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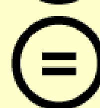

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EFFECTS OF EXERCISE ON APPETITE, FOOD INTAKE AND THE GASTROINTESTINAL HORMONES GHRELIN AND PEPTIDE YY

by

James Adam King

A Doctoral Thesis

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

September 2010

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified in acknowledgments or in footnotes, and that neither the thesis nor the original work contained therein has been submitted to this or any other institution for a degree.

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ABSTRACT

Gut hormones are implicated in the regulation of energy balance. The studies in this thesis have examined the effects exercise on gut hormones (acylated ghrelin and peptide YY_{3-36}), appetite and food intake, over extended durations. Sixty-nine young, healthy, predominantly Caucasian males were recruited to six studies. The age, height and body mass of the participants were: 22.4 ± 0.3 y, 1.80 ± 0.1 m, 76.2 ± 1.0 kg (mean ± SEM).

In study one, 90 min of resistance exercise did not influence appetite or energy intake over 24 h of assessment, yet stimulated a latent preference for carbohydrate rich foods. Study two demonstrated that appetite was suppressed during 60 min of swimming but was elevated after consuming a post-exercise meal. Plasma acylated ghrelin was suppressed during swimming but was unaltered after. Energy/macronutrient intake remained unchanged. In study three, 60 min of brisk walking (45 ± 2% of $\dot{V}O_2$ max) did not influence appetite, energy/macronutrient intake or plasma concentrations of acylated ghrelin during an eight hour observation period. Study four showed that 90 min of treadmill running (69 ± 1% of $\dot{V}O_2$ max) transiently suppressed appetite and acylated ghrelin but did not influence these variables, or energy/macronutrient intake within 22.5 h after exercise. The findings of study five suggest that the suppression and subsequent rebound in plasma acylated ghrelin after exercise may be related to a delayed voluntary decision to eat after. Finally, study six showed that appetite, food intake and circulating concentrations of acylated ghrelin and peptide YY_{3-36} are responsive to acute deficits in energy induced by food restriction but are not sensitive to equivalent energy deficits induced by exercise.

This thesis has shown that exercise transiently alters circulating levels of acylated ghrelin and peptide YY_{3-36} in directions expected to inhibit appetite however no changes are seen after exercise. Conversely, food restriction elicits marked compensatory changes in circulating acylated ghrelin and peptide YY_{3-36}. This thesis also demonstrates that resistance exercise, brisk walking and running do not stimulate appetite or energy intake over defined periods, even when the energy expenditure elicited is high. Swimming appears to increase appetite in the latter hours after exercise.

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

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J Sports Sci. (2008); 26(S2): S9.

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The influence of prolonged treadmill running on plasma acylated ghrelin, appetite and energy intake

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LIST OF ABBREVIATIONS

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

ANOVA (analysis of variance)

AUC (area under the concentration verses time curve)

BMI (body mass index)

CCK (cholecystokinin)

EDTA (ethylenediaminetetraacetic acid)

GH (growth hormone)

GHSR (growth hormone secretagogue receptor)

GLP-1 (glucagon-like-peptide-1)

OXM (oxyntomodulin)

PYY (peptide YY)

RPE (ratings of perceived exertion)

RQ (respiratory quotient)

SEM (standard error of the mean)

CHAPTER I

Introduction

Throughout the world the prevalence of overweight and obesity is increasing at an alarming rate in both developed and developing countries (Kelly et al, 2008). Recent World Health Organisation estimates indicate that 1.6 billion adults are overweight (body mass index 25 – 29.9 kg·m^{-2}) with an additional 400 million individuals qualifying as obese (body mass index $\geq$30 kg·m^{-2}) (WHO, 2006). In the United Kingdom (UK) the situation is no different with rates of obesity having more than doubled in the last 25 years. In England 42% of men and 32% of women are overweight with a further 25% of men and women being classified as obese (National Health Service Information Centre, 2010). Based on recent trends it has been predicted that approximately 60% of the UK population will be obese by 2050 (Government Office for Science, 2007). Only time will tell if this prediction is accurate but it is an alarming prospect.

Obesity develops when energy intake exceeds that expended over a defined period. Although hereditary may predispose certain individuals to becoming overweight or obese, the dramatic rate of increase in global prevalence during recent years indicates that genes are not the primary cause (Martinez et al, 2000; Farooqi and O'Rahilly, 2006). Instead, changes within the environment, most notably diets denser in energy and fat, combined with a reduction in physical activity during work and leisure, are more firmly implicated in the aetiology of overweight and obesity (Hill, 1998).

As the obesity epidemic has spread, concern about the significant health and economic ramifications has grown. Being overweight is associated with a range of adverse health outcomes such as heart disease, diabetes mellitus, hypertension, reproductive dysfunction, osteoarthritis, gall bladder disease and certain forms of cancer (Bray, 2004; Haslam and James, 2005). In England, the total costs attributed to overweight and obesity have been estimated at seven billion pounds annually, of which one billion is ascribed to the direct healthcare costs of treating associated health conditions (McCormick and Stone, 2007). Effective interventions are therefore needed to help individuals achieve and maintain a healthier body weight.

Bariatric surgery is the most effective long-term treatment for obesity however surgery should only be considered for individuals with a body mass index greater than 40 kg·m^{-2} or 35 kg·m^{-2} when associated with severe obesity related comorbidities (Bult et al, 2008). Furthermore, the defined risks associated with surgery, in addition to the expense endured, restricts its use only to those with the greatest need once all other therapeutic options have been exhausted. Consequently, bariatric surgery does not offer a viable therapeutic option for the majority of overweight or modestly obese individuals within any given population.

Over the last 100 years numerous pharmacological weight loss agents have been made available however nearly all of these have since been removed from the commercial market due a lack of efficacy or admits safety concerns (Bray, 2008). Sales of rimonabant, a cannabinoid receptor antagonist, were halted in October 2008 in light of research showing an increased risk of psychiatric disorders. More recently, the centrally acting noradrenaline-serotonin reuptake inhibitor – sibutramine, was removed from the commercial market in January 2010 in response to findings showing a significantly augmented risk of non-fatal myocardial infarction and stroke (Williams, 2010). Consequently, at present orlistat remains the only licensed weight loss medication available within the UK. Orlistat is a gastrointestinal lipase inhibitor which impairs the digestion and absorption of fat so that fewer calories are available to the body. Over six months, a 6 – 10% reduction in body weight can be expected with reasonably good maintenance for up to two years with continued use (Sjostrom et al, 1998; Davidson et al, 1999). Predictably though, given its mechanism of action, unpleasant side-effects are common, such as flatus, oily stools and faecal urgency and may over time lead vitamin deficiencies (Perrio et al, 2007; Wilding, 2008a). It is these side effects, in addition to the seemingly impossible feat of developing safe yet chronically effective drugs, that has limited the usefulness of non-surgical pharmacological therapies to date (Neary and Batterham, 2009a).

Dietary manipulation remains the most common method of weight control and a range of diets have been advocated including low calorie and fat restricted diets, and low carbohydrate diets (Malik and Hu, 2007). Although dietary interventions have the potential to induce short-term weight loss in determined individuals, weight regain

occurs over time in the vast majority of persons, rendering such practices relatively ineffective at producing sustained weight loss (Donnelly and Smith, 2005).

An increasing body of research suggests that physical activity is an important component of successful weight management (Donnelly and Smith, 2005; Donnelly et al, 2009). It has been explicitly stated that exercise on its own, regardless of dietary intervention, is an effective strategy for reducing obesity and its related health complications (Ross et al, 2000). Cross-sectional studies typically demonstrate an inverse relationship between body weight or body mass index and levels of physical activity across the lifespan i.e. greater amounts of physical activity are related to progressively lower body weight and/or body mass index (Martinez et al, 1999; Ball et al, 2001) and there is evidence indicating a dose-response relationship between these variables (McTiernan et al, 2007). These findings highlight an important role of physical activity in preventing weight gain.

