EFFECT OF EXERCISE AND DIFFERENT ENVIRONMENTAL CONDITIONS ON APPETITE, FOOD INTAKE AND THE APPETITE-REGULATORY HORMONES, GHRELIN AND PEPTIDE YY

by

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ABSTRACT

The role of gut hormones in the regulation of appetite and food intake is well established. The studies presented within this thesis have examined the effects of exercise and different environmental conditions on gut hormones (acylated ghrelin and total peptide YY), appetite and food intake. Forty-two young (mean ± SEM; 22.6 ± 0.4 y), healthy and generally lean (body mass index 23.7 ± 0.3 $kg \cdot m^2$) males were recruited into four studies.

In study one, 60 minutes of high intensity (70 % of $\dot{V}O_2$ max) running and cycling exercise suppressed concentrations of the appetite-stimulating hormone acylated ghrelin to a similar extent. Study two revealed that after 60 minutes running in the heat (30 °C), hunger is lower in the pre-prandial period, and energy intake lower over the 7 h trial duration compared with a similar trial conducted in temperate (20 °C) conditions. Acylated ghrelin was suppressed during running in the temperate and hot environment but this did not appear to mediate the lower energy intake observed during the hot trial. In study three, energy intake tended to be higher after 60 minutes running in a cool environment (10 °C) compared with a temperate (20 °C) environment. During and shortly after running in the cold, perceived ratings of fullness and satisfaction were lower. Acylated ghrelin concentrations appeared to be suppressed to a lesser extent during running in the cold which could mediate the elevated energy intake observed at the first meal. However, energy intake was also higher at the second meal in the cold trial when acylated ghrelin concentrations were higher in the temperate trial. Study four showed that energy intake and acylated ghrelin concentrations were lower, and total PYY tended to be lower, in normobaric hypoxia suggesting a possible role for acylated ghrelin, but not PYY, in mediating the decrease in energy intake observed in hypoxia.

This thesis confirms that exercise transiently suppresses acylated ghrelin concentrations regardless of the environmental conditions (temperature and altitude) exercise is performed in. The findings support anecdotal reports that appetite and energy intake are suppressed in the heat and stimulated in the cold. These responses may be partly mediated by acylated ghrelin immediately after running but other mechanisms are likely involved thereafter. Acute hypoxic exposure suppresses acylated ghrelin concentrations; an observation which may explain the decreased energy intake in hypoxia.

Key Words: exercise, acylated ghrelin, peptide YY, environment, energy intake

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PREFACE

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Chapter VII:
The influence of resting and exercising at a simulated altitude of 4000 m on appetite, energy intake and plasma acylated ghrelin concentrations
BASES 2010 annual conference – Glasgow (oral presentation)
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The following abbreviations are used throughout the chapters in this thesis. They are defined when they appear in text for the first time:

ANOVA – analysis of variance
AMS – acute mountain sickness
AUC – area under the concentration versus time curve
BMI – body mass index
CCK – cholecystokinin
EDTA – ethylenediaminetetraacetic acid
GHSR – growth hormone secretagogue receptor
GLP-1 – glucagon-like peptide-1
GOAT – ghrelin O-acyl transferase
PYY – peptide YY
RPE – ratings of perceived exertion
SEM – standard error of the mean
TAG – triacylglycerol

CHAPTER I

Introduction

Obesity arises when there is a persistent energy imbalance with energy intake exceeding energy expenditure. Although genetics are important in determining the susceptibility of an individual to weight gain (Elks et al 2010), underlying biological factors play a trivial role at best in the global obesity epidemic given its fairly rapid development in recent years (O'Rahilly and Farooqi 2008). Lifestyle and the environment play a part in determining food intake and energy expenditure (Lenard and Berthoud 2008). An increased consumption of energy dense foods with little nutritional value combined with a shift towards less physical activity in the workplace and leisure time likely steer the obesity epidemic. Obesity places an enormous economic burden on a country's health services because of its many associated co-morbidities such as hypertension, diabetes mellitus and heart disease. Thus it is essential that health care providers seek ways to both prevent and manage obesity cost-effectively (Trueman et al 2010). The prevalence of adult obesity (body mass index $\geq$ 30 $kg \cdot m^{-2}$) in the UK has increased by more than 10 % since 1994 and as of 2008, almost 25 % of adults aged sixteen years or over were classified as obese. This escalating problem is not just limited to adults, a third of under sixteens in the UK are now classified as overweight or obese (Source: Office for National Statistics). Globally, the situation is similar, but in many developing nations there also exists a scenario where obesity coexists with under-nutrition (Source: World Health Organisation). The problem is predicted to get worse and by the year 2015 the WHO estimate that 2.3 billion people worldwide will be overweight (body mass index 25 – 29.9 $kg \cdot m^{-2}$) and 700 million obese.

Treatments for the severely obese include pharmacological and surgical interventions. Bariatric surgery has been shown to be the only effective long term treatment for obesity and is also a cost-effective solution (Welbourn and Pournaras 2010). There are many types of surgical interventions but gastric banding and gastric bypass are the most commonplace (Buchwald and Oien 2009). The outcomes from surgical interventions appear to be superior to those from other interventions because firstly, gross weight loss is far greater, and secondly, weight loss is sustained in the longer term. With surgical interventions, individuals can lose up to 70 % of their excess weight after 1 y (Welbourn and Pournaras 2010) compared with approximately 8 % over the same duration with diet, diet and exercise, or meal replacement interventions (Franz et al 2007). However, surgery does not

come without its complications and Welbourn and Pournaras (2010) have suggested that gastric bypass can have a 10 times higher risk of death than gastric banding. As such, surgery is often considered a last resort when all other methods of weight loss have failed, and when there is an acceptable trade-off between the risks of the surgery and the beneficial outcomes that large amounts of weight loss will confer.

Drugs that cause weight loss are an appealing concept; however, their viability is questionable. Some drugs that make the market after clinical trials are subsequently withdrawn amid health concerns and/or limited efficacy (Wright and Aronne, 2011). The weight loss drugs rimonabant and sibutramine were recently withdrawn from the UK market because of unwanted side effects. Sibutramine reduces food intake by inhibiting the reuptake and degradation of serotonin and noradrenaline thus boosting their appetite suppressive actions (Williams, 2010). Data from the sibutramine cardiovascular outcomes trial, a 6 year trial of 10 000 patients, showed there was a 16 % increase in the risk of myocardial infarction or stroke amongst those individuals treated with sibutramine compared with controls (Williams, 2010). Given its modest effect on weight loss, this increased risk was deemed unsuitable and sibutramine was suspended from the market in Europe. Rimonabant, a cannabinoid receptor blocker, has been used for increasing weight loss. However, it was withdrawn amidst fears of causing adverse side effects, notably psychiatric effects. Despite this, a recent overview of the effects of rimonabant on weight loss and metabolic parameters showed that it was highly effective in decreasing body weight and importantly, it also caused favourable metabolic changes including increasing concentrations of high density lipoprotein which was partly independent of the loss in body weight (Christopoulou and Kiortsis 2011). Although these findings show there is some benefit of these drugs in decreasing obesity and its associated health problems, their undesirable side effects highlights the importance of developing drugs with a selective target. Only one anti-obesity drug remains on the market. Orlistat is a pancreatic lipase inhibitor that impairs the absorption of fat (Bray, 2010). Results from a 4 y, double blind, randomised controlled study showed that mean weight loss after 4 y was greater by 3 kg with orlistat than a placebo (Torgerson et al 2004). However, there was also a significant reduction in the relative risk of diabetes with orlistat treatment. Treatment with orlistat does not come without side effects; however, unlike rimonabant and sibutramine, the effects are more an inconvenience to the individual rather than causing any long term problems. Side effects include faecal fat loss and gastrointestinal problems (Bray, 2010).

There are some reports that orlistat may decrease the absorption of fat soluble vitamins (Filippatos et al 2008) although despite this, levels reportedly remain within the normal range (Bray, 2010). Given the economic cost of obesity and its associated health problems, any method that lessens the burden to the health service and to an individual's wellbeing must be welcomed. However, the viability of using drugs to combat obesity is potentially limited because the cost of bringing them to market along with their associated side effects on health may outweigh the beneficial impact on weight loss. Of note, in 2010, new drug applications for two of three obesity compounds were refused by the Food and Drug Administration because long term side effects were unknown (Wright and Aronne, 2011). In the efforts to combat the obesity epidemic, drug therapy may prove futile because of the complex system that regulates energy balance. Despite some drugs proving efficacious during clinical trials and when initially put on the market, the results of long term trials have indicated that the drugs are not so specific to simply reduce body weight, but also have other side effects that are often undesirable and detrimental to health.

Diet and physical activity interventions are common methods of controlling body weight. However, the efficacy varies depending on the type and/or combination of treatment. A comprehensive review of clinical weight loss trials observed that after successful weight loss during trials, many individuals regain weight at the end of treatment, albeit not back to baseline values (Franz et al 2007). Exercise interventions alone have revealed variable results with Franz et al (2007) reporting most exercise-alone interventions demonstrate minimal weight loss whereas a comprehensive review by Ross et al (2000) suggests exercise without a change in diet is effective for reducing both obesity as well as its related co-morbidities. Even in the absence of weight loss, it has been reported that regular exercise is associated with decreased total body fat (Lee et al 2005). The addition of exercise to a dietary intervention is associated with greater weight loss than a dietary intervention alone (Avenell et al 2004, Curioni and Lourenco 2005, Franz et al 2007). Collectively, these studies highlight the important role of exercise in the maintenance of a healthy body weight and body composition.

The conflicting findings regarding the effect of exercise on long term weight control may be due to several factors. Exercise has influence beyond its ability to expend energy, it also has effects on metabolic parameters that influence appetite and food intake and these effects may be specific to each individual (Hagobian and Braun, 2010). Recently, studies have

extended research past simply looking at the effect of an exercise intervention on a group's average weight loss to attempt to understand why some individuals lose weight and others do not. King et al (2008) undertook a study to examine the individual variability in weight loss in a group of overweight and obese male and female volunteers. After a 12 week exercise program where participants exercised for five days each week, body weight was significantly reduced suggesting that participants did not compensate for the energy they expended by increasing their energy intake. However, this study went further and looked at each individual's response to the exercise intervention. A large variability was observed in the change in body weight with some individuals losing almost 15 kg and others actually gaining weight. Based on this variability, the group was split into compensators and non-compensators. Compensators had increased hunger and an increase in energy intake in response to exercise, whereas non-compensators experienced no change in appetite but a decrease in energy intake. Thus, those that experienced a lower than expected weight loss were simply compensating for the energy expended through exercise by increasing their energy intake. The difference in response between individuals could not merely be explained by factors such as compliance to exercise, because in this study, exercise sessions were supervised. Given this large variability in exercise-induced weight loss, it is important to understand how exercise affects hunger and energy intake and the mechanisms behind the differences observed between individuals. This will facilitate more targeted methods of weight loss rather than a "one size fits all" approach.

There is a large body of research examining the effect of exercise on appetite. Most research suggests that acute bouts of high intensity exercise temporarily suppress hunger, a phenomenon termed "exercise-induced anorexia" (King et al 1994). This transient suppression of hunger does not generally affect post-exercise energy intake. Blundell and King (1998) suggest there is a loose physiological coupling between energy expenditure and energy intake. Indeed in the short (1 – 2 days) and medium term (7 – 16 days) it appears that individuals can tolerate large negative energy balances when undertaking exercise programs (Blundell et al 2003). Although energy intakes start to increase to make up the deficit, the compensation is only partial and accounts for about a third of the energy expended through exercise. To date, much of the current research examining the effects of exercise on appetite and food intake has primarily been of interest to those who are overweight or obese and seeking to lose weight through physical activity. However, the effect of exercise on appetite and food intake is not just of importance for those whose

primary goal is weight loss. It is also of significance to elite athletes who compete at a high level where an optimal body weight for their chosen sport is essential.

The American College of Sports Medicine highlights in its 2009 position statement on nutrition and athletic performance the importance of athletes consuming adequate energy when training for long periods of time or at a high intensity. Meeting their energy needs should be a top priority for athletes to enable them to maintain body weight and optimise any training effects. Inadequate intakes can cause loss of muscle mass and an increased risk of fatigue (Brouns, 1992). Furthermore, although not a predictor of optimal performance in a given sport, an individual's body composition and body weight may influence how successful they are. The composition of an athlete's diet may differ depending on the energy requirements of their chosen sport and the body composition desired. The environmental conditions exercise is performed in may also affect an individual's energy requirements. For example, when exercising in a hot environment, there is a shift in substrate utilisation towards increased reliance on carbohydrate stores (Starkie et al 1999). This is of particular importance for an athlete who may not habitually consume large amounts of carbohydrate but may need to increase their intake if training frequently in hot weather (Burke, 2001). Similarly, an enhanced reliance on carbohydrate utilisation at high altitude has been proposed (Brooks et al 1991, Roberts et al 1996). However these latter studies did not take into account the decrement in maximal oxygen uptake that occurs with increasing altitude in males and females (Miles et al 1980) and used the same absolute exercise intensity at sea level and altitude. When work rates at altitude are matched with the same relative intensity as sea level, substrate utilisation is unchanged under acute and chronic exposure (Lundby and van Hall 2002). It has been suggested that the type of energy store utilised during exercise could provide a stimulus to eat that particular nutrient at a meal (King, 1998). Although there appears to be no clear verdict regarding the effect of macronutrient preference after exercise, the effects of exercising in environmental extremes such as high altitude may indeed alter macronutrient preference (Boyer and Blume 1984, Rose et al 1988, Westerterp et al 1992) but further research is warranted.

Alterations in appetite and energy intake during exercise in environmental extremes could detrimentally affect an individual's ability to perform optimally. Time spent at high altitude results in body mass loss from both fat and lean stores (Boyer and Blume 1984).

At elevations less than about 5400 m almost three quarters of the body mass loss may be due to fat loss but at elevations above this, fat loss may account for only a quarter of the weight loss with the remainder coming from muscle wasting (Boyer and Blume 1984). The actual proportions may vary between individuals, dependent on their initial body composition, as well as the duration of exposure to high altitude (Westerterp and Kayser 2006). Body fat loss at altitude arises from high energy expenditures due to increases in exercise (Westerterp et al 1992, Westerterp et al 1994) and basal metabolic rate (Gill and Pugh 1964) that are not matched by comparable increases in energy intake. However, it has been suggested that the overriding determinant of weight loss at high altitude arises from insufficient energy intakes due to a lack of appetite and desire to eat (Westerterp and Kayser 2006). A loss of body mass at altitude is deleterious to performance and despite advances in clothing and equipment which improve an individual's chance of reaching the summit of high mountains (Winsdor and Rodway 2005), little progress has been made in understanding the mechanisms behind appetite loss at altitude, which in future could lead to the development of strategies to combat the problem. Hence more research is warranted to investigate the possible mechanism/s responsible for the loss of appetite and reduction in energy intake experienced at high altitude.

It is not just high altitude that poses a challenge to an individual's ability to perform optimally. There are an increasing number of demanding events that challenge human endurance (Brown, 2002). Many of these take place in environments where extreme ambient temperatures would add to the nutritional challenges such events would already likely place on an individual. Hot weather can suppress appetite (Burke, 2001), thus in a situation where large energy intakes are warranted to compensate for high exercise-induced energy expenditures, such as during endurance running, a lack of appetite would be undesirable. It may be a challenge for an athlete to consume enough calories to meet their requirement in a comfortable ambient environment, but this could be exacerbated by exercising in the heat. In short duration events, where athletes may be required to compete several times each day, recovery between events is essential (Burke, 2001). Poor appetite could hinder the recovery process if an athlete does not consume adequate intakes or the appropriate composition of the diet between events. Much of the evidence surrounding the effect of hot weather on appetite is anecdotal and more research is required to understand the effects of exercise in the heat on appetite and energy intake. It has been suggested that fluid-rich or liquid forms of carbohydrate may be useful in encouraging an individual to

obtain enough energy (Burke, 2001). However, it remains unclear whether provision of food that includes the option of liquid items would actually encourage an individual to obtain enough fuel and compensate for a lack of desire for solid foods.

Exercising in cold environments may stimulate appetite and increase energy intake (Dressendorfer, 1993, White et al 2005), however research is limited and quantification of energy intake has been limited to simply the post exercise meal. In sports such as open water swimming, water temperature will likely be lower than the thermoneutral temperature for humans (Pendergast and Lundgren 2009), hence heat will be rapidly conducted away from the body because of the high thermal capacity of water compared with air at equivalent temperatures (Weller et al 1997). This is evident from studies reporting that hypothermia is a common problem that affects participants in long distance open water swimming events (Nuckton et al 2000, Brannigan et al 2009), especially those taking longer durations to complete the race such as the recreational competitor (Brannigan et al 2009). The effects of such events on appetite are unknown, however, White et al (2005) show that appetite and energy intake are increased after cycling exercise on a modified ergometer whilst immersed in cold water. However, the applicability of this study to real-life situations is limited given the nature of exercise undertaken. A recent study shows that swimming in a pool at 28 °C induces a temporary suppression of appetite during exercise, but stimulates hunger in the hours after (King et al 2011a). Collectively, the limited evidence suggests that exercise in cold water stimulates appetite, but whether exercise in cold air elicits similar effects is unknown.

It has only been in recent decades that the processes by which appetite and food intake are regulated have become clearer. Although body weight can be maintained remarkably constant over time by adjustments in energy intake and energy expenditure (Stanley et al 2005) the homeostatic system appears to favour energy conservation and storage of body fat (Murphy and Bloom 2004). Appetite and food intake are regulated by the brain in response to peripheral signals arising from the gastrointestinal tract and adipose tissue (Stanley et al 2005). There are an extensive number of hormones secreted from the gut that influence food intake and the majority act to reduce food intake (Chaudhri et al 2006). Ghrelin is the only known gut hormone that stimulates appetite and as such is a central theme in appetite research. Ghrelin circulates in two forms, acylated and des-acylated ghrelin (Hosoda et al 2000) but acylation of ghrelin is essential for its appetite-stimulatory

effects. Although initial research focused on the effect of exercise on total ghrelin (acylated and des-acylated ghrelin), with the development of assays that can specifically measure acylated ghrelin, recent studies have investigated the effect of exercise on acylated ghrelin and how it may affect subsequent appetite and energy intake (Broom et al 2007, King et al 2010a, King et al 2010b). However, given these relatively recent developments, there is only limited research concerning the effects of exercising in environmental extremes on acylated ghrelin. This requires investigation given the unique role that acylated ghrelin holds in the control of appetite. Peptide YY (PYY) is an anorectic gut hormone that is released into the circulation in response to food ingestion. Recently, it has been observed that PYY concentrations are transiently elevated during exercise (Martins et al 2007, Broom et al 2009) and increased concentrations in response to exercise in the heat may be involved in lowering post-exercise energy intake (Shorten et al 2009). Although there is some research investigating the effects of altitude on other anorectic gut peptides the primary role of some of those hormones is not their inhibitory effect on appetite and food intake. Peptide YY plays a significant role in food intake yet there is no research that has investigated the effect of altitude on PYY to determine any involvement in appetite loss at high altitude. Adipose tissue plays a central role in long term energy balance and secretes the hormone leptin (Zhang et al 1994), which circulates in the blood in proportion to body fat mass (Considine et al 1996b) and acts to suppress food intake (Tuominen et al 1997). Leptin concentrations respond to periods of prolonged fasting and to weight loss and weight gain, but there is limited evidence of a role for leptin in the acute regulation of food intake (Jéquier, 2002). Similarly, studies have rarely shown any effect of leptin concentrations in response to exercise. However, leptin may be influenced by hypoxia (Grosfeld et al 2002) thus the effect of altitude on leptin concentrations warrants investigation.

Although it is evident that hormones from the gut and adipose tissue play an important role in body weight regulation, our understanding is still far from complete. The conflicting results from various interventions such as diet, exercise and drug therapy highlight how complex the appetite and energy intake regulating systems are and effective, long-term treatments for obesity still elude researchers. Much research now focuses on how appetite regulatory hormones are affected by exercise to increase our knowledge about the appetite and food intake responses to exercise. This may assist in understanding the variable effects of exercise programs on weight loss. Furthermore, evidence shows that the expected

appetite and food intake response to exercise can be perturbed after exercising in extreme environments. Thus there is a need to examine the effects of differing environmental conditions on appetite-regulatory hormones to determine any potential role for them in altering appetite and food intake.

The purpose of the studies within this thesis was to examine the effects of exercising in different environmental conditions such as ambient temperature and altitude on appetite and energy intake. The effects of those environments on the appetite regulatory gut peptides acylated ghrelin and PYY were also investigated in an attempt to enhance understanding of the involvement of gut hormones in mediating changes in appetite and energy intake. This is of relevance to both the overweight and obese population who seek to lose weight via exercise programmes and also to athletes competing and training in hot or high altitude environments where maintenance of energy balance is important for optimal performance. The mode of exercise used in many studies within the literature that have investigated the effect of exercise on appetite regulatory gut peptides has been running, however, for the overweight and obese this is likely an unrealistic option because of the weight bearing nature of this exercise mode. Cycling is a more acceptable form of exercise for this population because body mass is supported, however, whether the responses of hunger and acylated ghrelin concentrations differ between running and cycling has never been directly compared in the same study. Therefore, this will also be investigated in a study using normal weight volunteers.

CHAPTER II

Literature review

2.1 Introduction

In this review, the literature on the effects of exercise on appetite, energy intake and the appetite regulating hormones ghrelin and PYY will be discussed. The review will start by describing how hunger and appetite are regulated by gastrointestinal hormones and how this affects energy homeostasis. Thereafter, the effect of acute exercise on hunger and energy intake will be discussed and how acute exercise may alter concentrations of the hormones responsible for appetite control to modulate energy balance. Finally, the effect of resting and exercising in different environmental conditions on these variables will be examined.

2.2 Regulation of appetite by gastrointestinal hormones

Although the terms hunger and appetite are often used interchangeably, they can be defined differently. Hunger has been described as a motivational drive to consume food in order to eliminate the sensation, replenish the nutrients necessary for survival and achieve satiation (LaGraize et al 2004). Thus it arises from the physiological need for food to sustain life. Appetite encompasses feelings such as fullness and the urge to eat food (Blundell, 2006) and these factors can also be influenced by psychological and behavioural factors. Hunger is under the control of peripheral signals that act in the brain to alter an individual's perception of hunger or fullness depending on feeding status or in response to experimental manipulations (Blundell, 2006). These signals represent a well controlled although complex system by which acute changes in energy balance can be recognised and appropriate physiological responses coordinated to maintain energy balance. The precise control of this system is evident because even large fluctuations in daily energy intake and expenditure do not drastically alter body weight, because net energy balance over longer periods of time will be regulated close to zero (Abbott et al 1988).

The appetite regulatory system is highly complex and is under the control of two complementary drives; the hedonic system which is associated with the rewarding aspects of food, and the homeostatic system, which is primarily involved with the maintenance of energy balance (Lutter and Nestler 2009). These systems may interact with each other to control feeding or they may act independently (Saper et al 2002). Feeding can be initiated

by the taste and smell of food even in the presence of signals from the homeostatic system indicating a positive energy balance (Van Vugt, 2010). This disturbance of normal energy balance could clearly impact upon weight control. With the advance of new techniques, such as functional magnetic resonance imaging of the brain, it is now becoming possible to investigate how the homeostatic and hedonic systems interact to control feeding.

Energy homeostasis is regulated by short and long term signals which provide the brain with information regarding acute and chronic nutritional states. Short term signals are associated with eating episodes and originate mainly from the gastrointestinal (GI) tract (Blundell 2006). The GI tract is the largest endocrine organ (Ahlman and Nilsson 2001) and the hormones secreted from it are both orexigenic (ghrelin) and anorexigenic (eg: peptide YY, glucagon-like peptide-1, pancreatic polypeptide and oxyntomodulin). These hormones influence hunger prior to meal initiation and satiety in the postprandial period (Murphy and Bloom 2006) and thus are involved in the acute regulation of food intake. Long term regulation arises from signals outside of the GI tract such as leptin and insulin that are positively correlated with adiposity (Garfield et al 2009). Leptin is secreted from adipocytes in proportion to body fat mass and signals to the brain when energy stores are adequate or too low. Insulin, a peptide secreted by the beta-cells of the pancreas, circulates in high concentrations in obesity (Bonadonna et al 1990) in proportion to body fat stores (Polonsky et al 1988). These hormones that communicate both acute and chronic energy states are secreted into the circulation where they act in appetite regulating centres within the brain. They may also act via neural connections, such as the vagus nerve, that transmits signals from the gut to the brain (Berthoud, 2008).

The hypothalamus and the brainstem receive neural and hormonal inputs to coordinate a response to regulate energy homeostasis based upon adiposity and the acute nutritional state (Murphy and Bloom 2006). The arcuate nucleus (ARC) of the hypothalamus is an important area for appetite control and is ideally located by the median eminence (Cone et al 2001) which lacks an intact blood brain barrier (Peruzzo et al 2000). This enables neural cells to sample hormones circulating in the blood and influence neuronal activity in the hypothalamus (Peruzzo et al 2000). The ARC has two distinct subsets of neurons that act as sensors to regulate appetite. Orexigenic (appetite stimulating) neurons express neuropeptide Y (NPY) and agouti-related peptide (AgRP) and adjacent to these are anorexigenic (appetite suppressing) neurons that express pro-opiomelanocortin (POMC)

and cocaine and amphetamine related transcript (CART) (Morton et al 2006). It is these neurons that respond to changes in circulating hormone levels in the blood to alter appetite, energy intake and energy expenditure thus regulating energy homeostasis. A representation of this system is shown in Figure 2.1.

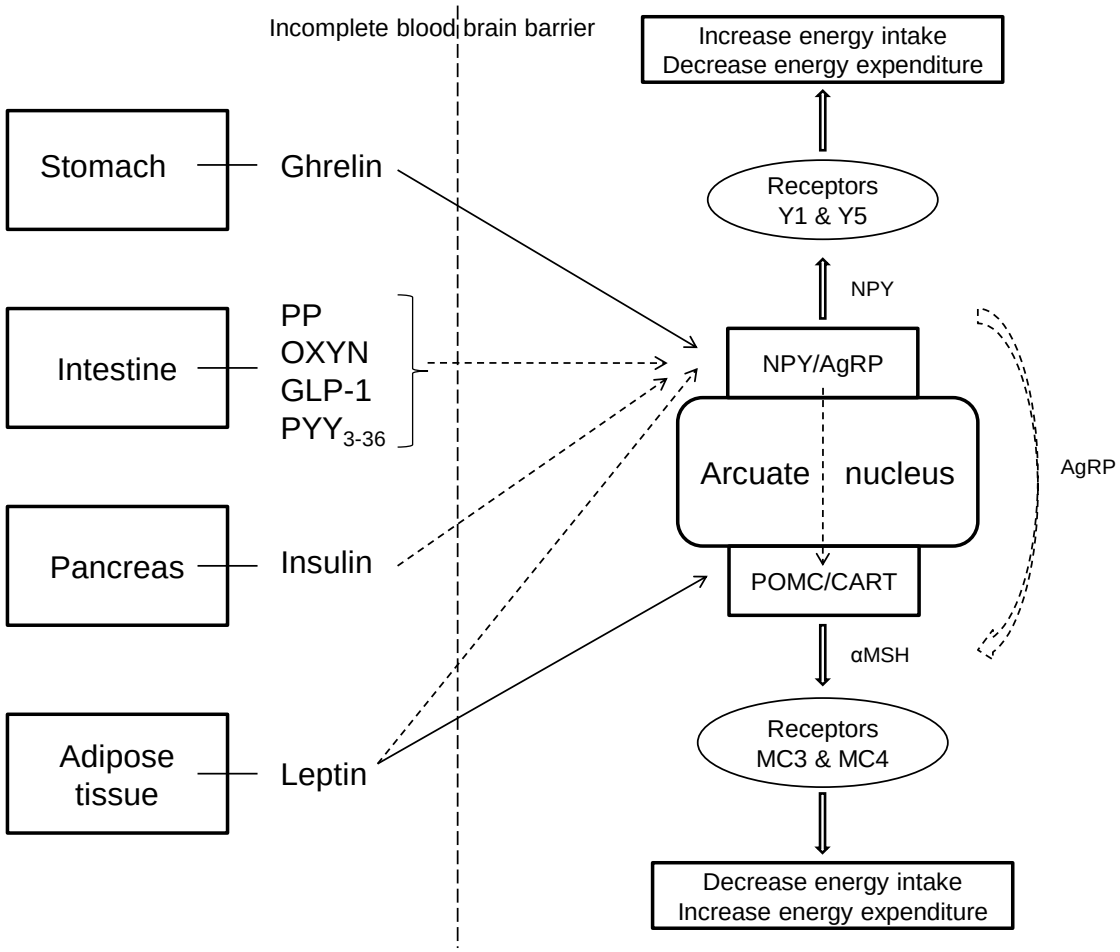

Figure 2.1 shows how the short term and long term signals that influence energy homeostasis are integrated in the arcuate nucleus and their effect on energy balance. The figure is adapted from Murphy and Bloom (2004). Solid black lines indicate stimulatory effects and dashed lines indicate inhibitory effects. PP – pancreatic polypeptide, OXYN – oxyntomodulin, GLP-1 – glucagon-like peptide-1, PYY_{3-36} – peptide YY_{3-36}, NPY – neuropeptide Y, AgRP – agouti-related peptide, POMC – pro-opiomelanocortin, CART – cocaine- and amphetamine-related transcript, αMSH – alpha-melanocyte-stimulating hormone, MC3/4 – melanocortin 3/4

2.3 Ghrelin

2.3.1 Identification, structure and function of ghrelin

Ghrelin is a gut hormone involved in the stimulation of appetite as shown in Figure 2.1. It was identified in 1999 by Kojima and colleagues as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). The name was derived from the term "ghre" which is the Proto-Indo-European root for the word "grow" in recognition of its role in the release of growth hormone. Ghrelin is a 28 amino acid hormone (Figure 2.2) generated from a 117 amino acid peptide called prepro-ghrelin (Kojima et al 1999). Ghrelin binds to the GHS-R1a which is mainly concentrated in the hypothalamic pituitary unit (Broglio et al 2003) but also distributed in other central and peripheral tissues including the heart, lung, pancreas and intestine suggesting diverse physiological roles for ghrelin (Kojima et al 1999, Gnanapavan et al 2002). However, its first observed function in humans was its ability to potently stimulate growth hormone release in a dose-dependent manner (Kojima et al 1999, Takaya et al 2000).

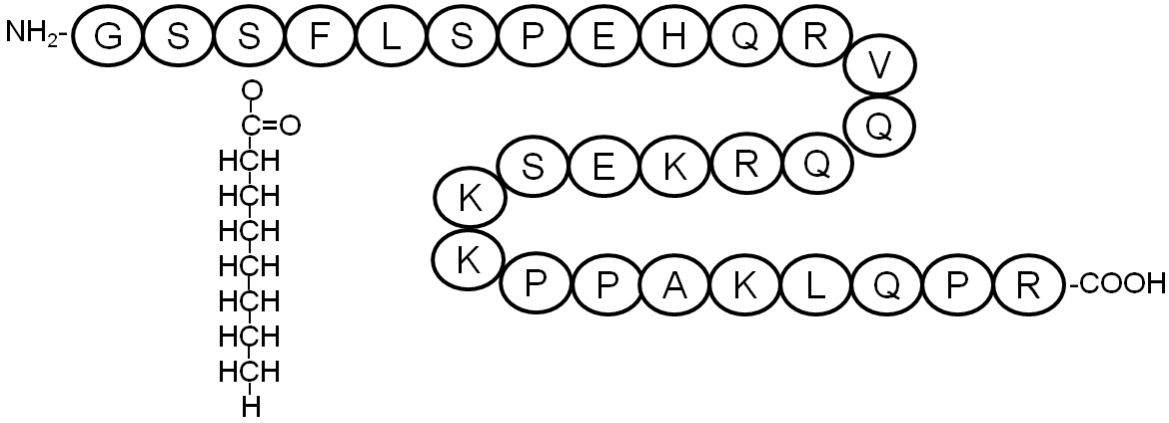

Figure 2.2 shows the structure of human ghrelin. The third amino acid, serine, is modified by *n*-octanoic acid. The figure is adapted from Kojima (2008).

Ghrelin is post-translationally acylated at serine 3 (Figure 2.2) under the influence of ghrelin O-acyltransferase (GOAT) (Yang et al 2008, Gutierrez et al 2008). Ghrelin is acylated predominantly by octanoic acid but also by other medium chain fatty acids

(Kirchner et al 2009) and this acylation is essential for ghrelin's ability to bind to its receptor and for most of it biological actions including its role in appetite stimulation (Kojima and Kangawa 2005). Although the site of ghrelin acylation by GOAT is unclear, research undertaken by Goodyear et al (2010) suggests that ghrelin acylation probably does not occur outside the parts of the gastrointestinal tract that are served by the portal venous system and the liver and their data tentatively suggests that the liver may be involved in the acylation of ghrelin. Ghrelin exists in two major forms - acyl and des-acyl ghrelin (Hosoda et al 2000) with up to 90 % of ghrelin in plasma being des-acyl ghrelin (Hosoda et al 2000, Broglio et al 2003). Des-acyl ghrelin was initially termed "inactive ghrelin" because it was not though to possess any biological activity (Kojima et al 1999). Recently, it has become apparent that des-acyl ghrelin is involved in some biological functions such as cell proliferation and cardiovascular activities (Broglio et al 2005) and may bind to an as yet, unidentified receptor (Kojima and Kangawa 2005, Gasco et al 2010). Aside from the early reports demonstrating the potent growth hormone secreting effect of ghrelin (Kojima et al 1999, Takaya et al 2000) it was found that ghrelin infusion stimulates appetite and food intake in humans (Wren et al 2001a) and the primary role of ghrelin is its orexigenic effect and role in energy homeostasis (Peeters, 2005). Subsequently, further discussion will be specifically in regard to the effects of ghrelin on appetite and energy balance.

2.3.2 Production of ghrelin

The X/A cells, or 'ghrelin cells' (Peeters, 2005) of the stomach are the major source of ghrelin production although smaller amounts may originate from the duodenum and jejunum (Date et al 2000, Ariyasu et al 2001). Consistent with these findings, fasting ghrelin levels are significantly reduced in patients who have undergone total gastrectomy (Jeon et al 2004, Kamiji et al 2010). However, Kamiji et al (2010) show ghrelin concentrations are far from negligible, suggesting other areas may compensate and produce ghrelin after total gastrectomy.

After production in the stomach, ghrelin is secreted into the portal vein and passes into the liver where it reaches the circulation (Goodyear et al 2010). Total ghrelin values are reportedly between 300 – 800 $pg \cdot mL^{-1}$ (Peeters, 2005) although studies now commonly report concentrations of acylated ghrelin as this is its active form. Ravussin et al (2001) showed that variations in ghrelin concentrations amongst individuals do not impact upon body weight regulation and suggested that variability in fasting levels most likely has a

genetic origin. Concentrations of plasma acylated ghrelin also show great inter-individual variability with Huda et al (2009) demonstrating a mean fasting ghrelin concentration of 762 $pg \cdot mL^{-1}$ in nine lean participants compared with a mean of 130 $pg \cdot mL^{-1}$ reported by King et al (2010a), also in nine lean individuals. The differences reported may be true physiological differences, but may also be due to specificity of the assay used and appropriate pre-treatment of blood samples for determination of ghrelin concentrations. The quantification of acylated ghrelin from plasma requires specific pre-treatment of the blood sample (section 3.14.2) to prevent the acyl modification being cleaved during sample extraction and the degradation of ghrelin by proteases (Kojima and Kangawa 2005). The two aforementioned studies used different methods of pre-treatment of blood samples for quantification of acylated ghrelin concentrations. Although the two methods are commonly used and recommended procedures, different methods may yield differences in acylated ghrelin concentrations (Chandarana et al 2009).

2.3.3 Ghrelin and short term energy regulation

To fully understand the appetite stimulatory effect of ghrelin, many studies have investigated the effects of ghrelin administration in rodents and humans. Intracerebroventricular and intraperitoneal administration of ghrelin in rodents increases food intake and body weight and also decreases fat utilisation (Tschöp et al 2000, Wren et al 2000, Nakazato et al 2001, Wren et al 2001b). Intravenous ghrelin administration at physiological and supraphysiological doses increases hunger and food intake in lean and obese humans (Wren et al 2001a, Akamizu et al 2004, Druce et al 2005, Akamizu et al 2008, Huda et al 2009) in a dose-dependent fashion (Akamizu et al 2004, Akamizu et al 2008). Even in individuals with diseases such as cancer where appetite and weight loss are reported, the orexigenic effect of exogenous ghrelin is also observed. Neary et al (2004) report a 31 % increase in energy intake after ghrelin infusion in 7 cancer patients experiencing appetite loss. This increase is comparable with the effect observed in healthy individuals (Wren et al 2001a). Collectively, these infusion studies suggest ghrelin is involved in the initiation of food intake.

Indicative of a role in the short term regulation of food intake, human ghrelin levels increase almost two fold before a meal and decrease rapidly postprandially (Cummings et al 2001, Tschöp et al 2001a). In support of this, in a study where ghrelin concentrations were measured 38 times over a 24 h period in humans receiving three meals a day, ghrelin

increased before each meal and fell to trough levels within one hour after meals (Cummings et al 2001). To eliminate the possibility that increases in ghrelin were in anticipation of habitual meal times another study showed that when all environmental cues usually associated with feeding were removed, ghrelin levels increased prior to spontaneous initiation of a meal and there was also a similar temporal profile between hunger scores and ghrelin levels (Cummings et al 2004). These findings support the notion that ghrelin is involved in the short term regulation of food intake.

Although ghrelin concentrations vary widely between individuals, the pattern of change is generally similar. Ghrelin concentrations exhibit diurnal variation with low concentrations seen at night and high concentrations seen in the morning with preprandial surges and postprandial slumps (Cummings et al 2001). There are certain instances where the usual pattern of ghrelin changes is not observed. Firstly, prolonged fasting of 61.5 h appears to abolish the expected changes in ghrelin levels around usual mealtimes and although total ghrelin levels are unchanged compared with the fed state, the proportion of des-acyl and acyl ghrelin are very different with a decrease in acyl ghrelin (Liu et al 2008). The balance between these forms of ghrelin might be mediated by the regulation of ghrelin acylation (Liu et al 2008) which has been shown to be influenced by dietary fatty acids (Nishi et al 2005). When overall energy content is kept the same, smaller more frequent feeding elicits a different acylated ghrelin response to larger infrequent meals (Solomon et al 2008). Secondly, partial and total gastrectomy abolishes the postprandial suppression of ghrelin seen in healthy individuals (Kamiji et al 2010). This is even though basal ghrelin levels are similar between partially gastrectomised individuals and healthy individuals, indicating the extent of gastrectomy may not be to blame for the altered response, which might be related to vagotomy (see section 2.3.6). Finally, compared with normal weight individuals obese individuals also exhibit reduced postprandial ghrelin suppression (le Roux et al 2005), but it is unclear if this reduced suppression is directly involved in the aetiology of obesity. In summary, the current available evidence indicates a role for ghrelin in the short term regulation of food intake; however, there are occasions where the normal ghrelin response to energy status is disturbed.

2.3.4 Ghrelin and long term energy regulation

Total ghrelin concentrations negatively correlate with the degree of adiposity and change appropriately in response to fluctuations in energy stores. Compared with age matched lean

controls, plasma ghrelin levels are lower in obese individuals and higher in individuals with anorexia nervosa (Tschöp et al 2001b, Shiiya et al 2002, Huda et al 2009). In rodents, repeated ghrelin administration induces hyperphagia (Wren et al 2001b). Daily ghrelin administration for 14 days in rodents induces adiposity as a result of increased food intake, as well as an increase in the respiratory quotient (RQ) which decreases fat utilisation and results in a gain in body mass (Tschöp et al 2000). This study also showed a reduction in energy expenditure suggesting ghrelin is involved in both aspects of energy balance.

Confirming the effect of increased body weight in regulation of ghrelin concentration a study in normal weight humans showed weight gain by chronic overfeeding decreases fasting plasma ghrelin levels compared with lean controls and blunts the usual responses to fasting and feeding in morbidly obese individuals (Tschöp et al 2001a, Engstrom et al 2007). In the Pima Indians, a population with high rates of obesity, ghrelin levels are significantly lower than in Caucasians (Tschöp et al 2001b). These studies suggest a down-regulation of ghrelin concentrations in the obese state is a consequence of chronic positive energy balance. It seems unlikely that ghrelin production plays a role in increased food intake in most obese individuals because circulating levels are low. However, it is unknown whether ghrelin levels are elevated in individuals that subsequently become obese, and gradually decrease over a prolonged period of weight gain.

Natural weight loss in obese individuals results in an increase in ghrelin levels in humans, independent of the method of weight loss (physical activity or food restriction). The increase in ghrelin is correlated with the extent of weight lost (Cummings et al 2002, Hansen et al 2002, Leidy et al 2004, Foster-Schubert et al 2005). Conversely, weight gain in anorexics reduces high circulating ghrelin levels back to normal values (Otto et al 2001). The effect of weight loss via gastric bypass surgery has revealed conflicting results regarding circulating ghrelin levels with increases (Holdstock et al 2003, Stratis et al 2006), decreases (Jeon et al 2004, Kamiji et al 2010) and no change (Mancini et al 2006, Olivan et al 2009) all observed. These discrepancies may be due to the differences in surgical techniques used and the length of time of follow up.

A study in mice fed high fat diets to induce obesity also demonstrates a reduction in plasma ghrelin levels (Perrault et al 2004). However, in this study it is possible that the decreased ghrelin levels were a consequence of the high fat diet and not just weight gain because

control rats were fed a low fat diet. This hypothesis was tested and the findings suggested ghrelin levels are suppressed in dietary induced obese mice as a consequence of obesity and not the macronutrient content of the diet. Substituting a high fat diet with a normal chow diet for 3 days prior to ghrelin administration resulted in a similar attenuation or ablation of ghrelin induced feeding. This suggests that exogenous ghrelin administration does not induce the usual feeding intake in dietary induced obese mice and this is not a consequence of a high fat diet (Briggs et al 2010). In these mice, obesity appears to promote hypothalamic ghrelin resistance and the authors propose that the positive state of energy balance is sensed by the hypothalamus which responds accordingly by suppressing the neuro-endocrine ghrelin axis. This resistance could also occur in humans and might explain the low levels of ghrelin that are associated with obesity. These findings suggest a role for ghrelin in the prevention of starvation but not in the aetiology of obesity.

2.3.5 Regulation of ghrelin secretion

Ghrelin is secreted from the stomach into the circulation in response to short term fasting. Plasma ghrelin concentrations are regulated by caloric intake with circulating concentrations falling after ingestion of food or glucose (Tschöp et al 2000). It is unlikely this suppression is caused by gastric distension because ingestion of a similar volume of water does not affect ghrelin concentrations (Tschöp et al 2000). The postprandial suppression of ghrelin is dependent on both the caloric and macronutrient content of the meal. Meals of increasing caloric load suppress ghrelin levels to a greater extent (Callahan et al 2004, le Roux et al 2005) and this suppression occurs within about an hour of the initiation of the meal (Cummings et al 2001). Carbohydrate meals elicit a greater postprandial suppression of ghrelin levels compared with either protein or fat (Monetelone et al 2003, Erdmann et al 2004, El Khoury et al 2006) and protein elicits a significant and prolonged suppression of acylated ghrelin compared with a balanced and high fat meal (Al Awar et al 2005). When the carbohydrate content of a meal is kept constant, acylated ghrelin is suppressed to a greater extent with a high protein low fat meal compared with a high fat low protein meal (El Khoury et al 2010). The effects of high fat meals on postprandial ghrelin levels are conflicting. It may be that total and acylated ghrelin respond differently to high fat meals because some research has shown high fat meals do not suppress acylated ghrelin (Tentolouris et al 2004) whereas they do suppress total ghrelin (Erdmann et al 2003, Monteleone et al 2003).

Insulin and glucose may be implicated in the regulation of ghrelin concentrations in rodents and humans. Negative correlations between insulin and ghrelin levels have been reported (Saad et al 2002, Cummings et al 2004, Al Awar et al 2005) and this appears to be mediated independently of the effect of insulin on glucose (Mohlig et al 2002, Flanagan et al 2003). Oral and intravenous administration of glucose results in a significant decline in circulating ghrelin concentrations (Nakagawa et al 2002). This suggests a role for plasma glucose in the suppression of plasma ghrelin levels. Ghrelin suppression by intraduodenal administration of glucose is independent of the glucose load and also unrelated to the plasma insulin or glucose level (Cukier et al 2008). However, Schaller and colleagues (2003) provide robust evidence to suggest that the meal related suppression of ghrelin is not regulated by either insulin or glucose. Ghrelin concentrations were unaffected by glucose administration of up to 11 $mmol \cdot L^{-1}$ and were only suppressed by insulin at supraphysiological levels. Other research has demonstrated that compared with food intake, administration of a short-acting insulin analog does not suppress ghrelin levels (Caixás et al 2002). Collectively, the data would suggest that although insulin and glucose are involved in the response of ghrelin to fasting and feeding, it is unlikely they explain the whole suppression of ghrelin with feeding.

