 1995; Erdmann *et al.*, 2007; Martins *et al.*, 2007) and strength training (Broom *et al.*, 2009). In terms of exercise intensity, some studies have evaluated low or moderate intensity exercise (King *et al.*, 2010b; Hagobian *et al.*, 2009) while others have examined high intensity exercise (Broom *et al.*, 2007, King and Blundell, 1995; Lluch *et al.*, 1998).

Some studies have examined appetite responses to exercise in males (King *et al.*, 2010a, 2010b; Broom *et al.*, 2007) while others have studied females (King *et al.*, 1996; Leidy *et al.*, 2004). Only a handful of studies have examined appetite responses to exercise in both males and females (Hagobian *et al.*, 2009; Burns *et al.*, 2007; Ambler *et al.*, 1998). Another methodological difference is the macronutrient composition of the test meals, some studies use high-fat meals such as Cheng and colleagues (2009), or low fat versus high-fat meals such as Tremblay and co-workers (1994), while others have used high carbohydrate meals

(Thomas *et al.*, 1991; Harrison *et al.*, 2009). In terms of time, some studies have investigated the time interval between exercise and eating such as Verger *et al.* (1992), others have examined whether exercise exerts different effects on appetite if performed before versus after a meal (Cheng *et al.*, 2009, Deighton *et al.*, 2012). Such differences may explain, at least in part, the inconsistencies in the study findings.

There is no clear evidence suggesting that there is an automatic increase in hunger and food intake in response to exercise. There are also consistent results in the literature indicating that there is a suppression of hunger during and after exercise; some studies indicate that hunger ratings return to control values within two hours after the cessation of exercise (Broom *et al.*, 2007; Blundell *et al.*, 2003; King and Blundell, 1995; King *et al.*, 1994). Imbeault and colleagues (1997) showed that energy intake tended to be lower after a high-intensity, rather than a low-intensity, exercise session but this difference did not reach statistical significance. Vigorous exercise (i.e.,  $\geq 60\%$   $\text{VO}_2\text{max}$ ) leads to hunger suppression (King *et al.*, 1994; Westerterp-Plantenga *et al.*, 1997). However, this induced suppression is short lived and has no subsequent effect on food intake (King *et al.*, 2010 a).

Sex differences may play an important role in compensation responses to physical activity. Imbeault and colleagues (1997) reported that in males there is no change in subsequent energy intake after acute exercise, whereas it was observed that the energy intake of females usually increases which, in turn, reduces or eliminates the effects of exercise on energy balance (Pomerleau *et al.*, 2004; Stubbs *et al.*, 2002b; Martins *et al.*, 2007). Other studies have shown that females, unlike males, do not demonstrate short-lived hunger suppression immediately after exercise and there may even be an increased sensory attraction to food (King *et al.*, 1996; King and Blundell, 1995).

Some studies have suggested that high intensity exercise increases energy intake sufficiently in females after an exercise session to compensate almost completely for exercise-induced energy expenditure (Hagobian *et al.*, 2009; Pomerleau *et al.*, 2004). Thus, for those wishing to use exercise to control body weight it is hypothesised that intensive exercise could be better suited to males and light exercise could be better in producing a negative energy balance in females (King *et al.*, 1996). Erdman and co-workers (2007) indicated that the exercise duration when undertaking moderate intensity

cycling was a factor in food intake. The study showed that for males and females food intake increased significantly after cycling for 120 min compared with control, 30 and 60 minutes of cycling. There was no difference between males and females.

Cheng and colleagues (2009) indicated that the timing of exercise around meal consumption might influence appetite and its hormonal regulators. Their study, which involved three trials included one trial where participants were given a meal without exercise; a second trial where exercise was performed one hour before the meal and in the third trial, exercise was performed two hours after the meal. Their findings indicated that post-meal exercise may extend the suppressive effects on appetite of meal consumption but it is presently unclear if these effects are similar when exercise is performed 3–4 hours after a meal (Cheng *et al.*, 2009). Typically, exercise-induced declines in hunger rebound relatively quickly (i.e. within approximately 15 minutes) after the cessation of exercise (King *et al.*, 1994). King *et al.* (1994) further discovered that although intense exercise increased the period of exercise-induced anorexia, it had no significant impact on food consumption. One reason for this could be that post-ingestive satiety signals are potent enough to prevent an increase in food intake, therefore the physiological system does not permit an rise in energy intake to match the higher energy expenditure from exercise, at least in the short term (King *et al.*, 1994).

Broom and colleagues (2009) compared hunger suppression during and immediately after vigorous treadmill running and resistance exercise. The research indicated that hunger was suppressed during and immediately after both forms of exercise but the suppression was not as great after resistance exercise as after running. King and co-workers (1995) demonstrated that exercise tended to delay the onset of eating but increase food intake slightly after an exercise session (involving cycling and running) compared with rest trials. Table 2.2 provides a summary of studies examining the acute effects of exercise on appetite and food intake.

**Table 2.2 Effects of acute exercise on appetite and food intake**

Reference	Subjects	Intervention	Energy intake	Hunger scores
Broom <i>et al.</i> (2007)	Normal weight male students	Treadmill running at 75% of maximum oxygen uptake.	Not tested	During the exercise period, hunger scores were significantly decreased; however, this effect disappeared in the post-exercise period
Broom <i>et al.</i> (2009)	Normal weight male students	<ol style="list-style-type: none"> <li>1. Resistance exercise: a 90-min free weight lifting session followed by a 6.5-h rest period</li> <li>2. Aerobic exercise: a 60-min run followed by a 7-h rest period</li> <li>3. Resting</li> </ol>	Not tested	Suppressed hunger during aerobic and resistance exercise. Post-exercise, hunger scores increased but remained suppressed compared with the control trial.
Cheng <i>et al.</i> (2009)	Moderately active normal weight young males	<p>Ergometer cycling at 60% of VO<sub>2</sub> max for 50min performed</p> <ol style="list-style-type: none"> <li>1. exercise 2h after a meal</li> <li>2. exercise 1h before a meal.</li> </ol>	High-fat meal	<p>Exercise performed 2h after a meal extended the appetite suppressing effect of food intake.</p> <p>Exercise prior to food intake decreased appetite</p>

**Table 2.2 Effects of acute exercise on appetite and food intake (Continued)**

<b>Reference</b>	<b>Subjects</b>	<b>Intervention</b>	<b>Energy intake</b>	<b>Hunger scores</b>
Erdmann <i>et al.</i> (2007)	Males & females normal weight	<ol style="list-style-type: none"> <li>1. cycling for 30 min at 50 W and then another 30 min at 100 W</li> <li>2. cycling at 50 W for 30, 60 and 120 min versus resting</li> </ol>	Food intake after 120 mins of cycling was significantly greater compared with control, 30 min and 60 min of exercise	No significant effect on hunger even though food intake was higher after 120 mins bouts of cycling exercise
Hagobian <i>et al.</i> (2008)	Healthy, active males & females	Exercise on a cycle ergometer or treadmill at 60% peak VO <sub>2</sub> .	Increased total energy intake	Not tested
Imbeault <i>et al.</i> (1997)	Young moderately active, normal weight men	Treadmill running at 35 % of VO <sub>2</sub> max and 75 % of VO <sub>2</sub> max for a duration allowing an exercise energy expenditure of 2050 kJ	No significant change in total energy and macronutrient intake	No significant change in post-exercise subjective levels of hunger and fullness

**Table 2.2 Effects of acute exercise on appetite and food intake (Continued)**

Reference	Subjects	Intervention	Energy intake	Hunger scores
King <i>et al.</i> (1994)	Lean healthy males	<p>Study 1:</p> <ol style="list-style-type: none"> <li>1. Control</li> <li>2. High intensity exercise (cycling on a bicycle ergometer at 70% of VO<sub>2</sub> max for 30 min)</li> <li>3. Low intensity exercise (cycling on the bicycle ergometer at 30% of VO<sub>2</sub> max for 60 min)</li> </ol> <p>Study 2:</p> <ol style="list-style-type: none"> <li>1. Control</li> <li>2. High intensity exercise of short duration(26 min)</li> <li>3. High intensity exercise of long duration(52 min)</li> </ol>	<p>Study 1:</p> <ul style="list-style-type: none"> <li>- No difference in food intake between the three trials</li> </ul> <p>Study 2:</p> <ul style="list-style-type: none"> <li>- Delayed onset of eating in both exercise trials compared with control.</li> </ul>	<p>Study 1:</p> <ul style="list-style-type: none"> <li>- High intensity exercise caused a significant suppression of hunger during and immediately after exercise.</li> <li>- Low intensity exercise did not suppress hunger</li> </ul> <p>Study 2:</p> <ul style="list-style-type: none"> <li>- Hunger was suppressed during and immediately after both high intensity exercise trials.</li> <li>- Suppression was greatest after the long duration exercise trial compared with the short duration exercise trial.</li> </ul>
King and Blundell (1995)	Lean, healthy males	Exercise session (at 70% of VO <sub>2</sub> max) (running or cycling) versus resting	Delayed onset of eating. No significant effect on the total amount of food eaten, but energy intake from high-fat/low-carbohydrate foods was significantly elevated	Transitory hunger scores decrease after exercise but recovered after 15 minutes

**Table 2.2 Effects of acute exercise on appetite and food intake (Continued)**

Reference	Subjects	Intervention	Energy intake	Hunger scores
King <i>et al.</i> (1997)	Lean males, regular exercisers	Treadmill running at approximately 70% of heart rate max for 50 min.	No significant effect	Average hunger scores lower on day of exercise compared to the day after exercise
King <i>et al.</i> (1996)	Dietary unrestrained, normal weight females	Exercise at 70% of VO <sub>2</sub> max versus resting	No significant effect	No significant effect
King <i>et al.</i> (2010a)	Healthy, normal weight, young males	Treadmill running at approximately 70% of VO <sub>2</sub> max for 90 min versus resting	No significant effect	During the exercise period, hunger scores were significantly decreased; however, this effect persisted for 30 minutes of the post-exercise period
King <i>et al.</i> (2010b)	Healthy, normal weight, young males	60-min brisk walk on a level-motorised treadmill at 33-55% of VO <sub>2</sub> max versus resting	No significant effect	No significant effect
Kissileff <i>et al.</i> (1990)	Obese & non-obese women	Exercising either strenuously (90 W) or moderately (30 W) on a cycle ergometer for 40 min versus resting	Intake of a liquefied test meal (1.04 kcal/g) 15 min after exercise was significantly decreased (g) in non-obese women	Not tested

**Table 2.2 Effects of acute exercise on appetite and food intake (Continued)**

Reference	Subjects	Intervention	Energy intake	Hunger scores
Hagobian <i>et al.</i> (2009)	overweight/obese males & females	<ol style="list-style-type: none"> <li>Four consecutive days bouts of exercise (50–65% of estimated VO<sub>2</sub> peak) with energy added to the baseline diet to maintain energy balance (BAL),</li> <li>Four consecutive days bouts of exercise without energy added to induce energy deficit (DEF).</li> </ol>	High fat test meal (fixed energy content)	In men, but not in women, appetite was inhibited after BAL relative to DEF.
Lluch <i>et al.</i> (1998)	Dietary restrained females, normal weight & regular exercisers	<ol style="list-style-type: none"> <li>High Fat buffet after 50 min of cycling at 70% of VO<sub>2</sub> max,</li> <li>High Fat buffet with no exercise (control)</li> <li>Low Fat buffet after 50 min of cycling at 70% of VO<sub>2</sub> max,</li> <li>Low Fat buffet with no exercise (control)</li> </ol>	No significant effect of exercise on total energy intake in both trials (high fat and low fat buffet) but energy intake increased during high-fat conditions compared to low-fat conditions.	No significant effect of exercise on hunger scores, exercise raised the perceived pleasantness of foods
Martins <i>et al.</i> (2007)	Healthy, normal-weight, volunteers (males & females)	Cycled on an ergometer for 60 min at 65% of maximal heart rate or rested.	Exercise significantly increased subsequent absolute EI, but produced a significant decrease in relative EI after accounting for the energy expended during exercise	During the exercise period, hunger scores were significantly decreased; however, this effect was short-lived



**Table 2.2 Effects of acute exercise on appetite hormones (Continued)**

Reference	Subjects	Intervention	Energy intake	Hunger scores
Pomerleau <i>et al.</i> (2004)	Moderately active, normal weight, females	Treadmill walking 1. 40% of VO <sub>2</sub> max 2. 70% of VO <sub>2</sub> max 3. Resting	High-intensity exercise increased energy intake in women	No significant effect
Verger <i>et al.</i> (1992)	Non-obese males	75 min of continuous swimming. Estimated (gross) expenditure of 500 kcal	No effect on food intake	Not tested
Verger <i>et al.</i> (1994)	Non-obese males	2h of athletic activity. Estimated (gross) expenditure of 800 kcal	Significant increase (440 kcal) in food intake after exercise compared to inactive control participants	Not tested
Westerterp-Plantenga <i>et al.</i> (1997)	Normal weight males	1. 2 h of cycling at 60% of VO <sub>2</sub> max versus resting 2. 3 x 20 min saunas at 80°C over a 2 h period.	1. Energy intake decreased, fluid intake increased 2. No change in food intake, fluid intake increased	1. Hunger scores decreased 2. No change in hunger

### 2.2.2 Effect of chronic exercise

Experiments that have continued over a long period of time (i.e. over weeks or months of exercise training) have reported inconsistent alterations in diet. These alterations include decreases in fat intake (Bryner *et al.*, 1997), increases in fat intake, and decreases in carbohydrate intake (Ambler *et al.*, 1998). Food intake surveys have shown that individuals with high levels of exercise as part of their habitual lifestyle select diets that are rich in carbohydrate and reciprocally low in fat (Deheeger *et al.*, 1997; Björntorp, 1976; Saris, 1989; Eaton *et al.*, 1995).

There is a lack of research on actual changes in food selection that accompanies varying levels of exercise. Deheeger and colleagues (1997) found that high levels of activity were associated with increases in carbohydrate intake and decreases in the percentage intake of fat in active 10 year old French children. Eaton and co-workers (1995) carried out a cross-sectional survey regarding physical activities and food choices in males and females. They found no difference in the daily energy intake of sedentary, moderately active and very active individuals; however, their diet composition was different. The two active groups consumed less fat, and especially less saturated fat, than the sedentary group. The regular practice of exercise seemed to be integrated within a 'healthy' lifestyle, including dietary aspects. However, another study showed that moderately intense exercise does not alter the macronutrient intake of young individuals (Donnelly *et al.*, 2003b).

There is no agreement on the effects of exercise interventions on energy intake. Some studies, such as that of Janssen *et al.* (1989), involving an 18 month running intervention programme aiming to prepare individuals for a marathon, report a significant increase in energy intake, with body fat increase in males but no significant change in the body fat composition of the female participants. The findings of another study carried out by Ambler *et al.* (1998) also revealed food intake increase in response to exercise training in males but no change was noted in females. However, no weight change was observed among the subjects. Martins and colleagues (2010) reported that 12 weeks of exercise training causes weight loss in both males and females and leads to an increase in fasting acylated ghrelin and hunger sensations, meaning that there is no difference between the sexes in response to exercise. The study also confirmed the findings of other studies that

exercise appears to balance this increased orexigenic drive by improving the satiety response to a meal and the sensitivity of the appetite control system. On the other hand, Snyder and colleagues (1997) argued that there is a little increase in food intake in females in response to exercise training. The findings of several studies conflict with this. Donnelly and colleagues (2000) found that the food intake decreased slightly in females after a period of aerobic exercise training regardless of mode. The findings of this study agreed with an earlier study carried out by Wood and colleagues (1991). Andersson and co-workers (1991) also showed a slight decrease in food intake after three months of exercise training in males and significant decrease in food intake in females.

One study involving a short-term (four day) intervention observed that hunger ratings in males were lower when energy intake matched energy expenditure than when they were in energy deficit. In contrast, females' appetite ratings were found not to decline significantly when energy intake was increased to replace exercise energy expenditure (Hagobian *et al.*, 2009). The study undertaken by Potteiger *et al.* (2003) examining the insulin and glucose response during an oral glucose tolerance test in overweight young adults before and after exercise training reported that males who ate *ad libitum* lowered both their body fat and body weight under supervised aerobic exercise performed 3 to 5 days per week for 16 months. In contrast no changes in body fat and body mass were observed in females during the same interval. Henderson and colleagues (2008; 2007) stressed that females match a higher energy expenditure caused by exercise by increasing their energy intake or by keeping their post-exercise fat oxidation unchanged; these, they suggest, are the main reasons why females may not lose body fat during an exercise intervention (Donnelly *et al.*, 2003a; Potteiger *et al.*, 2003). In contrast, males tend to lose body fat because they do not sufficiently increase their energy intake to match the higher exercise induced energy expenditure during interventions (Donnelly *et al.*, 2003a; Potteiger *et al.*, 2003). Table 2.3 provides a summary of studies examining the chronic affects of exercise on appetite and food intake.

The conflicting results obtained from these studies can be attributed to the different sample characteristics and the different methods employed by the researchers. No standard sample was used, and consequently results and outcomes will differ between the studies. Variables such as sample size, age, BMI and fitness level differ significantly between these studies.

Additionally, while in some studies food intake was measured by self-report or food record, others were weighed in the lab. The measurement of food intake by self-report is well documented to be subject to bias, usually towards the underestimation of habitual energy intake (Black *et al.*, 1991; Lichtman *et al.*, 1992; Samaras *et al.*, 1999; Kelly *et al.*, 1999), therefore weighing in the lab is more likely to produce more accurate results. Due to the variances in results, it is difficult to pinpoint the effect of exercise on food intake and body weight.

In summary, acute, high intensity exercise leads to suppressed hunger and a delayed onset of eating in males while in females there is some evidence that energy intake is increased to almost full compensation for exercise induced energy expenditure. The timing of exercise around meal consumption appears to play an important role in modifying appetite. Post meal exercise may prolong the suppression of appetite experienced after a meal. With respect to exercise duration, there is some evidence that this is positively related to subsequent food intake. Regarding chronic exercise, there is some evidence that this alters food preferences by increasing the intake of carbohydrate and decreasing the intake of fat. Chronic exercise training may or may not increase food intake although increases are possibly more likely in females than males according to some literature.

**Table 2.3 Effects on chronic exercise on appetite hormones and food intake (Adapted from Melzer *et al.*, 2005)**

Reference	Subjects	Intervention	Duration of the study	Observations
Ambler <i>et al.</i> (1998)	Non obese males & females aged 15-17 yrs	Running, aerobic dance, competitive sports (for example, basketball) and occasional weight-lifting.	5 weeks	Total energy expenditure was significantly greater than total caloric intake during the training period for both males and females. Fitness was positively associated with increased self-reported energy intake in males but not females, while exercise training led to alterations in food selection (greater fat intake and reduced carbohydrate intake) in females.
Donnelly <i>et al.</i> (2003b)	Overweight & moderately obese men & women.	Exercise of moderate intensity was performed for 45 min/d, 5 d/week	16 months	There were no significant differences for men or women between the exercise and control groups in fat, carbohydrate, or protein intake expressed as grams or as percentages of total energy intake.
Donnelly <i>et al.</i> (2000)	11 obese women BMI = 30.12 kg/m <sup>2</sup>	Exercise (559 kcal/week)	18 months	Slight decrease in food intake
	11 obese women BMI = 32.33 kg/m <sup>2</sup>	Walking (808 kcal/week)	18 months	Slight decrease in food intake
Martins <i>et al.</i> (2010)	overweight/obese men and women ( 8 men and 14 women).	Supervised exercise programme (five times per week, 75% of maximal heart rate)	12 weeks	Significant reduction in body weight and fasting insulin and an increase in plasma acylated ghrelin levels and fasting hunger sensations

**Table 2.3 Effects of chronic exercise on appetite hormones and food intake (Continued)**

<b>Reference</b>	<b>Subjects</b>	<b>Intervention</b>	<b>Duration of the study</b>	<b>Observations</b>
Wood <i>et al.</i> (1991)	Overweight men	Jogging	1 year	Slight decrease in food intake
Keim <i>et al.</i> (1990)	12 overweight women BMI = 27.4 kg/m <sup>2</sup>	No exercise, moderate duration exercise, long duration exercise	36 days	Slight increase in food intake in exercise trials compared to no exercise trials
Andersson <i>et al.</i> (1991)	22 overweight women 9 overweight men	Exercise three times/week, 60 min/session	3 months	Slight decrease in food intake in men Significant decrease in food intake in women
Snyder <i>et al.</i> (1997)	13 obese women BMI = 32.5 kg/m <sup>2</sup>	Moderate intensity exercise 30 min/d, 5 d/week	32 weeks	Slight increase in food intake
Mertens <i>et al.</i> (1998)	12 overweight subjects (8 males, 4 females) BMI = 29.5 kg/m <sup>2</sup>	Walking 1 h/d (6.4 km/h)	12 months	Slight increase in food intake

## 2.3 Exercise and gut hormones

### 2.3.1 Ghrelin

Ghrelin is a gastric hormone that plays an important role in appetite control. There is well established evidence in the literature strongly relating circulating ghrelin to appetite regulation. Plasma ghrelin levels increase pre-prandially and decrease post prandially (Cummings *et al.*, 2001; Tschöp *et al.*, 2001) and intravenous infusions of ghrelin stimulate both appetite and food intake (Wren *et al.*, 2001). Results have been mixed in studies that have examined how ghrelin is affected by exercise (See Table 2.4). Despite clear evidence in the literature related to ghrelin's role in appetite control, there is a lack of research that investigates whether there are differences in the ghrelin response to exercise differs between males and females, although there has been increased interest in this area in recent years in males.

A study conducted by Cheng and colleagues (2009) examined responses of ghrelin to exercise prior to and after a meal. The study revealed that, in the fasted state, the concentration of plasma total ghrelin increased after exercise. The study also revealed that plasma total ghrelin concentration increased after exercise in the fed state when exercise was undertaken 2 hours after a meal.

The response of ghrelin to different intensities of exercise has also been examined in some studies. The findings suggest that the intensity of exercise may be a factor that influences the ghrelin response. Erdmann and co-workers (2007) examined the effect of ghrelin release and *ad libitum* food intake in males and females exercising at different intensities (50 W and 100 W on a bicycle ergometer for 30 min) and different durations (50 W on a bicycle ergometer for 30, 60 and 120 min). The study observed an increase in ghrelin concentration when the exercise intensity was set at 50 W but not when the intensity was 100 W which suggests that low rather high intensity exercise stimulates increases in ghrelin concentration. In terms of exercise duration, a significant increase in acylated ghrelin responses occurred when participants completed 120 min bout of cycling, compared to 30 min and 60 min (all at 50 W) which showed no significant difference.

Another study conducted by Jürimäe and colleagues (2007b) investigated vigorous exercise in elite male rowers. They found that plasma ghrelin concentration was significantly increased immediately after high intensity exercise but decreased during the first 30 minutes of recovery, compared with the postexercise level. In contrast a study by Vestergaard and colleagues (2007) reported a significant decrease in basal and post-exercise concentrations of total ghrelin after 4 weeks of exercise training compared with pre-training responses in healthy males and females. Another study which investigated the impact of exercise on plasma ghrelin indicated no change in the total concentration of plasma ghrelin during and after a one-hour run for both males and females, despite hunger suppression (Burns *et al.*, 2007). Leidy and co-workers (2004) investigated the response of circulating ghrelin to an energy deficit elicited by a 3-month diet and exercise intervention in healthy females. They observed a significant increase in fasting plasma total ghrelin concentrations in exercising participants who lost weight during the intervention.

Acylated ghrelin (the active form of the ghrelin hormone) has been examined by several researchers in response to different types of exercise. Broom and colleagues (2009) found that acylated ghrelin was suppressed during resistance and aerobic exercise and, in another study; they found that the suppression of appetite during and immediately after exercise was related to a reduction in acylated ghrelin (Broom *et al.*, 2007). In both studies the suppression of acylated ghrelin coincided with a suppression of hunger. King and co-workers (2010a, 2010b) carried out two studies which examined acylated ghrelin in response to two different types of exercise. The first study showed that, despite inducing a considerable energy deficit, appetite and plasma acylated ghrelin were suppressed only for a brief period in high intensity exercise (treadmill running) (King *et al.*, 2010a). The second study showed that moderate intensity exercise (brisk walking) did not change plasma acylated ghrelin concentration (King *et al.*, 2010b).

The response of acylated ghrelin to exercise in overweight and obese individuals has attracted substantial attention. Martins and colleagues (2010) found a significant increase in the levels of fasting and postprandial plasma acylated ghrelin in overweight and obese individuals after a 12 week exercise programme; this outcome was consistent with a short term (five day) study carried out in adolescents by Mackelvie and co-workers (2007). They observed a significant increase in acylated ghrelin after exercise, and this increase was



higher in normal weight boys than in overweight boys. Hagobian and colleagues (2009) also examined the effect of exercise on acylated ghrelin in overweight and obese individuals; the novel finding of this study was a clear sex difference in the hormonal response to exercise; in females, acylated ghrelin concentration tended to increase to a greater extent post-exercise than in males regardless of whether participants were in energy deficit or energy balance.

### **2.3.2 Peptide YY**

Several studies have examined the influence of exercise on PYY to assess whether exercise induced anorexia might be related to increased concentrations of this satiety hormone. Broom and colleagues (2009) investigated the effects of resistance and aerobic exercise on PYY. The study observed an increase in PYY for prolonged periods throughout and after aerobic exercise, the latter result being believed to be a novel finding in the area of PYY. In another study, Cheng and co-workers (2009) found that PYY<sub>3-36</sub> (the biologically active form of the hormone) was higher before and after consuming a meal with prior acute exercise compared with a meal-only trial. For overweight adolescents (males and females), Jones and co-workers (2009), reported an increase in total fasting PYY after 32 weeks of aerobic training (various modes including: treadmill, Stairmaster, elliptical trainer, rowing machine and stationary cycle).

Weight status may influence the relationship between physical activity and PYY. Martins and colleagues (2007) found a significant increase in plasma PYY levels for healthy weight participants when they performed exercise of moderate to high intensity, despite the effect being short-lived. Another long-term study investigated the effect of high intensity exercise on fasting and postprandial plasma levels of PYY in overweight and obese individuals and found that exercise had no significant effect on plasma PYY levels (Martins *et al.*, 2010).

### **2.3.3 Other Hormones**

Several studies have examined the effect of exercise on other hormones, such as leptin, insulin, GLP-1, PP and CCK. Jürimäe and team examined the leptin response to a rowing training session in two studies. Both studies found significantly decreased leptin

immediately after the exercise (Jürimäe *et al.*, 2007a; 2007b). It is difficult to understand the reasons for these changes since leptin is thought to be secreted in proportion to fat cell size and fat cell size is unlikely to be altered significantly by a single bout of exercise. Martins and colleagues (2010) conducted a 12 weeks exercise intervention for overweight and obese individuals and observed a significant decrease in postprandial insulin levels after exercise. Similar findings arose from a short-term intervention conducted by Hagobian and co-workers (2009). They found a more pronounced decrease in insulin after exercise in females than males. Another study examined insulin responses to a 16 month exercise training programme, using *ad libitum* meals in males and females and reported an improvement in insulin sensitivity due to loss of body fat in males but no change in body fat or insulin sensitivity after exercise training in females (Potteiger *et al.*, 2003).

In a study lasting 10 weeks, Hurley and colleagues (1991) observed a significant decrease in fasting and peak insulin and a slight increase in fasting and peak PP plasma levels, in normal weight subjects when jogging. Consistent with these findings Sliwowski and co-workers (2001) identified a significant increase in plasma CCK and PP levels during a treadmill run to exhaustion and this was independent of feeding. In another study, O'Connor and colleagues (1995) investigated gut hormones in male and female marathon runners. They observed a significant increase in fasting plasma PP and GLP-1 levels after a race. Martins and team (2007) showed an increase in PP and GLP-1 plasma levels during and after acute bouts of moderate intensity exercise performed in a fed state. Another study of Martins and team (2010) demonstrated an increase in GLP-1 in the late postprandial phase after a 12 week exercise intervention in both males and females. In another study, Ueda *et al.* (2009a) indicated plasma levels of GLP-1 increased in obese and normal weight males during cycling exercise.

In summary, studies investigating total ghrelin responses to exercise have reported variable findings including increases, decreases and no change. Chronic exercise appears to increase total ghrelin concentrations when participants lose weight during the exercise training programme. The general consensus based on previous studies indicate that acylated ghrelin is suppressed during acute bouts of high intensity exercise and some evidence exist to show that acylated ghrelin is increased after chronic exercise training but the implications of these changes are uncertain. It is unclear if exercise induced changes in plasma total

ghrelin/acylated ghrelin affect subsequent food intake. Some of the variability in the findings reported in the literature may be due to the nature of the study participants (e.g. trained versus untrained, healthy weight versus overweight/obese, male versus female) and there is clearly scope for further research in this area. In contrast to research on ghrelin, the majority of studies examining appetite suppressing hormones suggest increases in plasma PYY, PP, CCK and GLP-1 in response to exercise while leptin and insulin tend to decrease in response to exercise training due to a loss of body fat and improvements in insulin sensitivity.

**Table 2.4 Effect of exercise on appetite hormones**

<b>Reference</b>	<b>Subjects</b>	<b>intervention</b>	<b>Results</b>
Broom <i>et al.</i> (2007)	Healthy males	60 min running versus resting	Plasma acylated ghrelin concentration was reduced during running
Broom <i>et al.</i> (2009)	Healthy males	Resistance exercise, aerobic exercise and control	Decreased ghrelin during aerobic and resistance exercise and increased PYY during aerobic exercise
Burns <i>et al.</i> (2007)	Healthy males & females	Treadmill running versus resting	No significant change in ghrelin plasma levels
Cheng <i>et al.</i> (2009)	Healthy males	Meal only without Exercise, Meal after Exercise., and Exercise after meal. Exercise = cycle ergometry	Increased PYY <sub>3-36</sub> during the meal after exercise and exercise after meal trials compared with meal only without exercise Increased ghrelin after exercise in the fasted state during the meal after exercise trial. Ghrelin also increased when the exercise was carried out after the meal, exercise after meal trial.
Erdmann <i>et al.</i> (2007)	Healthy males & females	1. Cycling for 30 min at 50 W and 30 min at 100 W 2. Cycling at 50 W for 30, 60 and 120 min 3. Resting	Ghrelin concentrations increased significantly at the lower intensity rather than the high intensity. Ghrelin level rose during all three durations of low intensity exercise.

**Table 2.4 Effect of exercise on appetite hormones (Continued)**

<b>Reference</b>	<b>Subjects</b>	<b>Intervention</b>	<b>Results</b>
Hagobian <i>et al.</i> (2008)	Healthy males & females	Cycle ergometer or treadmill	Ghrelin concentration decreased in response to glucose ingestion after exercise compared with no exercise.
Hagobian <i>et al.</i> (2009)	Overweight or obese males & females	<ol style="list-style-type: none"> <li>1. No ex. (energy balance),</li> <li>2. 4 days ex.( energy balance)</li> <li>3. 4 days ex. (energy deficit)</li> </ol>	Increased acylated ghrelin and decreased insulin responses to exercise in women compared with men.
Jürimäe <i>et al.</i> (2007a)	Highly trained male rowers	Rowing training session versus resting	<p>No change in plasma leptin</p> <p>Ghrelin concentration was increased 30-min post-exercise</p>
Jürimäe <i>et al.</i> (2007b)	Elite male rowers	Rowing ergometer test versus resting	<p>Ghrelin was significantly increased immediately after exercise</p> <p>Leptin decreased significantly immediately after exercise and remained significantly decreased after 30 min of recovery.</p>
Leidy <i>et al.</i> (2004)	Normal weight females	<ol style="list-style-type: none"> <li>1. Control (no ex.)</li> <li>2. Weight-stable exercisers</li> <li>3. Weight-loss exercisers</li> </ol>	Ghrelin concentrations increased significantly from pre- to post-intervention in the weight-loss exercisers. There was no significant change in ghrelin in the controls or weight-stable exercisers

**Table 2.4 Effect of exercise on appetite hormones (Continued)**

<b>Reference</b>	<b>Subjects</b>	<b>intervention</b>	<b>Results</b>
Martins <i>et al.</i> (2007)	Sedentary normal weight males & females	Cycling on a cycle ergometer for 60 min.	PYY, GLP-1 and PP levels were significantly increased during exercise and postprandially, No significant effect on postprandial levels of ghrelin.
Martins <i>et al.</i> (2010)	Sedentary overweight/obese individuals	Exercise 5 sessions/week, for 12 weeks	Significant decrease in fasting insulin and a significant increase in fasting plasma acylated ghrelin levels. Significant decrease in postprandial plasma insulin levels and a trend toward a rise in the late postprandial release of GLP-1 observed after exercise
Ueda <i>et al.</i> (2009b)	Obese & normal weight males	Cycling exercise versus resting	Increase in plasma levels of PYY and GLP-1, no change in plasma total ghrelin levels
Zoladz <i>et al.</i> (2005)	Normal-weight males	Incremental exercise test	No significant effect on plasma ghrelin and leptin levels

## **2.4 Exercise, appetite, food intake and gut hormones: studies comparing responses in males versus females.**

There are several studies that directly compared appetite responses to exercise in males and females. Staten (1991) investigated food intake responses to acute bouts of high intensity treadmill running in normal-weight male and female participants. The study observed a significant increase in energy intake in males but not in females although in both males and females the post-exercise energy intake was less than energy expended during exercise.