According to Donnelly and Smith (2005) more than half of individuals who lose weight regain it within a year, demonstrating a strong evolutionary drive to preserve body weight and thus an inherent resistance to weight loss. In addition to preventing weight gain, physical activity also appears to be a useful strategy which can facilitate successful weight loss maintenance (Catenacci and Wyatt, 2007; Donnelly et al, 2009). It has been suggested that an individual's level of physical activity is the best predictor of weight maintenance after weight loss (Tate et al, 2007; Catenacci et al, 2008). Perhaps the best example of this comes from the National Weight Control Registry (NWCR) in the United States. The NWCR has a cohort of over 6000 individuals who have maintained a minimum 13.6 kg weight loss for at least a year. Reports from this study demonstrate that physical activity is an integral component of successful weight loss maintenance, with individuals typically expending more than 2600 kcal per week though various forms of physical activity (Klem et al, 1997; Catenacci et al, 2008).

Although physical activity may attenuate initial weight gain and prevent weight regain after successful weight loss, the ability of physical activity to directly induce weight loss is more ambiguous (Donnelly and Smith, 2005). Some findings suggest that under strictly controlled conditions an increase in physical activity can induce significant weight loss (Ross et al, 2000; 2004) however if rigorous control is not imposed weight

loss may not occur. In this latter scenario it seems that failure to lose weight is due to the recruitment of adaptive metabolic and behavioural compensatory mechanisms which oppose weight loss, such as a reduction in exercise compliance, resting metabolic rate, and an increase in exercise efficiency (King et al, 2007). Each of these responses limit the potential for exercise to induce weight loss. In addition to this, the most important factor constraining weight loss appears to be a compensatory increase in energy intake to buffer that expended during exercise (King et al, 2008).

This notion was illustrated in a recent study conducted by King and Co-workers (2008). Thirty-five overweight and obese, sedentary men and women completed a 12 week supervised exercise intervention, expending 500 kcal on five days each week through aerobic activity. Body composition, resting metabolism, appetite and daily energy intake were assessed at baseline and at the end of the investigation. Over the 12 weeks body weight decreased by 3.7 kg however closer scrutiny of the data revealed large inter-individual variation in weight change between individuals (-14.7 kg to +1.7 kg). In an attempt to identity the characteristics of those who did and did not lose weight, participants were divided into compensators and non-compensators. Participants were labelled compensators if their actual weight loss was less than their predicted weight loss and non-compensators if their weight loss was equal to or greater than expected weight loss. In this study exercise compliance could not explain the differential in response as exercise sessions were supervised, nor could changes in resting metabolism. Instead, the researchers found that compensators experienced greater hunger at the end of the study and this was associated with a significant increase in daily energy intake (268 kcal). Conversely, non-compensators exhibited a reduction in daily energy intake (-130 kcal). These findings indicate that individuals who do not lose weight in response to exercise are compensating for the energy expenditure by increasing their energy intake. These findings highlight the importance of understanding the impact of physical activity on appetite and food intake.

Throughout the nineties researchers began to examine the influence of physical activity on appetite and energy intake. With specific regards to the influence on appetite, the most consistent effect reported from this work was that high intensity physical activity induces a transient suppression in appetite, a phenomena which has been termed ‘exercise induced anorexia’ (King et al, 1994) however this does not appear to influence

subsequent energy intake (King and Blundell, 1995; King et al, 1996). Similarly, in contrast to expectation, there appears to be a rather loose coupling between the energy expended through physical activity and energy intake in the short-term thereafter. Thus, physical activity does not appear to stimulate energy intake in the immediate hours after exercise (for reviews see Blundell et al, 2003; Martins et al, 2008).

Although the work highlighted above has provided a useful starting point for examining the influence of physical activity on appetite and food intake, there are notable limitations within this work and additional areas of enquiry yet to receive attention. On this latter point exercise mode is an important issue. Specifically, many forms of exercise are undertaken within the population yet studies that have examined the effects of physical activity on appetite and food intake have tended to use cycling and running as the exercise stimulus. Walking, swimming and resistance exercise are other popular forms of activity undertaken yet there is a lack of information about how these modes of activity influence appetite, food intake and energy homeostasis. The physiological and metabolic reactions to exercise are determined by the characteristics of the activity being performed e.g intensity, duration, muscle mass recruited – therefore it is possible that appetite and food intake responses may be specific to the mode of activity performed.

A second limitation of this previous work cited above concerns the duration of time over which responses have been examined. Typically, appetite and energy intake have been examined over a relatively brief period of time, commonly in response to single meals. Any effects of physical activity on appetite and energy intake may occur over a longer duration, therefore observations in response to additional meals, over a longer period of time, are necessary (Bilski et al, 2009).

A third issue concerns the meals from which energy intake responses have been examined. Meals have typically been provided to participants in a buffet format however the diversity of items presented has been severely limited, commonly to a handful of items (Kissileff et al, 1990; Verger et al, 1992; King et al, 1994; King et al, 1996; Ballard et al, 2009). Not only does this lack ecological validity, it also prevents the assessment of effects of physical activity on macronutrient preferences. The macronutrient composition of the food that we consume is a strong determinant of

energy consumption therefore it is important to examine the effects of physical activity on this variable (King et al, 1994). Provision of meals of sufficient content and macronutrient diversity would be necessary to assess this issue in greater depth (Arvaniti et al, 2000).

Until recently, attempts to understand the mechanisms responsible for changes in appetite and food intake after exercise have been limited to speculations based on circulating metabolites such as glucose, lactate and free fatty acids or stress hormones including corticotrophin releasing factor, adrenocorticotropic hormone, cortisol and the catecholamines (Scheurink et al, 1999). Over the last decade however, an increase in knowledge regarding the neuroendocrine regulation of appetite and food intake has promoted research interest within this area with more investigators examining the effects of physical activity on circulating peptides implicated in the regulation of appetite and feeding (Martins et al, 2008; Bilski et al, 2009).

As knowledge within the area of appetite control has developed over recent years it has become apparent that a complex system of afferent signals and efferent effectors operating between peripheral tissues and the central nervous system work synergistically to regulate appetite and energy intake (Moreton et al, 2006; Karra and Batterham, 2010). Specifically, the gastrointestinal tract, pancreas and adipose tissue secrete a diverse range of peptides which act centrally to inform the brain of acute and chronic energy stores and nutrient requirements. These signals are integrated within neurons located in brain regions implicated in the regulation of energy homeostasis, most notably the hypothalamic arcuate nucleus. These signals are then relayed to higher order neurons which mediate appetite perceptions and feeding behaviour (Murphy and Bloom, 2006).

Interest in this area was ignited by the discovery of leptin in the early nineties as a circulating factor responsible for informing the central nervous system of chronic energy reserves deposited within adipose tissue (Zhang et al, 1994). Since then, many peptides implicated in the regulation of appetite and feeding have received significant attention, most notably, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), pancreatic polypeptide, oxyntomodulin (OXN), ghrelin and peptide YY (PYY). In contrast to leptin, these peptides regulate appetite and food intake on an acute, meal to

meal basis, influencing the decision to start and stop eating (meal initiation and satiation) as well as the duration of time in between discrete meals (satiety). Of these peptides ghrelin remains conspicuous as a circulating factor which stimulates appetite and food intake, all other peptides cited above are secreted in response to nutrient ingestion and serve to inhibit appetite and feeding (Karra and Batterham, 2010).

The last decade has observed an increase in research seeking to characterise the effects of exercise on peptides implicated in the regulation of appetite and food intake (Martins et al, 2008; Bilski et al, 2009). Within this, ghrelin has received explicit attention (Kraemer and Castracane, 2007). Ghrelin is a 28 amino acid peptide that was discovered as the natural endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima et al, 1999). Soon after ghrelin's discovery the appetite stimulating properties of ghrelin were uncovered (Arvat et al, 2001; Wren et al, 2001), and as the only known circulating orexigenic peptide, this characteristic has identified ghrelin as an attractive target for investigation.

