The effects of exercise on hunger and the hunger-related hormones ghrelin and peptide YY

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THE EFFECTS OF EXERCISE
ON HUNGER AND THE HUNGER
RELATED HORMONES GHRELIN
AND PEPTIDE YY

by

David Broom

A Doctoral Thesis

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

July 2008

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ABSTRACT

Aerobic and resistance exercise have been promoted as a key component of exercise recommendations for weight control because exercise is an effective method of increasing energy expenditure and it may paradoxically, lead to a short term hunger suppression. This phenomenon has been termed 'exercise induced anorexia' but the mechanisms are unclear so the relationship between exercise and hunger has led to a need for investigators to study the role of gut hormones in mediating exercise-induced hunger changes. The effect of acute exercise bouts on total plasma ghrelin concentrations is controversial and no studies have reported the effects of exercise on acylated ghrelin. There is also a paucity of data on the effects of exercise on total PYY.

Several limitations are apparent in the research literature regarding exercise and gut hormones. Most studies have measured gut hormone responses for relatively short periods and few studies have assessed post-exercise gut hormone responses to feeding over a prolonged period or attempted to relate these responses to changes in hunger. Moreover, no studies have examined acylated ghrelin and PYY responses to resistance exercise. Therefore the purpose of the studies presented in this thesis is to investigate the acute effects of exercise on hunger and acylated ghrelin and the final study measured total PYY in healthy fasted participants to try and explain the mechanisms for 'exercise induced anorexia.'

The aim of study 1 (Chapter 4) was to confirm if high intensity exercise results in 'exercise induced anorexia.' The inclusion of a low intensity exercise and control trial would provide evidence as to whether there is an effect of exercise per se or if the suppression of hunger during exercise is merely the result of distraction. In addition problems were identified with the use of visual analogue scales (VAS) that measured hunger published by Flint et al (2000) during exercise. The study provided an opportunity to test the validity of the ratings of perceived hunger (RPH) scale developed by Burns et al (2007). Twelve healthy Caucasians participated in three, 5 hour trials (high intensity exercise, low intensity exercise and control). On the high intensity exercise trial participants ran for 60 minutes at 71 ± 2 % of maximum oxygen uptake and on the low intensity exercise trial participants ran for 60 minutes at 37 ± 1 % of maximum oxygen uptake resting for 4 hours after exercise. On the control trial participants rested for 5 hours. When using the RPH, hunger was suppressed as a result of exercise; main effect of trial ($P = 0.035$) main effect of time ($P < 0.0005$), trial × time interaction ($P < 0.0005$) and similar statistical findings and trends were found when using the VAS. Post hoc analysis shows that high intensity aerobic exercise leads to 'exercise induced anorexia' and there is a clear physiological role of exercise since suppressed subjective feelings of hunger is not merely the result of distraction as there was no suppression of hunger in the low intensity trial. Exercise intensity and energy expenditure is an important determinant and the RPH scale should be used to measure hunger during exercise.

Since causal mechanisms for 'exercise induced anorexia' remain to be elucidated the purpose of study 2 (Chapter 5) was to assess the effect of a single bout of treadmill running on the appetite stimulating hormone plasma acylated ghrelin. Nine healthy males participated in 2, 9 hour trials (exercise and control) in a random crossover design. On the exercise trial participants ran for 60 minutes at 72 ± 6 % of maximum oxygen uptake. After this they
rested for 8 hours and consumed a test meal at 3 hours (i.e. two hours after exercise). On the control trial participants rested for 9 hours and at 3 hours they consumed an identical test meal to that consumed on the exercise trial. Plasma acylated ghrelin concentration was suppressed during and following the run on the exercise trial and after feeding on both trials i.e. at 4 hours (two-way ANOVA, main effect of trial \( P = 0.022 \), main effect of time \( P = 0.048 \), trial x time interaction \( P = 0.043 \). Hunger declined during the run on the exercise trial and after feeding on both trials (two-way ANOVA, main effect of trial \( P = 0.19 \), main effect of time \( P < 0.0005 \), trial x time interaction \( P = 0.001 \). The findings show that acylated ghrelin is suppressed during prolonged, high intensity running and this coincides with suppressed feelings of hunger suggesting the suppression of hunger during exercise could be mediated by acylated ghrelin.

Having shown an association between acylated ghrelin and hunger during high intensity exercise study 3 (Chapter 6) sought to confirm these findings and examine the influence of low intensity exercise on acylated ghrelin. Whilst hunger was not suppressed during low intensity exercise in study 1 (Chapter 4), acylated ghrelin was not measured and also the energy expenditure of the two exercise trials was not matched. Nine healthy males participated in three trials being high intensity, low intensity and control in a random crossover design. Participants ran for 36 ± 2 minutes at 75.0 ± 1.0 % of maximum oxygen uptake on the high intensity trial and for 54.9 ± 2.0 minutes at 52.5 ± 1.0 % of maximum oxygen uptake on the low intensity trial and then rested thereafter. Exercise energy expenditure did not differ between trials being 2504 ± 55 kJ versus 2580 ± 51 in the high intensity and low intensity trials respectively (\( P = 0.376 \)). Participants rested on the control trial and each trial lasted 4 hours with runs performed at the start of this period. Two-factor ANOVA revealed no effect of trial (\( P = 0.747 \)), but a main effect of time (\( P < 0.0005 \)) and a trial x time interaction (\( P = 0.024 \)) for acylated ghrelin concentrations. At 0.5 hours plasma acylated ghrelin was lower on the high intensity trial compared with the low intensity and control trials (\( P = 0.065 \) after Bonferroni adjustment for high intensity versus control). Results confirm that high intensity running suppresses acylated ghrelin but there was no corresponding suppression of hunger, possibly due to the short duration of exercise. Acylated ghrelin was not suppressed during low intensity running despite matched energy expenditure highlighting that exercise intensity is a determinant of the exercise induced suppression of acylated ghrelin.

Studies 1 and 3 demonstrated that exercise intensity is a determinant of 'exercise induced anorexia' so Study 4 (Chapter 7) examined the effect of exercise duration on plasma acylated ghrelin concentrations. Nine healthy Caucasian males undertook three main trials, short duration run (45 minutes, 70 ± 0.7 % of maximum oxygen uptake), long duration run (90 minutes, 70 ± 0.7 % of maximum oxygen uptake) and control in a random crossover design with each trial lasting 9 hours. Two-factor ANOVA revealed a main effect of trial (\( P = 0.001 \)) for plasma acylated ghrelin, while time (\( P = 0.063 \)) and interaction (\( P = 0.097 \)) effects approached significance. For the effect of trial, Bonferroni post hoc tests indicated that control trial values differed significantly from values on the short duration (\( P = 0.041 \)) and the long duration (\( P = 0.005 \)) exercise trials. Acylated ghrelin was suppressed during both exercise trials with a longer lasting suppression on the long duration trial. Ratings of perceived hunger were also suppressed on the exercise trials (main effect of trial \( P = 0.039 \),
time $P < 0.0005$ and trial $\times$ time interaction $P < 0.0005$) and this suppression coincided with the suppression of acylated ghrelin. These findings demonstrate that the duration of suppression of hunger and plasma acylated ghrelin during high intensity treadmill running is proportional to the duration of exercise.

Since studies 1-4 focused on aerobic exercise it is important to clarify the effects of resistance exercise on hunger and gut hormones as contradictory effects have previously been reported (Kraemer and Castracane, 2007). Study 5 (Chapter 8) examined the effect of treadmill running and free weight resistance exercise on plasma acylated ghrelin and total PYY concentrations. Twelve healthy males undertook 3, 8 hour trials (aerobic, resistance and control) in a random crossover design. On the aerobic trial participants ran for 60 minutes at $68 \pm 2\%$ of maximum oxygen uptake then rested for 7 hours. On the resistance trial participants completed a 90 minute free weight session performing 3 sets of 12 reps of 10 different exercises at 80% of 12 rep max, then rested for 6.5 hours. On the control trial participants rested for 8 hours. Acylated ghrelin was suppressed during resistance and aerobic exercise (two-way ANOVA, time $P = 0.001$, trial $\times$ time $P = 0.035$). Total PYY increased during aerobic but not during resistance exercise (two-way ANOVA, trial $P < 0.01$, time $P < 0.01$, trial $\times$ time interaction $P = 0.027$). The findings show acylated ghrelin is suppressed and total PYY increases during high intensity treadmill running. Resistance exercise suppresses acylated ghrelin but there is no effect on total PYY. These hormonal changes are associated with hunger suppression in both modes of exercise.

In conclusion, high intensity treadmill running suppresses hunger and acylated ghrelin concentration and increases total PYY and whilst resistance exercise suppresses hunger and acylated ghrelin it has no effect on total PYY. There is a threshold of exercise intensity, duration and expenditure which needs to be reached before 'exercise induced anorexia' is experienced and these factors affect hunger and the change in gut peptide hormones. Whilst the findings of this thesis are useful in identifying potential causal mechanisms for the influence of exercise on hunger, more work is needed to address the effect of exercise on energy intake and how exercise can play a more effective role in the prevention of overweight and obesity, weight loss and the prevention of weight regain.

Key words: Acylated ghrelin, aerobic exercise, appetite, hunger, peptide YY, resistance exercise
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“Alone we can do so little; together we can do so much”

Helen Keller

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PREFACE

The findings presented in this thesis have been peer reviewed as follows:

Published Articles


In Press

The influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin and peptide YY levels in healthy males. *American Journal of Physiology - Regulatory, Integrative and Comparative*

Published Abstracts


Conference Presentations

Abstracts from all the studies presented in this thesis have been peer reviewed and have been accepted for conference presentations as follows:

Chapter 4:
Measurement of appetite during exercise using visual scales (Oral)
BASES 2007 annual conference – University of Bath

Chapter 5:
Acylated ghrelin concentrations are suppressed during aerobic exercise (Oral)
ACSM 2006 annual conference – Denver

Chapter 6:
The effect of exercise intensity on plasma acylated ghrelin (Poster)
BASES 2006 – University of Wolverhampton

Chapter 7:
The effect of treadmill running duration on plasma acylated ghrelin concentrations (Oral)
BASES 2008 – Brunel University
Chapter 8:
The effect of resistance and aerobic exercise on plasma total PYY and acylated ghrelin (Oral)
ACSM 2008 annual conference - Indianapolis
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LIST OF ABBREVIATIONS

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

ANOVA - Analysis of variance
AUC - Area under the concentration versus time curve
BMI - Body Mass Index
CCK - Cholecystokinin
CNS - Central Nervous System
GH - Growth Hormone
PYY - Peptide YY
RPE - Ratings of Perceived Exertion
RPH - Ratings of Perceived Hunger
RQ - Respiratory Quotient
SD - Standard Deviation
SEM - Standard Error
TAG - Triacylglycerol
VAS - Visual Analogue Scale
\( \text{VO}_2\text{ max} \) - Maximum oxygen uptake
CHAPTER I

Introduction

The Health Select Committees report (Health Select Committee, 2004) has highlighted that the prevalence of obesity has increased by almost 400% in England since 1980. This increase in prevalence reflects a worldwide trend which is most marked in, though not restricted to developed countries and has been described by the World Health Organization (WHO) as: ‘a global epidemic’ (WHO, 1998). A recent Health Survey for England examined overweight and obesity and found that 22.1% of men and 22.8% of women in England are classified as clinically obese (Sproston and Primatesa, 2003) which is defined as a body mass index (BMI) of \( \geq 30 \text{ kg}\cdot\text{m}^{-2} \) (calculated as weight in kilograms divided by the square of the height in metres). If recent rates of growth continue, the National Audit Office predicts a third of all adults will be obese by 2010 (National Audit Office, 2001).

Some people are genetically more susceptible to obesity than others, but it is unlikely that the gene pool has changed that dramatically in recent years to cause an obesity epidemic (Stunkard et al, 1986). Lifestyle choice is likely to be the causal factor as a result of an imbalance between energy expenditure and energy intake. Although the relative contributions of each at the individual level vary, obesity is thought to be the result of people being insufficiently active to balance the energy consumed from food and drink, leading to positive energy balance. This translates in the longer term to overweight and obesity.
Buchwald et al (2004) comment that diet therapy with and without support organisations is relatively ineffective in treating obesity in the long term and in severe cases bariatric surgery is recommended for the morbidly obese (BMI ≥ 40 or ≥ 35 kg·m⁻² in the presence of significant comorbidities). In conducting a systematic review of published observational and interventional trials, Buchwald et al (2004) found that bariatric surgery eliminates or significantly ameliorates diabetes, hyperlipidaemia, hypertension and obstructive sleep apnea. However, there are risks and the operative 30-day mortality rates were 0.1% for restrictive procedures, 0.5% for gastric bypass and 1.1% for duodenal switch.

Drug therapy can facilitate weight loss or long term weight maintenance. Orlistat is an anti obesity agent that works in the gastrointestinal tract to reduce dietary fat absorption. The mechanism of action is inhibition of the activity of gastrointestinal lipases decreasing the hydrolysis and subsequent absorption of ingested fat by up to 30% (Zhi et al 1994). O’ hill et al (1999) conducted a multicenter, double-blind, placebo-controlled study to test the hypothesis that orlistat is significantly more effective than a placebo in preventing weight regain. Obese participants who had lost ≥ 8% body weight during a 6 month lead in were randomly assigned to receive placebo, 30 mg orlistat, 60 mg orlistat, or 120 mg Orlistat 3 times daily for 1 year in combination with a maintenance diet to help prevent weight regain. After 1 year, participants treated with 120 mg orlistat 3 times daily regained less weight than did placebo-treated subjects. Moreover, more participants in the 120 mg orlistat group than in the placebo group regained ≤ 25% of lost weight, showing the use of orlistat during periods of attempted weight maintenance minimises weight
readjustment and facilitates long-term improvement in obesity-related disease risk factors.

Another drug, sibutramine (a serotonin-norepinephrine re-uptake inhibitor) is also frequently prescribed, as it appears to modify internal signals that control hunger and satiation (Barkeling et al, 2003). Many weight loss medications are prescribed with minimal or no lifestyle modification and the combined effect of lifestyle and pharmacological interventions is unknown (Phelan et al, 2002). Wadden et al (2005) therefore examined the effectiveness of sibutramine with or without lifestyle intervention. Two hundred and twenty four obese adults were randomly assigned to receive 15 mg of sibutramine per day alone; lifestyle-modification counselling alone, delivered in 30 group sessions; sibutramine plus 30 group sessions of lifestyle-modification counselling (i.e., combined therapy); or sibutramine plus brief lifestyle-modification counselling delivered by a primary care provider in eight visits of 10 to 15 minutes each. All participants were prescribed a diet of 1200 to 1500 Kcal per day and the same exercise regimen. At one year, participants who received combined therapy lost more weight than those receiving sibutramine alone, those treated by lifestyle modification alone and those receiving sibutramine plus brief therapy. The results underscore the importance of prescribing weight-loss medications in combination with lifestyle modification which includes the prescription of physical activity and exercise.

Although there are exceptions, there is general agreement from observational studies that overweight and obese individuals are less physically active than non-obese individuals
The role of exercise in reducing weight and preventing weight gain is widely accepted and it has been quoted that 'exercise alone is an effective strategy for reducing obesity and related comorbidities' (Ross et al, 2000, p1).

A recent review of lifestyle intervention studies concluded that the average weight loss from combined diet and exercise programmes is 3 to 5 kg compared with no treatment or usual care. Moreover, in most studies with long-term follow-up, the initial weight loss is followed by weight regain (Jain, 2005). Similarly Jakicic et al (1999) combined a diet and exercise intervention which resulted in a 13 kg weight loss in participants who performed an average of 285 minutes of exercise every week (approximately 40 minutes per day) over an 18 month period. Moreover, high physical activity levels (11.830 kJ/week) have been identified (along with low energy and fat intakes) as a common feature of individuals who are successful at long-term maintenance of substantial weight loss (Klem et al 1997; Wing and Phelan, 2005).

There is some evidence that genetic factors play a role in the extent to which weight loss occurs with exercise. Bouchard and colleagues (1994) studied seven pairs of monozygotic twins over a period of 93 days while they were kept on a constant daily energy intake. Participants cycled for close to an hour, twice a day, for 9 out of 10 days. This led to an estimated energy deficit of 244 MJ by the end of the training period. Although the average weight loss was 5 kg, weight loss varied between individuals from 1 to 8 kg. However, the variance between twin pairs was much greater than the variance within twin pairs. These findings suggest that exercise will be more effective as a means
of weight loss in some individuals compared with others and that this is due in part to genetic factors.

The benefits of exercise in preventing and treating obesity is the direct result of energy expenditure during exercise but there is potential for exercise to help control weight by suppressing hunger. Hunger is defined as a strong desire or need for food which has been used synonymously with the term appetite. Suppressing hunger during and in the immediate post-exercise period could modulate energy intake leading to a phenomenon which has been termed ‘exercise induced anorexia’ (Blundell et al, 2003; King et al, 1994) but the mechanisms are complex as the regulation of hunger and bodyweight includes neural, metabolic and endocrine signals from the periphery that communicate the short and long term status of energy balance. A central neural network centred in the hypothalamus receives and integrates signals from the adipostat factors leptin and insulin and the gut hormones, cholecystokinin (CCK), ghrelin and peptide YY (PYY), stimulating appropriate responses of hunger and expenditure.

Leptin was discovered by Zhang et al (1994) and is expressed and secreted by adipocytes in white adipose tissue and circulates in plasma at concentrations proportional to fat mass. Peripheral or Central Nervous System (CNS) administration of leptin to rodents reduces food intake and body weight and increases energy expenditure (Friedman & Halaas, 1998). The hyperphagic and obese ob/ob mouse lacks functional leptin (Zhang et al, 1994) yet the vast majority of obese humans have normally functioning ob genes and
high plasma leptin levels reflecting their high fat mass (Considine et al, 1996) so is not the main cause of obesity.

Insulin may play a similar role to leptin as an adiposity signal. Basal circulating levels of insulin correlate with body adiposity level (Polonsky, 1988; Woods & Seeley, 1998) and disruptions of insulin sensitivity are associated with both obesity and diabetes (Kahn & Flier, 2000). Insulin has been suggested to be involved in short-term hunger regulation (Flint et al, 2007) but the results of infusion studies have been conflicting. Rodin et al (1985) found increased insulin promoted hunger and subsequent energy intake yet Gielkinks et al (1998) found no such effect with insulin infusion.

The increase in hunger before and the reduction following a meal cannot be explained by the small changes in plasma leptin alone (Murphy and Bloom, 2003) and the acute postprandial rise in insulin does not appear to reduce hunger directly (Chapman et al, 1998). Postprandial satiety might be explained by a gut sensor system, signalling from the gut to the appetite centres in the brain since Murphy and Bloom (2003) highlight that CCK was the first gut hormone implicated in the control of hunger. The anorectic effects of CCK have been confirmed in rodent and human studies (Kissileff et al, 1981; Muurahainenn et al, 1988; Moran & Schwartz, 1994). The presence of digestive products in the intestinal lumen stimulates the release of CCK from the proximal small intestine and preprandial administration of CCK antagonists increases meal size in humans (Beglinger et al, 2001).
Ghrelin is a 28-amino acid peptide hormone that was identified through studies looking at the regulation of growth hormone (GH) secretion (Kojima et al, 1999). Ghrelin is secreted predominantly by the stomach (Kojima et al, 1999) and exhibits a diurnal rhythm, gradually rising throughout the day, reaching a zenith between 0100 and 0200 hours and a meal response, rising 1-2 hours before the initiation of a meal and falling to trough levels 1-2 hours after. This provides support for the involvement of ghrelin in hunger regulation (Cummings et al, 2001). This is supported by evidence that the infusion of ghrelin stimulates feeding (Wren et al, 2001a). Ghrelin has the opposite action of PYY which is a 36 amino acid hormone secreted from endocrine L-cells of the distal ileum and colon (Bottcher et al, 1984). It exists in two endogenous forms being PYY1-36 and PYY3-36 and increased concentrations of PYY3-36 inhibits food intake by altering central nervous system appetite circuits within the arcuate nucleus of the hypothalamus or area postrema (Batterham and Bloom, 2003).

The potential effect of exercise in suppressing hunger is not widely promoted because the evidence is conflicting and the causal mechanisms are not clear since the research evidence of how hunger related hormones respond to exercise is limited (O'Connor et al, 2006). Since exercise is a major component of the energy balance equation, examining how hunger related hormones respond to exercise is of importance. Despite the accumulation of a large body of research on ghrelin and PYY, few studies have examined the effects of exercise on these gut peptide hormones. The primary purpose of the studies in this thesis is therefore to examine hunger during and immediately post aerobic and resistance exercise to see if there are any co-inciding changes with plasma acylated
ghrelin and total PYY. It was hypothesized that exercise would cause a temporary suppression of hunger and that this would be associated with reduced concentrations of plasma acylated ghrelin and increased concentrations of total PYY. It was posited that the extent of these changes was likely to be dependent on the duration, intensity and/or mode of exercise.

Study 1 (Chapter 4) examined the effect of exercise intensity on hunger by looking at low and high intensity aerobic exercise compared with control. Study 2 (Chapter 5) measured acylated ghrelin and hunger responses to high intensity aerobic exercise compared with control. Study 3 (Chapter 6) measured acylated ghrelin and hunger responses to low and high intensity aerobic exercise compared with control. Study 4 (Chapter 7) measured acylated ghrelin and hunger responses to short and long duration aerobic exercise compared with control. Finally, study 5 (Chapter 8) measured acylated ghrelin, hunger responses and total PYY to high intensity aerobic and resistance exercise compared with control.

Reports indicate that obese people do not compensate for energy expenditure as a result of treadmill running by increasing energy intake (Pi-Sunyer and Woo, 1985) and Staten et al (1991) found normal weight young adults are in negative energy balance during and in the post exercise period. The physiological significance of an exercise-induced decrease in acylated ghrelin and increase in total PYY is whether it provides the added benefit of suppressing hunger and creating a negative energy balance. This is a novel area of research which is warranted to initially confirm an exercise induced effect on hunger
related hormones therefore contributing to the understanding of the effects of exercise on hunger suppression. This could have important implications regarding the role of exercise in weight control, weight regain and the prevention of overweight and obesity since exercise offers a safer, long term approach without the deleterious side effects associated with pharmacological interventions or surgery.
CHAPTER II

Review of Literature

2.1 Introduction and overview

This review will address the literature on the effects of exercise on hunger, ghrelin and PYY. The review begins by describing hunger regulation moving on to explaining the production and secretion of ghrelin and PYY and how these gut peptide hormones influence hunger and energy regulation. The potential role of exercise for suppressing hunger is highlighted and thereafter the influence of exercise on ghrelin and total PYY and how this may result in 'exercise induced anorexia is reviewed'. The purpose of the review is to present the findings of previous work and to make the case for undertaking the studies in this thesis and the manner in which they were undertaken.

2.2 Hunger regulation

Appetite is the desire to eat food, felt as hunger so the terms appetite and hunger will be used synonymously throughout this thesis. Hunger is controlled by a variety of peripheral signals that change in response to food intake or fasting which act in the brain to alter feelings of hunger and fullness (satiety) to determine meal initiation (hunger) and meal termination (satiation).

The hypothalamus is the key central nervous system (CNS) region involved in hunger regulation, though other brain regions, including the nucleus tractus solitarius and the area postrema, also play a role. Lesioning and stimulation experiments led to a 'dual centre' hypothesis which proposed that the ventromedial hypothalamic nucleus was a 'satiety centre' and the lateral hypothalamus a 'feeding centre' (Kalra et al, 1999). Over
time this simple picture has changed into a more sophisticated model in which a
number of discrete neuronal pathways within specific hypothalamic nuclei have been
shown to form a more complex, integrated neural network with numerous
neurotransmitters and modulators thought to be involved in the hypothalamic
regulation of appetite (Vettor et al, 2002).

The hypothalamus interprets and integrates a number of neural and humoral
(circulating in the blood) inputs to coordinate feeding and energy expenditure in
response to conditions of altered energy balance. Long-term signals communicating
information regarding the body's energy stores, endocrine status and general health
are predominantly humoral. Short-term signals, including gut hormones and neural
signals from higher brain centres and the gut, regulate meal initiation and termination.
Both short- and long-term signals can also affect energy expenditure via sympathetic
nervous outflow to brown adipose tissue and by affecting the secretion of various
pituitary hormones (Schwartz et al. 1999).

The hypothalamic arcuate nucleus (ARC), which is known as the infundibular nucleus
in man, appears to play a crucial role in receiving and integrating such signals. The
ARC is situated at the base of the hypothalamus and is incompletely isolated from the
general circulation by the blood–brain barrier, allowing direct access of circulating
factors to ARC neurones. There are two main populations of ARC neurones involved
in the regulation of food intake: appetite-inhibiting pro-opiomelanocortin (POMC)
neurones, and appetite-stimulating neuropeptide Y (NPY) and agouti-related peptide
(AgRP) coexpressing neurones (Williams et al. 2001).
Both the POMC and NPY/AgRP neuronal populations project to other hypothalamic nuclei, in particular the paraventricular nucleus (PVN), which is known to be critical in the regulation of food intake and energy expenditure. The PVN also assimilates inputs from other hypothalamic nuclei, including the lateral hypothalamic area, the ventromedial nucleus and the dorsomedial nucleus (Murphy and Bloom, 2004). Neurons containing alpha melanocyte-stimulating hormone (α-MSH) suppress hunger. α-MSH derives from POMC and acts to suppress hunger through a melanocortin receptor (MC4). Neurons containing NPY and AgRP stimulate feeding and weight gain by interfering with the actions of α-MSH.

Figure 2.1 illustrates the hormones that influence energy homeostasis via the arcuate nucleus. The experimental work of this thesis has included the measurement of the gut peptide hormones PYY and acylated ghrelin. In summary, the mechanism of ghrelin action asserts that within the hypothalamus ghrelin activates NPY and AgRP neurons while inhibiting POMC neurons in the ARC (Neary et al, 2004) increasing hunger. PYY exists in two forms PYY₁₋₃₆ and PYY₃₋₃₆. Whilst the mechanism of action for PYY is not completely understood, after crossing the blood-brain barrier, PYY₃₋₃₆ inhibits NPY and AgRP neurons in the ARC suppressing hunger. The effect is likely to be mediated through the Y2 receptor because its inability to inhibit feeding is abolished in Y2 receptor knockout mice (Tovar et al, 2004). In contrast to this PYY₁₋₃₆ acts through the Y1 and Y5 receptors increasing hunger.
Figure 2.1  Circulating hormones influencing energy homeostasis via the arcuate nucleus. Continuous lines indicate stimulatory effects e.g. ghrelin stimulates NPY via the Y1 and Y5 receptor increasing food intake. Key: AgRP, agouti-related peptide; aMSH, alpha-melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PYY, peptide YY. Adapted for human’s from Murphy and Bloom’s (2004) animal. There are limitations of inferring from animal studies since whilst hormonal responses may be similar, hormonal structures are different e.g. rat and human ghrelin differs at amino acids 11 and 12.
2.3 Ghrelin

Ghrelin is a gut peptide hormone that is involved in hunger regulation as shown in figure 2.1

2.3.1 Structure, production and secretion

Ghrelin is a 28 amino acid peptide that is acylated in its active form (acylated ghrelin) compared with its inactive form (des-acylated ghrelin) (Kojima et al, 1999; Hosoda, 2002). Modified at the amino acid ser 3 with an n-octanoic acid, acylated ghrelin (Figure 2.2) is the first known example of a bioactive peptide modified by an acyl acid (Kojima et al, 2001). Acylation of ghrelin is thought to be essential for ghrelin to bind to the growth-hormone-secretagogue receptor and to cross the blood brain barrier (Kojima et al, 1999; Murphy et al, 2006). The ratio of acylated to des-acylated ghrelin is approximately the same in tissues that synthesize ghrelin suggesting that acylation occurs in the cells of origin (Date et al, 2000). In humans des-acylated ghrelin does not possess the pituitary and pancreatic activity of acylated ghrelin (Broglio et al, 2003).

![Figure 2.2](image_url)  
*The structure of acylated ghrelin. Adapted from Kojima et al (2001)*
The stomach is the principal site of ghrelin synthesis producing approximately 10 times more of the hormone per gram of tissue than the next richest source, the duodenum (Kojima et al, 1999). Patients who have undergone gastric bypass surgery have a decreased plasma ghrelin concentration and changes in ghrelin concentration diminish with food intake in these patients, providing evidence that the stomach is the main site of production (Cummings et al, 2002). Research has also shown that ghrelin is secreted by the ARC of the hypothalamus (Kojima et al, 1999), the pituitary (Korbonitis et al, 2001), kidney (Mori et al, 2000), liver (Wang et al, 2002), pancreas (Wierup et al, 2002) and the small and large intestines (Date et al, 2000).

Ghrelin is secreted into blood vessels, circulating the whole body with one study showing normal fasting adult human plasma samples being 337.2 – 404.64 pg·mL⁻¹ (Kojima et al, 2001) although values as high as 800 pg·mL⁻¹ have been reported (Peeters et al, 2005). The majority (80-98%) of ghrelin in plasma is des-acylated (Broglio et al, 2003) and the ratio has been shown to be 3:1 (Hosoda et al, 2003).

2.3.2 Growth hormone releasing activity of ghrelin

Early research examined the effects of ghrelin on GH release since ghrelin is stimulated by hypothalamic GH releasing hormone (GHRH). GH-secretagogues (GHS) are synthetic compounds that are potent stimulators of GH release working through the GHS-receptor (GHS-R). Ghrelin is the endogenous ligand (a molecular group that binds to another chemical entity to form a larger complex) for the GHS-R and stimulates GH secretion more potently than GHRH (Kojima et al, 1999; De Vries et al, 2002). Further studies only examined the orexigenic properties of ghrelin as
volunteers reported an increase in appetite as a side effect of the administration of ghrelin (Wren et al, 2001b).

2.3.3 Ghrelin, hunger and short-term energy regulation

The most important role of ghrelin appears to be the stimulation of hunger and regulation of energy homeostasis (Peeters et al 2005) and ghrelin is the first peripheral orexigenic signal to be discovered (Muccioli et al 2002). Immunohistochemical analysis reveals that ghrelin responsive neuronal cells can be found in a very limited region in the hypothalamic ARC (Bagnasco et al, 2003; Kojima et al, 1999). As described in section 2.3 the arcuate nucleus is the target site of the appetite-stimulating peptides NPY and AgRP. Intracerebroventricular injection of ghrelin induces the expression of Fos protein (a deoxyribonucleic acid binding protein that regulates transcription) in NPY-containing neural cells, which increases the amount of NPY messenger ribonucleic acid (mRNA) in the ARC (Nakazato et al, 2001). Intravenous injection of ghrelin also stimulates neurons that contain NPY and/or AgRP (Cowley & Grove, 2004). Immunohistochemical analysis indicates that ghrelin neuron fibres directly protrude onto these neurons indicating that ghrelin increases feeding activity by stimulating NPY and AgRP containing neurons in the hypothalamus to promote the secretion of NPY and AgRP peptides. The standard hormonal model of ghrelin action asserts that circulating ghrelin derived primarily from the stomach accesses the ARC through a leaky blood-brain barrier (Banks et al, 2002; Casanueva et al, 2002).

Administration of ghrelin by systematic infusion in order to achieve levels 2-3 fold higher than baseline has been shown to increase perceptions of hunger by 48% and
energy intake by 38% in humans (Wren et al, 2001a). Ghrelin has been shown to stimulate gastric motility and acid secretion, both of which increase in anticipation of meals (Asakawa, 2001). Research shows a dramatic preprandial rise and postprandial fall in circulating ghrelin (Cummings et al, 2001) and healthy volunteers reported an intense feeling of hunger after ghrelin administration (Arvat et al, 2000) confirming ghrelin is a meal initiator. Ghrelin’s orexigenic actions are extremely rapid and short lived as required for a signal influencing meal related behaviour (Asakawa, 2001).

2.3.4 Ghrelin and long-term energy regulation

Ghrelin concentration correlates negatively with energy stores and displays compensatory changes in response to alterations of these stores (Cummings et al, 2005). In addition, ghrelin appears to be most sensitive to changes in body weight resulting from an overall energy deficit, independent of specific effects of reduced nutritional intake and/or physical exercise (Foster-Schubert et al, 2005; Garcia et al, 2006; Leidy et al 2006).

Evidence shows that ghrelin is involved in central energy balance regulation (Wren et al, 2001a), but the full extent of its effects in humans is unknown. In rodents, continual ghrelin administration promotes adiposity due to both increased caloric intake and increased respiratory quotient (RQ) favouring fat deposition, resulting in weight gain (Tschop et al, 2000). In humans, ghrelin secretion is suppressed by chronic over feeding and decreased concentrations of fasting ghrelin have been recorded in obese compared with age-matched lean subjects (Tschop et al, 2001a). This suggests that plasma ghrelin concentration may be down regulated in the obese as a possible consequence of chronic positive energy balance. Plasma ghrelin
concentrations have also been shown to be negatively correlated with body fat and are lower in Pima Indians (a population known for its susceptibility to obesity) compared with Caucasians (Ukkola and Poykko, 2002).

Ravussin et al (2001) overfed twelve pairs of monozygotic twins by 351.5 MJ over a 100-day period, whereas another seven pairs of monozygotic twins were subjected to a 221.8 MJ negative energy balance induced solely by exercise for 93 days. With overfeeding plasma ghrelin exhibited a non significant decrease of 205.7 ± 101.2 pg·mL⁻¹ (P = 0.18) while a non significant increase of 195.6 ± 114.6 pg·mL⁻¹ (P = 0.17) was observed with negative energy balance. Baseline plasma ghrelin concentration was negatively correlated with body mass and body fatness, but there was no relationship between baseline plasma ghrelin concentration and the magnitude of body weight change in both interventions providing evidence that ghrelin may not be involved in the aetiology of human obesity. In addition, the intraclass coefficient for twin resemblance (r = 0.75, P = 0.006) indicated that plasma ghrelin concentration is a familial trait i.e. plasma ghrelin concentration at baseline was more alike within pairs than between pairs of twins. This suggests that genetic differences among people could play a role in the production and/or clearance of ghrelin.

Circulating concentrations of ghrelin are reduced in obese participants, so hyperproduction of ghrelin (hyperghrelinemia) does not appear to be responsible for overfeeding in the obese. In addition ghrelin null mice have normal bodyweight, food intake and appetite (Sun et al, 2003). However, one study has shown that obese humans do not exhibit the decline in plasma ghrelin seen after a test meal in lean participants (English et al, 2002). This lack of suppression following a meal could
lead to increased food consumption and weight gain predisposing to obesity, raising the tempting hypothesis that impaired ghrelin suppression may lead to an impaired satiety after eating.

In contrast, increased ghrelin concentrations have been observed in patients with anorexia nervosa (Otto et al, 2001). This was confirmed by Ariyasu et al (2001) who found that ghrelin levels were markedly elevated in anorexia nervosa patients compared with healthy participants fasting for 12 hours. Two anorexic patients showed a seven fold higher plasma ghrelin concentration compared with control participants. Weight loss causes plasma ghrelin concentration to increase (Tschop et al, 2001b) suggesting that ghrelin may contribute to the drive to eat that makes long term success with dieting so difficult.

2.3.5 Regulation of ghrelin secretion

Ghrelin secretion is predominantly controlled by feeding, peaking before food intake, with feeding and hyperglycaemia suppressing its release. This ultimately leads to enhanced satiety (Cummings et al, 2001). In addition, temporal patterns of ghrelin and insulin surges are reciprocal. Figure 2.3 illustrates the ghrelin, insulin and hunger response to feeding.
Figure 2.3  Ghrelin response to feeding. Adapted from Pinkney and Williams (2002).

Yildiz et al (2004) measured circulating ghrelin for 24 hours in lean and obese participants and found significant ultradian (rhythms occurring in cycles more frequently than every 24 hours) fluctuations and an orderly pattern of release. There was a nocturnal rise that exceeded the meal associated increases in lean participants which was blunted in the obese suggesting this is a biological feature of human obesity. In lean participants, the highest ghrelin concentrations were recorded first thing in the morning, decreased after feeding, rising gradually throughout the day, increasing and decreasing in response to further meals.
It is not clear what other factors are responsible for ghrelin regulation, but blood glucose levels may be critical, since ghrelin concentrations rise during hyperglycaemia (Toshinai et al, 2001) and both Tschop et al (2000) and Nakagawa et al (2002) have reported that oral or intravenous administration of glucose decreases plasma ghrelin concentration. Whilst decreases in plasma ghrelin concentration may be attributable in part to hyperglycaemia, plasma insulin levels also increase after glucose administration which could also contribute to the decrease. Saad et al (2002) found that insulin infusion decreases plasma ghrelin concentration which was confirmed by Flanagan et al (2003) who reported that hyperinsulinaemia suppressed ghrelin.