Ghrelin secretion may also be affected by its interactions with other gut peptides such as PYY and cholecystokinin (CCK) that exert anorectic effects on appetite and food intake (Wisser et al 2010). Anorectic gut peptides may inhibit the secretion of ghrelin and also exert opposite effects in the hypothalamus to alter food intake. One study has demonstrated infusion of CCK suppresses ghrelin concentrations (Brennan et al 2007) however another has shown that perfusing isolated rat stomachs with CCK increases ghrelin secretion (Shrestha et al 2009). Batterham et al (2003) have demonstrated a decrease in ghrelin concentrations with PYY infusion in humans but there is limited other research available. Another appetite-regulatory hormone leptin, although not secreted from the gut, plays a role in satiety and has been shown to interact with ghrelin. Nakazato et al (2001) demonstrate a suppressive effect of leptin on ghrelin induced feeding and negative correlations between ghrelin and leptin have previously been observed (Tolle et al 2003, Shukla et al 2005). However, current evidence is conflicting and how these hormones interact to affect food intake still remains to be elucidated.

2.3.6 Mechanism of action

Ghrelin exerts its feeding activity by the stimulation of NPY/AgRP neurons in the hypothalamus. This stimulates both production and secretion of NPY and AgRP which increases appetite (Figure 2.1). Although peripheral administration of ghrelin stimulates neurons in the hypothalamus to increase appetite, it has been shown that ghrelin passes through the blood brain barrier at a low rate and hence it has been proposed that ghrelin may exert some of its appetite stimulating effects in the hypothalamus via an indirect path (Kojima and Kangawa 2005). The vagus nerve is thought to be involved because in vagomotised rats and humans the orexigenic effect of peripheral ghrelin is abolished (Date et al 2002, le Roux et al 2005). However, there is still controversy about the relative involvement of the circulation and afferent neurons in initiating the orexigenic effects of ghrelin in the hypothalamus. In patients with total gastrectomy and complete vagotomy, intravenous administration of ghrelin reduced the decline in post-operative body weight compared with a control group (Adachi et al 2010) suggesting ghrelin still exerted some orexigenic effect, although in another study intravenous administration of ghrelin after that procedure has not been shown to increase appetite and food intake (Huda et al 2009). Whether this lack of effect is a result of vagotomy or not is unclear, because a study in rats showed abdominal vagal afferents were not required for the appetite stimulating effects of peripherally administered ghrelin (Arnold et al 2006). Druce et al (2005) show ghrelin administration increases the palatability of available food in obese individuals which could also explain an increased food intake. Recent studies using brain imaging suggest that gut peptides such as ghrelin can influence areas in the brain associated with hedonic, or reward-driven hunger, meaning food intake can be regulated by reward-driven processes even when an individual is satiated (Gibson et al 2010).

2.4 Peptide YY

2.4.1 Identification, structure, production and function of PYY

Peptide YY was first isolated from porcine upper intestinal tissue (Tatemoto and Mutt 1980). Its name originated from the presence of a tyrosine (Y) residue at both the C- and N- terminus of the peptide (Tatemoto, 1982). Peptide YY is a 36 amino acid anorexigenic peptide released into the circulation from the endocrine L-cells of the gastrointestinal tract in response to food ingestion. Peptide YY circulates in two active forms in the human blood stream; PYY_{1-36} and PYY_{3-36} (Grandt et al 1994). Peptide $YY_{3-36,}$ a truncated 34

amino acid peptide, is produced as a result of tyrosine and proline amino acids being cleaved from the N terminus of PYY_{1-36} by the enzyme dipetidyl peptidase IV (DPP-IV) (Mentlein et al 1993). The major form of circulating PYY is the N-terminally truncated form, PYY_{3-36}, which is abundant in the fasted state constituting approximately 65 % of total circulating PYY in fasted normal weight and obese individuals (Batterham et al 2006) and comprising the larger proportion after a meal (Grandt et al 1994). Total PYY (PYY_{1-36} and PYY_{3-36}) and PYY_{3-36} have been shown to be highly positively correlated (r = 0.98) (Tsilchorozidou et al 2008**)** thus there is still value in studies that have solely measured total PYY**.**

Peptide YY is involved in various physiological functions including being an important mediator of the "ileal brake" mechanism (Lin et al 1996, Grudell and Camilleri 2007). The ileal brake was first identified by Read et al (1984) and Spiller et al (1984) as a slowing of gastric emptying and intestinal transit in response to lipids in the distal small intestine. Peptide YY also inhibits tumour growth and inflammation (Alosi and McFadden 2009), may be involved in renal function (Playford et al 1995) and could alter postprandial vascular resistance under physiological concentrations associated with disease (Playford et al 1992). However, one of the most well researched functions of PYY is its inhibitory effect on food intake and appetite (Batterham et al 2002) and its involvement in energy homeostasis. Thus, the literature concerning the appetite regulatory role of PYY will be the main focus of this section.

2.4.2 Peptide YY and short term regulation of appetite

Despite some controversy surrounding the anorexigenic effect of PYY in rodents (Tschöp et al 2004), exogenous administration of PYY in rodents does appear to reduce food intake (Batterham et al 2002, Challis et al 2004, Halatchev et al 2004, Pittner et al 2004, Chelikani et al 2005). In lean and obese humans, decreased appetite and energy intake is also seen after peripheral administration of PYY_{3-36} (Batterham et al 2002, Batterham et al 2003, Degen et al 2005, Sloth et al 2007). The effects are marked; a 36 % reduction in food intake is observed two hours after intravenous PYY_{3-36} infusion at doses to mimic postprandial concentrations (Batterham et al 2002). Furthermore, in the 24 hour period after intravenous PYY_{3-36} infusion, there was a cumulative reduction of 33 % in total calorie consumption (Batterham et al 2002). A comparable decrement in food intake of 30 % and a reduction in appetite were also observed in both lean and obese male and female

individuals after 90 minutes infusion of PYY_{3-36} (Batterham et al 2003). The decrease in food intake with PYY_{3-36} administration in obese individuals suggests that they are not resistant to the anorectic effect of PYY (Batterham et al 2003) and therefore incorporation of PYY_{3-36} into anti-obesity drugs could be an effective therapy for obesity. However, Degen et al (2005) showed physiological doses were insufficient to inhibit caloric intake. Although doses outside of the normal physiological range reduced caloric intake, this was associated with side effects thus questioning the efficacy of using PYY_{3-36} in treating obesity. Furthermore, repeated infusions of PYY_{3-36} were used in these studies rather than a single stimulus which suggests prolonged secretion may be required to elicit an effect. Further evidence supports this notion; when a single oral delivery of PYY_{3-36} was given with a delivery agent to mimic endogenous secretion, there was no significant change in energy intake (Steinert et al 2010). However, co-administration with glucagon-like peptide-1 (GLP-1) suppressed energy intake to a greater extent than GLP-1 alone suggesting a role for PYY_{3-36} in the reduction in food intake. In summary, evidence suggests that PYY reduces appetite and food intake.

2.4.3 Peptide YY and long term regulation of appetite and energy regulation

Compared with normal weight individuals, obese individuals have lower fasting and postprandial levels of PYY (Batterham et al 2003, Batterham et al 2006, le Roux et al 2006, Brownley et al 2010). In morbidly obese individuals, no increase in PYY levels is observed after a liquid meal (Morinigo et al 2006). Batterham et al (2003) showed that despite obese individuals consuming more calories at a test lunch they exhibited a diminished postprandial PYY response compared with lean individuals. To elicit a comparable increase in postprandial PYY concentrations in obese and lean individuals, an increase in calorie meal content is needed in the obese (le Roux et al 2006). Whether obesity is a direct result of low PYY levels or causes levels to be reduced as a consequence is unclear. Weight loss may reverse the reduced postprandial secretion of PYY and increase fasting levels but this is dependent on the method of weight loss (Roth et al 2005, Morinigo et al 2006, Valderas et al 2010). Weight loss from surgical interventions appears to result in exaggerated elevated PYY responses to feeding (Korner et al 2005, Chan et al 2006) even when individuals are still very obese (Morinigo et al 2006). Conversely, diet-induced weight loss causes a significant reduction in PYY levels in the fasting and postprandial states (Essah et al 2010) which the authors suggest as a mechanism behind weight gain on energy-restricted diets. Weight loss through a 12 week exercise program

does not alter fasting or postprandial PYY levels (Martins et al 2010) although after weight loss there was a tendency for a delayed increase in PYY two and three hours after feeding. In that instance, overall average weight loss of 3.5 kg might not be large enough to induce a compensatory PYY response in contrast with an average 15.4 kg weight loss from a surgical intervention (Morinigo et al 2006). The apparent failure for diet and exercise-induced weight loss to favourably increase PYY levels might represent a homeostatic mechanism to defend body weight. The mechanism behind the exaggerated postprandial increase in PYY with gastric surgery is unclear but could be due to bypassing the stomach leading to an increased rate of food entering the small bowel, thus earlier contact between ingested nutrients and the L cells responsible for PYY secretion (Korner et al 2005, Morinigo et al 2006). Currently, there are contradictory findings regarding the PYY response to weight loss which is possibly due to the method used to induce weight loss (surgical, diet, exercise or medicine induced).

2.4.4 Secretion of PYY

The circadian pattern of PYY secretion in humans has not yet been identified but the secretory response to various stimuli has been documented. After feeding, levels of PYY in the plasma increase within 15 minutes and reach a peak after about 90 minutes (Adrian et al 1985). These levels can remain high for several hours after feeding with the duration of elevation likely depending on the type and proportion of macronutrients ingested. Peptide YY is secreted in proportion to the amount of energy that is ingested (Adrian et al 1985) and recent findings suggest postprandial secretion is sensitive to the macronutrient composition of the meal. When examining the effect of single nutrients, fat appears to be the most potent of the macronutrients in stimulating PYY release (Adrian et al 1985, MacIntosh et al 1999) with the digestion of fat necessary for PYY secretion (Feinle-Bisset et al 2005). The findings are somewhat conflicting when assessing the effect of mixed meals on PYY levels. In obese men and women, a 7 d low carbohydrate high fat diet elicits a 55 % increase in postprandial PYY levels compared with a high carbohydrate low fat diet (Essah et al 2007). Conversely it has been shown that high protein meals result in the greatest increase in PYY levels compared with high fat and high carbohydrate meals in lean and obese men (Batterham et al 2006). In the same study, PYY levels were also significantly greater after the high fat meal compared with the high carbohydrate meal in the normal weight men suggesting carbohydrate is the least potent stimulator of PYY release. Peptide YY responses to high protein meals are similar to adequate protein meals

(Smeets et al 2008) and when carbohydrate content is kept constant, PYY levels are increased to a similar extent on high protein low fat diets and low protein high fat diets (Brownley et al 2010). Collectively these findings suggest a certain threshold of protein or fat is required for PYY secretion but amounts above this will not confer any additional increase in PYY secretion. When keeping the macronutrient composition of a meal constant, similar secretion of PYY is observed regardless of the glycaemic load of the meal (Brownley et al 2010). The available data indicates that the PYY response to macronutrient content is complex and probably influenced by many factors including the adiposity of the individual and whether single or mixed nutrient meals are provided.

Although ingested nutrients are necessary for the rise in PYY levels, Pilichiewicz et al (2007) propose that a certain amount of CCK release is required for the initial rise in PYY. They showed that PYY levels increased before nutrients would have reached the distal small intestine where the L cells responsible for PYY secretion are situated. Nutrients would have reached the proximal small intestine where cells that secrete CCK are located thus suggesting that an increase in CCK may initiate PYY secretion. Other regulatory gut peptides may also be implicated in the secretion of PYY. In rats, ghrelin infusion dose-dependently attenuates the anorexigenic response to intravenous PYY_{3-36} infusion (Chelikani et al 2006). In humans, infusion of GLP-1 inhibits the release of PYY into the circulation, a finding which the authors suggested may indicate a negative feedback of GLP-1 on L cell function, the cells which contain and release GLP-1 and PYY (Näslund et al 1999). Research thus far suggests that PYY release is stimulated by feeding but this response may not simply be a direct consequence of ingested nutrients stimulating secretion but could be influenced indirectly by alterations in concentrations of other gut regulatory hormones.

2.4.5 Mechanism of action

The mechanism by which PYY inhibits food intake is not clear but a variety of actions could be at work. Peptide YY mediates it effects through G protein coupled receptors Y1, Y2, Y4, Y5 and Y6. In humans and rodents, PYY_{1-36} binds all known receptor subtypes whereas PYY_{3-36} has a high affinity for the Y2 receptor, and a lesser affinity for Y1 and Y5 receptors (Dumont et al 1995). This difference in receptor binding between the 2 forms of PYY is due to the removal of tyrosine and proline from the N terminus by the action of DPP-IV to yield PYY_{3-36} which enables the hormone to bind predominantly to the Y2

receptor because the Y2 receptor only needs to recognise the C-terminal segment of PYY (Nygaard et al 2006). As PYY_{3-36} is a specific agonist for the Y2 receptor subtype (Keire et al 2000), DPP-IV represents a significant mechanism for regulating PYY receptor specificity (Ballentyne, 2006).

Most evidence suggests the effects of PYY on food intake are mediated via the hypothalamus. Firstly, feeding is inhibited with intra-arcuate injection of PYY_{3-36} but blockade of the Y2 receptor reduces this effect (Abbott et al 2005). Expression of c-Fos, a marker of neuronal activation, increases in the ARC after PYY_{3-36} administration (Batterham et al 2002) suggesting the site of action is in the hypothalamus. This group also showed that the actions of PYY_{3-36} were via a Y2 receptor dependent mechanism because in Y2 receptor-null mice, PYY_{3-36} did not inhibit food intake. Y2 receptors suppress appetite by inhibiting NPY release and increasing the activity of alpha MSH in the ARC (Ballantyne 2006). This acts to suppress the appetite through the melanocortin 4 receptor (MC4R) as represented in Figure 2.1. However, recent studies in mice have shown that in the absence of the MC4R (Halatchev et al 2004) or the POMC gene (Challis et al 2004) mice still retain the normal anorectic response to peripherally administered PYY_{3-36} thus indicating an alternative mechanism of action of PYY. It has been proposed that the vagus nerve may also play a role in mediating the anorectic effect of PYY_{3-36} with Koda et al (2005) demonstratating that PYY_{3-36} administration to vagotomised rats does not activate hypothalamic neurons whereas it does in control rats. In contrast, Halatchev and Cone (2005) show that vagotomised mice still demonstrate a reduced food intake with PYY_{3-36} administration and in fact, vagotomy appeared to prolong the inhibition of food intake compared with the control mice. Differences in findings may exist due to methodological differences or might be a species-specific feature which is why the result cannot definitively be extended to humans. Like ghrelin, the role of PYY_{3-36} in the short term regulation of food intake may not just be due to its effects on homeostatic brain areas, but might also relate to effects on hedonic brain areas. Using functional magnetic resonance imaging, Batterham and colleagues (2007) have shown that infusion of typical postprandial concentrations of PYY_{3-36} in normal weight volunteers modulates neuronal activity not just in the hypothalamus but also in regions of the brain involved in reward processing. Thus it is likely that PYY_{3-36} exerts its anorexigenic effects by acting on both homeostatic and hedonic brain circuits (Batterham et al 2007).

2.5 Leptin

2.5.1 Identification, structure, function and secretion of leptin

Leptin was first isolated in 1994 as the product of the obese (*ob*) gene in mice (Zhang et al 1994). In recognition of its effect on body mass, Zhang et al (1994) proposed the name leptin, from the Greek root leptós meaning thin. Leptin is a 167 amino acid adipocyte derived hormone (Zhang et al 1994) and its involvement in energy balance is depicted in Figure 2.1. Leptin is also secreted from the gastric mucosa (Bado et al 1998, Cinti et al 2000). Leptin secreted from these sites has specific roles with leptin secreted in a slow constant manner from adipocytes, but rapidly from gastric cells (Cammisotto et al 2010). Adipocyte leptin and stomach-derived leptin can be differentiated because leptin from the stomach increases rapidly after feeding and is secreted into the gastric lumen (Guilmeau et al 2004) where it is involved in the short term regulation of digestion such as delaying gastric emptying and the secretion of intestinal hormones (Cammisotto et al 2010). Leptin from adipocytes is secreted into the bloodstream where it is involved in the long term regulation of food intake and energy expenditure (Tuominen et al 1997) via its action in the hypothalamus.

In the obese *ob/ob* mouse, leptin administration reduces food intake and fat mass and increases energy expenditure (Halaas et al 1995). After the weight reducing effects of exogenous leptin administration in mice were observed, leptin administration in humans was proposed as an obesity cure until it was recognised that firstly, no mutations were observed in the *ob* gene sequence of obese humans (Considine et al 1996a) and secondly, leptin levels positively correlate with adiposity with elevated concentrations present in obese individuals (Considine et al 1996b, Clapham et al 1997, Korbonits et al 1997). Thus, obese humans are likely to be leptin resistant because elevated concentrations appear to be ineffective in preventing obesity progressing further. Leptin therapy to reduce body weight is efficacious in individuals with congenital leptin deficiency (Farooqi et al 1999) but does not elicit the same favourable effect in all obese individuals (Heymsfield et al 1999). However, recent research revealed that in obese leptin resistant individuals who lost 10 % of their initial weight through diet and exercise, circulating leptin was reduced and leptin sensitivity increased which would favour weight regain via changes in energy intake and expenditure, however, leptin therapy could prevent the weight regain by affecting neural activity in the brain related to feeding thus preventing a return to an obese state

(Rosenbaum et al 2008). Although adiposity is the largest determinant of inter-individual variations in leptin concentrations, gender accounts for 28 % of the variation, because for a given level of adiposity, females have higher leptin levels than men (Saad et al 1997). Leptin secretion into the blood is pulsatile with concentrations highest during the night and at their lowest point during the early afternoon with peak levels at night being up to 75 % higher compared with the lowest levels seen during the afternoon (Sinha et al 1996, Licinio et al 1997). Increased leptin concentrations in the obese are due to increased pulse height with other pulsatility parameters such as frequency being similar in lean and obese individuals (Licinio et al 1997, Yildiz et al 2004).

2.5.2 Leptin, hunger and energy regulation

It is generally believed that leptin secretion is not acutely affected by a single meal (Jequier, 2002). However, divergent responses to feeding between males and females have been observed with no change in leptin concentration reported in fasted lean and obese females (Clapham et al 1997, Korbonits et al 1997), an increase in lean men and a decrease in obese men (Imbeault et al 2001). However, rather than being meaningful observations, these contradictory findings may simply be a function of the circadian rhythm that leptin displays.

Leptin concentrations prior to an *ad libitum* meal provided 9 h after an isoenergetic meal high in fat or carbohydrate are not associated with subsequent food intake suggesting short term regulation of energy intake is not influenced by circulating leptin concentrations (Romon et al 1999). However, the suppression of leptin with 72 h of fasting appears to contribute to the increased caloric intake immediately post-fasting (Chan et al 2003). During sustained energy deficit, leptin concentrations fall at a rate that exceeds a decrease in fat stores (Considine et al 1996b) and the decline is proportionately greater in lean than obese individuals (Korbonits et al 1997). These findings suggest leptin is likely vital for the restoration of energy balance from large energy deficits occurring over several days but is not involved in satiation. In support of its role as a hormone involved in situations of famine rather than a hormone to limit excessive weight gain in those with plentiful food (Jequier, 2002), leptin is not associated with hunger in response to single feeding sessions (Karhunen et al 1997, Joannic et al 1998) but is correlated with hunger sensations in response to a sustained energy deficit (Keim et al 1998), an association that intensifies with increasing duration of energy restriction (Mars et al 2006).

2.5.3 Mechanism of action

Human leptin receptors are called LEPR and there are both long and short isoforms. Leptin binding to the long receptor isoform (Considine et al 1996a) is necessary for its effect on appetite and energy storage. Leptin receptors are most densely located on two populations of hypothalamic neurons in the ARC (Jéquier, 2002). These 2 populations of neurons that express leptin receptors are the orexigenic NPY and AgRP neurons, and the anorexigenic POMC and CART neurons. Leptin binding to its receptor decreases the expression of NPY and AgRP whereas it increases the expression of anorexigenic peptides POMC and CART (Jéquier, 2002). In states of leptin deficiency such as starvation, POMC and CART expression are decreased (Ahima et al 1999). Hence, the inhibition of feeding via leptin arises from the suppression of orexigenic, and the stimulation of anorexigenic neuropeptides (Ahima et al 1999, Jéquier, 2002).

2.6 Exercise, appetite and energy intake

2.6.1 Introduction

Energy balance relies on tight control of an individual's energy intake through food and drink, and their energy expenditure through physical activity and exercise. A mismatch in either of these will result in an increase in body weight when intake exceeds expenditure and a decrease in body weight when expenditure exceeds intake. Although the role of exercise and physical activity in preventing weight gain is commonly acknowledged in the field of obesity research, in the absence of energy restriction exercise surprisingly sometimes produces only modest weight loss (Franz et al 2007). This may indicate a strong homeostatic defence of energy balance and a temporary negative energy balance induced by exercise could be expected to increase appetite and energy intake immediately after exercise to restore energy balance (Blundell and King 1999). Indeed, this was thought to explain the observation that many people do not lose as much weight as expected through an exercise program (Epstein and Wing 1980). As a result, this has led to vast amounts of research investigating the effect of exercise on appetite and food intake. With increased understanding of the role that gut peptides play in appetite control, recent research has focussed on how exercise affects these hormones and their subsequent impact on appetite regulation. This section will review the literature that has examined these issues.

2.6.2 Appetite assessment

Visual analogue scales are commonly used to determine subjective feelings of hunger. These rating scales have been used in studies examining the effects of various foods on appetite and values were found to correlate well with energy intake and are sensitive to nutritional manipulations (Blundell and Hill 1986). Thus, visual analogue scales are deemed an appropriate tool to assess changes in subjective feelings of appetite with exercise and most studies have adopted this approach. A recent study tested the reproducibility and validity of using visual analogue scales and confirmed that they are a reliable tool in appetite research (Flint et al 2000).

2.6.3 Quantification of food intake

The measurement of food intake can be fraught with problems with different methodologies conferring an advantage depending on the setting. Food intakes are more likely to represent an individual's intake in a free-living environment, but outside of a laboratory this relies on self-report which can be subject to bias as individuals tend to underreport their intakes (Livingstone and Black 2003). Although eating in laboratory conditions could affect food intake and make it harder to separate the physiological and cognitive aspects of food intake (Blundell et al 2010), laboratory experiments provide the opportunity to more accurately quantify food intake in response to a stimulus when participants are naïve to the true purpose of the study.

2.6.4 Acute exercise and appetite

Contrary to early opinion, widespread evidence suggests that acute bouts of moderate to high intensity exercise transiently suppress appetite, a phenomenon termed "exercise-induced anorexia". This has been extensively replicated on many occasions in various activities including walking, running and cycling (Thompson et al 1988, King et al 1994, King and Blundell 1995, Westerterp-Plantenga et al 1997, Tsofliou et al 2003, Broom et al 2007, Ueda et al 2009b, King et al 2010a). The finding is not universal; some studies have shown intense exercise does not affect appetite or can even increase appetite (King et al 1996, Imbeault et al 1997, Hubert et al 1998, Melanson et al 1999, Maraki et al 2005).

Research is unanimous in confirming that exercise-induced anorexia is dependent on the intensity of the exercise bout. Low intensity bouts of exercise (approximately 35 % of $\dot{V}O_2$

max) are insufficient to elicit a suppression of appetite. This remains true even when energy expenditures are comparable with those elicited with high intensity exercise bouts (approximately 70 % of $\dot{V}O_2$ max) that do suppress appetite (Thompson et al 1988, King et al 1994). The threshold of exercise intensity required to suppress appetite is not well defined. Although a suppression of appetite has been observed with 30 minutes cycling exercise at 50 % $\dot{V}O_2$ max (Ueda et al 2009b), the majority of studies that have observed a suppression of appetite during exercise have used an exercise intensity of at least 60 % of $\dot{V}O_2$ max (Cheng et al 2009). The suppression of appetite during exercise does not seem to depend on the feeding status of the individual with the above studies being performed in both the fasted and fed states. However, one study showed that although hunger was suppressed during moderate intensity exercise performed in the fasted state, this was not the case when the same exercise was performed 2 hours after a meal but the exercise bout did prolong the depression of hunger ratings compared with a control trial (Cheng et al 2009). The lack of appetite suppression observed during the postprandial state could be related to the caloric intake of the meal although it is not possible to know because in the other studies where a standard meal was given the caloric content of the meal was not stated. Some studies report the suppression of hunger observed during intense exercise is only short-lived with hunger returning to values similar to those observed in control or low intensity exercise trials approximately 15 minutes after the end of high intensity exercise (King et al 1994). However, other studies show the suppression can last for several hours (Broom et al 2007). Collectively, current evidence suggests that moderate to high intensity exercise exerts a transient suppression of appetite.

2.6.5 Acute exercise and food intake

Most evidence suggests that energy intake is not affected by acute bouts of exercise (Thompson et al 1988, King et al 1994, King and Blundell 1995, King et al 1997, Hubert et al 1998). This is consistent with the majority of the studies that have also shown that exercise does not stimulate appetite. However, several studies have demonstrated contradictory findings with energy intake being increased (Verger et al 1992, Verger et al 1994, Pomerleau et al 2004, Martins et al 2007) and decreased (Kissileff et al 1990, Westerterp-Plantenga et al 1997, Ueda et al 2009b) after acute exercise bouts.

Although appetite appears to be sensitive to the intensity of an exercise bout, energy intake after exercise does not appear to be affected likely due to the transient nature of the suppression of appetite. A high intensity bout of cycling transiently suppressed appetite compared with a low intensity bout of cycling that was similar in energy expenditure, yet post-exercise energy intake at a meal given 1 h after exercise was no different between trials (Thompson et al 1988). Similarly, King et al (1994) demonstrate that compared with a control condition, energy intake was unaffected after low and high intensity cycling exercise which elicited comparable energy deficits. However, although there was no change in energy intake with intense exercise, there was a delay in the onset of eating compared with a control session (King et al 1994).

The mode of exercise and gender of participants does not appear to affect the energy intake response to intense exercise. King and Blundell (1995) investigated the effect of 50 minutes of intense running and cycling exercise in healthy males and showed that although there was a delay to the onset of eating, there was no effect of exercise on energy intake with either exercise mode. King et al (1996) examined the effect of cycling exercise in females, and as with males, no alteration in energy intake was observed between control and exercise trials.

It is important to note that many of the studies investigating the effect of exercise on energy intake tend to only look at the immediate post-exercise meal. Acute exercise does seem to produce a short term negative energy balance which could be important for weight management if this negative energy balance persists in the long term. However, the beneficial effect of a reduction in relative energy intake (total energy intake minus the energy expenditure of exercise) at the post-exercise meal would be negated if there were compensatory increases in energy intake at subsequent meals during the day. Regardless of weight status, moderate intensity exercise does not increase food intake 15 minutes after exercise or even in the 12 hours after exercise in sedentary normal weight or overweight male volunteers (Harris and George 2008). King et al (1997) investigated the effect of high doses of exercise with energy expenditures of approximately 1200 kcal on 48 h energy intake. Energy expenditure was calculated by collecting expired air in Douglas bags during the exercise bout. Energy intake on the same day or the day after exercise was not affected by a high dose of exercise when compared with the same period of observation with no exercise undertaken. However, energy intake was assessed using self-report thus the results

should be treated with caution due to possible reporter bias and imprecision (Schoeller, 1995).

King et al (2010a) investigated the effect of inducing a large energy deficit (via 90 minutes of treadmill running at 69 % of $\dot{V}O_2$ max) on appetite and food intake responses for the 22.5 h after exercise i.e.: looking at 24 h energy intake. Total daily energy intake was no different between the exercise and control trial, although after accounting for the energy cost of the treadmill run, participants remained in energy deficit compared with the control trial with a deficit of approximately 4912 kJ (1174 kcal). This study utilised two different methods of quantifying food intake - direct assessment in the laboratory followed by food diaries overnight at the end of the observation period in the laboratory. This enabled quantification of energy intake to be as accurate as possible whilst minimising disruption to participants and allowing them to continue a normal daily routine where possible. The findings indicate that substantial energy deficits induced by exercise do not automatically lead to compensatory increases in appetite or food intake on the day of exercise itself or increases in appetite on the morning after.

Even when 10 men and 10 women were subjected to 1 h a day of treadmill running at 68 % of $\dot{V}O_2$ max for 5 consecutive days, there was no total compensation for the energy expended during exercise (Staten, 1991). Men did increase their caloric intake by an average of 209 kcal each day compared with a control trial but this was insufficient to fully compensate for the average daily energy expenditure of 596 kcal. Conversely, women did not alter their caloric intake at all despite expending an average of 382 kcal each day. Hence, both men and women were in negative energy balance during the period of 5 days when exercise was undertaken. Clearly, the apparent lack of compensation in response to large energy deficits through aerobic exercise cannot be sustained indefinitely without a substantial loss in body mass. Homeostatic mechanisms must be in place to defend body fat stores so that at some point the body will respond by increasing appetite and energy intake equivalent to energy expenditure.

There are some instances where exercise has reportedly increased energy intake. Verger et al (1992) observed an increase in energy intake in response to 2 h of sub-maximal exercise. Furthermore, the longer the interval between cessation of exercise and provision of a meal,

the greater the energy intake with significant increases in energy intake observed 60 and 120 minutes after exercise compared with immediately post-exercise (Verger et al 1992). However, the increase in energy intake was comparable with the energy expended during exercise suggesting that individuals compensated for the energy cost of exercise. A limitation of the study was that energy expenditure was only estimated rather than directly measured. Pomerleau et al (2004) showed that in women, compared with a resting trial a high intensity bout of exercise increased absolute energy intake, whereas a low intensity bout of exercise expending the same number of calories did not. A recent study has demonstrated an increase in absolute energy intake at a meal given to 6 males and 6 females after 60 minutes of cycling exercise at 65 % of their age-predicted maximal heart rate (Martins et al 2007). Many studies that examine the effect of exercise on energy intake are limited in their provision of food items with only a few items available. In the study by Verger et al (1992) the choice of food on offer to participants was limited to eggs, ham, cheese, semolina and gelled fruits, so the authors completed another study whereby a greater choice of food was available to individuals. Their findings confirmed those from their previous study that energy intake was increased after exercise compared with a control condition (Verger et al 1994). However in their second study, different groups of individuals were used for the exercise and control trials. This study design limits the reliability of the results because differences in energy intake may have been due to differences between the participants rather than exercise.

King et al (1994) suggest that the effect of exercise on energy intake should be interpreted by accounting for the energy expenditure induced by exercise. These authors highlight that even if exercise stimulates energy intake even to a fairly substantial degree, this does not necessarily translate to positive energy balance. A short term negative energy balance can be attained if the energy cost of the exercise bout is taken into account, thus relative energy intake could be reduced, indicating exercise can be a beneficial modulator of energy intake. Where exercise has been reported to increase energy intake, after accounting for the energy cost of exercise, participants are actually in energy deficit (Pomerleau et al 2004, Martins et al 2007). However, in the study by Pomerleau et al (2004) when energy intake over a whole day was examined, energy intake was increased to almost totally compensate for the exercise induced energy expenditure arising from a high intensity exercise bout.

Several studies have observed decreases in absolute energy intake with exercise. Energy intake was significantly decreased at a meal given 10 minutes after the end of a 2 h cycling bout at 60 % of Wmax (Westerterp-Plantenga et al 1997). However, research suggests that even exercise of a shorter duration may be sufficient to decrease energy intake. In a study by Ueda et al (2009b), ten male subjects each performed three trials one week apart. These consisted of an *ad libitum* lunch provided to participants 30 minutes after completing 30 minutes of cycling at 50 % of $\dot{V}O_2$ max, 30 minutes cycling at 75 % of $\dot{V}O_2$ max and a resting trial of 30 minutes of rest. Compared with rest, absolute energy intake was significantly lower after exercise. This decrease in food intake was unrelated to the intensity of the exercise bout when exercise duration was kept constant suggesting that even moderate intensity exercise of 50 % of $\dot{V}O_2$ max is sufficient to reduce energy intake after exercise. Although this study used a fixed duration of exercise, previous research suggests that even if the energy expenditures of different intensity exercise bouts are matched thus increasing the duration of the moderate intensity exercise bout, energy intake is unaffected relative to a control trial (King et al 1994).

The discrepant findings regarding the effect of exercise on energy intake could be due to several factors. Firstly, the method used to quantify energy intake may either alter food intake or simply be an inaccurate estimation. Some studies rely on self-report of food intake where underreporting is a common theme. Therefore, direct quantification of energy consumed by participants in a controlled laboratory environment is preferable. However, this too is not without its limitations. Although intake can be covertly monitored, the unnatural environment may influence an individual's eating behaviour as well as the experimental manipulation thus it is not possible to unravel the individual effects of the experiment and the cognitive aspects on eating outcomes (Blundell et al 2010). The provision of food may also affect outcomes with some studies providing a wide range of foodstuffs (Verger et al 1994, Martins et al 2007) whereas others are limited to single items such as pasta or yoghurt (Kissileff et al 1990, Ueda et al 2009b). The latter method of quantifying energy intake is limited because provision of a single food could lead to boredom of taste which could influence termination of a meal (Tuomisto et al 1998). Moreover, the provision of single foodstuffs does not allow quantification of macronutrient preference. Conversely, the provision of a wide variety of palatable foods may encourage overconsumption. Secondly, the timing of the post-exercise meal and the duration of

follow up after exercise may explain discrepancies in energy intakes reported in the literature. Verger et al (1992) observed differences in energy intake depending on the length of time between cessation of exercise and meal provision. Early studies simply examined the energy intake response to one meal given shortly after exercise. However, energy intake may change over time during a day (Pomerleau et al 2004) thus with shorter studies, incorrect conclusions may be drawn. It is therefore important to assess the effect of several meals over the trial duration. Each method appears to confer its own advantages and disadvantages but used appropriately should provide beneficial data if interpreted suitably.

2.6.6 Exercise and macronutrient preference

The absolute amount of food intake will determine the total energy intake of an individual; however, the composition of the food is also important with each macronutrient yielding different amounts of energy per gram of food. As well as examining the energy intake response to exercise it is also prudent to investigate whether macronutrient preference is altered. When individuals are free to choose their own foods at *ad libitum* buffet meals, it enables calculation of spontaneous macronutrient preferences. Alterations in macronutrient preference could be a consequence of physiological needs to seek certain foods which replace specific energy stores used to fuel the exercise bout (King, 1998). The effect of exercise and physical activity on macronutrient preference is not fully established (Tremblay and Drapeau 1999). Exercise has been found to increase energy intake from carbohydrate (Verger et al 1992, Westerterp-Plantenga et al 1997), protein (Verger et al 1994) and fat (Pomerleau et al 2003) whilst several studies have observed no change in macronutrient preference after intense exercise (King et al 1994, Martins et al 2007, King et al 2010a). Conflicting findings may be explained by values being expressed in absolute terms in some studies, whereas in others, they are expressed as a percentage of total energy intake. However, the reason for differences observed may be due to the availability of the *ad libitum* foods which may be biased towards one particular macronutrient. The macronutrient composition of food given after exercise can determine the extent to which energy balance is restored. When a high fat diet is provided after 60 minutes of treadmill running, individuals appear not to just compensate for the energy cost of exercise, but are actually in positive energy balance for 48 hours (Tremblay et al 1994). This is in comparison with a low fat diet after exercise which resulted in an overall energy deficit. Thus the extent to which aerobic exercise can induce a beneficial energy deficit that would

be meaningful for the prevention of weight gain or to facilitate weight reduction is dependent on the composition of the chosen foods. Current findings are conflicting but it is likely that acute bouts of exercise do not influence macronutrient selection and the differences observed may be due to the increased provision of one particular macronutrient.

2.7 Exercise and the appetite regulating hormones

2.7.1 Introduction

The mechanisms behind the suppression of appetite with exercise and the lack of immediate compensation from exercise-induced energy expenditure are not fully clear. Likely mediators are exercise-induced changes in hormones involved in hunger and satiety. The gut peptides measured in the studies within this thesis are acylated ghrelin and PYY, thus the main focus of the review of the literature surrounding exercise and gut peptides will be on these hormones.

2.7.2 Exercise and ghrelin

The effect of exercise on circulating concentrations of ghrelin begun to receive attention soon after its appetite stimulatory effects were observed. However, studies were already investigating the effect of exercise on ghrelin levels to elucidate whether ghrelin was implicated in the well-documented increase in growth hormone with exercise. Several of these studies showed that despite increases in growth hormone, an acute bout of exercise did not affect plasma ghrelin levels regardless of the exercise intensity (Kallio et al 2001, Dall et al 2002, Kraemer et al 2004, Schmidt et al 2004). However, the study by Kraemer et al (2004) was the only one that included a resting control trial thus results may not be directly related to exercise but could be influenced by other factors such as circadian rhythms. It was proposed that ghrelin may be implicated in exercise-induced anorexia and also suggested that ghrelin concentrations may be elevated in the hours after exercise as a compensatory mechanism to restore energy balance after a large exercise-induced energy deficit. Subsequent studies examined the acute responses of hunger and ghrelin concentrations to an exercise bout and observed participants for varying durations to determine the post-exercise ghrelin response.

Burns et al (2007) examined the response of 60 minutes of high intensity treadmill running on hunger and ghrelin concentrations in lean males and females. They confirmed that high intensity running suppressed hunger and observed that plasma ghrelin concentrations were unchanged after running compared with a control trial indicating that exercise did not affect ghrelin concentrations. Studies using cycling as an exercise mode have reported contradictory results with no change (Martins et al 2007), an increase (Christ et al 2006, Erdmann et al 2007) and a decrease (Malkova et al 2007) in ghrelin all being observed. Martins et al (2007) showed that 60 minutes of cycling at 65 % of an individual's estimated maximum heart rate suppressed hunger but did not change ghrelin levels compared with a control condition whereas 60 minutes of cycling at 90 % of the lactate threshold has been shown to decrease plasma ghrelin levels during which time hunger ratings were increased (Malkova et al 2007). Conversely, an increase in ghrelin was observed after prolonged (3 h) moderate intensity cycling when a low fat diet had been consumed compared with a high fat diet (Christ et al 2006). Similarly, low intensity cycling exercise at 50 W for 30 to 120 minutes in duration increased plasma ghrelin concentrations, yet higher intensity cycling at 100 W for 30 minutes had no effect on ghrelin concentrations (Erdmann et al 2007). The latter findings suggest that low intensity but not high intensity cycling increases ghrelin concentrations and this is independent of the duration of the exercise bout. Collectively, these studies suggest that firstly, ghrelin concentrations are unaffected by acute bouts of exercise in lean adults and secondly, that ghrelin does not mediate either the increase of growth hormone during exercise or the transient suppression of appetite.

The aforementioned studies are limited in that only total ghrelin concentrations were quantified. Total ghrelin is comprised of both acylated and unacylated forms. The acyl-modified peptide is necessary for ghrelin's ability to bind to its receptor and affect appetite thus it is important to measure concentrations of circulating acylated ghrelin because measurement of total ghrelin alone could mask changes in acylated ghrelin. Research has shown that total ghrelin levels can remain unchanged but the proportion of acylated ghrelin can fall sharply during situations such as prolonged fasting (Liu et al 2008). In response to exercise, differential changes in acyl- and des-acyl ghrelin have been observed in normal weight and overweight adolescent males (Mackelvie et al 2007) again suggesting that measurement of total ghrelin would not appropriately reflect changes in acylated ghrelin. This was confirmed during a 12 week exercise program in overweight boys that showed in response to weight loss, total ghrelin increased but the proportion that was acylated ghrelin

remained unchanged (Kim et al 2008). Quantification of acylated ghrelin from plasma requires appropriate sample collection to preserve concentrations of acylated ghrelin, and it is only recently that studies have started to investigate the effects of exercise on the acylated form of this peptide, either uniquely, or in conjunction with total ghrelin.

Broom et al (2007) were the first authors to investigate the effects of intense exercise on acylated ghrelin. They observed a suppression of plasma acylated ghrelin concentrations during 60 minutes of treadmill running at 70 % of $\dot{V}O_2$ max. This coincided with a transient suppression of appetite. They concluded that the decrease in plasma acylated ghrelin concentrations observed during exercise may be implicated in exercise-induced anorexia. A similar response was observed with cycling exercise, although an incremental protocol to exhaustion was used rather than steady state exercise (Marzullo et al 2008). These authors demonstrated that at peak exercise, plasma acylated ghrelin concentrations were decreased and this response was observed in both lean and obese individuals with the magnitude of suppression in obese individuals greater than in lean individuals. This study further highlights the importance of measuring the acylated form of ghrelin because although decreases in acylated ghrelin were observed, no change in total ghrelin occurred. Recent research has confirmed a suppression of acylated ghrelin with other modes of exercise, namely weight lifting and swimming. Broom et al (2009) demonstrated that 90 minutes of resistance exercise suppressed acylated ghrelin concentrations to a similar extent as 60 minutes of treadmill running. A novel finding arising from that study was that hunger was also suppressed with resistance exercise supporting the possibility that acylated ghrelin may mediate exercise-induced anorexia. More recently, 42 minutes of swimming has been shown to suppress acylated ghrelin concentrations in 10 male participants with a concomitant suppression of appetite (King et al 2011a).

Several studies have not observed a suppression of acylated ghrelin in response to exercise. Forty to 60 minutes of walking in lean males or overweight females does not affect hunger or acylated ghrelin concentrations (King et al 2010b, Unick et al 2010). Similarly, no change in acylated ghrelin nor appetite has been observed in response to 60 minutes of cycling exercise at 50% of $\dot{V}O_2$ max in 7 obese and 7 control participants (Ueda et al 2009a). The reason for these discrepant findings compared with the majority of studies may be due to the lower intensity of the exercise bouts. In the study by King et al (2010b)

the intensity of walking was approximately 45% of $\dot{V}O_2$ max which is only slightly below the intensity of cycling (50% of $\dot{V}O_2$ max) used in the study by Ueda and colleagues. Thus the intensity of an exercise bout is likely an important determinant of the acylated ghrelin response to exercise. Furthermore, in the study by Ueda et al (2009a), participants were fed a 560 kcal meal 1 h prior to commencing exercise. Acylated ghrelin concentrations are suppressed with feeding (Cummings et al 2001) in proportion to the caloric load (Callahan et al 2004). The duration between feeding and exercise was likely too short for ghrelin concentrations to start increasing prior to exercise, thus ghrelin was already suppressed and the stimulus of exercise appeared to not suppress concentrations further. In the study by King et al (2011a), a snack was provided prior to exercise, but the caloric content for a 70 kg individual was less than half of that provided to participants in the study by Ueda et al (2009a) which may explain why compared with a resting condition acylated ghrelin was suppressed during a bout of swimming undertaken 1 h after a snack (King et al 2011a).

The decrease in acylated ghrelin concentrations observed during high intensity exercise is observed in both sedentary and well trained individuals and also in lean and obese subjects. However, research examining the effect of gender on the acylated ghrelin response to exercise is limited. Although some studies have used both male and female participants, they have been treated as a whole group when there could be gender-specific responses to exercise. Hagobian et al (2009) investigated the effect of four bouts of exercise on consecutive days in two groups of individuals; overweight females and overweight males. Exercise was undertaken so that participants remained in energy deficit or were in energy balance. Regardless of energy status, acylated ghrelin levels increased in females in response to a meal tolerance test after the four days of exercise compared with a control condition. However, in males, the acylated ghrelin response to the meal tolerance test was no different between the trials. The response in females may serve to defend body fat by increasing the drive to eat. In future, it is important that male and female subjects are treated as separate groups to ensure gender differences in the response of the appetite-regulating hormones to exercise can be fully established.

2.7.3 Exercise and PYY

Only a handful of studies have investigated the response of PYY to acute bouts of exercise (Martins et al 2007, Broom et al 2009, Shorten et al 2009, Ueda et al 2009a). Martins et al (2007) were the first authors to report the effects of exercise on PYY concentrations. Compared with a control trial, PYY concentrations were elevated in response to 60 minutes of cycling at 65 % of age-predicted maximum heart rate. Thirty minutes after the end of exercise, PYY concentrations were not significantly different from the control trial indicating a short-lived effect. During the exercise bout there was a transient suppression of appetite and compared with the control trial, there was a significant increase in energy intake at a buffet meal 1 h after exercise which suggests that the increase in PYY during exercise did not influence energy intake. These findings were confirmed by Broom et al (2009) who observed an increase in PYY and a suppression of appetite during 60 minutes of high intensity treadmill running. Peptide YY remained elevated 30 minutes after the end of the treadmill run compared with a control trial. Peptide YY appears to be insensitive to the effect of resistance exercise, with 90 minutes of weight lifting insufficient to alter PYY concentrations compared with a control trial (Broom et al 2009).