Within the circulation two forms of ghrelin exist, namely acylated and unacylated. Acylated ghrelin is made explicit by the post-translational addition of a medium chain fatty acid to its third amino acid residue. This modification is necessary in order for ghrelin to bind to the GHS-R and cross the blood-brain barrier, mechanisms which permit the role of ghrelin in the regulation of growth hormone release and energy metabolism (Kojima and Kangawa, 2005). Despite this, initial work examining the ghrelin response to exercise measured circulating concentrations of total ghrelin and primarily sought to examine the role of ghrelin in mediating exercise induced changes in growth hormone (GH) secretion. More recently, with an increase in knowledge regarding the neuroendocrine regulation of feeding, studies have sought to examine changes in ghrelin in response to exercise from an appetite regulatory perspective (Martins et al, 2008). Furthermore, with the development of commercially available assays specific for acylated ghrelin, investigators have begun to specifically measure acylated ghrelin (Broom et al, 2007; 2009; Marzullo et al, 2008; Ueda et al, 2009). Findings from these studies tend to show that circulating acylated ghrelin is suppressed by high intensity exercise however the physiological relevance of this remain unclear i.e. we do not know whether exercise-induced changes in acylated ghrelin influence food intake. The studies described in this thesis sought to examine this issue.

Peptide YY is a 36 amino acid peptide hormone that inhibits appetite and food intake (Batterham et al, 2002; 2003). Peptide YY is secreted from the distal intestine after nutrient ingestion in proportion to the caloric load and is an important determinant of satiation and satiety (Adrian et al, 1985a; Karra and Batterham, 2010). Two forms of PYY exist within the circulation, namely PYY_{1-36} and PYY_{3-36}. The latter is a truncated 34 amino acid peptide produced by cleavage of the N-terminal tyrosine and proline amino acid residues from PYY_{1-36} by the enzyme Dipeptidyl-peptidase IV (Mentlein et al, 1993) and is the main circulating form of PYY in both the fed and fasted state (Batterham et al, 2006). This structural modification is necessary for modulating digestive and feeding behaviour, as well as initiating satiety after a meal in response to an increase in circulating levels.

Circulating concentrations of PYY are inversely related to multiple measures of adiposity (Batterham et al, 2003; Roth et al, 2005; Guo et al, 2006) and attenuated levels have been linked with reduced satiety in overweight individuals (Le Roux et al, 2006). Consequently, the recognition that obese individuals remain sensitive to the anoretic effects of exogenous PYY (Batterham et al, 2003) has identified this hormone as an exciting therapeutic target. In 2007 Martins and co-workers were the first to publish findings regarding the effects of exercise on circulating levels of PYY. The researchers observed a significant increase in circulating levels immediately after exercise and since then other researchers have replicated this finding (Broom et al, 2009; Ueda et al, 2009). Unfortunately, circulating concentrations of total PYY were measured in these studies rather than $PYY_{3-36.}$ Only one investigation has examined the $PYY_{3-36.}$response to exercise (Cheng et al, 2009). Findings from this investigation suggest that PYY_{3-36} may not respond to exercise *per se*, but exercise may accentuate the PYY_{3-36} response to feeding. This would be a positive finding in the context of weight control, therefore further work is needed to better characterise the PYY_{3-36} response to exercise.

The primary rationales for conducting the studies described in this thesis were two-fold. The first objective was to characterise the effects of exercise mode (resistance exercise, swimming, walking and running) on appetite perceptions and *ad libitum* energy intake over a prolonged duration, rather than in response to single meals. The second aim was assess the effects of exercise on the gut peptides acylated ghrelin and PYY_{3-36}. Within

this, the association between these peptides, perceptions of appetite and *ad libitum* food intake were explored to determine the physiological relevance of exercise-induced changes in the circulating concentrations of these regulatory peptides.

The first study in this thesis (Chapter four) examined the effect of resistance exercise on appetite and *ad libitum* energy intake over an extended, 24 h period. Study two (Chapter five) sought to examine the influence of an acute bout of swimming on appetite, *ad libitum* energy intake and circulating concentrations of acylated ghrelin. Study three (Chapter six) assessed the effects of brisk walking on appetite, *ad libitum* energy intake and plasma acylated ghrelin concentrations. Study four (Chapter seven) examined appetite, *ad libitum* energy intake and circulating acylated ghrelin concentrations over 24 h in response to a prolonged bout of treadmill running – sufficient to induce a severe energy deficit. Study five (Chapter eight) assessed the effects of treadmill running on feeding latency and examined the potential role of acylated ghrelin in mediating this. The final study reported in this thesis (Chapter nine) examined the PYY_{3-36} response to treadmill running and compared appetite, energy intake, acylated ghrelin and PYY_{3-36} responses to energy deficits induced by exercise as compared with diet.

It is clear that over the last three decades overweight and obesity have developed into significant health and economic problems for nations across the globe. So far there is no ‘magic bullet’ which will halt the year on year increase in prevalence. Dietary, pharmacological and surgical therapies are available however it appears that these strategies are ineffective at controlling weight in the long-term. Studies show the potential of exercise to help in weight control however they also show that this can be undone through negative effects on appetite and food intake. Understanding the relationship between different types of exercise, appetite and food choices, and their mechanisms of regulation, may help us to optimise interventions to help individuals maintain a healthy body weight and composition.

CHAPTER II
Review of literature

2.1 Gastrointestinal regulation of appetite and energy intake

The observation that body weight remains remarkably constant over long periods of time despite large fluctuations in daily energy intake and expenditure indicate the presence of a system regulating appetite and energy intake (Wynne et al, 2005c). A complex system composed of afferent signals and efferent effectors operating within the central nervous system and peripheral tissues work synergistically to regulate appetite and energy intake on both an acute (meal to meal) and chronic basis (Wynne et al, 2005c; Moreton et al, 2006). The arcuate nucleus in the hypothalamus is the key central nervous system region governing the homeostatic regulation of appetite and energy intake although other brain regions located within the brain stem such as the nucleus tractus solitaries and area postrema are also important (Williams et al, 2000; Badman and Flier, 2005). Figure 2.1 provides a simplified schematic representation of this system.