There is some evidence to suggest that exercise exerts different effects on appetite hormones in males compared with females. Hagobian and colleagues (2009) carried out a study on overweight and obese males and females using treadmill running. The study found that appetite was inhibited in males after moderate intensity exercise when energy was balanced while there was no change in the appetites of females regardless of their energy state, whether in deficit or balanced. The concentrations of some hormones involved in appetite regulation were altered in a direction expected to stimulate appetite in females i.e. acylated ghrelin increased and insulin decreased in females in response to exercise. In contrast there was no change in acylated ghrelin in males and insulin was only lower after the exercise-energy deficit condition not after the energy balance condition as in females (Table 2.5).

A few studies have examined the influence of chronic exercise training on appetite in males and females. One example is a study by Ambler and colleagues (1998) which examined non obese adolescent males and females aged 15-17 performing exercise such as running, aerobic dance and weight lifting for 5 weeks. Ambler and colleagues (1998) found that total energy expenditure was significantly greater than total caloric intake during the training period for both males and females. Sex differences in response to exercise training were observed when examining alterations in food intake and food selection. Self-reported food intake was increased in response to exercise in males but not females. In contrast females increased fat and reduced carbohydrate intake but this change was not observed in males. However, a study by Donnelly and colleagues (2003b) failed to detect any difference between males and females in terms of macronutrient intake changes in response to a 16 month exercise intervention.

There is some evidence to suggest that exercise is more effective as a method of losing weight for males than for females (Donnelly *et al.*, 2003a; Donnelly *et al.*, 2005). In the 16 month exercise intervention referred to in the previous paragraph exercise produced weight loss in males but not in females although there were indications that the exercise intervention prevented weight gain in the females (Donnelly *et al.*, 2003a). In an earlier study, Hickey and co-workers (1997) showed that females, but not males, had lower fasting insulin concentrations and lower leptin levels after 12 weeks of exercise training. These changes suggest a greater motivation to eat after exercise intervention in females compared with males. Table 2.6 summarises the findings from studies which have directly compared appetite, energy intake and gut hormone responses to acute exercise in males and females

As mentioned earlier, only a few studies investigating the effect of exercise on appetite response and food intake have been carried out on females. This is because the sex hormones involved in the menstrual cycle can potentially alter appetite responses and influence the findings of the studies. Over the years, research has demonstrated that the menstrual cycle has effects on appetite and food intake. However, it is difficult to evaluate existing literature concerning the changes in food intake during the menstrual cycle. While some have ignored the phases when measuring food intake across the whole menstrual cycle (Pelkman *et al.*, 2001), others combined the luteal phase (where estradiol levels are lowest) and the late follicular phase (where estradiol is at its highest) in their analyses, thereby obscuring any potential differences between the phases (Dalvit, 1981; Tarasuk & Beaton, 1991).

Nevertheless, there is evidence that the menstrual cycle affects appetite, such that energy intake is lower during the follicular than the luteal phase (Brennan *et al.*, 2009). Significant changes in food intake during the menstrual cycle have been documented in both human and non-human primates. Menstrual cycle dependent changes in women and in rhesus monkeys documented by food diaries or food weighing show that food intake is reduced in the follicular phase and increased during the luteal phase (Czaja, 1978; Rosenblatt *et al.*, 1980; Dalvit, 1981; Kemnitz *et al.*, 1984; Lissner *et al.*, 1988; Gong *et al.*, 1989; Lyons *et al.*, 1989; Buffenstein *et al.*, 1995; Barr *et al.*, 1995; Dye and Blundell, 1997; Reimer *et al.*, 2005). Evidence has suggested that regardless of body weight, women tend to consume



more calories from carbohydrates and protein during the luteal phase than the follicular phase (Buffenstein *et al.*, 1995; Chung *et al.*, 2010 and Reed *et al.*, 2008).

Pliner and Fleming (1983) studied self-reported food intake, body weight, and sweetness preference in 34 women. They found that food intake and body weight were significantly higher during the luteal phase than during the follicular phase, as measured at the midpoint of each phase (Pliner and Fleming, 1983). The mean caloric intake in the luteal phase was approximately 223 calories greater than the mean caloric intake of the follicular phase (Pliner and Fleming, 1983). Sixty-six per cent of the women demonstrated an increase in food intake in the luteal phase and 71% exhibited an increase in body weight (Pliner and Fleming, 1983).

These fluctuations are thought to be due to the hormonal changes which occur during the menstrual cycle. Initially, elevated food intake in the luteal phase can be attributed to increased concentrations of progesterone (Gilbert and Gillman, 1956) while the decrease in food intake later can be attributed to the appetite-suppressant effects of estradiol (Dalvit, 1981). Many biological, physiological and psychological systems exert effects on appetite. However, the methodology used within a study can influence the outcomes and conclusions. For example when food intake was not measured and dietary information was self-reported, studies have evidently shown significant differences; they found a significantly higher food intake during the luteal phase than during the follicular phase (Johnson *et al.*, 1994; Barr *et al.*, 1995; Bryant *et al.*, 2006; Buffenstein *et al.*, 1995). When food intake was measured and weighed, there was no significant increase of energy intake during the luteal phase of the menstrual cycle in one study (Fong and Kretsch, 1993) although fewer studies of this nature are available. Therefore studies can show different outcomes, depending on the methodology implemented in each study. Food intake in the studies presented in this thesis was controlled and weighed, not self-reported.

In summary, there is some evidence that acute and chronic exercise may exert different effects on energy intake in males and females. There is evidence that males are more likely to increase their food intake in response to acute and chronic exercise than females. However, this contradicts the expectation that females are more likely to increase food intake in order to compensate for the energy expenditure during exercise, due to the

necessity of maintaining sufficient stores of body fat for pregnancy and child rearing (Frisch, 2004). Additionally, the monthly menstrual cycle and sex hormones has been a major reason why investigators have excluded females from exercise studies. In essence, some suggestions in the literature indicate that males are more likely to lose weight with exercise than females but evidence is very limited and it is clear that further research is required to clarify whether or not differences really exist between the sexes in terms of appetite, energy intake and gut hormone responses.

**Table 2.5 Comparison of appetite, energy intake and/or gut hormone responses to exercise in males and females**

<b>Reference</b>	<b>Subjects</b>	<b>Intervention</b>	<b>Food intake</b>	<b>Hunger</b>	<b>Hormones</b>
Burns <i>et al.</i> (2007)	Normal weight males & females	Treadmill running versus resting	Not tested	Suppressed during and 1h after exercise	No significant change in plasma total ghrelin or insulin for males, females or both sexes combined.
Hagobian <i>et al.</i> (2009)	Overweight/obese males & females	Moderate intensity treadmill running 1. Energy deficit 2. Energy balance	High fat test meal (fixed energy content)	Appetite inhibited in males after exercise when energy was balanced.  No change in females' appetite regardless of energy state.	Acylated ghrelin increased, insulin decreased in females in response to the initiation of exercise. No change of acylated ghrelin in males, insulin was lower after energy deficit
Staten (1991)	Normal weight males & females	5 days treadmill running for 1h/day (70% of VO <sub>2</sub> max)	Increased food intake for males; females' intake unchanged. Both males and females in negative energy balance.	Not tested	Not tested

**Table 2.6 Comparison of appetite, energy intake and/or gut hormone responses to chronic exercise in males and females**

Reference	Subjects	Intervention	Hormones & Hunger	Duration of the study	Food intake
Ambler <i>et al.</i> (1998)	Non obese males & females aged 15-17 yrs	Running, aerobic dance, competitive sports (for example, basketball) and occasional weight-lifting.	Not tested	5 weeks	Increased self-reported energy intake in males but not females, Increased fat intake and reduced carbohydrate intake only in females.
Donnelly <i>et al.</i> (2003a)	Overweight & moderately obese males & females	Treadmill walking at 55-70% of VO <sub>2</sub> max performed for 45 min/d, 5 d/week	Not tested	16 months	Exercise produced weight loss in males and prevented weight gain in females.
Donnelly <i>et al.</i> (2003b)	Overweight & moderately obese males & females.	Treadmill walking at 55-70% of VO <sub>2</sub> max performed for 45 min/d, 5 d/week	Not tested	16 months	There were no significant differences for men or women between the exercise and control groups in fat, carbohydrate, or protein intake
Hickey <i>et al.</i> (1997)	Healthy sedentary males & females	Over ground and/or treadmill walking and/or running for 45 min/d, 4 d/week at 85% of maximal heart rate	Leptin and fasting insulin decreased significantly in females, but not in males	12 weeks	Caloric intake increased in males No change in females

### **3 General methods**

This chapter describes the experimental methods used, the recruitment of participants and the biochemical analyses that were performed in the studies described in this thesis. Approval was sought for and received from Loughborough University's Ethical Advisory Committee for each study described in this thesis and the University's Code of Practice on Investigations Involving Human Participants was followed for each study. Moreover, written informed consent forms were distributed to and signed by all participating individuals before each study was carried out (Appendix A).

#### **3.1 Participants**

The participants involved within this research were primarily recruited from within a 3 mile radius of Loughborough University or from within the University itself. The following awareness-raising activities were conducted in order to recruit potential participants: posters were displayed in local areas (e.g. in University common rooms), emails were distributed and social networks (e.g. Facebook) were utilised; word of mouth was also used. The advertisements consisted of information on the purpose of study, the experimental procedures to be used and the potential risks and discomforts that might be experienced. It was also stressed to participants that this research was unpaid and undertaken on a voluntary basis and that they could withdraw from the research at any time without being obliged to give an explanation for their withdrawal.

Participants consisted of both males and females, between the ages of 18 – 50 years. Participants were recreationally active and had to have the ability to complete the exercise regimes employed within this research.

To identify risks, participants were screened using a health screen questionnaire and were asked to fill in a physical activity questionnaire. During this phase, participants were given a chance to ask any questions relating to the studies. Resting blood pressure was also measured prior to the exercise tests. Participants were also encouraged to warm up before each exercise trial and to cool down afterwards.

**In order for participants to be eligible, the following criteria had to be met:**

- Participants could be either male or female;
- They could have no history of cardiovascular disease, metabolic disease or dyslipidaemia;
- They could not currently be dieting, had to have a stable weight over the previous three months and could not display any extreme dietary habits;
- Only non-smokers were included;
- Female participants were not to be pregnant;
- They had to have the ability to meet and complete the study's demands;
- They had to be recreationally active (except for Study 1, Chapter 4);
- They could not be taking any medication or drug that might skew the results (e.g. anabolic steroids, marijuana, amphetamines, thyroid prescription drugs);
- They also required a BMI (Body Mass Index) of  $< 30 \text{ kg m}^{-2}$  (except for Study 1, Chapter 4).

### **3.2 Anthropometry**

It was necessary to measure participants' human physical characteristics i.e. height, weight, waist circumference, hip circumference, skinfold thicknesses and body fat.

Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca Ltd, Germany). Body mass was measured to the nearest 0.1 kg with the use of a digital scale (Seca Ltd, Germany) . Participants wore light clothing and bare feet during body mass measurements. The body mass index (BMI) was calculated by dividing participants' weight (in kilograms) by the square of their height (in metres). Waist circumference was measured using an inelastic poly fibre measuring tape; this was determined by taking the measurement at the end of expiration at the narrowest part of the torso.

Skin fold thickness was assessed to the nearest 0.2 mm utilising skinfold callipers (Harpenden skinfold caliper, Baty international, England). This was necessary in order to estimate total body fat and comprised subcutaneous fat samples from four anatomical locations: biceps, triceps, subscapula and suprailiac. This process was duplicated and was

achieved with participants in a standing position. Three samples were taken from the right side of the body, a mean of the two measurements closest together were used for the final value for each skinfold site. Body density was calculated by applying the predictive equations of Durnin and Womersley (Durnin and Womersley, 1974). The Siri equation (Siri, 1956) was then applied to calculate body fat percentage. The measurements were made by rotating through the sites in order for the skin to regain its texture before conducting the next set of samples.

### **3.3 Measurement of heart rate**

Heart rate was measured during preliminary exercise tests and during main exercise trials using short range telemetry (Polar F4, Polar Electro, Kempele). Values were recorded five times over the course of one minute sampling periods and the mean of the five values was used to represent the average heart rate at that point in time.

### **3.4 Rating of perceived exertion (RPE)**

Ratings of perceived exertion during exercise were assessed using the Borg Scale (Borg 1973), which measures exercise intensity on a scale ranging from 6 (rest/no exertion) to 20 (exhaustion/maximum exertion).

### **3.5 Arterial blood pressure assessments**

Resting arterial blood pressure measurements were taken in triplicate during the preliminary screening with participants in a seated position. This measurement was taken using an automatic sphygmomanometer (BP-Omran M5-I, Intelli Sense, Matsusaka, Japan) and was necessary to calculate average resting blood pressure after a relaxation period of 10 minutes in line with standard guidelines (Williams *et al.*, 2009).

## **3.6 Exercise tests**

### **3.6.1 Treadmill running: submaximal oxygen uptake test**

In studies 1, 3 and 4 (refer to Chapters 4, 6 and 7) an incremental submaximal treadmill running test was used to determine the relationship between running speed and oxygen consumption. This test involved a total of 16 minutes of treadmill running broken down into four, four-minute stages. These stages exercised participants through a range of intensities from moderate to hard effort but not maximum. The test began with participants running at a speed that was determined by their fitness ( $6.0+ \text{ km}\cdot\text{h}^{-1}$ ), with the speed gradually increasing after each 4-minute stage by  $0.5 - 1.5 \text{ km}\cdot\text{h}^{-1}$ . Within the final minute of each stage, expired air was collected into Douglas bags (Plysu Protection Systems, Milton Keynes, UK) and RPE was recorded. Heart rate was monitored and recorded continuously during the test. Upon completion of the test oxygen consumption was plotted against the running speed at each stage to determine the submaximal relationship between running speed and oxygen consumption.

### **3.6.2 Treadmill running: maximum oxygen uptake test**

The maximum oxygen uptake test commenced 15 - 20 minutes after the submaximal treadmill test. The test began at a gradient of  $3.5^\circ$  and this gradient was then increased by  $2.5^\circ$  at 3-minute intervals; this was repeated until the participant reached exhaustion. An expired air sample was collected for 1 minute, 1:45 minutes into each 3-minute interval: i.e. at 1:45 to 2:45, 4:45 to 5:45 and so on. Participants were instructed to signal one minute before they reached exhaustion in order to collect a final sample of expired air. Throughout this phase, strong verbal encouragement was given in order to capture the final sample with confidence. On completing the test, oxygen consumption and carbon dioxide production were determined from the collected expired air samples and the highest value attained in terms of oxygen uptake was noted. In order to confirm a true maximal value, the following criteria were used (Cooke, 2001):



- A plateau in oxygen consumption;
- A heart rate within plus or minus 10 beats·min<sup>-1</sup> of the age-predicted maximum heart rate (220 – age);
- A respiratory exchange ratio of  $\geq 1.15$ ;
- A rate of perceived exertion of 19 or 20;

Once the maximal oxygen uptake value was determined this value was used together with the running speed/oxygen consumption data from the submaximal treadmill test to determine the running speed necessary to elicit the desired percentage of maximum oxygen uptake during main trials. As a result participants began the main trials at that desired speed, adjusting the speed where necessary to maintain the required percentage of maximum oxygen uptake throughout.

### **3.6.3 Cycle ergometer: maximal oxygen uptake test**

In Chapter 5, Study 2, participants performed an incremental cycling exercise test, using a cycle ergometer (Excalibur, Lode, Groningen, The Netherlands), in which they were instructed to maintain 60 rpm in a seated position. The test involved participants undertaking a protocol consisting of 3-minute interval stages, at a starting intensity of 35 W and gradually increasing the work rate by 35 W at the end of each 3-minute stage; this was repeated until the participants experienced volitional fatigue. Expired air was collected using Douglas bags; this air was collected between minutes 1:45– 2:45 of each stage.

Each participant's heart rate and RPE were recorded (as described in Sections 3.3 and 3.4) during the collection of the expired air. Participants were instructed to indicate one minute before they reached exhaustion so that a final sample of expired air could be collected. On completion of the test, oxygen consumption and carbon dioxide production were determined from the collected expired air samples. The same criteria were used to determine maximum oxygen uptake as described for the treadmill running maximum oxygen uptake test.

### 3.7 Expired air analysis

Oxygen consumption and carbon dioxide production were assessed using Douglas bags (Plysu Protection Systems, Milton Keynes, UK). The collected expired air samples were analysed using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser (Series 1400, Servomex, Crowborough, East Sussex, UK). Prior to the analysis of the expired air, certified reference gasses were used to calibrate the analysers. Expired gas volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK). The gas meter was calibrated on a regular basis utilising a three-litre syringe (Series 5530, Hans Rudolph Ince, Kansas City, Missouri, USA). Expired air temperatures were measured using a thermistor during evacuation (Edale, type 2984, Model C, Cambridge, UK). A Fortin barometer was used in order to measure barometric pressures (F.D. and Company, Watford, UK). Oxygen consumption and carbon dioxide production values were corrected to standard temperature and pressure dry.

### 3.8 Calculation of energy expenditure

Energy expenditure and substrate oxidation were calculated by measuring oxygen consumptions and carbon dioxide productions (Frayn, 1983). The equations used were as follows:

Energy expenditure (kJ) = (fat oxidized (grams)\*39) + (CHO oxidized (grams)\*17) where,  
Fat oxidation rate ( $\text{g}\cdot\text{min}^{-1}$ ) =  $(\text{VO}_2 \text{ L}\cdot\text{min}^{-1} - \text{VCO}_2 \text{ L}\cdot\text{min}^{-1})/0.57$  and  
Carbohydrate oxidation rate ( $\text{g}\cdot\text{min}^{-1}$ ) =  $(\text{VO}_2 \text{ L}\cdot\text{min}^{-1} - (1.989*\text{fat oxidized in grams}))/0.828$

### 3.9 Physical activity and dietary control

Participants had to standardise their diets before they undertook the experimental trials because food and exercise can influence gut hormone levels, leading to participants' appetites being interrupted on the morning of the experiment (Chandarana *et al.*, 2009). Therefore, the experimental studies required participants to follow a specific diet where a replicate of food intake was consumed 24h prior to the experimental sessions. Participants

completed a weighed food record to identify the food items that were consumed. During this phase, participants were advised to refrain from consuming any beverages containing alcohol or caffeine and to refrain from undertaking physical activity. Participants were also instructed to refrain from eating after the hours of 22:00 – 23:00; however, water was an exception in order to prevent participants from becoming dehydrated.

Chapter 4 (Study 1) used the Global Physical Activity Questionnaire (GPAQ) version 2.0 as a method of data collection to assess the energy cost of participants' physical activity (Appendix B). It estimated the frequency by days and the time by minutes/hours spent doing moderate and high-intensity physical activity over a normal week in three domains: activity at work (paid and unpaid, including study/training and household chores), active form of transportation (walking and cycling), and physical activity in leisure time. It is derived from the International Physical Activity Questionnaire (IPAQ). GPAQ is validated and widely used to estimate physical activity patterns, especially in developing countries (Armstrong and Bull, 2006). For example, in a study comprising 8 developing countries, both GPAQ and IPAQ were used on at least two occasions. Criterion validity was assessed over 7 days, using an objective measure (pedometer or accelerometer). Concurrent validity between IPAQ and GPAQ revealed a moderate to strong positive relationship (.45 to .65). GPAQ demonstrated a moderate to strong positive correlation with IPAQ. The reliability coefficients were considered to be moderate to substantial in strength (Kappa ranged from .67 to .73; Spearman's rho .67 to .81) (Bull *et al.*, 2009). In another study by Trinh *et al.*, 2009 to investigate the reliability of the GPAQ, the score of the test-retest reliability revealed repeatability correlations of .69 after 2 weeks and .55 after 2 months.

### **3.10 Appetite assessment**

For each of the studies mentioned in this thesis, participants' appetites were assessed on a regular basis using visual analogue scales (Flint *et al.*, 2000). These scales involved a 100 mm continuum with descriptions of potential answers on either side. Appetite perceptions assessed included hunger, fullness, satisfaction and prospective food consumption. Once participants had rated their appetite on the scale, this was then quantified by measuring the horizontal distance from the left hand side of the scale to the point where the participant rated their appetite (Appendix C).

### 3.11 Ad libitum buffet meals and standardised snacks

Standardised snacks were made available during the various studies described within this thesis. These were standardised according to body mass. For an individual weighing 70 kg, the snacks provided 1092 kJ (260 kcal) of energy, 6 g of fat, 4 g of protein and 48 g of carbohydrate (Studies 3 and 4; Chapters 6 and 7). The amounts provided were adjusted up or down for participants who were heavier or lighter than 70 kg.

During the testing phase, participants were also given access to a full buffet from which they were allowed to consume as much food as they desired *ad libitum* (Studies 2, 3 and 4; Chapters 5, 6 and 7). The buffet was made up of items (Appendix D for full food list), which reflected participants' food preferences; they had previously stated their particular food preferences by completing a questionnaire (Appendix E). The food preference questionnaire included different types of food and was organised as a Likert scale, with the scale ranging from one (lowest figure/extreme dislike) to ten (highest figure/extreme liking). From the analysis of the questionnaire, foods that were selected by participants were presented to them when required. However, if participants did not like four or more items on the buffet list they would automatically be excluded from the study.

During testing, a cold buffet was presented to participants. The foods that were chosen and given out were a mix of those with protein, fat and carbohydrate content. This was necessary in order to meet the required macronutrient preferences. Once the buffet was accessible, participants were told that they had 30 minutes to consume food until they were satisfied. Participants were kept in isolation throughout the buffet process in order to limit social factors from influencing food intake.

Prior to the food consumption phase, all items were weighed. This was necessary in order to analyse the consumed food, which was the difference between the remaining weight and the initial weight of the food contents. Manufacturers' values were used to assess the energy and macronutrient content of the items consumed.

### **3.12 Time cues, environmental temperature and humidity**

For the preliminary tests and the main trials, environmental temperature and humidity were assessed using a hand-held hygrometer (Omega RH85, Manchester, UK). Participants were also told that mobile phones were not permitted during test sessions in the laboratory.

### **3.13 Blood sample collection**

Roughly 30 minutes before commencing the main trials, participants were relaxed in a semi-supine position whilst a cannula (Venflone, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein (Studies 3 and 4; Chapters 6 and 7). Furthermore, within Study 1 (Chapter 4), in order to minimise participants' discomfort in the main trial, a venepuncture was used to collect a single fasting blood sample. In Study 2, Chapter 5, four venepuncture samples were taken from participants in each trial they undertook.

With regard to venous blood samples, these were collected in pre-cooled 4.9 or 9 mL potassium-ethylenediamine tetra-acetic acid (EDTA)-coated monovettes (Sarstedt, Leicester, UK) with a multi-adaptor (Sarstedt, Leicester, UK). After the collection of each sample, the cannula was maintained by flushing it with 10 mL of non-heparinised saline (0.9% w/v sodium chloride, Baxter Healthcare Ltd, Norfolk, UK). In order to prevent any dilution of subsequent samples, any residual saline was removed directly prior to collection, utilising a 2 mL syringe. Five minutes prior to the collection of blood samples, participants lay down and maintained a semi-supine position; this was necessary in order to prevent postural changes in plasma volume. Exceptions to the blood sampling process occurred during the performance of the treadmill running exercise, in this situation participants straddled the treadmill whilst the procedure was carried out, this took no more than one minute.

Once blood samples were collected in 9 mL EDTA monovettes, they were immediately spun in a refrigerated centrifuge at 3000 rpm for 10 minutes (Heraeus Labofuge 400 R, Thermo Fisher Scientific Inc., Loughborough, UK) at four degrees Celsius; this caused the plasma to separate from the blood cells. The plasma supernatant was then divided into Eppendorf tubes (Sarstedt, Leicester, UK). Samples for glucose and triacylglycerol were stored at - 20°C; these were analysed at a later date.

Venous blood samples were collected separately and put into 4.9 mL monovettes for the assessment of plasma acylated ghrelin concentrations. In order to prevent protease enzymes from degrading the acylated ghrelin, these monovettes contained EDTA and a 50  $\mu$ L solution containing potassium phosphate buffer, p-hydroxymercuribenzoic acid and sodium hydroxide. Immediately after the collection of the samples, the monovettes were spun at 3500 rpm for 10 minutes in a refrigerated centrifuge (GS-15R Centriuge, Beckman Coulter, Fullerton, USA), again at four degrees Celsius. After this the supernatant was aliquoted into two plain storage tubes and 100  $\mu$ L of hydrochloric acid (1M) was added per mL of plasma. The samples were subsequently spun again for approximately five minutes at 3500 rpm to achieve thorough mixing; they were then stored at  $-20^{\circ}\text{C}$  for analysis at a later date.

Additional 2 mL blood samples were collected (Study 3; Chapter 6) at intervals to measure circulating concentrations of PYY<sub>3-36</sub>. In order to maintain peptide integrity, pre-chilled syringes consisting of dipeptidyl- peptidase-4 inhibitor (10  $\mu$ L.mL<sup>-1</sup>) (Millipore Ltd, Watford, UK) were utilised in collecting the samples. Once these samples had been collected, they were mixed by gentle inversion and dispensed into pre-chilled EDTA tubes which contained aprotinin (Nordic Pharma Ltd, Reading, UK) at a final concentration of 500 KIU.mL<sup>-1</sup>. The samples were then spun in a refrigerated centrifuge at 3500 rpm for approximately 10 minutes at 4°C. Plasma supernatant was then aliquoted into 2 mL Eppendorf tubes before being stored; the tubes containing the plasma supernatant were not stored until they were frozen (samples were frozen at  $-20^{\circ}\text{C}$  then stored at  $-80^{\circ}\text{C}$ ). All the frozen plasma samples that were taken during the studies were analysed within a six-month period.

Duplicated 20  $\mu$ L blood samples were collected in micropipettes at each sampling point and triplicate 20  $\mu$ L blood samples were placed in heparinised micro haematocrit tubes at each sampling point to assess blood haemoglobin and haematocrit concentrations respectively.

### **3.14 Blood sample analysis**

#### **3.14.1 Estimating changes in plasma volume**

Haemoglobin and haematocrit were measured in order to estimate changes in plasma volume (Dill and Costill, 1974). Haemoglobin was determined using the cyanmethaemoglobin approach employing an ultra-violet spectrophotometer (CECIL CE 1011, Cecil Instruments Ltd., Cambridge, UK); the samples were assessed in duplicate. Haematocrit was assessed using a micro-litre-haematocrit centrifuge (MIKRO, 20, Andreas Hettich GmbH and Co.KG, Tuttlingen, Germany) in triplicate.

#### **3.14.2 Glucose and triacylglycerol concentrations**

Plasma concentrations of triacylglycerol and glucose were obtained using an automated bench top analyser (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). In order to ensure precise analysis, internal quality controls exhibiting normal and pathological values were run prior to sample analysis.

#### **3.14.3 Plasma PYY 3-36 concentrations**

Plasma PYY<sub>3-36</sub> concentrations were analysed using a radioimmunoassay kit (LINCO Research, Missouri, USA.) In order to achieve precise results from the analysis phase, internal quality controls (high and low values) were used. This analysis was performed at University College London under the supervision of Dr Rachel Batterham.

#### **3.14.4 Plasma acylated ghrelin concentrations**

An enzyme-linked immune sorbent assay kit was used to determine plasma acylated ghrelin concentrations (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader (Expert Plus, ASYS, Eugendorf, Austria). Owing to the minimal plasma dilution (1:5), the limits of detection in the samples was 1.5 pg/mL. In order to achieve accurate analysis internal quality controls were used with each assay.

### **3.14.5 Precision of analysis**

Samples from each participant were analysed in the same run in order to eliminate any inter-assay variations. Some single plasma samples were analysed 5 times in order to calculate the coefficient of variation for each assay within the batch. Coefficient of variation values for each assay are stated within the methods' section of each experimental chapter.

## **3.15 Saliva sample analysis**

### **3.15.1 Progesterone and 17 $\beta$ -Estradiol**

Progesterone and 17 $\beta$ -estradiol concentrations were determined using commercially available enzyme immunoassay kits, (Salimetrics, Salivary progesterone enzyme immunoassay kit, USA and Canada) (Salimetrics High Sensitivity, Salivary 17 $\beta$ -estradiol enzyme immunoassay kit, USA and Canada) respectively.

The intra assay co-efficient of variation was 1.7% for progesterone and 2.2% for 17  $\beta$ -estradiol.

## **3.16 Statistical analysis**

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows (SPSS Inc., Chicago, IL, USA), software version 20.0. Where necessary, the 'Trapezoidal Rule' was applied to calculate area under the curve (AUC) values. Differences between fasting values or AUC values were examined using paired t-tests (for comparison of two sets of values) or one-way ANOVA (for comparison of three or more sets of values). Repeated measured two-way factor ANOVA was applied to examine differences between trials over time for circulating acylated ghrelin, PYY<sub>3-36</sub>, glucose and triacylglycerol concentrations, as well as for appetite responses. A three-way repeated measured ANOVA was used (Chapter 7; Study 4) to assess differences in variables among the trials with the three main effects being sex (male versus female), trial (exercise versus control) and time. Where appropriate, post-hoc comparisons were made using the



Bonferroni method. In order to examine relationships between variables, the Pearson product moment correlation coefficient was used. Statistical significance was accepted at the 5% level. Results are presented as mean  $\pm$  SD, unless stated otherwise.

The sample sizes used in the studies within this thesis were determined based on past literature. Specifically, in repeated assessments, eight participants were deemed sufficient for the detection of significant differences in appetite (AUC 10%) (Flint *et al.*, 2000) and differences in energy intake (240 kcal) (Gregersen *et al.*, 2008). Furthermore, a sample size of nine was deemed sufficient to detect significant differences in acylated ghrelin with exercise (Broom *et al.*, 2007).

Effect sizes are presented in some chapters within this thesis. Effect size can be defined as ‘the degree to which a phenomenon is present in the population’ (Cohen, 1988: 9 - 10). Given that studies are usually based on a sample of the entire population of interest to estimate the size of the relationship between variables, an effect size refers to a sample-based estimate of the size of the relationship between variables (Rosenthal, 1994). As the name suggests, an effect size estimate can place an easily interpretable value on the direction and magnitude of an effect of a treatment; the difference between 2 groups; or any other numerical comparison or contrast (Faraone, 2008).

There is no standard interpretation of an effect size but one of the most popular approaches used is Cohen’s *d*, where the value of the standardised mean difference statistic is obtained (Cohen, 1988). The effect size is then characterised based on the categories below:

< 0.2 = Trivial

0.2 – 0.49 = small

0.5 – 0.79 = moderate

0.8 + = large.

These categories were used to interpret the effect sizes presented within this thesis.

## **4 Study 1: Relationship between physical activity, physical fitness, body composition, resting metabolic rate, appetite perceptions, dietary restraint and plasma acylated ghrelin in men and women.**

### **4.1 Introduction**

A fundamental principle of nutrition and metabolism is that body weight change is associated with an imbalance between the energy content of food eaten (energy intake) and the energy expended by the body (energy expenditure) (Hall *et al.*, 2012). Energy intake consists of all ingested foods and beverages with an energy value while energy expenditure consists of resting metabolic rate (RMR), the thermic effect of foods (TEF), spontaneous physical activity (SPA), and exercise (Donnelly and Smith, 2005). Energy balance refers to an equal amount of energy intake and energy expenditure. Weight gain occurs when energy intake exceeds energy expenditure and to induce weight loss, a negative energy balance must be evoked (Donnelly and Smith, 2005).

Aerobic exercise is a tool used for body fat loss in both humans and animals, according to studies by Donnelly *et al.* (2003a) and Oscai *et al.* (1971). Interestingly, they discovered that the use of regular physical activity for fat loss is more effective in men than in women. This suggests that, in response to aerobic exercise training, women are able to more accurately match energy intake with energy expenditure than their male counterparts and thus preserve body fat (Hagobian and Braun, 2010). In contrast, during exercise training, men do not sufficiently increase energy intake to balance the new higher energy expenditure and therefore lose body fat (Hagobian and Braun, 2010). To address the questions on “exercise versus energy status” and the “sex differences in energy-regulating hormones”, Hagobian *et al.* (2009) assessed the effects of exercise on energy-regulating hormones in previously inactive, overweight/obese men and women. Results revealed sex differences in the way that exercise changed energy-regulating hormones and appetite.

In response to exercise without energy added back to the diet, women had higher concentrations of acylated ghrelin and lower concentrations of insulin, both of which would be expected to stimulate energy intake (Hagobian *et al.*, 2009). When dietary energy intake was increased to maintain energy balance, the pattern of response in acylated ghrelin and

insulin concentrations was diminished but persisted, implying some independent effects of exercise (Hagobian *et al.*, 2009). However, exercise had less impact on these hormones in men. Acylated ghrelin concentrations did not change in men, regardless of energy status (Hagobian *et al.*, 2009). Insulin concentrations were lower in the energy deficit condition in men, but this effect was completely absent when dietary energy was increased to restore energy balance (Hagobian *et al.*, 2009).