Research also shows that the energy content but not the volume of a meal is responsible for the postprandial decrease of plasma ghrelin concentrations (Callahan et al, 2004; Erdman et al, 2004). Calorie for calorie the magnitude of suppression based on ingested macronutrients is Carbohydrate – Protein – Lipid (Cummings et al, 2005). In addition, Tschop et al (2000) found that gastric distension caused by water intake does not alter ghrelin concentration or influence its release.

2.4 PYY

PYY is another gut peptide hormone involved in hunger regulation as shown in figure 2.1

2.4.1 Structure, production and secretion

Peptide YY is a 36 amino acid gastrointestinal hormone first isolated from porcine small intestine by Tatemoto (1980) and named PYY because of the presence of amino
acid terminal (Y) tyrosine and a carboxyl terminal tyrosine amide (Y). Studies have
identified PYY mRNA in the gastrointestinal tract, pancreas and the brainstem
(Broome et al, 1985; Ekblad and Sundler, 2002). The L cells found in the
gastrointestinal tract are the major source of PYY, which exists in two endogenous
forms PYY$_{1-36}$ and PYY$_{3-36}$. The latter is produced by the action of the enzyme
dipeptidyl peptidase IV and is a truncated 34 amino acid peptide generated by the
cleavage of two amino acids from the N terminus. The amino acid sequence of PYY$_{1-
36}$ and PYY$_{3-36}$ is as follows:

\[
\begin{align*}
\text{PYY$_{1-36}$:} & \quad \text{YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY} \\
\text{PYY$_{3-36}$:} & \quad \text{--AKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY}
\end{align*}
\]

PYY shares a common tertiary structure with neuropeptide Y (NPY) and pancreatic
polypeptide (PP) consisting of an α-helix and polyproline helix connected by a β-turn
resulting in a U-shaped peptide known as a PP fold (Glover et al, 1983). The PP fold
is important in the binding and activation of the six G protein-coupled receptor
subtypes – Y1, Y2, Y3, Y4, Y5 and Y6 (Keire et al, 2000). PYY$_{1-36}$ binds to and
activates at least four Y receptor subtypes (Y1, Y2, Y4 and Y5) whereas PYY$_{3-36}$ is
more selective for Y2 receptors (Ballentyne, 2006).

Both PYY$_{1-36}$ and PYY$_{3-36}$ are stored in the gut mucosal endocrine cells and are
secreted into the circulation following food intake (Grandt et al, 1994). The
percentage of the two forms in human blood differs depending on feeding status. In a
fasted state the concentration of PYY$_{1-36}$ predominates over PYY$_{3-36}$ (63% versus 37%
respectively) but after feeding the reverse is true (46% versus 54% respectively) (Grandt et al, 1994).

2.4.1 PYY, hunger and short term energy regulation

Peripheral administration of PYY was first reported in 1993 to decrease hunger (Okada et al, 1993). Peripheral administration of PYY$_{3-36}$ at doses mimicking postprandial levels activates neurones in the ARC and markedly inhibits food intake in rodents and man. In particular human subjects have been shown to reduce food intake by 30% (Batterham et al, 2002). Direct intra-ARC injection also reduces food intake in rats (Batterham et al, 2002).

After a meal PYY$_{3-36}$ is released into the circulation, and it is proposed that hunger is inhibited by PYY$_{3-36}$ acting directly on the arcuate nucleus via the Y2 receptor, a presynaptic inhibitory autoreceptor. POMC neurons are under a tonic γ-aminobutyric acid-mediated inhibition by NPY neurons, and thus decreased γ-aminobutyric acid-mediated tone, may lead to disinhibition of POMC neurons (Cowley et al, 2001; Batterham et al, 2002) and hence suppressed hunger. The binding of PYY$_{3-36}$ to the Y2 receptor leads to an inhibition of the NPY neurons and a possible reciprocal stimulation of the POMC neurons (Batterham et al, 2002). Reduced NPY mRNA expression levels and increased POMC mRNA levels are observed after peripheral PYY$_{3-36}$ administration (Challis et al, 2003). Evidence from these studies suggests that PYY physiologically inhibits hunger in humans and suggests that it is important in the everyday regulation of food intake.
Sloth et al (2006) confirmed the anorectic effects of PYY\textsubscript{3-36} infusion in lean and obese participants but noted feelings of nausea may confound subjective feelings of hunger. PYY\textsubscript{3-36} caused increase thermogenesis, lipolysis and increased postprandial glucose and insulin responses. Sloth et al (2006) also infused PYY\textsubscript{1-36} and found no effects of energy intake or hunger.

### 2.4.2 PYY and long term energy regulation

Obese individuals have a deficiency of PYY release, which is associated with reduced satiety which may reinforce obesity (Le Roux et al, 2004). Obese persons have a lower fasting basal PYY and exhibit a diminished postprandial rise (Batterham et al, 2003). Batterham et al (2003) also found that energy intake during a buffet lunch 2 hours after the infusion of PYY\textsubscript{3-36} was decreased by 30% in obese subjects and 31% in lean subjects leading to a cumulative reduction in 24 hour food intake. Batterham et al (2006) confirmed previous findings of a significantly lower fasting PYY concentrations in obese compared with lean subjects.

Chan et al (2006) found that complete fasting for 48 - 72 hours significantly decreases PYY concentrations in healthy participants. They also add that the fasting induced decrease in PYY concentrations is not mediated by falling leptin levels since restoration of leptin with exogenous recombinant human leptin does not alter the decline in PYY that is associated with acute energy deprivation.

Age does not impact on PYY levels. Macintosh et al (1999) studied PYY release following intra-duodenal infusion of lipid on PYY in seven younger (age 20 to 34 years old) and eight older (age 65 to 75 years old) volunteers. Basal and lipid
stimulated PYY levels were nearly identical in the two age groups and therefore age does not appear to affect PYY metabolism.

2.4.3 Regulation of PYY
In response to food ingestion, plasma PYY\textsubscript{3-36} concentrations rise within 15 minutes, peak at 60 minutes, plateau by approximately 90 minutes and remain elevated for up to 6 hours (Adrian et al, 1985; McGowan and Bloom, 2004). Postprandial PYY concentrations increase in both lean and obese subjects (Batterham et al, 2006). Batterham and Bloom (2003) suggest that PYY\textsubscript{3-36} is released in proportion to calories ingested and regulates subsequent food intake by modulating the activity of the NPY and POMC neurons in the ARC of the hypothalamus. PYY concentrations are influenced not only by energy intake but also by meal composition and higher concentrations are seen following fatty meals compared with meals containing high protein or carbohydrate (Adrian et al, 1985; Lin and Chey, 2003). This implies that due to a greater PYY release fatty meals should be eaten to suppress hunger but the downside of this is a greater energy intake so the effect of high fat meals on PYY, hunger, energy intake and subsequent energy intake on weight status remains to be elucidated. PYY release is also stimulated by gastric acid, cholecystokinin and infusion of bile acids into the ileum or colon, but not by gastric distension (Pedersen et al, 1996).

2.5 Exercise and hunger
The role of exercise in reducing weight and preventing weight gain is widely accepted and it has been quoted that 'exercise alone is an effective strategy for reducing obesity and related comorbidities' (Ross et al, 2000). This is the direct result of energy
expenditure during exercise, but there is potential for exercise to help control weight by suppressing hunger and reducing energy intake in the immediate post-exercise period, a phenomenon which has been termed 'exercise induced anorexia' (Blundell et al, 2003; King et al, 1994).

The inter-relationship between exercise and food intake is important because both are contributors to energy balance. The extra energy expended by exercise may stimulate hunger and increase energy intake to compensate for the energy used, possibly above and beyond that expended during exercise leading to positive energy balance. Though, contrary to widespread belief, there is no short-term increase in hunger and food intake after exercise (King and Blundell, 1995) and evidence suggests that an acute exercise-induced negative energy balance is not compensated by an increase in energy intake (Blundell et al, 2003). Negative energy balances of ≤ 4 MJ have been shown to be tolerable over periods of up to 16 days when performing exercise programmes. Although a subsequent increase in energy intake does eventually take place, evidence suggests that it only compensates for about 30% of the energy expended through exercise (Blundell et al, 2003).

Kissileff et al (1990) detected the suppression of feelings of hunger immediately following a vigorous bout of cycling (90W for 40 minutes). King et al (1994) assessed the differences between high and low intensity exercise with total energy expenditure remaining constant. Twelve healthy lean males either rested for ~45 minutes, cycled at 70% VO\textsubscript{max} for ~30 minutes or cycled at 30% VO\textsubscript{max} ~60 minutes on the experimental day before being allowed to eat test foods \textit{ad libitum} in their own time. Low intensity exercise did not produce any suppression of hunger either during nor
after the exercise session, but there was a significant suppression of hunger during and immediately after the high intensity exercise compared with the control session. Within 15 minutes of termination of the high intensity exercise session, subjective feelings of hunger had begun to return to control values. Absolute food intake was not affected by either exercise protocol.

If the suppression of hunger is short lived after single intense bouts of exercise this may only have a minor role to play in reducing food intake. Measuring food intake after exercise would be a better indicator of whether exercise is having an effect on energy intake than solely examining subjective feelings of hunger but this is beyond the scope of the studies in this thesis as the priority is to examine potential hormonal and metabolite changes that co-incide with any possible changes in hunger.

King et al (1994) also report a second study with a similar research design except participants underwent two separate high intensity exercise sessions, one of short duration (mean time = 26 minutes) and the other long duration (mean time = 52 minutes) and a resting control. The gross energy expenditure was significantly different and hunger was suppressed in both protocols, but the hunger suppression was greatest after the long duration session. Hunger values were not significantly different between any of the three treatments 10 minutes after the end of exercise.

The findings of 'exercise induced anorexia' are mixed since Imbeault et al (1997) and Stubbs et al (2002a; 2002b) found no effect of exercise on hunger. Gender differences are also apparent. In lean male humans the feeling of hunger is suppressed by intense exercise (King et al, 1994; King et al, 1997b; King and Blundell, 1995) but whilst an
intense exercise session leads to increased palatability rating of foods in lean women, there is no suppression of hunger (King, 1996a).

The mechanism through which intense exercise suppresses hunger is not understood, but Scheurink et al (1999) highlight that it could be due to an increase in blood lactate. Stress hormones such as corticotrophin releasing factor (CRF), adrenocorticotropic hormone (ACTH), cortisol and the catecholamines (all secreted in proportion to exercise intensity) could exert an anorexic effect (Borer, 2003). Growth Hormone is also secreted at increased exercise intensities and its releasing peptides, GHRH and ghrelin are associated with the stimulation and suppression of appetite (Borer, 2003). It is posited that the suppression of hunger may be the result of exercise induced changes to gut peptide hormones. The potential effect of exercise in suppressing hunger is not widely promoted because the evidence is conflicting and the causal mechanisms are not clear since the research evidence of how hunger related hormones respond to exercise is limited (O’Connor et al, 2006). Since exercise is a major component of the energy balance equation examining how hunger related hormones respond to exercise is of significant importance.

2.6 Exercise and ghrelin

The effect of acute exercise bouts on total plasma ghrelin concentrations are controversial with studies reporting no changes either during or post-exercise (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007a; Kallio et al, 2001; Kraemer et al, 2004a; Martins et al, 2007; Pomerants et al, 2006; Schmidt et al, 2004; Takano et al, 2005), as well as increases (Christ et al, 2006; Erdmann et al, 2007; Jürimäe et al, 2007b; Sartorio et al, 2008) and decreases (Ghanbari-Niaki, 2006; Kraemer et al,
The following studies have examined the ghrelin response to acute bouts of exercise. All have measured total ghrelin so there is no distinction between the changes in des-acylated and acylated ghrelin.

The first paper documenting the effects of exercise on ghrelin was by Kallio et al. (2001). They examined the ghrelin responses to cycling in 2 males and 7 females with the leucine 7 to proline 7 polymorphism (Leu7/Pro7 genotype) compared with normal pair matched controls (Leu7/Leu7 genotype). The Leu7/Pro7 genotype is associated with high blood lipid concentrations, accelerated atherosclerosis and increased birth weight. It was posited that this is due to increased NPY concentrations found in the Leu7/Pro7 genotype but the mechanisms are unclear. Ghrelin was measured since it has been shown to stimulate NPY and participants cycled since this would stimulate increases in GH that have also been shown to stimulate ghrelin and NPY. The cycling protocol progressed for 8 minutes until a workload of 80% of \( \dot{V}O_2\text{max} \) was reached which was maintained for a further 12 minutes before being reduced to 20% \( \dot{V}O_2\text{max} \) for 10 minutes as a cool down. Plasma ghrelin concentration did not change significantly during the exercise study period in either group and was similar among both groups. Ghrelin concentrations were suppressed initially as a result of a standard meal fed two hours prior to exercise which may have masked an exercise effect highlighting the importance for participants to be fasted in the studies described in this thesis.
Since ghrelin is the endogenous ligand for the GHSR, Dall et al (2002) studied plasma ghrelin concentration during submaximal aerobic exercise in GH deficient adult males studied both with and without GH replacement as well as age matched healthy adults acting as a control. GH deficient males exercised on two occasions. On one occasion GH replacement had been discontinued from the evening before, but on the other occasion they received their evening GH in addition to an intravenous infusion of GH during exercise the following day. The age matched healthy participants exercised on one occasion without GH. Blood samples were taken for 3 hours prior to, during and for 2.75 hours after exercise to measure plasma ghrelin concentration. Exercise consisted of 45 minutes of cycling at 'lactate threshold' (approximately 62% of \( \dot{VO}_2 \max \)). Despite exercise stimulating an increase in GH in healthy participants, plasma ghrelin concentration did not change significantly in any of the three trials before, during or after exercise. GH administration was associated with moderately reduced levels of ghrelin indicating that exercise induced GH release is not mediated via ghrelin.

The studies by Kallio et al (2001) and Dall et al (2002) both used cycling as the mode of exercise. The exercise intensity was high yet there was no reported change in plasma ghrelin. Kraemer et al (2003) therefore examined the ghrelin response to high intensity intermittent running. Six male participants completed an intermittent treadmill running protocol consisting of 10 minutes at 60%, 10 minutes at 75%, 5 minutes at 90% and 2 minutes at 100% of \( \dot{VO}_2 \max \). Blood was sampled at 40 and 10 minutes before exercise, after each exercise intensity and 4 times during a 1 hour recovery. Plasma ghrelin concentration changed significantly over time with a significant reduction from 100% \( \dot{VO}_2 \max \) to both 15 and 45 minutes post exercise.
When ghrelin concentrations were averaged for rest, exercise and recovery phases there was a significant decrease between exercise and recovery concentrations showing that high intensity running leads to a suppression of ghrelin. However the results are speculative since there was no control trial in the study and it is therefore difficult to ascertain whether the difference is the result of exercise or just by chance.

Due to the lack of a control group, a second aerobic exercise study by Kraemer et al (2004a) examined 6 well trained males (mean $\overline{\text{VO}_{2\text{max}}} 61.8 \pm 2.6 \text{ mL·kg}^{-1}\cdot\text{min}^{-1}$) who undertook the same intermittent treadmill run described previously (Kraemer et al, 2003) but who also completed a resting control trial one month later. Blood samples were taken at rest, during and for 1 hour post exercise. Plasma ghrelin concentration during exercise remained stable and was not different from the control trial. This was confirmed by subsequent area under the curve (AUC) analysis. The mean difference between ghrelin during exercise and control was small in that less than 1% of the variance was explained by the trial factor. The stronger research design discredits the findings of Kraemer et al (2003) and shows that high intensity running does not affect plasma ghrelin. However the exercise protocol was intermittent in nature and a continuous bout of running or resistance exercise had not been examined.

Kraemer et al (2004b) examined 9 males who completed two experimental resistance exercise trials. Participants performed the following resistance exercises in order: bench press, leg extension, military press and leg curl for four sets of 12 repetitions at 80% of 10 rep max either concentrically or eccentrically at the same absolute workload. Participants rested for 90 seconds between all sets and exercises. A blood sample was taken at rest, immediately post exercise and 15 minutes thereafter.
Ghrelin decreased significantly during the concentric trial both from pre to immediately post exercise and from the 15 minutes after the completion of exercise. No change was observed in the eccentric trial. This suggests that concentric muscle actions performed during resistance exercise leads to suppressions of ghrelin but again there was no control group. In addition ghrelin had been shown to possess orexigenic properties (Cummings et al, 2001) but it was not determined whether the magnitude of the small reduction in ghrelin concentration that occurred following the concentric trial would alter post exercise hunger or hunger feelings during exercise.

The aim of Schmidt et al's (2004) study was to determine the effect of exercise intensity on plasma ghrelin concentration. Eight healthy young male volunteers underwent treadmill exercise at 50% (10 minutes), 70% (10 minutes) and 90% (10 minutes intermittent i.e. 30 seconds on and 30 seconds off) of VO$_2$ max on different days. Plasma ghrelin concentrations were comparable between the three different exercise days at baseline but were not influenced by exercise at any of the three workloads. Analysis of blood samples at baseline, 10 and 20 minutes during exercise and in the recovery period at 40, 60 and 80 minutes showed no significant change confirming the findings of Kraemer et al (2004a) that treadmill running has no effect on plasma ghrelin. Again there was no resting control group.

Borer et al (2005) have only published preliminary findings but report that two bouts of low intensity walking (40% VO$_2$ max) with a net energy expenditure of 800 kcal prior to meals did not effect hunger ratings in a fed or fasted state and that exercise led to a significantly greater pre-meal plasma ghrelin concentration in the fasted state.
Takano et al (2005) highlight that heavy resistance exercise leads to a significant increase in GH which could potentially stimulate angiogenesis (capillary growth). Cardiac disease patients would benefit from such changes but it is unsuitable to apply heavy resistance loads due to the health risks yet low intensity resistance exercise with vascular occlusion has also been shown to lead to an increases in GH (Takarada et al, 2000). Takano et al (2005) therefore studied the hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with a reduction of muscle blood flow. Eleven untrained men performed bilateral leg extension on two separate occasions with or without the reduction of muscle blood flow using a specially designed belt. Participants performed 30 repetitions at 20% of one repetition maximum, and after a 20-seconds rest, they performed three sets again until exhaustion. Plasma concentrations of ghrelin and GH were measured at baseline, immediately post, 10 and 30 minutes after exercise. As a result of vascular occlusion the arterial flow was reduced to about 30% of the control. Vascular occlusion significantly increased GH but did not alter plasma ghrelin during the exercise. This contradicts the findings of Kraemer et al (2004b) who found a significant suppression of ghrelin as a result of resistance exercise. The different ghrelin response is likely because the exercise intensity of the resistance exercise in the Kraemer et al (2004b) study was much greater. Exercise intensity therefore appears to be a determinant of plasma ghrelin response to resistance exercise.

Zoladz et al (2005) posited that increases in plasma ghrelin concentration should be accompanied by decreases in heart rate during exercise so they compared incremental cycling starting at 30 W and increasing by 30 W every 3 minutes until exhaustion in a fed state with a submaximal incremental test up to 150 W (12 minutes) in a fasted
state. Despite a significant effect of overnight fast leading to a lower heart rate there was no difference pre exercise, during or post exercise ghrelin concentrations.

There is a paucity of data on the effect of meal composition on ghrelin so Christ et al (2006) investigated whether a short-term dietary intervention with high fat and low fat meals affects ghrelin and its modulators GH and insulin before and during aerobic exercise. Eleven healthy, endurance-trained male athletes were investigated twice in a randomized crossover design following two types of diet. The low fat diet comprised 0.5 g fat·kg⁻¹ body weight per day for 2.5 days whereas the high fat diet comprised of 0.5 g fat·kg⁻¹ bodyweight per day for 1 day followed by 3.5 g fat·kg⁻¹ bodyweight per day for 1.5 days. The protein and CHO content was identical. After a standardised carbohydrate snack in the morning, metabolites and hormones were measured before and at regular intervals throughout a 3 hour aerobic exercise test on a cycle ergometer at 50% of $W_{max}$. Diet did not significantly affect GH concentrations during exercise but resulted in a significant increase in ghrelin after low fat compared with high fat diet. In addition pre exercise levels of ghrelin were not influenced by diet modality. These data suggest that acute negative energy balance induced by exercise elicits a hormonal response of ghrelin and the response is modulated by the preceding intake of fat. Since a non-exercise control experiment was not performed it is difficult to separate the effects of exercise and the pre exercise snack but the study demonstrates the importance of dietary control in the days preceding to the main trials in the studies described in this thesis.

The aerobic studies described so far have failed to show suppressions of ghrelin during aerobic exercise. Both cycling and running have been examined at various
intensities. However the exercise protocols have been intermittent in nature and/or of short duration and thus whilst energy expenditures have not been reported these are assumed to be low. There was a need to examine continuous aerobic exercise of a longer duration for a greater energy expenditure. The author was involved in the study published by Burns et al (2007) in which 9 male and 9 female participants completed two 3 hour trials (exercise and control) on separate days in a randomized balanced design after overnight fasts. The exercise trial involved a 1 hour treadmill run at 73.5% of $\dot{V}O_2\text{max}$ followed by 2 hours of rest to determine the effect of a prolonged, continuous high intensity bout of treadmill running as this had not been examined previously. The control trial consisted of 3 hours of rest. Blood samples were collected at 0, 0.5, 1, 1.5, 2, and 3 hours and ghrelin concentrations were determined from plasma. In addition, despite its orexigenic properties, no study had yet to determine any associations between any observed changes in ghrelin and hunger responses so hunger was assessed following blood sampling using a 15-point scale. Hunger scores were significantly lower in the exercise trial compared with the control trial but plasma total ghrelin concentrations did not differ between trials. These findings indicate that treadmill running suppresses hunger but this effect is not mediated by changes in plasma total ghrelin concentration. However Burns et al (2007) comment that exercise may influence ghrelin concentrations in the ARC which cannot be measured and the responses of acylated and des-acyl ghrelin cannot be distinguished as all studies to date have measured total ghrelin.

Ghanbari-Niaki (2006) examined the effects of free weight circuit resistance exercise on ghrelin, plasma glucose and GH. Fourteen volunteer male physical education students completed a single bout of circuit resistance training (10 exercises, three
circuits at 60% of 1 rep max). Blood samples were collected before, immediately after exercise and for 24 hours following the exercise protocol. GH and glucose showed a significant increase immediately after exercise but returned to pre exercise values over time. Plasma ghrelin decreased significantly immediately after the resistance exercise and increased significantly during the 24 hours following the exercise. This supports the work of Kraemer et al (2004b) but contradicts the findings of Takano et al (2005).

The findings have been mixed possibly because exercise intensity is a determinant of the ghrelin response to resistance exercise which warrants further studies to confirm these findings.

Pomerants et al (2006) examined the changes in serum ghrelin concentrations during an acute aerobic cycle ergometer test in 60 boys at different stages of puberty. Boys were divided according to their pubertal status as group I (Tanner stage 1, n = 20), group II (Tanner stages 2 and 3, n = 20) and group 3 (Tanner stages 4 and 5, n = 20). Maximal oxygen consumption and individual ventilatory threshold of the participants were measured directly using increasing loads on a cycle ergometer followed by a second exercise test using a 30 minute constant load exercise on the same ergometer at the level of approximately 95% of the individual ventilatory threshold. Ghrelin was measured before, immediately after and after 30 minutes of recovery. At baseline, prepubertal children had significantly higher values for serum ghrelin compared to groups II and III, but acute exercise had no effect on ghrelin at different pubertal stages.

Previous studies have examined the plasma ghrelin response to cycling and running exercise protocols so Jüirimäe et al (2007a) investigated the effects of a single sculling
exercise performed above and below the individual ‘anaerobic threshold’ on total ghrelin concentration. Nine elite male rowers performed single scull rowing twice, below and above the individual ‘anaerobic threshold’ using a mean of 5 b.min\(^{-1}\). Ghrelin was measured before, immediately post exercise and after 30 minutes of recovery. Plasma ghrelin concentration did not increase significantly in either exercise trial but was approaching significance after 30 minutes of recovery \((P = 0.051)\) when the constant load sculling was performed at the intensity above the individual ‘anaerobic threshold’. Similar to cycling and running, rowing does not significantly effect plasma ghrelin levels and the evidence is accumulating that aerobic exercise that is short duration has no effect on plasma ghrelin regardless of the mode or intensity.

Jürimäe et al (2007b) evaluated the effects of maximal short-term ergometer rowing during a 6000 m all out test. It was hypothesized that maximal ergometer rowing would have an impact on plasma ghrelin as elite rowers present a relatively large body mass values and all extremities and trunk are involved in rowing compared with other endurance sports. Eight healthy well trained males performed a maximal 6000 m rowing ergometer test 2 hours after a standardised meal. Blood samples for the determination of plasma ghrelin were taken at baseline, immediately post exercise and 30 minutes into recovery. Ghrelin was significantly increased immediately after exercise and decreased during the first 30 minutes of recovery. This contradicts the previous studies that have investigated the effects of short term exercise (up to 45 minutes) on ghrelin concentrations after acute submaximal rowing (Jürimäe et al, 2007a), running (Jorgensen, 2003; Schmidt et al 2004), cycling (Dall et al, 2002; Kallio et al, 2001) and maximal running (Kraemer et al, 2004b). The absence of any
significant change may be due to the relatively low energy expenditure considering Christ et al (2006) witnessed an increase after 3 hours of moderate intensity cycling. The response may also differ since participants were more sedentary in the previous studies highlighted and/or the exercise protocols used fewer muscle groups.

Vestergaard et al (2007) looked at systemic ghrelin levels after exercise with and without concomitant GH administration to examine if plasma ghrelin is suppressed by the exercise-induced GH release. Twenty-nine elite athletes were studied after a maximal exercise test that was dependant on their sport. Blood samples were taken at baseline, immediately after the test and at 15, 30, 60, 90 and 120 minutes thereafter. In a double blind, placebo-controlled, parallel study, 32 healthy subjects were randomized to placebo, GH 0.1 IU/kg per day, or GH 0.2 IU/kg per day for 4 weeks. These participants performed a multistage fitness test to assess maximum oxygen uptake at baseline and after 4 weeks. Total serum ghrelin levels were measured before and immediately after exercise and frequently thereafter. The findings of the study show ghrelin levels decrease significantly after acute exercise in elite athletes and healthy participants by 21% and 34% respectively and that 4 weeks of high-dose GH suppresses ghrelin levels supporting the hypothesis that GH feedback inhibits ghrelin secretion. Ghrelin levels decreased significantly for up to 120 minutes after exercise in healthy participants but whether this is of physiological importance is unknown as there was no further analysis of subjective feelings of hunger, energy intake or macronutrient selection.

Erdmann et al (2007) examined the effects of both exercise intensity and duration on hunger sensations, food intake and the plasma ghrelin response. In Group A the effect
of exercise intensity was examined. Participants undertook three experimental trials being resting control, low intensity cycle ergometer exercise for 30 minutes at 50 W with an energy expenditure of 85.6 kcal and high intensity cycle ergometer exercise at 100 W for 30 minutes with an energy expenditure of 171.2 Kcal. During low intensity exercise ghrelin significantly increased compared with control but no differences were found in the high intensity trial. The postprandial ghrelin response to a standardised test meal showed no differences between the three trials. In Group B the effect of exercise duration was examined. Participants undertook 4 experimental trials including a resting control as well as cycling at 50 W for 30, 60 and 120 minutes with energy expenditures of 85.6, 171.2 and 342 kcal respectively. There was no difference in plasma ghrelin during exercise or postprandially between the four trials. The findings of both studies also show that low intensity exercise that is short duration 30-60 minutes has no effect on energy intake or hunger sensations. However a prolongation of exercise to 120 minutes led to an increase in food intake. Since ghrelin levels were increased during the low intensity trial but there was no increase in energy intake it is unlikely that ghrelin mediates changes to hunger as there was no increase in plasma ghrelin in the 120 minute cycling trial yet a subsequent increase in food intake.

Sartorio et al (2008) took two different groups of athletes to examine the ghrelin response to different training bouts. Group A athletes performed a 60-90 minutes training session at approximately 80% of $\dot{V}O_2_{\text{max}}$ on-the-field and ghrelin was measured immediately before and after. Group B athletes performed two consecutive 30 minute cycling sessions at 80% of individual $\dot{V}O_2_{\text{max}}$ at different time intervals between bouts (2 and 6 hours). In male athletes in Group A, ghrelin levels
significantly decreased after the training session while no significant changes were
found in females. In Group B athletes no changes in ghrelin were observed. The
findings show ghrelin responds to prolonged exercise bouts (60-90 minutes) in male
athletes only, while repeated exercise bouts of lower duration (30 minute) does not
change ghrelin concentrations.

Table 2.1 is a summary table of studies examining the acute effects of exercise on
ghrelin.
Table 2.1: Summary of research examining the acute effects of exercise on ghrelin.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Exercise</th>
<th>Results and Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kallio et al (2001)</td>
<td>9 participants with Leu7/Pro7 genotype 2 male 7 female and 9 pair matched controls with Leu7/LeuPro genotype</td>
<td>Cycling 30 min duration  Workload increased by 20% VO2 max every 2 minutes until 80% VO2 max reached at 8 minutes. Worked for further 12 minutes before cooling down at 20% VO2 max for 10 minutes</td>
<td>Ghrelin concentration did not change significantly during the exercise study period from values at -5 minutes in either group and was similar among both groups</td>
</tr>
<tr>
<td>Dall et al (2002)</td>
<td>8 healthy males and 8 hypopituitary males with verified GH deficiency</td>
<td>Cycling 45 minutes duration  Workload equivalent to lactate threshold (62% VO2 max)</td>
<td>Plasma ghrelin levels did not change significantly with time in either trial. No correlations were detected between ghrelin levels and parameters such as GH, age or body composition</td>
</tr>
<tr>
<td>Kraemer et al (2003)</td>
<td>6 males</td>
<td>Running 27 minutes duration  Intermittent treadmill protocol 10 minutes 60% VO2 max, 10 minutes 75% VO2 max, 5 minutes 90% VO2 max, 2 minutes 100% VO2 max</td>
<td>Ghrelin concentration changed significantly over time with a significant reduction from 100% VO2 max to 15 and 45 minutes post exercise. There was a significant difference between exercise and recovery concentrations but no resting control group</td>
</tr>
<tr>
<td>Kraemer et al (2004a)</td>
<td>6 well trained males</td>
<td>Running 27 minutes duration  Intermittent treadmill protocol 10 minutes 60% VO2 max, 10 minutes 75% VO2 max, 5 minutes 90% VO2 max, 2 minutes 100% VO2 max</td>
<td>There was no difference in the in the ghrelin response to the exercise and control trials. Ghrelin concentration during exercise remained stable</td>
</tr>
<tr>
<td>Kraemer et al (2004b)</td>
<td>9 males</td>
<td>Resistance  Bench press, leg extension, military press and leg curl for four sets of 12 repetitions at 80% of 10 rep max either concentrically or eccentrically at the same absolute workload. 90 second rest between sets</td>
<td>Ghrelin decreased significantly during the concentric trial both from pre to immediately post exercise and from the 15 minutes after the completion of exercise. No change was observed in the eccentric trial</td>
</tr>
<tr>
<td>Schmidt et al (2004)</td>
<td>8 young healthy male volunteers</td>
<td>Running 30 minutes duration  Treadmill running at 50% (10 minutes), 70% (10 minutes) and 90% (10 minutes intermittent) VO2 max on different days</td>
<td>Plasma ghrelin concentration remained unchanged at all three workloads</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Preliminary findings:</td>
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<tr>
<td>Borer et al (2005)</td>
<td>10 overweight postmenopausal women</td>
<td>Walking, treadmill walking 40% VO₂ max until a net energy expenditure of 400 Kcal repeated in the afternoon</td>
<td>No effect on hunger in fed or fasted state and exercise led to a significant greater pre-meal plasma ghrelin concentration in the fasted state</td>
</tr>
<tr>
<td>Takano et al (2005)</td>
<td>11 untrained men</td>
<td>Resistance, bilateral leg extension on two separate occasions with or without the reduction of muscle blood flow using a specially designed belt. 30 repetitions at 20% of one repetition maximum, and after 20-seconds rest three sets again until exhaustion</td>
<td>Vascular occlusion did not alter plasma ghrelin during the exercise</td>
</tr>
<tr>
<td>Zoldaz et al (2005)</td>
<td>8 healthy men</td>
<td>Cycling 2 bouts on separate occasions 1) Starting at 30 W and increasing by 30 W every 3 minutes until exhaustion in a fed state 2) a submaximal incremental test up to 150 W (12 minutes) in a fasted state</td>
<td>No difference pre exercise, during or post exercise ghrelin concentrations</td>
</tr>
<tr>
<td>Christ et al (2006)</td>
<td>8 endurance trained males</td>
<td>Cycling, 3 hours at 50% of Wmax</td>
<td>Significant increase in ghrelin</td>
</tr>
<tr>
<td>Ghanbari-Niazi (2006)</td>
<td>14 males</td>
<td>Resistance, circuit resistance training (10 exercises, three circuits at 60% of 1 rep max)</td>
<td>Plasma ghrelin decreased significantly immediately after the resistance exercise and increased significantly during the 24 hours following the exercise</td>
</tr>
<tr>
<td>Pomerants et al (2006)</td>
<td>60 boys at different pubertal stages</td>
<td>Cycling, 30 minute constant load exercise approximately 95% of the individual ventilatory threshold</td>
<td>Acute exercise had no effect on ghrelin at different pubertal stages</td>
</tr>
<tr>
<td>Burns et al (2007)</td>
<td>9 males 9 females</td>
<td>Running, 1 hour at 73.5% VO₂ max</td>
<td>Hunger scores were significantly lower in the exercise trial compared with the control trial but plasma total ghrelin concentrations did not differ between trials</td>
</tr>
<tr>
<td>Jùirimàe et al (2007a)</td>
<td>9 elite male rowers</td>
<td>Rowing, single scull rowing twice, below and above the individual 'anaerobic threshold' using a mean of 5 b.min⁻¹ during a graded exercise test for 30 minutes</td>
<td>Plasma ghrelin concentration did not increase significantly in either exercise trial but was approaching significance after 30 minutes of recovery</td>
</tr>
<tr>
<td>Jùirimàe et al (2007b)</td>
<td>8 elite male rowers</td>
<td>Rowing, 6000m all out test</td>
<td>Ghrelin was significantly increased immediately after exercise and decreased during the first 30 minutes of recovery</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Participants</td>
<td>Exercise Protocol</td>
<td>Results</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Vestergaard et al (2007)</td>
<td>29 elite athletes and 32 healthy controls</td>
<td>Max tests. Elite athletes performed maximal tests that were sport dependant and the controls performed the multi stage fitness test</td>
<td>Ghrelin levels decrease significantly after acute exercise in elite athletes and healthy participants by 21% and 34% respectively. Ghrelin levels decreased significantly for up to 120 minutes after exercise in healthy controls</td>
</tr>
<tr>
<td>Erdmann et al (2007)</td>
<td>Group A: 2 males 5 females</td>
<td>Group A Cycling. 30 minutes at 50 W as well as 30 minutes at 100W</td>
<td>Group A: During low intensity exercise ghrelin significantly increased compared with control but no differences were found in the high intensity trial</td>
</tr>
<tr>
<td></td>
<td>Group B: 2 males 5 females</td>
<td>Group B Cycling. Cycling at 50 W for 30, 60 and 120 minutes with energy expenditures of 85.6, 171.2 and 342 kcal respectively</td>
<td>Group B: There was no difference in plasma ghrelin during exercise or postprandially between the four trials</td>
</tr>
<tr>
<td>Sartorio et al (2008)</td>
<td>Group A: 19 males 18 females</td>
<td>Group A: Training session. 60-90 minutes training at 80% VO$_2$ max</td>
<td>Group B: Cycling. 30 minute cycling 80% VO$_2$ max</td>
</tr>
<tr>
<td></td>
<td>Group B: 4 males</td>
<td></td>
<td>In males ghrelin decreased significantly after training but no significant differences were found in females</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No significant changes in ghrelin</td>
</tr>
</tbody>
</table>

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2.6.2 Effect of repeated bouts (chronic response)

Having examined the ghrelin response to acute bouts of exercise the review now discusses the literature on the ghrelin response to chronic exercise and therefore an accumulation of exercise bouts.

Ravussin et al (2001) measured plasma ghrelin concentration in monozygotic twins. Twelve pairs of monozygotic twins were overfed by 84,000 kcal over a 100-day period whereas another 7 pairs were submitted to a 53,000 kcal negative energy balance induced by exercise over a 93-day period. In response to the overfeeding plasma ghrelin exhibited a non significant decrease and a non significant increase with exercise induced negative energy balance. Results do not therefore support that the physiological variability in ghrelin concentration has an impact on body weight regulation. A limitation of this study though is the small number of participants that actually completed the trials and details of the exercise induced negative energy balance in terms of mode, frequency, duration and intensity were not provided.