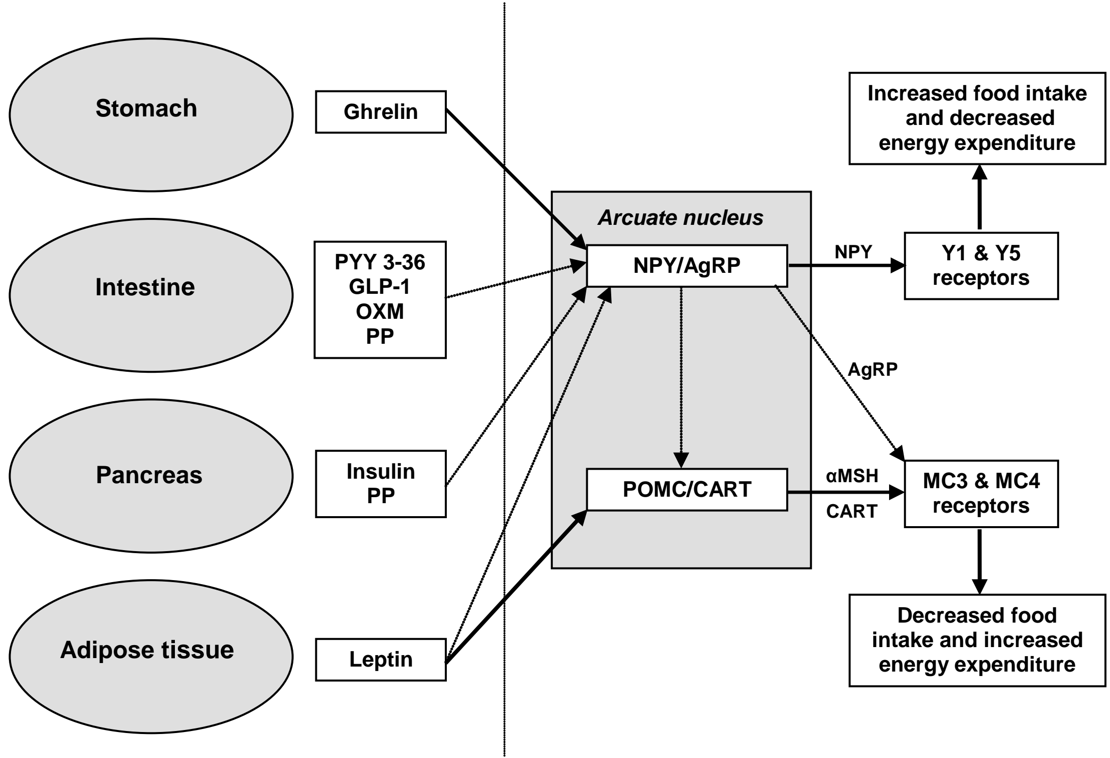

Figure 2.1: Peripherally derived hormones influencing energy homeostasis via the arcuate nucleus (adapted from Murphy and Bloom, 2004). Bold lines indicate stimulatory effects and dashed lines indicate inhibitory effects. AgRP, agouti-related peptide; CART, cocaine-and amphetamine-related transcript; GLP-1, glucagon-like-peptide 1; αMSH, alpha-melanocyte-stimulating hormone; NPY, neuropeptide Y; MC3/4, melanocortin receptors; NPY Y1 and Y5 receptors; OXM, oxyntomodulin; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PYY, peptide YY.

The system responsible for regulating appetite and energy intake is composed of neural and endocrine components. Peptide hormones communicating both acute and chronic energy status are secreted into the circulation from peripheral structures such as the gastrointestinal tract, pancreas and adipose tissue and reach appetite regulatory centres within the brain via entry through a partially permeable blood-brain barrier (Badman and Flier, 2005). These peripheral signals may also reach these sites via neural connections between the gut and brain, most notably the vagus nerve which connects the gut to the brainstem (Berthoud, 2008). The arcuate nucleus within the hypothalamus is the site where these signals are integrated. In this area two primary neuronal populations are responsible for integrating afferent signals and regulating the expression of appetite stimulating/inhibiting neuropeptides. Neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) stimulate higher order neurons which

promote appetite and energy intake whilst reducing energy expenditure. Conversely, neurons expressing pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) induce the expression of neuropeptides responsible for reducing appetite and food intake whilst increasing energy expenditure (Morton et al, 2006).

Kennedy (1953) first proposed the presence of a circulating factor responsible for informing the brain of adipose tissue reserves however at the time the exact mechanism remained unknown. In the early nineties this mechanism was elucidated with the discovery of leptin (Zhang et al, 1994). Leptin is a peptide derived primarily from white adipose tissue and is responsible for communicating information to the central nervous system regarding stored energy. Leptin is released into the circulation in direct proportion to levels of adiposity and operates as a long-term negative feedback signal, reducing appetite and increasing energy expenditure when circulating concentrations are elevated (Friedman, 2002). The initial discovery of leptin was met with optimism as it was thought that low circulating levels may have been implicated in the aetiology of obesity. Paradoxically, circulating concentrations of leptin are elevated in most obese individuals indicating obesity as a state of leptin resistance (Considine et al, 1996).

Insulin, produced by the beta cells of the pancreas, is a second factor which acts as an adiposity signal informing the brain of long-term energy reserves. Although circulating concentrations of insulin fluctuate in response to individual feeding episodes, over time, circulating levels directly represent adipose tissue mass (Wynne et al, 2005b). In rodents central administration of insulin reduced food intake and subsequently body weight (Ikeda et al, 1986). While both insulin and leptin are primarily thought of as long-term regulators of energy homeostasis these peptides may also have a subtle influence on the short-term control of feeding by mediating the sensitivity of the appetite regulatory system to gut peptides that are implicated in acute, meal to meal, control of appetite and food intake (Moreton et al, 2006).

In addition to chronic signals communicating information regarding long-term energy status, appetite and energy intake are also regulated in response to individual meals (Murphy and Bloom, 2006). Peptides secreted from the gastrointestinal tract and pancreas are important mediators of the acute (meal to meal) regulation of food intake.

Specifically, CCK, pancreatic polypeptide, GLP-1, oxyntomodulin and PYY are secreted in response to ingested nutrients and suppress appetite and feeding. Conversely, ghrelin remains the only known circulating gut peptide that stimulates appetite and feeding (Karra and Batterham, 2010). Figure 2.2 provides a schematic representation of the gastrointestinal tract illustrating where these hormones are concentrated and their physiological functions.

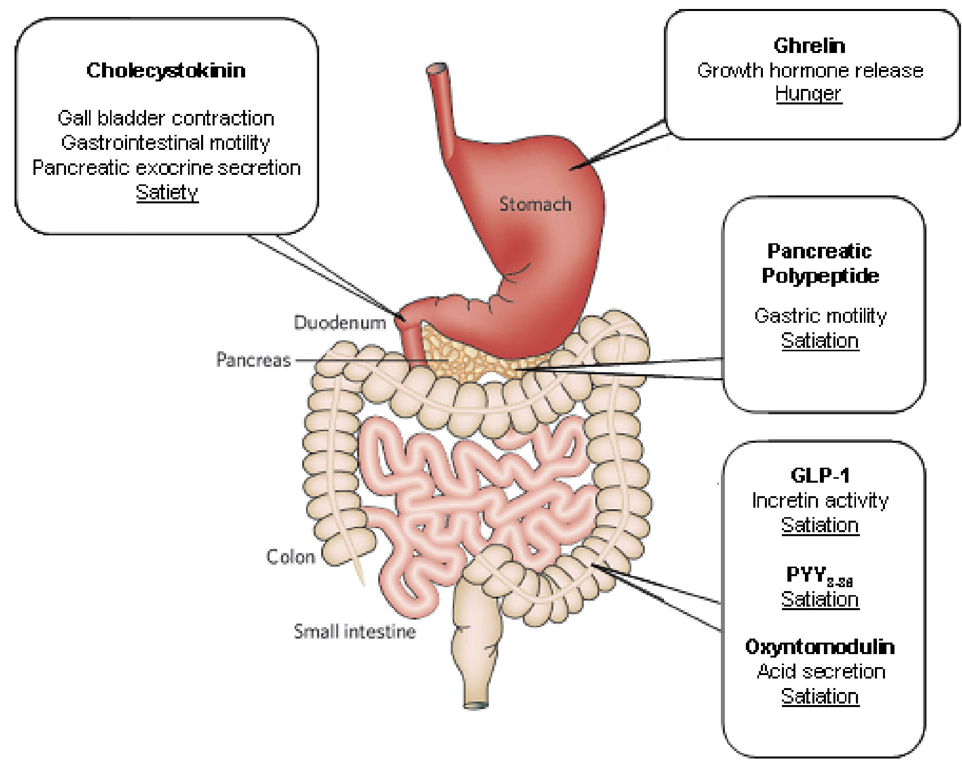

Figure 2.2: Schematic representation of the gastrointestinal tract illustrating the location and key functions of certain gut hormones implicated in the acute regulation of feeding. Adapted from Murphy and Bloom (2006).

Cholecystokinin is a peptide hormone produced by I-cells located in the duodenum. Approximately 15 min after nutrient ingestion, most notably in response to fat and protein, circulating concentrations of cholecystokinin start to increase, peak at ~25 min and remain elevated for approximately three hours (Paik et al, 2007). Cholecystokinin was the first gut hormone found to inhibit food intake when injected intraperitoneally into rodents (Gibbs et al, 1973). The appetite inhibitory effect of CCK has since been documented in both lean and obese humans (Kissileff et al, 1981; Lieverse et al, 1995).