Better matching of energy intake to energy expenditure in response to physical activity may be driven by the critical relationship between energy balance and reproductive success in women (Hagobian and Braun, 2010). For example, energy deficit in women suppresses ovulatory cycles, prevents gonadotropin-releasing hormone secretion, lessens pulsatility of luteinizing hormone, and stops copulatory behavior (Wade and Jones, 2004; Loucks and Thuma, 2003). In men, however, energy deficiency appears to have no major effect on reproductive success. Therefore, the higher acylated ghrelin concentrations and lower insulin/leptin concentrations that women experience in response to physical activity may be a mechanism to combat energy deficiency, defend body fat stores, and preserve reproductive function (Hagobian *et al.*, 2009). Although more work needs to be done, the evidence to date suggests that sex differences in the way regular physical activity impacts on energy regulating hormones and appetite may lead to different patterns of food intake and, subsequently, different effects on body fat loss (Hagobian and Braun, 2010).

Due to the orexigenic properties of ghrelin, it is reasonable to examine the association between exercise, hunger sensations, food intake and plasma ghrelin levels, since ghrelin could be part of a regulatory system that attempts restore energy balance after a preceding period of energy expenditure (Erdmann *et al.*, 2007). Many studies have also demonstrated that exercise does not excessively increase appetite and that vigorous exercise may lead to a temporary suppression of appetite, also known as ‘exercise-induced anorexia’, that leads to a short-term negative energy balance (Stensel, 2011). Due to ghrelin’s unique appetite-stimulating properties, several efforts were made to determine whether exercise-induced anorexia is related to lowered concentrations of ghrelin (Stensel, 2011). Initially, this did not appear to be the case because several studies observed that ghrelin concentrations were unaffected by acute bouts of exercise (Burns *et al.*, 2007, Dall *et al.*, 2002). However, these studies measured total ghrelin concentrations where more recent studies have shown that

acylated ghrelin (the form of ghrelin thought to be responsible for appetite stimulation) is suppressed during running and resistance exercise (Broom *et al.*, 2009; Broom *et al.*, 2007).

The majority of studies which have examined vigorous exercise have noted a reduction of subjective hunger sensations without associated changes of food and energy intake after exercise (Thompson *et al.*, 1988; Imbeault *et al.*, 1997; King *et al.*, 1996; King *et al.*, 1997). However, some studies showed that high-intensity exercise can stimulate food intake (Pormaleau *et al.*, 2004; Verger *et al.*, 1992; Verger *et al.*, 1994) while others have observed a suppressive effect of exercise (Westerterp-Plantenga *et al.*, 1997; Kissileff *et al.*, 1990). Exercise of low and moderate intensity does not appear to influence food intake or hunger sensations (Thompson *et al.*, 1988, Imbeault *et al.*, 1997, King *et al.*, 1994, Pomerleau *et al.*, 2004, Kissileff *et al.*, 1990, Reger *et al.*, 1986). In obese women, moderate intensity physical activity has been shown to reduce appetite and raise satiety and fullness perceptions while subsequent food intake was not affected (Tsofliou *et al.*, 2003).

Since in previous studies an increase of food intake was observed after 2 hours of exercise (Verger *et al.*, 1992, Verger *et al.*, 1994) and shorter time periods of 60 min or less were largely ineffective, Erdmann *et al.*, (2007) aimed to examine the influence of exercise intensity and duration on total ghrelin levels and food intake. It was discovered that moderate intensity exercise of 50 W has no effect on appetite/hunger sensations and food intake except for a stimulation of food intake after a prolonged exercise session of 2 hours (Erdmann *et al.*, 2007). However a study, in which subjects carried out bouts of brisk walking exercises had demonstrated that there was no elicited responses in acylated ghrelin, appetite or energy intake, despite influences on moderate energy deficit (King *et al.*, 2010b).

In addition to studies exploring the impact of exercise on hunger and energy intake, other studies have also examined its effects on body composition. For example, Donnelly *et al.*, (2003a) found that exercise prevented weight gain in women and produced weight loss in men. Men in the exercise group had significant decreases in weight, body mass index (BMI) and fat mass compared with controls while women in the exercise group maintained baseline weight, BMI, and fat mass, while the controls showed significant increases in

BMI, and fat mass at 16 months (Donnelly *et al.*, 2003a). There were no significant changes in fat-free mass in either men or women; but both had significantly reduced visceral fat (Donnelly *et al.*, 2003a).

Other studies have used skin-fold testing or hydrostatic weighing to measure the percent body fat of individuals participating in varying levels of exercise, ranging from sedentary individuals to competitive athletes (Elder *et al.*, 2007). Activities varied in intensity and duration, including running, bicycling, swimming, gymnastics, ice-skating, and weight lifting. Results showed that although men have lower percent body fat than women at any given level of exercise energy expenditure, the association is stronger in women such that the difference in body fatness between men and women is smaller in individuals who are more highly active (Elder *et al.*, 2007).

Resting metabolic rate (RMR) is an important factor when assessing the nutritional status in a patient: for example, it is used to calculate the energy requirements of a patient who needs parenteral or enteral nutrition (Brandi *et al.*, 1988, MacFie, 1984). The information on resting energy expenditure is also required to calculate the energy needs at a population level and for this, the Food and Agriculture Organization, World Health Organization and United Nations-UNICEF (FAO/WHO/UNU) have established formulas for predicting resting metabolic rate (Donnelly and Smith, 2005).

Although RMR decreases during energy restriction, there is substantial evidence that RMR is preserved when weight loss is caused by exercise (Wilmore *et al.*, 1998). Therefore, it is believed that the components of energy balance most likely to be responsible for compensation during exercise for weight loss are energy intake and spontaneous physical activity (Donnelly and Smith, 2005). For example, Donnelly *et al.* (2003b) observed a small increase in weight in response to several exercise sessions over a 16-month period in women, during which energy intake was monitored for six, 2-week periods in a cafeteria, and energy and macronutrient content were calculated using weigh-and-measure techniques. Additionally, 24-hour energy expenditure was measured using the doubly labeled water method, it was discovered that women had no changes in the energy or macronutrient content of their diet and experienced an increase in 24-hour energy expenditure, yet no weight loss occurred (Donnelly and Smith, 2005). Any compensatory

mechanisms for increased energy expenditure of exercise by other components of energy expenditure (i.e., decreased resting metabolic rate, SPA) were not sufficient enough to diminish the effects of exercise as evidenced by the increase in 24-hour energy expenditure and thus, in women, compensation through increases in energy intake is suspected (Donnelly and Smith, 2005).

In summary, exercise has an effect on weight loss, but there is some evidence to suggest that its impact may differ between males and females. This may be due to differences in the way plasma acylated ghrelin responds to exercise in men and women but limited research is available to support this hypothesis. The purpose of the present study was to compare fasting plasma acylated ghrelin concentrations in men and women, to see whether values differ between the sexes and to examine whether plasma acylated ghrelin concentrations are related to body composition, RMR, maximum oxygen uptake and appetite perceptions in men and women.

## **4.2 Methods**

### **4.2.1 Participants**

To investigate the relationship between physical activity, physical fitness, body composition, resting metabolic rate, appetite perceptions, and appetite hormones in men and women, tests were conducted on 34 male and 33 female volunteers. Participants were aged between 19 and 46 years. The selected volunteers were non-smokers, not following a strict diet, and had no personal history of cardiovascular disease, hypertension, metabolic disease or dyslipidaemia. Females were studied in the follicular phase (1 to 14 days) of the menstrual cycle based on their menstruation date. All examinations were performed with the approval of the Loughborough University Ethical Advisory Committee.

### **4.2.2 Testing day 1**

Participants visited the laboratory to undergo screening and exercise testing. Health status, and dietary habits were assessed using questionnaires.

#### **4.2.2.1 Anthropometry**

The participants' height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany) and body weight was measured to the nearest 0.01 kg using a balance-beam scale (Avery Industrial Ltd, Leicester, UK). Body mass index was calculated as the weight of the participant in kilograms divided by the square of their height in meters. Waist circumference was measured from the widest part of the torso between the xiphoid process of the sternum and the iliac crest. Measurements of skinfolds were taken according to the procedure mentioned within the methodology (Section 3.2). The samples were taken from 4 locations; biceps, triceps, subscapula and suprailiac.

#### **4.2.2.2 Eating behavior**

The three factor eating questionnaire was used to assess participants' eating behavior (Appendix F). The following values were used as cut-off points for dietary restraint (Stunkard *et al.*, 1985): 0-10 = low restraint, 11-13 = high restraint, 14-21 = clinical range (eating disorder i.e. borderline anorexia nervosa).

#### **4.2.2.3 Physical activity**

The global physical activity questionnaire (GPAQ) was used for collecting information about participants' physical activity (see the General Methodology, Section 3.9). Physical activity was assessed by a unit called METs (metabolic equivalents of task). One MET is defined as the energy required for a person to sit quietly or at rest.

#### **4.2.2.4 Exercise test**

A treadmill running maximum oxygen uptake test was done according to the procedure described in the general methodology (Section 3.6.2). This test was done to evaluate participants' physical fitness by determining her/his  $VO_2$  max. Participants were given at least two days to recover from the  $VO_2$  max test before undergoing the 2<sup>nd</sup> visit to lab (see details below).

#### **4.2.2.5 Dietary Control**

Refer to 'General Methodology (Section 3.9) for full details.

#### **4.2.3 Testing day 2**

The second visit took place not less than 48 hours and not more than 7 days after the first visit. Second visit tests started at approximately 9:30 am and finished at approximately 10:30 am.



#### **4.2.3.1 Blood samples**

Single blood samples were collected at the start of the second day of testing to determine levels of plasma acylated ghrelin. (Refer to the General Methodology, Section 3.13).

#### **4.2.3.2 Resting Metabolic Rate**

After blood samples were taken RMR was measured, this was carried out with participants positioned lying down for at least 5 minutes after blood collection. The room lights were dimmed, and the noise levels were kept to a minimum. The measurement was over a 20 minute period: five minute expired air samples were collected twice during this 20 minute period i.e. between minutes 5 and 10 and 15 and 20. Temperature and humidity were monitored using a handheld hygrometer. Barometric pressure was measured at the start of the trials using a barometer. Oxygen consumption and carbon dioxide production were assessed using Douglas bags See the General Methodology, Section 3.7 for further details).

#### **4.2.3.3 Assessment of appetite**

Appetite perceptions (hunger, fullness, satisfaction and prospective food consumption) were measured at the end of trial using visual analogue scales (Flint *et al.*, 2000). A breakfast meal was provided for participants at the end of testing. (General Methodology, Section 3.10).

#### **4.2.4 Biochemical analysis**

Refer to the General Methodology (Sections 3.14.2 and 3.14.4) for details about the analysis of acylated ghrelin, glucose and triacylglycerol (TAG). The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.9%, glucose 0.8% and TAG 3.9%.

#### **4.2.5 Statistical analysis**

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 for Windows (SPSS Inc., Chicago, IL). T-tests were used to assess differences between the sexes for every variable. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. Results are presented as mean  $\pm$  SD unless otherwise stated. Effect size was used to compare between males and females for all the variables examined in this study.

### 4.3 Results

The physical characteristics of the 67 participants (mean  $\pm$  SD) are displayed in Table 4.1 and in Figures 4.1 and 4.2. Male participants exhibited significantly higher values than female participants for all variables displayed in Table 4.1 and Figures 4.1 and 4.2.

**Table 4.1 Physical characteristics of the study participants**

<b>Characteristic</b>	<b>Females (range)</b>	<b>Males (range)</b>	<b><i>P</i></b>
Age (yr)	23.6 $\pm$ 5.4 (18 to 42)	28.2 $\pm$ 6.8 (21 to 46)	0.003
Height (cm)	163.1 $\pm$ 6.4 (152.8 to 177.6)	177.5 $\pm$ 6.6 (166.7 to 192.0)	<0.001
Weight (kg)	59.5 $\pm$ 8.1 (49.0 to 86.1)	76.5 $\pm$ 11.0 (57.9 to 103.5)	<0.001
BMI (kg.m <sup>-2</sup> )	22.3 $\pm$ 2.4 (18.3 to 29.2)	24.2 $\pm$ 2.9 (19.3 to 33.2)	0.004
Body fat (%)	21.6 $\pm$ 4.7 (15.6 to 29.4)	16.9 $\pm$ 4.5 (8.3 to 26)	<0.001
Waist circumference (cm)	72.7 $\pm$ 5.4 (64.7 to 84.0)	82.9 $\pm$ 7.5 (70.0 to 101.0)	<0.001
VO <sub>2</sub> max (mL.kg <sup>-1</sup> min <sup>-1</sup> )	43.9 $\pm$ 8.6 (24.6 to 55.8)	49.4 $\pm$ 8.4 (26.6 to 64.0)	0.010

Values are Mean  $\pm$  SD ( $n = 67$ , 34 males and 33 females). BMI = Body mass index; VO<sub>2</sub> max = maximum oxygen uptake.

### 4.3.1 Baseline parameters

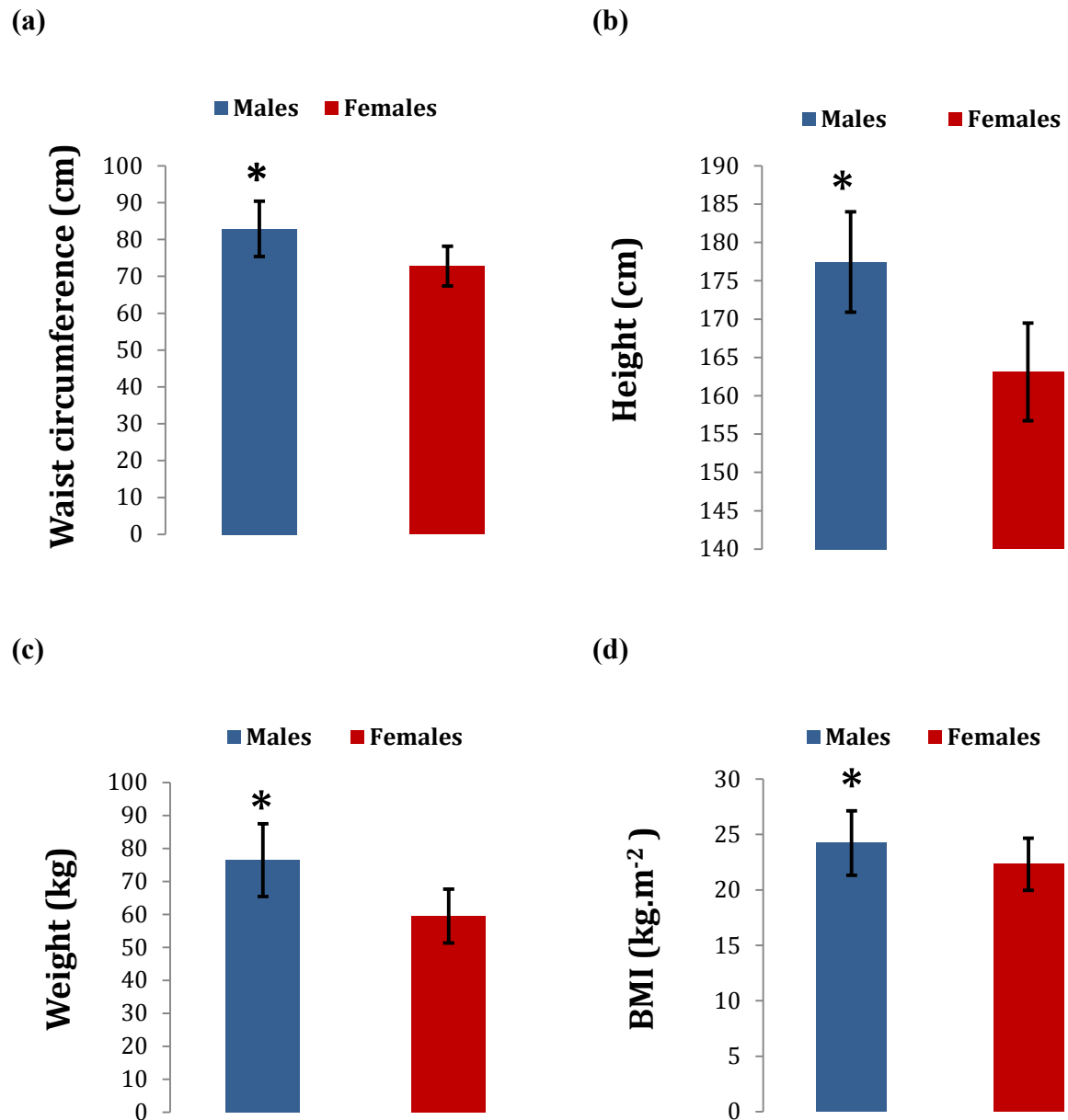
Table 4.2 displays values for appetite ratings, glucose, TAG and acylated ghrelin for males and females. Ghrelin, TAG and glucose data are also displayed graphically in Figure 4.3. Values did not differ significantly between males and females except for RMR which was significantly higher in males than females.

**Table 4.2 Fasting plasma acylated ghrelin, TAG, glucose, appetite, resting metabolic rate and dietary restraint values**

	<b>Females</b>	<b>Males</b>	<b><i>P</i></b>
Acylated ghrelin (pg.mL <sup>-1</sup> )	165 ± 103	175 ± 190	0.790
TAG (mmol.L <sup>-1</sup> )	0.9 ± 0.3	1.1 ± 0.6	0.093
Glucose (mmol.L <sup>-1</sup> )	5.3 ± 0.3	5.4 ± 0.3	0.520
Hunger (0-100)	47 ± 26	57 ± 22	0.106
Fullness (0-100)	24 ± 21	17 ± 17	0.137
Satisfaction (0-100)	30 ± 21	23 ± 16	0.173
PFC (0-100)	61 ± 19	68 ± 15	0.114
Resting metabolic rate (kJ.day <sup>-1</sup> )	5592 ± 471	7276 ± 563	0.000
GPAQ (METs-min.week <sup>-1</sup> )	2769 ± 2306	3508 ± 2837	0.250
Low restraint (0 – 10)	8.8 ± 1.4	9.4 ± 0.9	0.169
High restraint (11 – 13)	11.7 ± 0.9	12.2 ± 0.8	0.093
Clinical range restraint (14 – 21)	14.3 ± 0.6	14.8 ± 1.5	

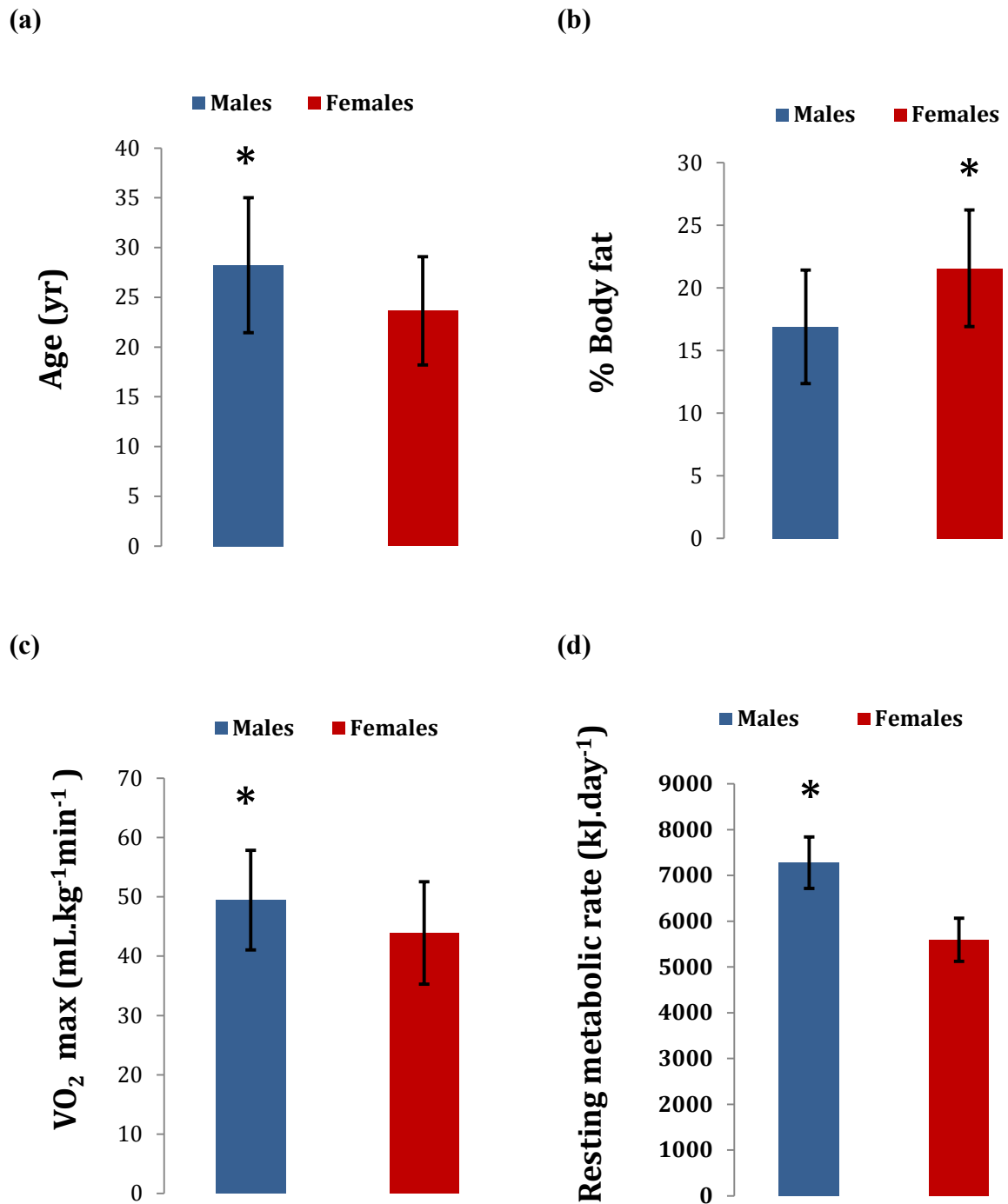
Values are mean ± SD (*n* = 67, 34 males and 33 females).GPAQ = global physical activity questionnaire, METs = metabolic equivalents.

Of the 67 participants (34 males and 33 females) 15 males and 15 females were low restraint, 15 males and 15 females were high restraint and 4 males and 3 females were considered within clinical range.



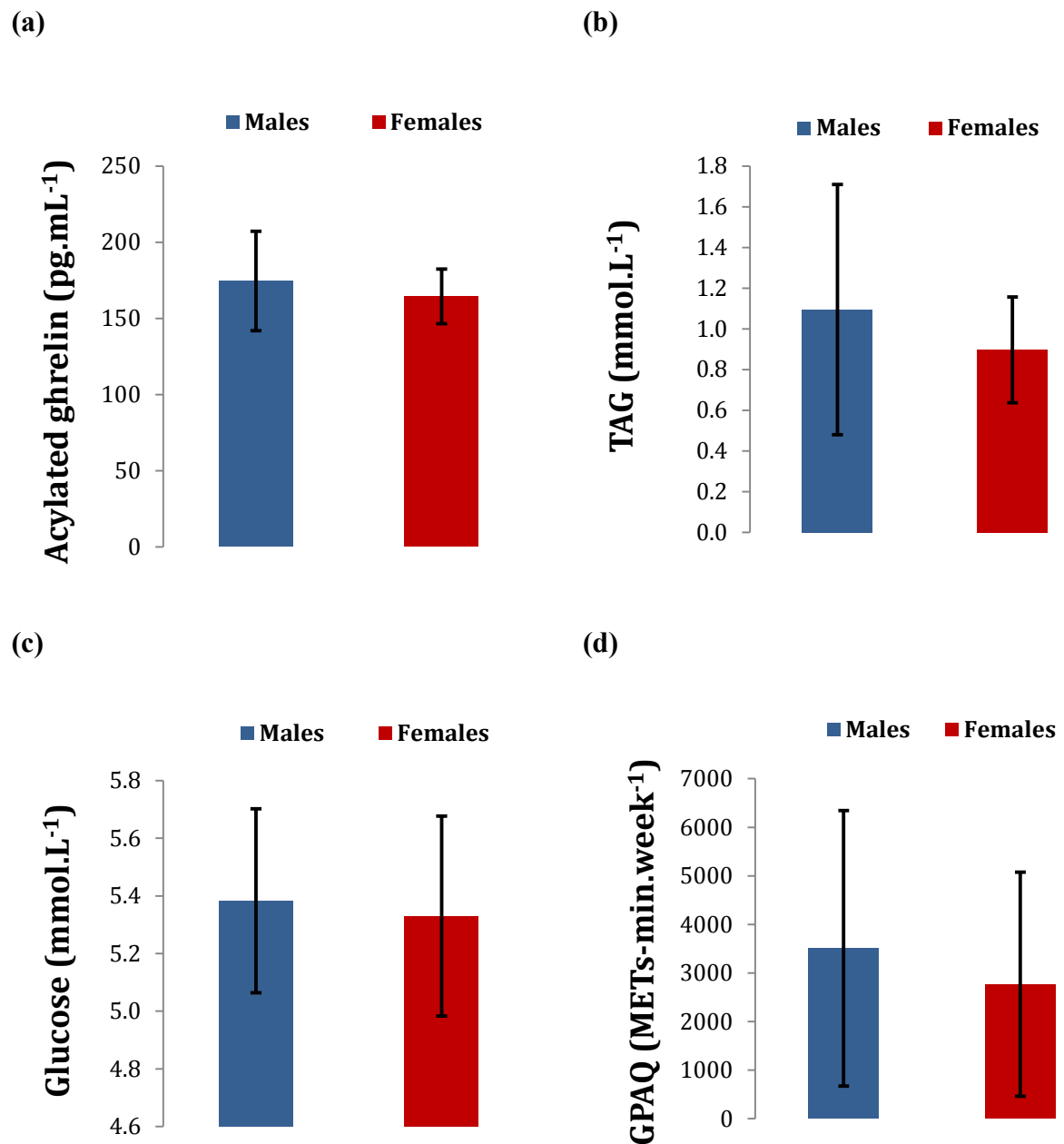
**Figure 4.1 Characteristic of the study participants**

Values for waist circumference (a), height (b), weight (c) and BMI (d) in males ( $n = 34$ ) and females ( $n = 33$ ). Values are mean  $\pm$  SD. \* significantly higher ( $P < 0.05$ ) in males than females.



**Figure 4.2 Age, body fat, VO<sub>2</sub> max and RMR in males and females**

Values for age (a), body fat percentage (b), VO<sub>2</sub> max (c) and resting metabolic rate (d) in males ( $n = 34$ ) and females ( $n = 33$ ). Values are mean  $\pm$  SD. \* significantly higher ( $P < 0.05$ ) in males than females except body fat percentage (% body fat) where female values were significantly higher than male values.



**Figure 4.3 Acylated ghrelin, triacylglycerol and glucose in males and females**

Values for plasma acylated ghrelin (a), triacylglycerol (TAG) (b), glucose (c) and global physical activity questionnaire (GPAQ) (d) in males ( $n = 34$ ) and females ( $n = 33$ ). Values are mean  $\pm$  SEM for acylated ghrelin, and mean  $\pm$  SD for other variables.

### 4.3.2 Correlations between acylated ghrelin and other variables

Fasting plasma acylated ghrelin concentration was negatively correlated with body fat ( $r = -0.0361$ ,  $P = 0.039$ ) in females, and with waist circumference ( $r = -0.346$ ,  $P = 0.045$ ) in males. This negative correlation indicates higher acylated ghrelin concentration in females who had a lower percentage body fat, and in males who had lower waist circumference. For males only, the correlation between fasting plasma TAG and physical activity (GPAQ) was negative and significant ( $r = -0.368$ ,  $P = 0.035$ ).  $VO_2$  max was significantly correlated in a negative way with the sum of skinfolds (the sum of four: biceps, triceps, subscapula and suprailiac) in females ( $r = -0.515$ ,  $P = 0.002$ ) and in males ( $r = -0.586$ ,  $P < 0.001$ ), and with body fat in females ( $r = -0.586$ ,  $P < 0.001$ ) and in males ( $r = -0.540$ ,  $P = 0.001$ ). There was a positive correlation between  $VO_2$  max and physical activity (GPAQ) in females ( $r = 0.584$ ,  $P < 0.001$ ) and in males ( $r = 0.448$ ,  $P = 0.009$ ), and between  $VO_2$  max and prospective food consumption in females ( $r = 0.493$ ,  $P = 0.004$ ) and in males ( $r = 0.357$ ,  $P = 0.038$ ). For females,  $VO_2$  max was correlated positively with hunger ( $r = 0.474$ ,  $P = 0.005$ ), and negatively with fullness ( $r = -0.348$ ,  $P = 0.047$ ). For males,  $VO_2$  max was correlated negatively with age ( $r = -0.362$ ,  $P = 0.036$ ), weight ( $r = -0.365$ ,  $P = 0.034$ ), BMI ( $r = -0.406$ ,  $P = 0.017$ ) and waist circumference ( $r = -0.381$ ,  $P = 0.026$ ). When examining RMR few significant relationships emerged. For males, RMR was significantly correlated with waist circumference ( $r = 0.504$ ,  $P = 0.002$ ), sum of skinfolds ( $r = 0.674$ ,  $P < 0.001$ ) and body fat percentage ( $r = 0.451$ ,  $P = 0.007$ ). Whereas for females, RMR was significantly (and negatively) correlated with fullness ( $r = -0.584$ ,  $P < 0.001$ ) and satisfaction ( $r = -0.509$ ,  $P = 0.002$ ).

### 4.3.3 Relationship between acylated ghrelin and restraint behaviour

Table 4.3 shows the relationship between fasting acylated ghrelin and dietary restraint classifications. There were 15 males and 15 females classified as having low dietary restraint (scores of 0 to 10), 15 males and 15 females classified as having high dietary restraint (scores of 11 to 13) and 4 males and 3 females classified in the clinical range (scores of 14 to 21). Fasting plasma acylated ghrelin values did not differ significantly between females classified as having low or high dietary restraint (Table 4.3). There was a borderline effect for males, with higher plasma acylated ghrelin values in males classified



as having high restraint compared with low restraint ( $P < 0.054$ , Table 4.3). Values of fasting plasma acylated ghrelin did not differ significantly between low dietary restraint males and females ( $P = 0.109$ ) or between high dietary restraint males and females ( $P = 0.201$ ).

**Table 4.3 Fasting plasma acylated ghrelin concentrations according to low or high dietary restraint classifications**

	<b>Low restraint</b>	<b>High restraint</b>	<b><i>P</i></b>
Females	163.0 ± 116.7 (15)	162.9 ± 96.6 (15)	0.988
Males	106.8 ± 62.9 (15)	254.4 ± 262.9 (15)	0.054
Combined	135.2 ± 96.5 (30)	208.7 ± 200.1 (30)	0.093

Values are mean ± SD. Values in brackets represent number of participants.

#### **4.3.4 Effect size**

Effect sizes for this sample were calculated for each of the variables by dividing the difference between the mean values (males versus females) with the standard deviation, (i.e. the average standard deviation from both trials). Table 4.4 shows the resulting effect size values for each comparison. (See General Methodology section 3.16.1).

**Table 4.4 Effect size comparisons between males and females for all variables examined in this study**

	Males	Females	SD	Effect Size	
AG (pg mL <sup>-1</sup> )	174.6	164.6	152.7	0.1	Trivial
VO <sub>2</sub> max (mL.kg <sup>-1</sup> min <sup>-1</sup> )	49.4	43.9	8.9	0.6	Moderate
TAG (mmol.L <sup>-1</sup> )	1.1	0.9	0.5	0.4	Small
Glucose (mmol.L <sup>-1</sup> )	5.4	5.3	0.3	0.2	Trivial
BMI (kg.m <sup>-2</sup> )	24.2	22.3	2.8	0.7	Moderate
Age (yr)	28.2	23.6	6.5	0.7	Moderate
Weight (kg)	76.5	59.5	12.9	1.3	Large
Height (cm)	177.5	163.1	9.7	1.5	Large
Waist circumference (cm)	82.9	72.7	8.3	1.2	Large
Sum of 4 Skinfolds (mm)	39.3	37.9	15.2	0.1	Trivial
Body density (g.cm <sup>-3</sup> )	1.1	1.0	0.0	1.1	Large
% Body fat	16.9	21.6	5.1	-0.9	Large
Hunger (0-100)	57.0	47.4	24.3	0.4	Small
Satisfaction (0-100)	23.4	29.7	18.9	-0.3	Small
Fullness (0-100)	16.6	23.6	19.0	-0.4	Small
PFC (0-100)	67.7	61.2	16.8	0.4	Small
RMR (kJ.day <sup>-1</sup> )	7276	5592	6447	1.7	Large
TFEQ 1	11.3	10.6	2.1	0.3	Small
TFEQ 2	7.5	8.5	2.2	0.4	Moderate
TFEQ 3	6.6	6.3	2.2	0.1	Small
GPAQ (METs-min.week <sup>-1</sup> )	3508	2769	2592	0.3	Small

AG = acylated ghrelin; TAG = triacylglycerol; BMI = body mass index; VO<sub>2</sub> max = maximum oxygen uptake; % Body fat = percentage of body fat; RMR = resting metabolic rate. TFEQ = three factor eating questionnaire, where TFEQ 1= dietary restraint, TFEQ 2= disinhibited eating, TFEQ 3= hunger, GPAQ = global physical activity questionnaire. (*n* = 67, 34 males and 33 females). Values are mean ± SD.