Ueda et al (2009a) have shown that in response to 60 minutes of cycling at 50 % of $\dot{V}O_2$ max , PYY concentrations increased to a similar extent in both lean and obese individuals, despite obese individuals having lower fasting levels of PYY. Energy intake was suppressed after exercise in both groups compared with a resting condition, but the suppression was even greater in the obese individuals, which is surprising given the similar increase in PYY concentrations with exercise. Therefore, it is unlikely that PYY solely explains the energy intake response after exercise. Despite observing no change in PYY concentrations after 40 minutes treadmill running at 70 % of $\dot{V}O_2$ peak at 25 ºC, Shorten et al (2009) demonstrated that the same exercise at 36 ºC elevated PYY concentrations compared with a control trial. This coincided with a decrease in relative energy intake at a subsequent meal. It is unclear why there was no change observed during running at 25 ºC, but it is unlikely to be related to exercise duration as PYY concentrations increased after the same exercise duration undertaken in the heat. Collectively, these studies demonstrate a transient increase in PYY concentrations with moderate to high intensity aerobic exercise.

Research investigating the effect of the more abundant form of PYY, PYY_{3-36}, in response to aerobic exercise confirm the findings of previous studies. Ueda et al (2009b) demonstrated that PYY_{3-36} increases in response to 30 minutes of cycling exercise. The response may be dependent on the exercise intensity with cycling at 75 % of $\dot{V}O_2$ max eliciting a greater increase compared with cycling at 50 % of $\dot{V}O_2$ max. However, the duration of exercise was the same so the greater increase in PYY observed with high intensity exercise may be due to the higher energy expenditure. This may explain the lack of response of PYY with resistance exercise in the study by Broom et al (2009). These authors had postulated that the energy expenditure of weight lifting may have been insufficient to evoke a response in PYY compared with a large energy expenditure induced by aerobic exercise.

2.7.4 Exercise and leptin

Compared with research regarding the effect of exercise on ghrelin and PYY concentrations, there have been even fewer studies that have investigated the effect of acute exercise on leptin concentrations. Leptin concentrations are unaffected by acute resistance exercise (Varady et al 2010) or acute aerobic exercise that is shorter than 60 minutes duration or induces an energy expenditure of less than 800 kcal (Bouassida et al 2010, Pop et al 2010). Exercise bouts lasting longer than 60 minutes in duration may decrease leptin concentrations (Boussida et al 2010) but whether this is an actual response to exercise, or merely a contribution of circadian rhythm remains unclear. When overweight and lean subjects are treated as distinct groups, a reduction in leptin after an acute bout of cycling has been observed in overweight females (Pop et al 2010). Reductions in leptin concentrations seen with chronic exercise are likely due to exercise-induced anthropometric changes and their recognised association with leptin levels. However, in the absence of a correlation between reduced leptin levels and weight loss with 4 weeks aerobic training (Sari et al 2007), other mechanisms cannot be ruled out. Current evidence suggests that acute exercise does not affect leptin concentrations and any changes seen with chronic exercise could simply be due to weight loss.

2.7.5 Summary of the effects of exercise on ghrelin, leptin and PYY

Collectively it appears that the appetite regulatory peptides, ghrelin and PYY, are transiently affected by acute bouts of high intensity exercise. Differences within the

literature are likely due to the intensity, duration or mode of exercise used. It remains unclear whether perturbations in concentrations of these hormones during exercise impact upon subsequent energy intake. Acute exercise does not appear to influence circulating leptin concentrations and this is likely due to its role in the long term regulation of energy balance.

2.8 Exercise and appetite in environmental extremes

Extreme environments can present challenging conditions to an individual through factors such as climate (heat and cold) or topography (high altitude). Exercising in extreme environmental conditions can perturb the normal physiological responses to exercise (Mazzeo, 2008). Participation in exercise challenges in extreme environments is becoming more widespread and attracts large numbers of individuals. Similarly, many well known sporting events are held in environments that are hostile to many competitors who are not adapted to such an environment, an example being the Mexico City Olympics in 1968. Exercising in extreme environments may also not just be a matter of choice but may be essential for an individual who finds themselves in such an environment by accident without the necessary equipment or clothing. Survival in extreme environments presents a challenge to any individual. However, humans are better suited to warm environments where they are adapted to dissipate heat, whereas in cold environments humans are not well adapted to retaining heat (Haman, 2006) and are therefore more reliant on clothing and shelter to minimise heat loss. Humans can acclimatise to high altitude and these physiological changes that take place occur over different time courses from immediate exposure up to several weeks (Ward et al 2001). The extent and speed with which people acclimatise differs between individuals (Ward et al 2001). It is well documented that altitude suppresses appetite, however, there is little empirical evidence regarding the affect of heat and cold on appetite. Physiological changes that occur during exercise may be further perturbed whilst exercising in environmental extremes and could subsequently affect the regulation of appetite, thus, some of the physiological changes that occur whilst exercising in these conditions will be discussed along with potential implications for appetite regulation.

2.8.1 Heat

The appetite responses to hot environments are not well understood and mainly based upon anecdotal evidence. Appetite is reportedly suppressed in the heat (Burke, 2001) and

exercising in the heat can decrease energy intake (Shorten et al 2009). Although the role of gut hormones in mediating the appetite response to extremes of environmental temperature has been proposed there is very limited evidence (Shorten et al 2009). Other physiological changes arising from exercising in the heat may be involved in this attenuation in appetite and food intake.

Cardiovascular adjustments during exercise are regulated by both neural and hormonal factors that act mainly upon the heart (Therminarias et al 1992). After exercising at a moderate to high intensity for approximately 15 minutes, a progressive but slow change occurs over time in cardiovascular measures such as heart rate, which gradually increases (Wingo et al 2005). This cardiovascular drift occurs in both thermoneutral and hot environments, but is greater in hot environments (Gliner et al 1975, LaFrenz et al 2008). Maximal oxygen uptake is affected by the heat with a reduction observed under conditions of extreme heat stress (LaFrenz et al 2008) and in these environments exercise time to exhaustion will be reduced (Galloway and Maughan 1997). When exercising in high ambient temperatures, body heat loss may be impaired because the gradient for the dissipation of heat is reduced (Febbraio, 2001, Wendt et al 2007). From an athlete's perspective, most importantly this could have immediate effects by impairing exercise performance (Wendt et al 2007), but other physiological changes may occur that could affect health after exercise. Changes in thermoregulatory mechanisms can affect the hormonal responses to exercise and impact upon substrate utilisation. Most literature suggests that there is a shift towards increased carbohydrate use during exercise in the heat (Burke, 2001, Febbraio, 2001). The nature of some sports such as endurance running lends itself to fluid and carbohydrate ingestion during exercise, whereas during team sports such as football, the opportunities to consume fluid may be more limited. However, during high intensity intermittent sports, there may also be more opportunity for heat dissipation compared with prolonged intense exercise (Burke, 2001), although some research has shown that variable intensity exercise in a hot environment is not advisable if an individual wishes to minimise physiological stress, because compared with constant exercise, variable intensity exercise increases thermal, metabolic and cardiovascular stresses (Mora-Rodriguez et al 2008). The consumption of fluids during exercise in the heat does not simply minimise dehydration but can provide an exogenous carbohydrate source to cope with the greater reliance on carbohydrate as a fuel source. Furthermore, unlike previous suggestions, ingesting carbohydrate during exercise in the heat does not elicit increases in

core temperature or metabolic rate (Horswill et al 2008). King (1998) suggests that macronutrient preference may be altered after exercise to replace the fuels used during the exercise bout, thus this enhanced reliance on carbohydrate during exercise in the heat may lead to increased carbohydrate consumption at a meal consumed after exercise in the heat.

During exercise in the heat, blood flow is directed to the muscles to support energy metabolism, to the skin for temperature regulation and to the brain to maintain central nervous system function (Cheuvront et al 2010). The circulation must meet these thermal and metabolic demands and these are affected by the intensity of the exercise bout, for example, splanchnic blood flow is reduced as the intensity of exercise increases and redistributed to working muscles (Rowell, 1974). This reduction in splanchnic blood flow is further attenuated at any given intensity whilst exercising in the heat (Rowell et al 1965). Broom et al (2007) speculated that suppressed acylated ghrelin concentrations during exercise may be related to a reduction in splanchnic blood flow that would impair oxygen delivery and could alter acylated ghrelin secretion. With a further reduction in splanchnic blood flow during exercise in the heat, acylated ghrelin concentrations may be attenuated to a greater degree during exercise in the heat, however this is purely speculative. Shorten et al (2009) demonstrate that although there is no difference in acylated ghrelin concentrations immediately prior to a meal after 40 minutes of high intensity treadmill running in the heat or a neutral environment, there was a tendency for acylated ghrelin to respond differently between trials with a decrease during running in the heat but not in the neutral environment suggesting that environmental temperature may affect acylated ghrelin concentrations.

Intense exercise increases circulating concentrations of cortisol, adrenaline and noradrenaline and concentrations of these hormones are further increased by exercise in the heat (Hargreaves et al 1996, Starkie et al 2005). Noradrenaline may interact with appetite regulatory peptides to modulate eating (Wellman, 2000) and it has been demonstrated that fasting ghrelin concentrations are positively correlated with noradrenaline and adrenaline (Schulpis et al 2004). However, in response to exercise, Toshinai et al (2007) observed negative correlations between plasma ghrelin and both plasma adrenaline and noradrenaline levels thus the role of catecholamines in ghrelin regulation is not clear. Even if catecholamines are only indirectly involved in appetite regulation, exercising in the heat could affect appetite via physiological changes designed to aid heat loss.

2.8.2 Cold

Evidence suggests that exercising in cold environments exerts the opposite effect of heat on appetite and energy intake. Energy intakes are increased in response to exercising in cold water (Dressendorfer, 1993, White et al 2005) although the mechanisms remain unclear. The appetite response to exercising in cold air could differ to that during exercise in cold water because other physiological differences are apparent between the two forms of cold exposure. For example, Haman et al (2002) concluded that available research suggested that in participants exposed to cold air, the predominant fuel used for heat production is carbohydrate, whereas in those cooled by immersion in cold water, lipid utilisation is the main source of heat production. The mechanisms for such differences remain unclear. Maintenance of core body temperature is of primary importance for a human exposed to the cold and mechanisms are in place to help prevent or abate decreases in core body temperature. Heat loss is minimised by peripheral vasoconstriction and heat is generated via shivering thermogenesis (Haman, 2006). Wilson et al (2007) show that in response to skin surface cooling, as well as peripheral vasoconstriction, there is also vasoconstriction of renal and splanchnic vessels. This is independent of changes in cardiac output and core body temperature. Changes in blood flow to the gut could affect secretion of hormones responsible for appetite control, many of which originate in the gastrointestinal tract. However, research is limited and although White et al (2005) suggest that increased energy intake after exercise in cold water may be mediated by changes in hormones responsible for appetite control, they did not measure concentrations of any gut hormones. Halse et al (2010) demonstrate that acylated ghrelin concentrations are increased after immersion in neutral temperature water, but not in response to immersion in cold water. This is in spite of increases in energy intake after both conditions (Halse et al 2010). Zeyl et al (2004) show that leptin concentrations are decreased in response to acute cold exposure. Decreased leptin concentrations would serve to reduce feelings of satiety. However, the same authors showed that in response to repeated cold water immersion, leptin concentrations increased compared with values pre-exposure. Collectively, these findings suggest that the hormonal response to ambient temperature is not straightforward.

Cold environments may affect the human body through the surface of the skin even when core temperature may rise (Graham et al 1991). Bridge et al (2003) demonstrate that some hormonal responses are affected by the temperature of the skin when rectal temperatures are similar. Thus, exercising in cold air may elicit different effects to that during cold water

on release of some hormones, and it remains to be elucidated whether energy intake would be increased after exercise in cold air as a consequence of changes in hormones released during exercise.

2.8.3 Altitude

At high altitude, energy requirements may be increased due to high energy expenditures arising from negotiating difficult terrain whilst spontaneous energy intakes can be reduced leading to a negative energy balance (Westerterp et al 1992). This may be undesirable for an individual in that setting. The effect of high altitude on appetite is well documented with evidence from field and laboratory studies demonstrating a loss of appetite and a reduction in energy intake at high altitude (Pugh 1962, Boyer and Blume 1984, Rose et al 1988, Tschöp et al 1998, Westerterp-Plantenga et al 1999). The mechanisms behind the reduction in appetite and energy intake at altitude are unclear but alterations in concentrations of gut hormones involved in appetite regulation may be responsible (Tschöp et al 1998, Shukla et al 2005, Benso et al 2007, Lippl et al 2010). However, research is sparse and often contradictory due to differences in methodologies or the presence of confounding factors such as weight loss that could explain observed changes in gut hormone concentrations.

A role for leptin, a key hormone involved in energy balance, in mediating the decrease in weight commonly reported at high altitude has been proposed. Conflicting findings have been reported, with Tschöp et al (1998) showing that leptin concentrations increased at high altitude. Appetite loss was also reported and the authors suggested that increased leptin may be associated with this appetite loss. However, in that study, individuals climbed to high altitude thus high levels of energy expenditure and a cold environment could have affected the findings. Furthermore, single measurements of leptin concentrations were made which may not give a true representation of leptin concentrations because leptin secretion occurs in a pulsatile fashion. A second study where individuals were transported to high altitude by helicopter, and several measurements of leptin concentrations were made, revealed similar findings (Tschöp et al 1998) thus confirming previous results and suggesting a role for leptin in appetite loss at high altitude. Conversely, Zaccaria et al (2004) demonstrate a significant reduction in leptin concentrations at high altitude which would indicate a reduction in satiety. However the authors acknowledge the role that perturbations in body composition likely play in mediating this effect, because the body

mass index of participants fell over time and was consistently correlated with leptin concentrations. Hamad and Travis (2006) suggested that further work in environmental chambers is required to elucidate a role for leptin in the loss of appetite at high altitude, where other environmental factors can be minimised and physical activity levels closely controlled.

There are few studies examining the effect of high altitude on concentrations of gut hormones involved in appetite regulation. The role of ghrelin in appetite regulation is well established but few studies have examined the effect of high altitude on ghrelin concentrations. Total ghrelin concentrations are decreased upon acute exposure to high altitude and therefore may be responsible for appetite suppression initially (Shukla et al 2005). However, total ghrelin concentrations then increase when individuals are acclimatised to high altitude which suggests that ghrelin may not be involved in the persistent appetite suppression observed at high altitude (Shukla et al 2005). Conversely, Benso et al (2007) report that chronic hypoxia does not affect ghrelin concentrations. These contradictory findings suggest that clarification is needed regarding the effect of altitude upon ghrelin concentrations; furthermore, the effect of altitude on the acylated form of ghrelin should be examined because acylated ghrelin is necessary for the stimulation of appetite.

Appetite regulating hormones such as ghrelin and leptin, act upon the brain to influence energy intake. The decrement in appetite observed at high altitude may be due to alterations in concentrations of these hormones but could also be due to the direct effect of altitude on the brain. Recently, the use of magnetic resonance imagining has enabled researchers to examine the effect of hypoxia in the brain and this research suggests a possible role for the blood brain barrier in the development of acute mountain sickness (Baneke, 2010). Given that hormones including acylated ghrelin must cross the blood brain barrier to bind with their receptors within the brain, changes in blood brain barrier permeability could influence how appetite regulatory hormones exert their effect on appetite and food intake. However, it remains important in the first instance to establish the effect of high altitude on concentrations of circulating appetite regulatory hormones to determine whether the suppression of appetite might be mediated by changes in gut hormone concentrations.

Other factors, such as alterations in basal metabolic rate and malabsorption have been proposed to explain the effect of altitude on energy balance. Basal metabolic rate may be increased after a week at high altitude and together with a reduction in food intake could contribute to the weight loss in obese individuals observed at high altitude (Lippl et al 2010). Butterfield et al (1992) have also demonstrated an increase in basal metabolic rate in participants who had spent 2 days at 4300 m. Basal metabolic rate at altitude decreased thereafter but remained above values obtained at sea level. However, not all studies report a sustained elevation in basal metabolic rate at altitude, some show that once individuals have acclimatised to altitude basal metabolic rates return to normal (Hannon and Sudman 1973, Mawson et al 2000) and thus cannot fully explain the decrease in body weight that occurs at high altitude. The increase in basal metabolic rate observed at high altitude has been partly attributed to the cold (Hamad and Travis 2006) with Nair et al (1971) suggesting that exposure to cold and altitude increases basal metabolic rate to increase heat production, however, more research is required before the increased basal metabolic rate can be explained. Malabsorption has been suggested to contribute to decreased body weight at high altitude (Pugh, 1962, Boyer and Blume 1984), however, this may only be true at altitudes above 5000 m and even then, findings are inconclusive (Westerterp et al 1994). Body weight loss occurs at altitudes below those at which malabsorption has been identified, thus, body weight loss occurs as a consequence of other factors related to reduced energy intake.

More research is needed to examine the effect of exercising in environmental extremes on the appetite response and the subsequent effect on energy intake. This is essential in helping athletes understand the effects they could encounter when competing in events in hot countries or at altitude that could impact on energy balance and detrimentally affect performance. Moreover, with the increasing global prevalence of obesity it is important to better understand how hormones from the gastrointestinal tract affect appetite and food intake to enable more targeted methods of controlling body weight whether it be via pharmacological methods or specific exercise programs. Knowledge of how exercise affects appetite via alterations in gut hormone concentrations is thus essential to expand current research within this area. Although the effect of exercise on gut hormones and their subsequent impact on appetite and energy balance is becoming clearer, the effects of exercise in extreme environmental conditions may alter the expected response and could affect subsequent appetite and energy intake thus further research is required. The use of

environmental chambers to mimic the conditions experienced in extreme environments enables researchers to investigate how appetite and energy intake may be altered in such conditions. Recently published research shows how various exercise modes suppress concentrations of acylated ghrelin but the effects of cycling exercise remains unclear. Given the appeal of this exercise mode to many individuals such as the overweight and obese, it is essential to determine whether the effect of cycling differs to that of running, the mode of exercise commonly used by researchers recruiting healthy lean individuals. Studies within this thesis thus contribute to this rapidly developing research area by extending current knowledge within the aforementioned fields.

CHAPTER III
General Methods

This chapter describes the participant recruitment, the experimental procedures and the biochemical analyses that were common to each of the studies presented within this thesis. Each of the studies had approval from the Loughborough University Ethical Advisory Committee and the Nottingham Trent University Ethical Advisory Committee with the exception of Study 1 (Chapter IV) which was conducted in its entirety at Loughborough University and hence has sole approval from Loughborough University Ethical Advisory Committee.

3.1 Participants

Participants were recruited from within both Loughborough and Nottingham Trent Universities by email, poster advertising and/or word of mouth. Full written information was given to potential study participants, this laid out the purpose of the study, the experimental procedures and any potential risks or discomfort that individuals could experience whilst participating in the study. After being fully briefed and having the opportunity to ask questions about the studies, participants signed a statement of informed consent (Appendix A) and completed a health screen questionnaire (Appendix B) to ensure their wellbeing during the study as well as ensuring they met the inclusion criteria. Participants also completed a physical activity questionnaire (Appendix C) to examine their habitual physical activity levels and a questionnaire to assess dietary restraint, disinhibition and hunger (Stunkard and Messick 1985, Appendix D). Most of the participants were undergraduate or postgraduate students who were involved with university-standard sport, or were at least recreationally active individuals undertaking moderate exercise at least 3 times per week.

To minimise risks to participants and ensure suitability for inclusion so that existing health status would not compromise findings, participants were recruited for the studies only if they met the following criteria:

- Were recreationally active
- Were non-smokers

- Were not obese i.e: body mass index (BMI) < 30 $kg \cdot m^{-2}$
- Were normo-tensive ie: resting blood pressure < 140/90 mm Hg
- Were not dieting and had been weight stable for at least 6 months
- Had no known cardiovascular disease or abnormalities
- Were not taking medication that influenced metabolism
- Had no known dyslipidaemia

3.2 Anthropometry

Measurements for height, weight, BMI and skinfold thickness were made in all studies in this thesis as follows:

- Height - measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany). Participants removed footwear and stood flat footed with their heels against a back plate.
- Weight - measured to the nearest 0.01 kg using a balance beam scale (Avery, Birmingham, U.K.). Participants removed footwear, wore light clothing and removed items from their pockets whilst being weighed.
- Body mass index - calculated by dividing the participant's weight in kilograms by the square of their height in metres.
- Skinfold thickness - To enable an estimation of percentage body fat, skinfold thicknesses were recorded to measure subcutaneous body fat. Callipers (Harpenden, Burgess Hill, U.K) were used to measure skinfold thickness at the following sites on the right hand side of the body whilst the participant was standing:

1) Tricep - Vertical fold; with the arm held freely to the side of the body, on the posterior midline of the upper arm, halfway between the acromion and olecranon processes
2) Bicep - Vertical fold; on the anterior aspect of the arm over the belly of the biceps muscle, 1 cm above the level used to mark the triceps site
3) Subscapular - Diagonal fold (45º to the vertical); 1 to 2 cm below the inferior angle of the scapula
4) Suprailiac - Diagonal fold; superior to the iliac crest in the mid axillary line in line with the natural angle of the iliac crest

All measurements were made on the right side of the body whilst the participant stood upright. Callipers were placed on the skin surface 1 cm above the site of measurement, perpendicular to the skinfold. The fold was maintained whilst reading the calliper and measurements were taken between 1 and 2 seconds of applying calliper pressure. Measurements were made in triplicate and a complete set was made at a time thus allowing the skin time to regain its normal thickness between each site measurement. An average of the three measurements at each site was calculated and taken as the skinfold thickness for that site. Body density was calculated from the sum of the four skinfolds using predictive regression equations proposed by Durnin and Womersley (1974). Body fat was calculated from body density using the Siri equation (1956).

3.3 Resting blood pressure

During health screening, arterial blood pressure was measured using an automatic blood pressure monitor (Omron M5-I, Omron Healthcare Europe). The participant rested for five minutes prior to measurement and two measurements were taken several minutes apart and the mean recorded.

3.4 Heart rate

Heart rate was measured using short range telemetry (Polar FS2, Polar Electro, Kempele, Finland) during the preliminary tests and main trial exercise tests in each study.

3.5 Ratings of perceived exertion

During the preliminary tests and main trial exercise tests in each study, ratings of perceived exertion (RPE) were used to obtain each participant's perception of effort using Borg's RPE scale which ranges from 6 (indicating no exertion) to 20 (indicating maximal exertion) (Borg, 1973).

3.6 Preliminary exercise tests

For each study, participants completed aerobic running tests on a motorised treadmill (RUNRACE, Technogym, Gambettola, Italy or Woodway ELG 55, Weil am Rhein, Germany) (Chapters IV, V, VI and VII). In Chapter IV only, participants completed an aerobic cycle test on a cycle ergometer (Monark Ergomedic 874E, Vansbro, Sweden). Participants were familiarised with all the exercise equipment prior to completing each test.

3.6.1 Sub-maximal incremental treadmill test

Participants were required to complete a sub-maximal incremental treadmill test to determine the relationship between running speed and oxygen consumption. The test was designed such that participants would run continuously at a range of different exercise intensities, but would not be maximal. The test was 16 minutes in duration and consisted of four, four minute stages. The test was completed on a flat running surface (i.e.: 0 % gradient). The initial treadmill speed was between 7 – 9 $km \cdot h^{-1}$ depending on the participant's reported fitness level. The treadmill speed was increased by 1 – 1.5 $km \cdot h^{-1}$ at the end of each four minute stage. During the final minute of each stage, expired air was collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, U.K.) for determination of oxygen consumption and carbon dioxide production. Heart rate was recorded throughout the test as described in section 3.4 and RPE was recorded as described in section 3.5 during the expired air collection. On completion of the test, running speed at each stage was plotted against oxygen consumption ($mL \cdot kg^{\cdot -1} min^{-1}$) at that speed to determine the sub-maximal relationship between speed and oxygen consumption.

3.6.2 Maximal oxygen uptake treadmill test

After allowing for sufficient recovery from the sub-maximal test, maximal oxygen uptake was measured directly. Running speed was kept constant whilst treadmill grade was increased by 1 % every minute from a starting gradient of 0 % until volitional fatigue (Jones and Doust 1996). Expired air was collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, U.K.) during the final minute of the test when the participant indicated that they could continue for only one more minute. Heart rate and RPE were recorded throughout the test. On completion of the test, the expired air sample was analysed for oxygen consumption and carbon dioxide production. Once maximal oxygen uptake was determined, using data from the sub-maximal running test it was possible to predict the speed that would elicit the required percentage of maximal oxygen uptake to be used during the main trial treadmill tests.

3.6.3 Sub-maximal incremental cycle ergometer test

In the study in Chapter IV, participants were required to complete a sub-maximal incremental test and maximal oxygen uptake test on a weight basket cycle ergometer as well as on a treadmill (as described previously). The sub-maximal test was 16 minutes in duration consisting of four, four minute stages. Participants pedalled continuously at a

cadence of 60 rpm with an initial starting mass of 1.5 kg. The applied mass was increased by 0.5 kg at the end of each four minute stage. During the final minute of each stage expired air was collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, U.K.) for the determination of oxygen consumption and carbon dioxide production. Flywheel revolutions were also measured during this time using an electrical revolution counter. Heart rate was recorded throughout the test as described in section 3.4 and RPE was recorded as described in section 3.5 during the expired air collection. Participants were verbally encouraged to keep a constant cadence of 60 rpm to ensure that work rates were common amongst the participants. On completion of the test, the work rate at each stage was calculated using the following equation to determine the relationship between work rate and oxygen consumption:

Work Rate (W) = [Applied mass (kg) x g ($m \cdot s^{-2}$)] x [Flyhweel circumference (m) x Flywheel revolutions] / Time (seconds)

3.6.4 Maximal oxygen uptake cycle ergometer test

After sufficient recovery, participants completed a maximal oxygen uptake test to exhaustion. Dependent on the ability of the individual, the initial mass applied to the cycle ergometer was between 2 - 4 kg. Participants were encouraged to keep to a pedal cadence of 60 rpm and were instructed to maintain a seated position throughout the test. The test consisted of three minute stages but was continuous in nature with the applied mass being increased by 0.5 kg at the end of each three minute stage. Expired air was collected between 1 minute 45 seconds and 2 minutes 45 seconds of each stage. Flywheel revolutions were also measured during this time using an electrical revolution counter. Heart rate was recorded throughout the test as described in section 3.4 and RPE was recorded as described in section 3.5 during the expired air collection. When the participant indicated that they could continue for only one more minute, a final sample of expired air was collected. At the end of the test, oxygen consumption and carbon dioxide production was determined from the expired air samples. The work rate at each stage was calculated as described in section 3.6.3. Once maximal oxygen uptake was determined, using data from the sub-maximal test, it was possible to calculate the work rate that would elicit the required percentage of maximal oxygen uptake to be used during the main trial cycle ergometer tests. Rearranging the equation in section 3.6.3, the applied mass that needed to

be added to the cycle ergometer that corresponded to the work rate at the required percentage of maximal oxygen uptake could be calculated.

3.7 Analysis of expired air samples

Expired air samples were collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, U.K.) during the preliminary and main trials. These samples were analysed for oxygen consumption and carbon dioxide production using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser respectively (Series 1400: Servomex, Crowborough, East Sussex, UK). Prior to use, these analysers were calibrated using gases of known concentration. The volume of expired air was measured with a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK). This value was corrected to standard temperature and pressure (dry).

3.8 Estimation of energy expenditure

Energy expenditure and substrate oxidation were calculated from measurements of oxygen consumption and carbon dioxide production (Frayn, 1983).

3.9 Plasma volume

Estimations of plasma volume change were made during all the studies included within this thesis. From each blood sample, triplicate 20 µL blood samples were collected into heparinised microhaematocrit tubes for the determination of haematocrit, and duplicate 20 µL blood samples were collected into micropippetes for the measurement of haemoglobin concentration using the cyanmethemoglobin method (Dill and Costill 1974).

3.10 Dietary and physical activity control

Each of the studies within this thesis included the assessment of appetite and measurement of gut hormones. Differences in subjective appetite scores and baseline concentrations of circulating gut hormones are affected by the caloric content of an evening meal consumed prior to an overnight fast (Chandarana et al 2009). In every study, participants were asked to weigh and record the quantity and timing of their food intake for the 24 h prior to the first main trial in an attempt to eliminate the effect of prior feeding status on the variables measured. Participants were then required to replicate this feeding pattern prior to each subsequent main trial within that study. Participants refrained from consuming alcohol and undertaking vigorous physical activity in the 24 hours prior to each trial. On the morning

of the main trials, participants were requested to come into the laboratory after a ten hour overnight fast. Water was permitted *ad libitum* during this time. It was not possible to standardise sleeping patterns for participants despite altered sleep patterns being known to alter the endocrine regulation of energy balance (Spiegel et al 2004, Copinschi 2005).

3.11 Standardised and *ad libitum* buffet meals

In Chapters IV and VII participants were fed standardised meals. These were provided at different times within the studies and also differed in macronutrient composition and energy content. Therefore, these meals are described where relevant, within each chapter. In Chapters V, VI and VII, participants were provided with cold buffet meals. These consisted of a wide variety of sweet and savoury cold items representative of foods consumed from breakfast, lunch and snacks in a typical Western diet. A list of items provided to participants is included in Appendix E. In Chapters V and VI, the option of liquid items including yoghurt and fruit juice was also available to distinguish between calories obtained from liquid or solid food sources.

3.12 Appetite assessment

In all studies included within this thesis, subjective sensations of hunger were assessed using visual analogue scales (VAS). The VAS consists of a 100 mm horizontal line preceded by the question, "How hungry do you feel?". This line is anchored at either ends with the words "I am not hungry at all" on the extreme left, and "I have never been more hungry" on the extreme right (Appendix F). Participants mark along the horizontal line with a small vertical mark at a point that corresponds to their feelings at that moment in time. Quantification of the hunger score is made by measuring the distance in mm from the left end of the line to the participant's mark. Participants were not permitted to refer back to their previous ratings when completing a VAS. In Chapters V, VI and VII, as well as quantifying subjective sensations of hunger, questions relating to perceived satisfaction, fullness and prospective food consumption were posed (Appendix F). Participants were asked "How satisfied do you feel?", underneath which was a 100 mm horizontal line anchored with the words " I am completely empty" on the extreme left and "I cannot eat another bite" on the extreme right. Relating to subjective feelings of fullness, participants were asked "How full do you feel?", underneath which was a 100 mm horizontal line anchored with the words "Not at all full" on the extreme left and "Totally full" on the extreme right. Finally, prospective food consumption was assessed by asking "How much

do you think you can eat?" underneath which was a 100 mm horizontal line anchored with the words "Nothing at all" on the extreme left and "A lot" on the extreme right. Participants marked along the horizontal line at a point corresponding to their feelings of each of the appetite perceptions. Scores were obtained by measuring the distance in mm from the left end of the line to the participant's mark.

3.13 Thermal sensations and core body temperature measurement

In Chapters V and VI environmental temperature was the independent variable thus the assessment of core body temperature was crucial for participant safety. Prior to the participant entering the environmental chamber at the start of each trial, a rectal probe (Grant Instruments Ltd, Cambridge, England) was self-inserted ~10 cm past the anal sphincter. Core temperature was measured with the use of a data logger (Squirrel 2020 data logger series, Grant Instruments Ltd, U.K). Rectal probes were calibrated prior to the start of the study using a water bath and a reference high resolution (0.1 ºC) mercury thermometer. Thermal sensations were recorded (Chapters V and VI) using a 9 point scale with 0.5 increments (Young et al 1987). The scale ranged from 0 (unbearably cold) to 8 (unbearably hot) with the midpoint 4 being "comfortable". For participant safety, if an individual's core temperature exceeded a threshold of 40 ºC then the protocol would be to remove them from the environmental chamber, appropriate measures taken to ensure their wellbeing and the trial would be terminated. However, this situation did not arise during any of the studies presented within this thesis.

3.14 Blood sampling

The collection and processing of blood samples obtained during each study is described below.

3.14.1 Blood sample collection

Venous blood samples were collected in all of the studies described within this thesis. However, the equipment used differed between studies due to alternative equipment at the institutions where the study was carried out. The main trials in Chapter IV were carried out at Loughborough University and the blood collection procedures used are detailed in section 4.2.4 of Chapter IV. The main trials in Chapters V, VI and VII were all carried out at Nottingham Trent University where equipment used was the same for all studies. These procedures are outlined below.

After arrival at the laboratory, participants rested in a supine position for five minutes before a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. This remained in place for the duration of the 7 h trials unless the cannula blocked, in which case it was removed and disposed of appropriately. In those instances, if the participant was willing, another cannula was inserted into an antecubital vein on the alternative arm. Venous blood samples were collected from the cannula using 10 mL disposable syringes (Plastipak, Becton, Dickinson U.K. Limited, U.K.), and immediately dispensed into pre-cooled 5 mL blood collecting tubes (Sarstedt, Leicester, U.K) coated with ethylenediaminetetraacetic acid (EDTA). The cannula was kept patent between sampling by flushing with a small amount (~10 mL) of non-heparinised saline (0.9%w/v Sodium Chloride, Baxter Healthcare Ltd, Norfolk, U.K) after each collection. The saline waste that was left in the connector tube was drawn off into a syringe and discarded prior to sample collection to prevent sample dilution. During control trials, each blood sample was collected with the participant in the supine position. This was also the case during exercise trials, with the exception of the 0.5 h sample which was taken whilst the participant stood on the treadmill. Small samples were drawn off from each venous blood sample using capillary tubes. These were used to estimate plasma volume changes as described in section 3.9. After collection, blood samples were spun at 3500 rpm in a refrigerated centrifuge at 4 ºC (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc) for 10 minutes. After this the plasma was dispensed into separate Eppendorf tubes which were stored overnight at -20 ºC before being transferred to -80 ºC until analysis of glucose and triacylglycerol (TAG) (Chapter V and VI) and glucose, TAG and total PYY (Chapter VII).

3.14.2 Pre-treatment and sample collection procedure for determination of acylated ghrelin

At each sample collection, separate venous blood samples were collected into 5 mL blood collection tubes (4.9 mL monovettes in Chapter IV) for the determination of plasma acylated ghrelin. These tubes contained EDTA and a 50 µL solution of sodium hydroxide, potassium phosphate buffer and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by proteases. These tubes were spun at 3500 rpm in a refrigerated centrifuge at 4 ºC (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc) for 10 minutes. The plasma was dispensed into plain storage tubes (Sarstedt, Leicester, U.K) and 100 µl of 1 M hydrochloric acid (HCl) was added per 1 mL of plasma. These samples were then spun at 3500 rpm in a refrigerated centrifuge (Fisher Scientific accuSpin

1R, Thermo Fisher Scientific Inc) at 4 ºC for 5 minutes before being dispensed into Eppendorf tubes and stored as described above until analysis.

In Chapter VII, venous blood samples were collected into 5 mL blood collection tubes with serum clotting activator (Sarstedt, Leicester, U.K). The sample was left to clot for 30 minutes prior to centrifugation at 3500 rpm in a refrigerated centrifuge (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc) at 4 ºC for 10 minutes. The serum was dispensed into Eppendorf tubes and stored overnight at -20 ºC before being transferred to -80 °C until analysis of leptin.

3.15 Analysis of blood samples

3.15.1 Glucose and triacylglycerol

Plasma samples were analysed for glucose and TAG concentrations via enzymatic, colorimetric methods using reagents from ABX Diagnostics (Montpellier, France) with the use of a Pentra 400 (Horiba ABX Diagnostics, France). Prior to analysis of samples, quality controls were run to ensure precision of analysis. Control samples exhibited both normal and pathological values.

3.15.2 Acylated ghrelin, total PYY and leptin

Plasma acylated ghrelin concentrations were determined using a commercially available enzyme immunoassay (SPI BIO, Montigny le Bretonneux, France supplied by Immuno Diagnostic Systems [IDS]) with the aid of a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria) to read the absorbance. To be sure of precision of analysis, quality controls supplied with the assay kit were run on each plate. Total PYY concentrations were determined by enzyme immunoassay (Millipore, Watford, U.K). Leptin concentrations were determined by enzyme immunoassay (Quantikine, R&D Systems, MN, U.S.A). Precision of analysis was ensured by assaying quality controls of low, medium and high leptin concentrations (Quantikine, R&D Systems, MN, U.S.A).

3.15.3 Precision of analysis

To eliminate inter-assay variation, all samples from the same participant were analysed on the same assay or in the same run. The within-batch coefficients of variation for the assays were as follows: acylated ghrelin 5.1%, total PYY 4.8 %, glucose 0.6 % and TAG 2.7%.

3.16 Statistical analysis

Data were analysed using the Statistical Package for the Social Science (SPSS) software (v17.0 for Windows, SPSS, Chicago, IL). Two-way repeated measures ANOVA was used to examine differences in variables between trials over time (Chapters IV, V and VI). In Chapter VII, three-way repeated measures ANOVA was used to examine differences in variables between trials with the three main effects being altitude, exercise and time. Area under the curve (AUC) values for variables including acylated ghrelin, total PYY, hunger and glucose were calculated using the trapezoidal rule. One-way repeated measures ANOVA was used to assess differences in AUC and fasting values for glucose, TAG, appetite ratings, acylated ghrelin and total PYY. Where appropriate, post-hoc analysis was performed using the Bonferroni method with adjustment for multiple comparisons. In Chapters V, VI and VII, one-way repeated measures ANOVA was used to assess differences in energy and macronutrient intake. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5 % level. Results are reported as means ± standard error unless stated otherwise. In Chapters IV and VII, some error bars are omitted on the figures for clarity where three or four trials are depicted.

CHAPTER IV

The influence of high intensity running and cycling exercise on plasma acylated ghrelin concentrations and hunger

4.1 Introduction

The last few decades have seen the identification of numerous gastrointestinal hormones that play a vital role in energy homeostasis by acting on the brain to regulate appetite and food intake (Chaudhri et al 2006). This has fuelled research into the precise roles that these hormones play in maintaining energy balance. Exercise and physical activity have long been considered important factors in the control of body weight and prevention of obesity (Grundy et al 1999). However, the beneficial effects of exercise may not solely be due to its ability to induce a negative energy balance through energy expenditure, but may be related to its effects on gastrointestinal hormones that modulate appetite (Martins et al 2008). Research is now being undertaken to determine how exercise affects concentrations of these hormones and whether any alterations lead to compensatory changes in appetite and energy intake in the hours after exercise (Martins et al 2007, Ueda et al 2009b, King et al 2010a).

Moderate and high intensity running and cycling exercise can induce a temporary suppression of appetite, termed "exercise-induced anorexia" (Thompson et al 1988, King et al 1994, King et al 1995, King and Blundell 1995). A potential role for ghrelin in mediating this effect has been proposed (Burns et al 2007). Of the numerous appetite-regulatory hormones secreted from the gastrointestinal tract, ghrelin remains unique in being the only circulating hormone that is orexigenic (Wisser et al 2010) whilst other gut hormones such as PYY and GLP-1 are anorectic (Verdich et al 2001, Batterham et al 2002). Early studies that examined the effect of exercise on ghrelin observed increases (Christ et al 2006, Erdmann et al 2007), decreases (Toshinai et al 2007, Vestergaard et al 2007) and no change (Burns et al 2007, Martins et al 2007). Collectively, these findings are not suggestive of a role for ghrelin in exercise-induced anorexia. However, the aforementioned studies investigated the effect of exercise on total ghrelin. Ghrelin is acylated with octanoate by the GOAT (Yang et al 2008, Gutierrez et al 2008) and this acylation is essential for its role in appetite stimulation (Broglio et al 2004). The importance of measuring the acylated form of ghrelin is made evident by Marzullo et al (2008) who demonstrate a suppression of

acylated ghrelin concentrations in lean and obese participants in response to exercise despite no change in total ghrelin levels.

It is now well-documented that acylated ghrelin concentrations are transiently suppressed during intense treadmill running (Broom et al 2007, Broom et al 2009, King et al 2010a) and this suppression may be implicated in exercise-induced anorexia. Whether acylated ghrelin concentrations are affected by cycling exercise remains unclear. Studies have reported no change (Ueda et al 2009a, Morris et al 2010) and a decrease (Marzullo et al 2008) in acylated ghrelin concentrations in response to cycling. The inconsistent findings regarding the response of acylated ghrelin during cycling may arise from the differing methodologies, such as the duration and intensity of exercise. However, although the research is limited, two of the three studies examining the effect of cycling on acylated ghrelin concentrations have seen no change in acylated ghrelin concentrations during exercise. This is in comparison with the suppression of acylated ghrelin commonly observed during running, thus current evidence would suggest that running and cycling exert different effects on acylated ghrelin concentrations. The differences observed may be due to the different methodologies used or may represent a true physiological difference because the two modes of exercise differ in that cycling is weight supported and potentially involves a smaller muscle mass than running (Millet et al 2009). Running and cycling also exert different effects on physiological parameters such as muscle recruitment, and on ventilatory responses including exercise-induced arterial hypoxaemia (Millet et al 2009). In response to 50 minutes of running or cycling at 70 % of $\dot{V}O_2$ max, similar profiles of appetite suppression are observed, suggesting that the appetite response is unaffected despite the physiological and mechanical differences between these exercise modes (King et al 1995). If acylated ghrelin is a mediator of exercise-induced anorexia, it seems surprising some studies do not observe a change in acylated ghrelin concentrations during cycling (Ueda et al 2009a, Morris et al 2010). Gastrointestinal distress is more commonly reported amongst runners than in cyclists and participants of other sports where the body is fairly stable (Brouns et al 1987). Given that ghrelin is produced mainly in the stomach (Ariyasu et al 2001), there is a possibility that the jostling of the internal organs that occurs during running but not cycling could interfere with the production or secretion of acylated ghrelin.

The effects of these two modes of exercise on plasma acylated ghrelin concentrations needs to be clarified because they are the most commonly used modes of aerobic exercise in studies investigating the effect of exercise on gut hormones and their impact on energy balance. Thus, it is important to undertake a study using a within-subjects design to examine whether the discrepancies in the literature are true physiological effects or simply due to methodological differences between studies. Firstly, this will enable comparisons to be made between studies that evaluate the impact of exercise on changes in gut hormones and how they may affect subsequent energy intake. Secondly, if cycling and running elicit similar effects, because cycling is a low impact form of exercise, it may be a more acceptable form of exercise to use for some population groups such as the overweight and obese. Therefore, the purpose of this study was to compare the effects of a 60 minute bout of high intensity treadmill running and cycling on plasma acylated ghrelin concentrations and ratings of perceived hunger.

4.2 Methods

4.2.1 Participants

Eleven healthy males aged 19 – 26 y from the student and staff population at Loughborough University volunteered to participate in this study. Table 4.1 shows the participant characteristics.

Table 4.1 Physical characteristics of participants

Characteristic	Mean ± SEM
Age (y)	22.7 ± 0.7
Height (m)	1.79 ± 0.03
Body mass (kg)	75.5 ± 3.8
BMI ($kg \cdot m^{-2}$)	23.4 ± 0.7
Body fat (%)	18.6 ± 1.4
Running $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	57.8 ± 3.0
Cycling $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	50.0 ± 2.9

Values are mean ± SEM (n = 11)

4.2.2 Experimental protocol

Participants were informed verbally and in writing about the study protocol and after this completed a health screen questionnaire. When no contraindications to exercise were identified, participants gave their written informed consent to take part in the study and anthropometric data was collected. At this visit, participants completed a sub-maximal incremental treadmill running test (RUNRACE, Technogym, Gambettola, Italy) and after sufficient rest, completed a maximum oxygen uptake ($\dot{V}O_2$ max) treadmill running test. After allowing several days for recovery, participants were required to attend the laboratory for a second preliminary visit to complete a sub-maximal incremental cycling test on a cycle ergometer (Monark Ergomedic 874E, Vansbro, Sweden) followed by a maximum oxygen uptake ($\dot{V}O_2$ max) cycling test.

After completion of the preliminary trials, participants visited the laboratory on three further occasions to undertake three main experimental trials each lasting four hours. Trials

were a resting control trial, a running trial and a cycling trial. These were undertaken in a randomised, crossover design with at least seven days between each trial.

For 24 h prior to the first main trial, participants weighed and recorded their food intake and replicated this before each subsequent main trial. During this time, participants refrained from consuming alcohol and from participating in vigorous physical activity. On the morning of each main trial, participants arrived at the laboratory at approximately 08:30 am after a 10 h overnight fast.

4.2.3 Main trials

This study comprised three, four-hour trials that were completed in a random order. The three trials were: 1) control, 2) running, and 3) cycling. During exercise trials, participants either ran or cycled for 1 h at an intensity predicted to elicit 70 % of mode-specific $\dot{V}O_2$ max. To monitor the exercise intensity, expired air samples were collected every 15 minutes and immediately analysed for oxygen consumption and carbon dioxide production. If oxygen consumption was higher or lower than the predicted value for 70% of $\dot{V}O_2$ max, running speed was adjusted appropriately in the running trial and the mass applied to the basket on the cycle ergometer increased or decreased during the cycling trial. Heart rate and RPE were recorded during expired air collections in the running and cycling trials. During the expired air collection in the cycling trial, flywheel revolutions were also recorded. After completion of the exercise bout, participants rested in the laboratory for the remainder of the 4 h trial where they were free to work, read or watch DVDs. During control trials, the protocol was identical with the exception that participants rested for the entire 4 h trial duration. Figure 4.1 shows a schematic representation of the main trial protocol.