Administration of cholecystokinin before a meal reduces meal size but does not reduce the number of subsequent feeding episodes. This indicates that CCK is short-acting signal important in determining meal termination and hence, meal size.

Pancreatic polypeptide is a 36 amino acid peptide produced by F-cells in the periphery of the pancreatic islets of Langerhans and also to a lesser extent in the colon (Ekblad and Sundler, 2002). Pancreatic polypeptide is released into the circulation after eating in proportion to the amount of energy consumed and remains elevated for up to six hours (Track et al, 1980). Peripheral administration of pancreatic polypeptide to rodents has been shown to reduce food intake (Asakawa et al, 1999). Moreover, intravenous infusion has been found to reduce appetite and food intake in humans (Batterham et al, 2003; Jesudason et al, 2007).

Glucagon-like peptide-1 and oxyntomodulin are both products of the preproglucagon gene which is expressed in the pancreas and intestine. In the pancreas, the preproglucagon gene product is post-translationally processed into the hormone glucagon whereas in the intestine GLP-1 and oxyntomodulin are produced (Murphy and Bloom, 2004). Glucagon-like-petide-1 is synthesised by intestinal L-cells located in the distal ileum and colon in two-forms: GLP-$1_{1\text{-}37}$ and GLP-$1_{1\text{-}36}$ amide, the latter being the major circulating form. Circulating GLP-1 concentrations increase ~10 min after nutrient ingestion, peak after ~30 min and remain elevated for several hours (Orskov et al, 1996). When secreted into the circulation GLP-1 is rapidly inactivated by the enzyme dipeptidyl-peptidase-4 (DPP4) yielding a half-life of approximately two min (Mentlein et al, 1993). Several studies have demonstrated appetite inhibiting effects of GLP-1. Both central and peripheral administration reduced food intake in rodents (Tang-Christiansen et al, 1996; Turton et al, 1996) whilst anorectic effects have also been seen with dose-dependant reductions in food intake in both lean and obese humans (Verdich et al, 2001). Glucagon-like-petide-1 is also a potent incretin, potentiating the production of insulin in response to elevations in blood glucose. Dipeptidyl-peptidase-4 resistant GLP-1 receptor agonists are showing promise as a treatment for diabetes with part of this success attributed to the associated weight loss resulting from a reduction in appetite (Chaudhri et al, 2008).

Oxyntomodulin is a 37 amino acid peptide released from intestinal L-cells after food ingestion in proportion to caloric load (Le Quellec et al, 1992). Oxyntomodulin is released into the circulation ~10 min after meal initiation with levels peaking after 30 min and remaining elevated for several hours (Le Quellec et al, 1992). Similar to GLP-1, oxyntomodulin is also a substrate for the protease enzyme DPP4. Several studies have shown suppressed energy intake in rodents after oxyntomodulin administration (Dakin et al, 2001; 2004; Baggio et al, 2004). Similarly, in humans, acute oxyntomodulin administration reduced food intake in lean volunteers (Cohen et al, 2003) and subcutaneous injection of oxyntomodulin induced weight loss over the course of four weeks in healthy overweight and obese individuals (Wynne et al, 2005b).

The experimental work in this thesis has included measurement of the gut hormones ghrelin and PYY therefore the following sections in this review will describe the structure, function and mechanisms of regulation in greater detail with particular emphasis on their roles in the regulation of energy homeostasis. Research has consistently described important effects of ghrelin and PYY in the regulation of appetite and energy intake. Currently ghrelin is the only known circulating gut hormone that increases appetite and stimulates meal initiation (Cummings et al, 2006). Conversely, PYY potently inhibits appetite and food intake and is an important determinant of inter-meal satiety (Batterham et al, 2002; Karra and Batterham, 2010). As a result of this both ghrelin and PYY have become significant targets for pharmaceutical development (Neary and Batterham, 2009a).

2.2 Ghrelin

2.2.1 Discovery of ghrelin

The discovery of ghrelin was preceded by work with synthetic compounds, the growth hormone secretagogues, which were shown to stimulate GH and food intake (Kojima et al 2001). In 1996 the growth hormone secretagogue receptor was identified as a G protein coupled receptor in the hypothalamus and pituitary. In 1999 the endogenous ligand for this receptor was identified and purified from rat stomach (Kojima et al, 1999). This ligand was named 'ghrelin'- from the Indo-European route *ghre* meaning to grow (Kojima, 2008).

2.2.2 Structure, production and secretion

Ghrelin is a 28 amino acid peptide formed by cleavage from its larger precursor, pre-proghrelin (Kojima and Kangawa, 2005). Ghrelin producing cells are primarily located in the x/a like-cells of the stomach fundus however smaller amounts are produced in the bowel, pituitary, kidney, placenta and hypothalamus (Kojima et al, 2001). Two forms of ghrelin exist within the circulation, namely acylated and unacylated. Acylated ghrelin is made explicit by the post-translational addition of a medium chain fatty acid, typically octanoate, to its third amino acid residue (serine), a modification which is catalysed by the recently identified enzyme – ghrelin O acyltransferase (GOAT) (Yang et al, 2008). This modification is necessary in order for ghrelin to bind to the growth hormone secretagogue receptor (GHS-R) and cross the blood-brain barrier, mechanisms which permit the role of ghrelin in the regulation of GH release and energy metabolism (Kojima and Kangawa, 2005).

Upon fasting, and/or low circulating levels of glucose and insulin, ghrelin is secreted into the circulation. Acylated ghrelin has a short circulating half-life (~25 min), being broken down by enzymes including butyrylcholinesterase and lysophospholipase 1 (De Vries et al, 2007). Consequently, in both the fed and fasted state the predominant form of ghrelin in the circulation is unacylated (75-90%) (Hosoda et al, 2004; Liu et al, 2008).

2.2.3 Physiological functions

Ghrelin is a multifaceted hormone with diverse biological functions (Van der Lely et al, 2004; Kojima and Kangawa, 2005). Both central and peripheral actions of ghrelin have been reported and include regulation of pancreatic function, gastric acid secretion and gastric motility (Masuda et al, 2000; Asakawa et al, 2001; Date et al, 2001), cardiovascular function (Nagaya et al, 2001; Sharma and McNeill, 2005), cell proliferation and apoptosis (Zhang et al, 2008), adipogenesis (Tschop et al, 2000) and sleep (Weikel et al, 2003). In addition, ghrelin was discovered as the endogenous ligand of the GHS-R and stimulates GH secretion. In rodents and humans intravenous ghrelin administration induces GH release with greater potency than GH releasing hormone (Takaya et al, 2000). Despite this, after the discovery of the orexigenic properties of ghrelin research interest shifted to investigating its role in energy homeostasis (Karra and Batterham, 2010).

2.2.4 Ghrelin, appetite and the acute regulation of energy intake

To date, ghrelin is the only circulating hormone that is known to stimulate appetite and food intake – all other circulating hormones implicated in the acute control of appetite serve to induce satiation and satiety. The appetite stimulating properties of ghrelin were first identified when three out of four participants reported an increase in appetite after receiving ghrelin intravenously during an investigation examining the effects of ghrelin on GH secretion (Arvat et al, 2001). A number of studies have since reported enhanced appetite and/or increases in food intake after exogenous ghrelin administration. In rodents, both intracerebroventricular and peripheral ghrelin administration increased feeding in a dose-dependent manner and led to weight gain in response to repeated administration (Wren et al 2000, Nakazato et al, 2001; Shintani et al, 2001; Wren et al, 2001b). These responses have also been observed at circulating concentrations typically observed when fasting, suggesting that these effects may be physiological meaningful (Wren et al, 2001b). Further evidence that ghrelin is orexigenic has been provided by studies which have induced diminished ghrelin action, by reducing peptide bioavailability or receptor activation (Nakazato et al, 2001; Asakawa et al, 2003). In these scenarios a decrease in food intake and body weight has been reported.