All of the variables showing the physical characteristics of the two groups have a moderate to large effect as may be expected. The effects are large for height, weight, waist circumference, body density and percentage body fat. The effects are moderate for age, BMI, RMR and VO<sub>2</sub> max. The effects are small for TAG and appetite perceptions. The effects are trivial for acylated ghrelin, glucose and sum of 4 skinfolds.

#### 4.4 Discussion

In this sample of volunteers, it was found that there was no significant difference in the values of acylated ghrelin, TAG, glucose and in fasting appetite ratings between the sexes. Men showed a slightly higher value for acylated ghrelin, which contradicts previous studies that have assessed the sex differences in energy-regulating hormones. Gayle *et al.* (2006) observed that ghrelin concentrations and *ad libitum* food intake were higher after a 12-hour fast in female rats compared with male rats. Hagobian and Braun (2010) also observed that there were clear sex differences in the way that exercise altered energy-regulating hormones and appetite; in response to exercise without energy added back to the diet, women had higher concentrations of acylated ghrelin than men. Barkan *et al.* (2003) indicated women have total ghrelin levels that are threefold higher than in men, which may be associated with the follicular stage of the menstrual cycle. Equally, Katsuki *et al.* (2004) noted that women have higher levels of acylated or active ghrelin levels than men, with no significant differences in total ghrelin levels.

Previous data suggest that exercise may stimulate larger changes to energy-regulating hormones in women than in men and this is consistent with the hypothesis that appetite will be stimulated more in women than in men (Hagobian and Braun, 2010). However, the current study did not find any differences between fasting acylated ghrelin or appetite ratings between the sexes. The means didn't differ significantly and the effect size was trivial, showing that there is no major difference between the two sexes based on acylated ghrelin concentrations. In fact, it shows the opposite of previous studies' results (Hagobian *et al.*, 2013). Men had a slightly higher concentration of acylated ghrelin ( $175 \pm 190 \text{ pg.mL}^{-1}$ ) than women ( $165 \pm 103 \text{ pg.mL}^{-1}$ ). Where Hagobian *et al.* 2013 found that women had a slightly higher concentration of baseline acylated ghrelin ( $549 \pm 330 \text{ pg.mL}^{-1}$ ) than men ( $498 \pm 305 \text{ pg.mL}^{-1}$ ). It is also worth noting that the acylated ghrelin concentrations in both women and men were much higher in the study of Hagobian and colleagues (2013) than in the present study. The reasons for this are unclear but may relate to the different procedures used to process and analyse the ghrelin samples and to differences in participant characteristics.

In addition to sex differences in acylated ghrelin concentrations, several studies have also noticed male-female differences in appetite in response to exercise. Based on the responses collected from questionnaires about appetite after exercise by Hagobian *et al.* (2009), it was discovered that men had less desire to eat and less perceived hunger than women (Hagobian *et al.*, 2009). Appetite responses to exercise were not assessed in the present study and no differences were detected in fasting appetite responses between males and females.

A previous study suggested that total ghrelin values are influenced by exercise intensity (Erdmann *et al.*, 2007). The study examined moderate intensity exercise and observed that with relatively short bouts (30 minutes, 60 minutes or 120 minutes) of exercise there were no significant effects on appetite and food intake, however after a 2h bout of exercise participants food intake was stimulated. The study also showed that during the exercise tests levels of total ghrelin were stimulated when participants carried out low intensity but not high intensity exercise (Erdmann *et al.*, 2007). Erdmann and colleagues (2007) concluded that increased ghrelin is not a major mediator of changes in appetite. It was also suggested that the effects of exercise could depend on sympathetic activation. However in recent studies King *et al.*, 2010b studied acylated ghrelin responses to moderate intensity exercise (brisk walking) and did not observe any increase during or after exercise.

Stubbs *et al.* (2002b) observed in their study that women increased *ad libitum* food intake to partially compensate for the higher energy expenditure due to exercise, but there was no change in perceived appetite. These results support those of Gayle *et al.* (2006) who observed that female rats had a higher *ad libitum* overnight food intake compared with male rats after a 12-hour fast. However, the current study did not observe any significant differences in appetite ratings between the sexes, which are confirmed by the small effect size values. It was also found that, although not that significant, men were hungrier and felt less satisfied and less full than women and had a higher value of prospective food consumption after fasting than women.

There is limited data and studies that investigated the association between age and acylated ghrelin. However the studies that are available suggest that there may be a slight relationship between the two factors (Hagobian *et al.*, 2009, Stubbs *et al.*, 2002b, Hagobian and Braun, 2010). This chapter and study showed that there was no correlation between age and ghrelin, thus may be due to the younger audience that were used to carry out this study. Therefore the range of ghrelin values brought limitations in detecting any age related differences to acylated ghrelin.

It should be noted and stressed that the participants fell into a wide age range (19 to 46 years) but this should not have an impact on the results when compared to other studies such as that of Hagobian *et al.* (2009) whose participants were aged between 19 and 57 years. However, most of the studies consisted of participants with a relatively homogenous fitness level, e.g. Hagobian *et al.* (2009) studied sedentary/obese individuals and the study of Stubbs *et al.* (2002b) involved lean females while volunteers for the current study exhibited a range of fitness (VO<sub>2</sub> max) values. This may be responsible for the differences in results that was found within this study to those previous studies. This study showed that there was a negative correlation with male participants and age associated with VO<sub>2</sub> max. There were no significant correlation values in females.

Studies relating to RMR and acylated ghrelin are limited; however there is a study that observed a significant inverse correlation between total ghrelin and RMR. The relation associated between ghrelin and RMR was found among lean, obese and hyperthyroid subjects (Marzullo *et al.*, 2004, Riis *et al.*, 2003). However, the study carried out within this chapter showed no significant value relating to acylated ghrelin and RMR.

A study that is related to RMR and appetite observed that participants were experiencing increased levels of hunger when RMR levels were high compared with those participants who had a lower level of RMR. It also stated that changes in RMR could be independent of sex, food energy density and energy intake (Caudwell *et al.*, 2013). Within this present study RMR relationship with appetite differed between the sexes. A significant association between appetite and RMR was observed for female participants (correlated fullness ( $r = -0.584$ ), satisfaction ( $r = -0.509$ )) and male participants not so significant. However, the

study showed that RMR in male participants had significant correlations relating to other variables; waist circumference, sum of skinfolds and body fat percentage, which female participants could not show.

Another factor assessed in the present study was the relationship between plasma acylated ghrelin concentrations and levels of dietary restraint. Within this study The Three Factor Eating questionnaire was used to assess dietary restraint scores and these did not differ significantly between males and females in the study. This study also investigated whether levels of dietary restraint were related to plasma acylated ghrelin concentrations.

Previous studies have observed weak correlations between ghrelin and dietary restraint. In some cases the relationship can be shown as positive and in some cases relationships can be shown as negative, however the correlation coefficients are not high and the relationships are not significant (Schur *et al.*, 2008, Vescovi *et al.*, 2008, Langlois *et al.*, 2011). Within this chapter and study there was no relationship observed in women but in men there was a trend for higher plasma acylated ghrelin concentrations in those exhibiting high levels of dietary restraint compared with those exhibiting low levels dietary restraint. This is logical because it might be expected that restrained eaters should feel hungrier than non-restraint eaters. Although this was an interesting finding found in this study, further investigations will need to be carried out in future studies.

#### **4.5 Conclusion**

In conclusion, the main finding of the present, cross-sectional, study is that fasting plasma acylated ghrelin concentrations did not differ significantly between males and females and neither did fasting appetite ratings although men tended to exhibit higher fasting values for hunger and prospective food consumption and lower values for fullness and satisfaction than women. Plasma acylated ghrelin concentrations did not differ significantly between women classified as having high or low levels of dietary restraint but a borderline effect was observed in men, with higher plasma acylated ghrelin concentrations observed in men classified as having high levels of dietary restraint. A limitation of the present study is the relatively small sample size for a cross-sectional study and these preliminary observations require confirmation/clarification in future studies with a much larger sample size.

## **5 Study 2: The effects of taking the contraceptive pill on appetite, acylated ghrelin and food intake responses to cycling exercises.**

### **5.1 Introduction**

Obesity has become a major issue around the world today with important consequences for health and society. It is a chronic disease that has reached epidemic proportions in both developed and developing countries (WHO, 2000). Among the several strategies for obesity treatment, healthy dietary and physical activity behaviours remain the key measures considered useful for losing weight and preventing weight gain in moderately obese adults (Curioni and Lourenço, 2005). One of the key factors for the maintenance of a healthy body mass is appetite and it is clear that both physical and psychological factors influence appetite regulation (Buffenstein et al., 1995). An important physical factor that can influence appetite is exercise and there is some evidence to suggest that the way that appetite hormones respond to exercise differs in males compared with females (Hagobian et al., 2009).

However, the majority of studies examining the effects of exercise on appetite, appetite regulating hormones and food intake have involved male participants (for some recent examples see Broom et al., 2007; Broom et al., 2009; King et al., 2010a; Deighton et al., 2012). One reason for the greater focus on male participants is because the menstrual cycle has the potential to alter appetite responses and hence ‘contaminate’ the findings of exercise studies in females. These influences are primarily due to changes in hormones; namely major female hormones progesterone and estradiol and biological interactions which control the outcome of a menstrual cycle. Changes in the concentrations of these hormones can cause differences in levels of energy intake, energy expenditure, mood, depression, irritability, breast tenderness and bloating. Estradiol (the main estrogen) is the hormone that stimulates



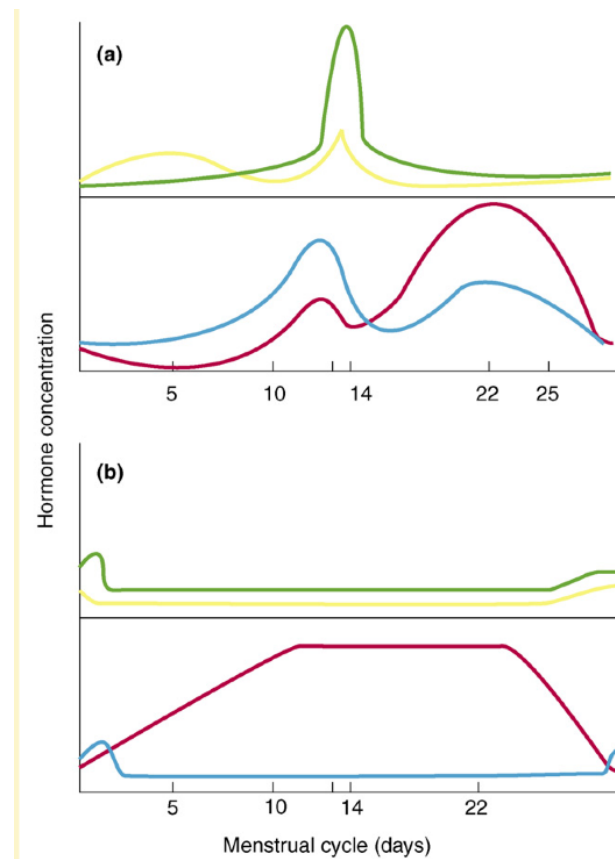
female growth (puberty) in breast development and causes the uterus and fallopian tubes to mature. Progesterone is secreted principally by the corpus luteum. It regulates several functions in reproduction such as maintenance of early pregnancy, induction of ovulatory heat, development of mammary glands, and many more (Al-Asmakh, 2007; Clarke and Sutherland, 1990).

A menstrual cycle is the result of a precise co-ordination of events that occurs in the ovaries. The monthly menstrual cycle prepares an egg for maturation, ovulation and fertilisation. Typically a cycle averages 28 days (Wilcox et al., 2000). The human menstrual cycle comprises three phases:

**Follicular Phase** – initiates the growth and maturation of an ovarian follicle, which actually begins during the last few days of the previous Luteal Phase. Occurs from days 1-14.

**Ovulatory Phase** – the interval in which the LH surge induces ovulation.

**Luteal Phase** – the last portion of the cycle that prepares the endometrium for implantation of a fertilised ovum. Occurs from days 16-28.



**Figure 5.1 Hormonal regulation of the menstrual cycle in (a) normally cycling women and (b) OCP users. (Alvergne & Lummaa, 2010)**

Through the administration of a steady daily level of both progestin (a substitute for progesterone; red lines) and oestrogen (blue lines), the OCP prevents the secretion of gonatropin-releasing hormone (GnRH) from the hypothalamus, thereby blocking a signal to the pituitary gland to secrete FSH (yellow lines) and LH (green lines). FSH stimulates the ovaries to grow egg follicles and LH triggers ovulation and in their absence, the ovary becomes relatively dormant, and no egg is produced to a point where it can be released. Thus, hormonal contraception maintains the menstrual cycle at the same late phase of the natural cycle on a continuous basis.

In conclusion, both (progesterone and estradiol) female sex hormones play vital roles in the menstrual cycle and the interaction between the two exerts a great deal of impact on a woman's food intake, energy expenditure and body weight. (Davidsen *et al.*, 2007). Thus, the

menstrual cycle should be taken into consideration as a factor in the physiology of energy balance (Bryant *et al.*, 2006). Considerable evidence exists to suggest that appetite and food intake vary across the menstrual cycle (Bancroft *et al.*, 1988; Dye and Blundell, 1997; Gilbert and Gillman, 1956 and Michell, 1975). These variations have been related to changes in the estradiol/progesterone ratio that occur during the menstrual cycle (Buffenstein *et al.*, 1995; Johnson *et al.*; 1994; Lyons *et al.*, 1989).

Concerning the relationship between the menstrual cycle and acylated ghrelin, there seems to be limited studies that have addressed this issue. Emerging evidence strongly indicated that ghrelin and the ghrelin receptor are present in the mammalian and non-mammalian ovary (Dupont *et al.*, 2010). For example, ghrelin is found in human, rat, pig, sheep, and chicken ovaries (Gaytán, *et al.*, 2003; Chen *et al.*, 2008; Miller *et al.*, 2005). Therefore, ovarian follicular and luteal cells are potential targets for systemic or locally produced ghrelin (Dupont *et al.*, 2010). Viani *et al.* (2008) found that, in cultured human granulosa luteal cells, ghrelin exerts an inhibitory effect on progesterone and estradiol production. In women during the menstrual cycle, administration of ghrelin did not affect LH and FSH secretion (Dafopoulos *et al.*, 2009).

The oral contraceptive pill (OCP) was first introduced in 1960s and were high in doses, however further research has helped reduce the component level of amounts of estradiol and progesterone (Bennell *et al.*, 1999) to minimize side effects and risks. The OCPs are commonly used for birth control and are the most effective form of contraception; these contraceptive pills are prescribed as either a combined hormonal contraceptive, containing synthetic versions of both estradiol and progesterone or progestins alone. A combined contraceptive pill can help eliminate any menstrual-cycle variability (Davidsen *et al.*, 2007, Fleischman *et al.*, 2010). Moreover, researchers are naming the combined OCP as a

‘monophasic’ pill, as the dosage has a slightly lower overall dose, with a constant and balanced dosage of both hormones (estradiol and progesterone). Thus, it mimicks ‘natural’ cycles more closely than the other contraceptive pills available i.e. progestin-alone pill (Frye, 2006). See Figure 5.1 for a comparison of hormonal levels during the menstrual cycle, with or without OCP.

The maintenance of energy balance and consequently, body weight stability, is largely influenced by the control of appetite and food intake (Jecquier, 1992, Prentice *et al.*, 1986). Appetite regulation is affected by many biological control systems, both physiological and psychological factors. These include factors such as neural signals, metabolites released during oxidation of macronutrient, neuropeptides and hormonal signals (Buffenstein *et al.*, 1995). However, these metabolic controls can also be influenced by environmental sensory information, cognitive learned behaviour and psychological variables (Blundell *et al.*, 1993). Furthermore steroid hormones, in particular the reproductive hormones estradiol and progesterone, also play a part in appetite control (Buffenstein *et al.*, 1995). Therefore, there have been mixed results from studies investigating the effect of OCP and food intake. While some studies found a significantly higher food intake in OCP users compared with non-OCP users (macronutrient intake was similar between the two groups) (Wallace *et al.*, 1987; Eck *et al.*, 1997), others found no difference in food intake between the two (McNeill *et al.*, 1988; Tucci *et al.*, 2010).

Another reported effect of OCP is an increase in snacking, caused by the suppression in release of postprandial CCK, which weakens post-meal satiety (Hirschberg *et al.*, 1996, Karlsson *et al.*, 1992). Oral contraceptive pill use may also reduce insulin sensitivity, and this impact on carbohydrate metabolism has been associated with the progestin component (Krauss & Burkman, 1992). This can lead to weight gain and impacts on performance in day-

to-day activities. As a result, there is a concern among athletes that these hormones may deteriorate exercise performance (Casazza *et al.*, 2002). A study by Jarrett & Spellacy (1983) found that the use of OCP by female athletes was lower compared to the general population, as OCP may have impacts on performance. However, the use of OCP has many benefits for athletic women, which include: i) a reliable and reversible method of contraception, ii) a decrease risk in iron deficiency anaemia by decreasing levels of blood-loss and iii) ability to manipulate the menstrual cycle, giving more opportunities to travel, compete and train for sporting events (Potter, 1997; Mishell, 1993; Tzingounis *et al.*, 1995). However, as with many medical pills, there are also disadvantages, i) headaches, ii) breast tenderness, iii) fluid retention iv) nausea and v) weight gain (Endrikat *et al.*, 1997). Athletes are very cautious about weight, as the side effect of weight gain is a negative impact on performance, therefore the OCP is not recommended to female athletes who are involved in low body weight sports such as distance running, lightweight rowing and gymnastics (Bennell *et al.*, 1999). However, further studies have discovered that, due to the availability of lower dose pills, female athletes are likely to use OCPs as often as the general public (White & Brukner, 1992; Shawdon, 1995). The likely impacts are discussed and stressed to athletes to ensure an informed decision but in general, the advantages that come with the OCP outweigh the disadvantages for sports women (Bennell *et al.*, 1999). Nevertheless, there is a demand for further research on the greater effects of the OCP on female athletes.

Very little work has been done to examine the effects of OCP on energy expenditure (EE), however previous research showed that there is a negative relationship between OCP use and EE (Eck *et al.*, 1997). Researchers discovered no change in EE for OCP users between pre-to post ovulation phases, whereas there was an increase in the 24-h EE between the two phases for OCP non-users (Webb, 1986). Furthermore, one of the OCP users that took part in the study was followed up a month after discontinuing the OCP, and it was discovered that EE

had risen between the two phases (Webb, 1986). This discovery, albeit in one individual, suggests that OCP has an effect and impact on EE.

Study one (Chapter four) examined fasting acylated ghrelin in female participants but the influence of exercise or the use of the OCP was not examined. Monophasic OCP users do not have follicular and luteal phases per se but for ease of description and interpretation the first day of bleeding was considered to be the 1<sup>st</sup> day of the OCP cycle, 5<sup>th</sup> – 11<sup>th</sup> day the follicular phase and 18<sup>th</sup> – 23<sup>rd</sup> day the luteal phase. The present study had three primary objectives. The first was to determine whether perceptions of appetite during and after cycling exercise differ between the ‘luteal’ and the ‘follicular’ phases of the OCP cycle. The second was to see whether *ad libitum* food intake after cycling exercise was influenced by the OCP during the OCP cycle phases. The third objective was to see whether the OCP influenced the concentration of the appetite-regulating hormone acylated ghrelin throughout the phases of the OCP cycle.

## 5.2 Methods

### 5.2.1 Participants

After the Loughborough University Ethical Advisory Committee had approved the study, 13 healthy, active female volunteers (aged 18 – 26 years) signed written informed consent forms to confirm their participation in the study. In order to ensure the safety of participants and to minimize confounding factors, participants had to conform to the following criteria: they had to be non-smokers; not currently taking any medication except for the combined hormone contraceptive pill (with both estradiol and progesterone); no personal history of cardiovascular disease, metabolic disease or dyslipidaemia; not pregnant (assessed by the health screen questionnaire); not taking any drugs (medical or illegal) known to affect digestion or metabolism (e.g., anabolic steroids, marijuana, amphetamines, thyroid prescription drugs); and all participants had to be able to cope with the exercise demands of the study. The physical characteristics of the participants are displayed in Table 5.1 below.

**Table 5.1 Physical characteristics of the study participants**

Characteristics	Mean	±	SD
Age (yrs)	21.8	±	2.1
Height (cm)	170.0	±	4.3
Weight (kg)	62.5	±	6.0
BMI (kg.m <sup>-2</sup> )	21.6	±	1.9
Body fat (%)	24.8	±	5.1
Waist circumference (cm)	71.0	±	4.6
Hip circumference (cm)	86.6	±	5.5
Resting SBP (mm Hg)	122	±	7
Resting DBP (mm Hg)	76	±	5
VO <sub>2</sub> max (mL.kg <sup>-1</sup> .min <sup>-1</sup> )	42.5	±	7.3

Data are displayed as mean ± SD, (*n* = 13). BMI = Body mass index; VO<sub>2</sub> max = maximum oxygen uptake; SBP = systolic blood pressure; DBP = diastolic blood pressure.

### 5.2.2 Preliminary sessions

Before participants took part in the main trials, they were invited to attend the laboratory on two separate occasions, i.e. for preliminary sessions. On the first visit, participants undertook screening, which lasted for 2 hours. This involved participants completing physical activity and health screening questionnaires, as well as a three factor eating questionnaire which helped in assessing whether each participant was a ‘restrained’ or ‘unrestrained’ eater. Once these questionnaires were completed, a series of measurements were taken including: resting blood pressure, height and weight, waist and hip circumferences and skinfold thickness at four different sites (biceps, triceps, subscapula and suprailiac). Once the screening phase was complete participants completed a preliminary exercise test to determine maximum oxygen uptake. This test consisted of continuous incremental cycling exercise beginning at a workload of 35 W with increments of 35 W every 3 minutes until participants reached volitional exhaustion. The exercise test was performed on a cycle ergometer (Excalibur, Lode, Groningen, The Netherlands). Expired air samples were collected using Douglas bags



for the last minute of each stage. Heart rate was measured throughout the test using short-range telemetry. Further details about this test are provided in the General Methodology (Section 3.6.3).

On the second preliminary session (familiarisation), participants were asked to familiarise themselves with the environment in which they would be tested. During this visit, participants were made aware of the protocols and were assured of the safety procedures. During this familiarisation visit, participants completed a 45-minute bicycle ride at 70% of their maximum oxygen uptake. They were also familiarised with all of the procedures to be used in the main trials.

### **5.2.3 Main trials**

One week after the preliminary visits, participants completed two main trials with an interval of one to two weeks between each trial; each trial lasted for approximately 3 hours (Figure 5.2). Participants were advised to use public transport when travelling to the laboratory in order to minimise their physical exertion on the days of the trials if they were travelling a long distance. Participants were asked to report/make contact on the first day of bleeding, and to calculate their follicular phase (5<sup>th</sup> – 11<sup>th</sup> day) and luteal phase (18<sup>th</sup> – 23<sup>rd</sup> day). Participants then arranged a day to attend the laboratory during the luteal phase of their OCP cycle and the follicular phase of their OCP cycle.

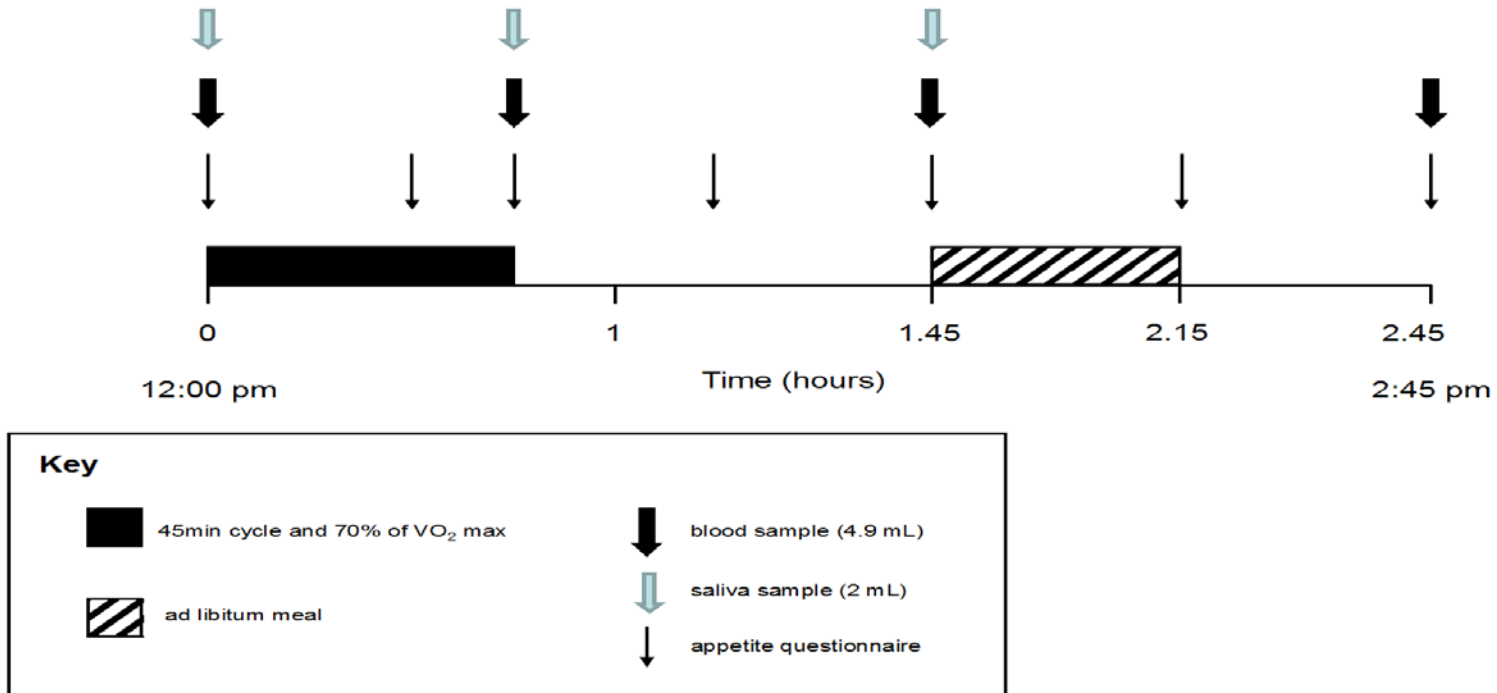
Each trial began at 12:00 noon and lasted for nearly 3 hours (i.e. from 12:00 noon to 2:45 pm). The trials began with the participants undertaking a 45-minute cycle ride, which was predicted to elicit 70% of maximum oxygen uptake (12:00 noon to 12:45 pm). During the trial, participants were requested to complete visual analogue scales every half an hour to assess their perceptions of hunger, satisfaction, fullness and prospective food consumption (Appendix C). Four venous blood samples and three saliva samples were taken during each main trial (Figure 5.2). During each exercise stage within the trials, expired air was collected into Douglas bags. After cycling, participants rested for the remainder of the trial (sitting reading, working at computer, writing, playing video games or watching a DVD).

Participants were asked to weigh and record everything they ate and drank for 24 hours prior to the first main trial and to consume identical amounts of the same food and drink prior to

their second main trial. Alcohol and caffeine were prohibited on the days when participants were recording their diet. Participants were required to finish eating by 11.00 pm on the evenings before the main trials. Water was allowed *ad libitum* prior to and during the main trials. On the morning of each main trial participants ate breakfast between 8.00 and 9.00 am and did not consume anything else before coming to the laboratory. Participants were asked to consume identical breakfasts prior to each main trial.

One hour after the completion of exercise participants were given access to an *ad libitum* meal, which consisted of three types of cereals, milk, tuna, ham, chocolate bar, cereal bar, two types of muffins, orange, banana, apple, cheese, butter, margarine, mayonnaise, white and brown bread. Once the buffet was accessible, participants were told that they had 30 minutes to consume food until they were satisfied. Participants were kept in isolation throughout the buffet process in order to limit social factors from influencing food intake. Prior to the food consumption phase, all items were weighed. This was necessary in order to determine the amount of food consumed, which was the difference between the initial weight of the food and the weight remaining after food consumption. Manufacturers' values were used to assess the energy and macronutrient content of the items consumed.

Blood samples were collected via venepuncture from an antecubital or forearm vein at four time points during the main trials as follows: 0 hours, 45 minutes, 1 hour and 45 minutes, 2 hours and 45 minutes. At each blood sampling point 4.9 mL of blood was collected. These samples were used for the measurement of plasma acylated ghrelin. Three 2 mL saliva samples were collected during each trial in sterile tubes using the passive dribble technique at time points 0 hours, 45 minutes and 1 hour and 45 minutes. Levels of progesterone and estradiol were assessed from the saliva samples. Further details about blood treatment are provided in the General Methodology (Section 3.13).



**Figure 5. 2** A schematic of the protocol for the main trials

#### **5.2.4 Biochemical analysis**

An enzyme immunoassay was used to determine the concentrations of plasma acylated ghrelin. In order to eliminate inter-assay, all samples from the same participant were run on the same plate. The within batch coefficient of variation for the assay was 5.0%. Further details about this test are provided in the General Methodology (Section 3.14.4).

#### **5.2.5 Saliva analysis**

Saliva concentrations of progesterone and 17 $\beta$ -estradiol were measured using commercially available ELISA kits (Salimetrics, Newmarket, UK). The intra assay co-efficient of variation was 1.7% for progesterone and 2.2% for 17beta-estradiol. See section 3.15.1 in the General Methodology.

#### **5.2.6 Statistical analysis**

The data were analysed using Statistical Package for the Social Sciences (SPSS) software, Version 20.0 for Windows. Repeated measures two-factor ANOVA was applied in order to examine differences between the follicular and luteal trials over time in terms of appetite perceptions and acylated ghrelin. The student t-test was used to examine differences between the follicular and luteal trials with regard to baseline acylated ghrelin, baseline appetite scores, AUC values for acylated ghrelin, and AUC values for appetite. Energy and macronutrient intake and exercise responses were also examined by utilizing t-test. The Pearson Product Moment Correlation Coefficient was used to examine relationships between variables.

## 5.3 Results

### 5.3.1 Responses to ergometer cycling

Table 5.2 presents participants' responses to 45 minutes cycling. There were no significant differences between the follicular and luteal phases in ratings of perceived exertion (RPE); work rate; heart rate;  $\text{VO}_2$ ;  $\% \text{VO}_2 \text{max}$ ; energy expenditure or respiratory exchange ratio. The ambient temperature was  $22.0 \pm 1.1^\circ\text{C}$  versus  $22.2 \pm 1.0^\circ\text{C}$  and the relative humidity was  $25.3 \pm 6.3\%$  versus  $25.6 \pm 6.2\%$  for the follicular and luteal trials respectively.

**Table 5.2 Participants' responses to exercise**

	<b>Follicular</b>	<b>Luteal</b>	<b><i>P</i></b>
RPE (6-20)	14 $\pm$ 2	14 $\pm$ 1	0.794
Work rate (W)	138.6 $\pm$ 19.1	139.7 $\pm$ 19.3	0.502
Heart rate (beats.min <sup>-1</sup> )	162 $\pm$ 10	165 $\pm$ 10	0.464
$\text{VO}_2$ (mL.kg <sup>-1</sup> .min <sup>-1</sup> )	30.5 $\pm$ 3.5	30.4 $\pm$ 5.1	0.941
$\% \text{VO}_2 \text{max}$	72.7 $\pm$ 7.3	72.1 $\pm$ 8.6	0.777
Energy expenditure (kJ)	1726 $\pm$ 159	1734 $\pm$ 252	0.859
Respiratory exchange ratio	0.95 $\pm$ 0.07	0.96 $\pm$ 0.08	0.667

Mean  $\pm$  SD ( $n = 13$ ). *P* values are from paired samples t-tests.

### 5.3.2 Baseline parameters

Table 5.3 displays baseline values for appetite ratings, acylated ghrelin and ovarian hormones in the follicular and luteal trials. Baseline acylated ghrelin and satisfaction did not differ significantly between the follicular and the luteal trials. However, baseline hunger and prospective food consumption values were significantly higher in the follicular than the luteal phase, while baseline fullness was significantly lower in the follicular than the luteal phase.  $17\text{-}\beta$  estradiol was significantly higher in the follicular phase than the luteal phase but progesterone concentrations did not differ significantly between the two

phases.

**Table 5.3 Baseline acylated ghrelin, ovarian hormone and appetite perception values in the follicular and luteal trials**

	<b>Follicular</b>	<b>Luteal</b>	<b><i>P</i></b>	<b>Effect size</b>
Acylated ghrelin (pg.mL <sup>-1</sup> )	150 ± 79	133 ± 87	0.121	0.2
Hunger (0–100)	50 ± 25	33 ± 24	0.045	0.7
Fullness (0-100)	31 ± 29	42 ± 24	0.023	0.4
Satisfaction (0-100)	34 ± 27	40 ± 21	0.366	0.2
PFC (0-100)	64 ± 22	45 ± 24	0.002	0.8
17-β estradiol (pg.mL <sup>-1</sup> )	2.78 ± 0.56	2.48 ± 0.53	0.018	0.5
Progesterone (pg.mL <sup>-1</sup> )	82.2 ± 36.7	87.2 ± 58.4	0.776	0.1

Values are mean ± SD (*n* = 13). *P* values are from paired samples t-tests.