Morpurgo et al (2003) identified ghrelin concentration in fasting conditions and after a standard meal test in obese participants before and after a 3 week integrated body weight reduction programme which included an energy restricted diet and exercise training. At baseline, 10 obese participants showed significantly lower ghrelin levels than controls which were not modified by standard meal test whereas a significant reduction was observed in controls. After the 3-week programme, obese participants significantly reduced their weight and BMI, but no significant changes were found in both fasting serum ghrelin and in the ghrelin response to a test meal. Results suggest that weight loss ~5% is not sufficient to normalise fasting ghrelin levels or to restore 44
the normal ghrelin suppression after a meal in obese subjects. It is therefore speculated that a more prolonged period and/or increased weight reduction to achieve a new set point after weight loss is required. The exercise component of the study described above consisted of 30 minutes of cycle ergometer exercise at a constant work load of 60 W eliciting an average heart rate corresponding to 30-45% of $\dot{V}O_{2\text{max}}$ on 5 days per week and outdoor walking for 50-70 minutes at 45-60% of $\dot{V}O_{2\text{max}}$ on 2 days per week. Also, participants undertook 30 minutes of indoor activity 5 days per week consisting of light jogging, dynamic standing and floor exercises. Unfortunately due to the multi faceted nature of the study the individual influence of exercise cannot be determined.

Leidy et al (2004) examined the effects of a 3-month energy deficit-imposing diet and exercise intervention on circulating ghrelin in normal weight, healthy women. Participants were regrouped after completion of the intervention into three groups. One: 7 female controls who performed no exercise and consumed a weight maintenance diet. Two: 5 exercising women who remained weight stable. Three: 10 exercising women who lost weight. Exercise training occurred five times per week at 70-80% of maximum heart rate and participants were fed a controlled diet. No significant differences were observed in plasma ghrelin between groups before the intervention but ghrelin increased significantly over time in the weight loss group compared with the control and weight stable group. In addition changes in ghrelin were negatively correlated with changes in body weight. The findings suggest that ghrelin responds in a compensatory manner to changes in energy homeostasis in healthy young women and highlights that the increase in ghrelin in the weight loss group was in response to the overall energy deficit created by the combination of
reduced food intake and exercise and not due to the endocrine or the metabolic effects of exercise. In addition the study shows that whilst ghrelin appears to be responsive to an energy deficit it is unclear what factors specifically modulate its secretion.

Whilst it was not a major component of the study by Langenberg et al (2005) they measured serum ghrelin in 848 men and 665 women and found that those who exercised at least three times a week had lower levels of serum ghrelin than those who did not. This association was strengthened and became statistically significant after adjustment for BMI.

Foster-Schubert et al (2005) randomised 173 sedentary, overweight, postmenopausal women to an aerobic exercise intervention or stretching control. Plasma ghrelin was measured at baseline, 3 and 12 months. Exercise resulted in weight loss which was associated with a progressive increase in ghrelin concentration in the presence of no significant change in energy intake. Ghrelin concentration increase was also dependant on the amount of weight loss since ghrelin levels increased in participants with more than a 3 kg weight loss but did not change in those without weight loss or when the weight loss was 0.5 – 3 kg. The findings support those of Leidy et al (2004) but it is still unclear as to the mechanisms which lead to an increase in ghrelin concentration and which components of body composition are detected by systems that regulate ghrelin.

Bobbert et al (2005) examined 8 healthy women and 11 healthy men who were training for a marathon and measured the ghrelin response to running a marathon. Ghrelin was measured 6 weeks and 10 days before the marathon 2 days and 10 days
after and then 6 weeks later. Whilst there was a trend towards higher ghrelin levels after the marathon no significant differences were seen. The slightly increased ghrelin levels 2 days after the marathon are likely the result of a negative energy balance as observed in the previous studies (Leidy et al, 2004; Foster-Schubert et al, 2005).

The aim of the study by Garcia et al (2006) was to investigate the relationship of plasma ghrelin concentrations to active weight loss and weight maintenance in obese participants. Obese Mexican-American women matched for age and BMI were randomized to a 12-month weight loss programme or a no intervention control. Interventions included diet, exercise, and orlistat. Fasting ghrelin concentrations were measured at baseline and 6 and 12 months. The weight loss group lost 8.5% of body weight after 6 months and maintained the new weight for the next 6 months. Ghrelin concentrations increased significantly at 6 months but returned to baseline at 12 months. Baseline ghrelin concentrations were directly related to the degree of weight loss achieved after 12 months. Controls experienced no change in BMI or ghrelin levels. Consistent with previous results (Leidy et al, 2004; Foster Schubert et al 2005), ghrelin rises in response to weight loss, perhaps as a counter regulatory mechanism. However, the present results indicate that ghrelin concentrations return to baseline with sustained weight maintenance, suggesting that its effects are unlikely to regulate long-term energy balance.

A later study by Leidy et al (2007) examined whether chronic energy deficiency achieved with caloric restriction combined with exercise is associated with changes in the 24-hour profile of ghrelin. Twelve non-obese, non-exercising women (age 18 to 24 years) were randomly assigned to a non-exercising control group or a diet and
exercise group. Repeated blood sampling over 24 hours to measure ghrelin occurred before and after the study. Significant increases in the following ghrelin features were found in only the deficit group: elevations in baseline, lunch peak, dinner peak, nocturnal rise and nocturnal peak. In addition there was a significantly larger dinner decline. The study is more informative than the previous research in that whilst elevation in ghrelin have been confirmed increases are seen throughout a 24 hour profile.

Mackelvie et al (2007) investigated the effects of exercise on acylated ghrelin and des-acylated ghrelin concentrations and hunger in a tertiary care centre. Seventeen normal-weight and overweight male adolescents participated in 5 consecutive days of aerobic exercise for one hour per day. Pre exercise total and des-acylated ghrelin concentrations were significantly lower in overweight compared with normal weight boys but acylated ghrelin was similar. Exercise did not significantly affect fasting total ghrelin concentrations but there was a differential effect of exercise on both acylated and des-acylated ghrelin at fasting and in response to a test meal. Exercise significantly increased fasting acylated ghrelin and the response to a test meal 30, 60 and 240 minutes later and this increase was greater in normal weight compared with overweight adolescents. In addition higher acylated ghrelin concentrations were correlated with an increase in markers of hunger. In normal weight boys exercise caused a decrease in des-acylated ghrelin concentrations but increased in overweight boys. Exercise differentially affects acylated and des-acylated ghrelin in normal weight and overweight male adolescents demonstrating the importance of measuring both types of ghrelin in future studies.
2.6.3 Exercise and ghrelin summary

When examining the ghrelin response to exercise, studies indicate that a single session of aerobic exercise has no influence on ghrelin concentrations (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007a; Kallio et al, 2001; Kraemer et al, 2004a, Martins et al, 2007, Schmidt et al, 2004; Zoladz, 2005) decreases (Kraemer et al, 2003; Vestergaard, 2007) increases (Christ et al, 2006; Jürimäe et al, 2007b) or the findings are mixed depending on the duration and intensity of exercise (Erdmann et al, 2007). With resistance exercise the findings are mixed with two studies showing a decrease in plasma ghrelin concentration (Kraemer et al, 2004b; Ghanbari-Niaki, 2006) and one showing no change (Takano et al, 2005).

Research designs need improving as there is a tendency not to include a resting control trial and other metabolites that may play a role in the ghrelin responses to exercise such as glucose or insulin have not been measured in all studies. Previous studies finding no significant effect of acute exercise on total plasma ghrelin concentration have predominantly used cycling as the mode of exercise and the acute bouts have mostly been short in duration and/or intermittent in nature so there is a need to examine longer duration exercise protocols of varying mode. However, the main limitation of research concerning exercise and ghrelin is that the studies reported have measured total ghrelin, but acylation of ghrelin is thought to be essential for ghrelin to bind to the GHSR and to cross the blood brain barrier (Kojima et al, 1999; Murphy et al, 2006). In humans des-acylated ghrelin does not possess the pituitary and pancreatic activity of acylated ghrelin (Broglio et al, 2003). Since acylated ghrelin is less stable than total ghrelin (Hosoda et al, 2004) this creates practical difficulties in sample collection and processing. This may therefore be why to the author's
knowledge no published study has examined the effects of an acute bout of exercise on acylated ghrelin.

Researchers have examined the role of ghrelin in hunger, energy balance and its relation to disorders including obesity in humans, because by suppressing ghrelin there is potential to suppress hunger and/or prevent a person from overeating. However, it may not be possible to cure obesity through the actions of ghrelin antagonists because plasma ghrelin concentrations have been shown to be decreased in obese individuals (Tschop et al, 2001b). Ghrelin antagonists could however play a role in prevention because a subset of obesity could be due to increased production or enhanced signalling through the GHSR pathway (Kojima et al, 2001). The development of a pharmacologic ghrelin antagonist is possible, but the blockade of GH secretion by a GHSR antagonist could cause problems. As well as promoting growth, GH has other important metabolic actions and the anti-obesity effects of a pharmacologic ghrelin antagonist might therefore be offset by impaired secretion of growth hormone resulting in negative mood and poor quality of life (Pinkney & Williams, 2002). Until effective, non-harmful pharmacological ghrelin antagonists become available, exercise could be an important intervention for preventing obesity because of its potential action on plasma acylated ghrelin concentration without having a negative impact on the actions of GH.

2.7 Exercise and PYY

2.7.1 Effect of a single bout of exercise (acute response)

To the authors knowledge only one study has examined the acute effect of exercise on PYY. Martins et al (2007) investigated the acute effects of exercise on the
postprandial levels of hunger-related hormones and metabolites, energy intake and subjective measures of hunger. PYY and ghrelin were measured in the fasting state and postprandially in 12 healthy, normal-weight volunteers (six males and six females) using a randomised crossover design. One hour after a standardised breakfast, participants either cycled for 60 minutes at 65% of their maximal heart rate or rested. Subjective hunger was assessed throughout the study using visual analogue scales and subsequent energy intake at a buffet meal was measured at the end (3 hours post-breakfast and 1 hour post-exercise). Exercise significantly increased mean PYY yet no significant effect of exercise was observed on postprandial levels of ghrelin. During the exercise period, hunger scores were significantly decreased, however, this effect disappeared in the post-exercise period. Exercise significantly increased subsequent absolute energy intake, but produced a significant decrease in relative energy intake after accounting for the energy expended during exercise. The study demonstrates that acute exercise, of moderate intensity, temporarily decreased hunger sensations and was able to produce a short-term negative energy balance. This impact on hunger and subsequent energy homeostasis was not explained by changes in postprandial levels of ghrelin however, 'exercise-induced anorexia' may potentially be linked to increased PYY.

2.7.2 Effect of repeated bouts (chronic response)

To the authors knowledge no published study has examined the effect of repeated bouts of exercise on PYY.
2.7.3 Exercise and PYY summary

It is clear that there is a paucity of data on the effects of exercise on PYY. The author felt it necessary to include the measurement of PYY in chapter 8 to confirm the findings of Martins et al (2007) and examine the effects of resistance exercise.

2.8 Summary

The rise in overweight and obesity has prompted researchers to critically examine the physiological mechanisms involved in the regulation of short and long term energy balance. The potential effect of exercise in suppressing hunger is not widely promoted because the evidence is conflicting and the causal mechanisms are not clear since the research evidence of how appetite related hormones respond to exercise is limited (O'Connor et al, 2006). Despite a large body of research literature on the gut peptide hormones ghrelin and PYY, there is still a paucity of data on the effects of acute exercise on these hormones. To the authors knowledge no studies have examined the effect of prolonged continuous bouts of treadmill running or resistance exercise on plasma acylated ghrelin or total PYY concentrations. Since exercise is a major component of the energy balance equation, examining how hunger related hormones respond to exercise is of significant importance and therefore measuring the response of acylated ghrelin and total PYY to different modes, intensities and durations of exercise is the focus of this thesis.
CHAPTER III

General Methods

The following chapter describes the experimental procedures employed in the studies described in this thesis as many are common to each. All studies were conducted with the approval of Loughborough University’s Ethical Advisory committee (Appendix A).

3.1 Participants

For each of the studies described in this thesis, participants were recruited from within Loughborough University and the local area by word of mouth and/or poster advertising. Volunteers were given written information about the studies including the purpose of the studies and the potential risks and discomforts (Appendix B). After an opportunity to ask questions regarding the studies, volunteers signed a statement of informed consent (Appendix C) and then each underwent a confidential health screening process using a health screen questionnaire (Appendix D). Participants also completed a physical activity questionnaire (Appendix E) which examined their physical activity levels. Most of the volunteers were sports science students studying at Loughborough University. All participants were physically active and most were involved in competitive sports such as soccer, rugby, tennis and hockey. Prior training status was not a prerequisite for any of the studies, but the physical demands of the studies ensured that all of the participants were reasonably fit.
To minimise risks participants were only recruited if they met the following criteria:

- Were non-smoking
- Were normally active
- Were free of any known cardiovascular disease or abnormalities, acute illness or active chronic systematic disease
- Were not taking any medication known to influence lipid or carbohydrate metabolism
- Had no orthopaedic or muscular contraindications to treadmill running or resistance exercise
- Had no known dyslipidaemia
- Had non-extreme dietary habits and were weight stable in the last 6 months
- Had resting arterial blood pressure <140/90 mmHg
- Had a BMI <30 kg·m⁻²
- Not undertaking shift work at night

3.2 Anthropometry

Anthropometric measurements were conducted in all studies described in this thesis as follows:

3.2.1 Height

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca, Hamburg, Germany). Participants stood barefoot with their heels together against a
wooden back plate. Participants kept their arms loosely by their side. The head was placed in the Frankfort Plane i.e. a horizontal line between the lower orbits of the eyes and the external auditory meatus. The stadiometer head plate was lowered onto the top of the head and then the height was recorded.

3.2.2 Weight

Weight was measured to the nearest 0.01 kg using a balance beam scale (Avery, Birmingham, U.K.). Participants wore light clothing, removed their shoes and jewellery and were told to remove anything from their pockets while being weighed.

3.2.3 Body mass index

Body mass index was calculated as weight in kilograms divided by the square of height in meters.

3.2.4 Waist circumference

Waist circumference was measured with an inelastic polyfibre tape measure (Hoechstmass Balzer GmbH, Sulzbach, Germany) placed directly on the skin while the participant stood balanced on both feet. The measurement was taken at the end of the expiration and was determined as the widest part of the torso between the xiphoid process of the sternum and the iliac crest.
3.2.5 Skinfold measurements

Measurements of subcutaneous fat were performed to estimate total body fatness. Skinfold thickness was measured using callipers (John Bull, British Indicators, West Sussex, U.K.). The measurements were taken at the following sites at the right hand side of the body with the subject standing:

1) Tricep - Vertical fold, on the posterior midline of the upper arm, halfway between the acromion and olecranon processes, with the arm held freely to the side of the body.

2) Bicep - Vertical fold, of the anterior aspect of the arm over the belly of the bicep muscle, 1 cm above the level used to mark the tricep site.

3) Subscapular - Diagonal fold (45° angle), 1 to 2 cm below the inferior angle of the scapula.

4) Suprailiac - Diagonal fold, in line with the natural angle of the iliac crest taken in the anterior axillary line immediately superior to the iliac crest.

Each skinfold was lifted by the experimenter's left hand thumb and index finger. The skinfold callipers were placed 1 cm above the site of measurement. The measurement was taken after 1-2 seconds of calliper pressure while still pinching the skinfold. Each site was measured in triplicate. The skin was allowed to regain its normal texture and thickness between measurements and the average of the three measurements for each site were calculated and used to represent the skinfold thickness for that site. The sum of the skinfolds was used to calculate body density using the predictive equations of Durnin and Womersley (1974) then body fat percentage was estimated using the Siri equation (Siri, 1956).
3.3 Heart rate measurement

Heart rate was measured during preliminary exercise tests and main trials (Chapters 4, 5, 6, 7, 8) using short range telemetry (Polar A3, Polar Electro, Kempele, Finland).

3.4 Ratings of perceived exertion

Ratings of perceived exertion (RPE) were used to obtain each individual’s perception of exercise intensity during preliminary exercise tests and main trials (Chapters 4, 5, 6, 7, 8) using the Borg Scale (Borg, 1973), with numbers ranging from 6 (no exertion at all) to 20 (maximal exertion).

3.5 Arterial blood pressure measurement

Arterial blood pressure was measured during health screening by auscultation using a random-zero sphygmomanometer (Hawksley MK. II, Hawksley and Sons Ltd, Sussex, U.K.) according to the guidelines of the British Hypertension Society (Ramsay et al, 1999; Williams et al, 2004). Participants were seated on a chair for 5 minutes before measurement. Two measurements were taken and the mean of these values recorded.

3.6 Exercise tests

For all studies participants completed aerobic exercise on a motorized treadmill (RUNRACE, Technogym, Gambettola, Italy). All participants were familiarised with treadmill running prior to testing.
3.6.1 Submaximal-incremental treadmill running test

Participants completed a submaximal-incremental treadmill running test in each study to determine the relationship between running speed and oxygen consumption. The test was designed to exercise participants through a range of intensities from moderate to vigorous, but not maximum. The test was 16-minutes in duration and was continuous in nature but was divided into four, four minute stages. The initial running speed was set between 6 and 8 km/h depending upon each subject’s fitness level. Treadmill speed was increased by 1 or 1.5 km/h at the end of each four min period depending upon each subject’s fitness level. Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) for the final minute of each four minute stage for the determination of oxygen consumption and carbon dioxide production. Four minute stages were used to ensure that subjects were in steady state during expired air collection periods. Heart rate was monitored continuously throughout the test using short-range telemetry (Polar A3, Kempele, Finland). Ratings of perceived exertion (Borg, 1973) were assessed simultaneously with the expired air collections during the tests. At the end of the test the oxygen consumption at each stage was plotted against the running speed at each stage to illustrate the submaximal running speed-oxygen consumption relationship.

Figure 3.1 shows a schematic representation of the submaximal-incremental treadmill running test protocol.
Expired air sample, heart rate and RPE.

Figure 3.1 Schematic representation of the submaximal-incremental treadmill running test protocol.

3.6.2 Maximum oxygen uptake test

Participants were given 20 to 30 minutes to recover from the submaximal treadmill test before they embarked on the maximum oxygen uptake test. Maximum oxygen uptake was measured directly using an incremental uphill protocol at a constant speed until the participants reached volitional fatigue (Taylor, 1955). The test was designed so that participants would reach volitional fatigue within 10 to 12 minutes as recommended by Taylor et al. (1955). The initial incline of the treadmill was set at 3.5%. Thereafter, treadmill gradient was increased by 2.5% every 3 minutes. Thus, treadmill incline was 3.5% during minutes 0 to 3, 6% during minute 4 to 6, 8.5% during minute 7 to 9 and 11% during minute 10 to 12. Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) between 1:45 and 2:45 minutes of each three minute stage and during the final minute of the test which occurred when participants
signaled that they could only continue for one more minute. Heart rate was monitored throughout these tests using short-range telemetry (Polar A3, Kempele, Finland). Ratings of perceived exertion (Borg, 1973) were assessed simultaneously with the expired air collections during each test. Strong verbal encouragement was given to participants throughout the test.

At the end of the maximum oxygen uptake test oxygen consumption and carbon dioxide production were determined from each expired air sample and the highest value was accepted as the maximum oxygen uptake. Criteria used to confirm a true maximum value included two or more of the following: 1) heart rate within ± 10 b·min⁻¹ of age-predicted maximum heart rate, 2) a respiratory exchange ratio value ≥ 1.15, 3) a plateau in oxygen consumption.

Once maximum oxygen uptake was determined the oxygen consumption (mL·kg⁻¹·min⁻¹) required to elicit the desired percentage of maximum oxygen uptake for each of the treadmill runs could be calculated. This value was used together with the data obtained in the submaximal-incremental test to estimate the running speed required to elicit the desired percentage of maximum oxygen uptake. This running speed was used but was adjusted if necessary i.e. if participants could not maintain the desired intensity and/or to account for cardiovascular drift.

Figure 3.2 shows a schematic representation of the maximal oxygen uptake test protocol.
Expired air sample, heart rate and RPE.

\( \dot{V}O_2 \) max collection, which could occur at any point throughout the

Figure 3.2 Schematic representation of the maximal oxygen uptake test protocol.

3.7 Analysis of expired air samples

Expired air samples were collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, U.K.). Oxygen consumption and carbon dioxide production were determined from these expired air samples using a paramagnetic oxygen analyzer and an infra-red carbon dioxide analyzer respectively (Series 1400; Servomex, Crowborough, East Sussex, U.K.). These analyzers were calibrated prior to analysis using gases of known concentration. Expired air volumes were measured using a dry gas meter (Harvard
Apparatus, Edenbridge, Kent, U.K.) and corrected to standard temperature and pressure (dry). The dry gas meter was calibrated regularly using a 3 litre calibration syringe (Series 5530, Hans Rudolph Inc, Kansas City, Missouri, USA).

3.8 Calculation of energy expenditure

For the aerobic trials oxygen consumption and carbon dioxide production values were used to calculate energy expenditure using indirect calorimetry (Frayn, 1983). Frayn (1983) notes that values of carbohydrate and fat oxidation calculated from standard formulas do not give true oxidation rates in the presence of metabolic processes such as gluconeogenesis or lipogenesis. In normal individuals it is reasonable to assume that rates of gluconeogenesis and lipogenesis are low, in which case standard formulas give almost true oxidation rates.

3.9 Dietary and exercise control

For one day prior to the first main trial in chapters 4, 5 and 6 participants weighed and recorded their food intake. Participants then replicated this food intake during the days prior to the one remaining trial (Chapter 4 and 5) or two remaining trials (Chapter 6). For more rigorous dietary control and after participant feedback that this would not be too inconvenient, participants weighed and recorded their food intake for two days prior to the first main trial in chapters 7 and 8 then replicated this food intake during the days prior to the two remaining trials.
Participants were asked to remain inactive and to avoid caffeine consumption and alcohol consumption in the 24 hours prior to each main trial for all experimental studies. On the mornings of the main trials, participants arrived at the laboratory having fasted for a minimum of 10 hours (no food or drink except water). Sleep was not controlled for.

3.10 Test meals

In all of the experimental studies described in this thesis, participants consumed test meals. The timing, number, composition and energy content of the meals varied and therefore the test meals will be described in each experimental study chapter.

3.11 Assessment of hunger

In each study described in this thesis, throughout each main trial participants rated how hungry they felt using a 16-point ratings of perceived hunger scale (RPH) (Appendix F) that ranged from 0 ‘Not Hungry’ to 15 ‘Very Hungry.’ In addition a previously validated visual analogue scale (VAS) (Flint et al, 2000) (Appendix F) was also used. To avoid bias all VAS were measured by independent researchers not associated with the studies and the data provided.

3.12 Environmental temperature and humidity

Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.) in chapters 5, 6, 7 and 8.
3.13 Blood sample collection

Prior to the start of all of the aerobic exercise and control trials, participants rested in a semi-supine position while a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 4.9 or 9 mL potassium-ethlenediamine tetra-acetic acid (EDTA)-coated monovettes (Sarstedt, Leicester, U.K.) via a multi-adapter (Sarstedt, Leicester, U.K.). Patency of the cannula was maintained by flushing with a small amount of non-heparinised saline (0.9% w/v Sodium Chloride, Baxter Healthcare Ltd, Norfolk, U.K.) after each collection. The saline waste remaining in the connector tube after flushing was drawn off with a 2 mL syringe immediately before the next blood sample was collected. All blood samples were obtained with participants resting in a semi-supine position for 5 minutes prior to collection except for samples collected during the aerobic exercise trials, where blood was collected while the participants straddled the treadmill. This took approximately 1 minute. For the resistance trial (Chapter 8), participants were venepunctured at 09:00 and at 09:45 since it was considered unwise for participants to undertake resistance exercise with a cannula inserted. At 10:30 a cannula was inserted and the remaining samples were extracted via this method as previously described.

Blood collected into the EDTA monovettes were immediately spun at 4000 revolutions per minute (1681 g) for 10 minutes in a refrigerated centrifuge (Koolspin, Burkard, Hertfordshire, U.K.) at 4°C. The plasma supernatant of no less than 0.5 mL (to minimize any freeze drying effect) was then aliquoted into Eppendorf tubes (Sarstedt, Leicester,
These were stored at -80°C for analysis of glucose and insulin (and total PYY in chapter 8) at a later date.

Separate venous blood samples were drawn into 4.9 mL monovettes for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 3500 revolutions per minute (1287 g) for 10 minutes in a refrigerated centrifuge (GS-15R Centrifuge, Beckman Coulter, Fullerton, U.S.A.) at 4°C. The supernatants were then aliquoted into storage tubes and 100 μL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 3500 revolutions per minute (1287 g) for 5 minutes in a refrigerated centrifuge (GS-15R Centrifuge, Beckman Coulter, Fullerton, U.S.A.) at 4°C before being transferred into Eppendorf tubes (Sarstedt, Leicester, U.K.). The samples were then stored at -80°C for analysis later as soon as possible as storage in warm environments is not recommended (Espelund et al, 2003; Groschl et al, 2002).

At each acylated ghrelin blood sampling point, duplicate 20 μL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and triplicate blood samples were collected into heparinised micro haematocrit tubes for the determination of haematocrit.
3.14 Blood Sample Analysis

3.14.1 Estimation of changes in plasma volume

Haemoglobin concentration and haematocrit were used to calculate changes in plasma volume (Dill and Costill, 1974). Haemoglobin concentration was assayed in duplicate by a cyanmethaemoglobin method using an ultraviolet-visible spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, England). Haematocrit was determined in triplicate using a microlitre-haematocrit centrifuge (MIKRO, 20, Andreas Hettich GmbH and Co.KG, Tutlingen, Germany).

3.14.2 Glucose

Plasma concentrations of glucose were determined by spectrophotometric assay using a commercially available kit (Randox Laboratories Ltd., County Antrim, U.K.) with the aid of an automated centrifugal analyzer (Cobas Mira Plus; Roche, Basel, Switzerland).

3.14.3 Insulin

For chapters 5 and 6, plasma insulin concentrations were determined by a solid-phase $^{125}$I radioimmunoassay available in a commercial kit (MP Biomedicals, Orangeburg, NY, U.S.A.) using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL, U.S.A.).

For chapters 7 and 8 plasma insulin concentrations were determined by enzyme-linked immuno sorbent assay using a commercially available kit (Mercodia, Sylveniusgatan,
Uppsala, Sweden) using a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria) to measure absorbance.

3.14.4 Acylated ghrelin

Plasma acylated ghrelin concentrations were determined by enzyme-linked immuno sorbent assay using a commercially available kit (SPI BIO, Montigny le Bretonneux, France supplied by Immuno Diagnostic Systems (IDS) using a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria) to measure absorbance.

3.14.5 Total PYY

Total PYY concentrations were determined by enzyme-linked immuno sorbent assay using a commercially available kit (Diagnostic System Laboratories, Texas, U.S.A.) using a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria) to measure absorbance.

3.14.6 Precision of analysis

To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 4.5%, total PYY 1.2%, glucose 1.8% and insulin (radioimmunoassay 8.2%; enzyme-linked immuno sorbent assay 4.0%).
3.15 Statistical analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.A.). Plasma acylated ghrelin, total PYY, hunger, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. One-way ANOVA was used to assess differences between fasting and area under the curve values for acylated ghrelin, total PYY, hunger, glucose and insulin. Repeated measures, two-factor ANOVA was used to examine differences between trials over time. Where appropriate, post-hoc pair wise comparisons were performed using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. Results are given as mean ± SEM unless otherwise stated. When a study has three trials, for graph clarity, error bars for one of the trials will not be shown.

3.16 Feeding pilot study

Due to the recent development of the kit by SPI BIO to measure acylated ghrelin, it was felt that a feeding pilot study was needed. The author collaborated with the kits provider (IDS) to make this possible. This served two purposes:

1) Confirm the acylated ghrelin response to feeding
2) Assess the kits reliability and validity
Procedures for the assay have been described previously. Despite the number of participants being low \((n = 5)\) statistical analysis has been performed to provide evidence statements.

3.16.1 Participants

Five healthy Caucasian males mean (±SEM) age (26.4 ± 1.5 years), weight (77.2 ± 3.2 kg), Body Mass Index (25.2 ± 1.2 kg·m\(^{-2}\)), body fat (15.2 ± 2.9 %), volunteered to take part in the study.

3.16.2 Main trials

After standardising exercise and feeding and fasting overnight, participants undertook a feeding trial and a control trial on a separate occasion, in a random, crossover design with an interval of at least 7 days. For the feeding trial participants consumed a mixed meal at baseline and then rested for 3 hours, but were allowed to participate in sedentary behaviour which included reading and watching television. For the control trial participants undertook exactly the same protocol but were not fed.

3.16.3 Test meal

For the feeding trial participants consumed a 4186 kJ (1000 kcal) test meal at baseline consisting of 95g of white bread, 90g of tuna, 15g of mayonnaise, 25g of potato crisps, a 52g chocolate bar, 80g of green apple and 100g of orange juice based on a 70 kg person. The macronutrient content was 56% carbohydrate (149g), 15% protein (37g) and 29% fat (33g). The amount of each food stuff was adjusted for each participant based on their
bodyweight on the day of first trial and identical amounts were consumed on the second trial. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times.

Water was available *ad libitum* during both trials and the volume ingested and time of presentation was recorded.

### 3.16.4 Blood sampling

Venous blood samples were drawn from a cannula at baseline, 0.5, 1, 1.5, 2 and 3 hours as described in section 3.13 (page 51)

### 3.16.5 Blood analysis

Plasma samples were analysed for acylated ghrelin in duplicate by enzyme immuno sorbent assay as described in section 3.14.4 (page 54). Two plates were completed.

### 3.16.6 Results

#### 3.16.6.1 Acylated ghrelin assay coefficient of variation

Table 3.1 shows the coefficient of variation (CV) of the standards for plate 1.

Table 3.2 shows the coefficient of variation (CV) of the standards for plate 2.
Table 3.1  Acylated ghrelin standards percentage coefficient of variation (CV) for plate 1.

<table>
<thead>
<tr>
<th>Standards (pg·mL⁻¹)</th>
<th>Absorbence 1</th>
<th>Absorbence 2</th>
<th>Absorbence 3</th>
<th>Mean Absorbence</th>
<th>SD</th>
<th>CV  (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (250)</td>
<td>2.308</td>
<td>2.313</td>
<td>2.321</td>
<td>2.314</td>
<td>0.006</td>
<td>0.280</td>
</tr>
<tr>
<td>2 (125)</td>
<td>1.693</td>
<td>1.716</td>
<td>1.608</td>
<td>1.672</td>
<td>0.057</td>
<td>3.400</td>
</tr>
<tr>
<td>3 (62.5)</td>
<td>0.785</td>
<td>0.774</td>
<td>0.782</td>
<td>0.780</td>
<td>0.006</td>
<td>0.778</td>
</tr>
<tr>
<td>4 (31.3)</td>
<td>0.379</td>
<td>0.402</td>
<td>0.394</td>
<td>0.392</td>
<td>0.012</td>
<td>2.957</td>
</tr>
<tr>
<td>5 (15.6)</td>
<td>0.201</td>
<td>0.234</td>
<td>0.204</td>
<td>0.213</td>
<td>0.018</td>
<td>8.648</td>
</tr>
<tr>
<td>6 (7.81)</td>
<td>0.100</td>
<td>0.099</td>
<td>0.075</td>
<td>0.091</td>
<td>0.014</td>
<td>15.442</td>
</tr>
<tr>
<td>7 (3.91)</td>
<td>0.067</td>
<td>0.06</td>
<td>0.061</td>
<td>0.063</td>
<td>0.004</td>
<td>5.697</td>
</tr>
<tr>
<td>8 (1.96)</td>
<td>0.061</td>
<td>0.029</td>
<td>0.035</td>
<td>0.042</td>
<td>0.017</td>
<td>41.172</td>
</tr>
</tbody>
</table>

Table 3.2  Acylated ghrelin standards percentage coefficient of variation (CV) for plate 2.

<table>
<thead>
<tr>
<th>Standards (pg·mL⁻¹)</th>
<th>Absorbence 1</th>
<th>Absorbence 2</th>
<th>Absorbence 3</th>
<th>Mean Absorbence</th>
<th>SD</th>
<th>CV  (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (250)</td>
<td>2.345</td>
<td>2.344</td>
<td>2.339</td>
<td>2.343</td>
<td>0.003</td>
<td>0.139</td>
</tr>
<tr>
<td>2 (125)</td>
<td>1.951</td>
<td>1.999</td>
<td>1.858</td>
<td>1.936</td>
<td>0.072</td>
<td>3.705</td>
</tr>
<tr>
<td>3 (62.5)</td>
<td>0.944</td>
<td>0.974</td>
<td>0.984</td>
<td>0.967</td>
<td>0.021</td>
<td>2.165</td>
</tr>
<tr>
<td>4 (31.3)</td>
<td>0.449</td>
<td>0.488</td>
<td>0.488</td>
<td>0.475</td>
<td>0.023</td>
<td>4.805</td>
</tr>
<tr>
<td>5 (15.6)</td>
<td>0.242</td>
<td>0.217</td>
<td>0.219</td>
<td>0.226</td>
<td>0.014</td>
<td>6.232</td>
</tr>
<tr>
<td>6 (7.81)</td>
<td>0.108</td>
<td>0.115</td>
<td>0.119</td>
<td>0.114</td>
<td>0.006</td>
<td>5.268</td>
</tr>
<tr>
<td>7 (3.91)</td>
<td>0.07</td>
<td>0.066</td>
<td>0.068</td>
<td>0.068</td>
<td>0.002</td>
<td>2.848</td>
</tr>
<tr>
<td>8 (1.96)</td>
<td>0.038</td>
<td>0.036</td>
<td>0.035</td>
<td>0.036</td>
<td>0.002</td>
<td>4.189</td>
</tr>
</tbody>
</table>

All samples were measured in duplicate and the mean CV was 6.6%.

3.16.6.2 Acylated ghrelin concentrations at baseline

Participants 1 - 4 baseline values for both the control and feeding trial ranged from 45.8 - 107.1 pg·mL⁻¹. However participant 5 displayed exceptionally high values for both the control and feeding trials (869.5 and 796.1 pg·mL⁻¹) respectively. These values remained high for the remainder of the trial, skewing the data. All analyses will include data for 4 (excluding participant 5) and 5 participants.
For 4 participants there was no difference ($P = 0.333$) in baseline acylated ghrelin concentration comparing the control (80.9 ± 12.8 pg·mL$^{-1}$) and feeding (68.9 ± 6 pg·mL$^{-1}$) trial. For 5 subjects there was no difference ($P = 0.174$) in baseline acylated ghrelin concentration comparing the control (238.6 ± 11.4 pg·mL$^{-1}$) and feeding (214.4 ± 5.9 pg·mL$^{-1}$) trial.

3.16.6.3 Acylated ghrelin response to feeding

For 5 participants (Figure 3.3) there was no main effect of trial ($P = 0.140$), no main effect of time ($P = 0.269$) and no trial × time interaction ($P = 0.325$) compared with control. Area under the curve values for acylated ghrelin confirm no difference ($P = 0.187$) when comparing control versus feeding (678.1 ± 29.6 pg·mL$^{-1}$·3h versus 596.0 ± 15.4 pg·mL$^{-1}$·3h respectively).
Figure 3.3  Acylated ghrelin concentration during a feeding and control trial. Values are mean ± SEM, $n = 5$. The black rectangle indicates consumption of the test meal.

Mean fasting plasma ghrelin concentrations (i.e. control trial concentration plus feeding trial concentration divided by two) were not significantly correlated with BMI, body mass, body fat percentage or waist circumference for 5 participants. This was expected due to the low sample size and therefore insufficient power to detect significant correlations.

For 4 participants acylated ghrelin (Figure 3.4) was lower after feeding: main effect of trial ($P = 0.007$), main effect of time ($P = 0.112$), trial × time interaction ($P = 0.054$) compared with control. Area under the curve values for acylated ghrelin confirm a
difference \( (P = 0.005) \) when comparing control versus feeding \((258.7 \pm 33.0 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h} \text{ versus } 126.0 \pm 17.2 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h} \text{ respectively})\).

![Graph of Acylated ghrelin concentration during a feeding and control trial.](image)

**Figure 3.4** Acylated ghrelin concentration during a feeding and control trial. Values are mean ± SEM, \( n = 4 \). The black rectangle indicates consumption of the test meal.