Exogenous ghrelin also stimulates food intake in humans. In a cross-over design, Wren and co-workers (2001a) infused ghrelin (5 $pmol{\cdot}kg{\cdot}min^{-1}$) or saline into nine healthy weight humans and recorded ratings of appetite and *ad libitum* food intake at a buffet meal provided towards the end of the infusion. Ratings of appetite were significantly higher during ghrelin infusion and this was associated with a 28% increase in energy intake at the buffet meal. Analysis of food diaries completed for 24 h after the infusion showed no evidence of compensatory under eating on the ghrelin trial. These results have been confirmed by other investigators in both lean and obese individuals (Druce et al, 2005; 2006). The orexigenic effect of ghrelin is not limited to healthy persons. Exogenous ghrelin enhanced appetite and food intake in patients with cancer cachexia (Neary et al, 2004), heart failure (Nagaya et al, 2004) and in malnourished patients on peritoneal dialysis (Wynne et al, 2005a). With further development ghrelin may therefore provide a useful therapeutic option in these circumstances.

The stimulatory effects of ghrelin on appetite and food intake are mediated through specific appetite related neural pathways within the brain, most notably within the

hypothalamic arcuate nucleus. In the circulation ghrelin can access these brain areas through a partially-permeable blood-brain barrier (Kojima and Kangawa et al, 2005). It is also possible that ghrelin may signal to the brain via the vagus nerve (Berthoud, 2008). In rodents, blockade of the gastric vagal afferent abolished ghrelin induced feeding and vagotomy abolished the typical rise in ghrelin in response to food deprivation (Date et al, 2002; Williams et al, 2003). Conversely, other work in rodents has shown that vagal afferents are not required to permit the eating-stimulatory action of ghrelin (Arnold et al, 2006) therefore uncertainty still surrounds the role of the vagus nerve in mediating the orexigenic action of ghrelin.

Within the arcuate nucleus ghrelin stimulates NPY and AgRP neuronal populations. These neuropeptides are anabolic, promoting positive energy balance by increasing appetite and reducing energy expenditure. Systemic ghrelin administration induces *c-fos* (a marker of neuronal activation) within NPY/AgRP neurons (Nakazato et al, 2001). Moreover, antibodies to, and antagonists of NPY and AgRP abolished ghrelin induced feeding (Nakazato et al, 2001) and in mice lacking these neuronal populations the stimulatory effect of ghrelin on food intake is absent (Chen et al, 2004; Bewick et al, 2005).

Recently, Malik et al (2008) have published findings which suggest ghrelin may also promote food consumption by enhancing the hedonic aspect of feeding. During ghrelin infusion, functional magnetic resonance imaging showed that food related images triggered neural responses in the amygdala, orbitofrontal cortex, anterior insula and the striatum (areas of the brain associated with pleasure and reward processing). These findings suggest that ghrelin participates in both the homeostatic and hedonic control of feeding.

2.2.5 Ghrelin and chronic energy homeostasis

Ghrelin also satisfies the criteria as a regulator of long-term energy homeostasis (Cummings, 2006). In rodents, repeated administration of ghrelin induces hyperphagia (Wren et al, 2000) and leads to weight gain if continued (Tschop et al, 2000). Furthermore, ghrelin peptide and receptor knock-out mice have been shown to display a lean phenotype and are resistant to diet induced obesity (Wortley et al, 2005; Zigman et al, 2005). In humans, circulating concentrations of ghrelin are inversely associated with

multiple measures of adiposity (Shiiya et al, 2002). Circulating concentrations of ghrelin are reduced in obese individuals (Vendrall et al, 2004) and elevated in those with anorexia/bulimia nervosa (Tanaka et al, 2002; Dostálová and Haluzík, 2009). These findings indicate that ghrelin is not causally implicated in the development of obesity, an exception to this being in individuals with Prada-Willi syndrome where hyperghrelinism precedes the onset of obesity (Feigerlová et al, 2008). Changes in body weight resulting from modifications in diet or exercise lead to inverse changes in ghrelin (Leidy et al, 2004; Foster-Schubert et al, 2005) and it is possible that a compensatory increase in circulating concentrations of ghrelin may explain why many individuals find dieting hard and weight loss difficult to maintain.

2.2.6 Regulation of ghrelin secretion

Plasma ghrelin levels rise and fall over the course of the day in relation to food intake (Cummings et al 2001, 2002; Liu et al, 2008) (Figure 2.3). Circulating concentrations of ghrelin rise during fasting, peak immediately prior to meals and fall shortly after food ingestion (Cummings et al, 2001; Cummings et al, 2002). The premeal elevation in circulating ghrelin has been interpreted as evidence of a role for ghrelin in determining meal initiation. This contention is supported by knowledge that the diurnal rhythm of ghrelin closely resembles changes in hunger (Cummings et al, 2001; Pinkney and Williams, 2002). Food intake is the most important variable determining circulating ghrelin levels however the exact mechanisms of this are not entirely clear (Hosoda et al, 2006; Yin et al, 2009). Mechanical distension of the gut does not appear to be important however (Shiiya et al, 2002).

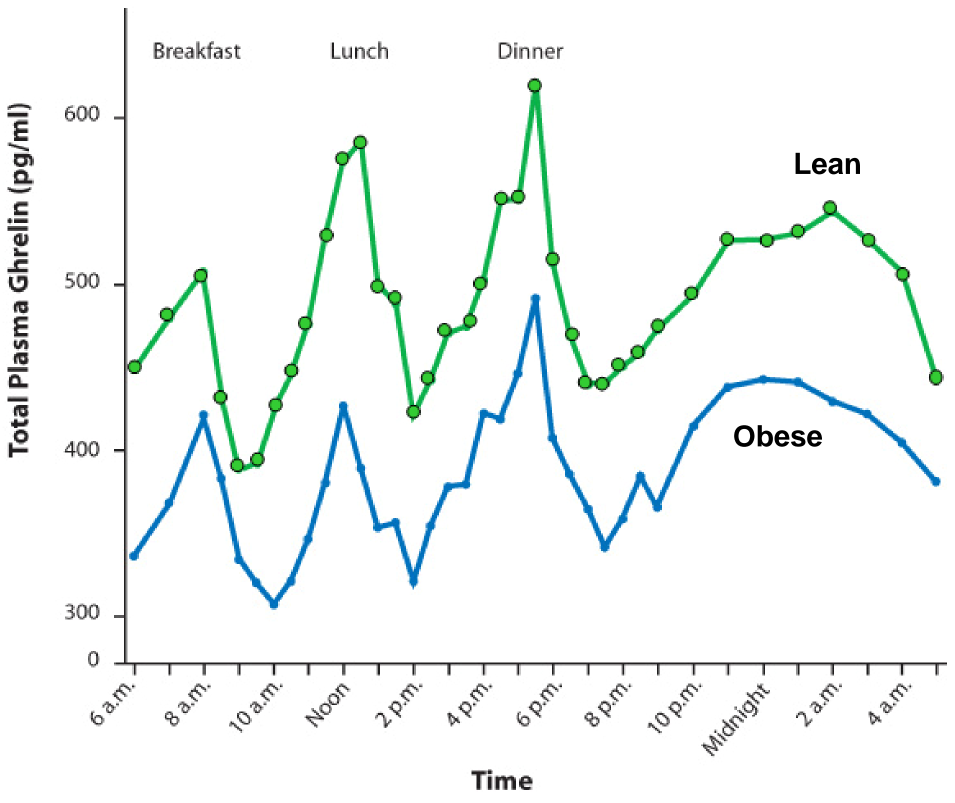

Figure 2.3: ghrelin response to feeding across 24 h in lean (large circles) and obese (small circles) individuals. Adapted from Cummings et al (2002).

Energy intake appears to be an important determinant of circulating ghrelin concentrations. The postprandial suppression of circulating ghrelin is directly proportional to the energy content of the ingested meal (Callahan et al, 2004; Leidy and Williams, 2006). Moreover, the meal related profile of ghrelin appears sensitive to the accumulation of calories across the day, with larger combined intakes at breakfast and lunch typically inducing a greater postprandial ghrelin suppression and subsequently leading to attenuated premeal ghrelin peaks before evening meals (Leidy and Williams, 2006).