### 5.3.3 Appetite

Two-way ANOVA revealed that time had a significant effect on hunger, satisfaction, fullness and prospective food consumption, indicating that appetite values change over time. Hunger and prospective food consumption tended to be higher in the follicular trial than in the luteal trial, while fullness and satisfaction were lower in the follicular than the luteal trial but there was no significant effect of trial and no significant time x trial interaction for the four appetite perceptions (Figure 5.3). Area under curve values over a time of 2 hours and 45 minutes of the follicular and luteal trials for hunger, satisfaction, fullness and prospective food consumption revealed that there was no significant difference between trials (Figure 5.5).

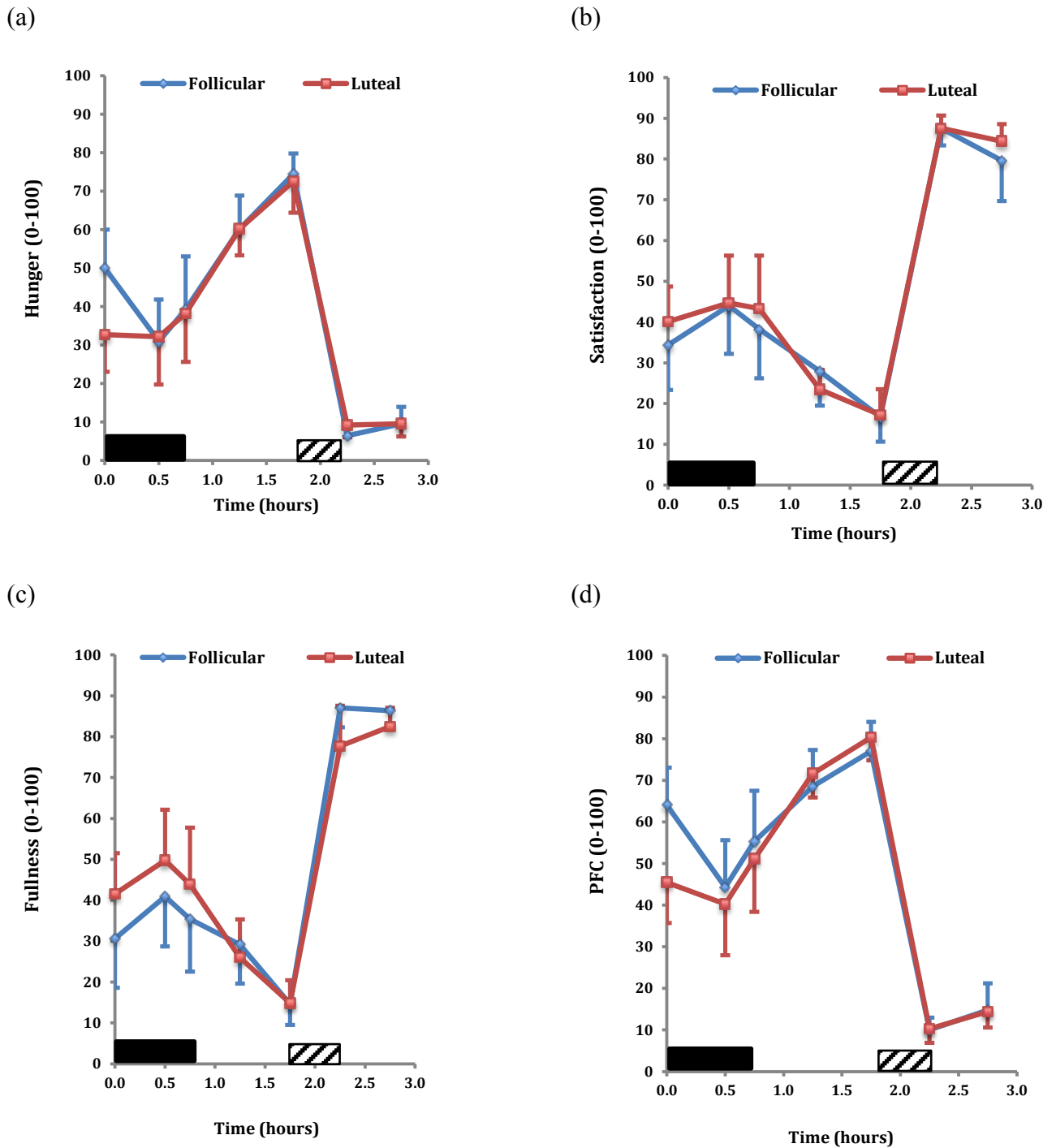
### 5.3.4 Food intake during *ad libitum* meals

There was no significant difference between carbohydrate, fat or energy intake during the *ad libitum* meals in the follicular and luteal phases (Table 5.4), indicating that responses did not differ significantly between trials. There was a tendency, however, for energy intake to be higher in the follicular phase than the luteal phase and protein intake during the *ad libitum* meals was significantly higher in the follicular than in the luteal phase.

**Table 5.4 Energy intake and macronutrient intake values during *ad libitum* meals in the follicular and luteal trials**

	Follicular	Luteal	<i>P</i>
<b>Energy intake</b>			
(g)	511 ± 251	449 ± 238	0.040
(kJ)	4615 ± 1736	4080 ± 1813	0.092
<b>CHO intake</b>			
(g)	120 ± 40	108 ± 50	0.269
(kJ)	2016 ± 677	1805 ± 831	0.269
<b>Protein intake</b>			
(g)	42 ± 19	35 ± 18	0.023
(kJ)	694 ± 312	589 ± 307	0.023
<b>Fat intake</b>			
(g)	49 ± 24	44 ± 27	0.236
(kJ)	1852 ± 895	1645 ± 1005	0.236

Values are mean ± SD, *n* = 13. CHO, carbohydrate.



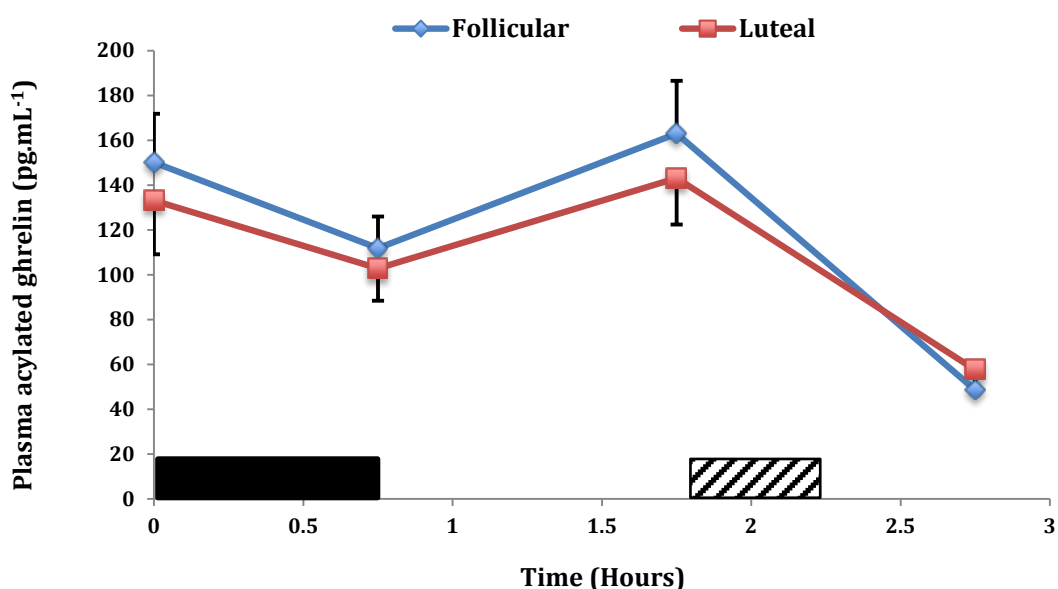
**Figure 5.3 Appetite perception values during the follicular and luteal trials**

Values are mean  $\pm$  SEM ( $n = 13$  females). The black rectangles indicate cycling; the diagonally shaded rectangles indicate the *ad libitum* meal. Comparisons are as follows: panel a) hunger; panel b) satisfaction; panel c) fullness; panel d) prospective food consumption (PFC).



### 5.3.5 Plasma acylated ghrelin

Two-factor ANOVA revealed that plasma acylated ghrelin concentrations changed significantly over time ( $P < 0.001$ ; Figure 5.4). There was no significant difference between trials and no significant trial  $\times$  time interaction effect for plasma acylated ghrelin. Thus there was no difference in the response of plasma acylated ghrelin between the follicular and luteal phases over time, or between trials). Area under curve values over the whole trial for acylated ghrelin showed that there was no significant difference between the follicular and luteal phases of the menstrual cycle (Figure 5.6).



**Figure 5.4 Plasma ghrelin during the follicular and luteal trials**

Values are mean  $\pm$  SEM ( $n = 13$ ). The black rectangle indicates cycling and the diagonally shaded rectangle indicates the *ad libitum* meal. Values are not significantly different between trials.

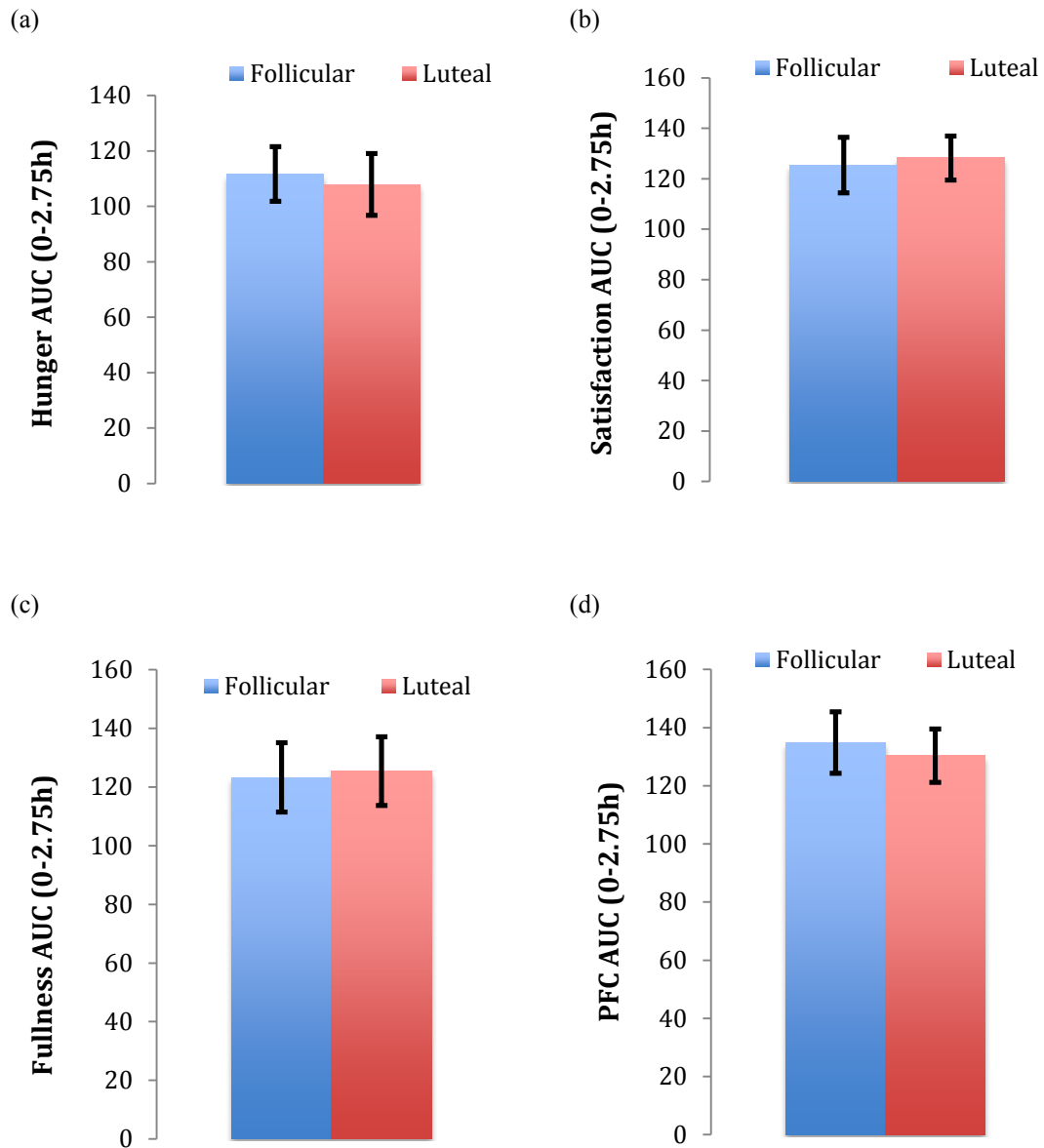
### 5.3.6 Correlations between acylated ghrelin concentration and other variables

Baseline plasma acylated ghrelin concentrations, in both the follicular and luteal trials, were not significantly correlated with body mass, BMI, percentage of body fat or maximum oxygen uptake. Changes in acylated ghrelin were correlated significantly and negatively

with changes in hunger before and after food intake in follicular phase ( $r = -0.636$ ,  $P = 0.019$ ), these changes were not significant in the luteal phase ( $r = -0.310$ ,  $P = 0.303$ ). On the other hand, there were no significant correlations between changes in acylated ghrelin before and after meals and energy intake in either phase (follicular,  $r = 0.697$ ,  $P = 0.119$ ; luteal,  $r = 0.255$ ,  $P = 0.401$ ).

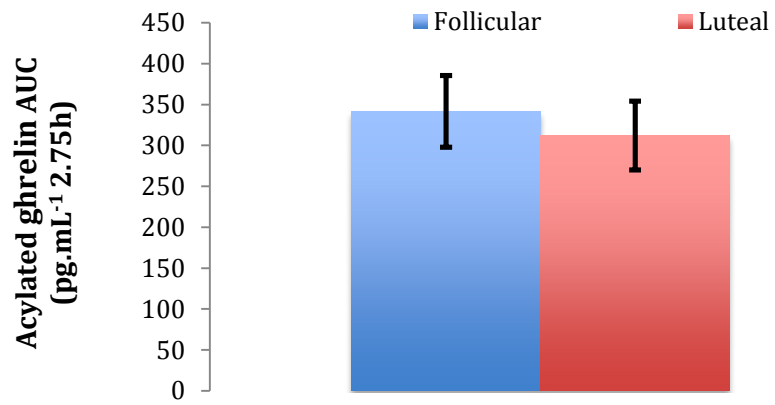
### **5.3.7 Ovarian hormones**

Paired samples t-tests revealed that 17- $\beta$  estradiol was significantly higher in the follicular phase than the luteal phase ( $P = 0.018$ ). There was no significant difference relating to the progesterone hormone ( $P > 0.05$ ).



**Figure 5.5 Area under the curve values for appetite perceptions**

Area under the curve values for appetite perceptions: (a) hunger, (b) satisfaction, (c) fullness and (d) prospective food consumption during the follicular and luteal trials ( $n = 13$ ). Values are mean  $\pm$  SEM. No significant differences.



**Figure 5.6 Acylated ghrelin AUC values during the follicular and luteal trials**

Values are mean  $\pm$  SEM, ( $n = 13$ )

## 5.4 Discussion

Sex steroids have been shown to interfere with appetite and metabolic functions (Rickenlund *et al.*, 2004). Estradiol inhibits feeding in animals (Geary, 2001) whereas high dose progestins are appetite stimulating (Maltoni *et al.*, 2001). However, to the author's knowledge no studies have documented the effect of the oral contraceptive pill on appetite, food intake and acylated ghrelin. The aim of this study was to determine whether food intake, perceptions of appetite and concentrations of acylated ghrelin during and after cycling exercise differ between the luteal and the follicular phases in females taking the OCP.

Concerning the menstrual cycle hormones, it was found that estradiol was significantly higher in the follicular phase than the luteal phase. Progesterone levels were high in both phases as expected in those taking the OCP. This can be attributed to the presence of synthetic hormones due to the administration of the oral contraceptive pill. Progesterone levels are high in both phases as this is the main mechanism used to prevent ovulation. Even though it was slightly higher in the luteal phase, there was no significant difference in the concentration of progesterone hormones in the two phases. These findings differ somewhat to those of Timmons *et al.* (2005) who observed that estradiol levels were low in both phases of the OCP cycle in women taking the OCP, while progesterone was higher during the luteal phase than during the follicular phase (Timmons *et al.*, 2005). The reason for this difference is not immediately clear although differences in the participant groups and/or the measurement procedures for estradiol and progesterone may be responsible.

Food intake was controlled and weighed to obtain dietary information. It is expected that food intake would be higher during the luteal phase than the follicular phase due to higher progesterone levels (and therefore a stimulatory effect). Conversely it was expected that food intake would be lower in the follicular phase due to higher estradiol levels which inhibit appetite. The findings of the present study show that there was no significant difference in the acylated ghrelin and energy intake response to cycling during the 'follicular' and 'luteal' phases but fasting hunger and PFC values tended to be higher while fasting satisfaction and fullness values tended to be lower in the follicular than the luteal

phase. Also, it should be noted that there was a borderline effect for energy intake ( $P=0.092$ ) in favour of a higher intake in the follicular phase. Previous results observed by authors such as Lyons *et al.* (1989) in their study of food consumption in 18 healthy women in the different phases of the menstrual cycle, carried out by means of the direct weighing of food and the production of a diet record, showed a significant rise in food consumption in the luteal phase. The same was observed by Barr *et al.* (1995), who examined the food intake of 42 women by means of a dietary record. Investigation by Li *et al.* (1999) involving 20 university students, found a greater caloric intake during the luteal phase than the follicular phase. Findings from Dalvit (1981) and Pliner and Fleming (1983) also confirm a higher energy intake in the luteal phase. The present study indicates contrary findings. Food intake was weighed and recorded and it did not differ significantly between the follicular and luteal phases of the OCP cycle. In addition, participants also reported feeling hungrier during the follicular phase rather than the luteal phase in the present study. The reason for these conflicting results is unclear but it may relate to the fact that participants were not taking the oral contraceptive pill in the previous study in contrast to the present study.

The present study also found that the consumption of carbohydrates was higher (although not significantly) during the follicular phase compared with the luteal phase. These findings differ from previous studies, such as that of Li *et al.* (1999), who detected a significant difference in the ingestion of carbohydrates and lipids during the luteal phase. Furthermore, Martini *et al.* (1994) similarly found significant increases in ingestion of carbohydrates, lipids and proteins within the luteal phase when compared to those values found in the follicular phase, on investigating the effect of menstrual cycle on food consumption in 18 women. Cross *et al.* (2001) also obtained similar results to those of these studies, reporting a significant increase in the consumption of lipids and carbohydrates in the luteal phase. Again, these contradictory results could be because the participants in these previous studies were not on the OCP, unlike in the current study. An increase in lipid consumption in the luteal phase has been described in the literature as being the consequence of the effect of steroid hormones on metabolism (Gil *et al.*, 2009). It is believed that progesterone promotes the accumulation of lipids in the interior of adipose cells, resulting in a reduction in plasma triacylglycerol and stimulating the consumption of meals containing higher

levels of fat (Gil *et al.*, 2009). Estradiol, by contrast, is thought to stimulate lipolysis, resulting in a greater quantity of substrate for energy production (Barr *et al.*, 1995). There was no significant difference in the consumption of carbohydrate and fat in the current study and food intake (in g but not in kJ) was higher in the follicular phase than the luteal phase.

This study has several limitations. First of all, the study was carried out on a relatively small sample size ( $n = 13$ ). The normal biological variations in the secretion of hormones among women will increase the variability to such a degree that a relatively large sample size is needed in order to minimize the statistical effects of these differences. It is recommended that further studies of this type with more representative samples be carried out to help get a better understanding of the relationships between female hormones and appetite, food intake and acylated ghrelin during the OCP cycle.

Limited studies have been carried out on the changes in acylated ghrelin over the OCP cycle. This study found that, while concentrations of acylated ghrelin changed over the course of the trial, there was no significant difference between the two phases. Concerning the acylated ghrelin results, the majority of past studies have focused on the effect of long duration, acute exercise on the concentration of acylated ghrelin. Two studies investigating the chronic effects of exercise (Kim *et al.*, 2008; Mirzaei *et al.*, 2009) have found that a long-term exercise regime has no impact on acylated ghrelin. Two studies (Broom *et al.*, 2009; Marzullo *et al.*, 2008) have focused on the acute effects of relatively intense exercise. Broom *et al.* (2009) found that 60 minutes of exercise on a treadmill at 70-72% of  $VO_2$  max caused a decrease in acylated ghrelin concentration. The other study also reported a decline in acylated ghrelin concentration in 8 obese males after a graded bicycle ergometer exercise test to exhaustion (Marzullo *et al.*, 2008). Contrary to these findings, Mackelvie *et al.* (2007) noted that 5 consecutive days of aerobic exercise (65% of maximum heart rate reserve) for one-hour duration increased the acylated ghrelin level in overweight and normal weight adolescent male subjects. The current study applied cycling as the exercise method, the session only lasted for 45 minutes and the results indicated suppression in acylated ghrelin immediately after exercise. It might be useful to obtain results from a control group that does not partake in exercise during the OCP cycle to gain a more in-

depth understanding of the relationship between of the OCP cycle and the concentration of acylated ghrelin. It would also be interesting to examine how the concentrations of other appetite regulating hormones respond during the different phases of the OCP cycle. However, due to time constraints, this could not be included in the current study.

Despite these limitations, this is one of the few studies to explore the relationship between OCP usage and acylated ghrelin concentrations and these preliminary findings indicate that plasma acylated ghrelin concentrations are suppressed immediately after exercise in female OCP users but are unaffected by the phase of the OCP cycle both at rest and during/after exercise. These findings suggest that in studies investigating appetite, appetite regulating hormones and food intake in female participants who are taking the OCP it may be unnecessary to account for OCP cycle phase.



## 6 Study 3: Appetite and hormonal responses to exercise and food restriction in women

### 6.1 Introduction

Obesity is now a global epidemic, especially in (but not restricted to) developed countries (WHO, 2003). The UK is not exempt, with an almost threefold increase in the prevalence of obesity in the last two decades and present numbers demonstrating that over 60% of the population are overweight (National Audit Office, 2001). As the prevalence in obesity continues to rise, effective strategies are needed to facilitate successful weight control (Kelly *et al.*, 2008). To achieve this, a better understanding of the mechanisms of energy balance regulation is required (King *et al.*, 2011a). In theory, energy balance is determined by the energy consumed as food and drink and that is expended during physical activity (Spiegelman and Flier, 2001). To induce weight loss, individuals must stimulate an energy deficit by either restricting their dietary intake or by increasing the amount of physical activity performed to create a negative energy balance (King *et al.*, 2011a). However, the steady increase in the prevalence of obesity has been accompanied by the opposite, with an increase in the consumption of energy-dense food and, on the other hand, by a reduction in physical activity levels (Varo *et al.*, 2003; WHO, 2003).

While it is widely accepted that physical activity plays a role in preventing weight gain (Haapanen *et al.*, 1997; Martinez-Gonzalez *et al.*, 1999), Miller *et al.* (1997) found that its impact on weight loss without a subsequent energy restriction appears to be only modest. This could be because the ability of exercise to create a negative energy balance does not only rely directly on its impact on energy expenditure, but also on its potential to indirectly control energy intake (King *et al.*, 1997). It has been suggested that the inefficiency of exercise on weight loss may originate from the energy deficit created by exercise being partially compensated for by an increase in energy intake (Blundell and King, 1999). However, most studies show no impact of acute exercise on appetite (King *et al.* 1996; Imbeault *et al.*, 1997; Hubert *et al.*, 1998) or subsequent energy intake (King *et al.*, 1996; King *et al.*, 1997; Imbeault *et al.*, 1997; Hubert *et al.*, 1998; Blundell and King 1999) and this remains a rather controversial area (Blundell and King 1999).

Different mechanisms operate in the very complex system that regulates appetite and energy balance, depending on the time frame of response; such as long-term signals that include leptin and insulin, intermediate post-absorptive signals associated with macronutrient oxidation and, finally, short-term mechanisms involving post-ingestion hormones such as ghrelin, cholecystokinin (CKK), peptide YY (PYY) and pancreatic polypeptide (PP) (Blundell, 1991; King *et al.*, 1997). These metabolic and endocrine signals are received and processed by specific areas in the hypothalamus and brainstem, leading to a subsequent, coordinated response that targets both energy intake and energy expenditure (Neary *et al.*, 2004). As exercise is a major factor in the energy balance equation, it is consequently extremely important to gain in-depth understanding of how these appetite-related hormones respond to exercise. However, past research in this area is rather limited (Sullivan, 1984; Greenberg *et al.*, 1986; Bailey *et al.*, 2001; O'Connor *et al.*, 2006) and as a result, the current study aims to investigate appetite perceptions, the appetite hormones acylated ghrelin and peptide YY<sub>3-36</sub> (PYY<sub>3-36</sub>), and energy intake responses to identical energy deficits imposed by food restriction and exercise.

The present study in females is a repeat of a study by King *et al.* (2011a) which investigated male participants. The study by King *et al.* (2011a) showed that there were compensatory ghrelin, PYY<sub>3-36</sub>, appetite and food intake responses to a dietary induced energy deficit which was not observed with an equivalent exercise induced energy deficit. In view of the evidence that appetite and hormonal responses to exercise may differ between females and males the present study was designed to assess whether similar compensatory responses to dietary induced energy deficits occur in women as have been shown to occur in men and also to assess whether the lack of a compensatory response to acute exercise in men is also observed in women.

## **6.2 Methods**

### **6.2.1 Participants**

After the Loughborough University Ethical Advisory Committee approved the study, 13 healthy, non-oral contraceptive user, female volunteers aged between 18 and 40 years old signed written informed consent forms to confirm their participation in the study. In order to ensure the safety of participants and to minimize confounding factors participants had to conform to the following criteria: have no personal history of cardiovascular disease, metabolic disease or dyslipidaemia (abnormal blood fat (triacylglycerol) or cholesterol); not dieting or following extreme dietary habits (assessed using a three-factor eating questionnaire); non-smoking; not taking drugs known to affect digestion or metabolism, medical or illegal (e.g. anabolic steroids, marijuana, amphetamines, thyroid prescription drugs) and not pregnant.

### **6.2.2 Study design**

Participants were required to attend the Exercise and Health laboratory (Clyde Williams building, HE1.11) on 5 occasions. On the first laboratory visit, the objectives and requirements of the study was explained to participants before they gave their written informed consent to participate. After this, screening tests were carried out. This included completing a health screen questionnaire to assess fitness to participate in the study; a physical activity questionnaire to assess current physical activity level and a three factor eating questionnaire to assess eating behaviour. After this, participants were given a chance to familiarise themselves with the testing procedures and equipment, with dietary recording and any questions they had were answered. Once these questionnaires were completed, a series of measurements were taken including resting blood pressure, height, weight, skinfold thicknesses at four sites, waist and hip circumferences. After the screening phase was completed, participants underwent a preliminary exercise test to determine maximum oxygen uptake. This involved a 16 minute submaximal treadmill running test followed by a maximum oxygen uptake test once participants had recovered from the submaximal test. (Please refer to Chapter III Sections 3.2 and 3.6.1 ‘General Methodology’)

During the second visit, participants completed a 90-minute familiarisation run. Participants also familiarised themselves with the testing procedures. The three subsequent occasions involved experimental trials each lasting for nine hours. One of these trials (exercise trial) involved a 90 minute treadmill run followed by seven and a half hours of rest; the other trials (control and diet-induced energy deficit trials) involved nine hours of rest. Participants were required to weigh and record all food and drink consumed in the 24 hours prior to each main trial and to abstain from alcohol, caffeine and structured physical activity during this time.

### **6.2.3 Main trials**

There were three main trials, each lasting nine hours. These were assigned in a random order and separated by at least one week. Participants had to be in the same phase of the menstrual cycle when completing each trial, therefore they completed two trials one month and then one trial the next month when in the same phase of the menstrual cycle as during the first two main trials.

### **6.2.4 Exercise trial**

Participants reported to the Exercise and Health Laboratory at 7.30 a.m. after having fasted overnight and not consumed breakfast. At 8.00 a.m. they performed a 90-minute treadmill run. After the run they rested (e.g. reading, working, listening to music, watching television, playing computer games) for the remainder of the trial (until 5.00 pm). Participants consumed standardised meals at 10.00 a.m. and 12.45 p.m. and they were given access to a free choice buffet meal at 4.00 p.m. Venous blood samples were collected at eight time points during the trial via a cannula inserted into an antecubital or forearm vein (Figure 6.1). Participants were asked to complete visual analogue scales (VAS) every half an hour (see below).

### **6.2.5 Control and diet-induced energy deficit trials**

Procedures during these two trials were identical to those during the exercise trial except that no exercise was performed. Participants therefore rested for the entire nine hours of the trials. Furthermore, during the diet-induced energy deficit trial, the breakfast and lunch meals had a reduced energy content (see below).

### **6.2.6 Appetite assessment**

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at baseline and then at 30-minute intervals throughout. Each VAS line was 100 mm long and contained a question above the line with answers to the question appearing at either end of the line e.g. How hungry do you feel right now? – ‘I am not hungry at all’ versus ‘I have never been more hungry’. Participants were asked to place a mark somewhere on the line that best described their feeling.

### **6.2.7 Test meals**

During main trials test meals were provided to participants at 10:00am and 12:45pm (2 and 4.75 h). Each meal was consumed within 15 minutes. The test meals consisted of a tuna and mayonnaise sandwich, salted crisps, chocolate muffin and green apple. The macronutrient content of the meal was balanced (fat 34%, protein 18%, carbohydrate 48%) and remained consistent at all test meals across main trials (Figure 6.1).

The energy content of the test meals was identical in the control and exercise trials and was calculated to be sufficient to meet each participant’s individual energy requirements. To calculate the amount to be provided for each participant resting daily energy requirements were estimated using validated predictive equations (Mifflin *et al.*, 1990). Then, the amount derived was multiplied by a physical activity level of 1.4 (this amount is deemed sufficient enough to meet the energy needs of individuals across a resting day). Participants received 70% of this amount divided equally across two identical test meals. The rationale for

providing this amount was based on pilot work, which showed that provision of this amount during a resting day was sufficient to induce a comfortable level of satiation.

In the food deficit trial, participants received a restricted amount of food at the test meals. The amount provided was calculated by deducting the net estimated energy expenditure of exercise from the energy provided at the meals in the control and exercise trials. The total amount deducted was divided equally across the two test meals. Consequently, as compared with control, after the second test meal in the food deficit trial participants were in an identical state of energy deficit in the exercise and food deficit trials, the only difference being the cause of the deficit (exercise verses food restriction).

### **6.2.8 Ad libitum buffet meals**

Eight hours into the main trials participants were given access to a buffet meal from which they were free to consume food *ad libitum* (Please refer to Chapter III ‘General Methodology’ Section 3.11 and Appendix D for full list of all foods). Participants were given 30 minutes to choose and consume food items from the buffet. At each meal food was presented in excess of expected consumption. Participants were told to eat until satisfied and that additional food was available if desired. Meals were consumed in isolation so that social influence did not affect food selection. Food consumption was determined by measuring the weighted difference in food items remaining compared to that initially presented. The energy and macronutrient content of the items consumed was determined using manufacturer values.

### **6.2.9 Blood sampling**

During the main trials venous blood samples were collected via a cannula inserted into an antecubital vein. The baseline sample on the exercise trial was an exception to this whereby blood samples were taken via venepuncture of an antecubital vein. In the exercise trial a cannula was inserted after the completion of exercise.

Blood samples were collected into 4.9 mL EDTA monovettes at baseline, 2, 3, 4.75, 6, 7, 8 and 9 h to measure circulating concentrations of acylated ghrelin. Additional 2 mL samples

were also collected at these times to measure circulating concentrations of PYY<sub>3-36</sub>. To maintain the integrity of the PYY<sub>3-36</sub> samples, blood was collected into pre-chilled syringes containing dipeptidyl-peptidase-4 inhibitor (10  $\mu\text{L}\cdot\text{mL}^{-1}$ ). After mixing by gentle inversion samples were then dispensed into pre-chilled EDTA tubes containing aprotinin at a final concentration of 500 KIU. $\text{mL}^{-1}$ . These samples were spun at 3500 rpm for 10 min in a refrigerated centrifuge at 4 °C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage at -80 °C.

All blood samples were collected with participants in a semi-supine position. For samples collected using a cannula patency was maintained by flushing with non-heparinised saline (0.9 % w/v sodium chloride). To avoid subsequent sample dilution residual saline was discarded using a 2 mL syringe before sample collection. To estimate changes in plasma volume, at each blood sampling point duplicate 20  $\mu\text{L}$  blood samples were collected into micropipettes and triplicate 20  $\mu\text{L}$  blood samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations, respectively.