Mean fasting plasma ghrelin concentrations (i.e. control trial concentration plus feeding trial concentration divided by two) were not significantly correlated with BMI, body mass, body fat percentage or waist circumference for 4 participants.
3.16.6.4 Discussion

The aim of the pilot study was to test a recently developed assay and examine the postprandial acylated ghrelin response to a mixed meal compared with control. It was hypothesised that acylated ghrelin concentrations would decrease post feeding and the findings of the study confirmed this was the case.

For plate 1 the 1.96 pg·mL⁻¹ had a very high CV of 41%. This highlighted the need for extreme care whilst pipetting and improvements were seen with the CV's in plate 2. The low coefficient of variation for the majority of the standards demonstrates that the assay is reliable.

All samples were measured in duplicate and a mean CV taken (6.6%). Unfortunately no samples were measured more than twice due to plate allocation, but the within batch coefficient for unknown samples was deemed acceptable compared with CV's published by researchers who were among the first to measure plasma total ghrelin:

Cummings et al (2001): 8.7%
Wren (2001a): 8.9%
Cummings et al (2002): 6.9%

Participant 5 displayed extremely high acylated ghrelin values. High ghrelin values of up to 800 pg·mL⁻¹ have been reported previously by Peeters et al (2005) but this is for total ghrelin. Participant 5 showed acylated ghrelin values ranging from 704.6 – 1015.3
pg·mL$^{-1}$. The reasons for this are unknown but participant 5 was dual heritage so ethnicity could be a factor and why this in turn may lead to hyperghrelinemia is unknown.

Figure 3.2 shows the results for 5 participants in which participant 5's results skew the graph and distorts the statistical findings. It is therefore necessary to remove persons who display atypically high ghrelin levels from the data analysis.

The finding that acylated ghrelin was suppressed post feeding yet increased slightly while fasting in 4 participants was assumed to be valid based on the findings found in the feeding literature on ghrelin (Erdmann et al, 2003; Shiya et al 2002) and acylated ghrelin (Al Awar et al, 2005; Lucidi et al 2004).

Lucidi et al (2004) compared the effects of a mixed meal ingestion (meal study) or of additional 240 min fasting (control study) on plasma concentrations of acylated and total ghrelin in 6 healthy participants. Compared with control, meal intake significantly suppressed (nadir at 90 minutes) acylated and total ghrelin by 38 ± 3 and 40 ± 3% of basal values, respectively.

The research of Al Awar (2005) confirmed the suppression of acylated ghrelin post feeding in a mixed meal in 11 healthy young women of normal body weight, but did not find a suppression with an iso-energetic high fat meal. However, in the high fat intervention the low baseline values of acylated ghrelin compared with the balanced intervention may have masked the effects of the high fat meal. Tentolouris et al (2004)
failed to find suppressed acylated ghrelin after a high fat breakfast in lean females so the
findings of a suppression of acylated ghrelin post high fat meals warrants confirmation.

Having successfully measured a post feeding suppression of acylated ghrelin, studies
measuring the acute effects of exercise on acylated ghrelin were undertaken.
CHAPTER IV

The measurement of hunger during exercise using visual scales

4.1 Introduction

The inter-relationship between exercise and food intake is important because both are contributors to energy balance. Energy expended by exercise may stimulate hunger and increase energy intake to compensate for the energy used, possibly above and beyond that expended leading to positive energy balance (Blundell et al., 2003). However, contrary to widespread belief, there is no short-term (a few hours after exercise) increase in hunger and food intake after exercise since only 19% of intervention studies show an increase in energy intake after exercise, 16% report a decrease whereas 65% report no change (Blundell and King, 1999).

Katch et al (1979) found food consumption and body weight gain was less after low and high intensity exercise compared with control in rodents. Studies in humans have shown that exercise can influence hunger through the immediate suppression of hunger during and post exercise leading to a phenomenon termed ‘exercise induced anorexia’ (Blundell et al., 2003; King et al., 1994; King et al., 1996b; Kissileff et al, 1990). However, the findings are mixed since other studies have reported no effect of exercise on hunger (Borer et al., 2005; Pomerleau et al., 2004; Stubbs et al., 2002a, 2002b).

Kissileff et al (1990) detected the suppression of feelings of hunger immediately following a high intensity bout of cycling but not low intensity cycling in non-obese women and demonstrated that food intake in a single meal is responsive to the effects
of exercise in humans. These findings were confirmed by King et al (1994) who compared a low and high intensity bout of cycling and only reported ‘exercise induced anorexia’ in the high intensity trial. The findings of no ‘exercise induced anorexia’ in response to low intensity exercise were also reported by Borer et al (2005) who found no suppression of hunger in postmenopausal women during or after treadmill walking. The research evidence shows that exercise intensity is an important factor in the determination of ‘exercise induced anorexia’.

Through the authors involvement in a previous study (Burns et al, 2007) problems were identified with the use of visual analogue scales (VAS) that measured hunger sensations published by Flint et al (2000) during exercise. As the participant needs to draw a vertical line on a horizontal scale, this was difficult while in motion during exercise. A number scale ranging from 0 ‘Not hungry’ to 15 ‘Very Hungry’ was therefore developed (See appendix F) so that participants only had to point to show how hungry they felt. There was no opportunity to test the validity of the scale so the main purpose of the present study was to examine whether the number scale was valid by comparing it with the previously published VAS (Flint et al, 2000).

Reviewers of the Burns et al (2007) paper suggested that 'exercise induced anorexia' is not the physiological response of exercise but merely distraction. The study would confirm if 'exercise induced anorexia' is the result of exercise by including a low intensity exercise bout and control trial. During low intensity exercise there is still distraction but any difference in hunger responses would show that there is an exercise intensity effect per se.
4.2 Methods

4.2.1 Participants

Twelve healthy Caucasians (6 males and 6 females) between the ages of 18 and 27 from the population at Loughborough University volunteered to participate in the study. Table 4.1 shows the physical characteristics of participants.

**Table 4.1 Physical characteristics of participants.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>20.7 ± 0.8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.5 ± 2.2</td>
</tr>
<tr>
<td>Sum of Skinfolds (mm)</td>
<td>45.9 ± 3.5</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.6 ± 0.6</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>19.7 ± 1.2</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>75.1 ± 1.4</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{ max} ) (mL·kg⁻¹·min⁻¹)</td>
<td>60.9 ± 2.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 12)

4.2.2 Study design

All experimental procedures were conducted in the Health and Paediatric Exercise Physiology Laboratory at Loughborough University. After being made aware of the protocol, health screened, then giving written consent to take part, anthropometric data was collected after which each participant undertook two preliminary exercise tests as follows: 1) submaximal-incremental treadmill running test, 2) maximum oxygen uptake (\( \dot{V}O_2 \text{ max} \)) treadmill running test. There was a 20 to 30 minute rest interval between the exercise tests.
During subsequent weeks participants undertook a high intensity exercise trial, a low
intensity exercise trial and a non exercise control trial on separate occasions, in a
random, crossover design with an interval of at least 7 days.

The day before the first main trial participants were asked to record their weighed
food intake using a food record diary. The same food intake was then consumed the
day prior to the next remaining trials. Participants were also asked to refrain from any
form of vigorous activity and ingesting caffeine or alcohol 24 hours prior to the main
trial. Participants were fasted for a minimum 10 hours, but were allowed to drink
water *ad libitum*. Participants attended the laboratory between the hours of 08:00 and
09:00 and were requested to use motorised transport where possible or ensure that
activity prior to attending the laboratory was light. There was no control for menstrual
cycle phase among the female participants in this study.

4.2.3 Main trials

For the high and low intensity exercise trials participants ran on a level treadmill for
60 minutes and results obtained from the submaximal-incremental treadmill running
test and $\text{VO}_2\text{max}$ tests were used to predict the running speed required to elicit a
workloads of 75% and 40% $\text{VO}_2\text{max}$. To ensure the speed was correct and adjust for
cardiovascular drift, 60 second expired air samples were taken at 14-15, 29-30, 44-45
and 59-60 minutes throughout the runs and analysed after each collection. Running
speed was adjusted after each expired air collection if the oxygen consumption was
above or below the predicted value or if the participant expressed that they were
experiencing difficulties. On completion of the treadmill run, participants rested for a
further 4 hours and were allowed to participate in sedentary behaviour which included
reading and watching television. For the control trial participants undertook exactly
the same protocol but rested throughout. Participants were not devoid of time cues
during the trials and a clock was on display in the laboratory throughout the trials.
Figure 4.1 shows a schematic representation of the study protocol.
Figure 4.1  Schematic representation of the main trial protocol

Exercise at 75% or 40% $\text{VO}_{2\text{max}}$ or rest

Baseline 1 2 3 4 5

Time (h)

Key:

$\uparrow$ Visual Analogue Scales

$\uparrow$ Samples of Expired Air
4.2.4 Subjective measurement of hunger

On arrival, participants completed a ratings of perceived hunger (RPH) scale developed by Burns et al (2007) which ranged from 0 ‘Not Hungry’ to 15 ‘Very Hungry’. Hunger measurements were recorded at baseline, 0.5, 1, 1.5, 2, 3, 3.5, 4, and 5 hours. At the same sampling points participants also completed a hunger visual analogue scale (VAS) which has been deemed to be reliable and valid (Flint et al, 2000) by placing a vertical mark on a 10 cm horizontal line ranging from 0 ‘Not at all hungry’ to 100 ‘Very Hungry.’ Other visual analogue scales of this type that were completed included fullness, ranging from 0 ‘Not at all full’ to 100 ‘Very full’, desire to eat ranging from 0 ‘Very weak’ to 100 ‘Very Strong’ and how much can you eat ranging from 0 ‘None at all’ to 100 ‘A large amount.’ These scales were then measured by researchers not involved in the study to remove bias.

4.2.5 Test meal

Participants were fed a 4186 kJ (1000 kcal) test meal three hours after baseline measurements for all trials. The meal consisted of 95g of white bread, 90g of tuna, 15g of mayonnaise, 25g of potato crisps, a 52g chocolate bar, 80g of green apple and 100g of orange juice based on a 70 kg person. The macronutrient content was 56% carbohydrate (149g), 15% protein (37g) and 29% fat (33g). The amount of each food stuff was adjusted for each participant based on their bodyweight on the day of their first trial and identical amounts were consumed on the second and third trials. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times. Water was available ad libitum during all trials and the volume ingested and time of presentation was recorded.
4.2.6 Oral temperature

Oral temperature was measured at 0, 0.5, 1, 1.5, 2, 3, 3.5, 4 and 5 hours using a digital thermometer.

4.2.7 Statistical analysis

Data were analysed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.A.). One-way ANOVA was used to assess differences in baseline hunger and weight and fluid intake for the three trials. Repeated measures ANOVA was performed to determine the differences between the effects of trial (high intensity versus low intensity versus control) and time (0, 0.5, 1, 1.5, 2, 3, 3.5, 4 and 5 hours) for hunger, fullness, desire to eat, how much you can eat and oral temperature and to assess whether there was any interaction effect (trial x time). The data for 0-15 hunger scale is discrete, but repeated measures ANOVA was used because there is no non-parametric equivalent. A 3 way repeated measures ANOVA was performed to determine the effect of gender (trial x time x gender). Post-hoc pair wise comparisons were made using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between the number and VAS. Statistical significance was accepted at the 5% level. Results are given as mean ± SEM unless otherwise stated.

4.3 Results

4.3.1 Responses to treadmill running

Table 4.2 shows the responses to treadmill running.
Table 4.2 Responses to treadmill running

<table>
<thead>
<tr>
<th>Exercise Trial</th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Percentage $\dot{V}O_2$ max (%)</strong></td>
<td>37 ± 1.0</td>
<td>71 ± 1.5</td>
</tr>
<tr>
<td><strong>Mean respiratory exchange ratio</strong></td>
<td>0.83 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td><strong>Gross energy expenditure (kJ)</strong></td>
<td>1835 ± 101</td>
<td>3488 ± 234</td>
</tr>
<tr>
<td><strong>Gross energy expenditure (kcal)</strong></td>
<td>438 ± 34</td>
<td>834 ± 56</td>
</tr>
<tr>
<td><strong>Percentage energy contribution from fat (%)</strong></td>
<td>61 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td><strong>Percentage energy contribution from carbohydrate (%)</strong></td>
<td>39 ± 4</td>
<td>71 ± 4</td>
</tr>
<tr>
<td><strong>Average heart rate (b·min⁻¹)</strong></td>
<td>112 ± 3</td>
<td>177 ± 3</td>
</tr>
<tr>
<td><strong>Median RPE</strong></td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td><strong>‘very light’ (range 9-10)</strong></td>
<td><strong>‘hard’ (range 15-16)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 12)

### 4.3.2 Fluid consumption and body mass

There was a difference (One factor ANOVA, $P = 0.002$) in water consumption during the control trial (672 ± 112 mL) compared with the low intensity (800 ± 118 mL) and high intensity (1110 ± 119 mL) exercise trials. Post hoc analysis showed that all were significantly different from each other. Body mass did not differ ($P = 0.262$) between the control, low intensity and high intensity exercise trials at baseline (66.8 ± 2.2 kg versus 66.5 ± 2.2 kg versus 66.4 ± 2.2 kg respectively).

### 4.3.3 Subjective ratings of hunger using the RPH and VAS scales

There was no difference (One factor ANOVA, $P = 0.780$) in baseline hunger between the control, low intensity and high intensity exercise trials (7 ± 1 versus 6 ± 1 versus 7 ± 1 respectively) when using the RPH. No difference was confirmed (One factor
ANOVA, \( P = 0.900 \) in baseline hunger between the control, low intensity and high intensity exercise trials (49 ± 8 versus 47 ± 7 versus 51 ± 6 respectively) when using the VAS scale.

Hunger scores were suppressed as a result of exercise; main effect of trial \( (P = 0.035) \) main effect of time \( (P < 0.0005) \), trial × time interaction \( (P < 0.0005) \) when using the RPH as shown in figure 4.2. Post hoc analysis revealed that differences were approaching significance in the high intensity compared with control \( (P = 0.066) \) but were significant in the high intensity compared with low intensity trials \( (P = 0.002) \). There was no difference in low intensity exercise compared with control.

Similar trends were reported when using the VAS as shown in figure 4.2. Hunger was suppressed as a result of exercise main effect of trial \( (P = 0.012) \) main effect of time \( (P < 0.0005) \), trial × time interaction \( (P < 0.0005) \). Post hoc analysis revealed that differences were reaching significance in the high intensity compared with control \( (P = 0.065) \) but were significant in the high intensity compared with low intensity trials \( (P = 0.035) \). There was no difference in low intensity exercise compared with control.
Subjective feeling of hunger using the RPH and VAS during control, low and high intensity exercise trials. Values are mean ± SEM, n = 12. The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. \(^a\) Control different from low intensity exercise \(P < 0.05\), \(^b\) Control different from high intensity exercise \(P < 0.05\), \(^c\) Low intensity different from high intensity \(P < 0.05\).
4.3.4 Hunger and gender

There was no effect of gender when using the RPH, main effect of trial x gender ($P = 0.617$), time x gender ($P = 0.583$) or interaction ($P = 0.612$) as shown in figure 4.2.

Figure 4.3 Subjective feeling of hunger using RPH during control, low and high intensity exercise trials by gender. Values are mean ± SEM, $n = 6$. The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. $^b$ Control different from high intensity exercise $P < 0.05$, $^c$ Low intensity different from high intensity $P < 0.05$. 
4.3.5 Fullness

Subjective feelings of fullness were higher as a result of exercise, main effect of trial ($P = 0.031$) main effect of time ($P < 0.0005$), and approaching significance for the trial $\times$ time interaction ($P = 0.053$) as shown in figure 4.3. However, post hoc analysis revealed there were only differences between trials at 1 hour.

![Graph showing fullness over time](image)

**Figure 4.4** Subjective feeling of fullness using VAS during control, low and high intensity exercise trials. Values are mean ± SEM, $n = 12$. The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. $^b$ Control different from high intensity exercise $P < 0.05$, $^c$ Low intensity different from high intensity $P < 0.05$.

4.3.6 Desire to eat

Subjective desire to eat was suppressed as a result of exercise main effect of trial ($P = 0.06$) main effect of time ($P < 0.0005$), trial $\times$ time interaction ($P < 0.0005$) as shown in figure 4.4. Post hoc analysis revealed that this was significant in the high intensity compared with control ($P = 0.037$) and in the high intensity compared with low
intensity trials \((P = 0.028)\). There was no difference in low intensity exercise compared with control.

![Graph showing desire to eat during control, low, and high intensity exercise trials.](image)

**Figure 4.5** Subjective desire to eat using a VAS during control, low and high intensity exercise trials. Values are mean ± SEM, \(n = 12\). The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. * Control different from low intensity exercise \(P < 0.05\), † Control different from high intensity exercise \(P < 0.05\), ‡ Low intensity different from high intensity \(P < 0.05\).

4.3.7 How much food

Subjective feelings of how much food could be eaten was suppressed as a result of exercise, main effect of trial \((P = 0.001)\) main effect of time \((P < 0.0005)\), trial \(\times\) time interaction \((P < 0.0005)\) as shown in figure 4.5. Post hoc analysis revealed that this was significant in the high intensity compared with control \((P = 0.009)\) and in the high intensity compared with low intensity trials \((P = 0.017)\). There was no difference in low intensity exercise compared with control.
Figure 4.6  Subjective feelings of how much food could be eaten using a VAS during control, low and high intensity exercise trials. Values are mean ± SEM, n = 12. The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. $^b$ Control different from high intensity exercise $P < 0.05$, $^c$ Low intensity different from high intensity $P < 0.05$.

4.3.8 Oral temperature

For the first hour of the trials oral temperature was lower as a result of exercise, main effect of trial ($P = 0.01$) main effect of time ($P < 0.0005$), trial × time interaction ($P = 0.008$). Post hoc analysis revealed that this was significant in the high intensity compared with control ($P = 0.011$) and in the low intensity compared with control ($P = 0.014$). There was no difference in low intensity exercise compared with high intensity exercise trials.
Figure 4.7  Oral temperature during control, low and high intensity exercise trials. Values are mean ± SEM, n = 12. The patterned rectangle indicates exercise during the exercise trials. The black rectangle represents consumption of the test meal. a Control different from low intensity exercise $P < 0.05$, b Control different from high intensity exercise $P < 0.05$.

4.3.9 Correlations between the RPH and VAS

Results for appetite using the RPH and VAS were correlated at all time points for all trials. All were significant ($P \leq 0.001$) and the $r$ value ranged from 0.806 - 0.967 as shown in table 4.3.
Table 4.3 Correlation coefficients between hunger values using the RPH and VAS.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Trial Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.911</td>
<td>0.825</td>
<td>0.935</td>
</tr>
<tr>
<td>0.5</td>
<td>0.957</td>
<td>0.908</td>
<td>0.875</td>
</tr>
<tr>
<td>1</td>
<td>0.946</td>
<td>0.902</td>
<td>0.930</td>
</tr>
<tr>
<td>1.5</td>
<td>0.956</td>
<td>0.878</td>
<td>0.875</td>
</tr>
<tr>
<td>2</td>
<td>0.967</td>
<td>0.920</td>
<td>0.841</td>
</tr>
<tr>
<td>3</td>
<td>0.908</td>
<td>0.806</td>
<td>0.819</td>
</tr>
<tr>
<td>3.5</td>
<td>0.925</td>
<td>0.947</td>
<td>0.924</td>
</tr>
<tr>
<td>4</td>
<td>0.839</td>
<td>0.875</td>
<td>0.942</td>
</tr>
<tr>
<td>5</td>
<td>0.835</td>
<td>0.940</td>
<td>0.953</td>
</tr>
</tbody>
</table>

(n = 12)

4.4 Discussion

The aim of the study was to confirm the existence of the phenomenon known as 'exercise induced anorexia' as an exercise effect and not merely the result of distraction as well as test the validity of a ratings of perceived hunger scale. It was hypothesised that any reported hunger suppression would be greater in the high intensity exercise trial. The main finding of a significant hunger suppression in the high but not the low intensity exercise trial compared with control shows that that this is the case and exercise intensity is an important determinant of 'exercise induced anorexia'. Both the VAS and RPH show similar trends and correlational analysis confirms that the RPH scale is valid and should be used in the future.

The present findings confirm previous research by Kissileff et al (1990) who detected the suppression of feelings of hunger immediately following a high intensity bout of exercise (cycling at 90W for 40 minutes) but not during a low intensity exercise bout (cycling 30W for 40 minutes). King et al (1994) assessed the differences between high and low intensity cycling but kept total energy expenditure constant. In
comparison with current findings low intensity (30% $\dot{V}O_2_{\text{max}}$) cycling did not produce any 'exercise induced anorexia' whereas high intensity (70% $\dot{V}O_2_{\text{max}}$) cycling did. Further support was provided in a second study which adopted a similar research design except that participants underwent two separate high intensity cycles, one of short duration (mean time = 26 minutes) and the other long duration (mean time = 52 minutes) and a resting control. The gross energy expenditure was significantly different and hunger was suppressed in both protocols, but suppression was greatest after the long duration session.

Similar to the present study, Burns et al (2007) examined the hunger response to a 1 hour high intensity (73.5 ± 0.8% $\dot{V}O_2_{\text{max}}$) treadmill run using the RPH. There was a significant suppression of hunger during and after exercise, but the magnitude of the suppression was less. At 0.5 hours hunger had decreased by a mean of 2 points from baseline and was not suppressed any further by the end of exercise. In the present study hunger decreased by a mean of 4 points at 0.5 hours and 1 more point by the end of exercise. The greater magnitude of suppression in the present study is unknown as males and females were examined in both studies yet the energy expenditure (3747 ± 207 kJ) and intensity (73.5 ± 0.8% $\dot{V}O_2_{\text{max}}$) was greater than that in the present study (3488 ± 234 kJ and 71 ± 1.5% $\dot{V}O_2_{\text{max}}$ respectively). This warrants further investigation.

King et al (1994) have demonstrated in two studies that hunger values were not significantly different between any of the three treatments ~15 minutes after the end of exercise and hunger had therefore begun to return to baseline values soon after the
termination of the exercise session. This contradicts the findings of the present study which showed significant differences at 30 minutes post exercise yet Burns et al (2007) and Thompson et al (1988) report that hunger is suppressed for up to 1 hour post exercise. This is possibly due to the greater energy expenditure but also during treadmill running there is greater gut movement than in stationery ergometer cycling.

Based on the findings of previous research and the outcome of the present study it would seem that there is a threshold of energy expenditure before hunger is suppressed during and post exercise which is influenced by intensity, duration and mode of exercise. Further research is needed to identify this threshold and other modes of exercise such as resistance work needs to be examined.

The reason for using two separate measures to assess hunger is because involvement in a previous study (Burns et al, 2007) identified difficulties in the use of the VAS particularly during exercise. Participants struggled to make a vertical line when running and measuring the scales is a time consuming process ideally being conducted by persons blinded to the hypothesised outcomes of the study to avoid bias. A RPH scale was developed and has been used previously (Burns et al, 2007) because the authors considered it to be more user friendly. The present study provided an opportunity to see whether it compared with previously published reliable and valid scales (Flint et al, 2000) and it is clear from figure 4.1 that trends are similar. Correlational analysis confirms this is the case, since when results for hunger using the number and VAS scale were correlated at all time points for all trials, all were significant \((P \leq 0.001)\) and the \(r\) value ranged from 0.806 - 0.967. In addition and as expected, both scales show a large suppression of hunger post feeding.
There was no difference in subjective feelings of fullness between the trials. This is possibly because the scale does not distinguish between feelings of fullness from food or water and water was available *ad libitum* throughout each trial. The fullness scale will not be used in future studies.

The desire to eat and how much food you can eat scales are other markers of hunger and showed exactly the same responses as the hunger scale for all three trials. As the author felt that enough information was provided by the hunger scales only these would be used in future studies.

In lean male humans, the feeling of hunger is suppressed by intense exercise (King et al, 1994; King et al, 1997; King and Blundell, 1995; Thompson et al, 1988) but there are gender differences. An intense exercise session leads to increased palatability rating of foods in lean women but there is no suppression of hunger (King, 1996a; Pomerleau et al 2004). There is a paucity of data and more studies are needed using female participants. Kissileff et al (1990) reported suppressed hunger in exercising women which contradicts the findings of Pomerleau et al (2004). Whilst the exercise intensities used in the present study were the same as that in Pomerleau et al (2004) study, contrasting results are likely because energy expenditure during the exercise trials was much lower (~1469 ± 46 kJ) compared with that in the present study (~3488 ± 234 kJ). Since the present study has shown that there is no difference between the hunger response to exercise in males and females, conflicting evidence warrants the need for further studies to confirm if this is the case.
During exercise core temperature increases and the extent of this change is greater in high intensity compared with low intensity exercise (Nielsen and Nielsen, 1962). Blundell and King (1999) suggest that changes in core temperature may be a causal mechanism for suppression of hunger and hence its measurement in the present study. A variety of measures were considered for measuring core temperature including rectal and esophageal probes, tympanic membranes and core temperature pills. The author felt that rectal or esophageal probes would discourage people from volunteering, core temperature pills are expensive and during a test run, tympanic membranes were annoying and painful. Although it is not as accurate a predictor of core temperature as the other methods previously highlighted, a digital thermometer was used to measure oral temperature. The results were not as expected showing a significant lowering of oral temperature during exercise. This finding cannot be fully explained, but is possibly due to the drinking of cold water *ad libitum* which occurred more frequently during the exercise trials. Oral temperature was measured at 0.5 and 1 h during exercise which took place on completion of the collection of an expired air sample. Having breathed through a mouthpiece it is commonplace for participants to drink water upon its removal, which may have affected the oral temperature reading and whether participants drank water prior to oral temperature measurements was not controlled for.

The strength of the research design reinforces the validity of the study but there are limitations. As the duration was fixed the extent to which 'exercise induced anorexia' is influenced by exercise intensity independently of energy expenditure cannot be distinguished. The author did not match the energy expenditure because when previous findings of 'exercise induced anorexia' were presented (Burns et al, 2007) it
was questioned whether it is not the physiological influence of exercise but the psychological distraction that causes subjective feelings of hunger suppression. A low intensity exercise trial was therefore included and since findings show that ‘exercise induced anorexia’ only occurred in the high intensity exercise trial, this provides evidence that exercise has the capacity to have a physiological influence and it is not merely psychological distraction. To determine an exercise intensity effect *per se* study 3 (Chapter 6) examined different exercise intensities but matched the energy expenditure.

Due to the large energy deficit caused by exercise it was assumed that hunger would be higher towards the end of the five hour observation period for the remainder of the trial compared with control and greater in high compared with low intensity exercise. It is posited that as there was no short-term increase in hunger there would have been no increased energy intake to compensate for the energy used supporting the findings of (Blundell and King, 1999). However, this cannot be stated with confidence as subsequent energy intake and macronutrient selection was not examined. Subsequent energy intake and macronutrient food selection by the provision of buffet meals is an area of interest and warrants further investigation.

In conclusion, high intensity exercise leads to ‘exercise induced anorexia’ supporting the findings of Burns et al (2007) and King et al (1994). There is a clear physiological role of exercise and suppressed subjective feelings of hunger is not merely the result of distraction. Responses are similar in males and females and exercise intensity is an important determinant of ‘exercise induced anorexia’. In addition ‘exercise induced anorexia’ seems to be influenced by duration, energy expenditure and mode of
exercise. The RPH scale developed by Burns et al (2007) is valid and will continue to be used in future studies. Finally, causal mechanisms for 'exercise induced anorexia' remain unclear and need to be studied.
CHAPTER V

Exercise induced suppression of hunger coincides with decreased acylated ghrelin in humans

5.1 Introduction

Having confirmed ‘exercise induced anorexia’ during and immediately after a bout of high intensity exercise, examining potential causal mechanisms was warranted. The mechanism through which intense exercise suppresses hunger is not understood, but Scheurink et al (1999) highlighted that it could be due to an increase in lactate since hunger and food intake has been shown to decrease in monkeys when lactate is infused in the portal vein. Stress hormones such as corticotrophin releasing factor (CRF), adrenocorticotrophic hormone (ACTH), cortisol and the catecholamines (all secreted in proportion to exercise intensity) could exert an anorexic effect (Borer, 2003). Growth Hormone (GH) is also secreted at increased exercise intensities and its releasing peptides, Growth Hormone Releasing Hormone and ghrelin are associated with the stimulation and suppression of hunger (Borer, 2003).

The potential effect of exercise in suppressing hunger is not widely promoted because the evidence is conflicting and the causal mechanisms are not clear since the research evidence of how hunger related hormones respond to exercise is limited (O'Connor et al, 2006). It is posited that the suppression of hunger may be the result of exercise induced changes to gut peptide hormones.

Ghrelin is a 28-amino acid peptide hormone which stimulates the release of growth hormone from the pituitary (Kojima et al, 1999). Ghrelin is secreted primarily from
cells within the stomach (fundus) but ghrelin is synthesized and secreted in many other tissues including the small intestine, pancreas, hypothalamus, cardiomyocytes, chondrocytes and placenta (Caminos et al 2005; Kojima and Kangawa, 2005). Plasma ghrelin concentrations rise prior to meals and decrease following meals suggesting that ghrelin is orexigenic (appetite stimulating) (Cummings et al, 2005; Cummings et al 2002). This suggestion is supported by the findings that intracerebroventricular ghrelin administration stimulates feeding in rats (Nakazato et al, 2001, Wren et al, 2001a) and intravenous ghrelin infusion increases food intake in humans (Druce et al, 2005, Wren et al, 2001b).

Several studies have investigated the influence of high intensity aerobic exercise bouts on plasma ghrelin concentration (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004a; Schmidt et al, 2004; Vestergaard et al, 2007). Most of these studies indicate that a single session of aerobic exercise has no influence on ghrelin concentrations (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004a; Schmidt et al, 2004) although one study reported that serum ghrelin levels are suppressed for up to an hour after the cessation of exercise (Vestergaard et al, 2007). In contrast, one study has reported that plasma ghrelin concentrations are increased during three hours of moderate intensity exercise but it is uncertain whether this is a true effect of exercise because this study did not include a control trial (Christ et al, 2006). Furthermore, fasting ghrelin concentrations appear to be unaffected by exercise training in the absence of concurrent weight loss (Leidy et al, 2004). This indicates that ghrelin may be a sensor of negative energy balance as suggested previously (Ravussin et al, 2001).
A limitation of research concerning exercise and ghrelin is that all of the studies performed so far have measured total ghrelin (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004a; Schmidt et al, 2004; Vestergaard et al, 2007). Ghrelin exists in des-acylated (non-acylated) and acylated forms with the majority (80 to 90%) being non-acylated (Ghigo et al, 2005; Hosoda et al, 2004). Acylation is thought to be essential for ghrelin to bind to the growth-hormone-secretagogue receptor and to cross the blood brain barrier (Kojima et al, 1999; Murphy and Bloom, 2006). In humans des-acylated ghrelin does not possess the pituitary and pancreatic activity of acylated ghrelin (Broglio et al, 2003). Therefore, des-acylated ghrelin is not considered to be important for appetite regulation although it may have other functions such as inhibiting cell proliferation (Cassoni et al, 2001) and stimulating adipogenesis (Thompson et al, 2004).

Relatively few studies have measured acylated ghrelin. Acylated ghrelin is less stable than total ghrelin (Hosoda et al, 2004) creating practical difficulties in sample collection and processing. Due to its short half life the sample needs to be processed immediately placing extra demands on resources. Two studies have shown that acylated ghrelin is suppressed after mixed meals providing evidence that macronutrient composition may affect the extent of this suppression (Al Alwar et al, 2005; El Khoury et al, 2006). Three studies have simultaneously measured acylated and total ghrelin responses to food consumption (Blom et al, 2006; Lucidi et al, 2004; Hosoda et al, 2004). These studies have confirmed that acylated ghrelin is suppressed after food intake and one of these studies found that acylated ghrelin responds more rapidly and dynamically than total ghrelin in response to glucose ingestion (Hosoda et al, 2004). In view of this latter finding and bearing in mind that hunger is suppressed
following exercise (King and Blundell, 1995; King et al, 1994) and that ghrelin is thought to be such a strong determinant of hunger, the author decided to re-investigate the influence of exercise on ghrelin by specifically measuring acylated ghrelin.

The primary purpose of the present study was to determine plasma acylated ghrelin concentrations during and following an intense bout of treadmill running. It was hypothesized that intense treadmill running would cause a temporary suppression of hunger and that this would be associated with reduced concentrations of plasma acylated ghrelin. The acylated ghrelin response to feeding was also assessed since limited information is available in this regard. Finally, concentrations of plasma glucose and insulin were measured during and after exercise and feeding because glucose and insulin have suppressive effects on concentrations of total ghrelin and are therefore important for ghrelin regulation (Flanagan et al, 2003; Murdolo et al, 2003; Shiya et al, 2002).

5.2 Methods

5.2.1 Participants

Nine healthy Caucasian males aged 19 to 25 years from the population at Loughborough University volunteered to participate in the study. Table 5.1 shows the physical characteristics of participants.
Table 5.1 Physical characteristics of participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.2 ± 0.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81 ± 0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.6 ± 2.0</td>
</tr>
<tr>
<td>Sum of Skinfolds (mm)</td>
<td>31.1 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.2 ± 0.7</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>14.2 ± 1.0</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>78.3 ± 1.7</td>
</tr>
<tr>
<td>VO₂ max (ml·kg⁻¹·min⁻¹)</td>
<td>63.3 ± 2.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 9).

5.2.2 Study design

All experimental procedures were conducted in the Health and Paediatric Exercise Physiology Laboratory at Loughborough University. After being made aware of the protocol, health screened, then giving written consent to take part, anthropometric data was collected after which each participant undertook two preliminary exercise tests as follows: 1) submaximal-incremental treadmill running test, 2) maximum oxygen uptake (VO₂ max) treadmill running test. There was a 20 to 30 minute rest interval between the exercise tests.

During subsequent weeks participants undertook two main trials (exercise and control) and these were performed in a randomized order with an interval of at least 7 days. The day before the first main trial participants were asked to record their weighed food intake using a food record diary. The same food intake was then consumed the day prior to the next remaining trials. Participants were also asked to refrain from any form of vigorous activity and ingesting caffeine or alcohol 24 hours prior to the main trial. Participants were fasted for a minimum of 10 hours, but were allowed to drink water ad libitum. Participants attended the laboratory between the
hours of 08:00 and 09:00 and were requested to use motorised transport where possible or ensure that activity prior to attending the laboratory was light.

5.2.3 Main trials

Participants were given at least one week to recover from the preliminary exercise tests before embarking on the main trials. Each main trial began in the morning and lasted for nine hours. At the start of the exercise trial participants ran on the treadmill for 60 minutes at a running speed predicted to elicit 75% of maximum oxygen uptake. One minute expired air samples were collected at 14-15, 29-30, 44-45 and 59-60 minutes during the run. Running speed was adjusted after each expired air collection if the oxygen consumption was above or below the predicted value or if the subject expressed that they were experiencing difficulties. After the run, participants rested for 8 hours (sitting reading, writing, working at a computer or watching television). For the control trial participants undertook exactly the same protocol but rested throughout. Participants were not devoid of time cues during the trials and a clock was on display in the laboratory throughout the trials. Due to participants being aware of the study protocol there may have been anticipatory changes.

Figure 5.1 shows a schematic representation of the study protocol.
Figure 5.1  Schematic representation of the main trial protocol

Ex 72% VO$_{2\text{max}}$ or rest

Baseline

1 2 3 4 5 6 7 8 9

Time (h)

Key:

Active Ghrelin

Insulin, Glucose, haematocrit and haemoglobin

↑ Visual Analogue Scales, Temperature and Humidity

↑ Samples of Expired Air
5.2.4 Subjective measurement of hunger

On arrival, participants completed an RPH scale compiled by Burns et al (2007) which ranged from 0 ‘Not Hungry’ to 15 ‘Very Hungry’. Hunger measurements were recorded at baseline, 0.5, 1, 1.5, 2, 3, 3.5, 4, 5, 6, 7, 8, and 9 hours.

5.2.5 Test meal

Participants were fed a 4201 kJ (1004 kcal) test meal three hours from baseline measurements for both trials. The meal consisted of 77g of white bread, 12g of butter, 12g of mayonnaise, 33g of cheddar cheese, 30g of potato crisps, a 52g chocolate bar, 150g of whole milk and 7g of milk shake powder based on a 70 kg person. The macronutrient content was 38% carbohydrate (103g), 10% protein (24g) and 52% fat (57g). The amount of each food stuff was adjusted for each participant based on their bodyweight on the day of first trial and identical amounts were consumed on the second trial. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times on both trials. Water was available ad libitum during trials and the volume and time of ingestion were recorded.