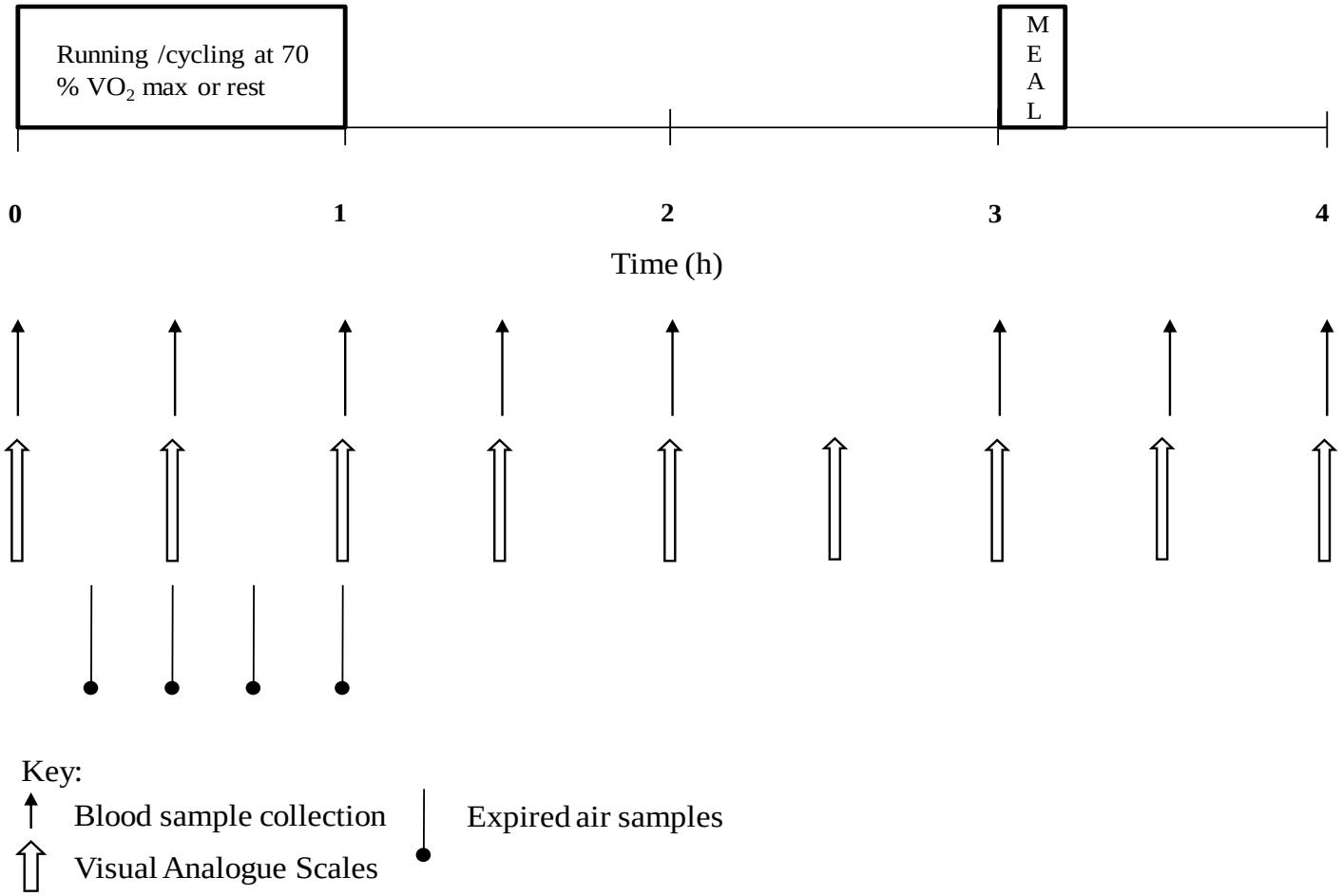

Figure 4.1 Schematic representation of main trial protocol

4.2.4 Blood sample collection

On arrival at the laboratory, participants rested in a semi-supine position and a cannula was inserted into an antecubital vein. Venous blood samples were collected into pre-cooled 9 mL EDTA monovettes at 0 (baseline), 0.5, 1, 1.5, 2, 3, 3.5 and 4 h for the determination of plasma glucose and TAG. Separate venous blood samples were drawn into pre-cooled 4.9 mL monovettes for the determination of plasma acylated ghrelin concentrations. The collection and processing of these blood samples is described in section 3.14.2. During the control trial, all blood samples were taken with the participant in a semi-supine position. In the exercise trials, all blood samples were collected with the participant in the semi-supine position with the exception of the sample at 0.5 h, which was collected whilst the participant straddled the treadmill in the running trial and with the participant seated on the cycle ergometer but not pedalling during the cycling trial.

Immediately after collection the 9 mL EDTA monovettes were centrifuged at 3500 rpm for 10 minutes at 4 °C (Heraeus Labofuge 400 R, Thermo Fisher Scientific Inc, U.K). The plasma supernatant was then aliquoted into separate Eppendorf tubes for storage at -20 °C prior to analysis of glucose and TAG. The 4.9 mL EDTA monovettes for determination of plasma acylated ghrelin were immediately centrifuged for 10 minutes at 3500 rpm at 4 °C (GS-15R Centrifuge, Beckman Coulter, Fullerton, U.S.A). The plasma was dispensed into plain storage tubes and 100 µL of 1 M hydrochloric acid (HCl) was added per 1 mL of plasma. These samples were centrifuged at 3000 rpm for 5 minutes at 4 °C before being transferred into Eppendorf tubes and immediately stored at -20ºC until analysis of acylated ghrelin concentrations.

Prior to centrifugation of venous blood samples, blood was collected in triplicate into 20 µL heparinised microhaematocrit tubes for the determination of haematocrit, and in duplicate into 20 µL micropippetes for the measurement of haemoglobin concentration. This enabled calculation of plasma volume change (Dill and Costill 1974).

4.2.5 Ratings of perceived hunger

Hunger was assessed at baseline and every 30 minutes thereafter using a validated 100 mm VAS which was anchored on the left with ‘not at all hungry’ and on the right with ‘very

hungry' (Flint et al 2000). Subjects indicated with a mark along the 100 mm line how hungry they were.

4.2.6 Standardised meal

A standardised meal was consumed by all participants at 3 h. The meal consisted of white bread, cheese, butter, full fat mayonnaise, salted potato crisps and a full fat strawberry milkshake. The meal provided 46.1 kJ per kilogram body mass and the macronutrient content of the meal was as follows: 0.69 g fat, 0.30 g of protein and 0.91 g carbohydrate per kilogram body mass. This provided 56 % of the calories as fat, 11 % as protein and 33 % as carbohydrate. Participants consumed the test meal within 15 minutes and the start and finish times were recorded and replicated for subsequent trials. The quantity of food provided to each participant was based on their body weight at the start of the first trial and identical quantities provided at each subsequent trial. Participants were free to consume water *ad libitum* throughout the trials and the volume consumed was recorded.

4.2.7 Environmental temperature and humidity

During the main trials, environmental temperature and humidity were monitored using a hand-held hygrometer (Omega RH85, Manchester, U.K) and the values recorded.

4.2.8 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria). Plasma samples were analysed for glucose and TAG concentrations via colorimetric methods using reagents from ABX Diagnostics (Montpellier, France) with the use of a Pentra 400 (Horiba ABX Diagnostics, France). To eliminate inter assay variation, samples from each participant were analysed in the same run. The within-batch coefficients of variation for the assays were as follows: ghrelin 7.2%, glucose 0.4% and TAG 2.7%.

4.2.9 Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A). Area under the curve (AUC) values were calculated for acylated ghrelin and hunger using the trapezoidal rule. Repeated measures ANOVA was used to assess differences in AUC between trials as well as differences between fasting measures of acylated ghrelin, hunger, glucose and TAG. Two-

factor repeated measures ANOVA was used to examine differences between the trials over time for acylated ghrelin, hunger, glucose and TAG. Where there were significant main effects, *post-hoc* analysis using the Bonferroni correction for multiple comparisons were performed. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5 % level. When plasma volume changes were adjusted for, statistical findings were not altered hence unadjusted values are reported. Results are reported as mean ± SEM.

4.3 Results

4.3.1 Responses to running and cycling exercise

Participants achieved a lower maximal oxygen uptake when they cycled compared with running on the treadmill (50.0 ± 2.9 *vs* 57.8 ± 3.0 $mL \cdot kg^{-1} min^{-1}$ respectively, $P < 0.001$). Participants expended more energy during the running trial compared with the cycling trial ($P < 0.001$). Table 4.2 shows the physiological responses to the running and cycling bouts.

Table 4.2 Physiological responses to 60 minutes of running and cycling

	Running	Cycling
Exercise Intensity (% $\dot{V}O_2max$)	71.7 ± 0.7	70.3 ± 1.1
Energy expenditure (kJ)	3746 ± 250*	3258 ± 254
Energy expenditure (kcal)	919 ± 63*	779 ± 61
Heart rate (bpm)	171 ± 3*	158 ± 4
Respiratory exchange ratio	0.93 ± 0.01	0.94 ± 0.01
Median RPE	14 (range 12 – 16)	14 (range 13 – 16)
Energy from fat (%)	24 ± 3	22 ± 3
Energy from carbohydrate (%)	76 ± 3	78 ± 3

Values are mean ± SEM ($n = 11$) * significantly different from cycling trial ($P < 0.05$)

4.3.2 Plasma acylated ghrelin concentrations

Fasting plasma acylated ghrelin concentrations did not differ at baseline ($P = 0.420$) between the control (173 ± 38 $pg \cdot mL^{-1}$), running (158 ± 33 $pg \cdot mL^{-1}$) and cycling (170 ± 38 $pg \cdot mL^{-1}$) trials. There was a main effect of trial ($P < 0.05$), time ($P < 0.005$) and a trial x time interaction ($P < 0.005$) for plasma acylated ghrelin (Figure 4.2). Post-hoc analysis showed that compared with the control trial, plasma acylated ghrelin concentrations were lower during both the running trial ($P = 0.06$) and the cycling trial ($P = 0.04$). There were significant differences between trials at 0.5 and 1 h, indicating suppressed acylated ghrelin concentrations during the running ($P < 0.005$) and cycling trials ($P < 0.001$) compared with the control trial.

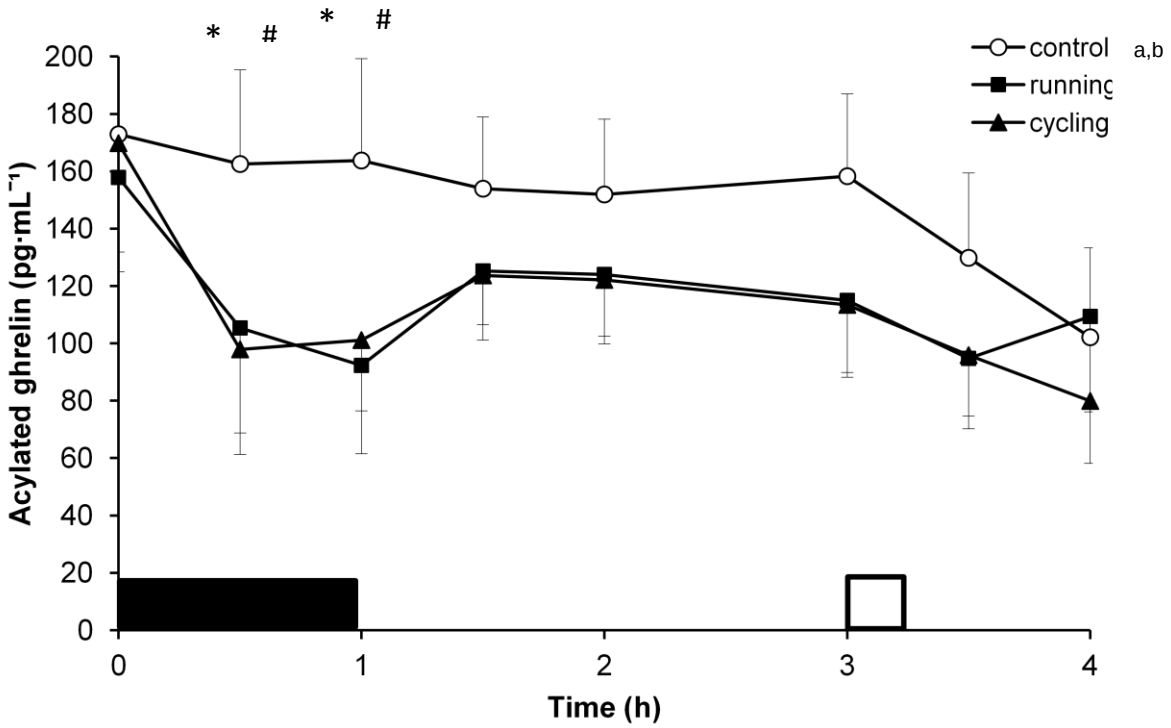

Figure 4.2 Plasma acylated ghrelin concentrations during the control (○), running (■) and cycling (▲) trials. Values are mean ± SEM (n = 11). The black rectangle indicates the treadmill run and the white square indicates test meal consumption. [*]running different from control (P < 0.005), [#]cycling different from control (P < 0.001). Main effect of trial, [a] control different from running (P = 0.06) [b] control different from cycling (P = 0.04).

Between trial differences in plasma acylated ghrelin concentrations were also assessed using AUC values (Table 4.3). Area under the curve values differed between trials during the exercise period (0 – 1 h; P < 0.05). Post-hoc analysis revealed acylated ghrelin AUC was lower in both the running and cycling trials compared with control (P = 0.04). Pre-prandial (0 – 3 h) AUC values were significantly different between trials (P = 0.007) and post-hoc analysis indicated that compared with the control trial, AUC values were lower during the running (P = 0.02) and cycling trials (P = 0.01). Finally, there was a significant difference in acylated ghrelin AUC values for the total trial duration (0 – 4 h; P = 0.01) with values for the running and cycling trials being lower than the control trial (P < 0.05).

Table 4.3 Acylated ghrelin AUC in the control, running and cycling trials

	Exercise or rest (0 – 1 h) pg·mL^{-1}·1 h	Pre-prandial (0 – 3 h) pg·mL^{-1}·3 h	Total trial (0 – 4 h) pg·mL^{-1}·4 h
Acylated ghrelin			
Control	165 ± 34	476 ± 88	606 ± 114
Running	119 ± 37*	351 ± 84*	455 ± 107*
Cycling	119 ± 29*	342 ± 70*	448 ± 95*

Values are mean ± SEM (n = 11). *significantly different from control (P < 0.05)

4.3.3 Subjective ratings of hunger

Fasting ratings of hunger did not differ at baseline between trials (P = 0.263). There was a main effect of time (P < 0.001) showing that hunger was suppressed in response to the test meal. There was a trial x time interaction (P < 0.05; Figure 4.3) indicating that hunger responses differed over time between the control, running and cycling trials. However, post-hoc analysis using the Bonferroni method revealed no differences in hunger between trials at any point. Between trial differences in hunger ratings were also analysed using AUC values for the entire 4 hour trial duration, during the exercise bout (0 – 1 h) and for the pre-meal interval (0 – 3 h). This analysis did not show any differences between trials during any of the time intervals assessed.

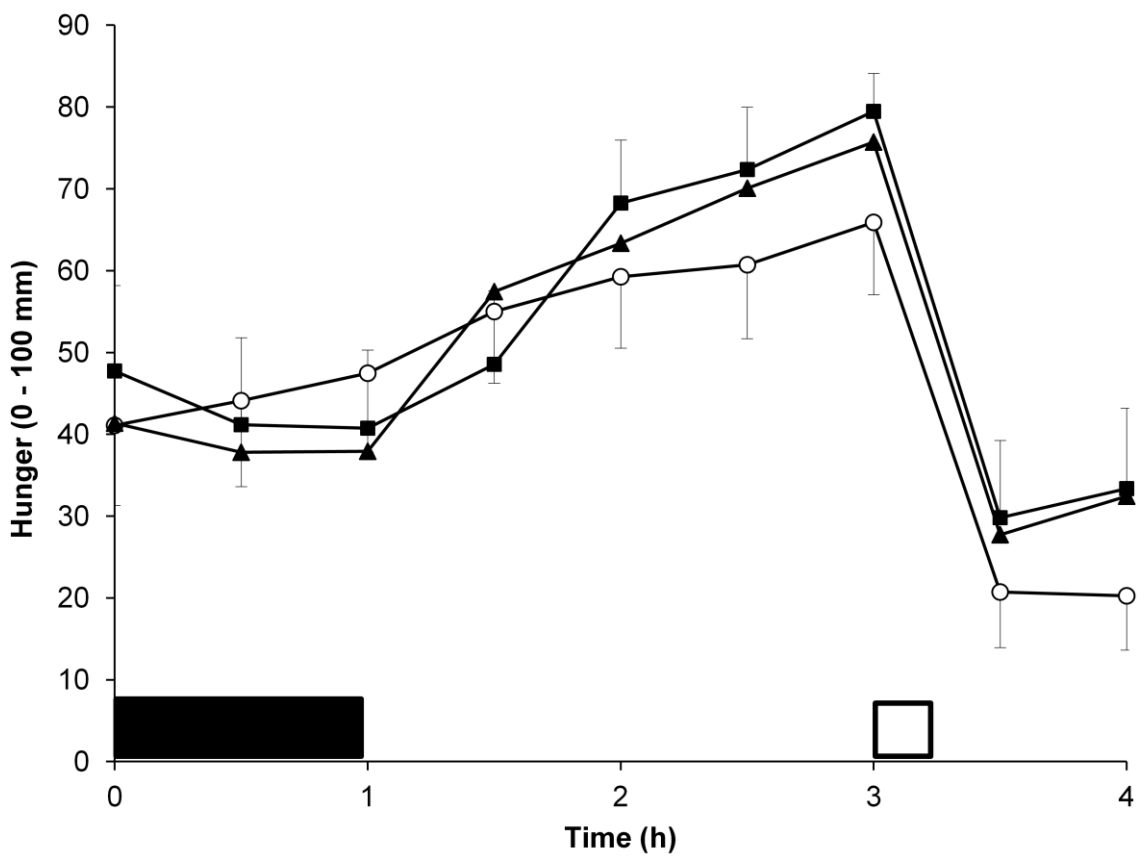

Figure 4.3 Subjective hunger ratings during the control (○), running (■) and cycling (▲) trials. Values are mean ± SEM, (n = 11). The black rectangle indicates the treadmill run and the white square indicates test meal consumption. Some error bars have been omitted for clarity (trial mean ± SEM; cycling 49 ± 8 mm)

4.3.4 Glucose and triacylglycerol concentrations

Fasting plasma glucose concentrations did not differ at baseline between trials (control 4.6 ± 0.1 mmol·L^{-1}, running 4.5 ± 0.2 mmol·L^{-1}, cycling 4.6 ± 0.1 mmol·L^{-1}; P = 0.563). There was a main effect of time (P < 0.001) and a trial x time interaction for plasma glucose (P < 0.001; Figure 4.4). Post-hoc analysis indicated that compared with the control trial, plasma glucose concentrations were elevated in the running and cycling trials at 4 h (P < 0.001).

Fasting plasma TAG concentrations were significantly higher at baseline on the control trial compared with the running and cycling trials (control 0.9 ± 0.1 mmol·L^{-1}, running 0.7 ± 0.1 mmol·L^{-1}, cycling 0.8 ± 0.1 mmol·L^{-1}; P < 0.05). There was a main effect of time (P <

0.001) and a trial x time interaction ($P < 0.001$) signifying that the responses to the trials differed over time (Figure 4.4). Post-hoc analysis indicated that the only difference between trials was at baseline ($P < 0.05$).

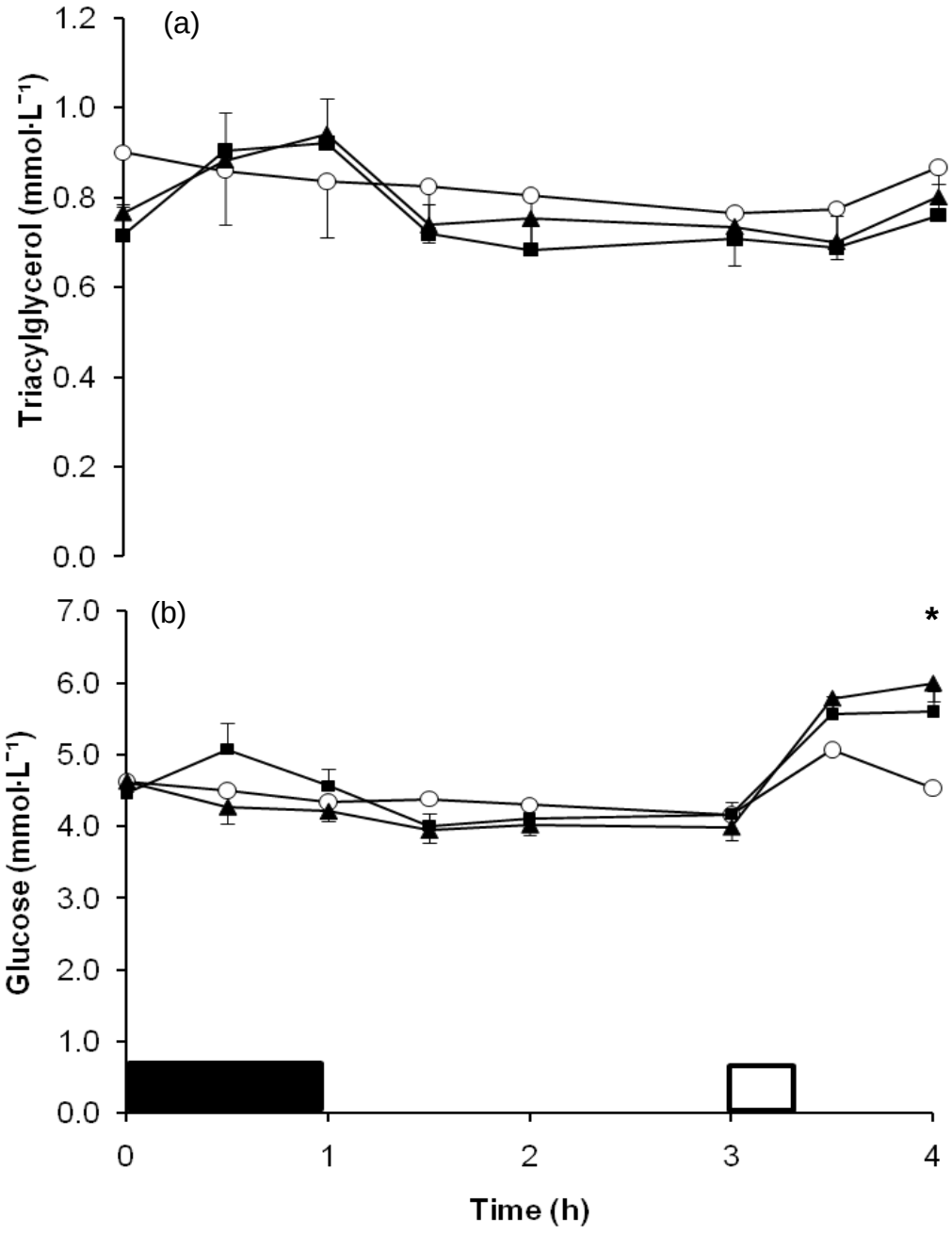

Figure 4.4 **(a)** Plasma triacylglycerol concentrations during the control (○), running (■) and cycling (▲) trials. Values are means ± SEM, (n = 11). The black rectangle indicates the treadmill run and the white square indicates test meal consumption. Error bars have

been omitted from the cycling trial for clarity (mean ± SEM; values for the cycling trial were 0.8 ± 0.1 mmol·L^{-1}). Trial x time interaction; control significantly different to running and cycling at 0 h ($P < 0.05$). **(b)** Plasma glucose concentrations during the control (○), running (■) and cycling (▲) trials. Values are mean ± SEM, ($n = 11$). The black rectangle indicates the treadmill run and the white square indicates test meal consumption. Error bars have been omitted from the control trial for clarity (mean ± SEM; values for the control trial were 4.5 ± 0.1 mmol·L^{-1}). *Running and cycling different from control ($P < 0.001$).

4.3.5 Body mass and fluid consumption

Body mass at baseline was similar between trials (control 75.8 ± 3.8 kg *vs* running 76.0 ± 3.7 kg *vs* cycling 75.4 ± 3.7 kg; $P = 0.133$). Participants consumed more water in the running (861 ± 114 mL) and cycling (865 ± 119 mL) trials than the control trial (411 ± 107 mL; $P < 0.05$).

4.3.6 Environmental temperature and humidity

Environmental temperature did not differ between trials (control 23.7 ± 0.5 ºC *vs* running 23.4 ± 0.4 ºC *vs* cycling 23.2 ± 0.4 ºC; $P = 0.585$). There was also no difference in relative humidity ($P = 0.999$) between the 3 trials (control 36.9 ± 1.9 *vs* running 36.9 ± 1.7 *vs* cycling 36.8 ± 2.1%).

4.3.7 Correlations between variables

Fasting plasma acylated ghrelin concentrations at baseline were not correlated with baseline hunger ratings in any trial. Similarly, acylated ghrelin was not correlated with hunger at subsequent times during the trials. There were no correlations between fasting plasma acylated ghrelin concentrations and BMI, body mass, percentage body fat, maximal oxygen uptake (running and cycling) or fasting plasma glucose concentrations.

4.4 Discussion

The main finding arising from this investigation is that plasma acylated ghrelin concentrations are suppressed in response to a high intensity bout of cycling exercise and the degree of suppression is similar to that observed with a bout of high intensity treadmill running that is equivalent in duration and intensity. Thus, despite the differing physiological demands of running and cycling, a suppression of acylated ghrelin is apparent during both modes of exercise.

Current evidence regarding the effects of cycling exercise on concentrations of plasma acylated ghrelin is inconclusive with two studies suggesting that cycling does not affect plasma acylated ghrelin concentrations (Ueda et al 2009a, Morris et al 2010) and one study demonstrating a decrease in acylated ghrelin concentrations (Marzullo et al 2008). It had been thought that the conflicting findings reported in the literature may be true physiological differences. Ghrelin is predominantly secreted from the stomach although smaller amounts are found in other parts of the gastrointestinal tract such as the duodenum and jejunum (Date et al 2000, Ariyasu et al 2001). During cycling, the upper body is relatively static and the body mass supported thus gastrointestinal disturbances are minimised, whereas during running intestinal jarring occurs and could affect the secretion of ghrelin from the stomach. Rehrer and Meijer (1991) measured the mechanical vibration of the body and found that acceleration/deceleration was more than doubled in running compared with cycling. Although we did not measure mechanical vibration we can presume there was a lower mechanical vibration of the body during high intensity cycling and despite this, acylated ghrelin is suppressed to a similar extent as with high intensity running. Thus the exercise-induced response of acylated ghrelin does not appear to be specific to the mode of exercise. This notion is further supported by recent studies demonstrating a transient suppression of acylated ghrelin during swimming (King et al 2011a) and resistance exercise (Broom et al 2009). The suppression of plasma acylated ghrelin concentrations during high intensity treadmill running is consistent with previous reports that demonstrate high intensity bouts of running lasting between 60 and 90 minutes transiently suppress plasma acylated ghrelin concentrations (Broom et al 2007, Broom et al 2009, King et al 2010a).

Differences in methodology likely explain the lack of suppression in acylated ghrelin during cycling exercise that is reported in the literature. Firstly, in the studies where no

change in acylated ghrelin with cycling has been observed, participants were fed test meals 60 minutes prior to exercise which significantly suppressed plasma acylated ghrelin concentrations compared with fasting (Ueda et al 2009a, Morris et al 2010). Thus, immediately at the start of exercise, acylated ghrelin concentrations were already suppressed (Ueda et al 2009a) and it appears that a single bout of cycling did not impact further upon acylated ghrelin concentrations after feeding. Secondly, the protocol used by Morris et al (2010) differs from other studies that have examined the plasma acylated ghrelin response to exercise in that the exercise bout took place in the evening. The lack of change in acylated ghrelin in response to exercise may be due to diurnal alterations in factors that influence ghrelin secretion (Morris et al 2010). However, the mechanisms responsible for the secretion of ghrelin are still not fully established. Finally, the intensity of the cycling bouts, which was approximately 50 % of maximal oxygen uptake (Ueda et al 2009a, Morris et al 2010), may have been insufficient to suppress acylated ghrelin concentrations. Compared with high intensity bouts of exercise, moderate intensity bouts of exercise such as walking do not suppress plasma acylated ghrelin concentrations (King et al 2010b, Unick et al 2010).

Ghrelin plays an important role in the acute regulation of energy balance and contributes to mealtime hunger and meal initiation (Cummings, 2006). The caloric load of a meal determines the extent of ghrelin suppression (Callahan et al 2004, le Roux et al 2005) and the preprandial increase in circulating ghrelin is determined by the energy content of the preceding meals (Leidy and Williams 2006). In response to chronic caloric deprivation, peak ghrelin concentrations are elevated prior to meals (Leidy et al 2007). Collectively, these findings suggest ghrelin is highly sensitive to changes in energy balance and acts to restore it by compensatory alterations in circulating concentrations. It may therefore be expected that after a large amount of energy is expended during exercise there would be compensatory increases in ghrelin in the hours after exercise to stimulate appetite and restore energy balance. However, in the present study, compared with the control trial, pre-prandial (0 – 3 h) AUC values of ghrelin were suppressed by approximately 26 % and 28 % in the running and cycling trials respectively, indicating that at least in the short term, acylated ghrelin is not stimulated after an acute bout of exercise. Similarly, King et al (2010a) show acylated ghrelin AUC is significantly suppressed over a 10 h trial when 90 minutes of prolonged treadmill running has been undertaken compared with a control condition. They also observed no change in energy intake over a 22.5 h period after

exercise compared with a control condition. In the present study, participants were fed a standardised meal thus it is not possible to examine the effect of this sustained suppression of acylated ghrelin on spontaneous energy intake.

Hunger was unaffected by running and cycling in the present study. This is surprising given that there is a wide body of literature showing that hunger is transiently suppressed as a result of moderate to high bouts of physical activity (Thompson et al 1988, King et al 1994, King and Blundell 1995, Broom et al 2007, Broom et al 2009, King et al 2010a). The intensity and duration of exercise are important determinants of exercise-induced anorexia (Thompson et al 1988, King et al 1994). Compared with other studies where participants experience a suppression of hunger during exercise, intensity and duration of exercise were similar, and in some cases greater, in the present study. It is therefore unlikely that the intensity of exercise was an insufficient stimulus for exercise-induced anorexia. The present findings are in agreement with some other research that does not demonstrate a suppression of appetite with moderate to high intensity exercise (King et al 1996, Maraki et al 2005, Morris et al 2010, Ueda et al 2009a). However, those studies differed with respect to gender of participants, the timing of appetite assessment after exercise, the time of day when exercise was performed and/or the feeding status of the individual. Thus the reason for a lack of exercise-induced anorexia in the present study is unclear. Baseline hunger ratings in the present study are lower than those reported in another study that used the same method of quantifying subjective ratings of hunger and observed a suppression of hunger during intense treadmill running (King et al 2010a). It is possible that when baseline hunger ratings are below a certain threshold they may be more resistant to change with exercise but this is purely speculative. Furthermore, there was a large inter-individual variability in maximal oxygen uptake values, particularly on the treadmill, with values ranging from 48 – 77 $mL \cdot kg^{-1} \cdot min^{-1}$. It may be that the appetite response of individuals with different aerobic fitness is altered. Those individuals with lower aerobic capacities are more likely to be unaccustomed to exercise and could believe there is an expectation for them to feel hungrier during intense exercise. However, not all of the participants with maximal oxygen uptake values above the mean of all the participants had hunger ratings that were lower than the mean of all the participants during exercise and there was no correlation between aerobic fitness and hunger during the treadmill run. Therefore, this notion is purely speculative and a greater number of

participants with varying values of maximal oxygen uptake would be required to investigate this.

The mechanisms responsible for alterations in appetite with exercise are not fully established. Given the role of the gut hormones in the regulation of appetite, the effect of exercise on circulating concentrations of many of these hormones has become the focus of attention (Broom et al 2007, Martins et al 2007, Broom et al 2009). Both hunger and concentrations of plasma acylated ghrelin were suppressed during 60 minutes intense treadmill running in one study (Broom et al 2007) and the authors postulated that a suppression of the orexigenic hormone, acylated ghrelin, mediated the transient suppression of hunger. Suppressed hunger and acylated ghrelin concentrations have also been observed with swimming and resistance exercise (Broom et al 2009, King et al 2011a). The findings from the present study are therefore surprising in that although acylated ghrelin concentrations were suppressed with running and cycling exercise, hunger was unaffected. Hunger is regulated by many gut hormones, but how these hormones interact in response to exercise remains unclear. Alterations in concentrations of anorexigenic gut peptides that were not measured in this study may also influence the appetite response and could override any inhibitory influence of suppressed acylated ghrelin concentrations. Conversely, although its role in appetite regulation and meal initiation is well established (Cummings, 2006) acylated ghrelin may not be involved in the control of appetite in response to exercise. Ueda et al (2009b) suggest that in response to different modes and intensities of exercise each gut hormone may display its own specific kinetics in the blood and that each hormone may play a different role in the appetite regulatory process after a bout of exercise.

In conclusion, this study shows that running and cycling exercise of equivalent duration and intensity suppress concentrations of plasma acylated ghrelin to a similar extent. In the future, this may enable more reliable conclusions to be drawn from different studies that examine the effect of exercise on gut hormones and energy intake using different exercise modes.

CHAPTER V

The influence of running in the heat on appetite, energy intake and plasma concentrations of acylated ghrelin

5.1 Introduction

There exists a large body of research investigating the effects of exercise on appetite and energy intake. Although there was no suppression of hunger observed during high intensity running and cycling exercise in the first study presented within this thesis, most evidence shows a transient suppression of appetite during high intensity exercise, and most research suggests post-exercise energy intake is not affected. With the identification of increasing numbers of gut hormones that regulate appetite, attention is now focusing on how exercise affects concentrations of these hormones and how this impacts upon regulation of appetite and energy intake during and after exercise. Most evidence shows that acute bouts of intense exercise affect circulating concentrations of appetite-regulatory hormones including acylated ghrelin, PYY and GLP-1 suggesting these hormones may be involved in the exercise-induced suppression of appetite (Broom et al 2007, Martins et al 2007). Ghrelin is an acylated peptide that is secreted predominantly from the stomach, and it is currently the only known circulating gut hormone that stimulates appetite (Karra and Batterham, 2010) giving it a unique role in energy homeostasis. Findings from the first study presented within this thesis, and from other evidence in the literature reveal a transient suppression of acylated ghrelin during aerobic exercise (Broom et al 2007, Marzullo et al 2008, Broom et al 2009, King et al 2010a) that is independent of exercise mode (Broom et al 2007, Marzullo et al 2008, King et al 2011a). Research is still emerging regarding the effect of any exercise-induced alterations in these hormones on post-exercise energy intake (Martins et al 2007, Ueda et al 2009b, King et al 2010a).

Exercise is a large determinant of energy balance, and in the absence of compensatory increases in food intake, can produce a short-term negative energy balance which may be efficacious for weight loss. However, there are situations where being in negative energy balance is undesirable for an individual. Many sporting events are held in countries where ambient temperatures are high and this can pose challenges beyond environmental heat stress and dehydration which can limit aerobic performance (Cheuvront et al 2010). Appropriate nutrition can have a large influence on an athlete's ability to perform optimally and when an athlete is competing frequently in events over the course of a day, or several

days, it is essential for them to be adequately refuelled for their next event. Anecdotal reports suggest that hot weather suppresses appetite (Burke, 2001); however, there is little empirical evidence despite Brobeck proposing in 1948 that ambient temperature is a strong metabolic regulator and high ambient temperatures will decrease food intakes. The majority of the research pertaining to exercise in the heat is concerned with an impairment of performance due to dehydration. However, a loss of appetite could be disadvantageous to an athlete competing in a hot climate and might impair performance if optimal refuelling does not take place.

Shorten et al (2009) compared the effect of exercise (40 minutes treadmill running at 70 % $\dot{V}O_2$ peak) in the heat (36 °C) and in a neutral temperature (25 °C) on energy intake and circulating concentrations of appetite-regulating hormones. Compared with a resting control trial at 25 °C, total energy intake was no different after exercise in the heat whereas it was increased after exercise in a neutral temperature. This suggests that exercise in the heat prevented a stimulation of appetite observed after exercise in a neutral temperature. Furthermore, when taking into account the energy cost of the exercise bout, relative energy intake was decreased after exercise in the heat compared with the control trial. Elevated concentrations of the anorectic gut hormone PYY were observed immediately prior to the meal after exercise in the heat, and the authors proposed that this could partly explain the lower relative energy intake after exercise in the heat. Core body temperature, assessed by tympanic temperature, was significantly elevated immediately before the meal during the exercise in the heat trial compared with the other trials. This was also postulated as a mechanism behind the lower energy intake. However, this study had several limitations. Firstly, subjective sensations of appetite were not directly measured and secondly, energy intake was only assessed on one occasion shortly after exercise. Alterations in energy intake at subsequent meals could compensate for any acute changes observed.

Given the important role that ghrelin plays in meal initiation (Cummings et al 2001) a simple study was undertaken by Tomasik et al (2005) to determine the effect of environmental temperature on total ghrelin. On separate occasions, participants spent 30 minutes resting in a room at a temperature of 20 °C prior to 30 minutes exposure to a temperature of either 2 °C or 30 °C. Blood samples were obtained for the determination of ghrelin concentrations immediately before and after exposure to the two conditions.

Compared with the neutral temperature of 20 °C, ghrelin concentration was decreased after short term exposure to an ambient temperature of 30 °C and increased after the same duration of time exposed to 2 °C. The authors concluded that ghrelin is one of the factors that modify appetite in response to alterations in environmental temperature. However, appetite sensations and energy intake were not measured in this study so it is unclear whether the decrease in total ghrelin concentrations after short-term heat exposure would translate into a decrease in appetite and food intake. Furthermore, the authors only measured total ghrelin, whereas it is the acylated form of the peptide that is necessary for exerting its appetite-stimulatory effect. It is known that exercise can cause a decrease in acylated ghrelin concentrations even though total ghrelin concentrations are unchanged (Marzullo et al 2008) thus it is important to assess the effect of a hot environment on concentrations of acylated ghrelin.

Collectively, the available research suggests that appetite and energy intake may be suppressed in hot weather and this may be mediated by changes in appetite-regulatory hormones. However, findings are not conclusive and there are several notable limitations associated with the current work. The aim of this study was to determine the effect of moderate intensity aerobic exercise performed in the heat on subjective ratings of appetite, energy intake and concentrations of acylated ghrelin over a 7 hour period. In this way, it is hoped that some of the limitations of currently available research can be addressed. The mechanisms responsible for the suppression of acylated ghrelin concentrations (whether it be a suppression of acylated ghrelin secretion or synthesis) after feeding are unclear, thus glucose and TAG concentrations were measured to examine a possible role in acylated ghrelin suppression in the postprandial state. A further aim was to examine the influence of environmental temperature on macronutrient preference and preference for energy intake from solid versus liquid food sources.

5.2 Methods

5.2.1 Participants

Eleven healthy, physically active males aged 19 to 23 y volunteered to participate in this study. Participants were recruited from the student and staff populations at Nottingham Trent University and Loughborough University. Table 5.1 shows the physical characteristics of the participants.

Table 5.1 Physical characteristics of participants

Characteristic	Mean ± SEM
Age (y)	21.1 ± 0.3
Height (m)	1.81 ± 0.02
Body mass (kg)	77.3 ± 2.2
BMI ($kg \cdot m^{-2}$)	23.6 ± 0.6
Body fat (%)	20.2 ± 0.7
Running $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	56.7 ± 1.5

Values are mean ± SEM (n = 11)

5.2.2 Experimental protocol

Each participant attended the laboratory on three occasions. At the first visit, participants were reminded verbally and in writing about the study protocol and gave their written informed consent to participate. Participants completed a health screen questionnaire and after their eligibility to participate was confirmed, anthropometric data were collected. Participants then completed two treadmill tests; the first was a sub-maximal incremental test to determine the relationship between running speed and oxygen consumption, the second was a maximal oxygen uptake ($\dot{V}O_2$ max) test. After sufficient recovery time from the preliminary trial, participants completed two main trials in a randomised counter-balanced order separated by at least one week. Main trials were 7 h in duration and were as follows: exercise in a hot environment (30.1 ± 0.0˚C; 48 ± 1% RH) and exercise in a temperate environment (20.3 ± 0.1˚C; 49 ± 1% RH). The main trials were carried out in the environmental chamber at Nottingham Trent University (Design Environmental WIR52-20HS, Design Environmental, Gwent, U.K).

5.2.3 Main trials

Participants weighed and recorded their food intake for 24 h prior to the first main trial and replicated the timing and quantity of this before the second trial. During this time, participants refrained from vigorous physical activity and alcohol consumption. On the morning of the main trials participants arrived at the laboratory after a 10 h overnight fast. Each participant completed the two, 7 h trials individually so that their food intake was not influenced by social factors. Upon arrival at the lab, participants body mass was recorded, and after a period of sitting an expired air sample was taken for measurements of resting energy metabolism. Participants then lay in a supine position prior to a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) being inserted into an antecubital vein. Prior to entry into the environmental chamber, a rectal probe (Grant Instruments Ltd, Cambridge, England) was self-inserted and a heart rate monitor (Polar Electro Oy, Kempele, Finland) attached (Design Environmental Ltd, Gwent, Wales, U.K). Upon entry into the chamber, participants rested for approximately 10 minutes, during which time resting values were obtained for heart rate, rectal temperature, thermal sensations and appetite perceptions. For the first hour, participants ran on a level treadmill at 65 % of $\dot{V}O_2$ max. Expired air samples were collected every 15 minutes for 60 seconds during exercise and running speed was adjusted if necessary to keep oxygen consumption as close to the value for 65 % of $\dot{V}O_2$ max as possible. Figure 5.1 shows a schematic representation of the main trial protocol.

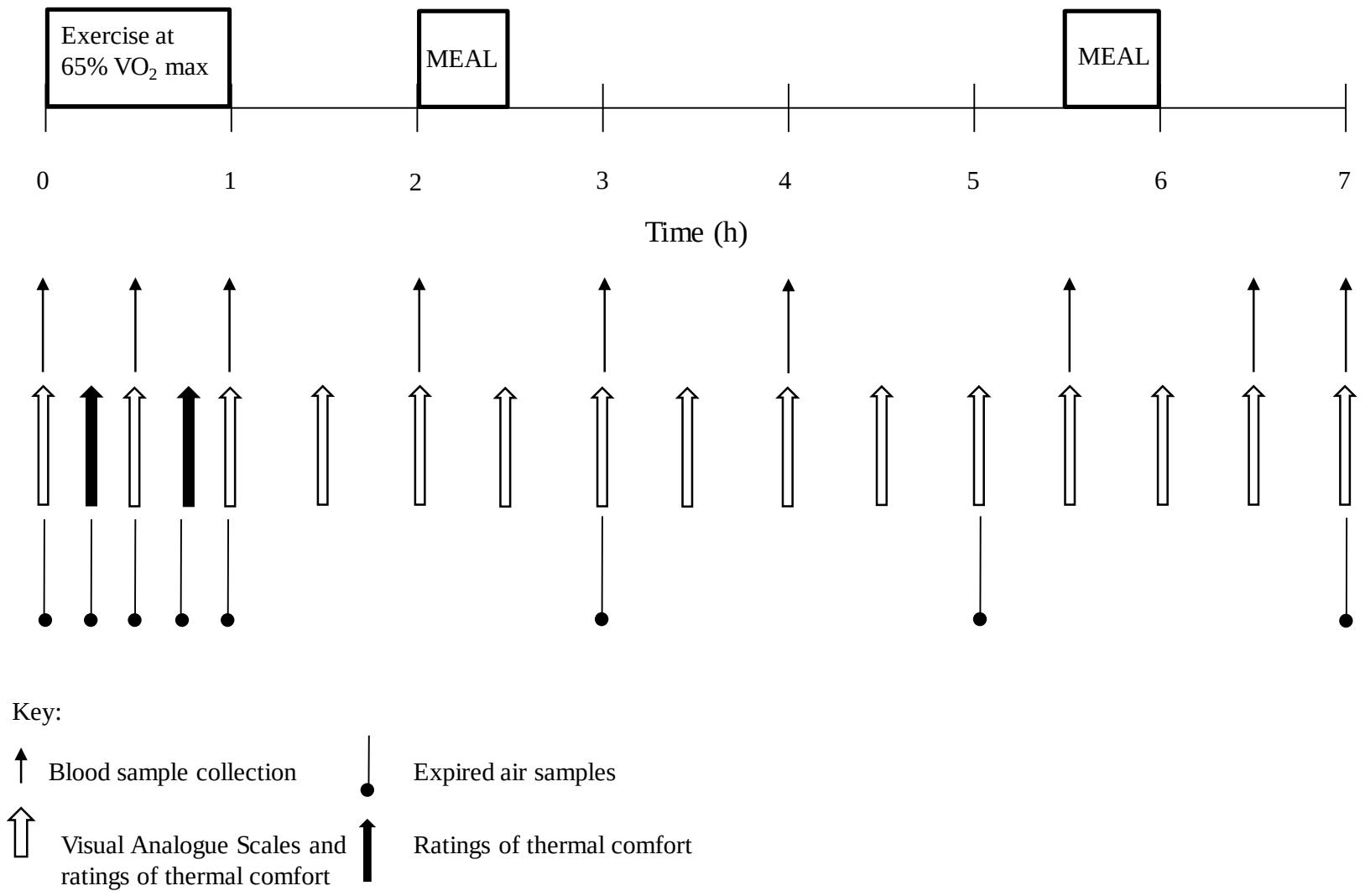

Figure 5.1 Schematic representation of main trial protocol

5.2.4 Blood sample collection

Venous blood samples were collected at baseline (0 h) and 0.5, 1, 2, 3, 4, 5.5, 6.5 and 7 h into pre-cooled 5 mL EDTA blood collecting tubes for the determination of plasma glucose and TAG concentrations. These samples were centrifuged at 3500 rpm for 10 minutes in a refrigerated centrifuge (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc, U.S.A). The plasma was dispensed into separate Eppendorf tubes which were stored overnight at -20 °C before being transferred to -80 °C until analysis. Additional venous blood samples were collected at these times into 5 mL EDTA blood collecting tubes for the determination of plasma acylated ghrelin concentrations. The collection and processing of the blood samples is described in section 3.14.2. Blood samples were collected with the participant in a supine position with the exception of the 0.5 h sample which was obtained whilst the participant stood stationary on the treadmill. Blood samples were not obtained from one participant due to problems with blood collection, thus, for all blood analyses $n = 10$. To estimate plasma volume changes, prior to centrifugation triplicate blood samples were collected into 20 µL heparinised microhaematocrit tubes and duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration.