#### **6.2.10 Biochemical analysis**

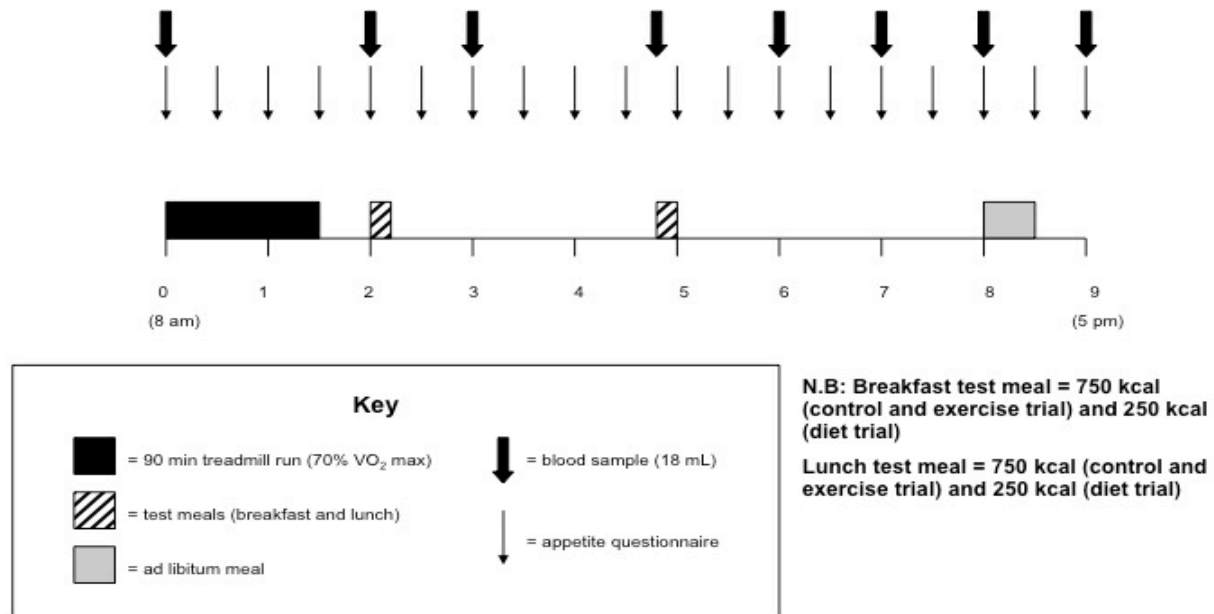
An enzyme-linked immune sorbent assay kit was used to determine plasma acylated ghrelin concentrations (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader (Expert Plus, ASYS, Eugendorf, Austria). To eliminate inter-assay variation, all samples from the same participant were run on the same plate. Plasma concentrations of PYY<sub>3-36</sub> were determined using a radio-immunoassay kit (LINCO Research, Missouri, USA.). The within batch coefficients of variation for the assays were as follows: acylated ghrelin 6.9%, PYY<sub>3-36</sub> (6.8%). (For further details of the blood sampling and analysis procedures see section 3.14 of the General Methodology).

### 6.2.11 Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 for Windows. All area under the concentration verses time curve calculations were carried out using the trapezoidal method. One-way repeated measures ANOVA was used to assess differences between trials in fasting and AUC values for acylated ghrelin, PYY<sub>3-36</sub>, and appetite perceptions. One-way ANOVA was also employed to assess between trial differences in buffet meal energy and macronutrient intake. Repeated measures, two-way ANOVA was used to examine differences between trials over time for appetite perceptions, acylated ghrelin and PYY<sub>3-36</sub>. Where significant main effects were found, post- hoc analysis was performed using the Bonferroni correction for multiple comparisons. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean  $\pm$  SD unless otherwise stated.



**Appetite and hormonal responses to exercise and food restriction in women**  
(Trial Schematic)



**Figure 6.1** A schematic of the protocol for the main trials

## 6.3 Results

### 6.3.1 Physical characteristics

The physical characteristics of the 13 participants involved in the study are presented in Table 6.1.

**Table 6.1 Physical characteristics of the participants**

Characteristic	Mean	SD
Age (yr)	23.2 ±	3.6
Height (cm)	165.7 ±	5.2
Weight (kg)	60.4 ±	4.0
BMI (kg.m <sup>-2</sup> )	22.0 ±	1.1
Body fat (%)	24.2 ±	2.7
VO <sub>2</sub> max (mL.kg <sup>-1</sup> min <sup>-1</sup> )	49.4 ±	5.4

(*n* = 13).

Table 6.2 shows participants' responses to exercise. They completed the 90-minute run at a speed of  $8.4 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$ . This produced a mean maximal oxygen consumption of  $49.4 \pm 5.4 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , which is equivalent of  $70.8 \pm 2.7\%$  of VO<sub>2</sub> max. Mean heart rate was  $176 \pm 4 \text{ beats}\cdot\text{min}^{-1}$  and energy expenditure averaged  $3833 \pm 446 \text{ kJ}$ . A mean RPE value of  $13 \pm 2$  denotes that the participants felt the intensity of the run was 'somewhat hard'.

**Table 6.2 Responses to treadmill running**

Variable	Mean	SD
RPE (6-20)	13 ± 2	
Speed (km.h <sup>-1</sup> )	8.4 ± 1.1	
Heart rate (beats.min <sup>-1</sup> )	176 ± 4	
VO <sub>2</sub> max (mL.kg <sup>-1</sup> min <sup>-1</sup> )	49.4 ± 5.4	
% VO <sub>2</sub> max	70.8 ± 2.7	
Energy expenditure (kJ)	3833 ± 446	

*n* = 13.

**Table 6.3 Baseline values in control, exercise and food deficit trials**

	Control	Exercise	Food deficit
Acylated ghrelin (pg·mL <sup>-1</sup> )	148 ± 100	140 ± 86	148 ± 96
PYY <sub>3-36</sub> (pg·mL <sup>-1</sup> )	75 ± 38	74 ± 33	76 ± 34
Hunger (0-100)	61 ± 23	65 ± 27	67 ± 21
Fullness (0-100)	16 ± 16	21 ± 22	11 ± 11
Satisfaction (0-100)	20 ± 15	25 ± 24	18 ± 12
PFC (0-100)	73 ± 16	73 ± 20	73 ± 14

Values are mean ± SD (*n* = 12 for acylated ghrelin and PYY<sub>3-36</sub>, *n* = 13 for other variables). There are no significant differences.

### 6.3.2 Ad libitum food intake

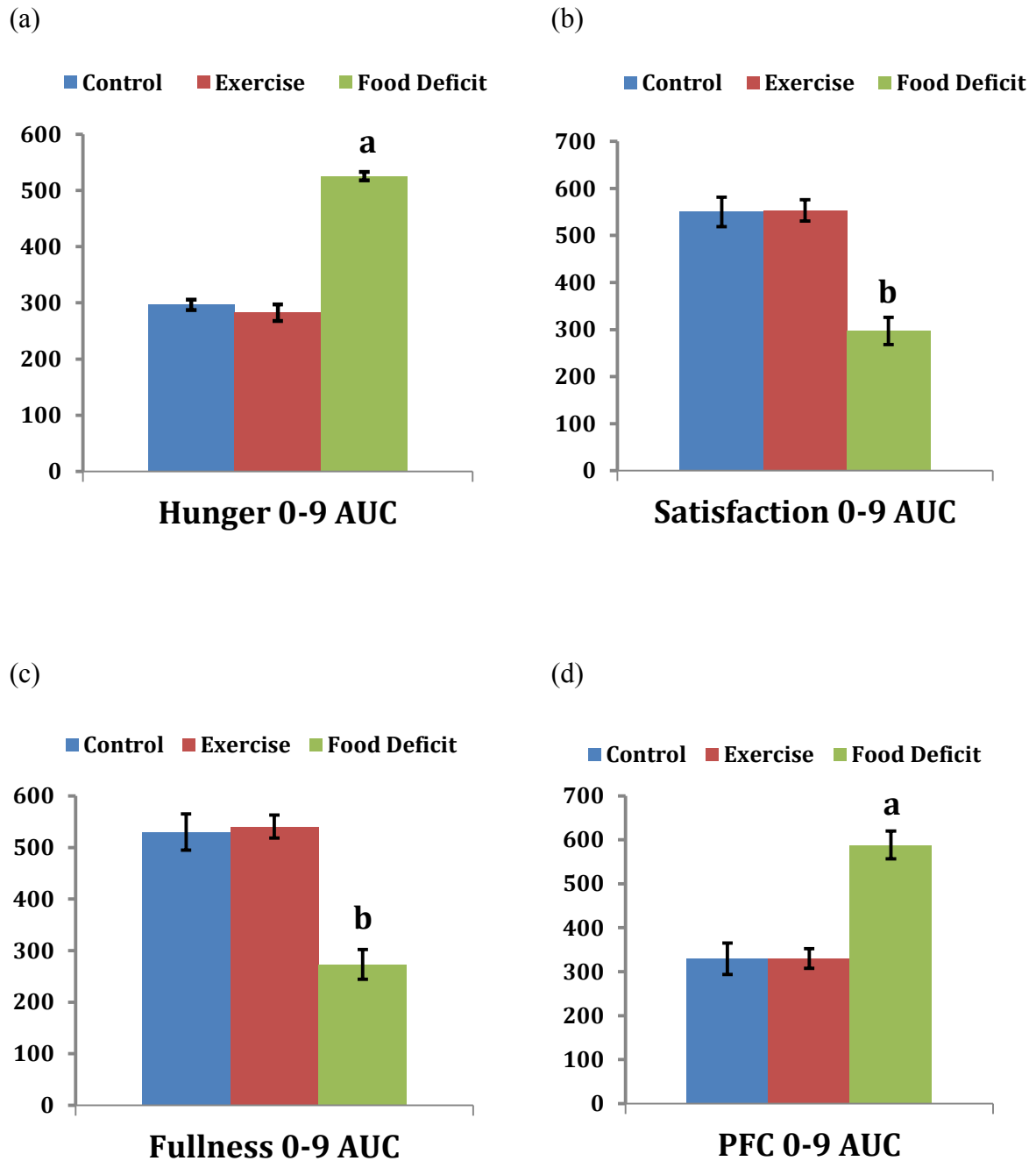
Table 6.4 presents the results of the food intake and macronutrient intake collected at the *ad libitum* meal. For food (energy) intake, one-way ANOVA revealed a significant main effect of trial (*P* = 0.000). Post hoc analysis showed that food intake, shown in kJ and grams, was significantly higher on the food deficit trial than the control trial (*P* < 0.03) and on the food deficit trial than the exercise trial (*P* < 0.05). One-way ANOVA was used to determine if there was a significant difference between the groups concerning

macronutrient intake. It indicated a significant difference in the amount of CHO ( $P = 0.005$ ), fat ( $P = 0.001$ ) and protein ( $P = 0.000$ ) consumed, with a significantly higher intake of each on the food deficit trial than on both the control and exercise trials. Water intake was higher in the exercise trial than in the control and food deficit trials ( $P = 0.000$ ).

**Table 6.4 Food and macronutrient intake value during *ad libitum* meal in the control, exercise and food deficit trials and water intake values during the whole of each trial**

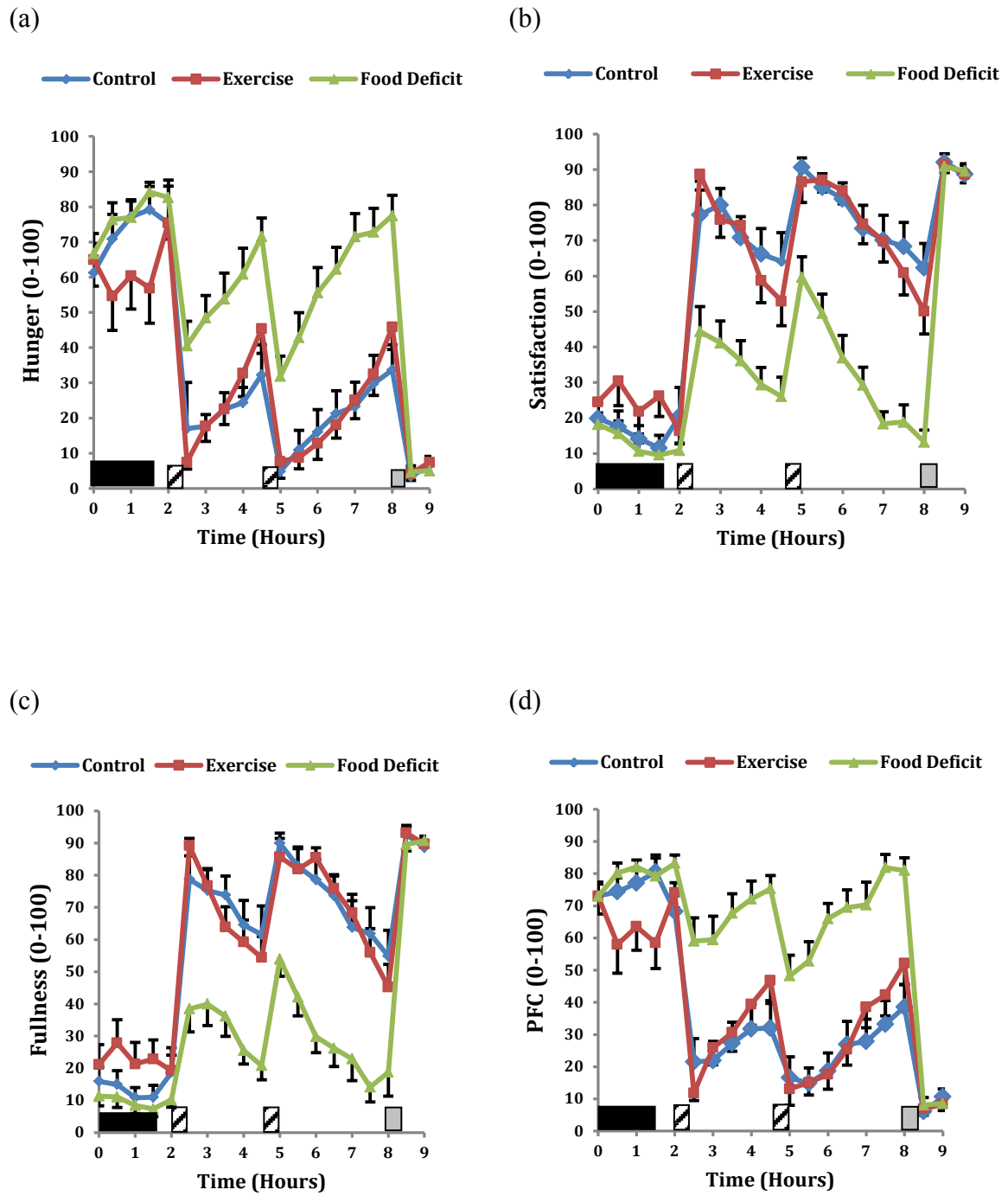
	Control	Exercise	Food Deficit	<i>P</i>
<b>Food intake</b>				
(g)	390 ± 150	415 ± 180	532 ± 163 <sup>ab</sup>	0.002
(kJ)	2517 ± 1076	2748 ± 1613	3897 ± 1371 <sup>ab</sup>	0.000
<b>CHO intake</b>				
(g)	81 ± 33	86 ± 37	111 ± 41 <sup>ab</sup>	0.005
(kJ)	1359 ± 552	1430 ± 616	1854 ± 689 <sup>ab</sup>	0.005
<b>Protein intake</b>				
(g)	20 ± 10	21 ± 15	33 ± 12 <sup>ab</sup>	0.000
(kJ)	327 ± 163	356 ± 253	547 ± 203 <sup>ab</sup>	0.000
<b>Fat intake</b>				
(g)	22 ± 17	25 ± 23	39 ± 19 <sup>ab</sup>	0.001
(kJ)	810 ± 650	942 ± 856	1457 ± 703 <sup>ab</sup>	0.001
<b>Water intake</b>				
(mL)	1163 ± 485	1567 ± 380 <sup>cd</sup>	1117 ± 497	0.000

Values are mean ± SD, *n* = 13. CHO=carbohydrate. *P* values in the right hand column represent values from one-way ANOVA tests. Post-hoc findings are highlighted by the letters a, b, c and d. <sup>a</sup> Significantly higher (*P* < 0.03) in food deficit trial than in the control trial. <sup>b</sup> Significantly higher (*P* < 0.05) in food deficit trial than in the exercise trial. <sup>c</sup> Significantly higher (*P* = 0.001) in exercise trial than in the control trial. <sup>d</sup> Significantly higher (*P* = 0.005) in exercise trial than in the food deficit trial.



**Figure 6.2 Appetite AUC in the control exercise and food deficit trials**

Area under curve values for appetite scores, hunger (a), satisfaction (b), fullness (c) and prospective food consumption (PFC) (d). for 0-9 hours of the control (Con), exercise (Ex) and food deficit (FD) trials. Values are mean  $\pm$  SEM ( $n = 13$ ). <sup>a</sup> Values significantly higher ( $P < 0.001$ ) in food deficit trial than in both the control and exercise trials, and <sup>b</sup> values significantly lower ( $P < 0.001$ ) in food deficit trial than both the control and exercise trials.



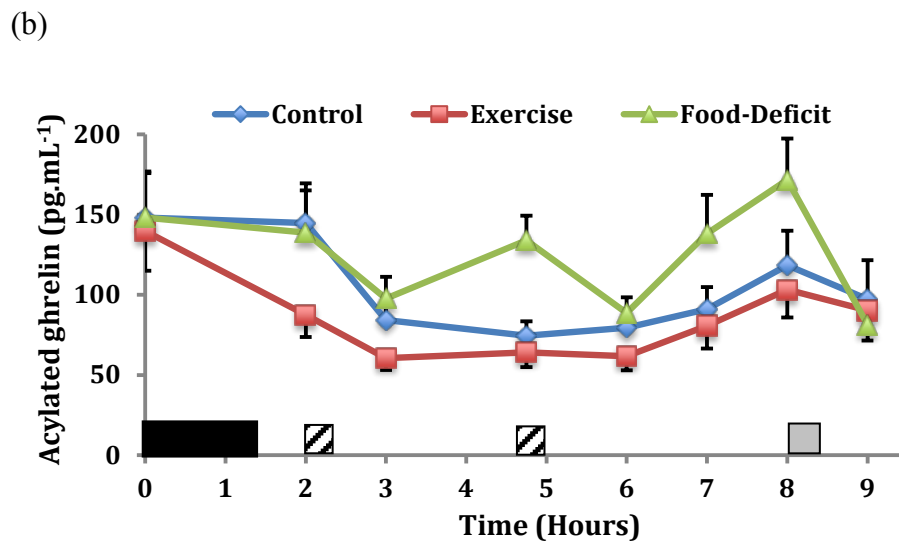
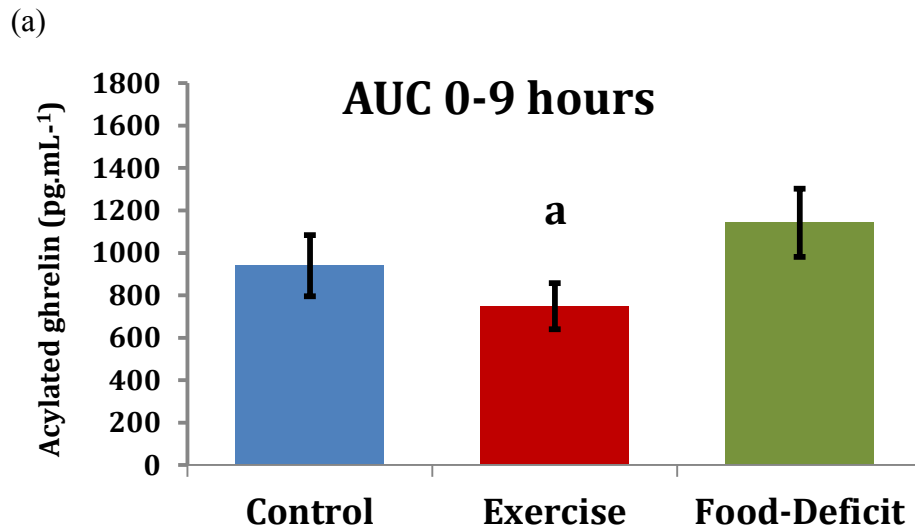
**Figure 6.3 Appetite ratings during the control, exercise and food deficit trials**

Comparisons are as follows: panel a) hunger; panel b) satisfaction; panel c) fullness and d) prospective food consumption (PFC). Values are mean  $\pm$  SEM ( $n = 13$ ). Black rectangle indicates running, diagonally shaded rectangles indicate standardised meals, shaded rectangle indicates the *ad libitum* meal.

### 6.3.3 Acylated ghrelin

Figure 6.4 shows acylated ghrelin responses during the trials. This was examined by calculating the AUC for the total trial (0 – 9 h). The analysis revealed significant differences in plasma concentrations of acylated ghrelin between the three trials ( $P < 0.001$ ) for the total trial. Two-way ANOVA revealed significant trial, time and trial x time (interaction) main effects (all  $P < 0.05$ ), showing that acylated ghrelin responses differed over time between the trials. Post-hoc tests showed that AUC values in the exercise trial were significant lower than in the control and food deficit trials, with exercise versus control ( $P = 0.016$ ), and exercise versus food deficit ( $P = 0.004$ ). This shows a suppressive effect of exercise on acylated ghrelin. At individual time points, post-hoc analysis shows significant differences between trials, at time point 2h acylated ghrelin values showed that it was lower in exercise trials than food deficit and control trials. At time point 7h acylated ghrelin values were higher in food deficit than exercise and control trials.





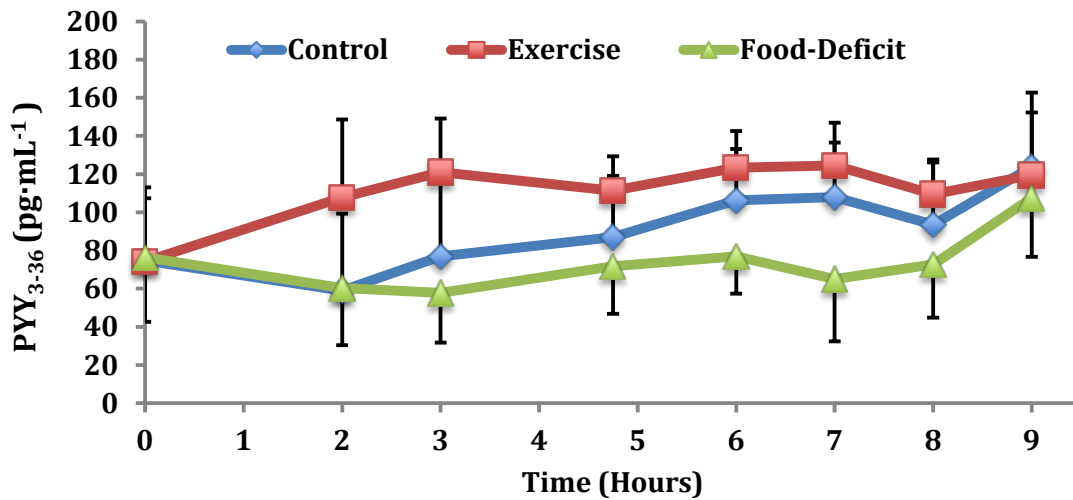
**Figure 6.4 Plasma concentrations of acylated ghrelin during the control, exercise and food deficit trials**

Panel a) Area under the curve values for acylated ghrelin 0-9 h; values are mean  $\pm$  SEM ( $n = 12$ ). <sup>a</sup> values significantly different between trials ( $P < 0.001$ ), post-hoc test showed that area under the curve value for acylated ghrelin in exercise trial was significantly lower than in the control and food deficit trials, exercise versus control ( $P = 0.016$ ), and exercise versus food deficit ( $P = 0.004$ ); panel b) acylated ghrelin values during the trials; values are mean  $\pm$  SEM ( $n = 12$ ). Black rectangle indicates running, diagonally shaded rectangles indicate standardised meal, shaded rectangle at 8 hours indicates the *ad libitum* meal.

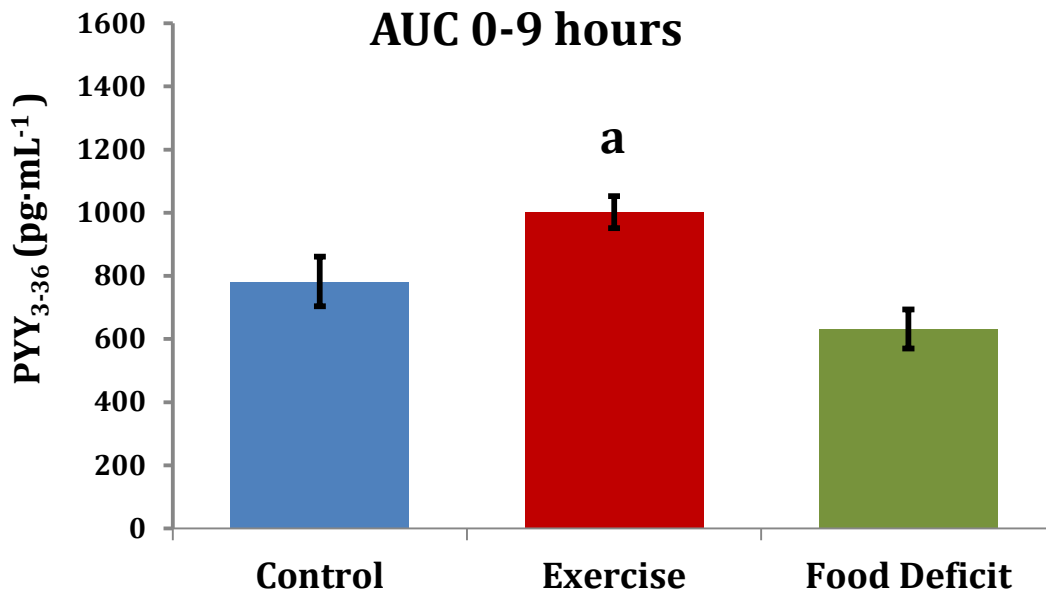
### 6.3.4 PYY<sub>3-36</sub> results

Results also show PYY<sub>3-36</sub> responses during the main trials. Two-way ANOVA revealed significant trial, time and trial x time (interaction) main effects (all  $P < 0.001$ ), showing that PYY<sub>3-36</sub> responses differed over time between the trials. Post-hoc analysis identified significantly higher circulating PYY<sub>3-36</sub> concentrations in the exercise deficit trial, compared with the control ( $P = 0.023$ ). There was also a significantly higher circulating PYY<sub>3-36</sub> concentration in the exercise trial when compared to the food deficit trial ( $P < 0.001$ ) (Figure 6.5). At individual time points, post-hoc analysis shows significant differences between all three trials, at time point 2h PYY<sub>3-36</sub> values showed that it was higher in exercise trials than in food deficit and control trials. However, at time point 7h and 9h PYY<sub>3-36</sub> values were lower in food deficit than exercise and control trials.

(a)



(b)



**Figure 6.5 Plasma concentrations of PYY<sub>3-36</sub> during the control, exercise and food deficit trials**

Panel a) PYY<sub>3-36</sub> values during trials; values are mean  $\pm$  SD ( $n = 12$ ). Black rectangle indicates running, diagonally shaded rectangles indicate standardised meal, shaded rectangle at 8 hours indicates the *ad libitum* meal; panel b) Area under the curve values for PYY<sub>3-36</sub> 0-9h; values are mean  $\pm$  SEM ( $n = 12$ ). <sup>a</sup> values significantly different between trials ( $P < 0.001$ ), post-hoc test showed that area under the curve values for acylated ghrelin in the exercise trial were significant higher than values in the control and food deficit trials, exercise versus control ( $P = 0.013$ ), exercise versus food deficit ( $P < 0.001$ ).

### 6.3.5 Correlations between acylated ghrelin concentration and other variables

Fasting plasma acylated ghrelin concentration on the control, exercise and food deficit trials were significantly correlated with height ( $r = 0.560$ ,  $P < 0.05$ ), but not significantly correlated with BMI, percent body fat or maximum oxygen uptake. This positive correlation indicates higher acylated ghrelin concentration in females who were taller. Hunger in the exercise trial was significantly but negatively correlated to acylated ghrelin levels at time 0h in the exercise trial ( $r = -0.586$ ,  $P = 0.045$ ). At 9 h, acylated ghrelin concentration was significantly, but negatively correlated with PYY<sub>3-36</sub> in control and exercise trials however not within the food deficit trial ( $r = -0.610$ ,  $r = -0.665$ , for control and exercise respectively,  $P < 0.05$ ).

## 6.4 Discussion

This aim of this study was to investigate appetite, PYY, acylated ghrelin and food/energy intake responses to energy deficits stimulated by exercise and food restriction. It was found that appetite and energy intake increased in response to an energy deficit induced by food restriction but remain largely unchanged in response to an energy deficit brought about by exercise. Additionally, it was found that exercise has a suppressive effect on acylated ghrelin levels, while it had a stimulatory effect on PYY<sub>3-36</sub>. The findings are consistent with the results of previous studies in this area that found that exercise has no significant impact on appetite and energy intake (King *et al.*, 1994; King and Blundell, 1995; King *et al.*, 1997) while other studies such as Lawton *et al.*, (1993) and Green *et al.*, (1994) corroborate the present findings that food restriction leads to an increase in appetite and subsequent food intake. These findings suggest an explanation of why dieting is often a difficult and unsuccessful method of weight control, due to the subsequent increase in energy intake that it induces (King *et al.*, 2011a). Exercise may be a more successful way to induce an energy deficit, as it involves a higher energy expenditure without a subsequent increase in food intake (Donnelly *et al.*, 2009).

There have been a multitude of studies in the past two decades examining the relationship between exercise and food intake (Martins *et al.*, 2008). Most of them have shown that acute exercise does not increase hunger or energy intake (Thompson *et al.*, 1988; King *et al.*, 1996; King and Blundell, 1995; Westerterp-Plantenga *et al.*, 1997; Lluch *et al.*, 1998 and Blundell and King, 1999), even at high intensities (King *et al.*, 1997) and therefore leads to the conclusion that exercise is able to induce a short-term negative energy balance. Studies involving vigorous exercise (high intensity cycling or running) found a temporary reduction in hunger, a phenomenon that is referred to as ‘exercise-induced anorexia’ (King *et al.*, 1994 and 1995), although this effect is temporary and likely has no significant impact on the subsequent energy intake (King *et al.*, 1994; King *et al.*, 1997; Bellisle, 1999).

Nevertheless, controversies still remain concerning the effects of exercise on objective and subjective measures of appetite, with a few studies indicating an increase in hunger (Maraki *et al.*, 2005) and energy intake (Verger *et al.*, 1992; Verger *et al.*, 1994 and

Pomerleau *et al.*, 2004) in response to acute exercise. However, these inconsistencies can be attributed to methodological differences among the studies, such as exercise intensity (Thompson *et al.*, 1988), gender (Imbeault *et al.*, 1997), macronutrient content of the test meal (Tremblay *et al.*, 1994) and the time interval between eating and exercising (Verger *et al.*, 1992). Very few studies have examined the impact of chronic exercise as the sole intervention on appetite responses (Martins *et al.*, 2008). In normal weight individuals (both men and women), a 7-day exercise programme (80 or 120 minutes of exercise per day), with *ad libitum* food intake, did not induce any significant changes in subjective feelings of hunger or fullness (assessed hourly during waking hours) compared with a control condition (Stubbs *et al.*, 2002a; Stubbs *et al.*, 2002b).

Some studies also concentrated on the influence of exercise on acylated ghrelin and found that moderate to high-intensity aerobic exercise briefly suppresses the concentration of acylated ghrelin, an effect that occurs alongside a suppression of appetite (King *et al.*, 2010a; Broom *et al.*, 2007; Broom *et al.*, 2009; Marzullo *et al.*, 2008). However, it was recorded that this effect was brief, with levels of acylated ghrelin quickly returning to control values and remaining the same for several hours after exercise (King *et al.*, 2011a). This lack of change in acylated ghrelin after exercise may be a reason why acute bouts of exercise do not induce compensatory appetite and energy intake responses (King *et al.*, 2011a).

Aside from ghrelin, recent studies have examined episodic gut hormones involved in appetite suppression to assess whether they may contribute to exercise-induced anorexia (Stensel, 2011). Most attention has been focused on PYY since it is a hormone known for its strong appetite-suppressing effects (Neary *et al.*, 2009). In the circulation PYY exists in two forms, namely PYY<sub>1-36</sub> and PYY<sub>3-36</sub>. PYY<sub>3-36</sub> is the major circulating form and primarily determines the appetite suppressing action of PYY (Batterham *et al.*, 2006; Karra *et al.*, 2009). Several studies reported that plasma PYY concentrations were increased during aerobic exercise both in lean (Broom *et al.*, 2007; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b) and obese (Ueda *et al.*, 2009b) participants. In a study where Broom *et al.* (2007) provided standardised meals to their participants 1 and 4 h after they had completed a 1-hour run, plasma PYY responses remained elevated for up to 5 h after exercise in comparison with responses on a resting control trial. The current study measured changes

in the concentration of PYY<sub>3-36</sub> in response to exercise and food deficit. PYY<sub>3-36</sub> increased with exercise, confirming results of studies carried out by Martins *et al.* (2007), King *et al.* (2011a) and Cheng *et al.* (2009).