5.2.6 Environmental temperature and humidity

Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.).

5.2.7 Blood sampling

Prior to the start of each trial subjects rested in a semi-supine position while a cannula was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 9 mL EDTA monovettes at baseline, 0.5, 1, 1.5, 2, 3, 3.5, 4,
5, 6, 7, 8, and 9 hours. During the exercise trial at 0.5 hours, blood was collected while the participants straddled the treadmill which took approximately 1 minute. All other blood samples were collected whilst subjects lay in a semi-supine position. The EDTA monovettes were spun at 1681 g (4000 rpm) for 10 minutes in a refrigerated centrifuge at 4°C. The plasma supernatant was then aliquoted into Eppendorf tubes. These were stored at -20°C for analysis of glucose and insulin later.

Separate venous blood samples were drawn into 4.9 mL monovettes at baseline, 0.5, 1, 3, 4 and 9 hours for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 1287 g (3500 rpm) for 10 minutes in a refrigerated centrifuge at 4°C. The supernatants were then aliquoted into storage tubes and 100 μL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 1287 g (3500 rpm) for 5 minutes in a refrigerated centrifuge at 4°C before being transferred into Eppendorf tubes. The samples were then stored at -20°C for analysis later.

At each blood sampling point, duplicate 20 μL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and triplicate blood samples were collected into heparinised micro haematocrit tubes for the determination of haematocrit.

5.2.8 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay. Plasma glucose concentrations were determined by enzymatic, colorimetric methods.
Plasma insulin concentrations were determined by radioimmunoassay. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 6.6%, insulin 7.4% and glucose 1.8%.

5.2.9 Statistical analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.). Plasma acylated ghrelin, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. Area under the curve (AUC) values for hunger versus time were also assessed using the same method. Student's t-tests for correlated data were used to assess differences between fasting and area under the curve values for acylated ghrelin, glucose and insulin on the control and exercise trials. Repeated measures, two-factor ANOVA was used to examine differences between the two trials over time for acylated ghrelin, glucose, insulin, hunger, body mass and plasma volume change. Post-hoc pair wise comparisons were performed using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. Adjustment of values for changes in plasma volume did not alter the statistical findings and hence for simplicity the unadjusted values are reported. Results are given as mean ± SEM unless otherwise stated.
5.3 Results

5.3.1 Responses to treadmill running

The mean percentage of maximum oxygen uptake elicited during exercise was $72 \pm 2.0\%$ and the mean respiratory exchange ratio was $0.94 \pm 0.01$. Gross energy expenditure during exercise was $3915 \pm 207 \text{ kJ} (935 \pm 50 \text{ kcal})$ with $15 \pm 4\%$ of energy provided from fat and $85 \pm 4\%$ of energy provided from carbohydrate. Average heart rate during exercise was $179 \pm 2 \text{ b·min}^{-1}$ and median rating of perceived exertion (RPE) was 15 i.e. 'hard' (range 15-16).

5.3.2 Body mass and fluid consumption

Two-factor ANOVA revealed a main effect of trial ($P < 0.009$) and a significant interaction ($P < 0.039$) for body mass. On the control trial body mass was $72.7 \pm 2.3 \text{ kg}$ at the start of the trial and $72.9 \pm 2.4 \text{ kg}$ at the end of the trial. On the exercise trial body mass was $72.3 \pm 2.3 \text{ kg}$ at the start of the trial and $72.1 \pm 2.3 \text{ kg}$ at the end of the trial. Bonferroni post hoc tests revealed that body mass at the end of the exercise trial was significantly ($P < 0.003$) lower than body mass at the end of the control trial. There was a trend (Student's $t$-test, $P = 0.061$) for water consumption to be higher during the exercise trial ($2168 \pm 335 \text{ mL}$) compared with the control trial ($1468 \pm 114 \text{ mL}$).

5.3.3 Temperature and humidity

There was no difference (Student's $t$-test, $P = 0.502$) in environmental temperature between the control and the exercise trials ($26.2 \pm 0.8$ compared with $25.6 \pm 1.0 \text{ °C}$ respectively). Likewise there was no difference (Student's $t$-test, $P = 0.960$) in
humidity between the control and the exercise trials (39.4 ± 3.1 compared with 49.2 ± 4.1% respectively).

5.3.4 Hunger

Two-factor ANOVA revealed a main effect of time ($P < 0.0005$) and a trial × time interaction effect ($P = 0.001$) for hunger indicating that responses differed over time between the exercise and control trials. Post hoc analysis indicated between trial differences at 0.5, 1 and 7 hours but after adjusting for multiple comparisons using the Bonferroni method the only difference to remain significant ($P = 0.003$) was that at 7 hours (Figure 5.2).

![Figure 5.2](image)

Figure 5.2 Subjective feelings of hunger using the RPH scale during the exercise and control trials. Values are mean ± SEM, $n = 9$. The diagonal patterned rectangle indicates the treadmill run. The black rectangle indicates consumption of the test meal. * Control different from exercise $P < 0.05$.

Between trial differences in hunger ratings were also evaluated using AUC values for the three hours prior to the meal (0 to 3 hours), the three hours after the meal (3 to 6
hours), the six hours after the meal (3 to 9 hours) and the full nine hours. A significant difference was found over the first three hours: 32 versus 24 (mean values) for the control and exercise trials respectively (Student’s t-test, \( P = 0.013 \)). The difference between AUC values for the six hours after the meal (11:00 to 17:00) approached significance: 44 versus 50 (mean values) for the control and exercise trials respectively (Student’s t-test, \( P = 0.056 \)).

5.3.5 Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ significantly between the control and exercise trials: control 150.3 ± 56.4, exercise 137.5 ± 46.8 pg·mL\(^{-1}\) (\( P < 0.274 \)). Two-factor ANOVA revealed a main effect of trial (\( P = 0.022 \)), a main effect of time (\( P = 0.048 \)) and a trial \( \times \) time interaction (\( P = 0.043 \)) for acylated ghrelin concentrations. Post hoc analysis indicated between trial differences at 0.5, 1 and 9 hours but after adjusting for multiple comparisons using the Bonferroni method the only difference to remain significant (\( P < 0.001 \)) was that at 0.5 hours (Figure 5.3).
Figure 5.3  Plasma acylated ghrelin concentration during the exercise and control trials. Values are mean ± SEM, n = 9. The diagonal patterned rectangle indicates the treadmill run. The black rectangle indicates consumption of the test meal. *Control different from exercise P < 0.05.

Total area under the concentration versus time curve for plasma acylated ghrelin was lower on the exercise trial compared with the control trial: 917.1 ± 342.2 compared with 1400.9 ± 521.0 pg·mL⁻¹·9 hours for the exercise and control trials respectively (Student's t-test, P = 0.033). The plasma acylated ghrelin AUC value was also lower for the first three hours of the exercise trial in comparison with the same time period in the control trial: 316.6 ± 135.1 compared with 509.6 ± 185.6 pg·mL⁻¹·3 hours for exercise and control respectively (Student's t-test, P = 0.021). In terms of percentage change area under the acylated ghrelin concentration versus time curve was 38% lower over the first three hours of the exercise trial and 35% lower over the full nine hours of the exercise trial compared with the control trial (Figure 5.4).
Fasting plasma acylated ghrelin concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting hunger, fasting plasma insulin concentrations or fasting plasma glucose concentrations.

5.3.6 Glucose

Fasting plasma glucose concentrations did not differ significantly (Student’s t-test, $P = 0.613$) between trials (control $5.4 \pm 0.1$ mmol·L$^{-1}$, exercise $5.3 \pm 0.1$ mmol·L$^{-1}$). Two-factor ANOVA revealed a main effect of trial ($P = 0.013$), a main effect of time ($P < 0.0005$) and a trial × time interaction ($P = 0.021$) for plasma glucose (Figure 5.5). Post hoc analysis indicated between trial differences at 0.5, 1 and 4 hours but after adjusting for multiple comparisons using the Bonferroni method none of these remained significant (Figure 5.5).
Figure 5.5  Plasma glucose concentration during the exercise and control trials. Values are mean ± SEM, n = 9. The diagonal patterned rectangle indicates the treadmill run. The black rectangle indicates consumption of the test meal.

Total area under the concentration versus time curve for plasma glucose was higher on the exercise trial compared with the control trial: 50.6 ± 1.0 compared with 47.3 ± 0.8 mmol·L⁻¹·9 hours for the exercise and control trials respectively (Student's t-test, P = 0.015). The glucose AUC value was also higher for the one-hour exercise bout in comparison with the same time period in the control trial: 6.5 ± 0.5 compared with 5.3 ± 0.1 mmol·L⁻¹·1 hour for exercise and control respectively (Student's t-test, P = 0.038).
5.3.7 Insulin

Fasting plasma insulin concentrations did not differ significantly (Student’s $t$-test, $P = 0.980$) between trials (control $208.4 \pm 20.1$ pmol·L$^{-1}$, exercise $207.7 \pm 34.0$ pmol·L$^{-1}$). Two-factor ANOVA revealed a main effect of time ($P < 0.0005$) for plasma insulin but there was no effect for trial and no interaction effect (Figure 5.6).

![Plasma insulin concentration during the exercise and control trials. Values are mean ± SEM, $n = 9$. The diagonal patterned rectangle indicates the treadmill run. The black rectangle indicates consumption of the test meal.](image)

**Figure 5.6** Plasma insulin concentration during the exercise and control trials. Values are mean ± SEM, $n = 9$. The diagonal patterned rectangle indicates the treadmill run. The black rectangle indicates consumption of the test meal.

Total area under the concentration versus time curve for plasma insulin showed no difference on the exercise trial compared with the control trial: $3889.5 \pm 405.5$ compared with $4339.7 \pm 608.1$ pmol·L$^{-1}$·9 hours for the exercise and control trials respectively (Student’s $t$-test, $P = 0.377$). The plasma insulin AUC value also showed no difference for the one-hour exercise bout in comparison with the same time period.
in the control trial: 183.6 ± 22.8 compared with 191.8 ± 24.9 pmol·L⁻¹·1 hour for exercise and control respectively (Student's t-test, \( P = 0.690 \)).
5.4 Discussion

To the author's knowledge, the present study is the first to examine plasma acylated ghrelin concentrations during exercise. The novel finding arising from this study is that there is a suppression of plasma acylated ghrelin during running. These findings suggest that acylated ghrelin responds differently to an exercise stimulus compared with total ghrelin, although a limitation of the study with regard to this latter finding is that total ghrelin concentrations were not measured due to cost implications. The present study also indicates that hunger is suppressed during exercise and to some extent in the immediate post-exercise period.

Although the present study is the first to examine plasma acylated ghrelin during exercise, several previous studies have investigated the response of plasma total ghrelin to exercise (Burns et al., 2007; Dall et al., 2002; Jürimäe et al., 2007; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2004). The findings of most of these studies indicate plasma total ghrelin concentrations are unaffected by a single session of exercise although one study has reported that serum total ghrelin levels are suppressed for up to an hour after the cessation of exercise (Vestergaard et al., 2007) while another has reported that plasma total ghrelin concentrations are elevated during prolonged (three hours) moderate intensity exercise (Christ et al., 2006). We did not measure plasma total ghrelin in the present study but in a previous study using a similar exercise protocol we found no change in plasma total ghrelin concentration during or after exercise (Burns et al., 2007).

It is possible that previous studies failed to detect changes in ghrelin because they measured total ghrelin and not acylated ghrelin. Hosoda and colleagues (2004) have
reported that acylated ghrelin responds more quickly than total ghrelin in response to glucose ingestion and more dynamically i.e. the percentage changes in acylated ghrelin are greater than those for total ghrelin in response to glucose ingestion. It is possible that the same applies during exercise. It is known that splanchnic blood flow is reduced during intense exercise (Rowell, 1974) and this would reduce oxygen delivery to the stomach and intestines. It is possible that this reduced oxygen delivery interferes in some way with the secretion of ghrelin, altering the ratio of total to acylated ghrelin but there is no evidence to support this and the suggestion is highly speculative.

The finding that there is a suppression of hunger during and to some extent after exercise is consistent with results from previous studies that have monitored subjective hunger ratings following vigorous exercise (above 60% of maximum oxygen uptake) (Blundell et al, 2003; King and Blundell, 1995; King et al, 1994). As in previous studies the suppression of hunger was short lived and hunger ratings had returned to control values within two hours of the cessation of exercise.

In addition to the decline in acylated ghrelin concentrations during exercise, acylated ghrelin concentrations declined after feeding in both the control and exercise trials. This is consistent with data from studies examining the response to feeding of both acylated plasma ghrelin (Al Alwar et al, 2005; Blom et al, 2005; El Khoury et al, 2006; Hosoda et al, 2004; Lucidi et al, 2004) and total plasma ghrelin (Blom et al, 2005; Cummings et al, 2002; Cummings et al, 2001; Hosoda et al, 2004; Lucidi et al, 2004). Macronutrient composition may influence the extent to which feeding suppresses ghrelin. There is evidence to suggest that carbohydrate has a greater
suppressive effect on plasma acylated ghrelin than protein or fat (El Khoury et al, 2006) and at least two studies have reported that plasma acylated ghrelin concentrations are not suppressed significantly after high fat meals (Al Alwar, 2005; Tentolouris et al, 2004). Our findings conflict with these latter findings since the meal employed in the present study was high in fat. However, this meal was also high in energy content (4200 kJ for an individual with a body mass of 70 kg) and this has been shown to impact on the suppression of total ghrelin (Callahan et al, 2004). Moreover high fat meals have been shown to suppress total serum ghrelin concentrations (Poppitt et al, 2006). Considering the similarity in the response of acylated and total ghrelin to feeding, it is difficult to understand why there is a divergent response to exercise i.e. no change in total ghrelin but a reduction in acylated ghrelin. The findings suggest that the mechanism by which exercise alters acylated ghrelin may differ from that by which feeding alters acylated ghrelin. This is feasible because factors other than feeding, such as circadian rhythms and body composition, have been demonstrated to influence total plasma ghrelin concentration (English et al, 2002; Natalucci et al, 2005).

The mechanisms by which feeding suppresses ghrelin concentration are thought to involve post gastric feedback. Intravenous infusion of insulin suppresses plasma total ghrelin concentrations in humans (Flanagan et al, 2003) as does intravenous infusion of glucose (Shiya et al, 2002). In individuals with type 1 diabetes meal intake suppresses total plasma ghrelin concentrations when insulin is administered but not in the absence of insulin (Murdolo et al, 2003). Moreover, at least one study has shown an inverse correlation between the percentage decrease in plasma total ghrelin concentration and the percentage increase in plasma insulin and glucose concentration.
after meals (Blom et al, 2005). In the present study the area under the curve values for plasma glucose concentration were elevated during the one hour run compared with the same time period during the control trial. This is consistent with the findings of previous studies which also demonstrate an increase in plasma glucose during exercise particularly in trained participants (Kjaer et al, 1986; Kraemer et al, 2002). This may in part explain the lower area under the curve values for plasma acylated ghrelin during the exercise trial. Plasma insulin concentrations were not elevated during exercise in the present study, in line with previous findings (Kjaer et al, 1986; Kraemer et al, 2002), and are therefore unlikely to contribute to the suppression of acylated ghrelin during exercise.

In the present study there was a tendency for higher hunger ratings over the last five hours of the exercise trial compared with the control trial. This is probably because participants were in energy deficit during the exercise trial compared with the control trial. Previous research has demonstrated an increase in energy intake after an acute bout of exercise (Pomerleau et al, 2004) and after a one-week period of exercise training (Stubbs et al, 2002a). This is not a universal finding since one study has reported no increase in energy intake over a two day period following acute bouts of exercise (King et al, 1997). Although there was a transient reduction in hunger after exercise in the present study, it is likely that hunger was elevated later in the day due to the 3915 kJ (930 kcal) energy expenditure resulting from the run. However, the nine hour values for plasma acylated ghrelin concentration do not support the notion that hunger was elevated at the end of the exercise trial. In fact, acylated ghrelin concentrations at nine hours suggest that hunger was still suppressed on the exercise
trial compared with the control trial. This apparent contradiction requires further study.

The present study had several limitations. Firstly the participants were young and well trained and therefore the findings may not apply to older subjects or to untrained participants. Secondly the low sample size may have limited the power to detect significant relationships between acylated ghrelin and other variables. Thirdly, acylated ghrelin was measured at only six time points over the nine hour observation period. More frequent measurements of acylated ghrelin would provide a clearer picture of the responses to exercise and feeding. Finally, total ghrelin concentration was not measured, limiting our ability to determine the extent of variation between the responses of acylated ghrelin and total ghrelin to exercise and feeding.

In conclusion, this study demonstrates that plasma acylated ghrelin concentration is reduced during an acute bout of treadmill running and this lends support for the role of acylated ghrelin in hunger suppression during and immediately after exercise. Further research is required to determine the influence of other modes, durations and intensities of exercise on plasma acylated ghrelin concentration and to document acylated ghrelin responses to exercise in different subject groups e.g. older, untrained and obese participants. Such research could have important implications regarding the role of exercise in weight management.
CHAPTER VI

The effect of exercise intensity on plasma acylated ghrelin

6.1 Introduction

Several studies have investigated the influence of high intensity aerobic exercise bouts on plasma ghrelin concentration (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004a; Schmidt et al, 2004; Vestergaard et al, 2007). Most of these studies indicate that a single session of aerobic exercise has no influence on ghrelin concentrations (Burns et al, 2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004a; Schmidt et al, 2004) although one study reported that serum ghrelin levels are suppressed for up to an hour after the cessation of exercise (Vestergaard et al, 2007). In contrast, one study has reported that plasma ghrelin concentrations are increased during three hours of moderate intensity exercise but it is uncertain whether this is a true effect of exercise because this study did not include a control trial (Christ et al, 2006). The study presented in the previous chapter found suppressed acylated ghrelin as a result of high intensity treadmill running but to the author’s knowledge no study has reported the effects of low intensity exercise on ghrelin or acylated ghrelin.

Chronic high intensity exercise suppressed food intake and associated weight gain in male rats more than low intensity exercise of the same expenditure (Katch et al, 1979). King et al (1994) found suppressed hunger was associated with high but not low intensity cycling. Thompson et al (1988) also inferred that exercise intensity is an important
variable mediating exercise effects on hunger as they found that hunger was briefly suppressed as a result of high intensity cycling compared to low intensity cycling and control. Causal mechanisms were not identified in the papers described but the results of the previous study (Chapter 5) suggest that acylated ghrelin is suppressed during and for a short period after a high intensity bout of treadmill running which coincides with suppressed hunger. An exercise induced effect on acylated ghrelin needs confirmation and there is a paucity data on how soon acylated ghrelin responds to an exercise stimulus. In the previous study the first blood sample was taken 30 minutes after the initiation of exercise so measurement of acylated ghrelin soon after exercise begins is warranted.

Similar to Katch et al (1979), King et al (1994) and Thompson et al (1988) the author has also demonstrated that exercise intensity is a determinant of ‘exercise induced anorexia’ as suppressed feelings of hunger were seen in the high but not the low intensity exercise trial in the study described in Chapter 4. However the energy expenditure in the high intensity exercise trial was much greater, so a true exercise intensity affect could not be determined. Energy expenditure was therefore controlled for in the present study.

To the author’s knowledge, no study has examined the influence of exercise intensity on plasma acylated ghrelin or its response soon after the initiation of exercise. Therefore, the purpose of the present study was to determine if acylated ghrelin concentrations are suppressed during and following a low and high intensity bout of treadmill running keeping the energy expenditure constant. Since associations between suppressed acylated ghrelin and ‘exercise induced anorexia’ have been demonstrated previously it was
hypothesized that intense treadmill running would be associated with a greater reduced concentration of plasma acylated ghrelin compared with low intensity exercise and that exercise would cause a temporary suppression of hunger which would again be greater in the high intensity exercise trial.
6.2 Methods

6.2.1 Participants

Nine healthy males aged 20 to 25 years from the population at Loughborough University volunteered to participate in the study. Table 6.1 shows the physical characteristics of the participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(Mean ± SEM)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>21.4 ± 0.6</td>
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<tr>
<td>Height (m)</td>
<td>1.80 ± 0.02</td>
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<tr>
<td>Weight (kg)</td>
<td>78.3 ± 3.2</td>
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<tr>
<td>Sum of Skinfolds (mm)</td>
<td>33.1 ± 1.7</td>
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<tr>
<td>BMI (kg·m⁻²)</td>
<td>24.5 ± 0.7</td>
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<tr>
<td>Body Fat (%)</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>77.7 ± 1.7</td>
</tr>
<tr>
<td>( \text{VO}_2 \text{ max} ) (ml·kg⁻¹·min⁻¹)</td>
<td>58.2 ± 1.6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 9)

6.2.2 Study design

All experimental procedures were conducted in the Health and Paediatric Exercise Physiology Laboratory at Loughborough University. After being made aware of the protocol, health screened, then giving written consent to take part, anthropometric data was collected after which each participant undertook two preliminary exercise tests as follows: 1) submaximal-incremental treadmill running test, 2) maximum oxygen uptake (\( \text{VO}_2 \text{ max} \)) treadmill running test. There was a 20 to 30 minute rest interval between the exercise tests.
During subsequent weeks participants undertook three main trials (low intensity exercise, high intensity exercise and control) and these were performed in a randomized order with an interval of at least 7 days.

The day before the first main trial participants were asked to record their weighed food intake using a food record diary. The same food intake was then consumed the day prior to the next remaining trials. Participants were also asked to refrain from any form of vigorous activity and ingesting caffeine or alcohol 24 hours prior to the main trials. Participants were fasted for a minimum 10 hours, but were allowed to drink water ad libitum. Participants attended the laboratory between the hours of 08:00 and 09:00 and were requested to use motorised transport where possible or ensure that activity prior to attending the laboratory was light.

6.2.3 Main trials

Participants were given at least one week to recover from the preliminary exercise tests before embarking on the main trials. Each main trial began in the morning and lasted for four hours. At the start of the low intensity exercise trial participants ran on the treadmill at a running speed predicted to elicit 50% of maximum oxygen uptake until a gross energy expenditure of 2510 kJ (600 kcal) was achieved. A 600 kcal energy expenditure was selected as analysis of results from previous studies showed that at this energy expenditure participants would be running for around one hour in the low intensity exercise trial and for 0.5 hours in the high intensity exercise trials. At the start of the high intensity exercise trial participants ran on the treadmill at a running speed predicted to
elicit 75% of maximum oxygen uptake until a gross energy expenditure of 2510 kJ (600 kcal) was achieved.

One minute expired air samples were collected frequently during the runs. Running speed was adjusted after each expired air collection if the oxygen consumption was above or below the predicted value or if the participants expressed that they were experiencing difficulties. After the runs, participants rested until 4 hours (sitting reading, writing, working at a computer or watching television). For the control trial participants undertook exactly the same protocol but rested throughout. Participants were not devoid of time cues during the trials and a clock was on display in the laboratory throughout the trials.

Figure 6.1 shows a schematic representation of the study protocol.
**Figure 6.1** Schematic representation of the main trial protocol

Ex 52% or 75% VO\textsubscript{2max} or rest until energy expenditure met

**Baseline**

<table>
<thead>
<tr>
<th>Time (h)</th>
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<th>4</th>
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</tbody>
</table>

**Key:**

- ♦ Acylated Ghrelin, haematocrit and haemoglobin
- Visual Analogue Scales, Temperature and Humidity
- Insulin and Glucose
- Samples of Expired Air
6.2.4 Subjective measurement of hunger

On arrival, participants completed an RPH scale compiled by Burns et al (2007) which ranged from 0 ‘Not Hungry’ to 15 ‘Very Hungry’. Hunger measurements were recorded at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 hours.

6.2.5 Test meal

Participants were fed a 4186 kJ (1000 kcal) test meal three hours from baseline measurements for all trials. The meal consisted of 95g of white bread, 90g of tuna, 15g of mayonnaise, 52g of chocolate bar, 25g of potato crisps, 80g of green apple and 100g of orange juice based on a 70 kg person. The macronutrient content was 56% carbohydrate (149g), 15% protein (37g) and 29% fat (33g). The amount of each food stuff was adjusted for each participant based on their bodyweight on the day of first trial and identical amounts were consumed on the remaining trials. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times on both trials. Water was available *ad libitum* during trials and the volume and time of ingestion were recorded.

6.2.6 Environmental temperature and humidity

Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.).
6.2.7 Blood sampling

Prior to the start of each trial participants rested in a semi-supine position while a cannula was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 4.9 mL EDTA monovettes at baseline, 0.5, 1, 1.5, 2, 3, 3.5 and 4 hours. During the exercise trials, if the participants were still running and a blood sample was due to be taken, blood was collected while the participants straddled the treadmill, which took approximately 1 minute. All other blood samples were collected whilst subjects lay in a semi-supine position. The EDTA monovettes were spun at 1681 g for 10 minutes in a refrigerated centrifuge at 4°C. The plasma supernatant was then aliquoted into Eppendorf tubes. These were stored at -20°C for analysis of glucose and insulin later.

Separate venous blood samples were drawn into 4.9 mL monovettes at baseline, 5 minutes, 0.5, 1, 3, 3.5 and 4 hours for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 1287 g for 10 minutes in a refrigerated centrifuge at 4°C. The supernatants were then aliquoted into storage tubes and 100 μL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 1287 g for 5 minutes in a refrigerated centrifuge at 4°C before being transferred into Eppendorf tubes. The samples were then stored at -20°C for analysis later.

At each blood sampling point, duplicate 20 μL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and triplicate blood
samples were collected into heparinised micro haematocrit tubes for the determination of haematocrit.

6.2.8 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay. Plasma glucose concentrations were determined by enzymatic, colorimetric methods. Plasma insulin concentrations were determined by radioimmunoassay. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.0%, insulin 8.9% and glucose 1.4%.

6.2.9 Statistical analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.). Plasma acylated ghrelin, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. One-way ANOVA was used to assess differences in baseline hunger, acylated ghrelin, weight and fluid intake for the three trials. Repeated measures ANOVA was performed to determine the differences between the effects of trial (high intensity versus low intensity versus control) and time (0, 0.5, 1, 1.5, 2, 3, 3.5 and 4 hours) for hunger, acylated ghrelin, glucose, insulin, temperature and humidity and to assess whether there was any interaction effect (trial x time). The data for the RPH is discrete, but repeated measures ANOVA was used because there is no non-parametric equivalent. Post-hoc pair wise comparisons were performed using the Bonferroni method. The
Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. Adjustment of values for changes in plasma volume did not alter the statistical findings and hence for simplicity the unadjusted values are reported. Results are given as mean ± SEM unless otherwise stated.
6.3 Results

6.3.1 Responses to treadmill running

Table 6.2 shows the responses to treadmill running.

Table 6.2 Responses to treadmill running.

<table>
<thead>
<tr>
<th>Exercise Trial</th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Percentage $\dot{V}O_2_{max}$ (%)</td>
<td>52 ± 1.0</td>
<td>75 ± 1.0</td>
</tr>
<tr>
<td>Mean respiratory exchange ratio</td>
<td>0.90 ± 0.01</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>Gross energy expenditure (kJ)</td>
<td>2580 ± 51</td>
<td>2504 ± 55</td>
</tr>
<tr>
<td>Gross energy expenditure (kcal)</td>
<td>617 ± 12</td>
<td>599 ± 13</td>
</tr>
<tr>
<td>Percentage energy contribution from fat (%)</td>
<td>32 ± 3</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Percentage energy contribution from carbohydrate (%)</td>
<td>68 ± 3</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>Duration of the run (minutes)</td>
<td>55 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Average heart rate (b-min$^{-1}$)</td>
<td>136 ± 5</td>
<td>164 ± 6</td>
</tr>
<tr>
<td>Median RPE</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are mean ± SEM ($n = 9$)

There was no significant difference in gross energy expenditure between the two trials ($P = 0.376$).

6.3.2 Body mass and fluid consumption

Two-factor ANOVA revealed changes in body weight were not significant. On the control trial body mass was 78.8 ± 3.4 kg at the start of the trial and 79.2 ± 3.6 kg at the
end of the trial. On the low intensity exercise trial body mass was 78.8 ± 4.1 kg at the start of the trial and 78.4 ± 4.0 kg at the end of the trial. On the high intensity exercise trial body mass was 78.9 ± 3.6 kg at the start of the trial and 78.9 ± 3.7 kg at the end of the trial. Water consumption was higher during the exercise trials compared with control (One-factor ANOVA, $P = 0.012$). When performing the Bonferroni method differences lay between the control (610 ± 104 mL) and high intensity exercise trial (883 ± 103 mL).

6.3.3 Temperature and humidity

There was no significant difference in environmental temperature between the control, low intensity and high intensity exercise trials (22.7 ± 0.1 °C versus 22.7 ± 0.3 °C versus 22.6 ± 0.2 °C respectively). However there was a significant trial x time interaction, with humidity between the control, low intensity and high intensity exercise trials (30.7 ± 1.7 % versus 34.0 ± 1.8 % versus 35.3 ± 1.1 % respectively). However post hoc analysis indicated no significant between trial differences after adjusting for multiple comparisons using the Bonferroni method.

6.3.4 Hunger

There was a significant effect of time ($P < 0.0005$) but no trial ($P = 0.742$) or trial x time interaction ($P = 0.239$) for hunger (Figure 6.2). This indicates that exercise did not stimulate ‘exercise induced anorexia.’
Figure 6.2  Subjective feelings of hunger using the RPH scale during the low intensity, high intensity and control trials. Values are mean ± SEM, n = 9. The checked rectangle indicates the low intensity treadmill run. The diagonal patterned rectangle indicates the high intensity treadmill run. The black rectangle indicates consumption of the test meal.

Between trial differences in hunger ratings were also evaluated using AUC values for the first hour of the trial (0 to 1 hours), the three hours prior to the meal (0 to 3 hours), and the full 4 hours. One-factor ANOVA confirmed no difference between the control, low and high intensity exercise trials for the first hour: 7 versus 6 versus 6 (mean values) respectively (P = 0.276). The difference between AUC values for the three hours prior to the meal and full four hours were also not significant.
6.3.5 Acylated ghrelin

One of the participants displayed exceptionally high acylated values throughout the trials ranging from 74 to 1489 pg·mL\(^{-1}\). The participants values were deemed to be an outlier and distorted the statistical findings so were removed from the data analysis.

Fasting plasma acylated ghrelin concentrations did not differ \((P = 0.473)\) between the control, low and high intensity exercise trials \((67.2 \pm 10.5 \textit{versus} 67.7 \pm 8.6 \textit{versus} 78.9 \pm 14.0 \text{ pg·mL}^{-1} \text { respectively})\). Two-factor ANOVA revealed no effect of trial \((P = 0.747)\), but a main effect of time \((P < 0.0005)\) and a trial × time interaction \((P = 0.024)\) for acylated ghrelin concentrations. At 0.5 hours plasma acylated ghrelin was lower on the high intensity exercise trial compared with the low intensity and control trials: 42.7 ± 11.2 \textit{versus} 59.3 ± 9.5 \textit{versus} 63.2 ± 9.7 pg·mL\(^{-1}\) (Figure 6.3). Post hoc analysis indicated this was approaching significance \((P = 0.065)\).
Acylated ghrelin concentration during the low intensity exercise, high intensity exercise and control trials. Values are mean ± SEM, n = 8. The checked rectangle indicates the low intensity treadmill run. The diagonal patterned rectangle indicates the high intensity treadmill run. The black rectangle indicates consumption of the test meal.

Between trial differences for acylated ghrelin were also evaluated using AUC values for the first hour of the trial (0 to 1 hours), the three hours prior to the meal (0 to 3 hours), and the full 4 hours. One-factor ANOVA confirmed no difference between the control, low and high intensity exercise trials for the first hour: 66.3 ± 9.7 versus 61.3 ± 9.4 versus 54.7 ± 11.5 pg·mL⁻¹·hour respectively (P = 0.559). The difference between AUC values for the three hours prior to the meal and full fours hours were also not significant (Figure 6.4).
Figure 6.4  Total area under the concentration versus time curve (AUC) for plasma acylated ghrelin (mean ± SEM, n = 9). Values are for the first hour of the trial (pg·mL⁻¹·1 hours), first three hours of the trial (pg·mL⁻¹·3 hours) and for the full four hours of the trial (pg·mL⁻¹·4 hours).

Fasting plasma acylated ghrelin concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting hunger, fasting plasma insulin concentrations or fasting plasma glucose concentrations.

6.3.6 Glucose

Fasting plasma glucose concentrations did not differ significantly (P = 0.451) between control, low and high intensity exercise trials (5.3 ± 0.2 versus 5.5 ± 0.2 versus 5.6 ± 0.4 mmol·L⁻¹ respectively). Two-factor ANOVA revealed a main effect of trial (P = 0.045), a main effect of time (P < 0.0005) but no trial × time interaction (P = 0.181) for plasma
glucose (Figure 6.5). Post hoc analysis identified trial differences were between the high intensity and low intensity exercise trial ($P = 0.023$).

![Figure 6.5](image)

**Figure 6.5** Glucose concentration during the low intensity exercise, high intensity exercise and control trials. Values are mean ± SEM, $n = 9$. The checked rectangle indicates the low intensity treadmill run. The diagonal patterned rectangle indicates the high intensity treadmill run. The black rectangle indicates consumption of the test meal.

Total area under the concentration versus time curve for plasma glucose confirmed that values were higher during the high intensity exercise trial compared with the low intensity exercise and control trials ($22.1 \pm 0.7$ versus $20.9 \pm 0.7$ versus $20.9 \pm 0.7$ mmol·L⁻¹·4 hours respectively, $P = 0.05$) for the 4 hours of the trial. Post hoc analysis showed that differences were between the low and high intensity exercise trials ($P = 0.004$).
6.3.7 Insulin

Fasting plasma insulin concentrations did not differ significantly \( (P = 0.218) \) between control, low and high intensity exercise trials (144.3 ± 18.0 \textit{versus} 188.6 ± 23.7 \textit{versus} 174.8 ± 17.2 pmol·L\(^{-1}\) respectively). Two-factor ANOVA revealed a main effect of time \( (P < 0.0005) \) but no effect of trial \( (P = 0.998) \) and no trial × time interaction \( (P = 0.559) \) for plasma insulin (Figure 6.6).

![Figure 6.6](image_url)

**Figure 6.6** Insulin concentration during the low intensity exercise, high intensity exercise and control trials. Values are mean ± SEM, \( n = 9 \). The checked rectangle indicates the low intensity treadmill run. The diagonal patterned rectangle indicates the high intensity treadmill run. The black rectangle indicates consumption of the test meal.

Between trial differences for insulin were also evaluated using AUC values for the first hour of the trial (0 to 1 hours), the 3 hours prior to the meal (0 to 3 hours), and the full 4 hours. One-factor ANOVA confirmed no difference between the control, low and high intensity exercise trials for the first hour: 159.7 ± 16.0 \textit{versus} 135.5 ± 12.0 \textit{versus} 203.7 ±
38.0 pmol·L⁻¹·h hours respectively ($P = 0.279$). The difference between AUC values for the 3 hours prior to the meal and full 4 hours were also not significant.
6.4 Discussion

To the author's knowledge, the present study is the first to examine the transient effect of exercise intensity on plasma acylated ghrelin concentrations during exercise. The novel finding arising from this study is that there is a suppression of plasma acylated ghrelin during high but not low intensity running.

At 0.5 hours plasma acylated ghrelin was lower on the high intensity exercise trial compared with the low intensity and control trials (42.7 ± 11.2 versus 59.3 ± 9.5 versus 63.2 ± 9.7 pg·mL⁻¹, P = 0.065). The findings therefore confirm those of chapter 5 that acylated ghrelin is suppressed in response to high intensity treadmill running. The findings also show that exercise intensity is a determinant of the acylated ghrelin response to exercise as there was no significant suppression of acylated ghrelin during the low intensity exercise trial even when the energy expenditure was kept constant.

To keep the energy expenditure matched the durations differed so that participants were running for 55 ± 2 minutes during the low intensity trial and for 36 ± 2 minutes for the high intensity exercise trial and the statistical findings show no significant suppression of acylated ghrelin during the low intensity trial. It is possible that acylated ghrelin is not suppressed during low intensity exercise compared with high intensity exercise due to a lower rate of energy expenditure, less gut upheaval and no significant increase in plasma glucose that was demonstrated in the high intensity trial.
A plasma acylated ghrelin measurement was taken at 5 minutes to examine if acylated ghrelin would respond to an exercise stimulus within this time. Although there is a trend for a decline in both the low and high intensity exercise trials (Figure 6.3) this was not significant. No previous study has measured acylated ghrelin so soon after the initiation of exercise so the findings need confirmation. However, the author felt that in subsequent studies acylated ghrelin should be measured at other time points due to the restricted number of plasma acylated ghrelin samples that can be assayed.