5.2.5 Appetite perceptions

Ratings of subjective feelings of hunger, fullness, satisfaction and prospective food consumption (PFC) were reported on 100 mm VAS (Flint et al 2000) at baseline and every 30 minutes thereafter.

5.2.6 Thermal sensations and rectal temperature

Thermal sensations were rated at baseline, every 15 minutes during exercise and every 30 minutes after exercise using a 9 point rating scale (Young et al 1987) described in section 3.13. Rectal temperature was recorded at baseline, at five minute intervals during exercise, and every 30 minutes thereafter (see section 3.13 for details of core temperature measurement).

5.2.7 Assessment of energy intake

Participants were given 30 minutes access to a cold buffet meal at 2 h and 5.5 h. Participants consumed food *ad libitum* whilst seated at a table in the environmental chamber and they completed each trial on their own so that their food intake and choices

were not influenced by social factors. Participants were instructed to eat until they were comfortably satisfied. The food presented consisted of a variety of food and drinks considered typical daytime meal choices for a Westerner (Appendix E). All items were provided in excess of expected consumption but participants could request more of any of the items provided if desired. To enable quantification of energy intake, all foods were covertly weighed prior to being given to the participants in the chamber and foods that were not consumed, as well as any waste, were removed from the environmental chamber after 30 minutes and were reweighed out of sight of the participants to the nearest gram. Energy and macronutrient intake were calculated using information from the food labels and in the case of non-packaged items such as fruit, values were obtained from the USDA National Nutrient Database. Participants were unaware that their food intake was being monitored but were fully briefed upon completion of the study. Participants were free to consume water *ad libitum* throughout the trials with the volume consumed recorded.

5.2.8 Environmental temperature and humidity

Temperature and humidity inside the environmental chamber were pre-set using climate control programming software (Contour Programming and Logging Software Package, Design Environmental Ltd, Gwent, Wales). The desired temperature and humidity within the chamber were confirmed using a handheld hygrometer (model RH85, Omega, Manchester, U.K.) every 30 minutes and the values recorded.

5.2.9 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Bretonneux, France). Plasma samples were analysed for glucose and TAG concentrations via enzymatic, colorimetric methods using reagents from ABX diagnostics (Montpellier, France) with the use of a Pentra 400 automated analyser (Horiba ABX Diagnostics, France). To eliminate inter-assay variation, all samples from the same participant were analysed in the same run. The within-batch coefficients of variation were as follows: acylated ghrelin 8.4 %, glucose 0.5 % and TAG 1.8 %.

5.2.10 Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Area under the curve (AUC) values were calculated for hunger, acylated ghrelin, glucose

and TAG using the trapezoidal rule. Differences in fasting and AUC values for appetite perceptions, acylated ghrelin, core temperature and thermal sensations were determined using Student's t-tests. Two-factor repeated measures ANOVA was used to examine differences between trials for appetite perceptions, energy and macronutrient intake, acylated ghrelin, core temperature and thermal sensations. Where significant interactions occurred, paired t-tests with Bonferroni adjustment for multiple comparisons were used to determine differences at individual time points. The Pearson product moment correlation coefficient was used to examine relationships between variables. When correcting values for changes in plasma volume, the statistical findings were unaltered so for simplicity unadjusted values are reported. Statistical significance was accepted at the 5 % level. Results are presented as mean ± SEM.

5.3 Results

5.3.1 Exercise responses

The physiological responses to treadmill running in a temperate and a hot environment are described in Table 5.2.

Table 5.2 Physiological responses to exercise in a temperate and hot environment

	Temperate	**Hot**
Exercise Intensity ($\%\dot{V}O_2max$)	65 ± 1	64 ± 1
Energy expenditure (kJ)	3427 ± 90	3386 ± 104
Energy expenditure (kcal)	819 ± 22	809 ± 25
Heart rate (bpm)	158 ± 4	168 ± 3*
Thermal sensations	5.3 ± 0.2	6.5 ± 0.1*
Respiratory exchange ratio	0.89 ± 0.01	0.90 ± 0.01
Energy from fat (%)	35 ± 5	35 ± 6
Energy from carbohydrate (%)	65 ± 5	65 ± 6

Values are mean ± SEM (n = 11). *significantly different from the temperate trial (P < 0.001).

5.3.2 Appetite perceptions

Baseline appetite ratings were not significantly different between the hot and temperate trials (P > 0.05). There was a main effect of time (P < 0.001) for each of the appetite perceptions assessed (hunger, satisfaction, fullness and PFC) (Figure 5.2) showing that appetite changed in response to the buffet meals. There were no trial or interaction main effects for hunger, satisfaction or fullness indicating that changes in each appetite perception were not significantly different over time between the two trials. There was no main effect of trial for PFC but there was a significant trial x time interaction (P < 0.05) indicating that the responses differed over time between the trials. Prospective food consumption tended to be lower over the first two hours of the hot trial compared with the

temperate trial but post-hoc analysis using the Bonferroni method did not indicate any differences between trials at individual time points.

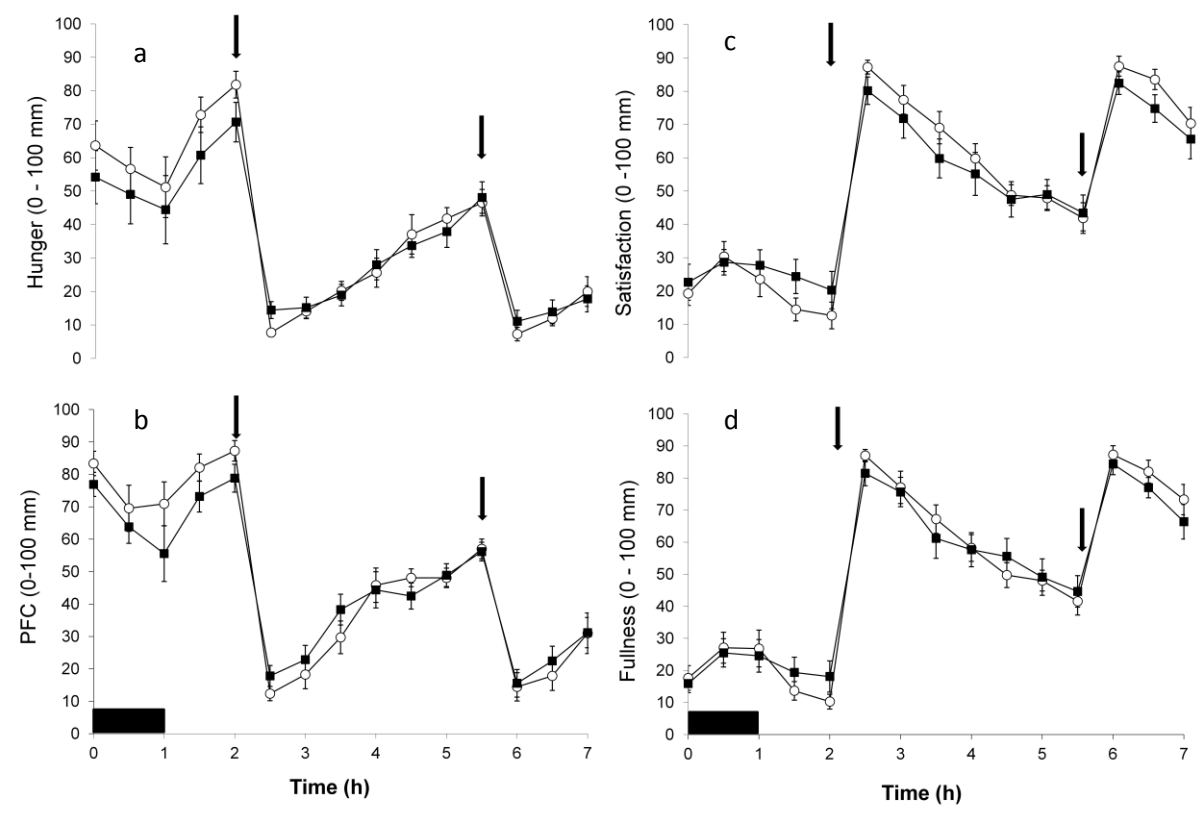

Figure 5.2 Subjective ratings of hunger (a), prospective food consumption (b), satisfaction (c) and fullness (d) during the temperate (○) and hot trials (■). Values are mean ± SEM, (n = 11). The black rectangle indicates the treadmill run and the solid black arrows indicate the buffet meals.

Differences in appetite perceptions between trials were also assessed using AUC values for the 2 h before the buffet meal and for the entire 7 h trial duration. For the first 2 h, AUC values for hunger were 15 % lower in the hot trial than the temperate trial (hot 108 ± 15 mm· 2 h, temperate 127 ± 10 mm· 2 h; $P < 0.05$). During this time, AUC values for PFC were suppressed by 12 % in the hot trial compared with the temperate trial (hot 135 ± 9 mm· 2 h, temperate 154 ± 10 mm· 2 h; $P < 0.005$). There were no other differences between trials for the times assessed.

5.3.3 Energy and macronutrient intake

Total energy intake from the buffet meals tended to be lower in the hot trial than the temperate trial ($P = 0.08$) (Figure 5.3). There was a main effect of time for energy intake ($P < 0.001$) with participants consuming an average of approximately 39 % more at the morning meal compared with the afternoon meal in both trials.

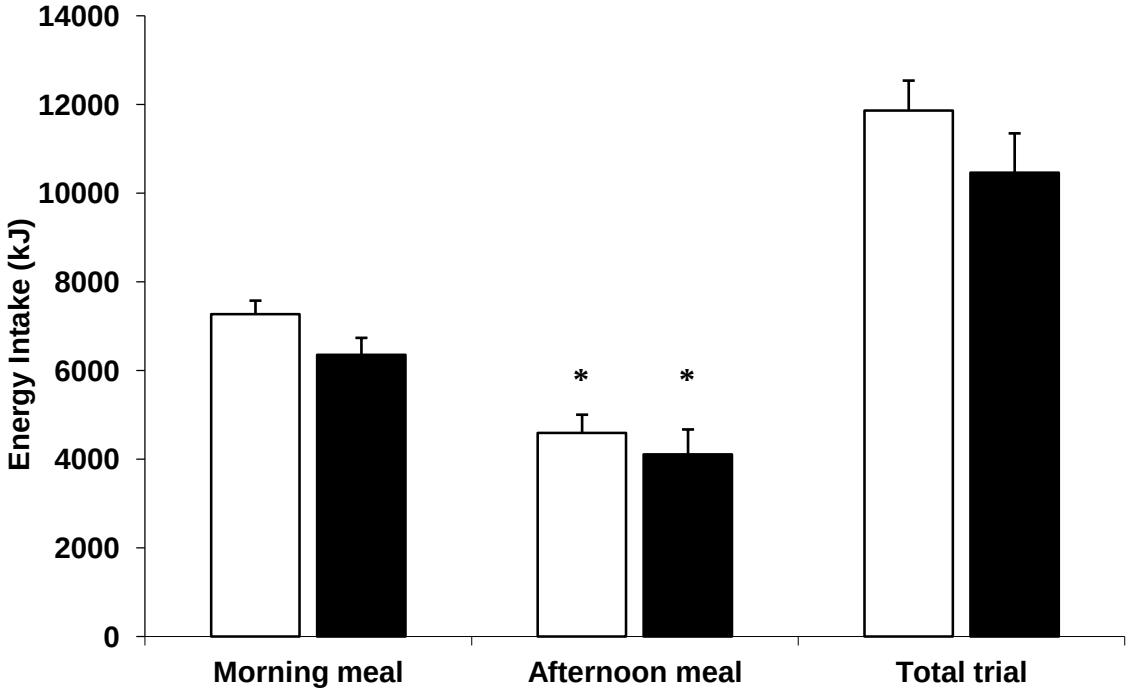

Figure 5.3 The effect of exercise in a temperate environment (□) and in a hot environment (■) on energy intake (kJ) at a morning and afternoon buffet meal, and for the total trial. Values are mean ± SEM ($n = 11$). *Significantly different from morning meal ($P < 0.001$).

Absolute carbohydrate intake tended to be greater in the temperate trial ($P = 0.059$), and was higher at the morning meal than the afternoon meal ($P < 0.001$; Table 5.3) and this was true for both trials. Intake of fat was similar between trials ($P = 0.238$) although again, intake was significantly higher at the morning meal than the afternoon meal ($P < 0.001$; Table 5.3). Protein intake was not significantly different between trials ($P = 0.101$) but intake was significantly greater at the morning meal compared with the afternoon meal ($P < 0.001$; Table 5.3).

Table 5.3 Effect of exercise in a temperate and hot environment on absolute macronutrient intake (g) and as a percentage of total energy intake (%).

		Carbohydrate	Fat	Protein
Temperate				
Morning	g	222 ± 12	66 ± 4	64 ± 5
	(%)	(51)	(34)	(15)
Afternoon	g	151 ± 13*	39 ± 5*	36 ± 5*
	(%)	(55)	(32)	(13)
Total Trial	g	373 ± 25	105 ± 9	100 ± 10
	(%)	(53)	(33)	(14)
Hot				
Morning	g	195 ± 11	58 ± 6	55 ± 5
	(%)	(52)	(34)	(14)
Afternoon	g	130 ± 19*	38 ± 6*	32 ± 6*
	(%)	(53)	(34)	(13)
Total Trial	g	325 ± 30	96 ± 12	87 ± 11
	(%)	(53)	(34)	(13)

Values are mean ± SEM (n = 11). * Significantly different from morning meal (P < 0.001).

Energy intake from liquid sources was not significantly different between trials (temperate 2475 ± 403 kJ and hot 2117 ± 439 kJ; P = 0.225). A significantly greater volume of plain water was consumed during the hot trial compared with the temperate trial (1864 ± 200 mL *vs* 924 ± 135 mL respectively; P = 0.001).

5.3.4 Plasma acylated ghrelin (n = 10)

Fasting plasma acylated ghrelin concentrations did not differ at baseline between trials (temperate 127 ± 14 pg·mL^{-1}, hot 125 ± 12 pg·mL^{-1}; P = 0.821). There was a main effect

of time ($P < 0.001$) and a trial x time interaction ($P < 0.05$) for plasma acylated ghrelin (Figure 5.4) indicating that the acylated ghrelin response differed between the temperate and hot trials over time. Post-hoc analysis using the Bonferroni method did not reveal differences between trials at any time points.

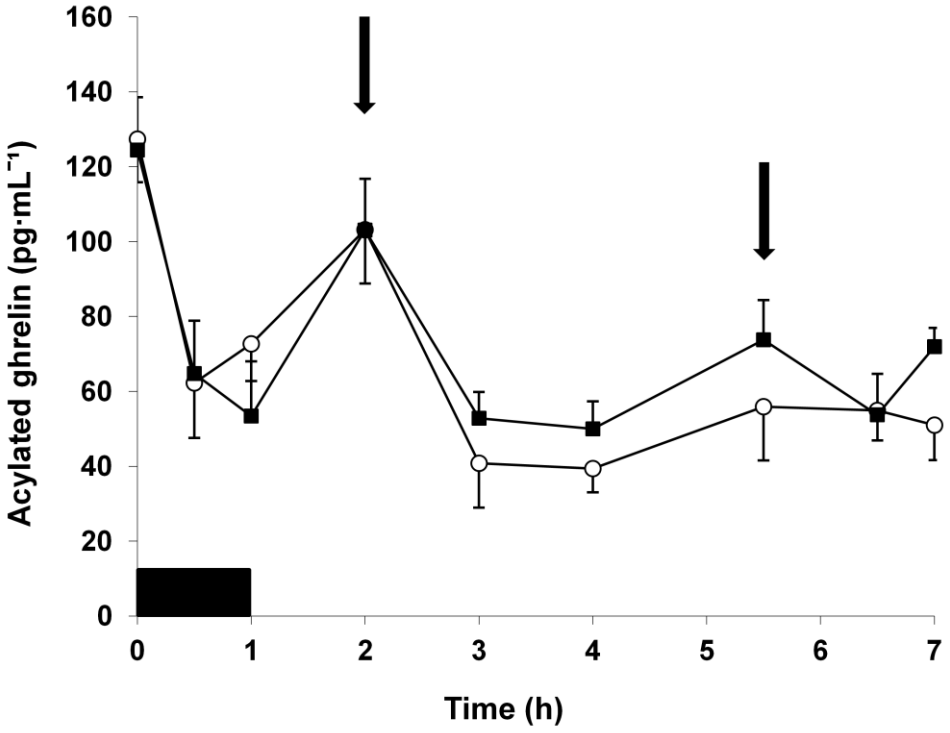

Figure 5.4 Plasma acylated ghrelin concentrations during the temperate (○) and hot (■) trials. Values are mean ± SEM, (n = 10). The black rectangle indicates the treadmill run and solid black arrows indicate the *ad libitum* buffet meals.

Area under the acylated ghrelin concentration versus time curve was not significantly different between trials for the total 7 hours (temperate 454 ± 73 pg·mL^{-1} ·7 h, hot 494 ± 74 pg·mL^{-1} ·7 h; P = 0.109). Similarly, there was no difference in AUC values for acylated ghrelin between trials for 0 – 2 h (temperate 169 ± 25, hot 155 ± 23 pg·mL-1· 2 h; P = 0.236).

5.3.5 Core temperature

Core temperature was similar at the start of the trials (temperate 36.9 ± 0.1 ºC *vs* hot 36.7 ± 0.1 ºC respectively; P = 0.270). There was a main effect of time ($P < 0.001$) and a trial

x time interaction for core temperature ($P < 0.001$). Post-hoc analysis revealed core temperature was higher upon completion of exercise in the hot trial compared with the temperate trial ($P < 0.001$; Figure 5.5).

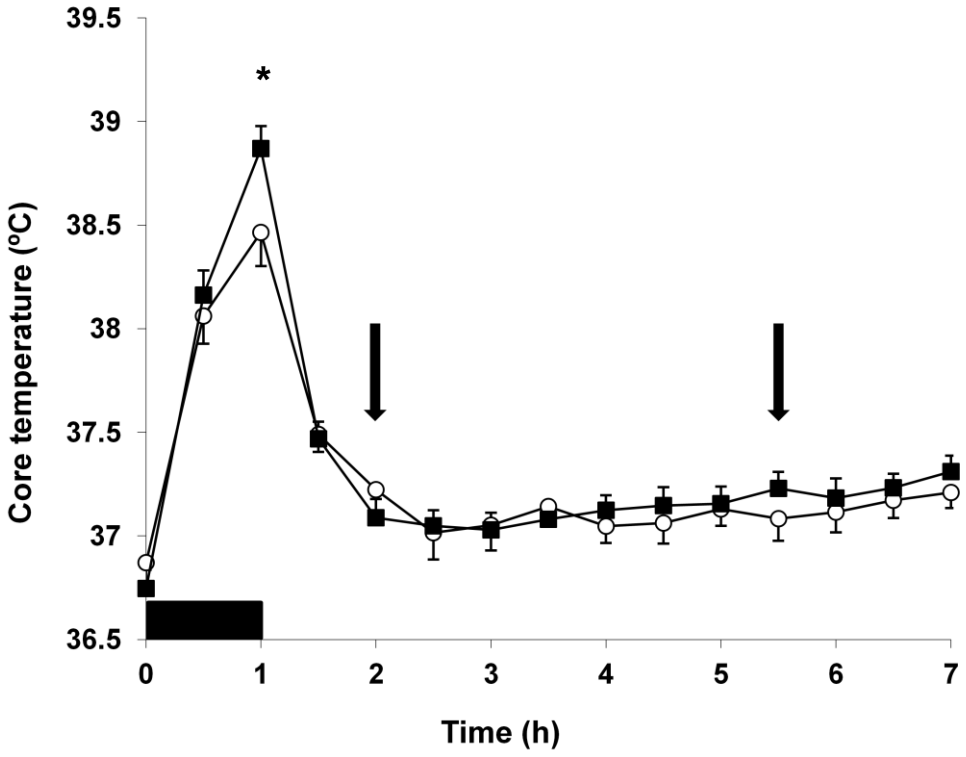

Figure 5.5 Core temperature in the temperate (○) and hot (■) trials. Values are mean ± SEM, (n = 11). The black rectangle indicates the treadmill run and solid black arrows indicate the buffet meals. *Significant difference between trials ($P < 0.001$).

5.3.6 Glucose and triacylglycerol (n = 10)

Fasting plasma glucose did not differ at baseline between trials (temperate 4.8 ± 0.2 mmol·L^{-1}, hot 4.5 ± 0.2 mmol·L^{-1}; $P = 0.111$). There was a main effect of time for glucose ($P < 0.05$) indicating elevated glucose concentrations during exercise. However, there were no trial ($P = 0.130$) or interaction main effects ($P = 0.089$). Fasting plasma TAG did not differ at baseline between trials (temperate 1.0 ± 0.1 mmol·L^{-1}, hot 1.0 ± 0.1 mmol·L^{-1}; $P = 0.723$). There were no main effects of trial, time or a trial x time interaction for plasma TAG concentrations.

5.3.7 Body mass and fluid consumption

Body mass was significantly lower after the treadmill runs in the temperate (pre versus post: 78.2 ± 2.3 kg versus 77.7 ± 2.3 kg; $P < 0.05$) and hot (78.6 ± 2.5 versus 78.3 ± 2.4 kg; $P < 0.05$) trials. Water consumption varied greatly between individuals during the treadmill runs (0 – 1375 mL) but was significantly greater during the run in the hot trial than the temperate trial (785 ± 80 mL *vs* 380 ± 71 mL respectively, $P = 0.001$).

5.3.8 Correlations between variables ($n = 10$)

Fasting plasma acylated ghrelin concentration at baseline was not significantly correlated with body mass, BMI, maximal oxygen uptake or percentage body fat. In both the temperate and hot trials there were no correlations between acylated ghrelin concentrations prior to a buffet meal and subsequent energy intake. In the hot trial, there was a significant positive correlation between the energy intake at the afternoon meal and the percentage change in acylated ghrelin ($r = 0.671$; $P < 0.05$). No other relationships were found between the energy intake at the meals and the percentage change in plasma acylated ghrelin. There were no correlations between acylated ghrelin and any of the appetite perceptions assessed in either trial.

5.4 Discussion

The purpose of this study was to examine the appetite, energy intake and acylated ghrelin responses to a bout of exercise undertaken in the heat compared with a temperate environment. The main findings are that firstly, hunger and prospective food consumption are lower in the preprandial period after exercising in the heat, secondly, energy intake tends to be lower after exercise in the heat and thirdly, acylated ghrelin concentrations respond differently over time between the hot and temperate trials.

Exercise in the heat creates its own nutritional needs (Burke, 2001) and until now, its effects on appetite were relatively unclear. The findings from the present study indicate that compared with exercise in a temperate environment, hunger and prospective food consumption are lower during, and for a short time after exercise in the heat. This is a novel finding, until now there have been only anecdotal reports that appetite is suppressed during exercise in hot weather. Exercising in the heat did not affect subjective ratings of appetite in the hours after exercise. Some research suggests that appetite may be stimulated in the recovery period after a bout of exercise (Malkova et al 2008), however, in the absence of a control trial it is not possible to know whether the same finding would occur in the present study. However, exposure to a high ambient temperature does not appear to affect subsequent ratings of appetite compared with a temperate environment.

Total energy intake tended to be lower in the hot trial with participants consuming on average 1400 kJ less from the buffet meals. This finding is in contrast with Shorten et al (2009) who show that 40 minutes of treadmill running in either 36 °C or 25 °C does not affect energy intake at a buffet meal provided 40 minutes after cessation of exercise. However, when accounting for the energy expenditure of the exercise bout, relative energy intake was lower after exercising in the heat compared with a resting control trial at 25 °C suggesting that participants did not compensate for the energy cost of exercise. A limitation of the study by Shorten et al (2009) was that energy intake was only assessed on one occasion soon after exercise. Alterations in energy intake at subsequent meals could compensate for the changes that occurred immediately after exercise. However, in the present study, approximately 60 % of the total energy intake from the two buffet meals was consumed at the first meal, and this was similar in both trials indicating that the trend for a lower energy intake during the hot trial compared with the temperate trial, persisted for the afternoon meal. This was despite appetite perceptions being no different between

trials after the first meal until the end of the trial. The temporary suppression of hunger observed during and shortly after exercising in the heat could be detrimental for an athlete competing in a hot country if they voluntarily consume less food at a subsequent meal as the present study suggests. This could lead to inadequate refuelling before ensuing events and could impair performance. Conversely, the findings may be of interest to those individuals wishing to lose weight via exercise and exercising in the heat could be advantageous in weight management programs. Hill et al (2003) report that a decrease of as little as 100 kcal per day, is sufficient to prevent weight gain. Providing that individuals do not overcompensate for the energy cost of exercise, a decrease in energy intake after exercise in the heat could be meaningful.

Carbohydrate requirements are increased whilst exercising in the heat because of a shift in substrate utilisation towards carbohydrate oxidation (Burke, 2001) and it has been suggested that macronutrient preference may be altered as a consequence of the need to replace specific energy stores used to fuel the exercise bout (King, 1998). However, in the present study carbohydrate oxidation rates and macronutrient preference were similar between trials. This confirms the findings of Shorten et al (2009) that macronutrient preference is unaffected by exercising in the heat.

The findings of the present study are in agreement with those of other studies that suggest the environmental temperature an exercise bout is performed in affects subsequent energy intake (Dressendorfer, 1993, White et al 2005). However, these studies focussed on exercise in a cold environment that was achieved by participants cycling on a modified cycle ergometer whilst immersed in cold water. A significant increase in food intake after exercise in cold water compared with the same exercise in warm water or on land was observed. Together with the findings from the present study, the available evidence suggests that energy intake is affected by the environmental temperature that a bout of exercise is performed in. The likely explanation for the difference in findings is that extremes of temperatures have opposing effects on appetite and energy intake for physiological purposes. For example, some evidence suggests that individuals from colder habitats have greater adiposity than those from warmer habitats (Katzmarzyk and Leonard 1998), although recently, changes in nutritional habits may have masked this observation. Of the prior studies directly investigating the effect of exercise in different environmental temperatures on appetite and energy intake, changes in core temperature

and gut hormone concentrations have been proposed to mediate the change in energy intake. A suppression of the usual exercise-induced rise in core temperature was associated with increases in energy intake in studies conducted in the cold (Dressendorfer, 1993, White et al 2005) and it was proposed that appetite after exercise was inversely related to core temperature. This notion is reinforced by Shorten et al (2009) who showed that compared with exercise in a moderate temperature, tympanic temperature was higher, and relative energy intake lower, after exercise in the heat. However, at least two of these studies measured core temperature using tympanic measurements. These do not provide a valid estimate of rectal temperature during laboratory exercise in the heat (Ganio et al 2009). Rectal temperature is valid and reliable for individuals at rest and exercise and as such is used as the reference standard (Ganio et al 2009). In the present study, rectal temperature at the end of exercise was higher in the hot trial than the temperate trial. Although energy intake tended to be lower at both meals after exercise in the heat, core temperature was no different prior to the meals suggesting that the proposed inverse relationship between core temperature and energy intake (Dressendorfer 1993, White et al 2005) may be more coincidental than causative. Furthermore, core temperature during exercise is no different if the same exercise is undertaken in hypoxia (Rowell et al 1982) and hypoxia is known to suppress appetite and energy intake (Westerterp-Plantenga et al 1999). Thus, the lower appetite and energy intake observed during the hot trial in the present study are likely mediated by factors other than core temperature.

The role of gut hormones in appetite regulation is well established. Recent research has sought to investigate how acute exercise affects circulating concentrations of these hormones. Although White et al (2005) proposed that hormonal factors may explain increased energy intake after exercise in cold water, they did not measure concentrations of any gut hormones. Only one study has sought to examine how gut hormones are affected by exercise in different environmental temperatures (Shorten et al 2009). Concentrations of the gut hormones, acylated ghrelin, pancreatic polypeptide and PYY were measured as well as concentrations of the adipokine leptin. Circulating concentrations of the anorectic hormone PYY were elevated prior to the meal in the hot trial compared with the control trial. The authors suggested increased PYY concentrations could explain the lower relative energy intake in the heat trial because no other changes in appetite-regulating hormones were observed. However, gut hormone concentration was measured using plasma from capillary blood rather than venous

samples. Whether the use of capillary samples to measure gut hormone concentration represents a valid method is unclear because excessive squeezing of the capillary site could increase the chance of lysis of red blood cells which could detrimentally affect measurement of the assay (Godfrey et al 2004). A study by Zeyl et al (2004) showed that concentrations of the appetite suppressing hormone leptin, were decreased in response to cold water immersion. Although no exercise was undertaken, and appetite perceptions were not measured, their findings suggest that leptin may be involved in the appetite response to exercise in cold water observed (Dressendorfer, 1993, White et al 2005). Given the unique role of acylated ghrelin in stimulating hunger and initiating meal times (Cummings et al 2001, Cummings et al 2004), it was thought that suppressed acylated ghrelin concentrations could explain the tendency for lower energy intake after exercise in the heat. However, no differences in acylated ghrelin concentrations were observed between trials. Our findings confirm those from previous studies that show acylated ghrelin is suppressed during moderate to high intensity aerobic exercise (Broom et al 2007, Broom et al 2009, King et al 2010a). The caloric load of a meal determines the extent of ghrelin suppression (Callahan et al 2004, le Roux et al 2005), however, the suppression of acylated ghrelin was only correlated with energy intake after the afternoon meal in the hot trial, and not at any other meal. The present findings suggest acylated ghrelin is not involved in the reduction in hunger and PFC during exercise in the heat and similarly, does not explain the tendency for the reduction in energy intake observed in the hot trial. Although the mechanisms are not known, a redistribution of blood flow away from splanchnic regions to working muscles could explain the suppression of acylated ghrelin concentrations (as measured from venous blood) observed during exercise. Given that exercising in the heat induces an additional physiological challenge, with blood also being distributed to the skin to aid in heat dissipation, it was thought that acylated ghrelin concentrations may be suppressed to a greater extent during exercise in the heat.

There are some strengths and limitations associated with this study. Energy intake was assessed over a longer period of observation compared with a single meal as in previous studies so it was possible to examine whether acute changes were compensated for at future meals. The longer period of observation enabled more blood samples to be obtained during trials to determine the effect of acylated ghrelin on appetite and energy intake. However, there are several limitations to this work. Firstly, there was no resting control trial, thus it is unclear whether energy intake after exercise was altered compared

with a control condition. Secondly, although acylated ghrelin is unique in that it is the only circulating appetite-stimulating gut hormone, there are many other gut hormones implicated in appetite regulation that are involved in satiety and may explain the differences in hunger and energy intake observed between trials. Additionally, a greater number of participants may have been needed to detect significant differences in the variables measured. Finally, participants in this study were young lean males, thus it is unclear whether the present findings can be generalised to other population groups such as the overweight/obese and females. Normal circulating concentrations of appetite-regulatory hormones are perturbed in the obese state (Tschöp et al 2001b, Batterham et al 2003) and Hagobian et al (2009) demonstrate clear gender differences in the way exercise affects appetite regulatory hormones and appetite perceptions.

In conclusion, this study demonstrates that an acute bout of exercise performed in the heat lowers preprandial hunger and prospective food consumption prior to the first meal after exercise. Energy intake tends to be lower after exercise in the heat, a finding which persists past the immediate post-exercise meal. It is unlikely that acylated ghrelin mediates this response because concentrations of acylated ghrelin were similar between trials.

CHAPTER VI

The influence of running in the cold on appetite, energy intake and plasma concentrations of acylated ghrelin

6.1 Introduction

Exercise is an important component of many weight management programs because in the absence of compensatory increases in appetite and energy intake it will favour negative energy balance. However, Franz et al (2007) suggest there is only minimal weight loss with most exercise-alone weight loss interventions. This modest weight loss could be related to compensatory increases in caloric intake (Hopkins et al 2010). Although most research suggests that appetite is transiently decreased during exercise, the findings regarding post-exercise energy intake are less clear. Most evidence suggests energy intake is unchanged after exercise, however some studies have demonstrated an increase in energy intake after exercise (Verger et al 1992, Verger et al 1994, Martins et al 2007). The favourable impact that exercise could have on energy balance would be negated if post-exercise energy intake increased above the energy expenditure of exercise. The limited available research suggests exercising in either hot or cold ambient temperatures has contrasting effects on appetite and food intake. Thus it is important to consider the environmental conditions exercise is performed in when assessing the effectiveness of exercise in the maintenance of energy balance.

Findings within the literature (Shorten et al 2009), and from the previous study presented in this thesis, suggest that appetite and energy intake are lower after exercising in the heat. Conversely, in cold weather environments, food intakes are reportedly increased compared with temperate environments. However, much of this evidence is anecdotal and based mainly upon either self-reported food intake records (which may be subject to bias), or alterations in body weight (Westerterp-Plantenga, 1999). Novel work by Johnson and Kark (1947) noted that voluntary daily energy intakes of troops in the Arctic was higher compared with those in the desert, implying that environmental temperature may be a determinant of energy intake. Similarly, Milan and Rodahl (1961) reported high intakes for scientists and sailors residing in the Antarctic for 12 months. All individuals gained weight over that period suggesting under those conditions the high intakes were actually surplus to caloric requirement. Whether the high energy intakes were due to physiological or psychological factors or a combination of both is unclear. High food intakes in subjects

on an Antarctic expedition have also been reported by Easty (1967). Seasonal variations in intakes were apparent and surprisingly, there was a reduction during winter months. However, this was likely due to subjects being largely confined within their living quarters where the ambient temperature could reach 20 °C and physical activity levels were low. Brobeck (1948) suggests that when environmental temperatures are low, food intake should be high to generate metabolic heat that can be used in defence against hypothermia. This is particularly true in animals where increases in energy intake compensate for increased energy expenditure from increased thermogenesis (Louis-Sylvestre, 1987) whereas in humans, increased energy needs do not consistently stimulate an immediate increase in appetite and food intake as characterised by exercise-induced anorexia (King et al 1994).

For many years after these initial reports, very little research was undertaken investigating the effect of cold temperatures on appetite and energy intake. A study by Dressendorfer (1993) demonstrated a 64 % increase in energy intake after exercise performed in cool water (22 °C) compared with the same exercise undertaken in warm water (34 °C). More recently, similar findings have also been reported by White et al (2005) who demonstrated that energy intake was increased in response to exercising in cold water. Compared with the same duration and intensity of exercise in water of 33 °C, cycling in cold water (20 °C) resulted in a 44 % increase in caloric intake at a meal provided 20 minutes after exercise. These recent studies suggest that energy intake is stimulated in response to a cold environment and the authors postulated that core temperature may mediate this increase in energy intake. In the study by Dressendorfer (1993), there was a suppression of the usual exercise-induced increase in core temperature when participants exercised in cold water and this was related to an increase in energy intake. Similarly, White et al (2005) observed that core temperature was lower during exercise in cold water, after which energy intake was increased. However, whether a lower core temperature stimulated energy intake is unclear because the actual difference in core temperature was only 0.3 °C which may have been an insufficient physiological stimulus for increased energy intake (White et al 2005). Westerterp-Plantenga et al (2002) suggest that overeating in a cooler ambient temperature attenuates a decrease in core temperature, rather than a reduction in core temperature stimulating an individual to consume more energy.

Whether it is the cold environment *per se* that stimulates energy intake in the studies by Dressendorfer (1993) and White et al (2005) is questionable. Recently, Halse et al (2011) investigated the acute effect of post-exercise cold water immersion on energy intake. They observed a significant increase in energy intake after 20 minutes of cold water immersion (15 ºC) compared with no immersion. However in this instance, it is unlikely that water temperature is the overriding factor in increasing energy intake because immersion in a neutral temperate (33 ºC) also triggered an increase in energy intake. The authors suggested that immersion itself was a sufficient stimulus for increasing energy intake. The contradictory findings amongst the aforementioned studies are likely due to different methodologies, such as the duration of immersion and whether or not exercise was undertaken during immersion. The mechanisms behind the increase in energy intake observed in response to cold exposure are unclear, but it has been proposed that the release of hormones responsible for appetite stimulation may explain the increase; however no studies have investigated this possibility.

Appetite and food intake are regulated by numerous hormones secreted from the gastrointestinal tract and adipose tissue which affect hunger and satiety as well as promoting energy conservation or increasing energy expenditure. Leptin, secreted predominantly from adipose tissue, regulates body fat stores by simultaneous effects on both appetite and energy expenditure (Havel, 2000). Zeyl et al (2004) demonstrated that leptin concentrations are suppressed in response to acute cold water immersion, and although appetite and energy intake were not assessed in that study, decreased leptin concentrations would serve to reduce satiety. Of note, leptin is not sensitive to acute perturbations in energy balance, with no changes in leptin concentrations observed in response to feeding or exercise (Jéquier, 2002, Pop et al 2010) accordingly it seems unlikely that leptin is involved in the increase in food intake observed shortly after exercise in the cold (Dressendorfer, 1993, White et al 2005). The effect of exercise in cold ambient temperatures on concentrations of other appetite regulatory peptides has not yet been examined. Acylated ghrelin is the only known circulating gut hormone that stimulates appetite and food intake and acylated ghrelin concentrations are transiently suppressed during high intensity exercise (Broom et al 2007, Broom et al 2009, King et al 2010a). Findings from the previous study within this thesis support these findings and extend them by suggesting that exercising in the heat does not differentially affect the acylated ghrelin response to exercise. Only one prior study has examined the effect of a

low ambient temperature on ghrelin concentrations. Total plasma ghrelin concentrations were significantly higher after exposure to acute cold (Tomasik et al 2005) and the authors concluded that ghrelin is one of the factors that modulate appetite in response to alterations in ambient temperature. Although this study is suggestive of a role for ghrelin in stimulating appetite after exercise in the cold, appetite and energy intake were not assessed. Furthermore, concentrations of total ghrelin were measured, and it is the acyl-modified peptide that is necessary for ghrelin to bind to its receptor and stimulate appetite.

To date, the applicability of the studies that have examined the effect of exercise in the cold on energy intake is limited because of the nature of the exercise undertaken which has involved participants cycling on a modified ergometer under water. It is likely that more people are exposed to cold temperatures in this country whilst undertaking sports such as running or walking outside than those exercising in cold water. Running is accessible to most individuals and is often a sport of choice when gross energy expenditure is important because it has a greater energy cost for the same distance compared with walking (Hall et al 2004). Additionally, the effect of exercise in the cold on concentrations of appetite regulatory hormones has not yet been examined. Therefore the purpose of this study was to investigate the effects of running in a cold environment on appetite, energy intake and concentrations of plasma acylated ghrelin.

6.2 Methods

6.2.1 Participants

Ten healthy physically active males aged 18 to 28 y volunteered to participate in this study. Participants were recruited from the student populations at Loughborough and Nottingham Trent Universities. Some of their physical characteristics are described in Table 6.1.

Table 6.1 Physical characteristics of study participants

Characteristic	Mean ± SEM
Age (y)	22.9 ± 0.8
Height (m)	1.79 ± 0.01
Body mass (kg)	73.9 ± 2.1
BMI ($kg \cdot m^{-2}$)	23.1 ± 0.5
Body fat (%)	15.7 ± 1.3
Running $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	57.9 ± 2.3

Values are mean ± SEM (n = 10)

6.2.2 Experimental protocol

Each participant attended the laboratory on three occasions. At the first visit, participants were reminded verbally and in writing about the study protocol and gave their written informed consent to participate. Participants completed a health screen questionnaire and anthropometric data were collected. Participants then completed two treadmill tests; the first was a sub-maximal incremental test to determine the relationship between running speed and oxygen consumption, the second was a maximum oxygen uptake ($\dot{V}O_2$ max) test. Participants then completed two main trials in a randomised counter-balanced order separated by at least one week. Main trials were 7 h in duration and were as follows: running in a temperate environment (20.5 ± 0.3˚C; 54 ± 1% RH) and running in a cool environment (10.7 ± 0.1˚C; 60 ± 2% RH). Main trials were carried out in the environmental chamber at Nottingham Trent University (Design Environmental WIR52-20HS, Design Environmental, Gwent, U.K).

6.2.3 Main trials

Participants weighed and recorded their food intake for 24 h prior to the first main trial and replicated the timing and quantity of this before the second trial. During this time, participants refrained from vigorous physical activity and alcohol consumption. On the morning of the main trials participants arrived at the laboratory after a 10 h overnight fast. Each participant completed the two, 7 h trials individually. Upon arrival at the lab, participants body mass was recorded, and after a period of sitting an expired air sample was taken for measurements of resting energy metabolism. Participants then lay in a supine position prior to a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) being inserted into an antecubital vein. Prior to entry into the environmental chamber, a rectal probe (Grant Instruments Ltd, Cambridge, England) was self-inserted and a heart rate monitor (Polar Electro Oy, Kempele, Finland) attached (Design Environmental Ltd, Gwent, Wales, U.K). Upon entry into the chamber, participants rested for approximately 10 minutes, during which time resting values were obtained for heart rate, rectal temperature, thermal sensations and appetite perceptions. For the first hour, participants ran on a level treadmill for 60 minutes at 65 % of $\dot{V}O_2$ max. Expired air samples were collected for 60 seconds every 15 minutes during exercise and running speed was adjusted if necessary to keep oxygen consumption as close to the value for 65 % of $\dot{V}O_2$ max as possible. After completion of the treadmill run, participants rested in the chamber for the remainder of the 7 h trial and consumed buffet meals at 2 and 5.5 h. Individuals wore the same clothing during the treadmill run in both trials but were free to wear whatever clothing they felt comfortable in for the remainder of the day. They were asked to wear sufficient clothing to ensure they did not shiver and spare clothing was available to participants if required. Figure 6.1 shows a schematic representation of the main trial protocol.