## 6.5 Conclusion

In conclusion, this study has shown that a 90-minute running session induces a brief suppression of appetite and plasma acylated ghrelin in women, yet does not have any significant effect on short-term energy and macronutrient intake, despite inducing a substantial energy deficit. In contrast, food restriction resulted in increased ghrelin concentrations and energy intake in female participants. These outcomes contribute knowledge regarding the role of exercise and diet in weight management (Stensel, 2011). The findings indicate that exercise can induce substantial deficits in energy in women without causing compensatory responses in acylated ghrelin, appetite and energy intake that would render exercise futile in weight management (although a clear limitation is that the present study was only conducted over a nine hour period and hence the long term effects of exercise were not assessed). The lack of a compensatory increase in food intake after exercise in these female participants may be due to the lack of change in ghrelin after exercise and/or a prolonging of satiety due to the exercise-induced stimulation of appetite-suppressing hormones such as PYY (Stensel, 2011). On the other hand, food restriction elicited a compensatory response in acylated ghrelin and PYY and consequently, led to increased energy intake, which could render food restriction futile in weight control. Further in-depth study is needed to pinpoint the link between exercise-induced changes in appetite hormones and changes in appetite and food intake. If a relationship can be identified and established, it may be possible to develop successful strategies for weight control, which would assist in combating the obesity epidemic.

## 7 Study 4 : Acute effects of exercise on appetite, energy intake and plasma acylated concentrations in males and females

### 7.1 Introduction

Obesity is a global epidemic in developed countries that is related to several serious chronic diseases including diabetes mellitus, coronary heart disease and cancer (Adams *et al.*, 2006; Hensrud *et al.*, 2006; Tiryaki-Sonmez *et al.*, 2013). One of the reasons for obesity and being overweight is an energy imbalance when individual energy intake exceeds energy expenditure over a sustained period of time (Bray and Champagne, 2005). Physical activity and diet are the two most discussed modifiable behaviours to regulate body weight and to prevent and/or reduce obesity (Tiryaki-Sonmez *et al.*, 2013). It can be argued that lack of physical activity leads to obesity. On the other hand there are studies suggesting that physical activity increases food intake, which may lead to a positive energy balance (Alméras *et al.*, 1991; Butterworth *et al.*, 1994). Despite this, there is evidence that regular exercise reduces the risk of obesity in men who are not undergoing caloric restriction (Ross *et al.*, 2000) and that increased physical activity is one of the best predictors of long-term success in weight reduction (Dalle Grave *et al.*, 2011).

There is some evidence that exercise has different effects on appetite in men and women. Women have been reported to increase food intake in response to exercise whereas most studies in men suggest that increased energy expenditure is not matched by increased energy intake in the short term (Hagobian *et al.*, 2009; Hickey *et al.*, 1997; Pomerleau *et al.*, 2004). Moreover, at least one study has shown that males do not sufficiently increase their energy intake to balance their new higher energy expenditure with exercise whereas females match energy intake with expenditure and as a result females maintain body weight and body fat (Hagobian *et al.*, 2008). Astrup (2001) asserted that an increase in fat intake by females correlated to an increase in calorie intake. This correlation is based on the type of macronutrient (fat instead of carbohydrate), rather the quantity of the food eaten. Some studies report that exercise training leads to weight loss in males but not in females – although exercise training may inhibit weight gain in females (Donnelly *et al.*, 2003; Potteiger *et al.*, 2003; Stubbs *et al.*, 2002a). However within this thesis; findings in study



three differed from those reports as it showed that female participant's food intake did not increase in response to 90 minutes of running at 70% of the VO<sub>2</sub> max.

The relationship between exercise and food intake may be mediated by a variety of energy-regulating hormones including ghrelin, peptide YY (PYY), glucagon-like peptide-1 (GLP-1), leptin and insulin (Leidy *et al.*, 2004 (ghrelin); Martins *et al.*, 2007 (ghrelin, PYY, GLP-1, PP), Hagobian *et al.*, 2009 (acylated ghrelin, leptin and insulin)). Of the hormones mentioned above, ghrelin is unique as the only known gut hormone which stimulates appetite. Ghrelin is a 28-amino acid peptide which is modified by an acyl side chain added to the serine at position 3. This acylation is essential for binding to the growth hormone secretagogue receptor (GHS-R) and for applying its effect on food intake (Kojima and Kangawa, 2005). Ghrelin acts as a 'hunger hormone', circulating plasma levels of ghrelin increase during a fast and decline after a meal (McGowan and Bloom, 2007). Ghrelin increases hunger sensations and subsequently food intake (Hotta *et al.*, 2009). It is possible that male-female differences in appetite, food intake and weight change in response to exercise may be explained by differential sex responses of these energy-regulating hormones.

Studies have investigated the influence of exercise on acylated ghrelin in males (Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 2011 a,b) and females (Hagobian *et al.*, 2008; Hagobian *et al.*, 2009, Martins *et al.*, 2007), while others have investigated the influence of exercise on PYY in males (Martins *et al.*, 2008; Broom *et al.*, 2009). These studies have reported that acylated ghrelin concentration is suppressed after exercise, and postprandial plasma PYY concentration is elevated for exercised subjects. One study showed that ghrelin concentration is reduced and appetite suppressed in males during an acute bout of treadmill running (Broom *et al.*, 2009).

This lends support for the role of acylated ghrelin in appetite suppression during and immediately after exercise (Broom *et al.*, 2007). Hagobian and colleagues (2009) investigated energy status (i.e. balance or deficit) and its influence on appetite hormones. The results of the study showed that, in females, acylated ghrelin was higher in both conditions after exercise compared with baseline (no exercise) while, in males, acylated

ghrelin did not change significantly in response exercise in both conditions compared with baseline.

The purpose of the present study was to compare plasma acylated ghrelin concentration and hunger ratings during and after an intense bout of treadmill running in healthy males and females feeding *ad libitum*. It was hypothesized at the beginning of the thesis that an intense bout of treadmill running would cause a suppression of hunger in males but not in females and that this would be associated with a suppression of plasma acylated ghrelin concentration before food intake to a much greater extent in males than females. However within the last study (Chapter six: Appetite and hormonal responses to exercise and food restriction in women) findings showed that in females there was a suppression in acylated ghrelin and appetite during exercise in women which confirmed the findings of a previous study that was carried out by King *et al.* (2011a) in male participants. In spite of several studies examining the effects of exercise on ghrelin and appetite in males and females separately, there is a lack of research that has directly compared the effects of exercise on ghrelin concentrations in males and females. Therefore, the present study will directly compare acylated ghrelin, appetite and energy intake responses to exercise in men and women in order to clarify whether or not there is any difference in responses between sexes.

## 7.2 Methods

### 7.2.1 Participants

After approval from the University Ethical Advisory Committee 10 male and 10 female participants aged 20-30 years of age volunteered to participate in this study. The participants were physically fit volunteers from among sports science students at Loughborough University. Females, non-oral contraceptive users, were studied in the follicular phase (1 to 14 days) of the menstrual cycle based on their menstruation date. The selected volunteers were non-smokers, not following a strict diet, and had no personal history of cardiovascular disease, hypertension, metabolic disease or dyslipidaemia. The physical characteristics of the participants (mean  $\pm$  SD) are displayed in Table 7.1.

**Table 7.1 Physical characteristics of the study participants**

Characteristic	Females	Males
Age (yr)	22.3 $\pm$ 2.5	22.6 $\pm$ 3.8
Height (cm)	166.6 $\pm$ 5.4 <sup>b</sup>	180.5 $\pm$ 6.2
Weight (kg)	61.9 $\pm$ 7.3 <sup>b</sup>	75.4 $\pm$ 9.4
BMI (kg.m <sup>-2</sup> )	22.3 $\pm$ 2.2	23.1 $\pm$ 2.1
Body fat (%)	22.4 $\pm$ 5.5 <sup>a</sup>	10.1 $\pm$ 4.2
VO <sub>2</sub> max (mL.kg <sup>-1</sup> min <sup>-1</sup> )	48.8 $\pm$ 6.1 <sup>b</sup>	66.1 $\pm$ 9.2

Values are Mean  $\pm$  SD ( $n = 20$ , 10 males and 10 females).

<sup>a</sup> Significantly higher ( $P < 0.01$ ) in females than males.

<sup>b</sup> Significantly lower ( $P < 0.02$ ) in females than males.

### 7.2.2 Anthropometry

The participants' height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany) and body weight was measured to the nearest 0.01 kg using a balance-beam scale (Avery Industrial Ltd, Leicester, UK). Body mass index was calculated as the weight of the participant in kilograms divided by the square of their height in metres. Waist

circumference was measured from the widest part of the torso between the xiphoid process of the sternum and the iliac crest. Refer to General Methodology section 3.2 for details.

### **7.2.3 Preliminary tests**

Participants underwent two preliminary exercise tests on a level motorised treadmill (RUNRACE, Techno gym, Gambettola, Italy). The first preliminary test was a submaximal-incremental treadmill running test (4 x 4 minute stages for a total of 16 minutes), and the second preliminary test was a maximum oxygen uptake test. Participants were given at least a 30 minutes rest interval between the two exercise tests. They were also given more than a week to recover from the preliminary exercise tests before undergoing the main exercise trials.

### **7.2.4 Submaximal-incremental treadmill running test**

This test was designed to ensure that participants experienced a range of exercise intensities; this was achieved by changing the speed (using a range from 7/8 to between 11 and 14 km/h) depending on the participants' fitness level. The participants ran for 16 minutes continuously during the test. These 16 minutes were divided into four, 4-minute periods and treadmill speed was increased at the end of each 4-minute period. The participants' expired air was collected into Douglas bags (Plysu Protection Systems, Milton Keynes, United Kingdom) between minutes 3 and 4 of each four minute period. Heart rate was monitored continuously throughout the test using short-range telemetry (Sports tester PE3000, Polar Electro, Finland). Oxygen consumption was plotted against running speed at each stage of the test to establish the running speed oxygen consumption relationship. This was accomplished using an Excel spread sheet. For further details please see the General Methodology (section 3.6.1).

### **7.2.5 Maximum oxygen uptake treadmill test**

Participants completed an open ended, incremental uphill protocol at a constant speed until volitional fatigue (which generally occurred in 10 to 12 minutes) (Taylor *et al.*, 1955). The running started at an inclination of 3.5% and the treadmill inclination increased by 2.5%

every 3 minutes. Expired air samples were collected between minutes 1:45 and 2:45 of each 3-minute stage and during the final minute of the test. The final minute of the test occurred when the participant signalled that he/she could only carry on for one more minute. Heart rate was monitored throughout the test using short-range telemetry. Ratings of perceived exertion (Borg, 1973) were assessed at the same time as the expired air collections during the test. Oxygen consumption and carbon dioxide production were determined at the end of the test from expired air samples and the highest value was taken as the maximum oxygen uptake.

The oxygen consumption ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) which would be elicited when working at 70% of the maximum oxygen uptake was then calculated. This value was used in conjunction with data from the submaximal-incremental test to estimate the running speed during level running, required to elicit 70% of maximum oxygen uptake. This running speed was used in the main trial. (Refer to the General Methodology Section 3.6.2 for details.)

### **7.2.6 Gas sampling**

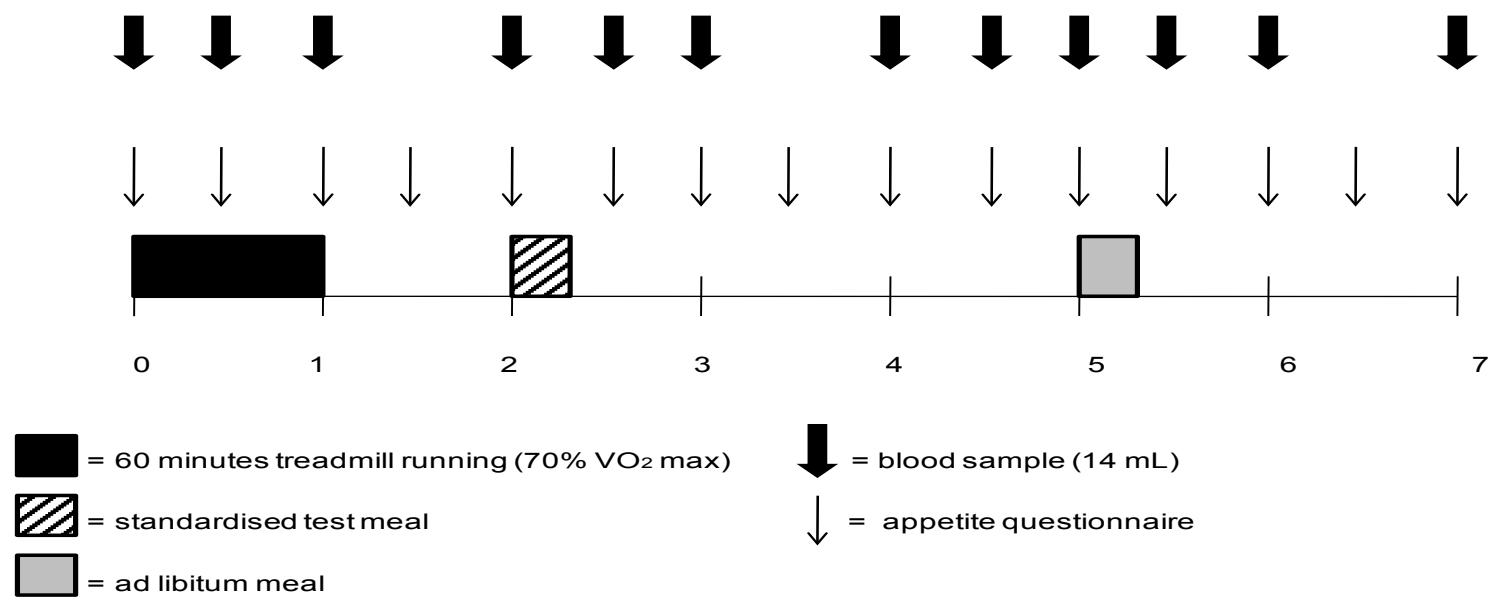
Oxygen consumption and carbon dioxide production were determined with the use of a paramagnetic oxygen analyzer and an infra-red carbon dioxide analyzer, respectively (Series 1400; Servomex, Crowborough, United Kingdom). These analyzers were calibrated before analysis using gases of known concentration. Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, United Kingdom) and corrected to standard temperature and pressure dry. (Refer to General Methodology Section 3.7 for details).

### **7.2.7 Dietary control**

Participants weighed and recorded their food intake the day before their first main trial and then replicated this food intake during the day before their second main trial. Participants were advised to remain inactive and to avoid alcohol and caffeine 24 hours before the main trials. The participants fasted, and took no food or drink except water, for a minimum of 10 hours before commencing each main trial. (Refer to the General Methodology Section 3.9 for details).

### 7.2.8 Main trials

One week after completing their preliminary exercise tests participants completed two main trials (exercise and control) with a one-week interval between trials. The exercise trial involved a one hour run followed by six hours of rest and the control trial comprised seven hours of rest. Trials started at approximately 9 am and finished at approximately 4 pm (Figure 7.1). The one-hour run during the exercise trial took place between 9.00 and 10.00 am. The run was completed at a constant speed predicted to elicit 70% of maximum oxygen uptake. One minute expired air samples were collected every 15 minutes, during running, at 09:14-09:15, 09:29-09:30, 09:44-09:45 and 09:59-10:00. Oxygen consumption and carbon dioxide production were determined from these samples to monitor exercise intensity during exercise trials and estimate energy expenditure (Frayn, 1983). The running speed was adjusted based on whether the oxygen consumption was above or below the predicted value. Participants rested for the remainder of the trial after the run; they were free to carry out light activities such as reading, writing or watching TV. In the control trial, participants rested for seven hours and the same procedure was repeated, without exercise. Temperature and humidity were monitored every hour during the main trials using a handheld hygrometer. Barometric pressure was measured at the start of the trials using a barometer.



**Figure 7.1** A schematic representation of the main trial protocol

### 7.2.9 Assessment of appetite

Appetite perceptions (hunger, fullness, satisfaction and prospective food consumption) were measured every 30 minutes during the main trials using visual analogue scales (VAS) (Flint *et al.*, 2000) as shown in Figure 7.1.

### 7.2.10 Test meals

Two meals were provided in the main trials: a standard meal two hours after the start of each trial, and an *ad libitum* buffet meal five hours after the start of each trial. The standard meal consisted of bread, ham, banana, salted crisps, and a Mars bar; participants were encouraged to consume this within 15 minutes. The macronutrient content for this meal was 9% protein, 63% carbohydrate and 28% fat; it provided 706 kcal (2954 kJ) for a 70 kg participant. The amount of food was adjusted based on the body weight of the participants. The buffet meal consisted of white and brown bread, semi-skimmed milk, cereals (Cornflakes, Coco Pops, Frosties and cereal bars), ham, tuna, mayonnaise, butter, margarine, Cheddar cheese, plain and chocolate muffins, chocolate rolls, cookies, Mars bars<sup>®</sup>, salted crisps, bananas, oranges, apples, and orange juice. Each item was weighed before and after the buffet meal to calculate the quantity of each item consumed. This information was used to calculate total the energy and macronutrient intake of participants.

### 7.2.11 Blood samples

Blood samples were collected throughout the main trials to determine levels of plasma acylated ghrelin. Venous blood samples were collected via a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) inserted into an antecubital vein. Samples were collected into pre-cooled EDTA monovettes (Sarstedt, Leicester, UK) at baseline: 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6 and 7 h. Blood samples for both trials were collected while the participants lay in a semi-supine position, except for the 0.5 h sample taken during the exercise trials. This sample was collected while participants straddled the treadmill. At each time point, two blood samples were collected in two separate EDTA collecting tubes i.e. a 4.9 mL EDTA monovette and a 9 mL EDTA monovette. The 9 mL EDTA monovettes were spun at 3000 revs.min<sup>-1</sup> for 10 minutes in a refrigerated centrifuge (Bukard, Hertfordshire, UK) at 4°C.



The plasma was then separated into eppendorf tubes and stored at  $-80^{\circ}\text{C}$  for analysis of glucose, triacylglycerol and insulin at a later date (NB These have not yet been analysed and therefore data for these is not presented here).

The 4.9 mL monovettes were used to collect samples from which acylated ghrelin would be measured. To prevent degradation of the acylated ghrelin, the 4.9 mL monovettes contained a 50  $\mu\text{L}$  solution containing potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH). Monovettes were spun at  $3500\text{ revs}\cdot\text{min}^{-1}$  for 10 minutes in a refrigerated centrifuge at  $4^{\circ}\text{C}$ . Then, the plasma supernatant was placed into a storage tube acidified with 100  $\mu\text{L}$  of 1M hydrochloric acid per mL of plasma. Samples were spun once more at  $3500\text{ revs}\cdot\text{min}^{-1}$  for five minutes in a refrigerated centrifuge at  $4^{\circ}\text{C}$  prior to storage at  $-80^{\circ}\text{C}$  for later analysis. At each blood sample point, duplicate samples of blood were collected into capillary tubes for the measurement of haemoglobin concentration and triplicate blood samples were collected into capillary tubes for the measurement of haematocrit. These samples were collected to assess any change in plasma volume (Dill and Costill, 1974).

#### **7.2.12 Biochemical analysis**

An enzyme immunoassay was used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France) using a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria). To eliminate inter-assay variation, samples from each participant were analysed in the same run. The within batch coefficients of variation for the assays was 7.8%.

#### **7.2.13 Statistical analysis**

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows (SPSS Inc., Chicago, IL). Area under the curve (AUC) calculations for plasma acylated ghrelin concentration and appetite ratings over time were performed using the trapezoidal method. Student's t-tests for correlated data were used to assess differences between fasting and area under the curve values for acylated ghrelin, caloric intake and appetite perceptions between the control and exercise trials. A three-way

(time x trial x sex) ANOVA was used to assess differences between trials (control versus exercise) and sexes over time. Where significant time x trial x sex interactions were observed further analysis was performed using repeated measures, two-factor ANOVA and Bonferroni post hoc tests to identify the exact nature of the differences. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean  $\pm$  SD unless otherwise stated.

## 7.3 Results

### 7.3.1 Responses to treadmill running

Male and female responses to treadmill running are shown in Table 7.2. The ambient temperature was  $23 \pm 0.3^\circ\text{C}$  and the relative humidity  $37 \pm 1.5\%$ .

**Table 7.2 Male and female responses to treadmill running**

	Females	Males
RPE (6-20)	12 $\pm$ 1	13 $\pm$ 2
Speed (km.h <sup>-1</sup> )	8.4 $\pm$ 1.0	10.7 $\pm$ 2.1
Heart rate (beats.min <sup>-1</sup> )	174 $\pm$ 11	163 $\pm$ 12
VO <sub>2</sub> (mL.kg <sup>-1</sup> min <sup>-1</sup> )	36.3 $\pm$ 4.1	44.6 $\pm$ 5.6
%VO <sub>2</sub> max	73.3 $\pm$ 2.0	70.1 $\pm$ 5.3
Energy expenditure (kJ)	2709 $\pm$ 400 <sup>a</sup>	4117 $\pm$ 600

(*n* = 20, 10 males and 10 females).

<sup>a</sup> Energy expenditure significantly lower (*P* < 0.01) in females than males.

### 7.3.2 Baseline parameters

Table 7.3 displays fasting values for appetite ratings and acylated ghrelin on the control and exercise trials for males and females. Fasting hunger, fullness, satisfaction and prospective food consumption did not differ significantly between males and females (Independent-sample T test) or between control and exercise trials (Paired-sample T test). Fasting plasma acylated ghrelin concentrations were significantly higher in females than males on the control trial. Values on the exercise trial were also higher in females but not significantly (*P* < 0.075). Fasting plasma acylated ghrelin concentration did not differ significantly between the control and exercise trials for males or females.

### 7.3.3 Appetite

Three-way ANOVA revealed a significant main effect of time and a significant effect of trial for hunger indicating that hunger values changed over time and tended to be higher on the control trial than the exercise trial – at least during first hour of the trial (Figure 7.2). There was no time x trial x sex interaction for hunger but there was a sex effect for hunger with values being higher in males than females ( $P = 0.023$ ). VAS ratings for prospective food consumption (data not shown) matched those for hunger i.e. values were higher in males than females and higher on the control than the exercise trial although differences were not significant. The reverse was true for satisfaction and fullness (data not shown) with values tending to be lower in males than females and lower on the control than the exercise trial although again these differences were not significant. There was no time x trial x sex interaction for prospective food consumption, satisfaction or fullness.

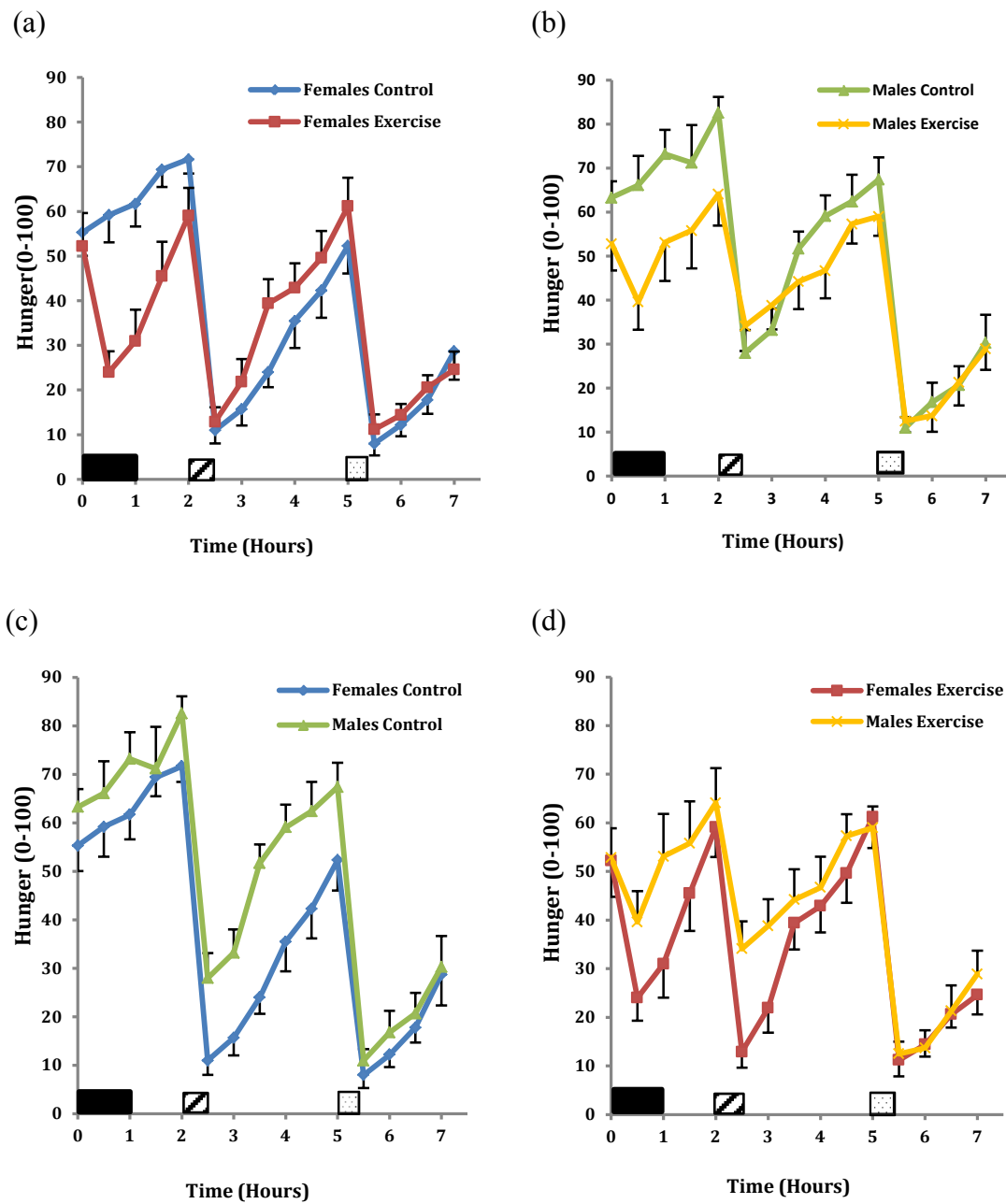
**Table 7.3 Baseline values in the control and exercise trials**

	Females			Males		
	Control	Exercise	<i>P</i>	Control	Exercise	<i>P</i>
acylated ghrelin (pg mL <sup>-1</sup> )	163 ± 63	145 ± 92	0.420	101 ± 56 <sup>a</sup>	82 ± 55	0.198
Hunger (0–100)	55 ± 17	52 ± 23	0.598	63 ± 12	53 ± 19	0.099
Fullness (0-100)	27 ± 15	33 ± 25	0.354	20 ± 12	19 ± 11	0.930
Satisfaction (0-100)	28 ± 12	38 ± 22	0.068	21 ± 10	23 ± 13	0.693
PFC (0-100)	58 ± 16	60 ± 22	0.707	74 ± 17 <sup>b</sup>	68 ± 13	0.380

Values are mean ± SD (*n* = 20, 10 males and 10 females).

<sup>a</sup> Acylated ghrelin significantly lower (*P* = 0.035) in males than females on the control trial.

<sup>b</sup> PFC significantly higher (*P* = 0.046) in males than females on the control trial.



**Figure 7.2 Hunger scores during the control and exercise trials**

Values are mean  $\pm$  SEM ( $n = 10$  males and 10 females). Black rectangle indicates running, diagonally shaded rectangles indicate standardised meal, dotted rectangle indicates the *ad libitum* meal. Comparisons are as follows: panel a) females exercise versus females control; panel b) males exercise versus males control; panel c) females control versus males control and panel d) females exercise versus males exercise.

### 7.3.4 Food intake during *ad libitum* meals

There was no significant difference between carbohydrate, fat, protein or energy intake during the *ad libitum* meals on the control and exercise trials for either males or females (two-way ANOVA,  $P < 0.05$  for each) (Table 7.4). There were no significant trial x sex interaction effects for carbohydrate, fat, protein and energy intake during the *ad libitum* meals indicating that responses did not differ significantly between trials for either sex. Energy and macronutrient intake during the *ad libitum* meals were higher in males than females (way ANOVA main effect of sex  $P < 0.05$ ).

### 7.3.5 Plasma acylated ghrelin

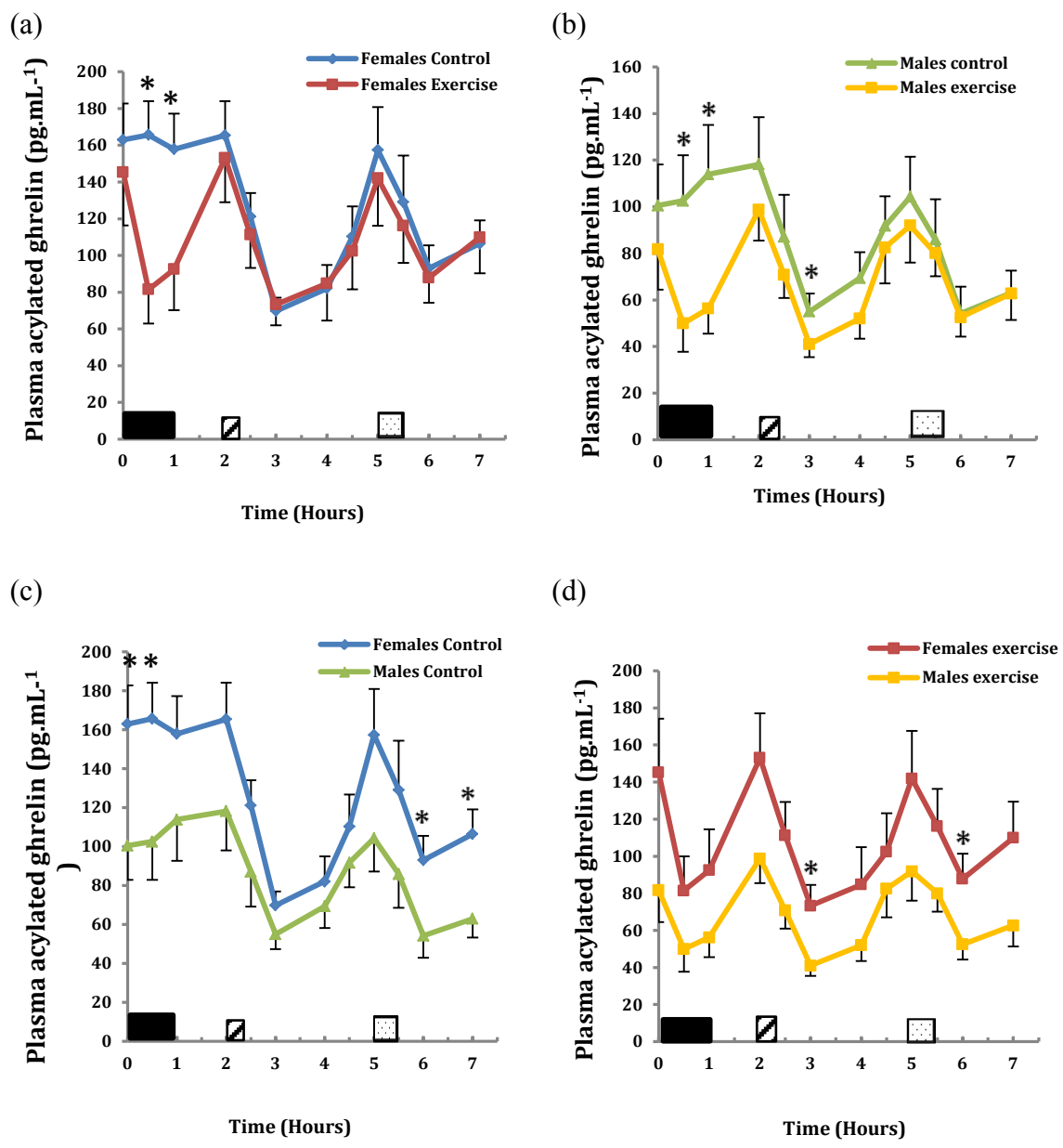
Three-factor ANOVA revealed a significant effect of time ( $P < 0.001$ ), trial ( $P = 0.002$ ) and trial  $\times$  time interaction ( $P < 0.001$ ) for plasma acylated ghrelin regardless of sex. This time x trial interaction indicated that acylated ghrelin concentrations were suppressed during running on the exercise trial compared with the control trial in both males and females (Figure 7.3). There was no significant trial  $\times$  sex or time  $\times$  sex interaction effect ( $P > 0.05$ ). This revealed no difference in the response of plasma acylated ghrelin between males and females over time, or between males and females on the control and exercise trials. Three-way ANOVA revealed a significant effect of sex for acylated ghrelin indicating that values were higher in females than males regardless of trial (Figure 7.3, panels c and d).