Circulating concentrations of nutrients and hormones also influence levels of ghrelin (Yin et al, 2009). Each of the macronutrients suppress ghrelin although with variable efficacy. Overduin et al (2005) demonstrated that isocaloric intake of each macronutrient suppressed circulating ghrelin concentrations with carbohydrate having the most potent effect and fat the least. This response may contribute to the role of high fat diets in obesity. The idea that insulin and/or glucose suppress ghrelin has received

significant attention. Blood glucose may influence ghrelin levels as circulating concentrations of ghrelin rise during hypoglycaemia (Toshinai et al, 2001) and both oral and intravenous administration of glucose suppress ghrelin (Shiiya et al, 2002). An inhibitory effect of insulin may underlie the suppression of glucose on ghrelin (Yin et al, 2009). While maintaining euglycemia insulin infusion significantly decreased circulating ghrelin (Saad et al, 2002; Flanagan et al, 2003). It is possible that hyperinsulinaemia may be responsible for suppressed circulating ghrelin in obesity.

One recent proposition which requires acknowledgement is the contention that ghrelin is influenced by available lipids and may act as a nutrient sensor, rather than predominantly as an acute hunger signal (Kirchner et al, 2009). These researchers propose that ghrelin informs the central nervous system about the availability, rather than absence, of available nutrients. In rodents, the investigators demonstrated that the mRNA for the enzyme responsible for ghrelin acylation (ghrelin-o-acyltransferase) is decreased during fasting (36 h) and also that the mRNA for the ghrelin peptide tended to be reduced. Moreover, the researchers found no change in circulating acylated ghrelin concentrations in these conditions. These responses are counter-intuitive to the traditional understanding of ghrelin where upon fasting an up regulation in the expression of these genes, and an increase in circulating peptide would be anticipated to promote the restoration of energy balance. Additionally, the researchers showed that dietary lipids are necessary for ghrelin acylation and that mice unable to make ghrelin-o-acyltransferase exhibited reduced body weight and fat mass. Conversely, mice over expressing ghrelin-o-acyltransferase demonstrated increased body weight and fat mass. These changes in body mass and composition were due to a reduction in energy expenditure and a decrease in the ability to oxidise lipid rather than to changes in food consumption. Collectively, these researchers suggest that ghrelin functions as a nutrient sensor, by using readily absorbable medium chain fatty acids to signal to the brain that high caloric food is available, leading to optimisation of nutrient partitioning and growth signals. Further research will shed more light on this issue.

2.3 Peptide YY

2.3.1 Discovery of PYY

Peptide YY was first isolated in 1980 from porcine intestinal mucosa (Tatemoto and Mutt, 1980). The porcine extract was named PYY due to the presence of tyrosine residues (the single letter amino acid code for tyrosine being Y) at the C- and N-termini of its amino acid structure (Karra et al, 2009).

2.3.2 Structure, production and secretion

Peptide YY is a 36 amino acid peptide hormone that is cleaved from its larger precursor pre-pro PYY. Peptide YY is structurally similar to pancreatic polypeptide and NPY and as a result, this collection of related peptides has been termed the PP-fold family (Vincent and Le Roux, 2008). Peptide YY is synthesised and secreted into the circulation from enteroendocrine L-cells located primarily in the distal intestine and colon (Adrian et al, 1985a). Two forms of PYY exist within the circulation, namely PYY_{1-36} and PYY_{3-36}. Peptide YY_{3-36} is a truncated 34 amino acid peptide produced by cleavage of the N-terminal tyrosine and proline amino acid residues from PYY_{1-36} by the enzyme DPP4 (Mentlein et al, 1993) and is the main circulating form of PYY in both the fed and fasted state (Batterham et al, 2006). Removal of the N-terminal residues changes the three dimensional conformation of PYY which alters its receptor specificity and biological effects. Five receptors mediate the effects of the PP-fold proteins (Y-1, Y-2, Y-4, Y-5 and Y-6) and these receptors differ in location and function. PYY_{1-36} binds to all receptors, however PYY_{3-36} shows a high affinity for the Y-2 receptor and a lesser affinity for the Y-1 and Y-5 receptors (Neary and Batterham, 2009b). This difference in receptor preference is necessary for modulating digestive and feeding behaviour.

2.3.3 Physiological function

The physiological effects of PYY are diverse. Peripheral infusion of PYY slows gastric emptying in humans (Allen et al, 1984). Moreover, postprandial administration of PYY inhibits secretions from the exocrine pancreas and stomach, and reduces the rate of gall bladder emptying (Adrian et al, 1985b; Hoentjen et al, 2001). Peptide YY is also an important mediator of the illeal break mechanism, slowing proximal gastrointestinal transit to facilitate the absorption of nutrients, fluid and electrolytes (Lin et al, 1996). Peptide YY may also have important effects on the cardiovascular system (Playford et

al, 1992). In addition to these functions, PYY has been shown to potently inhibit appetite and food intake, findings which stimulated interest concerning the role of PYY in the regulation of feeding (Batterham et al, 2002; 2003).

2.3.4 Peptide YY, appetite and the acute regulation of energy intake

Despite some initial contrasting results many researchers have now reproduced the finding that exogenously administered PYY reduces food intake in rodents (Batterham et al, 2002; Challis et al, 2003; Pittner et al, 2004; Abbot et al, 2005; Koda et al; 2005; Scott et al, 2005; Vrang et al, 2006; Unniappan and Kieffer, 2008). In humans, subsequent studies have also confirmed that appetite and food intake are suppressed in response to exogenous administration of PYY_{3-36} (Batterham et al, 2003; Degen et al, 2005; Batterham et al, 2007; Sloth et al, 2007). In healthy weight men, intravenous infusion of PYY_{3-36} in an amount sufficient to elicit typical postprandial circulating levels was associated with a significant decrease in hunger and energy intake (36%) at a buffet meal provided 2 h later (Batterham et al, 2002). Moreover, in an identical study this finding was subsequently replicated in obese humans, as both lean and obese study participants exhibited suppressed ratings of hunger and consumed approximately 30% less energy at a buffet meal provided 2 h after the end of a 90 min intravenous infusion of PYY_{3-36} (Batterham et al, 2003). These results indicate that obesity is not associated with PYY_{3-36} resistance and have provided impetus for research into PYY_{3-36} as a viable therapeutic target for obesity pharmacotherapy.

The anoretic effects of PYY_{3-36} are mediated through appetite regulatory neuronal populations within the hypothalamic arcuate nucleus (Batterham et al, 2002; Ortiz et al, 2007). The blood-brain barrier is permeable to circulating PYY_{3-36} which allows PYY_{3-36} to bind to Y-2 receptors located on NPY neurons and inhibit their orexigenic activity (Batterham et al, 2002). Specifically, electrophysiological studies have shown that PYY_{3-36} administration inhibits NPY neurons and reduces NPY mRNA expression (Batterham et al, 2002; Challis et al, 2003). Inhibition of NPY neurons also releases the tonic gama-aminobutyric acid mediated inhibition of anorexigenic pro-opiomelanocortin neurons. Thus, within the hypothalamic arcuate nucleus PYY_{3-36} directly inhibits orexigenic neurons and indirectly stimulates anorexigenic neurons.

Peptide YY may also influence feeding via a direct neural action. The Y-2 receptor is located on afferent terminals of the vagus nerve and it is possible that PYY_{3-36} communicates nutritional status via this route (Koda et al, 2005). Evidence supporting this is provided by studies showing that subdiaphagmatic vagotomy and trans-sectioning of brainstem-hypothalamic neuronal pathways abolished the anoretic effect of peripherally administered PYY_{3-36} (Abbott et al, 2005; Koda et al, 2005). In contrast to this, data from two studies in mice have not supported these findings as vagotomy (Halatchev and Cone, 2005) and pre-treatment with capsaicin (Talsania et al, 2005) did not inhibit the anorectic effects of PPY_{3-36} on food intake. Thus, uncertainty still surrounds the role of the vagus nerve in mediating the effects of PYY_{3-36} on food intake (Karra and Batterham, 2010).