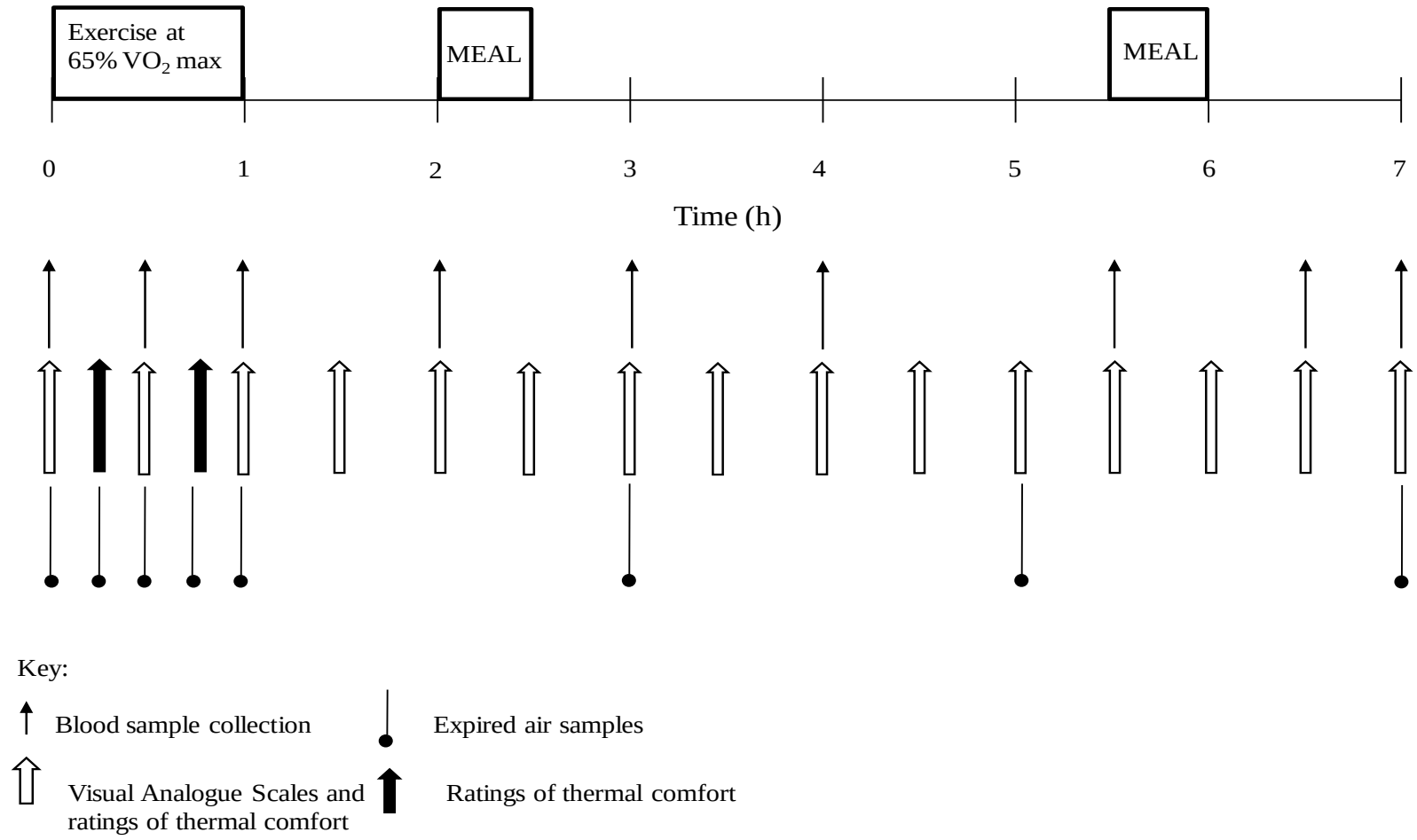

Figure 6.1 Schematic representation of main trial protocol

6.2.4 Blood sample collection

Venous blood samples were collected at baseline (0 h) and 0.5, 1, 2, 3, 4, 5.5, 6.5 and 7 h into pre-cooled 5 mL EDTA blood collecting tubes for the determination of plasma glucose and TAG concentrations. These samples were centrifuged at 3500 rpm for 10 minutes in a refrigerated centrifuge (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc, U.S.A). The plasma was dispensed into separate Eppendorf tubes which were stored overnight at -20 °C before being transferred to -80 °C until analysis. Additional venous blood samples were collected at these times into 5 mL EDTA blood collecting tubes for the determination of plasma acylated ghrelin concentrations. The collection and processing of these blood samples is described in section 3.14.2. Blood samples were collected with the participant in a supine position with the exception of the 0.5 h sample which was obtained whilst the participant stood stationary on the treadmill. To estimate plasma volume changes, prior to centrifugation triplicate blood samples were collected into 20 µL heparinised microhaematocrit tubes and duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration.

6.2.5 Appetite perceptions

Ratings of subjective feelings of hunger, fullness, satisfaction and prospective food consumption (PFC) were reported on 100 mm VAS (Flint et al 2000) at baseline and every 30 minutes thereafter.

6.2.6 Thermal sensations and rectal temperature

Thermal sensations were rated at baseline, every 15 minutes during exercise and every 30 minutes after exercise using a 9 point rating scale (Young et al 1987) described in section 3.13. Rectal temperature was recorded at baseline, at five minute intervals during exercise, and every 30 minutes thereafter (see section 3.13 for details of core temperature measurement).

6.2.7 Assessment of energy intake

Participants were given 30 minutes access to a buffet meal at 2 h and 5.5 h. Participants consumed their food whilst seated at a table in the environmental chamber and they completed each trial on their own so that their food intake and choices were not influenced by social factors. Participants were instructed to eat until they were comfortably satisfied. The food presented was the same as provided to participants in the previous study and a list

of items available is shown in Appendix E. All items were provided in excess of expected consumption but participants could request more of any of the items provided if desired. To enable quantification of energy intake, all foods were covertly weighed prior to being given to the participants in the chamber and foods that were not consumed, as well as any waste, were removed from the environmental chamber after 30 minutes and were reweighed to the nearest gram out of sight of the participants. Energy and macronutrient intake were calculated using information from the food labels and in the case of non-packaged items such as fruit, values were obtained from the USDA National Nutrient Database. Participants were unaware that their food intake was being monitored but were fully briefed upon completion of the study. Participants were free to consume water *ad libitum* throughout the trials with the volume consumed recorded.

6.2.8 Environmental temperature and humidity

Temperature and humidity inside the environmental chamber were pre-set using climate control programming software (Contour Programming and Logging Software Package, Design Environmental Ltd, Gwent, Wales). The desired temperature and humidity within the chamber were confirmed using a handheld hygrometer (model RH85, Omega, Manchester, U.K.) every 30 minutes and the values recorded.

6.2.9 Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Bretonneux, France). Plasma samples were analysed for glucose and TAG concentrations via enzymatic, colorimetric methods using reagents from ABX diagnostics (Montpellier, France) with the use of a Pentra 400 automated analyser (Horiba ABX Diagnostics, France). To eliminate inter-assay variation, all samples from the same participant were analysed in the same run. The within-batch coefficients of variation were as follows: acylated ghrelin 7.4 %, glucose 0.5 % and TAG 1.8 %.

6.2.10 Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Area under the curve (AUC) values were calculated for hunger, acylated ghrelin, glucose and TAG using the trapezoidal rule. Differences in fasting and AUC values for appetite perceptions, acylated ghrelin, core temperature and thermal sensations were determined using Student's

t-tests. Two-factor repeated measures ANOVA was used to examine differences between trials for appetite perceptions, energy and macronutrient intake, acylated ghrelin, core temperature and thermal sensations. Where significant interactions occurred, paired t-tests with Bonferroni adjustment for multiple comparisons were used to determine differences at individual time points. The Pearson product moment correlation coefficient was used to examine relationships between variables. When correcting values for changes in plasma volume, the statistical findings were unaltered so for simplicity unadjusted values are reported. Statistical significance was accepted at the 5 % level. Results are presented as mean ± SEM.

6.3 Results

6.3.1 Responses to exercise

Table 6.2 shows some of the physiological responses to treadmill running in the temperate and cold environments.

Table 6.2 Physiological responses to exercise in a temperate and cold environment

	Temperate	**Cold**
Exercise Intensity (% $\dot{V}O_2$max)	64 ± 2	66 ± 2
Energy expenditure (kJ)	3357 ± 98	3431 ± 70
Energy expenditure (kcal)	802 ± 23	820 ± 16
Heart rate (bpm)	155 ± 6	156 ± 7
Thermal sensations	5.2 ± 0.2	4.2 ± 0.3*
Respiratory exchange ratio	0.90 ± 0.01	0.88 ± 0.01
Energy from fat (%)	33 ± 4	40 ± 3
Energy from carbohydrate (%)	67 ± 4	60 ± 3

Values are mean ± SEM (n = 10). *Significantly different from the temperate trial (P < 0.005).

6.3.2 Appetite perceptions

Baseline ratings of hunger, satisfaction and fullness were not significantly different between trials (P > 0.05) but ratings of PFC were significantly elevated at baseline in the cold trial compared with the temperate trial (cold 73 ± 3 mm, temperate 66 ± 3 mm; P < 0.05). There was a main effect of time (P < 0.001) for each of the appetite perceptions assessed (hunger, satisfaction, fullness and PFC) (Figure 6.2) indicating that appetite changed in response to the buffet meals. There were no trial or interaction main effects for hunger, fullness or PFC indicating that these responses did not differ over time between the trials. For satisfaction, there was no main effect of trial but there was a significant trial x time interaction (P < 0.001) showing that satisfaction tended to be higher over the first 2

hours of the temperate trial than the cold trial, but post-hoc analysis using the Bonferroni method did not indicate differences between trials at any time.

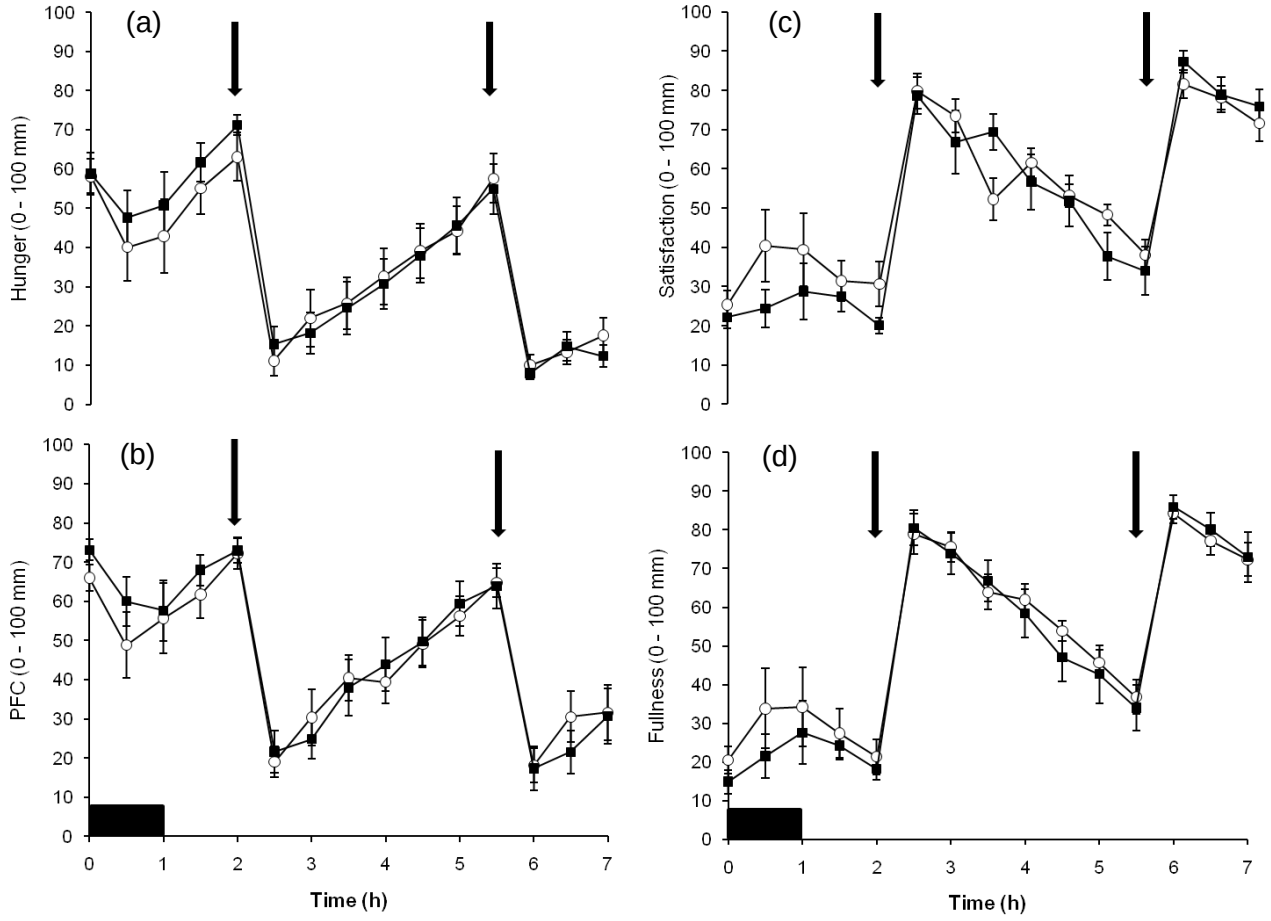

Figure 6.2 Subjective ratings of hunger (a), prospective food consumption (b), satisfaction (c) and fullness (d) during the temperate (○) and cold (■) trials. Values are mean ± SEM (n = 10). The black rectangle indicates the treadmill run and the solid black arrows indicate the buffet meals.

Between trial differences in appetite perceptions were also assessed using AUC values for the 2 h before the buffet meal, and for the total 7 h trial duration. There were no differences in AUC values for any of the appetite perceptions assessed for the whole 7 h trial duration. For the first 2 h, AUC values for satisfaction were significantly lower ($P < 0.05$) in the cold trial (51 ± 7 mm·2 h) than the temperate trial (70 ± 13 mm·2 h) and AUC values for fullness for the same period, also tended to be lower in the cold trial compared with the temperate trial (cold 50 ± 8 mm·2 h; temperate 58 ± 14 mm·2 h; $P = 0.07$).

6.3.3 Energy and macronutrient intake

Energy intake from the buffet meals was higher in the cold trial compared with the temperate trial although this did not quite reach significance (P = 0.08) (Figure 6.3). Energy intake was greater by an average of approximately 1453 kJ (348 kcal) in the cold trial compared with the temperate trial. There was no effect of time or a trial x time interaction indicating that food intake was similar between the morning and afternoon meals in both trials.

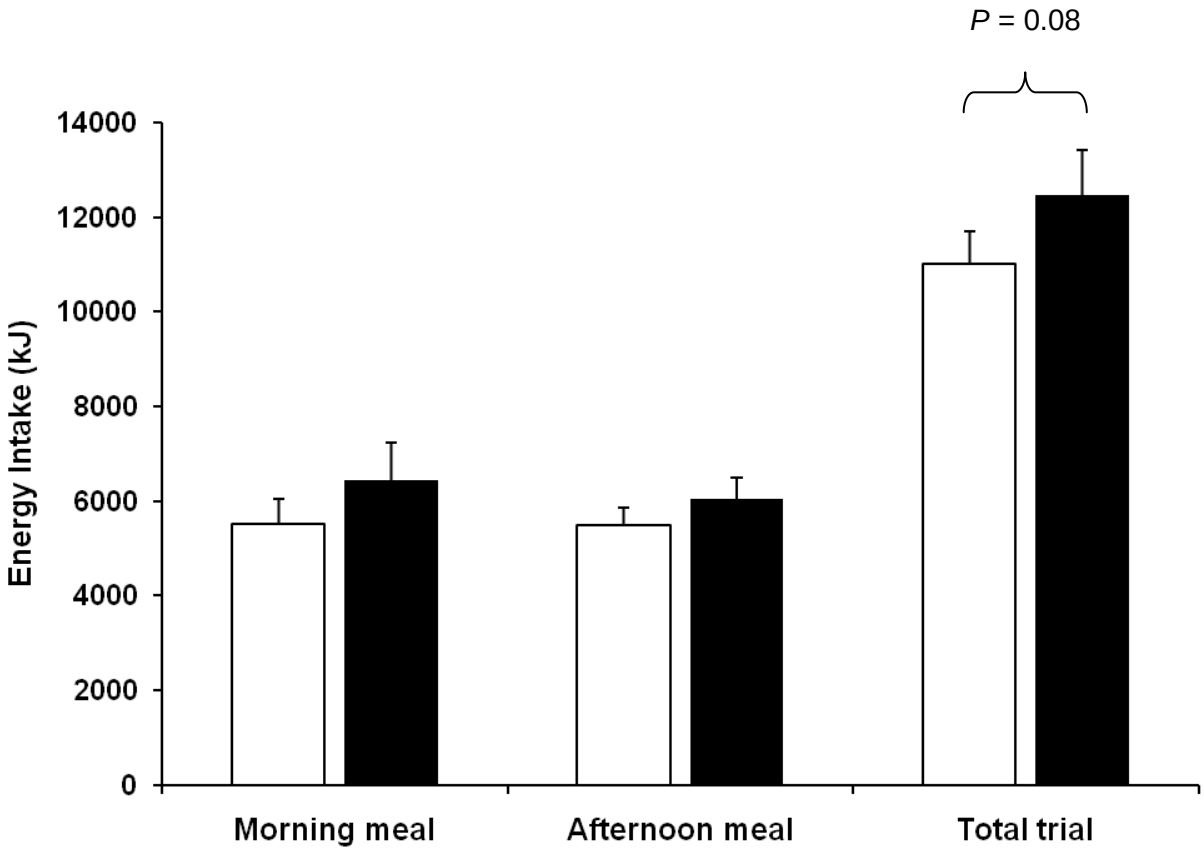

Figure 6.3 The effect of exercise in a temperate (□) and cold (■) environment on energy intake (kJ) at a morning and afternoon buffet meal, and for the total trial. Values are means ± SEM (n = 10).

Two-factor ANOVA was used to examine the absolute and percentage macronutrient intake from the buffet meals during the trials (Table 6.3). Absolute intake of fat (g) was higher in

the cold trial than the temperate trial (cold 113 ± 9 g and temperate 90 ± 8 g; $P < 0.05$). Similarly, the percentage of the energy derived from fat was significantly higher in the cold trial than the temperate trial (cold 33 ± 2 %, temperate 31 ± 2 %, $P < 0.05$). There were no differences in absolute carbohydrate or protein intake between trials. The percentage of the energy derived from carbohydrate was not significantly different between trials but was higher at the morning meals than the afternoon meals ($P = 0.052$). There was no significant main effect of trial, time or a trial x time interaction for the percentage of energy derived from protein.

Table 6.3 Effect of exercise in a temperate and cold environment on absolute macronutrient intake (g) and as a percentage of total energy intake (%).

		Carbohydrate	Fat	Protein
Temperate				
Morning	g	206 ± 19	41 ± 5	36 ± 4
	(%)	(63)	(28)	(11)
Afternoon	g	179 ± 16	49 ± 4	42 ± 4
	(%)	(54)	(34)	(13)
Total Trial	g	384 ± 29	90 ± 8	78 ± 6
	(%)	(58)	(31)	(12)
Cold				
Morning	g	214 ± 26	57 ± 9	43 ± 6
	(%)	(57)	(32)	(11)
Afternoon	g	197 ± 15	56 ± 6	47 ± 6
	(%)	(55)	(34)	(13)
Total Trial	g	412 ± 34	113 ± 9*	90 ± 9
	(%)	(55)	(34)*	(12)

Values are mean ± SEM ($n = 10$). * Significantly greater than temperate trial ($P < 0.05$)

6.3.4 Plasma acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ at baseline between trials (temperate 87 ± 8 pg·mL^{-1}, cold 83 ± 7 pg·mL^{-1}; $P = 0.672$). There was a main effect of time ($P < 0.001$) indicating that acylated ghrelin concentrations were suppressed during exercise and in response to feeding. There was also a trial x time interaction ($P = 0.05$) for

acylated ghrelin concentrations (Figure 6.4). It appears concentrations were higher during exercise, and lower between meals, in the cold trial than during the temperate trial but post-hoc analysis using the Bonferroni method did not reveal differences between trials at any point. Area under the curve values for acylated ghrelin were not significantly different between trials for the total 7 h trial duration (temperate 337 ± 24 pg·mL^{-1}·7 h, cold 298 ± 29 pg·mL^{-1}·7 h; P = 0.197) or in the first pre-prandial period (0 – 2 h) (temperate 120 ± 11 pg·mL^{-1}·2 h, cold 133 ± 18 pg·mL^{-1}·2 h; P = 0.365). Similarly, there was no significant difference between trials when AUC values were assessed for the exercise period (0 – 1 h; P = 0.390).

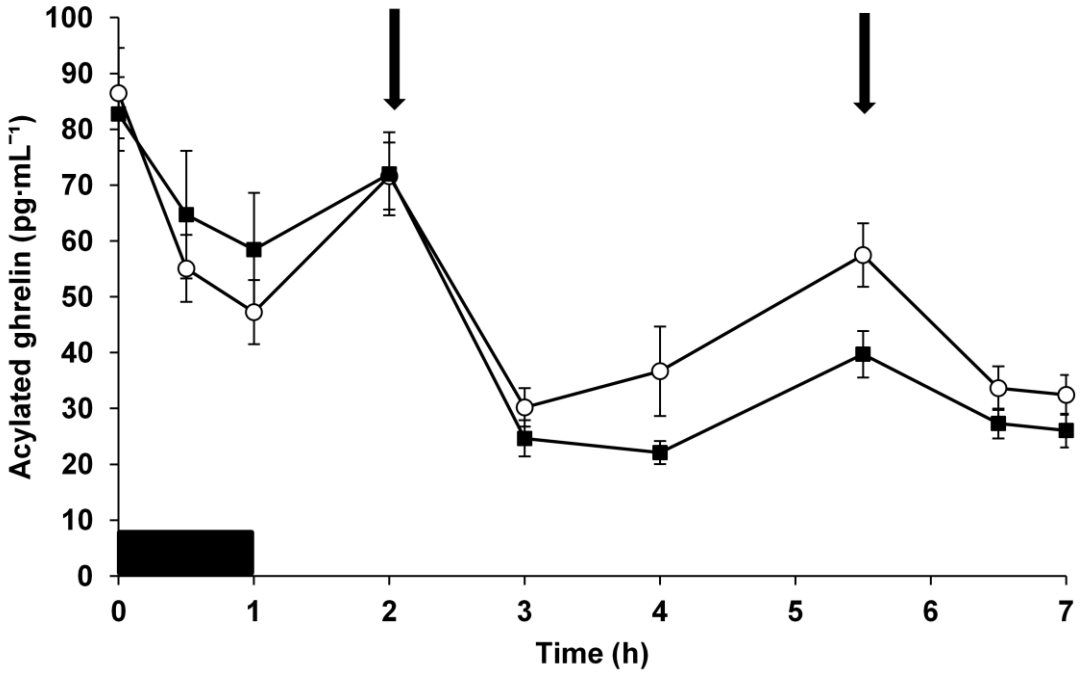

Figure 6.4 Plasma acylated ghrelin concentrations during the temperate (○) and cold (■) trials. Values are mean ± SEM, (n = 10). The black rectangle indicates the treadmill run and solid black arrows indicate the buffet meals.

6.3.5 Core temperature

Rectal temperature was not significantly different at baseline between the trials (temperate 36.7 ± 0.1 °C, cold 36.5 ± 0.2 °C; P = 0.322). There was a main effect of time (P < 0.001) indicating elevated rectal temperature during and on completion of exercise (Figure 6.5) but there was no significant main effect of trial nor was there a trial x time interaction.

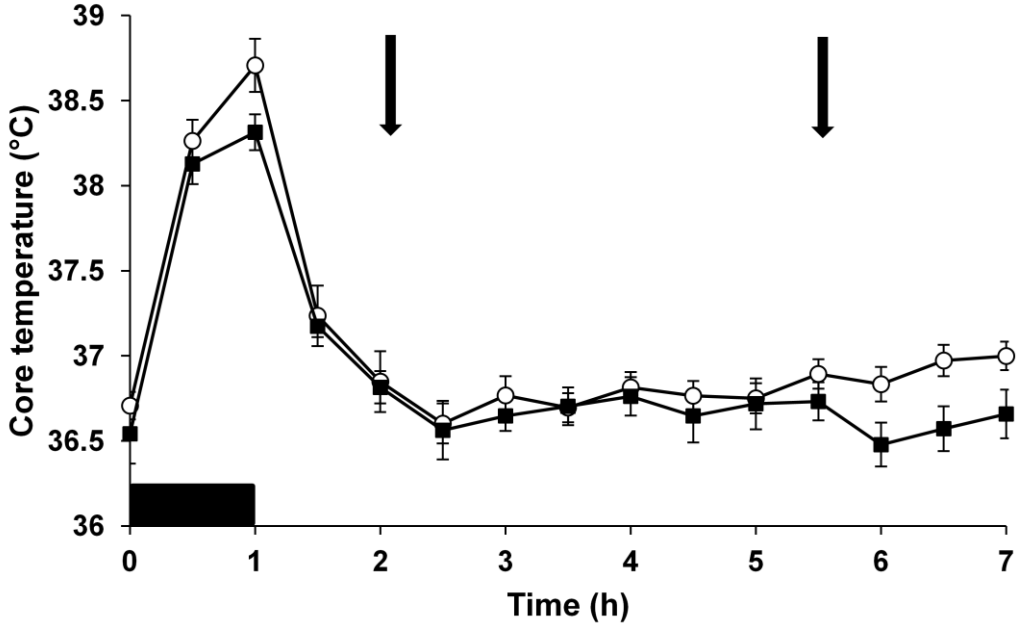

Figure 6.5 Core temperature in the temperate (○) and cold (■) trials. Values are mean ± SEM, (n = 10). The black rectangle indicates the treadmill run and solid black arrows indicate the buffet meals.

6.3.6 Glucose and triacylglycerol

Fasting plasma glucose was similar at baseline between trials (temperate 4.8 ± 0.1 mmol·L^{-1} and cold 4.8 ± 0.1 mmol·L^{-1}; P = 0.431). There was a main effect of time (P < 0.005) indicating elevated glucose concentrations during exercise compared with baseline. There was no effect of trial (P = 0.715) nor a trial x time interaction (P = 0.520). Fasting plasma TAG did not differ at baseline between trials (temperate 0.9 ± 0.1 mmol·L^{-1} and cold 1.0 ± 0.1 mmol·L^{-1}; P = 0.331). There was a main effect of time (P < 0.001) indicating that compared with baseline TAG concentrations were elevated during exercise and after consumption of the buffet meals. There was no effect of trial (P = 0.172) and there was no trial x time interaction (P = 0.359).

6.3.7 Correlations between variables

Baseline plasma acylated ghrelin concentrations were not significantly correlated with body mass, BMI, percentage body fat or maximal oxygen uptake. Plasma acylated ghrelin concentrations prior to the buffet meals were not correlated with subsequent energy intake at either meal in both trials. There was no correlation between the energy intake at a meal

and the percentage change in acylated ghrelin concentrations. There were no correlations between hunger prior to a meal and subsequent energy intake. In the cold trial, acylated ghrelin was negatively correlated with satisfaction (r = -0.746) and fullness (r = -0.703) at 4 h ($P < 0.05$). There were no other correlations at any other times between acylated ghrelin and any of the appetite perceptions assessed. Acylated ghrelin was positively correlated with TAG at 6.5 h in the cold trial (r = 0.723; $P < 0.05$). There were no other correlations between acylated ghrelin and TAG or glucose at any other times during the trials.

6.4 Discussion

This study investigated the effects of running in a cold environment on appetite, energy intake and concentrations of the orexigenic gut hormone acylated ghrelin. The main findings are that energy intake tends to be higher after running in the cold, perceived ratings of satisfaction and fullness are lower in the pre-prandial period after running in the cold and finally, although plasma acylated ghrelin concentrations are not different between trials, concentrations appear to be higher whilst running in the cold and lower between meals than in the temperate trial. Thus, exercise followed by a period of rest in a cold environment tends to increase energy intake which may be due to individuals feeling less full and satisfied and although might be related to higher acylated ghrelin concentrations during exercise prior to the first meal, is likely not due to differences in acylated ghrelin concentrations prior to the second meal.

The tendency for increased energy intake in the present study is in accordance with evidence that demonstrates an increase in energy intake after exercise in cold water (Dressendorfer, 1993, White et al 2005). However, the present study differs from previous studies in that exercise was undertaken in cold air, rather than in cold water. Whether exercising in cold air would affect energy intake was unknown because most evidence suggesting energy intake is increased in cold environments is anecdotal. Furthermore, a recent study by Halse et al (2011) showed increases in energy intake after both cold and neutral water immersion compared with a control condition, suggesting that water immersion itself may mediate the increase in energy intake observed in the former studies, and not the temperature of the water. Furthermore, exercising in water has been shown to differentially affect some hormonal responses (Wiesner et al 2010) although no hormones responsible for appetite control were measured. Given that energy intake is mainly regulated by hormones secreted from the gastrointestinal tract, it is possible that there could be differences in the energy intake response to exercise in cold air and exercise in cold water. However, findings from the present study not only indicate exercising in cold air may stimulate energy intake but also suggest this increase in energy intake persists when individuals remain resting in the cold environment, because energy intake in the cold tended to be elevated to a similar extent at both buffet meals compared with in the temperate environment. It has been suggested that ambient temperatures of approximately 11 °C, similar to that used within the present study, can be advantageous to performance when undertaking prolonged exercise of moderate intensity (Nimmo, 2004). Therefore, the

present findings are an important consideration for athletes competing in a cold environment, because energy consumption above their requirements may have a detrimental effect on their post-exercise nutritional strategy. Furthermore, if an increased energy intake persisted in the long term, it could have important implications for weight management. However, the increase in energy consumption may be a consequence of the physiological need to increase metabolic rate to remain warm, in which instance, energy intake may not be excess to requirement.

In addition to examining the effect of exercise in the cold on energy intake, the effect on macronutrient selection was also investigated. Total intake of fat, and percentage of calories consumed from fat were higher in the cold trial. This is in keeping with the study by White et al (2005) who observed an increase in calories from fat and absolute fat intake, but no changes in carbohydrate or protein intake. It has been suggested that macronutrient preference may be altered as a consequence of the physiological need to consume foods that replace the specific energy stores used to fuel the exercise bout (King, 1998). In the present study, although not statistically significant, there was a tendency for a greater proportion of fat to be used to fuel the bout of exercise during the cold trial. Based on the assumption by King (1998) this could explain why a greater proportion of energy was obtained from fat during the cold trial. The tendency for increased reliance on fat as an energy source during the cold trial may be surprising because carbohydrate is identified as the preferred substrate oxidised in individuals exposed to the cold in the absence of any exercise (Weller et al 1997). However, exercise in the cold has been shown to increase fat oxidation (Timmons et al 1985), reduce fat oxidation (Layden et al 2002) and have no effect on substrate oxidation (Sink et al 1989). The differences in findings likely reflect the absolute temperatures used in the studies, the intensity of exercise, and how much clothing was worn during exercise. The findings of Layden et al (2002) confirm other research demonstrating no differences in fuel utilisation when comparisons are made between ambient temperatures of 0 °C to 20 °C. Therefore, given the ambient temperature used in the cold trial, the mechanism behind the tendency for increased energy from fat in the present study is unclear. Findings from the previous study presented within this thesis, and from the study by Shorten et al (2009), suggest that in response to exercise in the heat, macronutrient preference is unchanged thus it appears that high and low ambient temperature may differentially affect macronutrient preference.

The mechanisms behind the tendency for increased energy intake after exercise in the cold are unclear. Changes in core temperature have been proposed to explain alterations in energy intake in response to different ambient temperatures (Johnson and Kark 1947, Brobeck, 1948, Dressendorfer, 1993, White et al 2005). Indeed, both Dressendorfer (1993) and White et al (2005) showed that an attenuation of an exercise-induced rise in core temperature during exercise in cold water was associated with increases in post-exercise energy intake suggesting that appetite after exercise may be inversely related to core temperature. However, it has been proposed that a reduction in core temperature *per se* does not explain the increased energy intake (Westerterp-Plantenga et al 2002). An increased energy intake compared with energy requirements was observed at 16 °C and this attenuated the decrease in core temperature that was observed when individuals were kept in energy balance at the same ambient temperature. Since there was no difference in energy intake between 16 °C and 22 °C when participants could eat *ad libitum*, it is unlikely that core temperature *per se* explains the increased energy intake (Westerterp-Plantenga et al 2002). The authors suggest that in practice, maintenance of core temperature can be preserved in the cold by overeating as well as by increasing physical activity or insulation. In the present study, participants were allowed to wear whatever clothing they wished so that they remained in thermal comfort and core temperature was no different between trials. Thus, the combination of wearing clothing that they felt comfortable in and the tendency for increased energy intake may have jointly contributed to the maintenance of core temperature in the cold trial. Despite this, ratings of thermal comfort were significantly lower during the cold trial compared with the temperate trial, with participants feeling "cool" during the cold trial and "comfortable" during the temperate trial. It has been shown that thermal perceptions are important inputs in the self-selection of exercise intensity (Schlader et al 2011), hence thermal status may also be involved in other behavioural responses such as feeding.

Together with current evidence and from the results presented thus far within this thesis, the present study confirms that acylated ghrelin concentrations are suppressed in response to a bout of moderate to high intensity exercise. Furthermore, the present findings do not indicate a role for acylated ghrelin in the increase in energy intake observed after exercise in cold environments. Plasma acylated ghrelin concentrations tended to be elevated between meals in the temperate trial. Ghrelin concentrations are suppressed in proportion to the caloric load of a meal thus the higher acylated ghrelin concentrations between meals

in the temperate trial may be due to the lower energy intake at the first meal. The present findings are not in line with those of Tomasik et al (2005) who observed a significant increase in ghrelin after short-term exposure to 2 °C and suggested increased ghrelin may be responsible for increased appetite in response to cold environmental temperatures. However, the authors did not measure subjective ratings of either appetite or energy intake, so it is unclear whether the changes they observed in total ghrelin affected appetite. Additionally, they only measured concentrations of total ghrelin, which is comprised of both acylated and des-acyl ghrelin. Changes in total ghrelin may not accurately reflect changes in acylated and des-acyl ghrelin, thus it is important to quantify concentrations of acylated ghrelin because acylated ghrelin is involved in the stimulation of appetite. Collectively, findings from this study and from the previous study presented within this thesis indicate that it is unlikely that acylated ghrelin is involved in the differential response in energy intake to extremes of environmental temperature.

Although acylated ghrelin is unique in being the only known circulating gut hormone that stimulates appetite, there are numerous other hormones secreted from the gut, as well as other regions such as adipose tissue, that are involved in satiety and reducing energy intake. Shorten et al (2009) observed increases in the anorectic gut hormone, PYY, after exercise in the heat and they postulated that the reduction in relative energy intake after exercise in the heat was mediated by increased PYY concentrations. Whether alterations in PYY concentrations, or the concentrations of any other anorectic gut peptides, are involved in the increase in energy intake in response to a cold environment is unknown, thus further research is warranted. Concentrations of leptin, an adipokine involved in satiety, are decreased in individuals 48 h after arriving in Antarctica (Vats et al 2005). The authors of this study suggested that this could mediate the increase in appetite and body weight observed. However, after a 1 month stay in this environment, leptin levels were elevated above those observed at baseline and at 48 h. Zeyl et al (2004) also demonstrated a decrease in leptin concentrations in response to acute cold-water immersion which could explain the evidence in the literature showing an increase in energy intake after exercise in cold water (Dressendorfer 1993, White et al 2005). However, these authors also showed that with repeated cold exposure, plasma leptin concentrations actually increase. Collectively, the findings from these studies suggest that although leptin may mediate a short-term increase in food intake, other mechanisms are likely involved in the longer term. Whether the findings of Vats et al (2005) represent true physiological effects to the cold is

unclear, because leptin concentrations can also be affected at high altitude (Shukla et al 2005) and elevation at Antarctica can reach almost 5000 m (Source: CIA World Factbook). Thus their findings should be interpreted with some caution. Vats et al (2005) also observed an increase in plasma concentrations of orexigenic neuropeptide Y (NPY) after 48 h in Antarctica. Neuropeptide Y is produced and secreted from neurons within the hypothalamus in response to alterations in concentrations of appetite regulatory hormones including acylated ghrelin, thus the change in plasma NPY may be a result of changes in concentrations of other gut hormones. Conversely, research by Bing et al (1998) found no significant alterations in hypothalamic NPY concentrations in cold-exposed rats, suggesting that NPY neurons are not activated during cold exposure. The authors suggest that other neuronal pathways may mediate the increase in food intake observed in response to cold exposure. Acylated ghrelin exerts its feeding activity by stimulating NPY/AgRP neurons in the hypothalamus, hence findings from the present study showing that acylated ghrelin concentrations are unchanged during cold exposure, and from the findings of Bing et al (1998), may indicate that other appetite-regulatory hormones that influence different neurons within the hypothalamus are involved in the appetite response to cold. Given that many hormones from the gut and adipose tissue are involved in the regulation of energy balance via their stimulatory or inhibitory effects on neurons within the hypothalamus, it is unlikely that changes in just one hormone mediate the alterations in energy intake observed in response to different ambient temperatures. The present study suggests that although acylated ghrelin might be involved in the lower ratings of satisfaction and fullness observed during exercise in the cold and the higher energy intake observed at the first post-exercise meal, alterations in acylated ghrelin concentrations do not explain the tendency for increased energy intake observed at the second meal during the cold trial.

Due to the differing methodologies and results within the literature, some clarity was needed regarding the effect of exercise in the cold on appetite and energy intake. This study sought to examine these effects; however, more research is required because it was not possible to cover every aspect within this study. Firstly, the absolute temperature used in the cold trial may not have been low enough to induce physiological effects related to extreme cold. In future, the effect of more extreme environmental temperatures on appetite and energy intake should be examined. Furthermore, the effect of exercise in the cold on other appetite-regulatory peptides such as PYY and leptin should be examined to determine possible mechanisms behind the elevated energy intake observed after exercise in the cold.

The present study benefited from the longer period of follow-up than in other studies to enable the impact of exercise in the cold on energy intake after the immediate post-exercise meal to be examined. However, this warranted individuals being able to remain comfortable over the 7 h duration. Thus participants could wear adequate clothing to prevent shivering. In future, the effect of exercise in lower ambient air temperatures on the immediate post-exercise meal should be examined because in the literature, this has only been investigated after exercise in cold water.

In conclusion, the present study demonstrates a tendency for increased energy intake in response to exercise in a cold environment. Perceived ratings of fullness and satisfaction were lower in the first preprandial period in the cold trial during which time acylated ghrelin concentrations appeared to be higher than in the temperate trial indicating a possible link between elevated acylated ghrelin concentrations and increased appetite and energy intake. There were no differences in ratings of appetite thereafter and acylated ghrelin concentrations appeared to be elevated in the temperate trial prior to the second meal, at which time energy intake was higher in the cold trial, thus in this instance data are not supportive of acylated ghrelin being the sole mediator in the elevated energy intake in the cold trial.

CHAPTER VII

The effect of rest and exercise at a simulated altitude of 4000 m on appetite, energy intake and plasma concentrations of acylated ghrelin and peptide YY

7.1 Introduction

Lowlanders who are acutely exposed to high altitude (> 2500m) (Barry and Pollard, 2003), often suffer from a loss of appetite termed 'high altitude anorexia'. A loss of body mass, dependent on the duration of exposure and altitude reached (Kayser, 1994), is evident after several days or more and appears to be an inevitable consequence of hypobaric hypoxia (Boyer and Blume 1984, Guilland and Klepping 1985, Rose et al 1988, Westerterp et al 1992). Energy balance is most likely disturbed at altitude by an increase in daily energy expenditure through physical exertion, which is not matched by a comparable increase in energy intake due to a reduced desire to eat and/or lack of availability of food (Westerterp-Plantenga et al 1999). Although appetite loss has long been recognised as a side effect of high altitude (Pugh, 1962), there is limited empirical evidence, but recently this has been confirmed in both laboratory (Westerterp-Plantenga et al 1999) and field studies (Kalson et al 2010). Most studies attribute the observed decrease in energy intake to a loss of appetite associated with acute mountain sickness (AMS), symptoms of which include headache and anorexia. Symptoms of AMS usually manifest within 6 – 12 hours of arrival at a new high altitude and will resolve without medical intervention over several days providing individuals do not ascend further (Barry and Pollard 2003). However, loss of appetite cannot be merely a by-product of AMS because anorexia and weight loss still persist when symptoms of AMS have subsided (Tschöp and Morrison 2001c).

Energy intakes are reported to decrease by between 17 % and 57 % at altitudes ranging from 3600m to 6000m (Surks et al 1966, Janoski et al 1969, Consolazio et al 1972, Guilland and Klepping 1985, Rose et al 1988, Major and Doucet 2004, Vats et al 2007). It is inconclusive whether food preferences change at altitude. An increased preference for carbohydrate has been reported (Pugh, 1962, Boyer and Blume 1984, Rose et al 1988, Westerterp-Plantenga et al 1999). However, this could simply reflect greater availability of certain foods at altitude, or the relative ease of food preparation, because other studies have shown no change in diet composition (Westerterp et al 1992, Westerterp et al 1994). A lack of palatable foods available at altitude may explain the reduced energy intake often reported. However, studies conducted in hypobaric chambers where other environmental

stressors that could affect appetite can be eliminated (ie: extreme cold and physical exertion) and a variety of plentiful foods are available, demonstrate that individuals lose body mass because of a reduced energy intake in spite of the availability of palatable foods (Rose et al 1988, Westerterp-Plantenga et al 1999). Thus it appears hypoxia *per se* causes the reduced appetite and energy intake and although findings are inconclusive, this decrement in energy intake could be related to changes in endocrine factors responsible for the control of appetite.

Recently, much research has investigated the role that appetite-regulating hormones play in the acute and chronic regulation of energy homeostasis. Concentrations of these hormones may be affected by environmental factors such as temperature to alter appetite and energy intake (Shorten et al 2009). However, the role that episodic appetite regulating hormones play in high altitude anorexia is not well established. Levels of total ghrelin, an appetite stimulating hormone, decrease at high altitude in the short term ($\leq$ 2 days) (Shukla et al 2005) but are unchanged in the long term (7 days to 7 weeks) (Shukla et al 2005, Benso et al 2007). No studies have examined the effect of altitude upon acylated ghrelin concentrations, the posttranslationally modified form of this peptide essential for its appetite-stimulatory effects (Broglio et al 2004). The effect of hypoxia on leptin, an adipokine involved in the long term regulation of body weight and energy intake, is equally contentious. Studies show increased (Tschöp et al 1998, Grosfeld et al 2001, Shukla et al 2005, Snyder et al 2008), decreased (Vats et al 2007, Zaccaria et al 2004) and unchanged (Barnholt et al 2005, Benso et al 2007) leptin levels at high altitude. The contradictory findings likely arise from differing methodologies, such as the mode of transportation (foot or vehicle) and the presence of confounding factors such as physical exertion and cold, thus further investigation is warranted. No studies have investigated the effect of altitude on the anorectic gut hormone PYY. This study sought to confirm the exact role, if any, that these appetite-regulating hormones play in the pathogenesis of high altitude anorexia.

If the mechanisms behind high altitude anorexia are understood, and the corresponding effects on body mass subsequently minimised, then this could have a profound impact on not just the safety of climbers, mountaineers and other individuals who ascend to high altitude, but also on their exercise capacity and performance. In the medical field, knowledge of the effects of hypoxia on various physiological variables including appetite regulatory hormones could assist in the treatment of sick people where chronic hypoxia is a

primary problem (Hamad and Travis 2006, Khosravi and Grocott 2009). However, a reduction in energy intake and a loss of body weight at altitude may not always be an undesirable outcome. A recent study has observed a reduction in body weight in overweight/obese individuals exposed to hypoxia with weight loss being maintained in the follow up period (Lippl et al 2010). This further highlights the importance of understanding the mechanisms responsible for the reduction in appetite and food intake in hypoxia.

To isolate the effects of normobaric hypoxia from other confounding factors present in real altitude situations this study was undertaken in an environmental chamber where temperature, humidity and physical activity levels could be controlled. Participants were exposed to normobaric hypoxia designed to mimic an altitude of 4000 m. Both a resting control trial and an exercise trial were undertaken in normoxia and hypoxia to determine whether exercise in hypoxia would exacerbate any effects of hypoxia on appetite, energy intake and the appetite regulating hormones. Exercise was undertaken at the same relative exercise intensity in normoxia and hypoxia. Compared with normoxia, it was expected that hypoxia would suppress appetite and that energy intake at an *ad libitum* buffet meal would be decreased. We hypothesised that this would be mediated by an alteration in concentrations of acylated ghrelin and PYY.

7.2 Methods

7.2.1 Participants

Ten healthy young males aged between 19 and 28 y gave their written informed consent to participate in this study. The participants were all recreationally active individuals recruited from the student and staff populations at Nottingham Trent University and Loughborough University. Participants were non-smokers, normotensive, not taking any medication, free from food allergies and residents at an elevation between 50 – 150 m above sea level. Furthermore, a prerequisite was that participants must have had no history of travel to altitude 3 months prior to commencing the study. Table 7.1 shows the physical characteristics of the participants.

Table 7.1 Physical characteristics of participants

Characteristic	Mean ± SEM
Age (y)	23.8 ± 0.8
Height (m)	1.75 ± 0.02
Body mass (kg)	76.3 ± 3.0
BMI ($kg \cdot m^{-2}$)	24.7 ± 0.7
Normoxic $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	56.9 ± 2.1
Hypoxic $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	37.5 ± 1.4

Values are mean ± SEM (n = 10)

7.2.2 Experimental protocol

Prior to the main trials, participants were required to attend two preliminary sessions. During the first, anthropometric measurements were made and participants were familiarised with the laboratory equipment and treadmill protocols. Participants completed questionnaires regarding their current and past health status, habitual physical activity levels, dietary habits and food preferences. Participants then completed a sub-maximal incremental treadmill running test and a maximum oxygen uptake treadmill running test on a motorised treadmill (Woodway ELG 55, Weil am Rhein, Germany). The second preliminary trial was undertaken in an environmental chamber (Design Environmental, Gwent, U.K) where participants were exposed to normobaric hypoxia with a gas mixture containing 12.7 % O_2 (simulating an altitude of approximately 4000 m) and completed the

same sub-maximal and maximal treadmill running tests as before. The four main trials were carried out in a randomised four way crossover design with each trial separated by at least 7 days. All trials were completed in an environmental chamber (Design Environmental, Gwent, U.K). The trials were as follows: a resting control trial in normoxia, an exercise trial in normoxia, a resting control trial in hypoxia, and an exercise trial in hypoxia. Participants were exposed to normobaric hypoxia as in the preliminary trial. Oxygen levels within the chamber were closely monitored throughout trials with a gas analyser and adjusted if necessary. Exercise trials commenced with a 60 minute treadmill run followed by 6 h of rest and control trials involved 7 h of rest. Relative humidity was set at 50 % and temperature at 23 ˚C with the exception of the 60 minute exercise period where for participant comfort temperature was set at 18 ˚C and increased on cessation of exercise.