Acylated ghrelin values reported are similar to that reported in chapter 5 apart from one of the participants whose acylated ghrelin data was removed from the analysis due to extremely high values. His fasting acylated ghrelin concentration during the control trial was 584 pg.mL$^{-1}$ which is not a result of measurement error as values were high throughout all trials. Atypically high acylated ghrelin values have been reported previously in Chapter 2. The reasons for this are unclear but it is interesting to note that both participants were dual heritage and/or ethnic minority. High total ghrelin values have been reported previously (Peeters et al, 2005) but to the author's knowledge no study has previously reported atypically high acylated ghrelin values or given reasons for high ghrelin values per se in lean participants without genetic defects.

In addition to the decline in acylated ghrelin concentrations during exercise, acylated ghrelin concentrations declined after feeding. This is consistent with data from studies examining the response to meals of both acylated plasma ghrelin (Al Alwar et al, 2005; Hosoda et al, 2004; Lucidi et al, 2004) and total plasma ghrelin (Blom et al, 2005;
Cummings et al, 2002, Lucidi et al, 2004) and the study presented in chapter 5. The present study measured acylated ghrelin 30 minutes after the initiation of feeding and found a 12% suppression in the control trial, a 7% suppression in the low intensity trial and 2% reduction in the high intensity trial. The study described in Chapter 5 used a high fat test meal fed at 3 hours in which the macronutrient content was (38% CHO, 10% protein and 52% fat) providing 4201 kJ (1004 kcal) based on a 70 kg person. This led to a 30% decline in plasma acylated ghrelin concentration 1 hour after the meal in the control trial and a 27% decline in the exercise trial. In the present study a mixed meal was fed at 3 hours in which the macronutrient content was 56% CHO, 15% protein and 29% fat providing 4186 kJ (1000 kcal) based on a 70 kg person. This led to a 44% decline in plasma acylated ghrelin concentration 1 hour after the meal in the control trial, a 42% decline in the low intensity exercise trial and a 39% decline in the high intensity exercise trial. Whilst analysis of acylated ghrelin’s feeding response to varying meal composition in two different participant groups is highly speculative, meal composition could affect the suppression of acylated ghrelin which has been shown by Al Awar et al (2005) who found that a mixed meal led to a greater acylated ghrelin suppression than an isoenergetic high fat meal.

Another key finding from the present study was the failure of the exercise stimulus to suppress hunger during and following both high and low intensity treadmill running. This is consistent with results from previous studies that have monitored subjective hunger ratings (King et al, 1996a; Pomerleau et al 2004), but contradicts other work where the
high intensity exercise bout was above 60% of maximum oxygen uptake (Blundell et al., 2003; King and Blundell, 1995; King et al., 1994; Thompson et al., 1988).

Thompson et al. (1988) assigned 16 males aged 19 to 29 years to undertake low intensity cycling at 35\% \text{VO}_2\text{max}, high intensity cycling at 68\% \text{VO}_2\text{max} and a resting control matching the energy expenditure at 4.1 kcal-kg\(^{-1}\) bodyweight. The mean body weight of participants was 75.9 kg so the predicted energy expenditure for each trial was 311 kcal and participants were cycling for a mean of 57.5 minutes for the low intensity exercise trial and a mean of 28.7 minutes for the high intensity exercise trial. There was a brief suppression of hunger immediately after high but not low intensity exercise or control. The duration of exercise is similar to that undertaken in the present study, but the energy expenditure is nearly half. It is therefore surprising that the high intensity exercise trial in the present study did not suppress hunger considering participant characteristics are also similar.

King et al. (1994) also examined the effect of exercise intensity keeping the total energy expenditure constant in twelve lean males aged 21-27 years. In the high intensity exercise trial participants cycled at 70\% \text{VO}_2\text{max} for a mean time of 27 minutes with a mean energy expenditure of 340 kcal. For the low intensity exercise trial participants cycled at 30\% \text{VO}_2\text{max} for a mean time of 63 minutes with a mean energy expenditure of 340 kcal. A resting control was also undertaken where participants rested throughout. Similar to Thompson et al. (1988) low intensity exercise did not produce any suppression of hunger either during or after the exercise session whereas high intensity exercise did. These
findings contradict those of the present study despite the exercise intensity being similar. However the exercise challenge was greater in the present study due to a larger energy expenditure and considering that cycling is weight supported it is posited that there was greater gastro intestinal upheaval during treadmill running.

The finding of no ‘exercise induced anorexia’ during high intensity running was unexpected considering that acylated ghrelin was suppressed in the high intensity exercise trial which contradicts the association found between the suppression of acylated ghrelin and ‘exercise induced anorexia’ seen in chapter 5. Participants undertaking high intensity treadmill running in the study described in chapter 5 and in the present study were still running at 0.5 hours and the exercise intensity was similar (74.0 ± 1.5 % \( \dot{VO}_{2\text{max}} \) present study versus 71.9 ± 2.1 % \( \dot{VO}_{2\text{max}} \) Chapter 5). In the present study, from baseline to 0.5 hours there was a mean decline of plasma acylated ghrelin concentration of 42% yet in chapter 5 the decline was only 30% which was temporarily associated with ‘exercise induced anorexia.’ Baseline hunger values reported in chapter 5 were higher than that in the present study and may have therefore been more susceptible to change. An exercise intensity and energy expenditure threshold for acylated ghrelin cannot be stated with confidence at this time as more work is needed.

The mechanism by which exercise suppresses acylated ghrelin concentration is uncertain. Growth hormone is secreted at increased exercise intensities and its releasing peptides GHRH are associated with changes in acylated ghrelin (Borer, 2003). It is impossible to ascertain links between changes in acylated ghrelin and GH in the present study as GH
was not measured. Future studies should therefore consider the measurement of GH but this is not feasible with the remaining studies of this thesis due to the cost implications.

The finding of a significant increase in plasma glucose during the high intensity treadmill running confirms the findings described in chapter 5 and since there was no significant increase in plasma glucose during low intensity exercise and no suppression of acylated ghrelin the exercise induced increase in glucose during high intensity treadmill running could be a mechanism for acylated ghrelin suppression during exercise. However figure 6.5 shows that post feeding there were similar increases in glucose which coincided with similar suppressions of acylated ghrelin (Figure 6.3) in all three trials. Therefore the mechanism by which exercise influences the relationship between glucose and ghrelin may differ from the mechanism by which feeding alters the relationship between glucose and ghrelin. The finding of no significant difference in plasma insulin concentration between the trials confirms the findings of the study described in chapter 5 and therefore changes in insulin cannot be responsible for the exercise induced changes to acylated ghrelin.

In conclusion, this study demonstrates that plasma acylated ghrelin concentration is reduced during an acute bout of high intensity but not low intensity treadmill running. Exercise intensity is therefore a key determinant of the acylated ghrelin response to exercise. Trends show that acylated ghrelin responds within 5 minutes to an exercise stimulus but this needs confirmation. Increases in glucose concentration during high intensity treadmill running is temporarily associated with a decline in acylated ghrelin but
the mechanism by which exercise alters acylated ghrelin may differ from that by which feeding alters acylated ghrelin as similar increases in glucose were seen in all three trials post feeding. Findings of no ‘exercise induced anorexia’ were surprising and contradict previous findings of previous studies presented in this thesis.
CHAPTER VII

The effect of exercise duration on acylated ghrelin

7.1 Introduction

The findings of the studies presented in this thesis so far indicate that acylated ghrelin is suppressed during high intensity treadmill running (Chapters 5 and 6) and exercise intensity is a determinant as no suppression was seen in a low intensity exercise trial (Chapter 6). The author has also demonstrated that exercise intensity is a determinant of 'exercise induced anorexia' as suppressed feelings of hunger were seen in a high but not a low intensity exercise trial (Chapter 4) and high intensity trial compared with control (Chapter 5). However no 'exercise induced anorexia' was seen in either high or low intensity exercise trials (Chapter 6) which was surprising since there was a suppression of acylated ghrelin in the high intensity exercise trial and associations between the suppression of acylated ghrelin and 'exercise induced anorexia' have been shown previously (Chapter 5). It was posited that despite a suppression of acylated ghrelin subjective feelings of hunger were not suppressed post exercise as the energy expenditure was only 600 kcal since the duration of the high intensity exercise was only short.

The effect of exercise duration on 'exercise induced anorexia' has been examined previously. King et al (1994) recruited twelve participants who undertook two high intensity cycling bouts of short duration (mean time 25.9 minutes; mean 77% \( \dot{V}O_2 \) max), long duration (mean time 51.8 minutes; mean 74% \( \dot{V}O_2 \) max) and a resting control. Compared with control hunger was suppressed during both the short and long duration exercise sessions and remained suppressed immediately post exercise but
returned to baseline values after 10 minutes. As expected the hunger suppression was
greatest after the long duration exercise session showing that exercise duration is a
determinant of the extent of 'exercise induced anorexia'. Potential changes to
hormones and metabolites could not be identified since blood samples were not taken
so these responses need to be examined.

Despite numerous studies examining the exercise response to ghrelin (Burns et al,
2007; Dall et al, 2002; Jürimäe et al, 2007; Kallio et al, 2001; Kraemer et al, 2004;
Schmidt et al, 2004; Vestergaard et al, 2007) and accumulating evidence of the
plasma acylated ghrelin response to exercise as a result of the work presented in this
thesis, to the author's knowledge no study has examined the effect of exercise
duration on plasma acylated ghrelin whilst keeping the exercise intensity constant. As
suppressions of acylated ghrelin had been shown previously with high intensity
exercise (Chapter 5 and Chapter 6) the intensity of the exercise would be kept high in
the present study. In both Chapters 5 and 6 a target exercise intensity of 75% $V_{O_2}^{\text{max}}$
was set so it was decided that a target exercise intensity of 70% $V_{O_2}^{\text{max}}$ would be set
in the present study to examine if a lowering of the exercise challenge would result in
a different acylated ghrelin response. In chapters 5 participants ran for 60 minutes and
a mean time of 36 minutes in the high intensity trial in chapter 6, so short duration
exercise was set at 45 minutes and long duration exercise was set at 90 minutes.

It had been drawn to the author's attention that changes in self reported hunger ratings
may not reflect a decrease or increase in hunger because other factors affecting
hunger sensations such as feelings of nausea which have been found after long
duration vigorous exercise (Halvorsen and Ritland, 1992) may influence responses.
To confirm if any feelings of nausea were influencing subjective feelings of hunger a nausea VAS was compiled and implemented.

The findings presented in this thesis suggest a threshold of exercise intensity, duration and energy expenditure which when reached, exercise will stimulate changes in gut peptide hormones which could influence subjective feelings of hunger. Evidence is accumulating on the effects of exercise intensity and energy expenditure on acylated ghrelin but to the author’s knowledge, no study has examined the influence of exercise duration on plasma acylated ghrelin. Therefore, the purpose of the present study was to determine if acylated ghrelin concentrations are suppressed during and following a short and long duration bout of high intensity treadmill running. It was hypothesized that the longer duration treadmill running would be associated with a greater reduced concentration of plasma acylated ghrelin compared with the short duration exercise and that exercise would cause a temporary suppression of hunger which would again be greater in the long duration trial. The inclusion of a nausea VAS would confirm if feelings of sickness confound any suppressions of hunger seen during exercise.
7.2 Methods

7.2.1 Participants

Nine healthy males aged 20 to 28 years from the population at Loughborough University volunteered to participate in the study. Table 7.1 shows the physical characteristics of participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.2 ± 0.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.0 ± 1.6</td>
</tr>
<tr>
<td>Sum of Skinfolds (mm)</td>
<td>26.1 ± 1.3</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.8 ± 0.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>76.7 ± 0.6</td>
</tr>
<tr>
<td>(\text{VO}_2\text{max}) (mL·kg⁻¹·min⁻¹)</td>
<td>63.4 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 9)

7.2.2 Study design

All experimental procedures were conducted in the Health and Paediatric Exercise Physiology Laboratory at Loughborough University. After being made aware of the protocol, health screened, then giving written consent to take part, anthropometric data was collected after which each participant undertook two preliminary exercise tests as follows: 1) submaximal-incremental treadmill running test, 2) maximum oxygen uptake (\(\text{VO}_2\text{max}\)) treadmill running test. There was a 20 to 30 minute rest interval between the exercise tests.
During subsequent weeks participants undertook three main trials (short duration exercise, long duration exercise and control) and these were performed in a randomized order with an interval of at least 7 days.

For two days before the first main trial participants were asked to record their weighed food intake using a food record diary. The same food intake was then consumed for the two days prior to the next remaining trials. Participants were also asked to refrain from any form of vigorous activity and ingesting caffeine or alcohol 24 hours prior to the main trial. Participants were fasted for a minimum 10 hours, but were allowed to drink water *ad libitum*. Participants attended the laboratory between the hours of 08:00 and 09:00 and were requested to use motorised transport where possible or ensure that activity prior to attending the laboratory was light.

### 7.2.3 Main trials

Participants were given at least one week to recover from the preliminary exercise tests before embarking on the main trials. Each main trial began in the morning and lasted for 9 hours. At the start of the short duration exercise trial participants ran on the treadmill at a running speed predicted to elicit 70% of maximum oxygen uptake for 45 minutes. After the run, participants rested for 8.25 hours (sitting reading, writing, working at a computer or watching television). At the start of the long duration exercise trial participants ran on the treadmill at a running speed predicted to elicit 70% of maximum oxygen uptake for 90 minutes. After the run, participants rested for 7.5 hours (sitting reading, writing, working at a computer or watching television). For the control trial participants undertook exactly the same protocol but
rested throughout (9 hours). Participants were not devoid of time cues during the trials and a clock was on display in the laboratory throughout the trials.

One minute expired air samples were collected frequently during the runs. Running speed was adjusted after each expired air collection if the oxygen consumption was above or below the predicted value or if the subject expressed that they were experiencing difficulties.

Figure 7.1 shows a schematic representation of the study protocol.
Figure 7.1  Schematic representation of the main trial protocol

Exercise 70% \( \text{VO}_{2\text{max}} \) for 45 min, 90 min or rest

Baseline 1 2 3 4 5 6 7 8 9

Key:

- Acylated Ghrelin, Insulin haematocrit and haemoglobin
- Glucose
- Visual Analogue Scales, Temperature and Humidity
- Samples of Expired Air
7.2.4 Subjective measurement of hunger and nausea

On arrival, participants completed an RPH scale compiled by Burns et al (2007) which ranged from 0 'Not Hungry' to 15 'Very Hungry'. Hunger measurements were recorded at baseline, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 hours. At the same sampling points participants also completed a nausea VAS by placing a vertical mark on a 10 cm horizontal line ranging from 0 'Not at all nauseous' to 100 'Very nauseous.'

7.2.5 Test meal

Participants were fed two 3230 kJ (772 kcal) test meals at 2 and 6 hours from baseline measurements for all trials. Each meal consisted of 77g of white bread, 33g of cheddar cheese, 12g of mayonnaise, 12g butter, 30g of potato crisps, 7g of milkshake powder and 150g of whole milk based on a 70 kg person. The macronutrient content was 33% carbohydrate, 11% protein and 56% fat. The amount of each food stuff was adjusted for each participant based on their bodyweight on the day of first trial and identical amounts were consumed on the remaining trials. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times on both trials. Water was available ad libitum during trials and the volume and time of ingestion were recorded.

7.2.6 Environmental temperature and humidity

Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.).
7.2.7 Blood sampling

Prior to the start of each trial participants rested in a semi-supine position while a cannula was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 9 mL EDTA monovettes at baseline, 0.75, 1.5, 2, 2.5, 3, 4, 5, 6, 6.5, 7, 8 and 9 hours. During the exercise trials, when participants were still running and a blood sample was due to be taken, blood was collected while the participants straddled the treadmill, which took approximately 1 minute. All other blood samples were collected whilst participants lay in a semi-supine position. The EDTA monovettes were spun at 4000 revs.min⁻¹ (1681 g) for 10 minutes in a refrigerated centrifuge at 4°C. The plasma supernatant was then aliquoted into Eppendorf tubes. These were stored at -80°C for analysis of glucose and insulin later.

Separate venous blood samples were drawn into 4.9 mL monovettes at baseline, 0.75, 1.5, 2, 3, 6, 7 and 9 hours for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 3500 revs.min⁻¹ (1287 g) for 10 minutes in a refrigerated centrifuge at 4°C. The supernatants were then aliquoted into storage tubes and 100 µL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 3500 revs.min⁻¹ (1287 g) for 5 min in a refrigerated centrifuge at 4°C before being transferred into Eppendorf tubes. The samples were then stored at -80°C for analysis later.

At each acylated ghrelin blood sampling point, duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and
triplicate blood samples were collected into heparinised micro haematocrit tubes for the determination of haematocrit.

7.2.8 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay. Plasma glucose concentrations were determined by enzymatic, colorimetric methods. Plasma insulin concentrations were determined by enzyme immunoassay. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 2.2%, insulin 4.73% and glucose 0.6%.

7.2.9 Statistical analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.). Plasma acylated ghrelin, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. One-factor ANOVA was used to assess differences in baseline hunger, nausea, acylated ghrelin, weight and fluid intake for the three trials. Repeated measures, two-factor ANOVA was used to examine differences between the three trials over time for acylated ghrelin, glucose, insulin, hunger, body mass and plasma volume change. Post-hoc pair wise comparisons were performed using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. There was no statistically significant change in plasma volume and hence for simplicity the unadjusted values are reported. Results are given as mean ± SEM unless otherwise stated.
7.3 Results

7.3.1 Responses to treadmill running

Table 7.2 shows the responses to treadmill running.

Table 7.2 Responses to treadmill running.

<table>
<thead>
<tr>
<th></th>
<th>Exercise Trial</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Short Duration</td>
<td>Long Duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Percentage $\overline{V}O_2_{\text{max}}$ (%)</td>
<td>70.1 ± 0.7</td>
<td>69.7 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean respiratory exchange ratio</td>
<td>0.93 ± 0.02</td>
<td>0.89 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross energy expenditure (kJ)</td>
<td>2918 ± 110</td>
<td>5949 ± 218</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross energy expenditure (kcal)</td>
<td>697 ± 26</td>
<td>1422 ± 52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage energy contribution from fat (%)</td>
<td>24 ± 3</td>
<td>33 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage energy contribution from carbohydrate (%)</td>
<td>76 ± 3</td>
<td>67 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average heart rate (b•min$^{-1}$)</td>
<td>169 ± 4</td>
<td>169 ± 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Median RPE | 13 | 14 | ‘fairly hard’ (range 13-14) | ‘fairly hard’ (range 13-14).

Values are mean ± SEM (n = 9)

There was no significant difference in exercise intensity between the two trials ($P = 0.630$).

7.3.2 Body mass and fluid consumption

Two-factor ANOVA revealed changes in body weight which were not significant. On the control trial body mass was 71.9 ± 1.7 kg at the start of the trial and 72.2 ± 1.7 kg at the end of the trial. On the short duration exercise trial body mass was 72.0 ± 1.8 kg at the start of the trial and 72.0 ± 1.2 kg at the end of the trial. On the long duration exercise trial body mass was 72.0 ± 1.7 kg at the start of the trial and 71.7 ± 1.8 kg at...
the end of the trial. There was a trend (One-factor ANOVA, \( P = 0.001 \)) for water consumption to be higher during the exercise trials compared with control. When performing the Bonferroni method differences lie between the control (1433 ± 237 mL) and long duration exercise trial (2348 ± 315 mL) (\( P = 0.001 \)) as well as the long duration exercise trial and short duration exercise trial (1532 ± 240 mL) (\( P = 0.048 \)).

7.3.3 Temperature and humidity

There was no significant difference in environmental temperature between the control, short duration and long duration exercise trials (23.9 ± 0.3 versus 23.7 ± 0.3 versus 24.4 ± 0.5 °C respectively). Likewise there was no significant difference in humidity between the control, short duration and long duration exercise trials (42.3 ± 1.6 versus 40.3 ± 0.9 versus 39.1 ± 1.9 % respectively).

7.3.4 Hunger

Fasting hunger did not differ (\( P = 0.943 \)) between the control, short and long duration exercise trials (7 ± 1 versus 7 ± 1 versus 7 ± 1 mean values respectively). Two-factor ANOVA revealed a main effect of trial (\( P = 0.039 \)), time (\( P < 0.0005 \)) and a trial × time interaction effect (\( P < 0.0005 \)) for hunger indicating that responses differed over time between the exercise and control trials. Post hoc analysis indicated between trial differences between the control and short duration trial (\( P = 0.003 \)). Post hoc analysis indicated between trial differences between control and short duration at 0.5, 0.75, 1, 1.5, and 2 hours (Figure 7.2). There were further between trial differences between the control and long duration exercise trial at 0.5, 0.75, 1, 1.5 and 2 hours (Figure 7.2). At 2 hours the difference between short duration and long duration exercise was approaching significance (\( P = 0.052 \)).
Subjective feelings of hunger using the RPH scale during the short duration, long duration exercise and control trials. Values are mean ± SEM, n = 9. The checked rectangle indicates the short duration treadmill run. The diagonal patterned rectangle indicates the long duration treadmill run. The black rectangles indicate consumption of the test meals. a Control different from short duration exercise \( P < 0.05 \), b Control different from long duration exercise \( P < 0.05 \).

Between trial differences in hunger ratings were also evaluated using AUC values for the first 0.75 hour of the trial (0 to 0.75 hours), the first 1.5 hours of the trial (0 to 1.5 hours), and the full 9 hours. One-factor ANOVA confirmed a difference between the control and short duration exercise trial for the first 0.75 hours: 6 versus 4 (mean values) respectively \( (P = 0.006) \) as well as between control and long duration exercise: 6 versus 4 (mean values) respectively \( (P = 0.018) \). One-factor ANOVA confirmed a difference between the control and short duration exercise trial for the first 1.5 hours: 14 versus 9 (mean values) respectively \( (P < 0.0005) \) as well as between control and long duration exercise: 14 versus 7 (mean values) respectively \( (P = 0.004) \). One-factor ANOVA confirmed a difference between the control and short
duration exercise trial for the full 9 hours: 66 versus 50 (mean values) respectively ($P = 0.011$) but there was no difference between control and long duration ($P = 0.141$) and short duration and long duration exercise ($P = 1.00$).

### 7.3.5 Nausea

Fasting nausea did not differ ($P = 0.512$) between the control, short and long duration exercise trials ($12 \pm 4$ *versus* $11 \pm 4$ *versus* $13 \pm 3$ mean values respectively). Two-factor ANOVA revealed no significant effect of trial, time or trial x time interaction (Figure 7.3) but time was approaching significance ($P = 0.078$). The data was examined further using a planned contrast using the Helmert statistical test to examine the difference between control and both runs and the difference between runs. There was no significant difference between the control and both runs ($P = 0.133$) and between short and long duration runs ($P = 0.460$).

![Figure 7.3](image)

**Figure 7.3** Subjective feelings of nausea using a VAS during the short duration, long duration exercise and control trials. Values are mean ± SEM, $n = 9$. The checked rectangle indicates the short duration treadmill run. The
Between trial differences in nausea ratings were also evaluated using AUC values for the first 0.75 hour of the trial (0 to 0.75 hours), the first 1.5 hours of the trial (0 to 1.5 hours), and the full 9 hours. One-factor ANOVA confirmed no differences between the control, short duration and long duration exercise trial for the first 0.75 hours: 8 versus 11 versus 12 (mean values) respectively ($P = 0.163$). One-factor ANOVA confirmed no differences between the control, short duration and long duration exercise trial for the first 1.5 hours: 15 versus 24 versus 32 (mean values) respectively ($P = 0.071$). One-factor ANOVA confirmed no differences between the control, short duration and long duration exercise trials for the full 9 hours: 95 versus 118 versus 129 (mean values) respectively ($P = 0.198$).

A repeated measures ANOVA was also undertaken for the first 1.5 hours. There was no significant effect of trial or trial x time but these were approaching significance ($P = 0.76$ and $P = 0.64$ respectively). The main effect of time was significant ($P = 0.006$) so a planned contrast using the Helmert statistical test to examine the effect of control versus runs with a 'repeated on time' analysis to examine differences over time. There was a significant difference between control and both runs ($P = 0.016$) but no difference between runs ($P = 0.371$). There was a significant trial x time interaction ($P = 0.009$) so changes over time were examined between 0-0.5, 0.5-0.75, 0.75-1 and 1-1.5 hours and there was a significant ($P = 0.017$) increase in nausea between 0.5 and 0.75 hours showing that nausea did increase during the run.
7.3.6 Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ \( (P = 0.775) \) between the control, short and long duration exercise trials \( (158.6 \pm 46.6 \text{ versus } 162.9 \pm 46.8 \text{ versus } 153.4 \pm 42.7 \text{ pg·mL}^{-1} \text{ respectively}) \). Two-factor ANOVA revealed a main effect of trial \( (P = 0.001) \), a main effect of time that was approaching significance \( (P = 0.063) \) but no trial \( \times \) time interaction \( (P = 0.097) \) for acylated ghrelin concentrations (Figure 7.4). Post hoc analysis indicated between trial differences between the control and short duration trial \( (P = 0.041) \) and between the control and long duration trial \( (P = 0.005) \). Post hoc analysis indicated between trial differences between control and short duration at 0.75 hours (Figure 7.2). There were further between trial differences between the control and long duration exercise trial at 0.75 and 1.5 hours and differences that were approaching significance at 3 \( (P = 0.077) \) and 6 \( (P = 0.070) \) hours (Figure 7.2). At 1.5 hours the difference between short duration and long duration exercise was significant.
Figure 7.4  Acylated ghrelin concentration during the short duration, long duration exercise and control trials. Values are mean ± SEM, n = 9. The checked rectangle indicates the short duration treadmill run. The diagonal patterned rectangle indicates the long duration treadmill run. The black rectangles indicate consumption of the test meals. a Control different from short duration exercise $P < 0.05$, b Control different from long duration exercise $P < 0.05$, c Short different from long duration exercise $P < 0.05$.

Between trial differences for acylated ghrelin were also evaluated using AUC values for the first 0.75 hour of the trial (0 to 0.75 hours), the first 1.5 hours of the trial (0 to 1.5 hours), and the full 9 hours. One-factor ANOVA confirmed a difference between the control and long duration exercise trial for the first 0.75 hours: $123.3 \pm 30.0$ versus $95.4 \pm 26.1$ pg·mL$^{-1}$·0.75 hours respectively ($P = 0.032$). One-factor ANOVA confirmed a difference between the control and long duration exercise trial for the first 1.5 hours: $248.3 \pm 57.9$ versus $170.6 \pm 52.6$ pg·mL$^{-1}$·1.5 hours respectively ($P = 0.004$). One-factor ANOVA confirmed a difference between the control and short duration exercise trial for the full 9 hours: $1360.4 \pm 279.8$ versus $968.9 \pm 196.9$ pg·mL$^{-1}$·9 hours respectively ($P = 0.024$) as well as between the control and long...
duration exercise trial for the full 9 hours: 1360.4 ± 279.8 versus 961.9 ± 218.3 pg·mL⁻¹·9 hours respectively ($P = 0.018$) (Figure 7.5).

![Graph showing AUC for acylated ghrelin](image)

**Figure 7.5** Total area under the concentration versus time curve (AUC) for plasma acylated ghrelin (mean ± SEM, $n = 9$). Values are for the first 0.75 hours of the trial (pg·mL⁻¹·0.75 hours), for the first 1.5 hours of the trial (pg·mL⁻¹·1.5 hours) and for the full 9 hours of the trial (pg·mL⁻¹·9 hours). *a* Control different from short duration exercise $P < 0.05$, *b* Control different from long duration exercise $P < 0.05$.

Fasting plasma acylated ghrelin concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting hunger, fasting plasma insulin concentrations or fasting plasma glucose concentrations.
7.3.7 Glucose

Fasting plasma glucose concentrations did not differ significantly ($P = 0.946$) between control, short duration and long duration exercise trials ($5.0 \pm 0.1$ versus $5.1 \pm 0.1$ versus $5.1 \pm 0.2$ mmol·L$^{-1}$ respectively). Two-factor ANOVA revealed no main effect of trial ($P = 0.084$), a main effect of time ($P < 0.0005$) and a trial x time interaction ($P = 0.017$) for plasma glucose (Figure 7.6). Post hoc analysis indicated between trial differences between control and short duration at 0.75 hours (Figure 7.6). There were further trial differences between the control and long duration exercise trial at 0.75 hours and a difference that was approaching significance at 3 hours ($P = 0.077$) (Figure 7.6). There was no significant differences between the short duration and long duration exercise.

![Figure 7.6](image)

**Figure 7.6**  Plasma glucose concentration during the short duration, long duration exercise and control trials. Values are mean ± SEM, $n = 9$. The checked rectangle indicates the short duration treadmill run. The diagonal patterned rectangle indicates the long duration treadmill run. The black rectangles indicate consumption of the test meals. $^a$ Control different from short duration exercise $P < 0.05$, $^b$ Control different from long duration exercise $P < 0.05$. 

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Between trial differences for plasma glucose were also evaluated using AUC values for the first 0.75 hour of the trial (0 to 0.75 hours), the first 1.5 hours of the trial (0 to 1.5 hours), and the full 9 hours. One-factor ANOVA confirmed a difference between the control and short duration exercise trial for the first 0.75 hours: 3.7 ± 0.1 versus 4.2 ± 0.1 mmol·L⁻¹·0.75 hours respectively (\(P = 0.045\)) and between the control and long duration exercise trial: 3.7 ± 0.1 versus 4.0 ± 0.1 mmol·L⁻¹·0.75 hours respectively (\(P = 0.045\)). One-factor ANOVA confirmed no differences between the control, short and long duration exercise trials for the first 1.5 hours: 7.4 ± 0.1 versus 8.0 ± 0.2 versus 7.7 ± 0.2 mmol·L⁻¹·1.5 hours respectively (\(P = 0.059\)). One-factor ANOVA confirmed no difference between the control, short and long duration exercise trials for the full 9 hours: 43.1 ± 0.7 versus 45.3 ± 0.6 versus 45.2 ± 0.6 mmol·L⁻¹·9 hours respectively (\(P = 0.151\)).

7.3.8 Insulin

Fasting plasma insulin concentrations did not differ significantly (\(P = 0.834\)) between control, short duration and long duration exercise trials (26.55 ± 5.42 versus 24.1 ± 4.85 versus 24.35 ± 7.35 pmol·L⁻¹ respectively). Two-factor ANOVA revealed a main effect of time (\(P < 0.0005\)) but no effect of trial (\(P = 0.461\)) and no trial × time interaction (\(P = 0.440\)) for plasma insulin (Figure 7.7). Post hoc analysis indicated between trial differences between control and short duration at 0.75 hours (Figure 7.7). There were further between trial differences between the short and long duration exercise trial at 2 hours (Figure 7.7). There were no differences between control and long duration exercise.
Figure 7.7  Plasma insulin concentration during the short duration, long duration exercise and control trials. Values are mean ± SEM, \( n = 9 \). The checked rectangle indicates the short duration treadmill run. The diagonal patterned rectangle indicates the long duration treadmill run. The black rectangles indicate consumption of the test meals. \(^a\) Control different from short duration exercise \( P < 0.05 \), \(^c\) Short different from long duration exercise \( P < 0.05 \).

Between trial differences for plasma insulin were also evaluated using AUC values for the first 0.75 hour of the trial (0 to 0.75 hours), the first 1.5 hours of the trial (0 to 1.5 hours), and the full nine hours. One-factor ANOVA showed a difference between the control and short duration exercise trial for the first 0.75 hours: \( 18.8 \pm 3.0 \) versus \( 28.1 \pm 4.4 \) pg·mL\(^{-1}\)·0.75 hours respectively \( (P = 0.044) \). One-factor ANOVA showed a difference between the short and long duration exercise trials for the first 1.5 hours: \( 53.9 \pm 8.4 \) versus \( 29.7 \pm 5.7 \) pg·mL\(^{-1}\)·1.5 hours respectively \( (P = 0.036) \) and a difference between the control trial and short duration trial that was approaching significance: \( 34.7 \pm 5.6 \) versus \( 53.9 \pm 8.4 \) pg·mL\(^{-1}\)·1.5 hours respectively \( (P = 0.055) \).
One-factor ANOVA confirmed no difference between the control, short and long duration exercise trials for the full 9 hours: 679.6 ± 90.7 versus 671.8 ± 80.2 versus 616.5 ± 81.4 pg·mL⁻¹·9 hours respectively (P = 0.799).
7.4 Discussion

To the author's knowledge, the present study is the first to examine the effect of exercise duration on plasma acylated ghrelin concentrations during exercise. The novel finding arising from this study is that there is a suppression of plasma acylated ghrelin during both short and long duration exercise and the suppression occurs whilst exercise is ongoing.

At 0.75 hours plasma acylated ghrelin was lower on the short and long duration exercise trials compared with control. (94.8 ± 43.2 versus 100.9 ± 38.2 versus 170.2 ± 46.3 pg·mL⁻¹ respectively). The findings therefore confirm those of chapter 5 and 6 that acylated ghrelin is modestly suppressed in response to high intensity treadmill running even though the exercise intensity was slightly lower at 70% VO₂ max. In the short duration exercise trial participants ceased exercise at 0.75 hours at which point plasma acylated ghrelin values began to return to baseline and were approaching control values at 1.5 hours (142.9 ± 42.4 versus 163.3 ± 40.8 pg·mL⁻¹ respectively). However, in the long duration exercise trial participants remained exercising for a further 0.75 hours and plasma acylated ghrelin concentration remained suppressed. The findings therefore show that exercise duration is a determinant of the extent of the acylated ghrelin response to exercise when the exercise intensity is kept constant.

Findings also show that the suppression of acylated ghrelin plateaus after 0.75 hours during the long duration treadmill run. Whilst acylated ghrelin values remain suppressed there is no further decline and values remain similar at 0.75 and 1.5 hours (100.9 ± 38.2 versus 99.6 ± 44.6 pg·mL⁻¹ respectively). This shows that depending on the exercise stimulus, acylated ghrelin declines to a point after which if there is no
change to the exercise intensity there will be no change in the acylated ghrelin response. All the previous studies in this thesis measuring acylated ghrelin have shown that values begin to return to baseline upon the cessation of exercise so it is assumed that if the exercise intensity was reduced but participants continued to exercise, acylated ghrelin concentration would remain suppressed but values would begin to return to baseline. It is also posited that an increase in exercise intensity would result in a greater suppression of acylated ghrelin but this is highly speculative and warrants further investigation as a plateau of acylated ghrelin suppression may exist after which no increase in exercise intensity, duration or expenditure will increase the suppression of acylated ghrelin further.

Acylated ghrelin values reported are similar to that reported in chapters 5 and 6 and no participant displayed atypically high acylated ghrelin values. It has been suggested in chapter 6 that participants who are of ethnic origin have a tendency to display higher acylated ghrelin values possibly due to genetic differences and this is supported by the findings of the present study as all participants were Caucasian.

In addition to the decline in acylated ghrelin concentrations during exercise, acylated ghrelin concentrations declined after feeding. The present study used two test meals fed at 2 and 6 hours and acylated ghrelin was measured 1 hour after the initiation of feeding. One hour after the initiation of the first test meal there was a 32% suppression in the control trial, a 39% suppression in the short duration trial and a 35% suppression in the long duration trial. One hour after the initiation of second test meal there was a 36% suppression in the control trial, a 40% suppression in the short duration trial and a 35% suppression in the long duration trial. As a result of exercise
plasma acylated ghrelin concentrations are lower than resting control values but exercise does not seem to affect the acylated ghrelin response to meals.

The study described in Chapter 5 used a high fat test meal fed at 3 hours in which the macronutrient content was 38% CHO, 10% protein and 52% fat providing 4201 kJ (1004 kcal) based on a 70 kg person. This led to a 30% decline in plasma acylated ghrelin concentration 1 hour after the meal in the control trial and a 27% decline in the exercise trial. The macronutrient content of each test meal in the present study was 33% CHO, 11% protein and 56% fat providing 3230 kJ (772 kcal). Despite a lower energy content of the test meals employed in the present study the percentage of fat is greater which co-incides with declines in plasma acylated ghrelin concentration that are greater than that reported in chapter 5. This provides further evidence that meal composition could affect the suppression of acylated ghrelin.