**Table 7.4 Food intake and macronutrient intake values during *ad libitum* meals in the control and exercise trials**

	Females			Males		
	Control	Exercise	<i>P</i>	Control	Exercise	<i>P</i>
<b>Food intake</b>						
(g)	580 ± 223	645 ± 289	0.278	889 ± 428	940 ± 372	0.663
(kJ)	2914 ± 1510	2977 ± 1583	0.858	4971 ± 2650	5386 ± 2420	0.388
<b>CHO intake</b>						
(g)	108 ± 44	111 ± 49	0.846	170 ± 81	197 ± 81	0.252
(kJ)	1813 ± 729	1852 ± 812	0.847	2844 ± 1349	3298 ± 1350	0.253
<b>Protein intake</b>						
(g)	22 ± 19	24 ± 17	0.547	37 ± 28	37 ± 25	0.986
(kJ)	365 ± 315	399 ± 285	0.548	621 ± 467	622 ± 415	0.984
<b>Fat intake</b>						
(g)	19 ± 16	19 ± 15	0.837	40 ± 30	39 ± 27	0.818
(kJ)	732 ± 593	703 ± 572	0.840	1485 ± 1138	1458 ± 1021	0.825

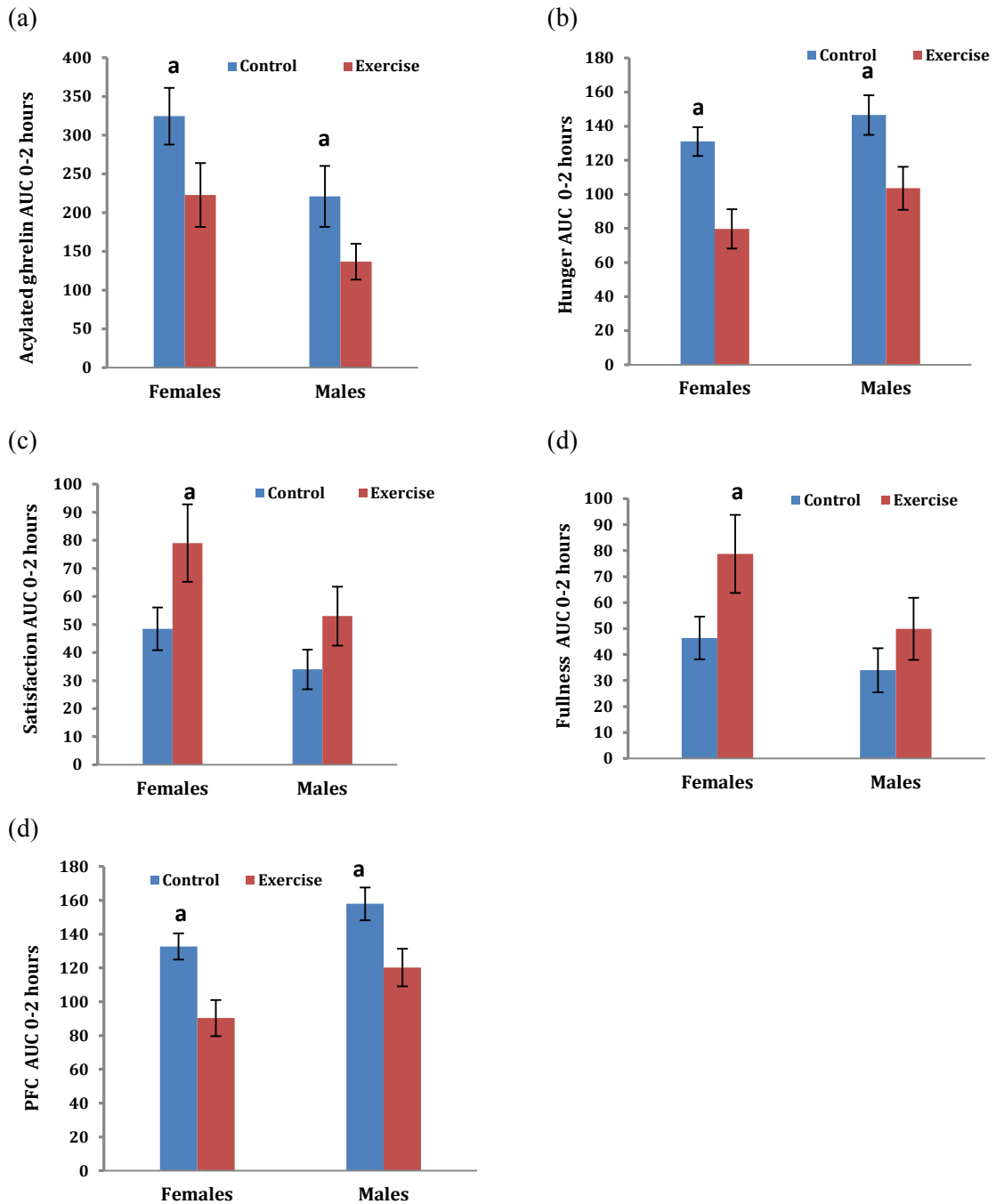
Values are mean ± SD, *n* = 20 (10 males and 10 females). CHO, carbohydrate. (*P* values indicate t-test comparisons between control and exercise values from males and females separately).





**Figure 7.3 Ghrelin during the control and exercise trials for males and females**

Figure 7.4 illustrates AUC values over the first two hours of the control and exercise trials for hunger, satisfaction, fullness, prospective food consumption and acylated ghrelin in males and females. In each case the values are significantly different (Student's t-test) between trials with the exception of fullness ( $P < 0.207$ ) and satisfaction ( $P < 0.067$ ) in males.



**Figure 7.4 Area under the curve values for ghrelin and appetite in the control and exercise trials**

AUC values over the first two hours of the control and exercise trials for hunger, satisfaction, fullness, prospective food consumption and acylated ghrelin in males and females. In each case the values are significantly different (Student's t-test) between trials with the exception of fullness ( $P < 0.207$ ) and satisfaction ( $P < 0.067$ ) in males.

### 7.3.6 Correlations between acylated ghrelin concentration and other variables

Fasting plasma acylated ghrelin concentration, on both the control and exercise trial, was not significantly correlated with body mass, BMI, percent body fat or maximum oxygen uptake within males or females or when both groups were combined. For females only the correlation between percent body fat and fasting plasma acylated ghrelin in the exercise trial was almost significant ( $P = 0.051$ ,  $r = -0.629$ ). This negative correlation indicates higher acylated ghrelin concentration in females who had a lower percentage body fat. On the other hand, there was a significant correlation between fasting acylated ghrelin concentration in the exercise trial and maximum oxygen uptake ( $P = 0.045$ ,  $r = -0.453$ ) within the whole group combined (males and females) indicating higher acylated ghrelin concentration in those who had lower  $VO_2$  max values. When examining correlations at individual time points, few significant relationships emerged. There was a negative correlation between acylated ghrelin and fullness for the whole group combined (i.e. males and females) in the exercise trial at time points 4.5 and 5 h which were immediately before the *ad libitum* meal (-0.455 and -0.464). These correlations indicate higher ghrelin concentrations in those who were less full. There was also a negative correlation between ghrelin and satisfaction at 5 h (-0.522) indicating higher ghrelin values in those who were less satiated.

## 7.4 Discussion

The purpose of this study was to ascertain whether exercise has a different impact on the concentration of plasma acylated ghrelin in males and females. To the author's knowledge, the present study is one of only two to compare the influence of exercise on plasma acylated ghrelin concentration in normal weight males and females (the other one being that of Hagobian *et al.* 2013). All female participants were studied in the follicular phase of their menstrual cycle to ensure that fluctuations in menstrual cycle hormones would not confound the outcome. The key finding of this study is that there was no significant difference between males and females in the plasma acylated ghrelin response to exercise or feeding although a sex effect for acylated ghrelin was observed indicating higher values in females than males across both trials. In contrast to the findings for acylated ghrelin, hunger scores tended to be higher in males than females although again there was no difference in the response to exercise. Finally *ad libitum* energy intake was higher in males than females regardless of trial.

The findings of this study confirm the suppression of acylated ghrelin during exercise both in males and in females as demonstrated previously in males (Broom *et al.*, 2007, Marzullo *et al.*, 2008, Broom *et al.*, 2009, King *et al.*, 2010a, King *et al.*, 2011a and Wasse *et al.*, 2012). The results of this research also indicate a suppression of hunger in both sexes during and immediately after exercise. This finding is consistent with findings from previous studies that have studied subjective hunger ratings after high intensity exercise (above 60% of maximum oxygen uptake), (Burns *et al.*, 2007, in males and females; Blundell *et al.*, 2003, in males and females; King and Blundell, 1995, males; King *et al.*, 1994, males; Broom *et al.*, 2007, males). This suppression of hunger was short lived and hunger returned to control values within two hours of the termination of exercise. The hunger ratings generally were higher in males than females but there were no significant differences between males and females in the exercise and control trials i.e. no trial or interaction effects. Similar to this, a recent study that consisted of normal weight participants was carried out by Hagobian *et al.* (2013) found that acute acylated ghrelin responses to moderate-high intensity exercise did not differ between sexes.

Acylated ghrelin concentration decreased significantly during exercise and after feeding in both trials. This finding is consistent with several studies in showing a decline in acylated ghrelin concentration after feeding (Al Awar *et al.*, 2005; Blom *et al.*, 2005; Hosoda *et al.*, 2004). However, Hagobian *et al.* (2009) found that plasma acylated ghrelin concentration and appetite ratings differed by sex in a study involving overweight/obese males and females. They found the females had a higher ghrelin concentration after short-term training, compared with males.

This study compared appetite, energy intake and plasma acylated ghrelin responses to exercise concurrently in males and females. The current research showed that females responded to the beginning of the trials (control, exercise) with higher acylated ghrelin concentration than males which might be expected to stimulate higher energy intake. This study also demonstrated that ghrelin concentration was not significantly correlated with body mass, BMI, percent body fat or maximum oxygen uptake. It was also observed that at individual time points acylated ghrelin was not correlated with hunger, fullness, satisfaction and prospective food consumption. The initial fasting plasma acylated ghrelin concentrations indicated differences between trials in both sexes. During the first hour of the trials fasting plasma acylated ghrelin concentration decreased during exercise and increased in control. This study also demonstrated that the concentration of plasma acylated ghrelin was higher generally (i.e. throughout the trials) in females than males. This finding agrees with the results obtained by Hagobian *et al.* (2009), who measured the level of acylated ghrelin after a 4-day exercise trial was used to achieve an energy deficit. They found a compensatory increase in levels of acylated ghrelin in women but not in men. Similarly, a study comparing male rats to female rats by Gayle *et al.* (2006) demonstrated that ghrelin concentrations were higher after a 12-h fast in females than in males. An exercise-induced energy deficit sufficient to cause weight loss resulted in an increase in the levels of ghrelin in women (Leidy *et al.*, 2009), but not in men (Ravussin *et al.*, 2001). In contrast hunger was generally higher in males than females against expectations. It can be argued that the participants' responses to their hunger may be influenced by their general disposition to eating i.e. unrestrained versus restrained versus disinhibited (Hill *et al.*, 1995).

Previous studies have observed an increase in energy intake in females after an acute bout of exercise (Pomerleau *et al.*, 2004) and after a one-week period of exercise training (Stubbs *et al.*, 2002a). Gayle *et al.*, (2006) also found that *ad libitum* food intake was higher after a 12-h fast in female than in males. On the other hand, a series of studies on males showed no increase in energy intake or a suppression in energy intake over 24 hours after an acute bout of exercise (King *et al.*, 1997, 2010, 2011; Stensel, 2011). Moreover, within study three (Chapter 6) of this thesis, there was no significant difference in food intake between exercise and control trials in females. Consistent with this, the current study found no significant difference between males or females' food intake after exercise. This is consistent also with the recent findings of Hagobian *et al.* (2013).

The present study had several limitations. Firstly, the participants were young and physically active and therefore the findings may not be generalized to sedentary or older subjects. Secondly, the study was based on relatively a low sample size which may have failed to detect significant relationships between acylated ghrelin and other variables. Furthermore, the participants in the present study were lean and hence these findings may not apply to overweight and obese participants. Fourthly, acylated ghrelin was the only appetite-regulating hormone examined in the present study and hence exercise may have caused differences in the concentration of other appetite regulating hormones (e.g. peptide YY, glucagon like peptide-1 and/or pancreatic polypeptide) which were not detected in the present study. Another possible limitation to this study is that, perceptions of liking and wanting, that could have an influence on energy intake and appetite hormones, was not measured (Finlayson *et al.*, 2009). It is likely that perceptions of liking and wanting, unlike other appetite ratings such as hunger, fullness and prospective food consumption that was measured in this study, could have given better information about the suppressed *ad libitum* energy intake.

## **7.5 Conclusion**

In conclusion, this study indicates that plasma acylated ghrelin concentration is reduced in both sexes during an acute bout of treadmill running. This lends support to the role of plasma acylated ghrelin in hunger suppression during exercise although this could of course be coincidental. This study revealed that exercise has an impact on plasma acylated

ghrelin concentration in both sexes together with a short lived suppression of hunger during exercise but no differences were evident in the appetite, food intake or plasma acylated ghrelin responses to exercise between males and females and hence a sex difference in response to exercise is not supported.

## **8 General discussion**

### **8.1 Introduction**

This thesis has so far examined the effects of exercise on appetite and food intake. The four studies presented have attempted to extend knowledge of the reactions of appetite, food intake and gut hormones to exercise in women. In the first study fasting appetite, plasma acylated ghrelin and dietary restraint questionnaire values were compared between 34 men and 33 women. No significant differences were found between sexes for these variables. The second study focused only on females and no differences were found in the responses of appetite, plasma acylated ghrelin and food intake to exercise between the follicular and the luteal phases in a group of women taking the oral contraceptive pill. The third study revealed that (as with men) women undergo compensatory appetite, gut hormone and food intake responses to dietary-induced energy deficits but not to exercise-induced deficits. The fourth and final study showed suppressions of both hunger and plasma acylated ghrelin during exercise; however once again no significant differences were noted between males and females during or after exercise.

### **8.2 Summary of the main findings**

To summarise the studies presented here do not support the hypothesis that exercise has different effects on appetite, appetite hormones or food intake in men and women. So far, however, these studies have mainly focused on finding significant differences between males and females. These studies as well as the similar studies in the literature have paid little attention to the actual values of acylated ghrelin and energy intake across experiments. Indeed there are no established reference ranges for acylated ghrelin in men and women at present. As the result, additional conclusions could be drawn from comparing the actual values found across studies within this thesis and within the literature. Table 8.1 below summarises the protocols used in the current thesis



**Table 8.1 Summary of the four studies presented in this thesis (AG: acylated ghrelin; PYY: peptide YY; EI: energy intake)**

<b>Study (Chapter)</b>	<b>Trials</b>	<b>Participants</b>	<b>Exercise mode</b>	<b>Intensity %VO<sub>2</sub>max</b>	<b>Exercise duration</b>	<b>Measurements</b>
<b>1 (4)</b>	Control	34 males	Rest			AG/TAG/Glucose
	Control	33 females	Rest			AG/TAG/Glucose
<b>2 (5)</b>	Exercise: follicular	13 females	Cycling	72.7 ± 7.3	45 minutes	AG/EI
	Exercise: luteal	13 females	Cycling	72.1 ± 8.6	45 minutes	AG/EI
<b>3 (6)</b>	Exercise	13 females	Running	70.8 ± 2.7	90 minutes	AG/PYY <sub>3-36</sub> /EI
	Food deficit	13 females	Running	70.8 ± 2.7	90 minutes	AG/PYY <sub>3-36</sub> /EI
	Control	13 females	Rest			AG/PYY <sub>3-36</sub> /EI
<b>4 (7)</b>	Exercise	10 males	Running	70.1 ± 5.3	60 minutes	AG/EI
		10 females	Running	73.3 ± 2.0	60 minutes	AG/EI
	Control	10 males	Rest			AG/EI
		10 females	Rest			AG/EI

### **8.3 Comparison of acylated ghrelin baseline values between the four studies**

Firstly, fit women as chosen in study 4, and women chosen with mixed levels of fitness (study 1) had similar mean (fasting) levels of plasma acylated ghrelin; 163 pg/mL in the former case and 165 pg/mL in the latter. Studies 1, 2, 3 and 4 provided similar average values of acylated ghrelin for females (between  $133 \pm 87$  and  $165 \pm 103$  pg/mL). Fit men (study 4) had on average lower fasting levels of acylated ghrelin than men with lower levels of fitness (study 1) i.e.  $101 \pm 56$  pg/mL in study 4 and  $175 \pm 90$  pg/mL in study 1 (Table 8.2).

### **8.4 Comparison of the postprandial acylated ghrelin values between studies**

The acylated ghrelin values one hour after ad libitum eating for females in the exercise trial were lower in study 2 (follicular:  $49 \pm 28$ ; luteal:  $58 \pm 29$  pg/mL) than in studies 3 ( $90 \pm 65$  pg/mL) and 4 ( $88 \pm 43$  pg/mL). This difference might be because food (energy) intake was higher in study 2 than in studies 3 and 4 (Table 8.3). On the other hand the acylated ghrelin values for studies 3 and 4 were very similar in both the control and the exercise trials. The participants in the food deficit trial in study 3 exhibited lower mean values of acylated ghrelin ( $81 \pm 36$  pg/mL) than the participants in study 4 ( $88 \pm 43$  pg/mL) but higher values than the participants in study 2. This would be expected since food intake at the buffet meal was highest in study 2 and lowest in study 4. As far as males are concerned their values were much lower than those of the females in study 4 (Table 8.2). This may be due to the fact that the males ate more than the females in this study but it should be noted that the males also had lower fasting ghrelin values than the females.

### **8.5 Comparing the baseline versus the postprandial acylated ghrelin values**

Study 2 showed that the postprandial acylated ghrelin values were much lower than at baseline for both the follicular ( $49 \pm 28$  against  $150 \pm 79$  pg/mL) and the luteal ( $58 \pm 29$  against  $133 \pm 87$  pg/mL) phases. However in the luteal phase the difference between the baseline and the postprandial is slightly smaller than in the follicular phase. In study 3 the largest drop between the baseline (fasting) and the postprandial values occurs in the food

deficit trial; from  $148 \pm 96$  to  $81 \pm 36$  pg/mL. Finally study 4 showed that the postprandial acylated ghrelin values decreased to a similar extent after feeding in both women and men.

However the largest decline occurred in the control trial for women: from  $163 \pm 63$  to  $93 \pm 40$  whilst for men the values dropped from  $101 \pm 56$  to  $54 \pm 36$  pg/mL. To conclude, the comparison between baseline and postprandial values did not suggest any major difference between men and women.

**Table 8.2 Baseline and postprandial AG values from the four studies in this thesis**

AG values (pg/mL)		Baseline Control	Baseline Exercise	Baseline Food Deficit	Postprandial Control	Postprandial Exercise	Postprandial Food deficit
Study 1	Female	$165 \pm 103$	NA	NA	NA	NA	NA
	Male	$175 \pm 90$	NA	NA	NA	NA	NA
Study 2	Follicular	NA	$150 \pm 79$	NA	NA	$49 \pm 28$	NA
	Luteal	NA	$133 \pm 87$	NA	NA	$58 \pm 29$	NA
Study 3	Female	$148 \pm 100$	$140 \pm 86$	$148 \pm 96$	$97 \pm 84$	$90 \pm 65$	$81 \pm 36$
Study 4	Female	$163 \pm 63$	$145 \pm 92$	NA	$93 \pm 40$	$88 \pm 43$	NA
	Male	$101 \pm 56$	$82 \pm 55$	NA	$54 \pm 36$	$53 \pm 26$	NA

## 8.6 Comparison of ghrelin values with the literature

Initially most studies examining the effects of exercise on ghrelin measured total ghrelin but in recent years several studies have assessed acylated ghrelin. Among the studies providing values of acylated ghrelin most only provide values for men with two exceptions.

Firstly, Hagobian *et al.* (2009) studied the effects of exercise on energy regulating hormones and appetite in men and women. Their results exhibited a mean baseline value for acylated ghrelin of 31.6 pg/mL. This is lower than the values found in the present thesis. This is possibly because the participants in the study of Hagobian and colleagues (2009) were 18 overweight/obese individuals (9 men and 9 women). Weight gain has been shown to suppress ghrelin levels in some previous studies (Williams *et al.* (2006), Robertson *et al.* (2004) and Lindqvist *et al.* (2005)). Another possibility is that the lower ghrelin values in the study of Hagobian and colleagues (2009) are due to the use of a different assay (a competitive binding radioimmunoassay; Millipore, St. Charles, MO) than that used in the present study (an ELISA).

Secondly, Hagobian *et al.* (2013) studied the effects of acute exercise on appetite hormones and *ad libitum* energy intake in 11 healthy men and 10 healthy women (the women were on an oral contraceptive regimen). The acylated ghrelin values for both men and women were quite high. For women the mean acylated ghrelin value was  $549 \pm 330$  pg/mL in the rest trial and  $467 \pm 247$  pg/mL in the exercise trial whilst for men the values reached  $498 \pm 305$  pg/mL and  $495 \pm 365$  pg/mL in the rest and exercise trial respectively. The reason for these high values is unclear since both studies employed healthy participants. A possible explanation is the use of a different ELISA (Phoenix Pharmaceuticals, Inc., Burlingame, California, or Millipore Corporation, Billerica, Massachusetts) compared to the one used in the studies reported in this thesis.

In recent years an increasing number of studies have focused on the effects of exercise on acylated ghrelin in male subjects. Among these studies most provided values that are similar to those reported in this thesis. For instance, King *et al.* (2010a), King *et al.* (2011b), Wasse *et al.* (2012), Wasse *et al.* (2013a,b), and Broom *et al.* (2007) all provide values that lie in the same range as those reported in this thesis (it should be noted that all of these studies were performed in the same laboratory as the studies reported in this thesis). The baseline values reported in these published studies generally lie in the range between 82 and 175 pg/mL, which agree well with the range of mean values reported here. Different exercise types appear to exert a similar suppression of acylated ghrelin (provided the exercise intensity is sufficient) although it is noted that running may exert a stronger/larger suppression of acylated ghrelin than cycling (Wasse *et al.*, 2013b).

## 8.7 Comparison of food intake between the present thesis' studies

Studies 2, 3 and 4 presented in this thesis provide values for energy intake for the different trials. The comparison between the values observed in each study could provide further evidence on how food intake is influenced by different factors. This section effectively enables us to draw some conclusions. Firstly, the participants in study 2 who were taking oral contraceptive pills exhibited higher values of food intake than the other two studies (3 and 4) which involved participants who were not taking oral contraceptive pills (Table 8.3). When comparing between studies 3 and 4 for females there were no obvious differences in food intake in the control trials. Likewise, the exercise trials did not show significant differences in food intake for females between the two studies (Table 8.3).

As mentioned in previous chapters, study 3 has shown that women experienced compensatory food intake responses to dietary induced energy deficits but not to exercise induced energy deficits (over the course of a nine hour observation period). Finally, study 4 showed that the food intake for males was significantly higher than for females (Table 8.3).

**Table 8.3 Food intake values observed in studies 2, 3 and 4**

Food intake (kJ)		Control	Exercise	Food Deficit
<b>Study 2</b>	Follicular	NA	4615 ± 1736	NA
	Luteal	NA	4080 ± 1813	NA
<b>Study 3</b>	Female	2517 ± 1076	2748 ± 1613	3897 ± 1371
<b>Study 4</b>	Female	2914 ± 1510	2977 ± 1583	NA
	Male	4974 ± 2650	5386 ± 2420	NA

## 8.8 Comparison of energy intake levels with the literature

Staten (1991) studied the effect of acute exercise on caloric intake in normal-weight young people. Ten men and 10 women were monitored during consecutive five day periods; one period without exercise and one period with exercise. Energy intake during the control period was  $7666 \pm 431$  kJ/day for women and  $10329 \pm 691$  kJ/day for men. During the exercise period the men increased their energy intake to  $11128 \pm 787$  kJ/day whilst energy intake remained unchanged in the women at  $7662 \pm 381$  kJ/day. The important difference between the energy intake values reported in this thesis and the values reported by Staten (1991) is that the participants in Staten's study consumed two meals during the course of a day whereas the values reported in this thesis came from a single buffet meal. Hagobian *et al.* (2009) investigated food intake of the 18 obese/overweight males and females in 3 trials: control, exercise and food deficit. They found that for females the food intake was  $8449 \pm 1160$  kJ/day in the control trial which increased to  $10898 \pm 1135$  kJ/day in the exercise trial and decreased to  $8206 \pm 992$  kJ/day in the food deficit trial. As far as men are concerned the food intake in the control trial was  $10777 \pm 1574$  kJ/day, went up to  $13687 \pm 1695$  kJ/day in the exercise trial and declined to  $10634 \pm 1260$  kJ/day in the food deficit trial. The relatively high values of food intake reported by Hagobian *et al.* (2009) are due to the fact that three meals were provided: breakfast, lunch and dinner. Nevertheless the values are very similar to those of Staten (1991) although the latter provided only two meals.

Hagobian *et al.* (2013) compared the food intake of healthy men and women in two trials: control and exercise. This study differs from those of Staten (1991) and Hagobian, *et al.* (2009) in that it provides only one buffet meal, which is the protocol used in the studies in this thesis. The energy intake of females in the control trial was  $2470 \pm 967$  kJ and it remained stable at  $2474 \pm 766$  kJ in the exercise trial. On the other hand, men's energy intake was much higher and increased from the control trial ( $5091 \pm 2650$  kJ) to the exercise trial ( $6900 \pm 3977$  kJ) in Hagobian's (2013) study. In general the values for food intake provided by Hagobian *et al.* (2013) are similar to those reported in this thesis.

Other recent research with males (King *et al.* (2011b), King *et al.* (2013), Wasse *et al.* (2012) and Deighton *et al.* (2012)) provided values between 7,000 kJ for one buffet meal

and 13,500 kJ for up to 3 buffet meals as some of these studies analysed food intake for up to 10 hours. An outlier is found in King *et al.* (2010a) with values over 17,000 kJ over a 10 hour period for one participant. In general, men's food intake is relatively similar across studies with around 5,000 to 7,500 kJ for one standardised + 1 buffet meal, between 7,500 and 10,000 kJ over 8 hours and between 13,000 and 18,000 kJ over 10 hours. These values are higher than the values observed for women in this thesis (Table 8.3).

## 8.9 Limitations

Although this thesis has attempted to provide further insights into the effects of exercise on appetite, gut hormones and food intake, the following limitations are associated with its different studies:

- The sample size in the studies was small
- Participants were young, healthy and fit and hence are not representative of the general population
- Appetite hormone measurements were limited to acylated ghrelin and PYY<sub>3-36</sub>
- Food intake was assessed in the laboratory i.e. an artificial environment
- Only acute responses to exercise were monitored – not the chronic response
- The long term effects of exercise on appetite, food intake and gut hormones and whether these differ in men and women remain uncertain.

## 8.10 Future directions for research

Notwithstanding, the above limitations the findings of the studies reported here suggest that males and females do not differ in their appetite regulatory responses to acute exercise and they indicate some useful avenues for future research as follows:

- Compare appetite, hormonal and food intake responses to exercise in overweight women and men;
- Examine appetite, hormonal and food intake responses to exercise in older women and men;

- Examine appetite, hormonal and food intake responses to exercise in female and male adolescents;
- Compare appetite, hormonal and food intake responses to exercise in women and men classified as restrained versus unrestrained eaters;
- Examine responses to chronic as well as acute exercise in women and men;
- Measure a greater variety of appetite related hormones in addition to other related metabolites when comparing responses in women and men;
- Include control (non-exercise) trials when examining appetite, gut hormone and food intake responses to exercise in different phases of the menstrual cycle in women.
- Use larger sample sizes



## 9 References

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## Appendix A : Informed consent form

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 Advisory 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.

I agree to participate in this study.

Your name

---

Your signature

---

Signature of investigator

---

Date

---



## Appendix B: Global physical activity questionnaire (GPAQ version 2)

<b>Physical Activity</b>			
<p>Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.</p> <p>Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. <i>[Insert other examples if needed]</i>. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.</p>			
Questions	Response	Code	
<b>Activity at work</b>			
<b>1</b>	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like <i>[carrying or lifting heavy loads, digging or construction work]</i> for at least 10 minutes continuously?	Yes 1  No 2 <i>If No, go to P 4</i>	P1
<b>2</b>	In a typical week, on how many days do you do vigorous intensity activities as part of your work?	Number of days    ___	P2
<b>3</b>	How much time do you spend doing vigorous intensity activities at work on a typical day?	Hours : minutes    ___ : ___ hrs            mins	P3
<b>4</b>	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking <i>[or carrying light loads]</i> for at least 10 minutes continuously?	Yes 1  No 2 <i>If No, go to P 7</i>	P4
<b>5</b>	In a typical week, on how many days do you do moderate intensity activities as part of your work?	Number of days    ___	P5
<b>6</b>	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minute    ___ : ___ hrs            mins	P6
<b>Travel to and from places</b>			
<p>The next questions exclude the physical activities at work that you have already mentioned.</p> <p>Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. <i>[insert other examples if needed]</i></p>			
<b>7</b>	Do you walk or use a bicycle ( <i>pedal cycle</i> ) for at least 10 minutes continuously to get to and from places?	Yes 1  No 2 <i>If No, go to P 10</i>	P7
<b>8</b>	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days    ___	P8

*Continued on next page .....*

<b>Physical Activity (Travel to and from places) contd.</b>		
<b>Questions</b>	<b>Response</b>	<b>Code</b>
9	How much time do you spend walking or bicycling for travel on a typical day? Hours : minutes    ___ : ___ hrs        mins	P9 (a-b)
<b>Recreational activities</b>		
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), [insert relevant terms].		
10	Do you do any vigorous-intensity sports, fitness or recreational ( <i>leisure</i> ) activities that cause large increases in breathing or heart rate like [ <i>running or football,</i> ] for at least 10 minutes continuously? Yes 1 No 2 <i>If No, go to P 13</i>	P10
11	In a typical week, on how many days do you do vigorous intensity sports, fitness or recreational ( <i>leisure</i> ) activities? Number of days    ___	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? Hours : minutes    ___ : ___ hrs        mins	P12 (a-b)
13	Do you do any moderate-intensity sports, fitness or recreational ( <i>leisure</i> ) activities that causes a small increase in breathing or heart rate such as brisk walking (cycling, swimming, volleyball) for at least 10 minutes continuously? Yes 1 No 2 <i>If No, go to P16</i>	P13
14	In a typical week, on how many days do you do moderate intensity sports, fitness or recreational ( <i>leisure</i> ) activities? Number of days    ___	P14
15	How much time do you spend doing moderate intensity sports, fitness or recreational ( <i>leisure</i> ) activities on a typical day? Hours : minutes    ___ : ___ hrs        mins	P15 (a-b)
<b>Sedentary behaviour</b>		
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.		
16	How much time do you usually spend sitting or reclining on a typical day? Hours : minutes    ___ : ___ hrs        mins	P16 (a-b)

## Appendix C: Appetite scales (Visual Analogue Scales)

Subject Number: \_\_\_\_\_ Trial \_\_\_\_\_ Date: \_\_\_\_\_

**Visual Analogue Scale**  
Time: 0

**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**

**Place a mark on the horizontal lines below after considering the following questions:**

I am not hungry at all	<b>How hungry do you feel?</b> 	I have never been more hungry
I am completely empty	<b>How satisfied do you feel?</b> 	I cannot eat another bite
Not at all full	<b>How full do you feel?</b> 	Totally full
Nothing at all	<b>How much do you think you can eat?</b> 	A lot
Not at all nauseous	<b>How nauseous do you feel?</b> 	Very nauseous

Temperature (°C)	Humidity (%)

## **Appendix D: Full buffet meal items**

<b>Frosties</b>
<b>Corn Flakes</b>
<b>Co-co Pops</b>
<b>Milk</b>
<b>Orange Juice</b>
<b>White Bread</b>
<b>Brown Bread</b>
<b>Cheese</b>
<b>Ham</b>
<b>Tuna</b>
<b>Banana</b>
<b>Apple</b>
<b>Orange</b>
<b>Walkers Salted</b>
<b>Butter</b>
<b>Margarine</b>
<b>Mayonnaise</b>
<b>Nutri-grain</b>
<b>Mars</b>
<b>Cookies</b>
<b>Chocolate Muffins</b>
<b>Plain Muffins</b>
<b>Mini-rolls</b>

### **Appendix E: Food preference questionnaire**

Please complete questionnaire by rating each food item using a 10-point scale system. Please circle and rate each item; 1 = dislike extremely; 10 = like extremely

Coco-pops

1 2 3 4 5 6 7 8 9 10

Cornflakes

1 2 3 4 5 6 7 8 9 10

Frosties

1 2 3 4 5 6 7 8 9 10

Nutri-grain bars

1 2 3 4 5 6 7 8 9 10

White bread

1 2 3 4 5 6 7 8 9 10

Brown bread

1 2 3 4 5 6 7 8 9 10

Ham

1 2 3 4 5 6 7 8 9 10

Tuna

1 2 3 4 5 6 7 8 9 10

Cheese

1 2 3 4 5 6 7 8 9 10

Butter

1 2 3 4 5 6 7 8 9 10

Mayonnaise

1 2 3 4 5 6 7 8 9 10

Margarine

1 2 3 4 5 6 7 8 9 10

Crisps (ready salted)

1 2 3 4 5 6 7 8 9 10

Apple

1 2 3 4 5 6 7 8 9 10

Orange

1 2 3 4 5 6 7 8 9 10

Banana

1 2 3 4 5 6 7 8 9 10

Muffins

1 2 3 4 5 6 7 8 9 10

Cookies

1 2 3 4 5 6 7 8 9 10

Chocolate rolls

1 2 3 4 5 6 7 8 9 10

Milk

1 2 3 4 5 6 7 8 9 10

NB: rating four or more items less than four resulted in participant non-inclusion

## Appendix F: 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 5lbs 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 calories 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 what 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 that 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)