7.2.3 Main trials

Participants weighed and recorded their food intake for 24 h prior to the first main trial; they then replicated the quantity and timing of this before each subsequent trial. During this time, participants refrained from vigorous physical activity and alcohol consumption. Participants arrived at the lab after a 10 h overnight fast and were weighed wearing light clothing and no footwear. After resting for five minutes, a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein to enable collection of venous blood samples during the trials. Upon entry to the chamber on exercise trials, participants ran for 60 minutes at a speed predicted to elicit 70 % of altitude-specific $\dot{V}O_2$ max. Expired air samples were collected for 60 seconds every 15 minutes during exercise and running speed was adjusted if necessary to keep oxygen consumption as close to the value for 70 % of $\dot{V}O_2$ max as possible. Values for energy expenditure were estimated from oxygen consumption and carbon dioxide production using indirect calorimetry (19). During exercise, heart rate was measured by short range telemetry (Polar Electro FS1, Kempele, Finland), oxygen saturation was monitored via fingertip pulse oximetry (Nonin 3100 Series, Nonin Medical Inc, Plymouth, USA) and RPE were recorded. After the treadmill run, participants rested (working, reading or watching DVDs) in the chamber for the remaining 6 h. During the normoxic and hypoxic control trials the protocol was identical to that of the exercise trials, except participants rested (working, reading or watching DVDs) in the chamber for the entire 7 h trial duration. Expired air samples were

collected during the first 60 minutes of the control trials to enable estimation of resting metabolic rate.

7.2.4 Standardised and *ad libitum* buffet meals

A standardised test meal was provided for participants at 2 h and participants were instructed to consume the meal within 15 minutes. The meal consisted of white bread, ham, a chocolate bar, a banana and plain salted crisps. The macronutrient content of the meal was as follows: 1.62 g carbohydrate, 0.3 g fat, 0.21 g protein and 42 kJ per kg body mass. This provided 65 % of the calories as carbohydrate, 27 % as fat and 8 % as protein. The meal was prescribed according to each participant's body weight on the first trial and the quantity replicated for subsequent trials. A cold buffet meal was provided at 5.5 h. Participants had 30 minutes access to this where they could eat *ad libitum* and they were instructed to eat until satisfied. Food was presented in excess of expected consumption but extra food could be requested if desired. Foods were covertly weighed before and after being given to participants to enable calculation of energy intake. Energy and macronutrient intake were estimated using manufacturer values. Participants were unaware that their energy intake was being monitored but were briefed upon completion of all the trials.

7.2.5 Appetite perceptions and symptoms of AMS

Subjective feelings of hunger, fullness, satisfaction and PFC were reported on 100 mm visual analogue scales (Flint et al 2000) at baseline and every 30 minutes thereafter. Heart rate and oxygen saturation were also recorded at these times. Symptoms of AMS were assessed every hour from baseline using the Lake Louise Consensus AMS scoring system (Roach et al 1993). However, question 5 of this questionnaire was omitted because it was not relevant in this setting as it concerns the difficulty an individual had in sleeping the night before whilst at altitude (Appendix G). This shortened version of the questionnaire correlates highly with other AMS scoring systems as well as a clinical AMS assessment (Savourey et al 1995). Total scores of ≥ 3 which include a headache are suggestive of AMS.

7.2.6 Blood sample collection

During the main trials venous blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5.5, 6.5 and 7 h. All samples were collected in the chamber with the exception of the baseline (0 h)

sample which was obtained immediately prior to the participant entering the chamber. Samples were collected into 5 mL pre-cooled EDTA tubes (Sarstedt, Leicester, UK) for the determination of plasma glucose, TAG and PYY concentrations. These samples were centrifuged at 3500 rpm for 10 minutes in a refrigerated centrifuge (Fisher Scientific accuSpin 1R, Thermo Fisher Scientific Inc). After this the plasma was dispensed into separate Eppendorf tubes which were stored overnight at -20 ºC before being transferred to -80 ºC until analysis. Samples were collected into 5 mL pre-cooled serum tubes (Sarstedt, Leicester, UK) for the determination of serum leptin. Samples were left to clot for 30 minutes prior to centrifugation, and were dispensed and stored as described above. Additionally, samples were collected into 5 mL pre-cooled EDTA tubes for determination of plasma acylated ghrelin concentrations. The collection and processing of these samples is described in section 3.14.2. Blood samples were collected with the participant in a supine position with the exception of the 0.5 h sample during the exercise trials which was taken with the participant standing up on the treadmill. Figure 7.1 shows a schematic representation of the main trial protocol.

7.2.7 Blood biochemistry

An ELISA was used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Watford, U.K) and serum leptin (Quantikine, R&D Systems, MN, USA). For serum leptin, samples from two participants for all trials could be analysed on one assay. After the first assay, it was evident that many of the samples fell below the dynamic range of the assay. Thus for subsequent assays, dilution of the samples was adjusted from a 100 fold dilution to a 50 fold dilution. However despite this, complete sets of data were only obtained for 3 participants with several samples during trials for the remaining participants falling below the dynamic range with a minimum detectable dose of leptin being < 7.8 $pg \cdot mL^{-1}$. Plasma samples were analysed for glucose and TAG concentrations via enzymatic, colorimetric methods using reagents from ABX Diagnostics (Montpellier, France) with the use of a Pentra 400 (Horiba ABX Diagnostics, France). To eliminate inter-assay variation, all samples from the same participant were analysed in the same run. The within batch coefficients of variation were as follows: acylated ghrelin 5.1 %, PYY 4.8 %, glucose 0.6 % and TAG 2.7 %.

7.2.8 Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software (v17.0 for Windows, SPSS, Chicago, IL). Three-factor repeated measures ANOVA was used to examine differences between trials for the appetite perceptions, acylated ghrelin, glucose, TAG, PYY and leptin with the factors being altitude, exercise and time. Area under the curve (AUC) values were calculated for acylated ghrelin, hunger, glucose, TAG and PYY using the trapezoidal rule. Repeated measures ANOVA was used to assess differences in AUC values between trials, as well as differences between fasting measures of hunger, acylated ghrelin, TAG, glucose and PYY, and differences in energy and macronutrient intake. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5 % level. When plasma volume changes were adjusted for, statistical findings were not altered hence unadjusted values are reported. Results are reported as means ± SEM.

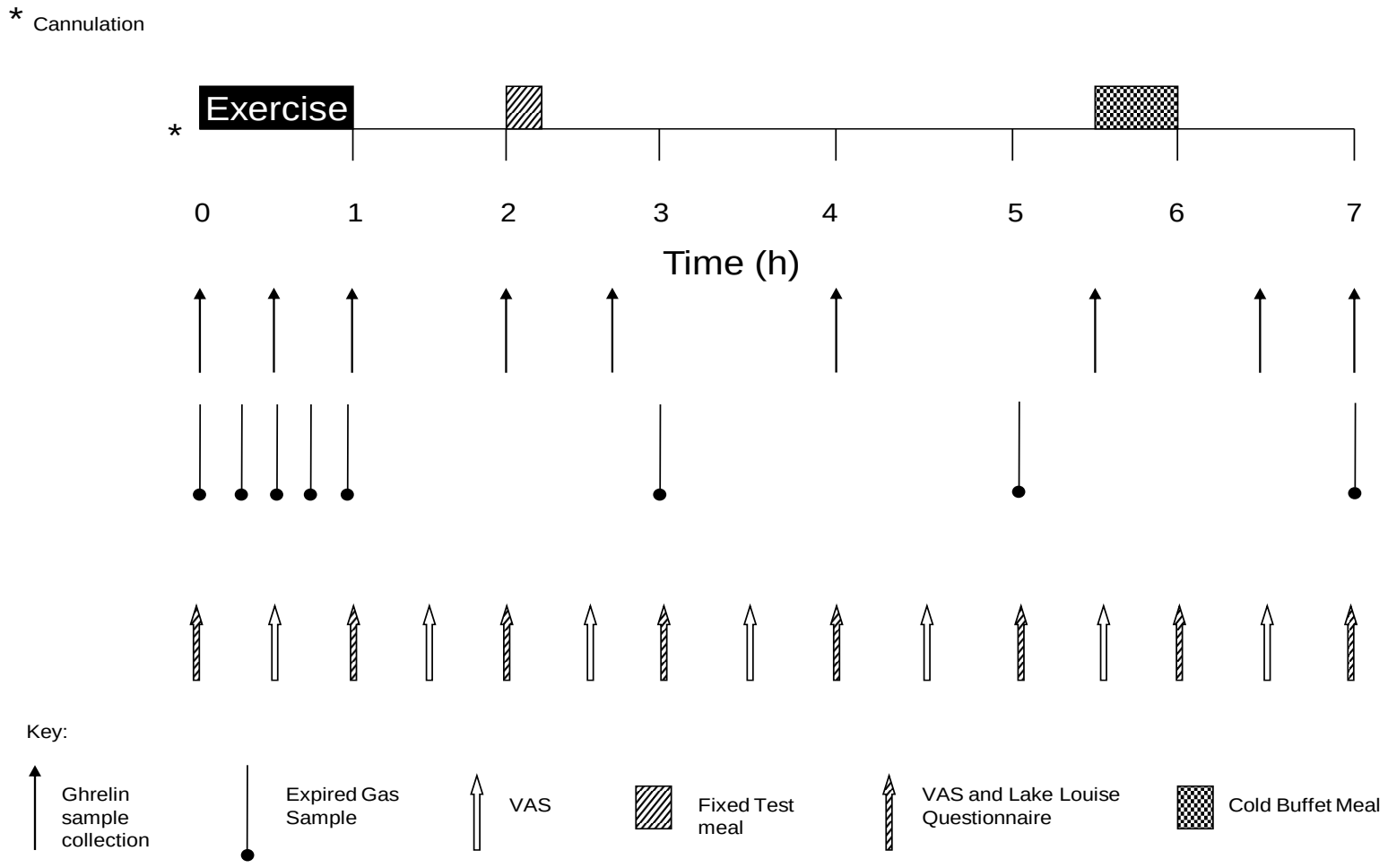

Figure 7.1 Schematic representation of main trial protocol

7.3 Results

7.3.1 Exercise responses

Maximum oxygen uptake was significantly reduced in hypoxia compared with normoxia (56.9 ± 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ and 37.5 ± 1.4 $mL \cdot kg^{-1} \cdot min^{-1}$ respectively; $P < 0.001$). Participants ran at 71.2 ± 1.8 % of normoxic $\dot{V}O_2$ max in the normoxic exercise trial and 74.1 ± 1.6 % of hypoxic $\dot{V}O_2$ max in the hypoxic exercise trial ($P = 0.279$). Gross energy expenditure was 3663 ± 148 kJ (875 ± 35 kcal) in the normoxic exercise trial and 2647 ± 135 kJ (633 ± 32 kcal) in the hypoxic exercise trial ($P < 0.001$). In the normoxic exercise trial, 38 ± 6 % of the energy was derived from fat and 62 ± 6 % of the energy from carbohydrate, and in the hypoxic exercise trial, 36 ± 4 % of the energy was derived from fat and 64 ± 4 % of the energy from carbohydrate.

7.3.2 Appetite perceptions

There was a main effect of time (all $P < 0.001$) for each of the appetite perceptions assessed (hunger, satisfaction, fullness and prospective food consumption) (Figure 7.2). Compared with normoxia, ratings of hunger and PFC were lower in hypoxia although these trial effects did not reach significance (both $P < 0.062$). Conversely, ratings of fullness and satisfaction tended to be higher in hypoxia compared with normoxia though again these trial effects did not reach significance (both $P < 0.086$). Altitude x time interaction effects were observed for satisfaction, fullness and PFC (all $P < 0.05$) and exercise x time interaction effects were observed for hunger, satisfaction and fullness (all $P < 0.05$) indicating differences in responses between trials over time (Figure 7.2). For the total 7 h trial duration, AUC values for hunger were 20% lower in hypoxia than normoxia (hypoxia 265 ± 35 mm·7h, normoxia 333 ± 29 mm·7h; $P = 0.071$).

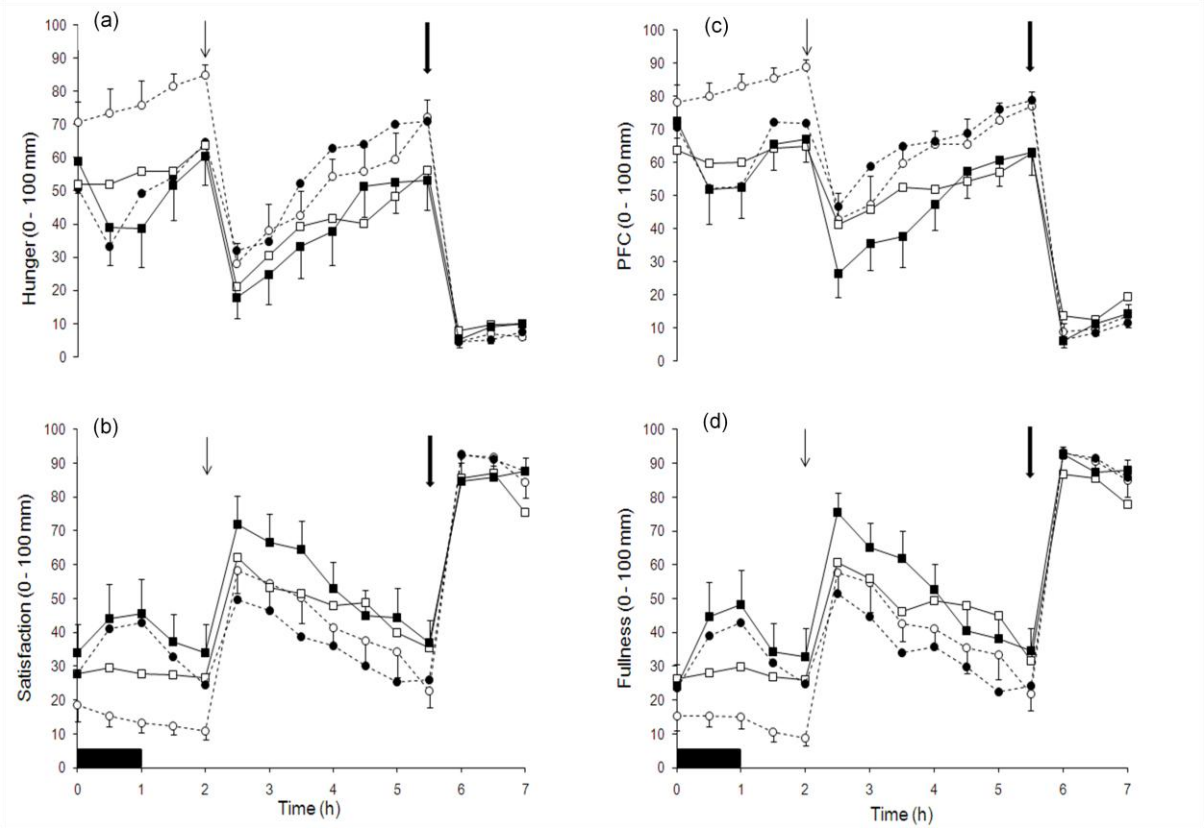

Figure 7.2 Changes in appetite perceptions during the normoxic control trial (--○--), normoxic exercise trial (--●--), hypoxic control trial (—□—), and hypoxic exercise trial (—■—). Values are means ± SEM, n = 10. Some error bars have been omitted for clarity Black rectangle indicates treadmill running, thin downward arrow (⇓) indicates test meal consumption, bold downward arrow (⇓) indicates buffet meal consumption. Repeated measures ANOVA revealed a main effect of time (P < 0.001) for each appetite perception, an altitude x time interaction for satisfaction, fullness and PFC (all P < 0.05) and an exercise x time interaction for hunger, satisfaction and fullness (all P < 0.05).

7.3.3 Energy and macronutrient intake

Energy intake at the buffet meals was reduced in hypoxia compared with normoxia (hypoxia 5291 ± 565 kJ [1265 ± 135 kcal], normoxia 7718 ± 675 kJ [1845 ± 161 kcal]; P = 0.001) but was similar between the control and exercise trials (6516 ± 612 kJ [1557 ± 146 kcal] and 6493 ± 586 kJ [1552 ± 140 kcal] respectively; P = 0.950, Figure 7.3).

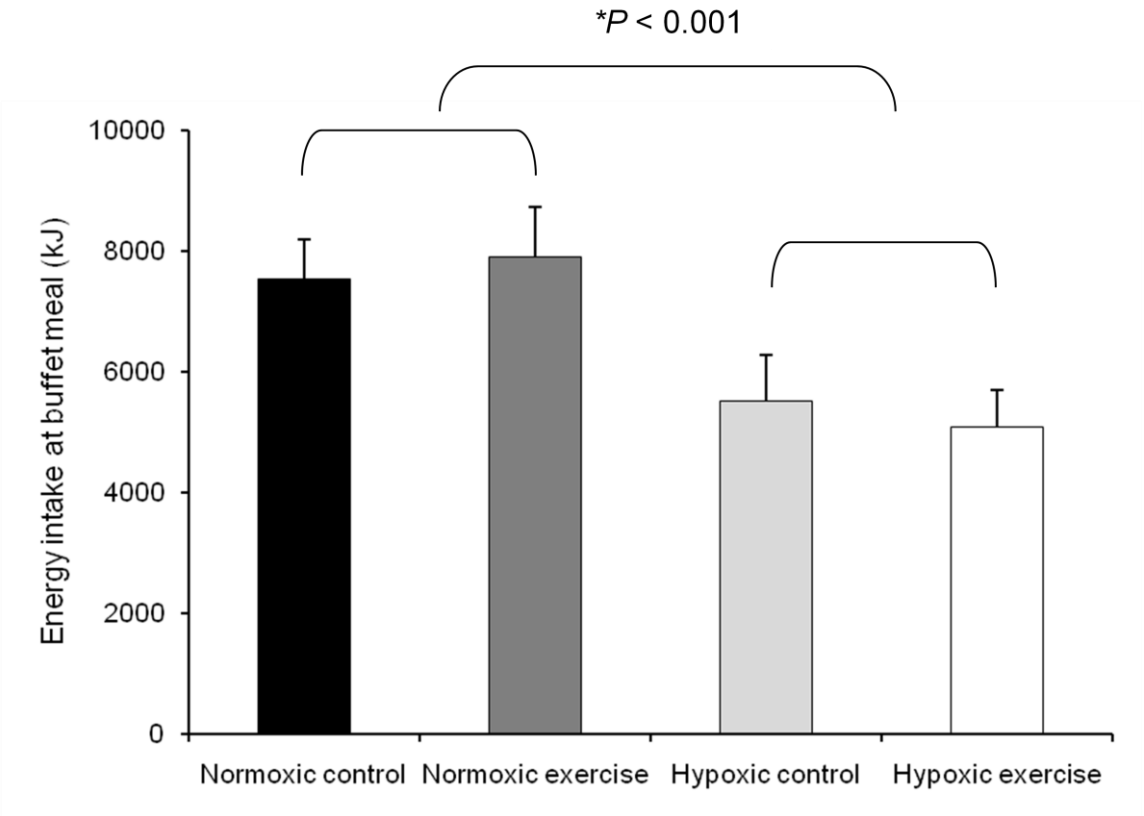

Figure 7.3 Total energy intake at the buffet meal (kJ). Values are means ± SEM, *n* = 10. *Hypoxic trials significantly different from normoxic trials (*P* < 0.001).

When expressed as a percentage of overall intake, macronutrient composition at the buffet meal differed between trials with a greater proportion of fat consumed during exercise compared with control (40 ± 2 % versus 38 ± 2 % respectively; *P* = 0.006) which appeared to be at the expense of protein where the proportion of protein was higher during the control trials compared with exercise (15 ± 1 % versus 13 ± 1 % respectively; *P* = 0.031). However, hypoxia did not influence macronutrient preference (Table 7.2).

Table 7.2 Macronutrient intake at the buffet meal expressed as total intake (g) and percentage (%) of total caloric intake

	Carbohydrate	Fat	Protein
Normoxic control			
g	215 ± 26	74 ± 7	70 ± 5
%	47 ± 2	37 ± 2	16 ± 1
Normoxic exercise			
g	207 ± 21	90 ± 12	66 ± 8
%	45 ± 2	41 ± 2	14 ± 1
Hypoxic control			
g	151 ± 19	59 ± 10	48 ± 7
%	47 ± 2	39 ± 2	14 ± 1
Hypoxic exercise			
g	144 ± 17	55 ± 8	39 ± 5
%	49 ± 2	39 ± 2	12 ± 1

Values are mean ± SEM, n = 10. NB: Mean percentage fat intake significantly higher during exercise versus control trials: 40 ± 2 % versus 38 ± 2 %; P = 0.006. Mean percentage protein intake significantly higher in control versus exercise trials: 15 ± 1 % versus 13 ± 1 %; P = 0.031. There are no significant differences in percentage intake of carbohydrate, fat or protein between hypoxic and normoxic trials.

7.3.4 Plasma acylated ghrelin

Fasting plasma acylated ghrelin concentration did not differ between trials at baseline (P = 0.219). There was a main effect of altitude (P = 0.005), exercise (P = 0.001) and time (P < 0.001) and an exercise x time interaction (P < 0.05) for plasma acylated ghrelin (Figure 7.4). *Post hoc* tests revealed mean plasma acylated ghrelin concentrations were suppressed in hypoxia compared with normoxia (82 ± 21 pg·mL^{-1} and 100 ± 22 pg·mL^{-1} respectively; P = 0.005) and lower during exercise trials than resting trials (86 ± 20 pg·mL^{-1} versus 97 ± 22 pg·mL^{-1} respectively; P = 0.001). Acylated ghrelin AUC values (Figure 7.5) were 18 % lower in hypoxia than normoxia (573 ± 153 pg·mL^{-1}·7 h versus 700 ± 146 pg·mL^{-1}·7 h

respectively; $P < 0.05$) and 10 % lower during exercise trials than control trials (602 ± 143 pg·mL^{-1}·7 h versus 671 ± 154 pg·mL^{-1}·7 h respectively; $P = 0.001$)

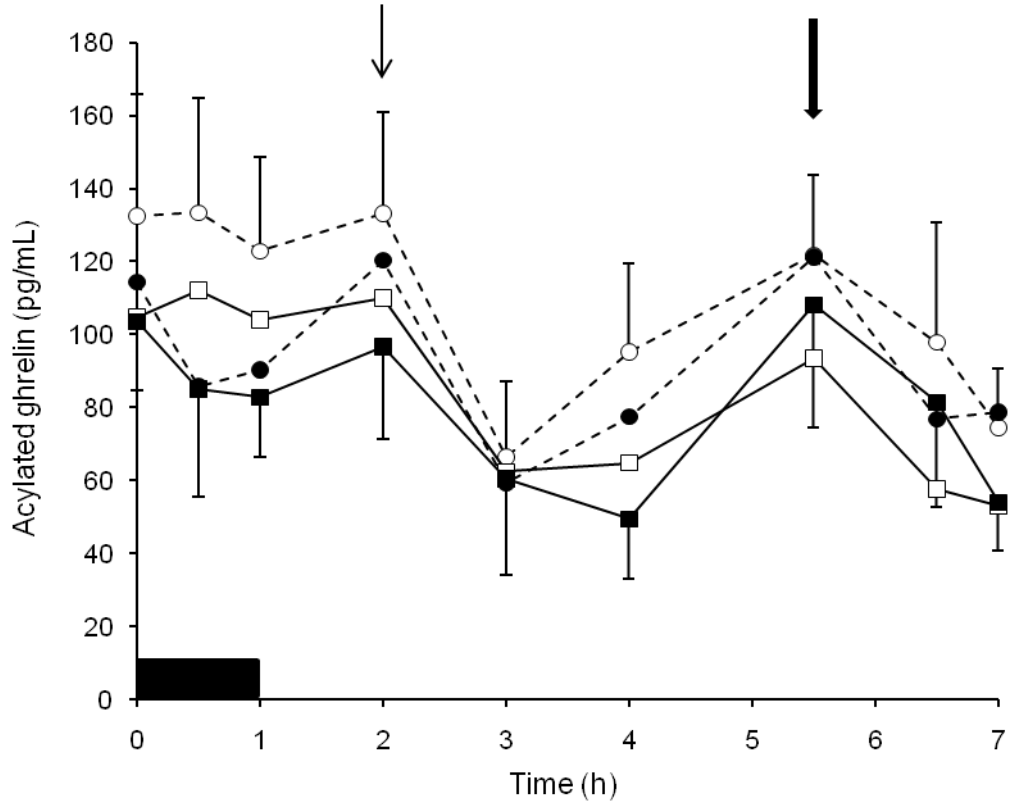

Figure 7.4 Plasma acylated ghrelin concentrations during the normoxic control trial (--○--) normoxic exercise trial (--●--), hypoxic control trial (—□—), and hypoxic exercise trial (—■—). Values are means ± SEM, $n = 10$. Some error bars have been omitted for clarity. Black rectangle indicates treadmill running, thin downward arrow (⇓) indicates test meal consumption, bold downward arrow (⬇) indicates buffet meal consumption. Repeated measures ANOVA revealed a main effect of altitude ($P = 0.005$), exercise ($P = 0.001$) and time ($P < 0.001$) and an exercise x time interaction ($P < 0.05$).

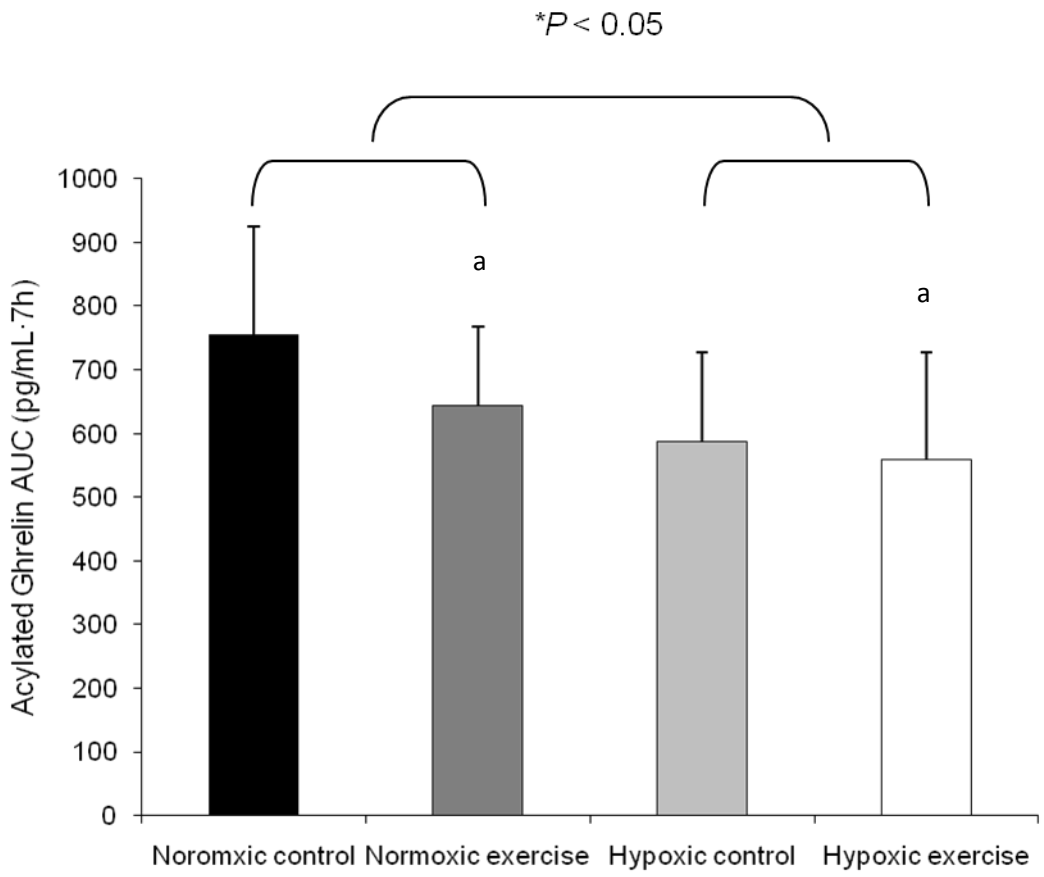

Figure 7.5 Total area under the concentration versus time curve (AUC) for plasma acylated ghrelin. Values are means ± SEM, n = 10. *Hypoxic trials significantly different from normoxic trials (P < 0.05). [a]Mean of exercise trials significantly lower than control trials (P = 0.001).

7.3.5 Peptide YY

Fasting plasma total PYY concentration did not differ at baseline between trials (P = 0.526). There was a main effect of exercise (P = 0.044), time (P < 0.001) and an altitude x time interaction (P = 0.016) for plasma total PYY (Figure 7.6). *Post hoc* tests showed that mean total PYY concentrations were higher in the exercise trials than the control trials (32.3 ± 3.2 pmol·L^{-1} versus 29.8 ± 2.7 pmol·L^{-1},; P = 0.044) and higher in normoxia (32.3 ± 2.8 pmol·L^{-1}) than hypoxia (29.6 ± 3.1 pmol·L^{-1}) although this latter difference did not quite reach significance (P = 0.059).

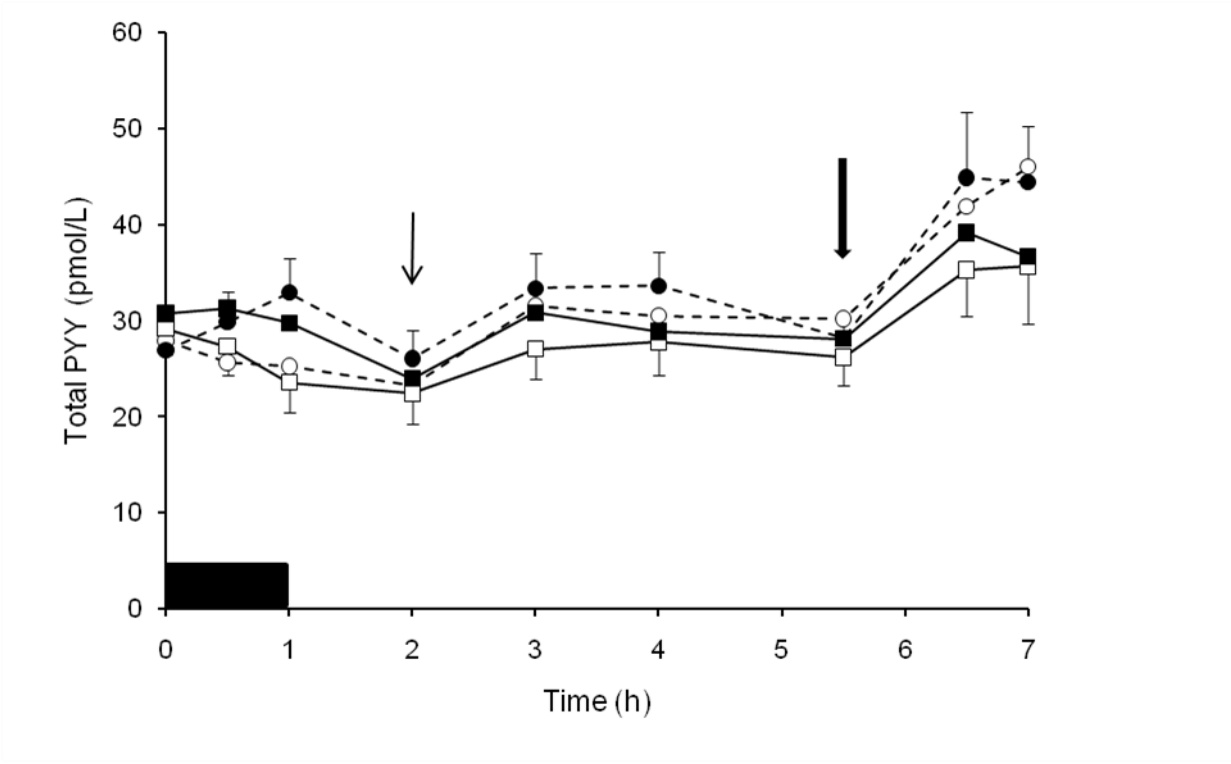

Figure 7.6 Plasma total PYY concentrations during the normoxic control trial (--○--), normoxic exercise trial (--●--), hypoxic control trial (—□—), and hypoxic exercise trial (—■—). Values are means ± SEM, n = 10. Some error bars have been omitted for clarity. Black rectangle indicates treadmill running, thin downward arrow (⇓) indicates test meal consumption, bold downward arrow (⬇) indicates buffet meal consumption. Repeated measures ANOVA revealed a main effect of time ($P < 0.001$), a main effect of exercise ($P < 0.05$) and an altitude x time interaction ($P = 0.016$).

7.3.6 Leptin

Incomplete sets of data were obtained for serum leptin concentrations. In the present study fasting leptin concentration at baseline was determined for all participants during the hypoxic control trial. Serum leptin concentrations ranged from 1199 – 6295 pg·mL^{-1} with a mean of 2899 pg·mL^{-1}. Where leptin concentration could not be determined, samples fell below the lowest standard of 15.6 pg·mL^{-1} when samples were diluted 100 fold. There was a trend for a positive correlation between fasting leptin concentrations at baseline and BMI (r = 0.607; $P = 0.063$) (Figure 7.7).

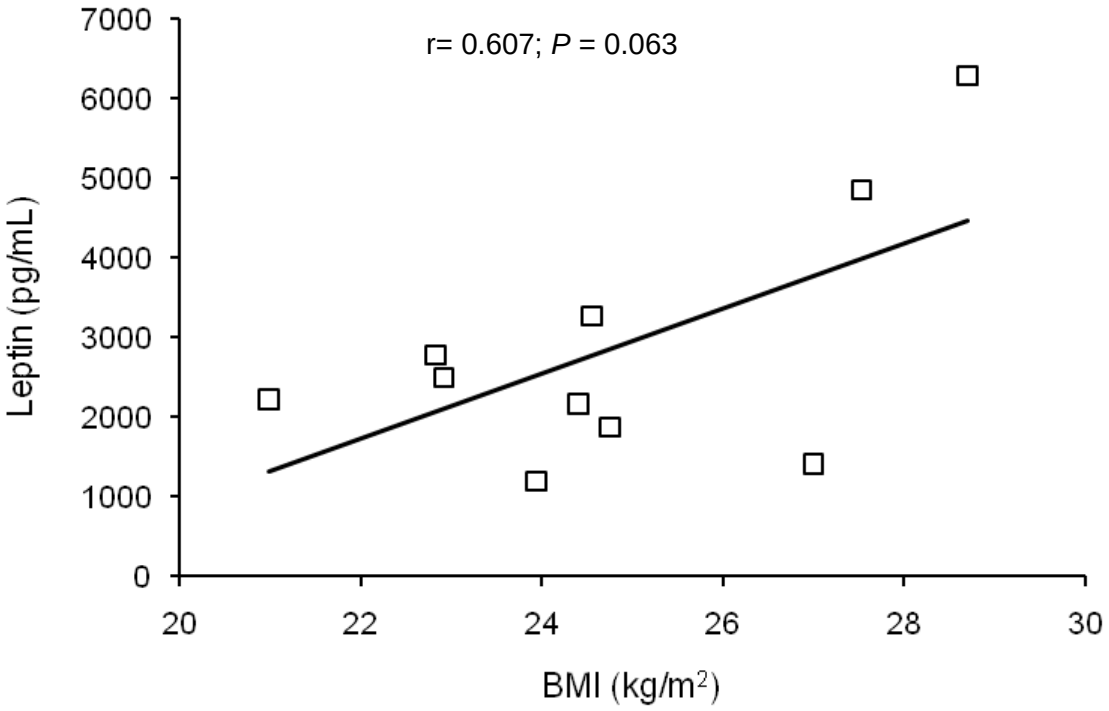

Figure 7.7 Correlation between BMI and fasting leptin concentrations at baseline in the hypoxic control trial (n = 10).

7.3.7 Glucose and triacylglycerol

Fasting plasma glucose did not differ at baseline between the trials (P = 0.646). There was a main effect of time (P < 0.001), altitude (P = 0.048) and exercise (P = 0.008) for glucose. Plasma glucose was higher during the hypoxic trials (5.1 ± 0.2 mmol·L^{-1}) than the normoxic trials (4.9 ± 0.1 mmol·L^{-1}; P = 0.048). This finding was confirmed by analysis of AUC values for glucose for the entire 7 h trial duration (hypoxia 36.5 ± 1.2 mmol·L^{-1}·7 h, normoxia 34.5 ± 0.6 mmol·L^{-1}·7 h; P = 0.02). Plasma glucose was higher during the exercise trials than the control trials (5.2 ± 0.1 mmol·L^{-1} versus 4.9 ± 0.1 mmol·L^{-1} respectively; P = 0.008) and this was confirmed by analysis of AUC values (exercise 36.7 ± 1.0 mmol·L^{-1}·7 h, control 34.4 ± 0.9 mmol·L^{-1}·7 h; P = 0.015). Fasting plasma TAG concentrations were not significantly different between trials (P = 0.154). There was a significant effect of time (P < 0.001) but no other main effects (data not shown).

7.3.8 Oxygen saturation and AMS

There was a main effect of time ($P < 0.001$), altitude ($P < 0.001$), exercise ($P < 0.05$) and an altitude x time interaction ($P < 0.001$) for oxygen saturation. Post-hoc tests indicated oxygen saturation was significantly lower in hypoxia (82 ± 1 %) than normoxia (98 ± 0 %; $P < 0.001$) confirming the hypoxic nature of these trials. Mean capillary blood oxygen saturation was significantly lower in the exercise trials than the resting trials (89 ± 1 % versus 91 ± 1% respectively; $P < 0.05$). The decrease in capillary blood oxygen saturation during the hypoxic trials varied widely amongst participants, with the mean individual oxygen saturation across the trial duration ranging from 70 – 88 %. Exposure to hypoxia resulted in increases in Lake Louise Score with the participants reporting varying degrees of AMS. Symptoms across the trial duration ranged from 0 to 6 on the hypoxic control trial and 0 to 7 on the hypoxic exercise trial. On the normoxic control trial symptoms ranged from 0 to 2 and on the normoxic exercise trial symptoms ranged from 0 to 4. Symptoms of headache and AMS were prevalent in 6 out of 10 participants in the hypoxic control trial and 5 out of 10 participants during the hypoxic exercise trial on at least one occasion during the trials.

7.3.9 Correlations between appetite-regulating hormones and other variables

Acylated ghrelin concentrations were positively correlated with hunger at 0.5 h in the normoxic exercise trial (r = 0.632; $P < 0.05$). Acylated ghrelin concentrations were negatively correlated with fullness at 4 h (r = -0.649; $P < 0.05$) and positively correlated with PFC at 4 h (r = 0.655; $P < 0.05$) and 5.5 h (r = 0.787; $P = 0.007$) on the normoxic control trial. There were no correlations between acylated ghrelin and appetite ratings at any other timepoints on any of the trials. Acylated ghrelin concentrations immediately prior to the buffet meal (5.5 h) were positively correlated with energy intake in the normoxic control trial (r = 0.677, $P < 0.05$) but not in the other trials. Acylated ghrelin was negatively correlated with glucose during the normoxic control trial at 7 h (r = -0.657, $P = 0.039$) but not at any other times. Energy intake at the buffet meal was positively correlated with pre-meal hunger in all trials, correlation coefficients ranged from 0.670 – 0.770 ($P < 0.05$). Peptide YY was not correlated with hunger or glucose at any times, nor with subsequent energy intake at 5.5 h. Peptide YY concentrations at 0.5 h on the normoxic control trial, were positively correlated with fullness (r = 0.650; $P < 0.05$) and negatively correlated with PFC (r = -0.637; $P < 0.05$). In the hypoxic control trial at 2 h, PYY was positively correlated with satisfaction (r = 0.647; $P < 0.05$) and fullness (r =

0.680; $P < 0.05$) and negatively correlated with PFC (r = -0.673; $P < 0.05$). Peptide YY was negatively correlated with PFC immediately prior to the buffet meal at 5.5 h on the hypoxic control trial (r = -0.671; $P < 0.05$). Peptide YY was negatively correlated with TAG at 2 h in the hypoxic exercise trial (r = -.0.745; $P = 0.013$).

7.4 Discussion

This study investigated the effects of rest and exercise in hypoxia on appetite, energy intake and concentrations of the gut hormones ghrelin and total PYY. The main findings are: 1) energy intake is suppressed in normobaric hypoxia; 2) acylated ghrelin concentrations are suppressed in normobaric hypoxia and 3) total PYY has a tendency to be suppressed in normobaric hypoxia. Thus short-term exposure to hypoxia, in the absence of cold and other stressors, suppresses plasma acylated ghrelin concentrations and this suppression may be implicated in high altitude anorexia.

High altitude anorexia, characterised by a reduction in appetite and food intake, is commonly reported during high altitude sojourns and in controlled laboratory studies mimicking high altitude (Rose et al 1988, Westerterp et al 1994, Westerterp-Plantenga et al 1999, Benso et al 2007, Vats et al 2007). The role of endocrine factors in the control of appetite and food intake is now well established and recent studies have investigated possible roles for appetite regulating hormones in high altitude anorexia (Tschöp et al 1998, Shukla et al 2005). However, clarification is needed because current findings are inconclusive and contradictory, largely due to methodological differences.

Only a handful of studies have examined the effect of altitude on ghrelin levels and findings are inconsistent with no change (Benso et al 2007, Lippl et al 2010) and a decrease (Shukla et al 2005) reported. Possible reasons for these discrepancies in findings are the differences in altitude at which measures were taken, the method of transportation (foot or vehicle) and the body fatness of the individuals. Furthermore, these studies only measured total ghrelin, although it is the acylated form of this peptide which is responsible for its appetite stimulating effects (Broglio et al 2004). Quantification of total ghrelin alone may mask any changes in the proportion of acylated ghrelin; accordingly measurement of acylated ghrelin concentrations at altitude is important to ascertain any possible role in high altitude anorexia. Our results are indicative of a role for acylated ghrelin in high altitude anorexia in the short term at least which is in accordance with the decrease in total ghrelin observed previously (Shukla et al 2005).

We can only speculate about the mechanisms underlying the suppression of plasma acylated ghrelin in hypoxia. For ghrelin to bind to its receptor and exert its appetite-stimulating effects, it must be acylated by an 8 carbon fatty acid (octanoate) at its serine 3

residue by the enzyme GOAT (Gutierrez et al 2008, Yang et al 2008). Kirchner and colleagues (2009) posited that ghrelin acylation and the secretion of acylated ghrelin are likely two different processes (Kirchner et al 2009). Ghrelin acylation by GOAT is dependent on feeding status and availability of dietary lipids. The complexity of the ghrelin/GOAT system means that explaining the exact mechanism behind the suppression of acylated ghrelin in hypoxia is difficult. It is possible that hypoxia might interfere with the ghrelin/GOAT system in some way, causing an impairment of the acylation process by GOAT, an inhibition of GOAT secretion, or an altered secretion pattern of ghrelin after its enzyme-requiring modification. However, this study did not measure concentrations of total ghrelin or des-acyl ghrelin so it is not possible to know whether changes in acylated ghrelin observed occur independently of changes in total ghrelin or whether there was a change in the ratio of des-acyl and acyl ghrelin.

Ghrelin is secreted primarily from the stomach into the bloodstream (Ariyasu et al 2001) and can reach the food-regulating centres of the hypothalamus by passing through the blood brain barrier (Banks et al 2002). Ghrelin produced from the stomach will pass through the liver from the portal vein where it will then reach the peripheral circulation. Data from a study by Goodyear et al (2010) tentatively suggests that the liver may be involved in the acylation of ghrelin. Wolff (2007) suggests that the splanchnic area may be vulnerable to the needs of blood flow elsewhere in the body and thus the decrease in oxygen saturation in hypoxia may result in blood being "stolen" from the splanchnic area so that oxygen delivery can be maintained elsewhere. Impaired gut blood flow has been proposed as a mechanism behind high altitude anorexia. Research has shown 2 h at a simulated altitude of 4800 m significantly reduced superior mesenteric artery blood flow before and after food ingestion (Loshbaugh et al 2006). However, a recent study employing a similar protocol shows that increases in arterial and venous blood flow in the gut after a food challenge are similar at sea level and after a 3 day exposure to hypobaric hypoxia (Kalson et al 2010). In this study all participants experienced hunger loss with hypobaric hypoxia suggesting that impaired gut blood flow was not responsible for high altitude anorexia after several days as had initially been proposed. However, the suppression of acylated ghrelin we observed in hypoxia over 7 h could be involved in high altitude anorexia, and this might be linked to the observation of altered gut blood flow in response to acute hypoxia (Loshbaugh et al 2006). In the longer term different mechanisms may exist because Kalson et al (2010) observed no differences in blood flow in the gut after 3 day exposure to hypoxia compared with sea

level. More research needs to be undertaken that examines the effects of both acute and chronic hypoxia on plasma acylated ghrelin concentrations to establish the time course of any changes.

Ghrelin concentrations increase preprandially and decrease postprandially (Cummings et al 2001). The mechanisms by which ghrelin is suppressed postprandially are unclear, although a glucose induced suppression has been proposed (Nakagawa et al 2002). However, one study has shown that hyperglycaemia of 11 $mmol \cdot L^{-1}$ does not suppress plasma ghrelin concentrations (Schaller et al 2003). In the present study, glucose concentrations were elevated and acylated ghrelin concentrations were suppressed in the hypoxic trials compared with the normoxic trials. The observed elevation in plasma glucose in the hypoxic trials compared with the normoxic trials, although significant, was only 0.2 $mmol \cdot L^{-1}$ which is likely insufficient to suppress plasma ghrelin concentrations. In the post-prandial period there are many other hormones released in response to nutrient ingestion (Koliaki et al 2010) and these may be involved in the post-meal ghrelin response at altitude.