Another key finding from the present study is that both short and long duration exercise lead to ‘exercise induced anorexia’ and the suppression of subjective feelings of hunger is present whilst exercise is ongoing. This is consistent with the findings of King et al (1994) who also found that compared with control, hunger was suppressed during both short and long duration exercise sessions and occurred whilst exercise was ongoing. Findings therefore provide further evidence that exercise duration is a determinant of the extent of ‘exercise induced anorexia.’ King et al (1994) used a different VAS making comparisons difficult but trends are similar. In both studies hunger declines during exercise and begins to return to baseline values post exercise yet ‘exercise induced anorexia’ is not short lived in the present study. Whereas King et al (1994) reported that feelings of hunger had returned to baseline values within 10-15 minutes after the cessation of exercise in the present study hunger had not returned
to baseline 1.25 hours after short duration exercise or for 0.5 hours after long duration exercise. Unfortunately the time it would have taken for values to return to baseline cannot be assessed as a test meal was given. Whilst the exercise intensity was lower in the present study the more prolonged 'exercise induced anorexia' reported in the present study is likely because treadmill running as opposed to cycling was used for a longer duration, greater energy expenditure and greater gut upheaval. The exercise challenge in the present studies short duration trial was greater than that of the long duration trial in King et al (1994) since participants were exercising for a mean 51.8 ± 2.4 minutes with a gross energy expenditure of 541 ± 52 kcal whereas the short duration trial in the present study was 45 minutes with a gross energy expenditure of 697 ± 26 kcal.

It had been drawn to the author’s attention that suppressed feelings of hunger may not be a result of exercise per se but due to other sensations such as feelings of nausea which have been found after long duration vigorous exercise (Halvorsen and Ritland, 1992). To confirm if any feelings of nausea were influencing subjective feelings of hunger, a nausea VAS was compiled and implemented and the findings show that there is a significant increase in nausea during exercise. The finding of a significant difference of nausea in the presence of a significant hunger suppression shows that during exercise, 'exercise induced anorexia' may be confounded by feelings of nausea.

In terms of causal mechanisms the findings confirm those of chapter 5 that suppressed acylated ghrelin is temporarily associated with 'exercise induced anorexia'. It cannot be stated with confidence that suppressed acylated ghrelin is the sole mechanism for
'exercise induced anorexia' since no association was seen in chapter 6 and since hunger regulation is complex involving the interplay of many other gut peptide hormones more work is needed particularly for the hormone PYY as to the authors knowledge there is only one published study. Likewise the mechanism in which acylated ghrelin is suppressed is not fully understood but the finding of a significant increase in plasma glucose during both the long and short duration treadmill runs confirms the findings described in chapter 5 and 6. Increase in glucose concentrations during high intensity treadmill running could therefore be a mechanism for acylated ghrelin suppression. However, figure 7.6 shows that post feeding there were similar increases in glucose which coincided with similar suppressions of acylated ghrelin in all three trials providing further evidence that the mechanism by which exercise alters acylated ghrelin may differ from that by which feeding alters acylated ghrelin as discussed previously in chapter 6.

The finding of a significant increase in plasma insulin concentration during the short duration trial at 0.75 hours is perplexing considering that participants were working at similar exercise intensities and energy expenditures as in the long duration trial. Regardless a suppression of acylated ghrelin has arisen with an increase in insulin during the short duration trial and no increase in the long duration trial confirming the findings of chapters 5 and 6 that is likely that changes in insulin cannot be responsible for the exercise induced changes to acylated ghrelin.

In conclusion, this study demonstrates that plasma acylated ghrelin concentration is reduced during an acute bout of high intensity short and long duration treadmill running and is more prolonged in the long duration exercise trial. Suppressions of
acylated ghrelin co-incide with ‘exercise induced anorexia’ which again is more prolonged in the long duration exercise trial and the effect may not be short lived as hunger was suppressed for some time after exercise. Exercise duration is therefore a key determinant of the acylated ghrelin and hunger response to exercise. Whilst associations have been seen causal mechanisms cannot be stated with confidence as the findings are mixed and more work is needed. Finally, suppressed hunger may be confounded by associated feelings of nausea.
CHAPTER VIII

Effects of resistance and aerobic exercise on total PYY and acylated ghrelin

8.1 Introduction

The majority of studies examining the effect of exercise on ghrelin have used aerobic exercise protocols using cycling (Dall et al, 2002; Kallio et al, 2001; Schmidt et al, 2004; Vestergaard et al, 2007), rowing (Jürimäe et al, 2007) or treadmill running (Burns et al, 2007; Kraemer et al, 2004a) including the studies reported in chapters 5, 6 and 7 which have measured acylated ghrelin. However there is some evidence that hormonal responses may vary depending on the mode of exercise (Kraemer and Castracane, 2007) and few studies have looked at resistance exercise. Kraemer et al (2004b) found a reduction in ghrelin following concentric muscle contractions which was supported by Ghanbari-Niaki (2006) who found that ghrelin decreased significantly after three circuits of 10 resistance exercises at 60% of 1 rep max. However, Takano et al (2005) found no change with short term low intensity resistance exercise so the findings are mixed and need confirmation. To the author’s knowledge the influence of resistance exercise on acylated ghrelin has not been examined.

Despite a growing evidence base on the influence of exercise on total and acylated ghrelin research measuring other gut peptide hormones is scarce. PYY is a 36 amino acid hormone secreted from endocrine L-cells of the distal ileum and colon (Bottcher et al, 1984). It exists in two endogenous forms, PYY1-36 and PYY3-36. Increased concentrations of PYY3-36 inhibits food intake by altering central nervous system appetite circuits within the arcuate nucleus of the hypothalamus or area postrema.
PYY is a potent inhibitor of food intake and increased concentrations leads to reduced food intake.

To the author's knowledge only one study has examined the effects of exercise on PYY. Martins et al (2007) showed an increase in total PYY with intermittent moderate intensity cycling. This coincided with suppressed hunger suggesting that exercise induced changes to total PYY also leads to 'exercise induced anorexia'. There appears to be no evidence on the effects of treadmill running or resistance exercise on PYY. Therefore, the primary purpose of the present study was to determine plasma acylated ghrelin and total PYY concentrations during and following treadmill running and resistance exercise to see if there are any associations between subjective feelings of hunger and the exercise induced response of these gut peptide hormones. It was hypothesized that exercise would cause a temporary suppression of hunger and that this would co-incide with reduced concentrations of plasma acylated ghrelin and increased concentrations of total PYY.
8.2 Methods

8.2.1 Participants

Twelve healthy Caucasian males aged 19 to 23 years from the population at Loughborough University volunteered to participate in the study. Table 8.1 shows the physical characteristics of participants.

Table 8.1: Physical characteristics of participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(Mean ± SEM)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>21.2 ± 0.3</td>
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<tr>
<td>Height (m)</td>
<td>1.79 ± 0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.4 ± 2.4</td>
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<tr>
<td>Sum of Skinfolds (mm)</td>
<td>29.2 ± 1.7</td>
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<tr>
<td>BMI (kg·m⁻²)</td>
<td>23.2 ± 0.4</td>
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<tr>
<td>Body Fat (%)</td>
<td>13.5 ± 0.8</td>
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<tr>
<td>Waist Circumference (cm)</td>
<td>78.6 ± 1.1</td>
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<tr>
<td>(\dot{V}O_{2\text{max}}) (mL·kg⁻¹·min⁻¹)</td>
<td>62.0 ± 1.8</td>
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</table>

Values are mean ± SEM (n = 12)

The physical characteristics of the subjects (mean ± SEM) were as follows: age 21.2 ± 0.3 years, height 1.79 ± 0.02 m, body mass 74.4 ± 2.4 kg, BMI 23.2 ± 0.4 kg·m⁻², waist circumference 78.6 ± 1.1 cm, maximum oxygen uptake 62.0 ± 1.8 mL·kg⁻¹·min⁻¹ (4.58 ± 0.13 L·min⁻¹).

8.2.2 Study design

All experimental procedures were conducted in the Health and Paediatric Exercise Physiology Laboratory at Loughborough University. After being made aware of the protocol, health screened, then giving written consent to take part, anthropometric data was collected after which each participant undertook two preliminary exercise tests in one session as follows: 1) submaximal-incremental treadmill running test, 2)
maximum oxygen uptake ($\dot{V}O_2_{\text{max}}$) treadmill running test. There was a 30 minute rest interval between the exercise tests. Participants also undertook a 12 rep max resistance test and resistance familiarisation prior to starting the main trials on separate days with an interval of at least 3 days.

During subsequent weeks participants undertook three main trials (aerobic exercise, resistance exercise and control) and these were performed in a randomized order with an interval of at least 7 days. For two days before the first main trial participants were asked to record their weighed food intake using a food record diary. The same food intake was then consumed for the two days prior to the next remaining trials. Participants were also asked to refrain from any form of vigorous activity and ingesting caffeine or alcohol 24 hours prior to the main trials. Participants were fasted for a minimum 10 hours, but were allowed to drink water ad libitum. Participants attended the laboratory between the hours of 08:00 and 09:00 and were requested to use motorised transport where possible or ensure that activity prior to attending the laboratory was light.

8.2.3 12-repetition maximal test

The 12-repetition maximal test was completed for each of the 10 resistance exercises employed in the study. The order in which each resistance exercise was performed was the same for each participant i.e. squat, dumbbell lateral raise, bench press, upright row, lunges, bicep curl, barbell pullover, seated shoulder press, tricep extension and bent over row. The 12-repetition maximal values were determined by trial and error by adding or removing weights after each attempt, as required. Participants were allowed to take as long as they felt necessary to recover from each
8.2.4 Resistance familiarisation session

On a separate visit participants undertook the 90 minute resistance exercise session to ensure completion of 3 sets of each resistance exercise at 80% of 12 rep max and confirm fatigue from overload by the end. This was verified by visual inspection and verbal feedback from the participants and adjustments were made if necessary.

8.2.5 Main trials

Participants were given at least one week to recover from the preliminary exercise sessions before embarking on the main trials. Each main trial began in the morning and lasted for 8 hours.

8.2.5.1 Aerobic trial

At the start of the aerobic exercise trial participants ran on the treadmill for 60 minutes at a running speed predicted to elicit 70% of maximum oxygen uptake. One minute expired air samples were collected at 14-15, 29-30, 44-45 and 59-60 minutes during the run. Running speed was adjusted after each expired air collection if the oxygen consumption was above or below the predicted value or if the subject expressed that they were experiencing difficulties. After the run, participants rested for 7 hours (sitting reading, writing, working at a computer or watching television).
8.2.5.2 Resistance trial

At the start of the resistance trial participants completed a free weight session for 90 minutes performing three sets of 12 repetitions of 10 different weight lifting exercises at 80% of 12 repetition max. Three sets of 12 repetitions was used to maximise total energy expenditure and increase the likelihood of an exercise effect. Pilot work for a previous study (Burns et al, 2006) revealed that a higher exercise intensity would have prevented most participants from completing the session. Participants were given 3 minutes in which to complete each set. On completion of the 12 repetitions, participants rested for the remainder of the 3 minutes. An expired air sample was taken for 3 minutes during the third set for each exercise. Thus the whole exercise session lasted for 90 minutes (10 exercises x3 sets x 3min). Exercises were completed in the order described for the preliminary resistance sessions. All sets for one exercise were completed before moving onto the next exercise. After the resistance session, participants rested for 6.5 hours (sitting reading, writing, working at a computer or watching television).

8.2.5.3 Control trial

For the control trial participants undertook exactly the same protocol but rested throughout.

Participants were not devoid of time cues during the trials and a clock was on display in the laboratory throughout the trials.

Figure 8.1 shows a schematic representation of the study protocol.
Figure 8.1 Schematic representation of the main trial protocol

Exercise 68% VO\(_{2}\text{max}\) for 60 min, 90 min resistance or rest

Baseline

<table>
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</table>

Key:

- Acylated Ghrelin, PYY, insulin haematocrit and haemoglobin
- Hunger, Temperature and Humidity
- Glucose
- Samples of Expired Air during aerobic exercise
8.2.6 Calculation of energy expenditure

For the aerobic trial oxygen consumption and carbon dioxide production values were used to calculate energy expenditure using indirect calorimetry (Frayn, 1983). The short duration intermittent nature of weight lifting invalidates the typical assumptions of indirect calorimetry because the respiratory exchange ratio is consistently equal to or greater than 1.0. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per liter of oxygen (McArdle, 2001). This reflects the assumption that that energy was derived from carbohydrate rather than fat and assumes no protein contribution to energy provision during the exercise. This assumption may not be entirely valid and as no attempt was made to quantify the energy contribution from anaerobic sources, this would lead to underestimations of the energy expended during exercise.

8.2.7 Subjective measurement of hunger and nausea

On arrival, participants completed an RPH compiled by Burns et al (2007) which ranged from 0 ‘Not Hungry’ to 15 ‘Very Hungry’. Hunger measurements were recorded at baseline, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 hours. At the same sampling points participants also completed a nausea VAS by placing a vertical mark on a 10 cm horizontal line ranging from 0 ‘Not at all nauseous’ to 100 ‘Very nauseous’.

8.2.8 Test meal

Participants were fed a 3230 kJ (772 kcal) test meal at 2 hours and another at 5 hours. Each meal consisted of 77g of white bread, 12g of butter, 12g of mayonnaise, 33g of cheddar cheese, 30g of potato crisps, 150g of whole milk and 7g of milk shake powder based on a 70 kg person. The macronutrient content was 33% carbohydrate.
(133g), 11% protein (43g) and 56% fat (96g). The amount of each food stuff was adjusted for each participant based on their bodyweight on the day of first trial and identical amounts were consumed on the second trial. Participants were encouraged to consume the meal within 15 minutes and kept to the same start and finish times on both trials. Water was available ad libitum during trials and the volume and time of ingestion were recorded.

8.2.9 Environmental temperature and humidity

Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.).

8.2.10 Blood sampling

Prior to the start of the aerobic and control trials, participants rested in a semi-supine position while a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 9 mL EDTA monovettes (Sarstedt, Leicester, U.K.) at 0, 0.75, 1.5, 2, 2.5, 3, 4, 5, 5.5, 6, 7, 8 hours. During the aerobic trial at 0.75 hours, blood was collected while the participants straddled the treadmill which took approximately 1 minute. All other blood samples were collected whilst participants lay in a semi-supine position. For the resistance trial participants were venepunctured at baseline and at 0.75 hours and were given a few minutes to recover before continuing with the resistance session. At 1.5 hours a cannula was inserted and the blood sample was taken within 5 minutes. Remaining blood samples were taken via the cannula at the same sampling points as the other main trials.
The EDTA monovettes were spun at 1681 g (4000 revs·min⁻¹) for 10 minutes in a refrigerated centrifuge (Burkard, Hertfordshire, U.K.) at 4°C. The plasma supernatant was then aliquoted into Eppendorf tubes. These were stored at -80°C for analysis of total PYY, glucose and insulin later.

Separate venous blood samples were drawn into 4.9 mL monovettes at 0, 0.75, 1.5, 2, 2.5, 5, 5.5 and 8 hours for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 1287 g (3500 revs·min⁻¹) for 10 minutes in a refrigerated centrifuge at 4°C. The supernatants were then aliquoted into storage tubes and 100 µL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 1287 g (3500 revs·min⁻¹) for 5 min in a refrigerated centrifuge at 4°C before being transferred into Eppendorf tubes. The samples were then stored at -80°C for analysis later.

At each acylated ghrelin blood sampling point, duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and triplicate blood samples were collected into heparinised micro haematocrit tubes for the determination of haematocrit.

8.2.11 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay. Plasma glucose concentrations were determined by enzymatic, colorimetric methods. Plasma insulin concentrations were determined by radioimmunoassay. To eliminate inter-assay variation, samples from each participant were analyzed in the same run.
The within batch coefficients of variation for the assays were as follows: acylated ghrelin 4.8%, PYY 1.2%, glucose 3.3% and Insulin 3.3%.

8.2.12 Statistical analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.). Plasma acylated ghrelin, total PYY, hunger, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. One-factor ANOVA was used to assess differences between fasting and area under the curve values for acylated ghrelin, total PYY, hunger, glucose and insulin for the three trials. Repeated measures, two-factor ANOVA was used to examine differences between the three trials over time for acylated ghrelin, total PYY, glucose, insulin, hunger, body mass and plasma volume change. Post-hoc pair wise comparisons were performed using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. There was no statistically significant change in plasma volume and hence for simplicity the unadjusted values are reported. Results are given as mean ± SEM unless otherwise stated.
8.3 Results

8.3.1 Responses to treadmill running
The mean percentage of maximum oxygen uptake elicited during aerobic exercise was 68 ± 2.0% and the mean respiratory exchange ratio was 0.93 ± 0.01. Gross energy expenditure during aerobic exercise was 3847 ± 93 kJ (920 ± 22 kcal) with 25 ± 3.9% of energy provided from fat and 75 ± 3.9% of energy provided from carbohydrate. Average heart rate during running was 167 ± 3 beats·min⁻¹ and the median rating of perceived exertion (RPE) was 15 i.e. ‘hard’ (range 13-16).

8.3.2 Responses to resistance exercise
The mean weight lifted for each participant during the 90 minute resistance exercise session was 10 824 ± 645 kg working at 80 ± 0.3% of 12 rep max. The gross energy expenditure from resistance exercise was estimated to be 1488 ± 110 kJ (356 ± 26 kcal).

8.3.3 Body mass and fluid consumption
Two-factor ANOVA revealed no effect of trial (P = 0.217) for body mass. On the control trial body mass was 75.1 ± 2.6 kg at the start of the trial and 75.3 ± 2.6 kg at the end of the trial. On the resistance trial body mass was 74.7 ± 2.8 kg at the start of the trial and 74.8 ± 2.8 kg at the end of the trial. On the aerobic trial body mass was 74.5 ± 2.5 kg at the start of the trial and 74.8 ± 2.6 kg at the end of the trial. There was a difference (One-factor ANOVA, P < 0.001) in water consumption between the trials. Post hoc analysis revealed water consumption during the aerobic exercise trial (2089 ± 205 mL) was greater than that consumed during the control trial (1216 ± 189 mL).
mL, \( P < 0.001 \) but was not significantly different to that consumed during the resistance exercise trial (1572 ± 237 mL, \( P = 0.082 \)).

8.3.4 Temperature and humidity

There was no difference (Two-factor ANOVA, \( P = 0.457 \)) in environmental temperature between the control, resistance exercise and aerobic exercise trials (23.0 ± 0.5 versus 22.5 ± 1.3 versus 23.1 ± 0.4 °C respectively). Likewise there was no difference (Two-factor ANOVA, \( P = 0.654 \)) in humidity between the control, resistance exercise and aerobic exercise trials (37.3 ± 1.9 versus 38.4 ± 1.4 versus 36.0 ± 2.1% respectively).

8.3.5 Hunger

Fasting hunger did not differ significantly (One-factor ANOVA, \( P = 0.401 \)) between the control, aerobic and resistance trials (8 ± 1 versus 7 ± 1 versus 6 ± 1 respectively). Two-factor ANOVA revealed a main effect of time (\( P < 0.0005 \)) and a trial × time interaction effect (\( P < 0.001 \)) for hunger indicating that responses differed over time between the exercise and control trials. Post hoc analysis indicated between trial differences for aerobic exercise and control at 0.75, 1, 1.5 and 2 hours (Figure 8.2). Post hoc analysis indicated between trial differences for resistance exercise and control at 0.5 hours and differences were approaching significance at 1.5 (\( P = 0.051 \)) and 2 (\( P = 0.054 \)) hours (Figure 8.2).
Post hoc analysis indicated between trial differences for aerobic exercise and resistance at 0.5, 0.75 and 1 hours.

![Graph showing subjective feelings of hunger using the RPH scale during the aerobic exercise, resistance exercise, and control trials.](Image)

**Figure 8.2** Subjective feelings of hunger using the RPH scale during the aerobic exercise, resistance exercise, and control trials. Values are mean ± SEM, n = 12. The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals. *a* Control different from resistance exercise $P < 0.05$, *b* Control different from aerobic exercise $P < 0.05$, *c* Resistance different from aerobic exercise $P < 0.05$.

Between trial differences in hunger ratings were also evaluated using AUC values for the 2 hours prior to the first meal (0 to 2 hours), the 6 hours after the first meal (2 to 8 hours) and the full 8 hours (0 to 8 hours). A significant difference was found from 0 to 2 hours ($P = 0.005$) and post hoc analysis identified the difference was in the control compared with aerobic trial (19 versus 10 mean values respectively, $P = 0.004$). The difference nearly but did not quite reach significance in the resistance compared with
the control trial (13 versus 10 mean values respectively, \( P = 0.067 \)). There was no difference between AUC values for the 6 hours after the first meal (2 to 8 hours) or for the whole trial (0 to 8 hours) in all three trials.

8.3.6 Nausea

Fasting nausea did not differ \(( P = 0.231 \)) between the control, resistance and aerobic exercise trials (\( 15 \pm 6 \) versus \( 9 \pm 4 \) versus \( 7 \pm 3 \) mean values respectively). Two-factor ANOVA revealed a main effect of time \(( P = 0.007 \)) but no significant main effect of trial or trial x time interaction. The data was examined further using a planned contrast using the Helmert statistical test to examine the difference between control and both exercise modes combined and the difference between the run and resistance training. There was so significant difference between the control and both exercise modes \(( P = 0.754 \)) but there was a difference between aerobic exercise and resistance training \(( P = 0.049 \)). Using a repeated analysis these differences occurred significantly over time between 2 - 2.5 hours \(( P = 0.036 \)) and 4.5 - 5 hours \(( P = 0.016 \)).

8.3.7 Acylated ghrelin

It was not possible to read participant 9's acylated ghrelin values as these were greater than the highest standard and due to cost implications these could not be diluted and re assayed. Acylated ghrelin values are therefore only available for 11 participants. Fasting plasma acylated ghrelin concentrations did not differ significantly between the control, resistance and aerobic trials (\( 111.0 \pm 34.7 \) versus \( 103.7 \pm 28.8 \) versus \( 115.7 \pm 33.6 \) pg.mL\(^{-1} \) respectively, \( P = 0.648 \)). Two-factor ANOVA revealed a main effect of
time \( (P = 0.001) \) and a trial \times \text{time} \text{ interaction} \( (P = 0.035) \) for acylated ghrelin concentrations. Post hoc analysis indicated between trial differences for aerobic exercise and control at 0.75 hours (Figure 8.3). Post hoc analysis indicated between trial differences for resistance exercise and control at 0.75 and 1.5 hours (Figure 8.3). There were no differences between resistance and aerobic exercise.

![Acylated ghrelin concentrations during the aerobic exercise, resistance exercise and control. Values are mean ± SEM, \( n = 11 \). The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals. a Control different from resistance exercise \( P < 0.05 \), b Control different from aerobic exercise \( P < 0.05 \).](image)

**Figure 8.3** Acylated ghrelin concentrations during the aerobic exercise, resistance exercise and control. Values are mean ± SEM, \( n = 11 \). The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals. a Control different from resistance exercise \( P < 0.05 \), b Control different from aerobic exercise \( P < 0.05 \).

There was no significant difference (One-factor ANOVA) in the area under the acylated ghrelin concentration versus time curve for the first 0.75 hours of the trials. Area under the acylated ghrelin concentration versus time curve was 26% lower over the first two hours of the resistance exercise trial (One-factor ANOVA \( P = 0.014 \)) and 14.2% \( (P = 0.388) \) lower over the full 8 hours compared with the control trial (Figure 8.4). For the first two hours of the aerobic exercise trial, area under the acylated...
ghrelin concentration versus time curve was 17% lower \((P = 0.415)\) and 9% lower \((P = 0.949)\) over the full eight hours compared with the control trial.

![Acylated Ghrelin AUC](image)

**Figure 8.4** Total area under the concentration versus time curve (AUC) for plasma acylated ghrelin (mean ± SEM, \(n = 11\)). Values are for the first 0.75 hours of the trial (pg·mL\(^{-1}\)·0.75 hours), first 3 hours of the trial (pg·mL\(^{-1}\)·3 hours) and for the full 9 hours of the trial (pg·mL\(^{-1}\)·9 hours). \(^a\) Control different from resistance exercise \(P < 0.05\).

Fasting plasma acylated ghrelin concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting hunger, fasting plasma insulin concentrations or fasting plasma glucose concentrations.

### 8.3.8 Total PYY

Fasting total PYY concentrations did not differ significantly between the control, resistance and aerobic trials (127.7 ± 18.1 \(versus\) 142.7 ± 26.5 \(versus\) 145.8 ± 20.8 pg·mL\(^{-1}\) respectively, \(P = 0.525\)). Two-factor ANOVA revealed a main effect of trial
(P = 0.005), time (P < 0.0005) and a trial × time interaction (P = 0.027) for total PYY concentrations. Post hoc analysis indicated between trial differences between control and aerobic exercise (P = 0.038) and resistance and aerobic (P = 0.028).

Post hoc analysis revealed between trial differences for aerobic exercise and control at 1.5 and 2.5 hours (Figure 8.5). Post hoc analysis indicated between trial differences for resistance exercise and aerobic exercise at 1.5 and 2 hours and was approaching significance at 5.5 hours (P = 0.07). There were no significant differences between control and resistance exercise.

![Figure 8.5](image-url)  
Figure 8.5  Total PYY concentrations during the aerobic exercise, resistance exercise and control. Values are mean ± SEM, n = 12. The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals. Control different from aerobic exercise P < 0.05, Resistance different from aerobic exercise P < 0.05.

There was no significant difference (One-factor ANOVA P = 0.177) in the area under the total PYY concentration versus time curve for the first 0.75 hours of the trials.
Area under the total PYY concentration versus time curve was 38% higher over the first 2 hours of the aerobic exercise trial (One-factor ANOVA $P = 0.036$) and 22% higher over the full 8 hours ($P = 0.056$) compared with the control trial (Figure 8.6). There was no significant difference in the area under the total PYY concentration versus time curve over the first two hours or for the full eight hours of the control trial compared with resistance exercise.

Figure 8.6 Total area under the concentration versus time curve (AUC) for total PYY (mean ± SEM, $n = 12$). Values are for the first 0.75 hours of the trial (pg·mL$^{-1}$·0.75 hours), first 2 hours of the trial (pg·mL$^{-1}$·3 hours) and for the full 8 hours of the trial (pg·mL$^{-1}$·9 hours). $^b$ Control different from aerobic exercise $P < 0.05$.

Fasting total PYY concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting hunger, fasting plasma insulin concentrations or fasting plasma glucose concentrations.
8.3.9 Correlations between acylated ghrelin and total PYY

When removing 2 outliers fasting plasma acylated ghrelin concentrations were not significantly correlated with fasting total PYY \((r = 0.196 \, p = 0.614)\) (Figure 8.7).

![Figure 8.7](image)

**Figure 8.7**  Fasting acylated ghrelin and total PYY concentrations. \(n = 9\).

8.3.10 Glucose

Fasting plasma glucose concentrations did not differ significantly (One-factor ANOVA, \(p = 0.847\)) between the control, resistance and aerobic trial (5.0 ± 0.1 \(versus\) 5.1 ± 0.1 \(versus\) 5.0 ± 0.1 mmol·L\(^{-1}\) respectively). Two-factor ANOVA revealed a main effect of trial \((p < 0.0005)\), a main effect of time \((p < 0.0005)\) and a trial \(\times\) time interaction \((p = 0.016)\) for plasma glucose. Post hoc analysis indicated between trial differences between control and aerobic exercise \((p = 0.011)\) and resistance and aerobic exercise \((p = 0.001)\). Post hoc analysis indicated between trial differences for aerobic exercise and control at 0.75 hours (Figure 8.8). Post hoc analysis indicated
between trial differences for resistance exercise and aerobic exercise at 0.75, 3 and 4 hours. There were no significant differences between control and resistance exercise.

![Figure 8.8](image)

**Figure 8.8** Glucose concentrations during the aerobic exercise, resistance exercise and control. Values are mean ± SEM, n = 12. The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals. \(^{b}\) Control different from aerobic exercise \(P < 0.05\), \(^{c}\) Resistance different from aerobic exercise \(P < 0.05\).

For the first 2 hours, total area under the concentration versus time curve for plasma glucose was significantly higher on the aerobic exercise trial compared with the resistance trial (10.8 ± 0.5 versus 9.7 ± 0.2 mmol·L\(^{-1}\)·2 hours respectively, \(P = 0.039\)) but did not differ from the control trial (10.8 ± 0.5 versus 10.0 ± 0.2 mmol·L\(^{-1}\)·2 hours respectively, \(P = 0.150\)).

**8.3.11 Insulin**

Fasting plasma insulin concentrations did not differ significantly (One-factor ANOVA, \(P = 0.751\)) between the control, resistance or aerobic trials (22.3 ± 3.4
versus 24.7 ± 4.0 versus 23.6 ± 3.3 pmol·L⁻¹ respectively). Two-factor ANOVA revealed a main effect of time (P < 0.0005) with no significant differences between trials or trial x time interaction (Figure 8.9).

![Graph of insulin concentrations during exercise](image)

**Figure 8.9** Insulin concentrations during the aerobic exercise, resistance exercise and control. Values are mean ± SEM, n = 12. The checked rectangle indicates the treadmill run. The diagonal patterned rectangle indicates the resistance exercise. The black rectangles indicate consumption of the test meals.

For the first 2 hours, total area under the concentration versus time curve for plasma insulin was significantly higher on the resistance exercise trial compared with the control trial (58.7 ± 7.4 versus 38.4 ± 4.9 pmol·L⁻¹·2 hours respectively, P = 0.004) but did not differ from the aerobic trial (58.7 ± 7.4 versus 63.1 ± 20.1 pmol·L⁻¹·2 hours respectively, P = 1.0).
8.4 Discussion

To the author's knowledge, the present study is the first to examine plasma acylated ghrelin and total PYY concentrations during resistance exercise. The novel findings arising from this study are that there is a suppression of plasma acylated ghrelin and hunger during resistance exercise but no increase in total PYY. These findings confirm those found in chapters 5, 6 and 7 that acylated ghrelin is suppressed during high intensity treadmill running and that of Martins et al (2007) that total PYY is increased during aerobic exercise.

Findings show that acylated ghrelin is suppressed during aerobic exercise compared with control confirming the findings of that found in chapter 5. Whilst the duration of the run was the same (60 minutes) the exercise intensity of aerobic running in the present study (68 ± 2.0% V\textsubscript{O\textsubscript{2}}\text{max}) was slightly lower than that previously reported in chapter 5 (72 ± 2.0% V\textsubscript{O\textsubscript{2}}\text{max}) but similar suppressions were seen. The present study also confirms that acylated ghrelin concentrations begin to return to baseline values soon after post exercise.

Kraemer et al (2004b) found no suppression of total ghrelin during resistance exercise. Whilst Kraemer et al (2004b) do not report the energy expenditure of their resistance protocol, 4 sets of 12 repetitions for four whole body exercises were completed at 80% of 10 rep max so whilst it is assumed the energy expenditure is greater in the present study, the protocol is still quite challenging. A novel finding from this study is that resistance exercise causes a suppression of acylated ghrelin during and immediately post exercise returning to baseline values within 0.5 hours.
The evidence is accumulating that acylated ghrelin responds differently to an exercise stimulus compared with total ghrelin, although a limitation of the study with regard to this latter finding is that total ghrelin concentrations were not measured. Despite plasma total ghrelin not being measured in the present study, in a previous study using a similar aerobic exercise protocol we found no change in plasma total ghrelin concentration during or after aerobic exercise (Burns et al 2007).

Since acylated ghrelin was suppressed during resistance exercise despite a low energy expenditure of $1488 \pm 110$ kJ it would appear that movement and energy expenditure per se regardless of mode suppresses acylated ghrelin as long as it is high intensity. There is still a paucity of data on low intensity resistance exercise which needs be explored and duration could also be a determinant as the duration of the resistance bout was long at 1.5 hours. Chapter 6 shows that acylated ghrelin is suppressed in high but not low intensity exercise of the same energy expenditure and there is a greater suppression in long duration compared with short duration exercise when the energy expenditure is greater as shown in chapter 7. A threshold of intensity and/or duration and/or energy expenditure may exist and it is plausible that acylated ghrelin may not have been suppressed had the resistance session been much shorter performing less sets and/or at a lower rep max.

Findings show that total PYY is increased during aerobic but not resistance exercise. This confirms the findings of Martins et al (2007) who found an increase in total PYY after one hours intermittent cycling at 65% of maximum heart rate. They did not report an exercise energy expenditure but it is assumed to be somewhat less than the exercise energy expenditure of treadmill running reported in the present study ($3848 \pm$
92 kJ) because of the weight bearing nature of cycling, lower exercise intensity and the inclusion of rest intervals. Martins et al (2007) reported their findings in pmol.L\(^{-1}\) and in converting our findings to SI units (pg.mL\(^{-1}\) x 0.25 = pmol.L\(^{-1}\)) whilst similar exercise trends are seen the values for the present study (including fasting) are higher. The reasons for this are unknown, but may be due to different methods of determination as Martins et al (2007) used a radio-immunoassay. Martins et al (2007) also included female participants and females have been shown to display higher PYY values than males (Kim et al, 2005).

Similar to Martins et al (2007) only total PYY concentration was measured limiting the author’s ability to determine the extent of variation between the responses of PYY\(_{1-36}\) and PYY\(_{3-36}\) to both exercise and feeding. PYY\(_{3-36}\) is the more biologically active form as it contributes to a greater percentage of circulating PYY post feeding (Grandt et al, 1994) but it is unclear as to whether PYY\(_{1-36}\), PYY\(_{3-36}\) or both increase during aerobic exercise. Only the measurement of total PYY was commercially available at the time but as assays to measure both PYY\(_{1-36}\), and PYY\(_{3-36}\) become available these should be used to clarify the exact PYY exercise response.

To the author’s knowledge this is the first study to examine the effects of resistance exercise on total PYY. Since acylated ghrelin and hunger were suppressed it is unclear why total PYY did not increase during resistance exercise. PYY did not respond and stayed low so it is speculated that exercise induced changes in total PYY are more dependant on mode, energy expenditure and exercise intensity than acylated ghrelin.
The present study confirms that hunger is suppressed during aerobic exercise and to some extent in the immediate post-exercise period. This is consistent with results from previous studies that have monitored subjective hunger ratings following vigorous aerobic exercise (above 60% of maximum oxygen uptake) (Blundell et al 2003; King and Blundell, 1995; King et al 1994; Martins et al, 2007; Tsofliou et al 2003) and the findings in chapter 5 and 7. As in previous studies the suppression of hunger was short lived and hunger ratings began to return to control values within an hour of the cessation of exercise.

To the author’s knowledge no studies have examined the hunger response to resistance exercise and the present study shows the presence of ‘exercise induced anorexia’ as a result of resistance exercise. This is likely the result of the suppression of acylated ghrelin.

Our findings confirm previous research that following the ingestion of food, plasma levels of total PYY increase (Adrian et al, 1985). PYY levels have been shown to reflect meal size and the nature of food, fat being the most potent nutrient in releasing PYY (Adrian et al, 1985) hence why there was a large increase in PYY post feeding due to the high fat content of the test meals.

In the present study the area under the curve values for plasma glucose concentration were elevated during the one hour run compared with the same time period during the control and resistance trials. This is consistent with the findings of previous studies which also demonstrate an increase in plasma glucose during aerobic exercise (Kjaer et al 1986; Kraemer et al 2002) but contradicts the previous resistance exercise
findings in studies which found an increase (Ghanbari-Niaki, 2006; Kraemer et al, 2004b; Robergs et al 1991). A rise in glucose may in part explain the lower area under the curve values for plasma acylated ghrelin during the aerobic exercise trial but cannot explain the suppression of acylated ghrelin during the resistance trial.

It has been suggested that insulin may have a role in ghrelin regulation (Haqq et al 2003). Plasma insulin concentrations were not elevated during either aerobic or resistance exercise in the present study, in line with previous findings of aerobic exercise (Kjaer et al, 1986; Kraemer et al. 2002) but contradicts the findings of (Ghanbari-Niaki, 2006) and therefore insulin is unlikely to contribute to the suppression of acylated ghrelin during exercise.

Riediger et al (2004) reported that in the ARC electrophysiological studies have shown ghrelin excitation and PYY3-36 inhibition of neurons. Neurons of the ARC are post-synaptically inhibited by PYY3-36, suggesting that direct action of PYY3-36 on receptive neurons may cause the suppressive effects on food intake. In man infusion of PYY3-36 markedly decreases circulating ghrelin levels and attenuates the preprandial rise (Batterham et al, 2003). The action of PYY3-36 may lead to a reduction in the direct inhibitory effect on ghrelin-stimulated neurons as well as an attenuation of circulating levels. The findings of the present study show that this may not be the case of endogenous PYY as concentrations did not increase during resistance exercise yet acylated ghrelin was suppressed.

Evidence is accumulating that potentially there is a relationship between the response of gut peptide hormones and 'exercise induced anorexia'. The findings presented in
Chapter 4, 5 and 7 and the present study shows that hunger is suppressed during aerobic exercise which coincides with a suppression of acylated ghrelin. Martins et al (2007) and the present study shows that hunger is suppressed during aerobic exercise which coincides with an increase in total PYY. In addition the present study shows a suppression of acylated ghrelin and hunger in resistance exercise to a similar extent of that found in the aerobic trial but no significant change PYY. The regulation of hunger and energy balance is complex incorporating an interplay of many systems and other hormones including glucagon-like peptide-1 (GLP-1) and pancreatic polypeptide (PP). It is clear that more research is needed to determine how much reduction in acylated ghrelin and/or increase in total PYY is required to suppress hunger and potentially alter eating behaviour as well as the responses of many others.

In conclusion, this study demonstrates that plasma acylated ghrelin concentration is reduced and total PYY concentration is increased during an acute bout of high intensity treadmill running which lends support for the role of acylated ghrelin and total PYY in hunger suppression during and immediately after exercise. Resistance exercise suppresses acylated ghrelin and hunger but there appears to be no effect on total PYY. Further research is required to confirm the influence of other modes, durations and intensities of exercise on total PYY as well as to document the individual exercise response of PYY$_{1-36}$ and PYY$_{3-36}$ when the assays become commercially available.
CHAPTER IX
General Discussion

9.1 Introduction

The aim of this chapter is to integrate the findings from all experimental studies described in this thesis.

The balance of evidence from previous research suggests that acute exercise does not increase hunger or energy intake in the hour after exercise yet high intensity exercise may lead to a suppression of hunger during and immediately post exercise. The main aim of this thesis was to examine the hunger and hunger related hormone responses to exercise as it is of interest to know whether the effects of exercise on hunger are mediated by acylated ghrelin and total PYY. The studies presented were examined in a systematic way to address questions including the effect of exercise intensity, duration and mode and begin to identify potential causal mechanisms. Table 9.1 shows a summary of the study exercise protocols and the hormones, metabolites and subjective feelings measured.
Table 9.1: Summary of the exercise protocols and the hormones, metabolites and subjective feelings measured. Values listed as Mean ± SEM

<table>
<thead>
<tr>
<th>Study (chapter)</th>
<th>Trials</th>
<th>Mode</th>
<th>Intensity (% VO$_{2\text{max}}$) or (% 12 rep max)</th>
<th>Duration of exercise (min)</th>
<th>Energy expenditure of exercise kJ (kcal)</th>
<th>Hormones, metabolites and subjective feelings measured during the study</th>
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<tbody>
<tr>
<td>1 (4)</td>
<td>Control</td>
<td>Rest</td>
<td>37 ± 1.0</td>
<td>60</td>
<td>1835 ± 140 (438 ± 34)</td>
<td>Hunger, fullness, desire to eat, how much food, and oral temperature</td>
</tr>
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<td></td>
<td>Low Intensity</td>
<td>Running</td>
<td>71 ± 1.5</td>
<td>60</td>
<td>3488 ± 234 (834 ± 56)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High Intensity</td>
<td>Running</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (5)</td>
<td>Control</td>
<td>Rest</td>
<td>72 ± 2.0</td>
<td>60</td>
<td>3915 ± 207 (935 ± 50)</td>
<td>Hunger, acylated ghrelin, glucose and insulin.</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>Running</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (6)</td>
<td>Control</td>
<td>Rest</td>
<td>52 ± 1.0</td>
<td>55 ± 2</td>
<td>2580 ± 51 (617 ± 12)</td>
<td>Hunger, acylated ghrelin, glucose and insulin.</td>
</tr>
<tr>
<td></td>
<td>Low Intensity</td>
<td>Running</td>
<td>75 ± 1.0</td>
<td>36 ± 2</td>
<td>2504 ± 55 (599 ± 13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High Intensity</td>
<td>Running</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (7)</td>
<td>Control</td>
<td>Rest</td>
<td>70 ± 0.7</td>
<td>45</td>
<td>2918 ± 110 (697 ± 26)</td>
<td>Hunger, nausea, acylated ghrelin, glucose and insulin</td>
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<tr>
<td></td>
<td>Short Duration</td>
<td>Running</td>
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<tr>
<td></td>
<td>Long Duration</td>
<td>Running</td>
<td>70 ± 0.7</td>
<td>90</td>
<td>5949 ± 218 (1422 ± 52)</td>
<td></td>
</tr>
<tr>
<td>5 (8)</td>
<td>Control</td>
<td>Rest</td>
<td>68 ± 2.0</td>
<td>60</td>
<td>3848 ± 110 (920 ± 22)</td>
<td>Hunger, nausea, acylated ghrelin, glucose, insulin and total PYY</td>
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<tr>
<td></td>
<td>Aerobic</td>
<td>Running</td>
<td></td>
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<tr>
<td></td>
<td>Resistance</td>
<td>Free Weights</td>
<td>80 ± 0.3 (with rest)</td>
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The main aim of the initial study was to confirm if high intensity exercise results in suppressed hunger termed ‘exercise induced anorexia’ during and immediately post exercise. This was the case and since subsequent studies also found a suppression, the effect of exercise on hunger is warrants discussion.
9.2 Hunger

The findings from the studies presented in this thesis suggest that exercise can suppress hunger, but this is dependent on the intensity of exercise, duration, energy expenditure and mode. Study 1 showed that high intensity treadmill running $71 \pm 1.5 \% \text{VO}_{2\text{max}}$ with an energy expenditure of $3488 \pm 234 \text{kJ} (834 \pm 56 \text{kcal})$ led to 'exercise induced anorexia' compared with low intensity exercise $37 \pm 1.0 \% \text{VO}_{2\text{max}}$ with an energy expenditure of $1835 \pm 140 \text{kJ} (438 \pm 34 \text{kcal})$. The duration of both runs was 60 minutes. 'Exercise induced anorexia' was seen in study 2 as a result of 60 minutes treadmill running at $72 \pm 2.0 \% \text{VO}_{2\text{max}}$ with an energy expenditure of $3915 \pm 207 \text{kJ} (935 \pm 50 \text{kcal})$ compared with resting control. There was no evidence of 'exercise induced anorexia' in study 3 as a result of high intensity treadmill running at $75 \pm 1.0 \% \text{VO}_{2\text{max}}$ with an energy expenditure of $2505 \pm 55 \text{kJ} (599 \pm 13 \text{kcal})$. This contradicts previous findings with high intensity treadmill running but the duration ($36 \pm 2 \text{minutes}$) was shorter resulting in a much lower total energy expenditure. This may also be because initial baseline hunger values were lower and possibly less subject to change. Similarly to study 1 there was no significant hunger suppression in the low intensity exercise trial.

Study 4 showed 'exercise induced anorexia' in both high intensity exercise trials. The 45 minute short duration trial elicited an energy expenditure of $2918 \pm 110 \text{kJ} (697 \pm 26 \text{kcal})$ working at an intensity of $70.1 \pm 0.7 \% \text{VO}_{2\text{max}}$ compared with the 90 minute long duration run eliciting an energy expenditure of $5949 \pm 218 \text{kJ} (1422 \pm 52 \text{kcal})$ working at an intensity of $69.7 \pm 0.7 \% \text{VO}_{2\text{max}}$. Finally study 5 showed that high intensity exercise 60 minutes long, at a workload of $68 \pm 2.0 \% \text{VO}_{2\text{max}}$ with an
energy expenditure of 1488 ± 110 kJ (920 ± 22 kcal) lead to ‘exercise induced anorexia’. Based on the findings of this thesis it could be said that high intensity treadmill running ≥ 68% ≥ 45 minutes that elicits an energy expenditure of at least 2918 kJ (697 kcal) will result in transient ‘exercise induced anorexia’. Study 5 highlighted that resistance exercise of a high intensity also results in ‘exercise induced anorexia’ but a range of intensities and durations needs to be examined.

Hunger may be suppressed for longer than 15 minutes which contradicts the findings of King et al (1994). When solely looking at the high intensity treadmill running responses to exercise in studies 1 and 2, subjective feelings of hunger took up to 2 hours to return to baseline. In studies 4 and 5 subjective feelings had not returned to baseline at 1 hour post exercise at which point a test meal was given masking any further effect. It is posited that the greater severity of ‘exercise induced anorexia’ found in the present studies is due to the greater exercise intensity, duration of exercise and therefore energy expenditure compared with previous studies examining the effects of exercise on hunger.

There is no effect of exercise on the hunger response to meals. Studies 1, 2, 3, 4 and 5 all show similar declines in hunger 30 minutes post feeding and there is no difference between trials as time progresses. This could be due to similar changes in the concentration of gut peptide hormones as shown in studies 2, 3, 4 and 5.

Having confirmed ‘exercise induced anorexia’ as a result of high intensity treadmill running in study 1 the focus of the thesis moved towards identifying potential changes
in gut peptide hormones. The findings of study 2 identify 'exercise induced anorexia' occurs when acylated ghrelin is suppressed.

9.3 Acylated ghrelin

On the balance of the evidence from previous research studies it would appear that exercise has no effect on total ghrelin. In particular Burns et al (2007) undertook the same exercise protocol to that shown in study 2 and found no change in total ghrelin. Since measurements of total ghrelin may mask important changes in acylated ghrelin and this had yet to be studied the focus of the thesis was on the measurement of acylated ghrelin and it is clear from the studies presented in this thesis that high intensity treadmill running suppresses acylated ghrelin.

To the author's knowledge no published study had examined the hunger and acylated ghrelin response to exercise. Study 2 showed that a 60 minute treadmill run \(72 \pm 2.0\%\) \(\dot{V}O_2\max\) with an energy expenditure of \(935 \pm 50\) kcal suppressed acylated ghrelin during and post exercise which coincided with suppressed hunger 30 minutes into the run. Similarly to feelings of hunger, concentrations of acylated ghrelin began to return to baseline values after the termination of exercise and there was a clear suppression of acylated ghrelin post feeding after the test meal in both trials. The response of acylated ghrelin could therefore be a causal mechanism for 'exercise induced anorexia.'

Having shown the acylated ghrelin response to high intensity treadmill running and from an awareness that exercise intensity is a determinant of 'exercise induced anorexia', study 3 examined the effect of exercise intensity by matching the energy
expenditure of two runs of differing exercise intensity. At 30 minutes, high intensity treadmill running (75 ± 1.0% \( \dot{V}O_2\text{max} \)) led to a decline in acylated ghrelin that was approaching significance. There was no significant decline in the low intensity (52 ± 1.0% \( \dot{V}O_2\text{max} \)) trial and neither the low or high intensity treadmill runs lead to a significant suppression of hunger putting the findings of study 2 into question but the sample size was small and energy expenditure was lower. However, the findings of study 4 clearly show suppressions of acylated ghrelin in both high intensity treadmill runs of short and long duration which again co-incided with ‘exercise induced anorexia’. Acylated ghrelin remains suppressed throughout the duration of both runs and returns to baseline levels after the termination of exercise. Finally study 5 showed that acylated ghrelin was suppressed as a result high intensity treadmill running which was again associated with suppressed feelings on hunger. Resistance exercise also lead to a significant decline in acylated ghrelin which was associated with ‘exercise induced anorexia’.

It would be assumed that higher fasting acylated ghrelin would be associated with higher subjective feelings of hunger so due to the number of participants measured throughout the studies within this thesis, correlation analysis between fasting acylated ghrelin and hunger as well as a number of other variables was performed. The number examined was 36 as one participant volunteered for three of the studies and ghrelin data was not available for one of the participants in study 5. There were no significant correlations as shown in Table 9.2 and is likely that a larger data set would be needed to improve the statistical power. The only correlation that was approaching statistical significance was the relationship between fasting acylated ghrelin and weight and a negative correlation was found. This supports the work of Cummings et al (2002) who
found that after weight loss, ghrelin levels rise so that lighter individuals have higher acylated ghrelin values which may be a defense mechanism which leads to a drive to eat.

Table 9.2 Correlation between fasting acylated ghrelin and other variables

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<tr>
<td>Hunger</td>
<td>0.204</td>
<td>0.226</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.205</td>
<td>0.222</td>
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<tr>
<td>Weight</td>
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<td>0.091</td>
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<tr>
<td>Body Fat (%)</td>
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<td>0.132</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>-0.231</td>
<td>0.169</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>0.228</td>
<td>0.176</td>
</tr>
</tbody>
</table>

n = 36

The findings of study 2 show there was a tendency for higher hunger ratings over the last five hours of the aerobic exercise trial compared with the control trial. This was probably because participants were in energy deficit during the exercise trial compared with the control trial. However, the nine hour value for plasma acylated ghrelin concentration did not support the notion that hunger was elevated at the end of the exercise trial. In fact, acylated ghrelin concentrations at nine hours would suggest that hunger was still suppressed on the exercise trial compared with the control trial which was not the case. This apparent contradiction required further confirmation and subsequently hunger and ghrelin responses for all three trials were similar at 8 hours
in study 3. This may be the result of a 500 kcal greater energy intake and being fed a second test meal later in the day influencing hunger responses in study 3.

Comparisons with the literature are not possible since to the author’s knowledge no published study has examined the acylated ghrelin response to exercise. The findings of this thesis demonstrate temporal associations between the response of acylated ghrelin and ‘exercise induced anorexia’ in high intensity treadmill running and resistance exercise. Since decreasing acylated ghrelin is potentially a causal mechanism for suppressed feelings of hunger it is unclear why there was no reported suppressed feelings of hunger in study 3. The control of hunger is a complex process involving an interplay of many different hormones and therefore the response of other hormones may have resulted in a failure of exercise to stimulate ‘exercise induced anorexia’ in these cases. Therefore study 5 also measured the exercise response to the gut peptide hormone total PYY.

### 9.4 Total PYY

The decision to measure PYY as opposed to other hormones such as CCK or GLP-1 was made because PYY was receiving attention in the feeding literature and at the time of compiling the study to the author’s knowledge no published study had examined the influence of exercise on PYY. Study 5 measured the PYY response to 60 minutes high intensity treadmill running (68 ± 1.0% $\dot{V}O_2$ max) with an energy expenditure of 920 ± 22 kcal, 90 minutes of resistance exercises working at 80 ± 0.3% 12 rep max with an energy expenditure of 356 ± 26 kcal and a resting control. There
was a significant increase in total PYY during the treadmill run but no significant difference in the resistance or resting control trials.

A limitation of the PYY findings is that total PYY was measured so the individual changes of PYY \textsubscript{3-36} and PYY \textsubscript{1-36} cannot be distinguished. PYY \textsubscript{3-36} is the more biologically active form as it contributes to a greater percentage of circulating PYY post feeding (Grandt et al, 1994) and is more closely associated with inhibition of hunger than total PYY (Batterham et al, 2003) however only the measurement of total PYY was commercially available at the time. Future studies should measure the influence of exercise on PYY \textsubscript{3-36}.

9.5 The effect of glucose and insulin on acylated ghrelin and total PYY

Glucose and insulin were measured because they may be important in the regulation of ghrelin and PYY concentrations (Boey et al, 2007; Flanagan et al, 2003; Shiya et al, 2002). In all high intensity treadmill runs there was a surge in plasma glucose which could potentially lead to a suppression of acylated ghrelin and increase in total PYY as these changes are also seen postprandially. It is also interesting to note that in study 3 there was no increase in glucose during low intensity exercise which is possibly why there was no suppression of acylated ghrelin. Research supports that glucose suppresses ghrelin since Tschop et al (2000) found a decrease when filling the stomach with a 50% solution but not with an equal volume of water and Flanagan et al (2003) showed a suppression as a result of hyperglycaemic clamp. This is mimicked during exercise since prolonged high intensity exercise begins to lower blood glucose.
concentration stimulating the release of glucagon which elicits an almost instantaneous release of glucose from the liver so that it is possible that exercise induced hyperglycemia may be responsible for subsequent changes in gut peptide hormones. This is supported by Banasch et al (2006) who found suppressions of ghrelin as a result of the infusion of glucagon like peptide 2. However exercise induced glucose release may not be the sole mechanism for suppression of acylated ghrelin during exercise since the resistance trial of study 5 demonstrated a suppression of acylated ghrelin but no change in plasma glucose.

The findings of this thesis would indicate that insulin does not contribute to changes in gut peptide hormones as there was only a significant change in insulin during the short duration exercise trial of study 4 but in all other high intensity treadmill running trials there was a suppression of acylated ghrelin in the absence of any change to plasma insulin.

9.6 Other potential causal mechanisms influencing hunger, ghrelin and PYY

Exercise has been shown to increase feelings of nausea (Halvorsen and Ritland, 1992) and it was suggested that suppressed hunger may be the result of increased nausea as opposed to an exercise effect *per se*. The inclusion of a nausea VAS in studies 4 and 5 show a significant increase in nausea during exercise showing that nausea may confound ‘exercise induced anorexia’.

It was initially thought that the reduction in gut blood flow during aerobic exercise could limit supply leading to a suppression in acylated ghrelin. Whilst there is no
supporting evidence it is assumed that gut blood flow is not reduced during resistance exercise to as great an extent as aerobic exercise and as a result of a suppression of acylated ghrelin, reductions in gut blood flow as a mechanism is unlikely.

There is some evidence to support a suppressive effect of GH on ghrelin from patients with GH deficiency (Dall et al. 2002; Eden et al. 2003). Exercise of even moderate intensity stimulates GH release after a lag phase of 10 to 20 minutes and levels usually remain elevated for at least 1 hour after exercise (Lassarre, 1974). Vestergaard et al. (2007) hypothesized that circulating ghrelin is suppressed by exercise induced GH release. Findings confirmed this was the case when examining healthy and fit subjects exercising with and without GH administration. An exercise induced rise in GH during both aerobic and resistance exercise in the studies presented in this thesis could have suppressed acylated ghrelin. Since GH was not measured, this cannot be stated with confidence and to the authors knowledge no trials have examined the acylated ghrelin response to exercise induced GH secretion with or without additional GH administration and this as a potential acylated ghrelin suppressing mechanism remains to be elucidated.

Corticosteroids (a class of steroid hormones that are produced in the adrenal cortex) have been shown to inhibit ghrelin production (Proulx et al. 2005). Ebal et al. (2007) found reduced food intake in rats who exercised for 5 weeks. They propose a regulatory feedback loop whereby glucocorticoids (such as cortisol) inhibit ghrelin secretion since they increase in the exercising rat which reflects an activation of the hypothalamo-hypophysoadrenal axis which interferes with food intake. The response
of the glucocorticoids to exercise is reported by Lac et al (1999) and is another potential mechanism that needs to be examined.

9.7 Hyperghrelinemia

A small number of participants studied displayed atypically high ghrelin values. Initially this phenotype was seen only in participants of ethnic origin but was then displayed in a Caucasian male in the study described in Chapter 8. An examination of the participants BMI, body mass, body fat %, fitness and hunger responses were not different from other participants so the reason for hyperghrelinemia is unknown. Beaumont et al (2003) have shown that ghrelin can bind to high density lipoprotein (HDL) so potentially people who have high HDL may have higher ghrelin values. The measurement of HDL in participants who display atypically high ghrelin values compared with participants with normal ghrelin values therefore warrants investigation.

9.8 Limitations

"An expert is a man who has made all the mistakes which can be made in a very narrow field"

Niels Bohr

The studies in this thesis have a number of limitations:

1) Participants were generally male, young, fit and healthy. How well these findings apply to female, old and unhealthy populations is unknown.
2) Low sample size in each study may have limited the power to detect significant associations between acylated ghrelin, total PYY and other variables.

3) A clock was on show at all times. As the participants had an awareness of the study protocol and the timing of meals, hunger ratings may have been affected if participants anticipated being fed.

4) Total ghrelin concentration was not measured, limiting the author's ability to determine the extent of variation between the responses of acylated ghrelin and total ghrelin to exercise and feeding.

6) Total PYY concentration was measured limiting the author's ability to determine the extent of variation between the responses of PYY$_{1,36}$ and PYY$_{3,36}$ to exercise and feeding.

7) Ghrelin concentration may be influenced by the duration of sleep (Steiger, 2007) yet amount of sleep was not controlled for in any of the studies nor were participants questioned on whether they had slept well on arrival at the laboratory.

8) Participants began every trial in the fasted state. As well as to control any potential effects of food intake it was anticipated that ghrelin levels and hunger would be high and therefore more susceptible to change. However this is likely to reduce ecological validity since people are most likely to have some form of breakfast prior to undertaking exercise and it has been shown that timing of exercise to meal
consumption may influence hunger and its hormonal regulators (Cheng et al, 2008). Participants undertook three trials in a random crossover design 1) meal followed by exercise 2) exercise followed by meal consumption 3) meal consumption only. Fifty minutes of moderate intensity exercise diminished hunger in the initial post exercise period of the exercise followed by meal trial and suppressed the post meal rise in hunger that was appear not during the meal and the exercise then meal trials. PYY tended to be higher when participants exercised either before or after consuming a meal in comparison to the meal only trial (Cheng et al, 2008).

9.9 Future research recommendations

"Science never solves a problem without creating ten more"

George Bernard Shaw

Further research is required to confirm the influence of other modes, durations and intensities of exercise on hunger, plasma acylated ghrelin and total PYY concentrations and to document responses in different groups including females, older adults, the untrained and the overweight and obese.

The completion of this thesis arose from personal interest in the effects of exercise on suppressed feelings of hunger post vigorous running and team sports. However through personal experience and in discussion with others, the author has noted that after swimming, hunger is not suppressed and may even increase. The reasons for this are unknown and swimming as a mode of exercise in particular warrants
investigation. Nor has any work on solely upper body exercise such as arm cranking been undertaken which would be particularly important in wheelchair users.

The focus of this thesis has been the measurement of acylated ghrelin during and post exercise. More frequent measurements and longer trials are needed in particular to determine how soon gut peptide hormones respond to exercise and whether there is any prolonged effects that are not masked by feeding. There is still little or no data on the effects of exercise on other hormones implicated in the regulation of hunger. The role of cholecystokinin (CCK) in pancreatic secretion and gallbladder contraction was already well established when it was demonstrated that CCK also reduced meal size in a dose-dependent manner in rats. CCK was the first gut hormone implicated in the control of hunger and the anorectic effects of CCK have since been confirmed in human studies (Kissileff et al. 1981; Muurahainen et al. 1988; Moran & Schwartz, 1994). A number of other hormones and metabolites could be measured. Glucagon-like (GLP-1) and oxyntomodulin (Oxm) are produced by posttranslational processing of the preproglucagon gene in the CNS and the intestine and colon. Both peptides are released into the circulation in response to nutrient intake and appear to function as satiety signals.

There is still a paucity of data on the effects of exercise on subsequent energy intake and how soon food would be consumed and what is the relationship between acylated ghrelin and PYY. This would involve the provision of buffet style meals as opposed to test meals in which participants are free to eat as and when they choose. Numerous research questions would include:
1) Does exercise influence the timing of energy intake post exercise?

2) Does exercise influence the macronutrient selection of foods consumed shortly after exercise?

3) What is the effect of exercise on energy intake and macronutrient selection the day after exercise?

4) Is energy intake lower if eating is undertaken immediately post exercise?

There are also other findings in the studies presented in this thesis that warrant further investigation, in particular whether the macronutrient content of the test meal influences the suppression of acylated ghrelin. It also needs to be identified whether there is a plateau at which acylated ghrelin is suppressed as a result of exercise. Referring to the acylated ghrelin response to high intensity treadmill running in study 4 the same exercise intensity was maintained for both runs. It would be interesting to see if acylated ghrelin would be suppressed any further with an increase in intensity and to what extent would the concentration of acylated ghrelin return to baseline levels if the exercise intensity was lowered as opposed to terminated.

Confirmation of the findings presented in this thesis and perusing the above research topics would have important implications regarding the role of exercise in manipulating hunger and weight management.
9.10 Conclusion

The growing obesity epidemic together with the advances in the understanding of the complex physiology that regulates bodyweight have lead to increased interest in hormonal signals implicated in weight homeostasis and as such a need has arisen to examine the effect of exercise on hunger related hormones.

The main findings from the studies presented in this thesis are:

1) High intensity treadmill running transiently suppresses hunger and acylated ghrelin concentration and increases total PYY.

2) Resistance exercise suppresses hunger and acylated ghrelin but has no effect on total PYY.

3) There is a threshold of exercise intensity, duration and expenditure which needs to be reached before 'exercise induced anorexia' is observed and these factors affect hunger and the change in gut peptide hormones.

4) Changing concentrations of acylated ghrelin and total PYY in response to feeding and exercise are possibly related to changes in glucose but it is unlikely they are mediated by changes in insulin.

5) Whilst the findings of this thesis are useful in identifying potential causal mechanisms for the influence of exercise on hunger, more work is needed to
address the effect of exercise on energy intake and how exercise can play a
more effective role in the prevention of overweight and obesity, weight loss
and the prevention of weight regain.

"The hunger and thirst for knowledge, the keen delight in the chase, the good
humored willingness to admit that the scent was false, the eager desire to get on with
the work, the cheerful resolution to go back and begin again, the broad good sense,
the una"

Frederic William Maitland
REFERENCES


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Title: The Influence of aerobic and resistance exercise on appetite.
Applicant: Dr DJ Stensel, DR Broom, C Mathers, J Zakrzewski, N Whitehead, J King
Department: SSES
Date of clearance: 16 November 2006

Comments of the Committee:
The Committee agreed to issue clearance to proceed.
The influence of resistance and aerobic exercise on appetite

INVESTIGATORS

Mr. David Broom, Mr. James King, Miss Nichola Whitehead, Miss Julia Zakrzewski, Miss Charlotte Mathers and Dr. David Stensel
School of Sport and Exercise Sciences

BACKGROUND

There is evidence to suggest that appetite is suppressed after continuous bouts of high intensity aerobic exercise, a phenomenon known as exercise induced anorexia. Appetite is regulated by several factors including active ghrelin, which is a hormone that is secreted by the stomach. Very little is known about the effects of aerobic and resistance exercise on active ghrelin. The proposed study will therefore investigate the effect
of a treadmill run and free weight resistance exercise session on active ghrelin.

INCLUSION CRITERIA

Volunteers will: i) be male ii) have no personal history of cardiovascular disease, metabolic disease or dyslipidaemia, (iii) not be dieting or have any extreme dietary habits (iv) be non-smoking (v) not be taking drugs known to affect digestion or metabolism; (vi) have a BMI < 30 kg·m⁻² i.e. not classified obese (vi) have experience of resistance exercise.

STUDY DEMANDS

If you volunteer to participate in this study you will be required to visit the laboratory on 6 occasions as follows:

- Aerobic preliminary procedures and screening including measurement of height, weight, blood pressure, body composition and activity status
- Determination of 12 rep max for 10 resistance exercises
- Resistance familiarisation
- Three Main Trials
  - Aerobic Exercise trial
  - Resistance Exercise trial
  - Control trial

PRELIMINARY PROCEDURES

You will be asked to attend the laboratory for a preparatory session during which we will:

- explain the objectives of the study and its requirements;
- ask you to complete a confidential questionnaire regarding your health;
- familiarise you with the testing procedures and equipment;
- familiarise you with dietary recording;
- answer any questions you may have.

Following this you will be asked to complete several exercise tests as follows:
Submaximal treadmill test: You will run on a treadmill for 16 minutes. The speed of the treadmill will be increased every four minutes. You will periodically be asked to breathe through a mouthpiece while wearing nose clips during the run.

Maximum oxygen uptake (VO2 max) test: You will run on a treadmill until volitional fatigue which will take between 8 and 12 minutes. The incline of the treadmill will be increased every three minutes. You will periodically be asked to breathe through a mouthpiece while wearing nose clips during the run.

Determination of 12 rep max: On a separate day you will lift free weights to determine your 12 rep max for 10 exercises (in order: squat, dumbbell lateral raise, bench press, upright row, lunges, bicep curl, barbell pullover, seated shoulder press, triceps extension, bent over row). You can rest between each exercise for as long as you like.

Resistance session familiarisation: On a separate day you will undertake the full resistance exercise session to ensure that you can complete it. Resistance exercise will be performed using free weights and will involve three sets of 12 repetitions of the 10 exercises. Each will be performed at 80% of 12 repetition maximum with a 3 minutes work and rest interval giving a duration of 90 minutes.

MAIN TRIALS

There will be three main trials. The order of these will be randomly assigned:

AEROBIC EXERCISE trial - you will report to the laboratory at 9.00 am having fasted overnight. You will spend the day (9.00 am to 5.00 pm) in the laboratory. Shortly after arrival a cannula will be placed in a vein in your arm so that blood samples can be extracted. Thirteen venous blood samples will be collected throughout the day and appetite and motivation to eat will be measured using questionnaires. You will perform high intensity exercise (70% of maximum oxygen uptake) for 60 minutes. Samples of your expired air and heart rate will be collected during exercise. At 11 am you will be provided with a test meal consisting of a cheese, butter and mayonnaise sandwich, ready salted crisps, and strawberry milkshake. You will receive an identical test meal at 2 pm.
RESISTANCE EXERCISE trial - you will report to the laboratory at 9.00 am having fasted overnight. You will spend the day (9.00 am to 5.00 pm) in the laboratory. Shortly after arrival a baseline blood sample will be taken via venepuncture. You will then undertake the same resistance exercise session that you undertook during the resistance familiarisation. Mid way through this session you will be venepunctured again. Once resistance exercise is completed you will be cannulated and a further eleven venous blood samples will be collected throughout the day and appetite and motivation to eat will be measured using questionnaires. Samples of your expired air and heart rate will be collected during exercise. At 11 am you will be provided with a test meal consisting of a cheese, butter and mayonnaise sandwich, ready salted crisps, and strawberry milkshake. You will receive an identical test meal at 2 pm.

CONTROL trial - you will report to the laboratory at 9.00 am having fasted overnight. You will spend the day (9.00 am to 5.00 pm) in the laboratory. Shortly after arrival a cannula will be placed in a vein in your arm so that blood samples can be extracted. Thirteen venous blood samples will be collected throughout the day and appetite and motivation to eat will be measured using questionnaires. At 11 am you will be provided with a test meal consisting of a cheese, butter and mayonnaise sandwich, ready salted crisps, and strawberry milkshake. You will receive an identical test meal at 2 pm.

PREPARATION FOR THE TESTS

• **Recording your diet:** you will be asked to weigh and record everything you eat and drink for two day’s prior to any of the main trials. You will then consume identical amounts of the same food and drink prior to the next main trials. This is very important in order to control for diet and we will discuss this with you prior to the main trials. No alcohol should be consumed on the days when you are recording your diet.

• **Controlling physical activity:** this is crucial to the success of the experiment and you should undertake exactly the same type and amount of activity the day before the start of each main trial.

• **Ten-hour overnight fast:** you will finish eating by 11 pm on the evenings before the main trials. You may continue to drink water after this time. You will report to the laboratory at 9 am the following morning without eating breakfast.
• **Travelling to the laboratory:** if you live within 400 m of the laboratory you should walk in slowly on the morning of each main trial. Please do not run or cycle. If you live more than 400 m from the laboratory then you should drive in. If you do not have access to a car please inform us and we will arrange for you to be collected.

**HOW MUCH TIME WILL IT TAKE?**

- Aerobic preliminary procedures including screening and Q + A - 1 hour 30 minutes maximum
- Determination of 12 rep max - 1 - 1 hour 30 minutes
- Resistance familiarisation session - 1 hour 30 minutes
- Main trials: three days in total - 8 hours for each trial

You will be encouraged to bring work and reading material with you for the main trials. Alternatively you may watch television or listen to music.

**POSSIBLE RISKS AND DISCOMFORTS**

High intensity treadmill exercise and resistance exercise using free weights will cause breathlessness. The maximum oxygen uptake test will lead to physical exhaustion, but you should recover within a few minutes. In a tiny minority of individuals, even in young adults, the possibility exists that such exercise triggers disturbances to normal physiology: these include abnormal blood pressure, fainting or a change in the normal rhythm of the heart. There are also risks associated with performing exercise including musculoskeletal injury but these are minimal and measures will be taken to prevent any occurrences.

Venous cannulation can cause air or plastic embolism, but good practice minimises this risk and personnel conducting the procedure have been trained and are very experienced. Cannulation can also lead to local thrombophlebitis (inflammation) in a superficial vein but the absolute level of risk is low.

**BENEFITS OF THE STUDY**

The study will provide important information regarding the effects of resistance and aerobic exercise on the hormonal control of appetite. We
will provide you with feedback on your own results and will be happy to discuss these with you.

CONFIDENTIALITY

Although information will be stored on computer, each subject will be entered as a number rather than by name, in accordance with the Data Protection Acts of 1984 and 1998. Data will be used for research purposes only and confidentiality will be maintained in any publications arising from the study. Participants are able to access any data on themselves held by the investigators on request. Data shall not be kept longer than is necessary for the purposes of this investigation.

CONTACTS

Please feel free to ask any questions, at any stage. Contact information for the staff involved in the study is as follows:

Mr. David Broom, D.R.Broom@lboro.ac.uk, tel: 01509 226351
Dr David Stensel, D.J.Stensel@lboro.ac.uk, tel: 01509 226344
Miss Charlotte Mathers, C.L.Mathers-04@student.lboro.ac.uk
Miss Julia Zakrzewski, J.Zakrzewski-04@student.lboro.ac.uk
Miss Nichola Whitehead, N.J.Whitehead-04@student.lboro.ac.uk
Mr. James King, J.A.King-04@student.lboro.ac.uk
APPENDIX C

THE INFLUENCE OF RESISTANCE AND AEROBIC EXERCISE ON APPETITE

INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical 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 D

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name or Number .................

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:
   (a) on medication, prescribed or otherwise ......................... Yes □ No □
   (b) attending your general practitioner ............................... Yes □ No □
   (c) on a hospital waiting list ........................................... Yes □ No □

2. In the past two years, have you had any illness which require you to:
   (a) consult your GP ..................................................... Yes □ No □
   (b) attend a hospital outpatient department .................... Yes □ No □
   (c) be admitted to hospital .......................................... Yes □ No □

3. Have you ever had or been diagnosed with any of the following:
   (a) Convulsions/epilepsy ............................................. Yes □ No □
   (b) Asthma ............................................................... Yes □ No □
   (c) Eczema ............................................................... Yes □ No □
   (d) Diabetes ............................................................. Yes □ No □
   (e) A blood disorder .................................................. Yes □ No □
   (f) Head injury ......................................................... Yes □ No □
   (g) Digestive problems .............................................. Yes □ No □
   (h) Heart problems .................................................... Yes □ No □
   (i) Problems with bones or joints ................................. Yes □ No □
   (j) Disturbance of balance/coordination ......................... Yes □ No □
   (k) Numbness in hands or feet ................................... Yes □ No □
   (l) Disturbance of vision .......................................... Yes □ No □
   (m) Ear / hearing problems ...................................... Yes □ No □
   (n) Thyroid problems ............................................... Yes □ No □
   (o) Kidney or liver problems ..................................... Yes □ No □
   (p) Allergy to nuts .................................................. Yes □ No □
   (q) High cholesterol ............................................... Yes □ No □
   (r) High triacylglycerol or any other form of dyslipidaemia ... Yes □ No □

4. Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ........ Yes □ No □
5. Are you:

(a) Currently active .................................................... Yes ☐ No ☐
(b) Dieting .............................................................. Yes ☐ No ☐
(c) A smoker or have you ever smoked ......................... Yes ☐ No ☐

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

Thank you for your cooperation!

Loughborough University
APPENDIX E

LOUGHBOROUGH UNIVERSITY, SCHOOL OF SPORT AND EXERCISE SCIENCES

PHYSICAL ACTIVITY QUESTIONNAIRE

During one week, how many times on average do you do the following kinds of exercise for more than 15 minutes?

(a) Strenuous Exercise (heart beats rapidly)
   For example; running, jogging, squash, hockey, football, basketball, vigorous swimming, vigorous long distance cycling.

   _______ times per week.

(b) Moderate Exercise (not exhausting)
   For example; fast walking, tennis, easy cycling, badminton, easy swimming, dancing.

   _______ times per week.

(c) Mild Exercise (minimal effort)
   For example; yoga, archery, fishing, bowling, golf, easy walking.

   _______ times per week.
APPENDIX F

Subject No.:  
Trial:  
Time:  

Appetite

Please indicate how hungry you are now by circling the relevant number.

<table>
<thead>
<tr>
<th>Not Hungry</th>
<th>Fairly Hungry</th>
<th>Hungry</th>
<th>Very Hungry</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

Place a vertical mark on the horizontal line depending on the intensity of your hunger based on the following question. Consider the two extreme anchors (not at all and very) to be the most extreme. That is, if you place a mark on the not at all (equal to 0) then this is the least hunger you have ever felt.

How hungry do you feel?

Not at all hungry  

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Hungry</td>
</tr>
</tbody>
</table>

How nauseous do you feel?

Not at all nauseous  

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Very nauseous</td>
</tr>
</tbody>
</table>