Peptide YY is released in response to caloric ingestion in a dose-dependent manner (Adrian et al 1985) and infusion of postprandial concentrations of PYY_{3-36} in humans decreases appetite and food intake (Batterham et al 2002). The effect of altitude on PYY concentrations has not been examined. In the present study, concentrations of total PYY tended to be higher in normoxia than in hypoxia suggesting that total PYY does not play a role in high altitude anorexia. The reason for the suppression of total PYY in hypoxia is unknown but might be related to changes in concentrations of other appetite-regulatory peptides that we did not measure, such as CCK or GLP-1. Acute exercise in normoxia has been shown to increase concentrations of the satiety peptide CCK (Bailey et al 2001). However, the same response did not occur in acute normobaric hypoxia, with concentrations being suppressed compared with a resting trial (Bailey et al 2001). In response to feeding at least, it has been proposed that a certain amount of CCK release is needed for the initial rise in PYY levels (Pilichiewicz et al 2007). Although we did not measure CCK, the tendency for lower levels of PYY during hypoxia in the present study, might be related to the previously observed decrease in CCK during acute exercise in hypoxia. Peptide YY release is inhibited by infusion of GLP-1 (Näslund et al 1999) hence alterations in concentrations of GLP-1 may mediate the PYY response to hypoxia.

Research is limited with only one study investigating the effect of hypoxia on GLP-1 concentrations. This study demonstrated a tendency for a meal-induced increase in GLP-1 concentrations after 17 h of exposure to hypoxia simulating an altitude of approximately 4100 m (Snyder et al 2008). However, before the meal GLP-1 concentrations were unchanged after hypoxic exposure compared with sea level suggesting GLP-1 is not affected by hypoxia in the absence of feeding. Finally, the finding from the present study that total PYY concentrations are higher in the exercise trials than in the resting trials confirms previous findings that an acute bout of aerobic exercise increases PYY concentrations, this holds true in both the fed (Martins et al 2007, Ueda et al 2009b) and fasted states (Broom et al 2009). A limitation of our study is that we only measured total PYY. Peptide YY circulates in two forms; PYY_{1-36} and PYY_{3-36}. Peptide YY_{1-36} is released post-prandially and is converted to PYY_{3-36} by the enzyme dipeptidyl peptidase-IV (Mentlein et al 1993). When infused peripherally, PYY_{3-36} is more potent than PYY_{1-36} in suppressing hunger (Chelikani et al 2005) and infusion of PYY_{3-36} in humans reduces both appetite and caloric intake (Batterham et al 2002, Degen et al 2005). Although it is important to verify the effects of hypoxia on PYY_{3-36} concentrations in the future, total PYY and PYY_{3-36} concentrations are highly positively correlated (Tsilchorozidou et al 2008) and hence changes in total PYY should reflect changes in $PYY_{3-36.}$ Given the limited research available concerning the effect of hypoxia on concentrations of the appetite regulating hormones, more studies need to be undertaken to not only examine the effects of hypoxia on hormonal concentrations but to investigate how changes may be mediated by alterations in the other gut hormones.

Leptin is produced from adipose tissue (Zhang et al 1994) and circulates in proportion to body fat with higher concentrations observed in overweight and obese individuals (Considine et al 1996b, Clapham et al 1997). Fasting leptin concentrations in the present study are similar to the values reported in the literature in lean individuals. Muscelli et al (1996) report mean fasting leptin concentrations of 6400 $pg \cdot mL^{-1}$ in 12 lean participants with a mean BMI of 22.2 $kg \cdot m^2$ and Hickey et al (1996) report mean leptin concentrations of 2190 $pg \cdot mL^{-1}$ in 13 lean male runners with a mean BMI of 23.1 $kg \cdot m^2$. This is in contrast with the elevated leptin concentrations of 26 600 $pg \cdot mL^{-1}$observed in 12 obese individuals with a mean BMI of 34 $kg \cdot m^2$ (Muscelli et al 1996). Although participants in this study were a relatively homogenous group based on their BMI, there was still a tendency for increased leptin concentrations in those participants with a higher BMI. It is

likely that many of the samples tested were below the range of the lowest standard because the participants were generally lean. To evaluate for leptin in the assay, the assay manufacturers drew samples from 16 male healthy volunteers and observed a range of 2205 – 11 149 $pg \cdot mL^{-1}$ with a mean of 4760 $pg \cdot mL^{-1}$. However, when they used the recommended 100 fold dilution, 5 additional samples were below the lowest standard of 15.6 $pg \cdot mL^{-1}$. The body fat of those individuals tested was not described so it is unknown what the body fat percentage of the volunteers who had leptin values below the lowest standard was. As leptin concentrations correlate with adiposity it is likely that the body fat of those individuals was quite low.

In the present study, spontaneous energy intake at a buffet meal, in the presence of *ad libitum* palatable food, was decreased by approximately 31 % in hypoxia. This response confirms previous findings from studies using hypobaric chambers and supports the notion that hypoxia alone is a sufficient stimulus for causing a reduction in energy intake (Rose et al 1988, Westerterp-Plantenga et al 1999). When decreased energy intake at altitude persists over some time, body mass loss is inevitable (Boyer and Blume 1984, Guilland and Klepping 1985, Rose et al 1988, Westerterp-Plantenga et al 1999). Given the acute nature of this study, it is not possible to know whether this decreased energy intake would persist over time and translate into a loss of body mass. The simulated altitude participants were exposed to in our study is low enough for total acclimatisation to occur after several days (Ward et al 2000). It is therefore probable that the decrease in energy intake we observed in hypoxia would disappear over time unless a higher altitude was simulated. Also, meals were prescribed at set times in this study so it is unclear when individuals would have initiated feeding. An increase in meal frequency and reduction in meal size has been reported at altitude (Westerterp-Plantenga et al 1999). This style of eating frequency may be adopted in an attempt to meet energy intake requirements.

Body mass loss at altitude has only been prevented when individuals were instructed to consume enough food to match energy expenditure (Butterfield et al 1992). If body mass loss due to energy imbalance can be prevented by simply increasing calorie intake then it is important to understand the mechanisms behind high altitude anorexia so that methods to increase voluntary calorie intake in free-living conditions can be sought. At the very least, individuals should maintain an awareness of their own caloric needs so that they can attempt to match their intake with expenditure to minimise body mass loss. Butterfield and

colleagues (1992) reported that individuals in their study had no difficulty in consuming the food presented to them, suggesting that provided sufficient food is available at altitude, individuals can maintain body mass. Appetite is regulated by psychological as well as physiological factors with the rewarding nature of food sufficient to stimulate feeding (Wynne et al 2005). In the study by Butterfield et al (1992) body mass may be maintained because the individuals may have simply overridden a lack of desire to eat despite not being hungry, however, appetite sensations were not measured in their study so this is purely speculative. Despite the common assumption that appetite is decreased at altitude, Westerterp-Plantenga et al (1999) were the first researchers to quantify this. Confirming this, in the present study, hunger was lower by 20 % in hypoxia than in normoxia. Furthermore, the amount of food participants thought they could eat at a given time (prospective food consumption) tended to be lower in hypoxia. It is clear that a reduction in energy intake is consistently observed at high altitude and when individuals remain at high altitude this leads to a decrease in body mass which is undesirable for mountaineers and climbers.

Given the persistence of high altitude anorexia over time, in future, studies should examine the effects of chronic hypoxia on plasma acylated ghrelin and PYY_{3-36} responses. The participants used in this study were young healthy males; whether our findings extend to other population groups including females, older individuals and the overweight and obese in whom circulating ghrelin levels are already decreased (Tschöp et al 2001b), are unclear. Hypoxia exposure has been postulated as a potential therapy for obesity (Quintero et al 2010). In the absence of acclimatisation, our findings could certainly lend support to this idea and exercise in hypoxia may be an effective weight loss strategy. Limited data are available regarding the effects of hypoxia on energy intake and weight loss in obese individuals, but recently it has been demonstrated that 1 week of moderate hypoxic exposure (2650 m) in obese participants results in a significant, albeit modest, sustained weight loss (Lippl et al 2010). Hence the combined effect of exercise and hypoxia on weight loss in overweight and obese individuals clearly warrants attention. Finally, other physiological factors such as other appetite regulatory hormones, psychological and behavioural factors and alterations in taste perception or the perceived palatability of food could also play a part in the decreased energy intake at altitude and future studies should attempt to address these issues.

In conclusion, acute exposure to normobaric hypoxia causes a reduction in spontaneous energy intake that may be related to suppressed plasma acylated ghrelin concentrations. Exercise also suppresses plasma acylated ghrelin concentrations but does not appear to exacerbate the suppression noted in hypoxia. It has been suggested that studying the physiological effects of hypoxia in healthy individuals voluntarily exposing themselves to hypoxia may enhance understanding of the effects of hypoxia in clinical disease (Khosravi and Grocott 2009). Hence, the present findings may be relevant not just for mountaineers and climbers who sojourn to high altitude for recreational purposes and suffer from high altitude anorexia, but potentially for individuals who suffer from disordered eating due to illness and disease.

CHAPTER VIII

General Discussion

8.1 Introduction

The complex mechanisms by which hormones secreted from the gastrointestinal tract regulate appetite and food intake are becoming evident with an ever expanding body of research dedicated to such topics. The research highlights the multifaceted nature of this regulatory system and demonstrates how it is far from straightforward and influenced by factors such as exercise. Acute exercise affects concentrations of circulating gut hormones and transiently suppresses hunger, although appears not to affect subsequent energy intake. The research presented within this thesis complements current knowledge surrounding the effect of exercise and exercise mode (running and cycling) on appetite, energy intake and appetite-regulatory peptides (acylated ghrelin and total PYY), and provides novel evidence demonstrating how environmental factors (ambient temperature and altitude) may perturb the expected physiological responses. This chapter will bring together the findings from the studies presented within thesis. In all the studies within this thesis, measurements of acylated ghrelin concentrations were made and hunger sensations were measured. With the exception of Chapter IV where a standardised test meal was provided, spontaneous energy intake was quantified from *ad libitum* meals. Table 8.1 briefly summaries each study design and the variables measured, followed by a summary of the main findings from the studies.

Table 8.1 Summary of trial protocols and variables measured for each study presented in this thesis

	Chapter IV *Modality*	**Chapter V** *Heat*	**Chapter VI** *Cold*	**Chapter VII** *Altitude*
Participant number (*n*)	11	11	10	10
Trials	*3 x 4 h trials* Control (rest) Running Cycling	*2 x 7 h trials* Running in 20°C Running in 30°C	*2 x 7 h trials* Running in 20°C Running in 10°C	*4 x 7 h trials* Normoxic control (rest) Hypoxic control (rest) Normoxic exercise Hypoxic exercise
Exercise duration and intensity	60 min @ 70 % of mode-specific $\dot{V}O_2$ max	60 min run @ 65 % of $\dot{V}O_2$ max	60 min run @ 65 % of $\dot{V}O_2$ max	60 min run @ 70 % of altitude-specific $\dot{V}O_2$ max
Measurements	Hunger Acylated ghrelin	Appetite Energy intake Acylated ghrelin (*n* = 10) Core temperature	Appetite Energy intake Acylated ghrelin Core temperature	Appetite Energy intake Acylated ghrelin Total peptide YY

The main findings from the above studies are as follows:

- Acylated ghrelin concentrations are suppressed to a similar extent during high intensity cycling as they are during high intensity running
- Ambient temperature affects appetite perceptions with a transient lowering of hunger and prospective food consumption observed after running in a hot environment and a transient lowering in fullness and satisfaction is observed after running in a cold environment
- Normobaric hypoxia tends to suppress hunger and prospective food consumption
- Energy intake is suppressed in normobaric hypoxia and is modulated by ambient temperature with a tendency for reduced energy intake in a hot environment and increased energy intake in a cold environment
- Acylated ghrelin concentrations are suppressed in normobaric hypoxia which may be implicated in the lower energy intake observed in hypoxia
- Acylated ghrelin concentrations respond differently over time in hot and cold temperatures compared with a neutral temperature but likely do not play a major part in perturbations in energy intake observed in these environments with the mechanisms responsible remaining unclear

8.2 Appetite

There is a large body of literature demonstrating a transient suppression of appetite during moderate and high intensity exercise. Evidence suggests that appetite returns to control values shortly after exercise and remains no different in the hours after, even in the presence of large energy deficits induced by exercise (King et al 2010a). Although there exists this large body of data, most research has been undertaken in standard laboratory conditions. However, the environmental conditions exercise is undertaken in may affect the expected appetite response (Westerterp-Plantenga et al 1999, White et al 2005, Shorten et al 2009). One of the aims of the studies presented within this thesis was to examine the effect of exercising and resting in different environmental extremes on appetite and to provide empirical evidence to support the mainly anecdotal reports within the literature.

It is widely accepted that appetite is suppressed at high altitude, but it is only recently that this has been confirmed in laboratory and field studies (Westerterp-Plantenga et al 1999, Kalson et al 2010). Chapter VII shows that hunger and prospective food consumption are suppressed in normobaric hypoxia whilst ratings of fullness and satisfaction tend to be elevated. Furthermore, this effect manifests within a short time of exposure to altitude ($\leq$ 7 h). Given both the altitude simulated, and the acute nature of the study presented within Chapter VII, whether this suppression of appetite in hypoxia would persist in the long term is unclear because it is suggested that below altitudes of 4000 m appetite returns to normal after several days (Ward et al 2000). In the aforementioned studies that have demonstrated a suppression of appetite at altitude, measurements were obtained after 24 h at 4392 m (Kalson et al 2010) and during a simulated 31 d ascent of Mount Everest (Westerterp-Plantenga et al 1999), suggesting that this is not just a transient response at altitudes above 4000 m. Exercise-induced anorexia, characterised by a transient suppression of appetite in response to acute bouts of moderate to high intensity exercise, is also a well-documented phenomenon. The intensity of exercise is an important determinant of exercise-induced anorexia and most evidence suggests that an intensity of 60 % of $\dot{V}O_2$ max is sufficient to suppress appetite. When exercising at altitude, there are three independent stressors on an individual; exercise, cold and hypoxia. These may affect normal physiological responses (Mazzeo, 2008). The findings in Chapter VII reveal that a bout of exercise in hypoxia (70 % of $\dot{V}O_2$ max) suppresses hunger to a similar extent as the same exercise undertaken in normoxia suggesting that the inclusion of another appetite-suppressive stimulus (hypoxia) does not have an additive effect on the hunger response. However, this is likely because the same relative intensity of exercise was used.

Mainly anecdotal reports exist concerning the effect of exercising in different ambient temperatures on appetite. The findings presented in Chapters V and VI suggests that subjective perceptions of appetite are differentially affected by exercising in hot or cold ambient temperatures. Specifically, during and shortly after running in the heat, hunger and prospective food consumption are reduced, whereas ratings of satisfaction and fullness are reduced during the same period after running in the cold. Appetite sensations are a reliable and valid means of measuring subjective states of motivation to eat prior to and in response to meals (Flint et al 2000). Thus the present findings regarding altered perceptions of appetite lend support for the notion that individuals eat less in hot environments, and more in cold environments. After the first post-exercise meal, appetite

sensations were no different between trials in a hot or cold temperature compared with the neutral temperature suggesting that appetite may only be responsive to environmental temperature during exercise. However in these studies, individuals were required to wear the same clothing during exercise in each trial, but were permitted to wear whatever they wished thereafter to remain in thermal comfort. Exercise itself may be an additional thermal challenge particularly during exercise in the heat where heat dissipation could be impaired if clothing prevented the evaporation of sweat. Thus, whether appetite would be altered if individuals were required to wear the same clothing whilst they remained resting in each ambient temperature is unclear. In Chapter VII, hunger was lower in hypoxia than in normoxia, and unlike in the heat or cold when participants could wear less or more clothing, individuals could not alter their behaviour to suit the environment in hypoxia which may explain why appetite was altered in hypoxia over the entire 7 h trial duration compared with just the first 2 h of the trials undertaken in the heat and cold.

To summarise, hypoxia significantly affects perceptions of appetite, and ambient temperature during a bout of exercise also tends to transiently affect appetite compared with a neutral temperature. Hypoxia was likely a strong enough stimulus to affect appetite whereas the extremes of environmental temperature used within the present studies may not have differed enough from the neutral temperature to exert significant differences in appetite ratings, and the option for participants to wear what clothes they wanted to after the exercise bout in the hot and cold trials possibly negated any effect of temperature on appetite thereafter.

8.3 Energy intake

The effect of hypoxia on energy intake is well documented whereas the response to environmental temperature is less well established. Although evidence is unanimous in concluding that energy intakes are lower at high altitude, the extent to which they are lower is variable and likely due to the duration of altitude exposure, the altitude attained, and also the availability of foods at altitude (Rose et al 1988, Kayser, 1994, Westerterp-Plantenga et al 1999, Vats et al 2007). Many studies have been conducted in field environments where other factors such as extreme cold or physical exertion could influence food intake, and the duration of altitude exposure has been prolonged. With respect to environmental temperature, the limited empirical evidence suggests exercise in the cold increases energy

intake (Dressendorfer, 1993, White et al 2005) and exercise in the heat may decrease energy intake (Shorten et al 2009). However, energy intake in these studies was quantified from a single meal provided shortly (between 20 and 35 minutes) after the exercise bout. A longer period of observation could reveal alterations in energy intake at subsequent feeding opportunities that compensate for the initial perturbation from control values. Therefore, the studies presented within this thesis examined the effect of hypoxia and ambient temperature on energy intake. With regard to altitude, the duration of observation was shorter than has previously been reported, and for temperature, was longer. Furthermore, exercise in a cold environment had previously been undertaken via water immersion, thus in Chapter VI, the effect of exercise in cold air was investigated which is likely a more common experience for most individuals than exercising in cold water.

The findings from Chapters V, VI and VII indicate that energy intake is affected by the environmental conditions that exercise and/or rest is undertaken in. In Chapter VII, there was no difference in energy intake between control and exercise conditions suggesting that energy intake is unaffected by exercise. This is in accordance with other literature demonstrating no change in energy intake even after large energy deficits induced via exercise (Martins et al 2008, King et al 2010a). However, it must be acknowledged that the *ad libitum* meal in Chapter VII was provided 4.5 h after the end of exercise, and the effects of exercise on appetite and energy intake are known to be transient. When comparing the effect of hypoxia and normoxia, energy intake at an *ad libitum* meal provided 5.5 h after the start of the trials was lower in hypoxia which confirms findings within the literature. Thus it is apparent that this response occurs even in acute hypoxic exposure. In Chapters V and VI respectively, energy intake tended to be lower after exercise in the heat and tended to be elevated after exercise in the cold. When comparing data between trials, total energy intake was approximately 1953 kJ (466 kcal) higher in the cold trial (10 °C) than in the hot trial (30 °C) and although not significant when comparing independent groups ($P = 0.15$), the results indicate energy intake is responsive to extremes in ambient temperature. These findings have implications for the many individuals who undertake physical activity for both recreational and competitive purposes in environmental extremes. Although not a predictor of success, optimal body composition for specific sports is often advantageous for performance. Although these studies were acute in nature, if these perturbations in energy intake persisted in the longer term, changes in energy balance (whether positive or negative) could detrimentally affect performance.

Although humans can become adapted to high altitude, there can be variability in the extent to which individuals acclimatise. Thus it seems unlikely that adaptive responses in all physiological functions occur after some time in hypoxia. For example, Yan et al (2011) show that in high altitude residents who have are chronically exposed to hypoxia, there is decreased activation in the neural circuit for food craving and these individuals also have a lower body mass despite similar height to matched sea level resident controls. Thus this may indicate these individuals do not consume as much energy as their high matched sea level controls, possibly due to lower food craving. Furthermore, chronic mountain sickness of which appetite loss is a symptom is common in high altitude residents (Moore et al 2007). Additionally, in sojourners to high altitude, a loss in body mass occurs which is dependent on the duration of exposure, and altitude reached (Kayser, 1994). The effects of long-term exposure to extremes in ambient temperature are less clear because individuals can acclimatise to the heat, and although there does not appear to be an adaptive response to the cold, most humans are not habitually exposed to prolonged cold and if they are, clothing and centrally heated buildings can limit the effects.

It is unclear what the physiological relevance of alterations in energy intakes are in these situations. Changes in food intake in response to ambient temperatures seems to serve a logical purpose as Brobeck (1948) suggests that food intakes should be high when the ambient temperature is low to generate metabolic heat, and vice versa when the ambient temperature is high. This is true, at least in the short term, although if food intakes are consistently low in the heat to minimise metabolic heat production then an individual may be in negative energy balance and subsequently lose weight. There does not appear to be a physiological advantage of the loss of appetite and energy intake that occurs at high altitude, because when it persists over several days or more, body mass is lost. Although individuals can become acclimatised after several days to high altitude, and appetite and energy intake can return to normal below altitudes of approximately 4500 m (Ward et al 2000), above these altitudes a loss of appetite and decreased energy intake persist. It is likely that these responses reflect an individual existing in an environment that they were not designed to inhabit. The duration and degree of acclimatisation will vary between individuals with some people never being able to fully acclimatise to high altitude, thus there will likely be large inter-individual variability to the extent and duration of appetite loss and reduced energy intake.

It has previously been suggested that core temperature may be a determinant of subsequent energy intake. In the studies presented within this thesis where ambient temperature varied between trials (Chapter V and Chapter VI), although core temperature differed between trials at the end of exercise in Chapter V, there were no other differences in core temperature between trials, despite differences in energy intake between a temperate environment and a hot and cold environment. The more prolonged period of observation in these studies allowed quantification of energy intake at two meals provided during a 7 h trial. Previous studies have only examined the effect of exercise on the immediate post-exercise meal (Dressendorfer, 1993, White et al 2005, Shorten et al 2009) and alterations in energy intake at the immediate post-exercise meal may have been compensated for at subsequent meals during the day. Core temperature was not assessed in Chapter VII where the ambient temperature was approximately 23 °C, however, it has been shown that core temperature at ambient temperatures between 15.5 and 26.5 °C is depressed during hypoxic exposure (Cipriano and Goldman 1975, Robinson and Haymes 1990). If, as suggested by White et al (2005), a suppression of core temperature increases subsequent energy intake, an increase in energy intake at altitude would be the expected outcome. Thus, it is likely that alterations in core temperature are not directly responsible for changes in energy intake in hypoxia. Skin temperature measurements were not made in these studies, so in future, measurements of skin temperature could be made to determine whether changes in perceptions of thermal sensations from exposed skin may influence an individual's appetite perhaps via an interaction between neurons in the hypothalamus that are involved in maintenance of core temperature and in energy homeostasis.

In Chapter VII, energy intake at the buffet meal was correlated with pre-meal hunger in all trials, confirming the notion that appetite sensations are a reliable method of measuring subjective desire to eat both before and in response to meals (Flint et al 2000). However in Chapters V and VI, there were no correlations between energy intake and appetite immediately prior to a meal. This lack of relationship between energy intake and appetite sensations may not be surprising. Food intake at a meal can be influenced by other variables including the palatability of the food, gender, body weight, genes and other external factors (Drapeau et al 2007). Although we tried to limit the extent of other external factors influencing food intake, this was not always possible. It is known that social factors, such as eating in the presence of others, can influence food intake (Wansink, 2004), and for this reason all participants completed trials on their own. However, it

remains possible that the environment within the chamber may have affected an individual's food intake. It has been described that factors such as lighting and noise may affect the extent to which an individual pays attention to what they consume, for example, when noise is loud or discomforting individuals may consume their meal quickly without paying attention to what or how much they are eating (Wansink, 2004). Although the environment within the chamber was unfamiliar and noisy for the participants, the study design should have eliminated any effect because in every study that utilised the environmental chamber, each trial was undertaken in the chamber. Furthermore, trials were completed in a randomised, counterbalanced order, which should have eliminated any trial order effect. Other studies have observed changes in energy intake in the absence of changes in appetite perceptions. Little et al (2005) suggested that appetite perceptions may be regulated by mechanisms that are different to those involved in the acute regulation of energy intake. It may be that energy intake is more sensitive to change in response to stimuli, whereas appetite perceptions may be more resistant to change and thus may need a stronger stimulus before differences are reported.

In Chapter IV a fixed test meal was provided to participants, and in the other studies presented within this thesis (Chapters V, VI and VII), participants were fed meals (either fixed test meals or *ad libitum* buffet meals) at predetermined times and in Chapters V and VI, both meals available to participants were *ad libitum* buffet meals. The lack of relationship between hunger and energy intake could be in part due to participants not being given the option when to initiate feeding, but upon being presented with food, consuming more than they may have otherwise consumed and actually overriding the physiological need or desire for food. It is important to acknowledge this hedonic aspect of feeding which is associated with the rewarding nature of food. Even in the presence of homeostatic signals that indicate an individual is full, feeding can still be initiated by the taste or smell of food (Van Vugt, 2010). These factors aside, in Chapter VII, hunger was correlated with energy intake prior to the *ad libitum* meals. Hypoxia may have been a strong enough stimulus to be the main affector of appetite and subsequent energy intake and overcome any signals pertaining to the rewarding aspect of food. It has been suggested that in rats, in response to hypoxic stress, feeding behaviour shifts from predominantly metabolic signals to sensory signals (Singh et al 1997) and a fMRI study in high altitude residents shows decreased activation in the neural circuit for food craving (Yan et al 2011). Thus, the decrease in appetite and energy intake in hypoxia could be in

part due to a change in the rewarding aspect of food. Some evidence has shown that subjective perceptions of appetite are associated with energy intake in a controlled laboratory environment but not with energy intake in a free living environment (Mattes, 1990, Parker et al 2004). In the absence of consistent correlations between appetite perceptions and energy intake in the studies presented within this thesis, the benefit of studies that use alterations in appetite as a proxy measure of how energy intake would be affected may be limited. It has been shown that using an *ad libitum* meal to measure spontaneous energy intake is reproducible (Gregersen et al 2008) and furthermore using the buffet type meal design, as utilised in Chapters V, VI and VII, may result in more reliable *ad libitum* energy intakes rather than a fixed meal design because of the increased variety of foods available (Gregersen et al 2008).

Although appetite sensations are reported to be a reliable method of measuring subjective desire to eat before a meal, researchers should be cautious in their interpretation of assuming that changes in subjective ratings of appetite may automatically translate into alterations in energy intake. Eating behaviour is influenced by many factors other than just appetite such as social factors and habitual behaviour as well as an unfamiliar laboratory environment. In summary, this research confirms that energy intake is suppressed in hypoxia and this effect manifests with even acute hypoxic exposure. Ambient temperature also tends to influence energy intake with cold temperatures increasing energy intake and hot temperatures decreasing energy intake. It remains unclear whether more extremes of temperature would further exacerbate the perturbations in energy intake observed here.

8.4 Factors influencing the effect of exercise on acylated ghrelin and total PYY

Broom et al (2007) were the first authors to demonstrate a suppression of acylated ghrelin concentrations during high intensity treadmill running. More recently, suppressed acylated ghrelin concentrations have also been observed during resistance exercise (Broom et al 2009) and swimming (King et al 2011a). Walking does not suppress acylated ghrelin concentrations (King et al 2010b, Unick et al 2010) suggesting that the intensity of exercise is important in this suppression. Studies examining the effect of cycling on acylated ghrelin concentrations are equivocal (Marzullo et al 2008, Ueda et al 2009b, Morris et al 2010) but findings from the first study presented within this thesis (Chapter VI) demonstrate a similar suppression of acylated ghrelin concentrations during high

intensity running and cycling exercise. The findings from Chapters V and VI, confirm that acylated ghrelin concentrations are suppressed during moderate intensity exercise and indicate that hot and cold temperatures during running may differentially modulate this suppression. Comparing acylated ghrelin concentrations during the hot (30 °C) and cold (10 °C) trials for the period prior to the first meal when all participants were fasted did not reveal any significant differences. However, caution must be taken when comparing independent groups because acylated ghrelin concentrations can vary greatly between individuals even those exhibiting normal appetite responses. Exercising in hypoxia exerts a similar suppression of acylated ghrelin as in normoxia suggesting that hypoxia does not differentially affect plasma acylated ghrelin concentrations. However, findings from Chapter VII also reveal that hypoxia alone suppresses acylated ghrelin concentrations over a 7 h period compared with normoxia suggesting that unlike environmental temperature, hypoxia is a sufficient stimulus for suppressing acylated ghrelin concentrations even at rest. In this study, a test meal was consumed at 2 h and an *ad libitum* buffet meal at 5.5 h. The study design enabled acylated ghrelin concentrations to be compared after the first meal to examine the effect of hypoxia on acylated ghrelin concentrations without the confounding influence of participants consuming meals of different caloric and macronutrient content in each trial.

The present findings are in contrast to that of Tomasik et al (2005) who observed significant changes in ghrelin concentrations in response to acute exposure to cold and hot ambient temperatures. However, those authors measured concentrations of total ghrelin rather than the acylated form that is involved in appetite regulation. Total ghrelin concentrations are reportedly unchanged during exercise (Burns et al 2007), yet a decrease in acylated ghrelin is observed (Broom et al 2007), indicating that concentrations of total ghrelin do not likely reflect changes in acylated ghrelin. Tomasik et al (2005) proposed that the changes observed in total ghrelin levels during acute exposure to the heat and cold were in a direction expected to suppress and stimulate appetite respectively. Although in the present studies, no significant differences in acylated ghrelin concentrations were observed between trials in different ambient temperatures, energy intake tended to be influenced by environmental temperature suggesting that other factors, such as other appetite regulatory peptides may mediate this effect. Until now, no studies have examined the effect of hypoxia on acylated ghrelin concentrations, although decreased total ghrelin concentrations have been observed after 48 h exposure to high altitude (Shukla et al 2005).

It has previously been suggested that a decrease in acylated ghrelin concentrations may mediate the suppression of appetite observed during high intensity exercise (Broom et al 2007) because these authors observed a correlation between hunger and acylated ghrelin concentrations during exercise. Given the unique role of acylated ghrelin in stimulating appetite, it was thought that the suppression of appetite and energy intake reported at high altitude may be a consequence of suppressed acylated ghrelin concentrations. In Chapter VII, acylated ghrelin concentrations were suppressed in hypoxia, and both appetite and energy intake were also lower. This may be suggestive of a role for acylated ghrelin in high altitude anorexia, however, no correlations between acylated ghrelin and appetite were observed during the hypoxic trials. The precise role, if any, that acylated ghrelin plays in mediating energy intake in the present studies is not fully clear. Despite no difference in energy intake between control and exercise trials in Chapter VII, acylated ghrelin concentrations were lower in the exercise trials. Similarly, in Chapter IV, although energy intake was not assessed, acylated ghrelin concentrations were lower over the 4 h exercise trials compared with the control trial. A logical expectation would be for acylated ghrelin concentrations to be elevated after exercise to stimulate appetite to compensate for the energy expended during exercise. However, similar to the present findings, evidence suggests that this is not the case, with King et al (2010a) showing that there is no compensatory increase in acylated ghrelin concentrations in response to a large energy deficit (5324 kJ) induced by exercise. In a subsequent study, the same authors show that again, although acylated ghrelin is not stimulated by a large energy deficit induced by exercise, it is stimulated in response to an equivalent energy deficit induced by food restriction (King et al 2011b).

The mechanisms responsible for the transient suppression of acylated ghrelin during exercise, and for the lower values during hypoxia remain unknown and different mechanisms may play a role in these situations. Although reductions in gut blood flow may be involved in the decrease in acylated ghrelin concentrations observed during exercise as blood gets shunted to the working muscles, the same may occur in hypoxia when blood flow could get shunted away from the gastrointestinal system to ensure adequate blood and hence oxygen delivery to the brain. However, limited research suggests gut blood flow is unaffected in hypoxia, but more research should be undertaken to confirm or disprove this finding. Insulin and glucose have been thought to play in a role in ghrelin regulation but evidence suggests that although they may be involved in ghrelin

regulation in response to fasting and feeding, it is unlikely that they explain the whole postprandial ghrelin response. In the present studies, no consistent correlations were observed between glucose and acylated ghrelin concentrations. Furthermore, when acylated ghrelin concentrations were suppressed during exercise, circulating levels of glucose were not always elevated. Although glucose concentrations were elevated in hypoxia whilst acylated ghrelin concentrations were suppressed, the small increase in glucose concentrations was thought insufficient to trigger the suppression of acylated ghrelin. Ghrelin suppression after feeding is influenced by the macronutrient content of the meal with carbohydrate being thought to suppress acylated ghrelin concentrations to a greater extent than fat or protein (El Khoury et al 2006). In Chapter VII, participants were fed a standardised test meal, thus, differences in acylated ghrelin concentrations between the hypoxic and normoxic trials would not have been a consequence of macronutrient content. In Chapters V and VI, acylated ghrelin concentrations were no different between trials, yet there was a tendency for macronutrient preference to be altered, again suggesting that macronutrient content does not play a large role in acylated ghrelin regulation. Acylated ghrelin secretion is regulated by numerous factors that may play major or minor roles dependent on energy balance and nutrient status. With the relatively recent discovery of the enzyme, GOAT, responsible for the acylation of ghrelin (Gutierrez et al 2008, Yang et al 2008) research is still emerging as to how it is influenced by various factors such as nutrient availability. Thus, it is still unclear what effect hypoxia has on GOAT and its involvement, if any, in decreased acylated ghrelin concentrations observed in acute hypoxic exposure however, research in this area is warranted. It also remains unclear what mechanisms are responsible for controlling the rate at which acylated ghrelin becomes des-acylated ghrelin. It is also important in future to investigate other aspects of the ghrelin system in defining its role in energy homeostasis, such as ghrelin and GOAT mRNA in the stomach which is the main site of ghrelin production, as well as how its receptor in the brain is affected by different environmental conditions. If the entire ghrelin/GOAT axis can be affected by obesity arising from nutritional manipulations (Briggs and Andrews 2011) then it is feasible this system may be affected by environmental factors.

In the last study presented within this thesis (Chapter VII), circulating concentrations of the anorectic gut hormone PYY were measured. Peptide YY is one of a handful of gut hormones involved in satiety. However, although there is a large body of literature highlighting the role of PYY in appetite regulation (Neary and Batterham 2009), studies

examining the effects of exercise on PYY concentrations are still emerging (Martins et al 2007, Broom et al 2009, Shorten et al 2009). Furthermore, knowledge of the effect of exercising in environmental extremes on PYY concentrations is limited, with one study examining the effect of exercise in the heat on PYY (Shorten et al 2009) but no research investigating the effect of exercise in the cold, or exercise in hypoxia. Thus, the choice was made to measure concentrations of PYY rather than other anorectic gut peptides such as GLP-1 or CCK because some research already exists concerning the effect of environment on these hormones (Bailey et al 2001, Snyder et al 2008). Findings from Chapter VII demonstrated elevated PYY concentrations in response to exercise, an effect previously reported (Martins et al 2007, Broom et al 2009, Shorten et al 2009, Ueda et al 2009a). Peptide YY concentrations tended to be elevated in normoxia, a finding which would refute a role for PYY in mediating the loss of appetite observed at high altitude. Shorten et al (2009) observed elevated concentrations of PYY after exercise in the heat which they proposed may be responsible for the lower energy intake observed in that trial. Thus, the present findings confirm that PYY is influenced by exercise but is likely not involved in the disturbed appetite regulation in hypoxia whereas it may have a role in the reduction in energy intake observed after exercise in the heat (Shorten et al 2009).

The leptin gene is activated in response to hypoxia (Guerre-Millo et al 2002, Yingzhong et al 2006), however, studies conducted in the field have reported conflicting results concerning the effect of high altitude on circulating leptin concentrations (Tschöp et al 1998, Shukla et al 2005, Barnholt et al 2006, Benso et al 2007, Zaccaria et al 2004). Leptin concentrations were measured in Chapter VII, however, possibly due to the homogenous group of lean individuals who participated in the study, many sample concentrations were below the dynamic range of the assay. Therefore, it was not possible to examine the effect of acute hypoxic exposure on leptin concentrations.

Collectively, the studies presented within this thesis demonstrate that the appetite regulatory peptides acylated ghrelin and PYY are affected by exercise and environmental conditions such as temperature and hypoxia. Given the changes in energy intake observed, these perturbations in gut hormone concentrations are not always in a direction that would be expected which highlights the complex mechanisms by which appetite is regulated.

8.5 Limitations and direction for future research

There were some limitations associated with the studies presented within this thesis and some of these issues could be addressed in future work in this area. Study participants were young, generally lean and healthy males who were predominantly recruited from within the student populations at Loughborough and Nottingham Trent Universities. As such, the homogenous nature of the group may limit the applicability of the findings to other population groups such as females or the overweight/obese. It has been suggested that the ease and convenience of recruiting undergraduate students may detract from the relevance of the research question (Caudwell et al 2011). Thus future studies should examine the effect of environmental extremes on different population groups, in whom, the regulation of appetite may be perturbed. The studies presented in this thesis provide novel, descriptive data concerning the effects of environmental extremes on some appetite regulatory peptides but future work should not simply examine the effects of these environments on concentrations of other hormones implicated in appetite regulation, but should also seek to understand the mechanisms responsible for any changes observed.

The studies presented provide a sound base from which to continue further research in this area particularly regarding the effect of ambient temperature on energy intake and appetite regulatory peptides. Although the ambient temperatures used in the chapters within this thesis were suitable to expose an individual for a prolonged duration and thus examine energy intake at two meals, in future, participants should be exposed to more extreme environmental temperatures over shorter durations to fully establish the effect of temperature on appetite regulation. Furthermore, concentrations of other anorexigenic gut peptides such as GLP-1 should be quantified to try and understand the complex interplay between the appetite-regulating hormones. Similarly, with regard to the effect of altitude, a study with several trials over increasing altitudes should be conducted to examine whether there is a dose-response effect of altitude on energy intake and appetite-regulatory peptides.

Whilst the study of appetite regulatory gut peptides in plasma allows quantification of circulating concentrations of these hormones, increases or decreases in concentrations may not reflect the subsequent action of the hormone; for instance it is important to elucidate the interaction of the hormones with their receptors under such environmental conditions.

With regard to acylated ghrelin, future studies should also measure concentrations of total ghrelin (unacylated and acylated ghrelin) or unacylated ghrelin so that the ratio of acylated ghrelin to unacylated ghrelin can be established. If total ghrelin was unchanged in the presence of changes in acylated ghrelin, this would indicate a change in acylated ghrelin possibly due to changes in substrate (usually octanoate) or GOAT activity, whereas if total ghrelin concentrations were altered, changes in acylated ghrelin may merely reflect a change in total ghrelin secretion. Given the relatively recent identification and characterisation of GOAT (Gutierrez et al 2008, Yang et al 2008), there is still much more research to be done examining how various factors such as hypoxia may affect its expression or activity and thus might be involved in the decrease in acylated ghrelin concentrations observed in hypoxia reported within this thesis.

In summary, the research presented in this thesis firstly, provides a valuable insight into how environmental factors such as hypoxia and ambient temperature affect the regulation of energy homeostasis and secondly, lays the foundations for further work and development within this area. Furthermore, findings from the first study demonstrating similar profiles of acylated ghrelin changes in response to running and cycling exercise pave the way for similar research to be undertaken in populations such as the obese but using more appropriate exercise modes.

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APPENDIX A

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 B

Loughborough University

Participant ID .....

Health Screen Questionnaire for Study Volunteers

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

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

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

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

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

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

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

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

(a)	Convulsions/epilepsy	Yes		No	
(b)	Asthma	Yes		No	
(c)	Eczema	Yes		No	
(d)	Diabetes	Yes		No	
(e)	A blood disorder	Yes		No	
(f)	Head injury	Yes		No	
(g)	Digestive problems	Yes		No	
(h)	Heart problems	Yes		No	
(i)	Problems with bones or joints	Yes		No	
(j)	Disturbance of balance/coordination	Yes		No	
(k)	Numbness in hands or feet	Yes		No	
(l)	Disturbance of vision	Yes		No	
(m)	Ear / hearing problems	Yes		No	
(n)	Thyroid problems	Yes		No	
(o)	Kidney or liver problems	Yes		No	
(p)	Allergy to nuts	Yes		No	

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

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

.....

5. Allergy Information

(a)	are you allergic to any food products or alcohol?	Yes ☐	No ☐
(b)	are you allergic to any medicines?	Yes ☐	No ☐
(c)	are you allergic to plasters?	Yes ☐	No ☐

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

……..

One of questions 6 – 8 was asked as applicable for each of the heat, cold and altitude studies.

6. Have you suffered from any form of cold injury prior to taking part in this study?

Yes ☐ No ☐

7. Have you suffered from any form of heat illness prior to taking part in this study?

Yes ☐ No ☐

8. Have you resided at altitude during the previous 3 months?

If so, at what altitude and for how long:

9. Are you currently fit and healthy and able to undertake the exercise this study requires?

Yes ☐ No ☐

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

Name and relationship to participant:

…….

Telephone Number:

…….

Work ☐ Home ☐ Mobile ☐

11. Are you currently involved in any other research studies at the University?

Yes ☐ No ☐

If yes, please provide details of the study

……

……

APPENDIX C

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, volleyball, 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 D

Part 1 : Please circle either true or false

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

9) When I feel anxious, I find myself eating

True False

10) Life is too short to worry about dieting

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

20) When I feel blue, I often overeat.

True False

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

True False

22) When I see real delicacy, I often get so hungry I have to eat right away.

True False

23) I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.

True False

24) I get so hungry that my stomach often seems like a bottomless pit.

True False

25) My weight has hardly changed at all in the last 10 years.

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

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

True False

Part 2.
Please answer the following questions by circling the number above the response that is appropriate to you.

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

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
Not at all	slightly	moderately	very much

39) How often do you feel hungry?

1	2	3	4
Only at meals	sometimes between meals	often between meals	almost always

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

1	2	3	4
Never	rarely	often	always

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

1	2	3	4
Easy	slightly difficult	moderately difficult	very difficult

42) How conscious are you of what you are eating?

1	2	3	4
Not at all	slightly	moderately	extremely

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

1	2	3	4
Almost never	seldom	usually	almost always

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

1	2	3	4
Unlikely	slightly unlikely	moderately likely	very likely

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

1	2	3	4
Never	rarely	often	always

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

1	2	3	4
Unlikely	slightly likely	moderately likely	very likely

47) How frequently do you skip dessert because you are no longer hungry?

1	2	3	4
Almost never	seldom	at least 1 a week	almost every day

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

1	2	3	4
Unlikely	slightly likely	moderately likely	very likely

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

1	2	3	4
Never	rarely	sometimes	at least 1 a week

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

0
Eat whatever you want, whenever you want it

1
Usually eat whatever you want, whenever you want it

2
Often eat whatever you want, whenever you want it

3
Often limit food intake, but often 'give in'

4
Usually limit food intake, rarely 'give in'

5
Constantly limiting food intake, never 'giving in'

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

1	2	3	4
Not like me	little like me	pretty good description of me	describes me perfectly

APPENDIX E

A list of the items available for participants at the *ad libitum* meals

- Milk
- Breakfast cereal (3 varieties)
- Bread (white and brown)
- Fruit (apples, bananas and oranges)
- Cheddar cheese
- Ham
- Tuna
- Margarine
- Mayonnaise
- Muffins
- Salted crisps
- Chocolate bars
- Chocolate rolls
- Chocolate chip cookies
- Nutri-grain bars
- Yoghurt
- Orange Juice
- Cola
- Nesquik

} Available in Chapter V and Chapter VI

APPENDIX F

Visual Analogue Scale

Time……

Subject Number: …… **Trial: ……..…..** **Date:…….**

Core Temp (˚C) ……

HR (bpm) ……..

Thermal Sensations…...

Temperature (˚C) ……

Humidity (%) …….

Please indicate how hungry you feel by circling the number which corresponds to your feeling of hunger, with 0 being not at all hungry and 15 being the most hungry you have ever felt

Not Hungry				Fairly Hungry				Hungry				Very Hungry			
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

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

How hungry do you feel?

Not at all hungry ——— Very Hungry

How satisfied do you feel?

I am completely empty ——— I cannot eat another bite

How full do you feel?

Not at all full ——— Totally full

How much do you think you can eat?

Nothing at all ——— A lot

APPENDIX G

Subject ID …….. **Trial:** SL con /SL ex / AL con / AL ex **Date ….....**

Time…….. **Oxygen Saturation……**

Lake Louise Acute Mountain Sickness Self-Report Questionnaire

Please complete the following questions 1 -4 by circling the number that corresponds most closely with how you feel.

1) Headache	0	No Headache
	1	Mild Headache
	2	Moderate Headache
	3	Severe Headache, incapacitating
2) Gastrointestinal symptoms	0	None
	1	Poor appetite or nausea
	2	Moderate nausea and/or vomiting
	3	Severe nausea and/or vomiting
3) Fatigue and/or weakness	0	Not tired or weak
	1	Mild Fatigue/weakness
	2	Moderate Fatigue/weakness
	3	Severe Fatigue/weakness
4) Dizziness/Lightheadedness	0	Not Dizzy
	1	Mild Dizziness
	2	Moderate Dizziness
	3	Severe Dizziness/incapacitating

Total Score:……