The role of PYY_{3-36} in the short-term regulation of energy homeostasis appears to be mediated through both homeostatic and hedonic mechanisms. In a novel study which combined exogenous PYY_{3-36} administration with functional magnetic resonance imaging, Batterham et al (2007) showed that PYY_{3-36} modulates neural activity in brain regions associated with cognition, emotion and reward, in addition to regions known to be implicated in the homeostatic control of food intake. In response to food related questions, the researchers found that in the presence of low circulating PYY_{3-36} (fasted participants receiving saline infusion) brain activity within hypothalamic areas (associated with homeostatic regulation of food intake) strongly predicted energy intake at a buffet meal. Conversely, in response to PYY_{3-36} infusion (mimicking typical postprandial levels) neural activity within the orbital frontal cortex (a brain region associated with reward processing) was the strongest predictor of energy intake. Thus, it appears that in the presence of elevated PYY_{3-36} the regulation of food intake switches from homeostatic to hedonic control mechanisms. These findings underscore the complexity of food intake regulation and highlight the interplay between physiological, psychological and behavioural influences governing feeding.

2.3.5 Peptide YY and chronic energy homeostasis

Mounting evidence suggests that PYY_{3-36} may contribute to the regulation of long-term energy balance. In both children and adults it has been observed that plasma concentrations of PYY_{3-36} correlate inversely with various measures of adiposity (Roth et al, 2005; Guo et al, 2006; Le Roux et al, 2006). In addition, investigations using

rodents demonstrated that chronic PYY_{3-36} administration attenuates gains in body weight and adiposity (Batterham et al, 2002; Pittner et al, 2004; Vrang et al, 2006). Moreover, a lack of PYY_{3-36} induces hyperphagia and weight gain (Batterham et al, 2006). Specifically, Batterham and co-workers (2006) generated transgenic mice lacking PYY_{3-36}. These PYY_{3-36} knock-out animals were hyperphagic and ate significantly more than their wild-type littermates when exposes to a fast re-feed protocol. Furthermore, these mice were heavier and exhibited increased levels of subcutaneous and visceral adiposity. These defective characteristics were reversed following exogenous PYY_{3-36} replacement. These findings suggest that PYY_{3-36} deficiency causes weight gain and that exogenous replacement can ameliorate this.

Although changes in body weight and adiposity in response to PYY_{3-36} administration are strongly determined by effects on appetite, PYY_{3-36} may also modulate chronic energy homeostasis by influencing thermogenesis and fuel partitioning. Transgenic mice over-expressing PYY_{3-36} exhibit increased body temperature suggesting that PYY_{3-36} stimulates thermogenesis (Boey et al, 2008). Moreover, in humans it has been demonstrated that peripheral PYY_{3-36} administration increases body temperature and fat oxidation (Sloth et al, 2007). Effects of PYY_{3-36} on fuel partitioning have also been suggested by others who found a significant inverse association between peak postprandial circulating PYY_{3-36} concentrations and 24 h respiratory quotient (Guo et al, 2006). These researchers also found an inverse relationship between peak postprandial circulating PYY_{3-36} concentrations and body weight change over six months. Thus, it appears that PYY_{3-36} may be involved in the long-term regulation of energy homeostasis through effects on appetite, energy expenditure and lipid metabolism.

Basal and postprandial circulating PYY_{3-36} concentrations have been reported to be reduced in obese individuals and this has been linked to reduced feelings of satiety (Batterham et al, 2003; Le Roux et al, 2006). The direction of this relationship remains unclear however i.e. are low circulating PYY_{3-36} levels implicated in weight gain or does weight gain cause a reduction in circulating concentrations. The former explanation is strengthened by recent evidence demonstrating that mice resistant to diet induced obesity exhibited significantly higher circulating PYY_{3-36} concentrations than those susceptible to weight gain (Rahardio et al, 2007). Nonetheless, obese individuals remain sensitive to the anorectic effects of exogenous PYY_{3-36} therefore identifying

$PYY_{3\text{-}36}$ as a potential target for pharmacological intervention (Batterham et al, 2003; Sloth et al, 2007).

2.3.6 Regulation of PYY secretion

To date there is little data regarding the 24 h profile of circulating PYY (Karra et al, 2009). Circulating PYY concentrations fluctuate in response to individual feeding episodes. Circulating values are low in the fasted state, increase within 15 min after nutrient ingestion, peak after 1-2 h and remain elevated for several hours (Adrian et al, 1985a). This temporal profile, particularly the sustained elevation, identifies PYY as a determinant of inter-meal satiety, postponing the initiation of the next meal even more so than determining within meal satiation.

The secretion of PYY is related to the energy content of meals and their macronutrient composition. Circulating concentrations of PYY increase in direct proportion to the energy content of ingested food. In 25 healthy weight humans, Adrian et al (1985a) examined PYY responses to meals of step-wise increasing caloric content and observed reciprocal elevations in circulating peptide levels. Studies examining the influence of the macronutrients on PYY release suggest that PYY is secreted in response to each nutrient however data comparing the relative potency has produced mixed findings. Initial reports suggested that fat stimulated PYY secretion with greater efficacy than either protein or carbohydrate (Lin and Chey, 2003) however more recent evidence has identified protein as the most potent stimulator of PYY (Batterham et al, 2006). Gastrointestinal peptides may also modulate PYY release. Circulating concentrations of PYY increase before nutrients reach the distal intestine and this is thought to be stimulated by CCK (McFadden et al, 1992). Moreover, vasoactive intestinal peptide has been shown to stimulate PYY (Ballantyne et al, 1993) release whilst gastrin inhibited PYY release (Greeley et al, 1989). Gastric distension and water ingestion has no influence (Pedersen-Bjergaard et al, 1996).

2.4 Exercise, appetite and food intake

Energy balance is the product of energy consumed and that expended. When in balance, body weight remains stable. Conversely, mismatches between these variables induce reciprocal changes in body mass and composition. It is known that exercise has an important influence on energy balance (Donnelly et al, 2009; Seagle et al, 2009).

Regular exercise increases energy expenditure and can therefore facilitate successful weight control. In addition to this, exercise may also have an indirect effect on energy balance by influencing appetite and subsequently, food intake. These effects are less well recognised and understood. The next section reviews studies which have sought to examine the effects of exercise on appetite and food intake.

2.4.1 Appetite Assessment

Before reviewing studies which have examined the effects of exercise on appetite, first it is necessary to define what is meant by ‘appetite’ and describe how it can be quantified. Blundell et al (2010) suggest that appetite refers to the sensory and qualitative aspects of eating and includes the responsiveness to both physiological and environmental influences. It has also been noted that appetite is a subjective construct and is therefore not open to direct measurement (Mattes et al, 2005). It is possible however to make an indirect assessment of appetite by using questionnaires. Visual analogue scales are the most common response format to assess subjective ratings of appetite. These questionnaires require participants to place a mark on a horizontal line, typically 100 or 150 mm in length, that are anchored at either end with descriptive statements (Raben et al, 1995; Flint et al, 2000). Initial questionnaires tended to focus on measurement of hunger however it has been suggested that this fails to recognise the multi-dimensional aspect of appetite (Hill and Blundell, 1982). Instead, a more valid assessment of appetite is likely to be gained from taking measurement of additional appetitive sensations, including fullness, satisfaction and prospective food consumption (Mattes et al, 2005).

2.4.2 The effects of exercise on appetite

Consciously restricting food intake leads to an increase in appetite and subsequently, food intake (Hubert et al, 1998). Although it would be logical to presume that the same response would be observed when energy is expended through exercise, the evidence available suggests that this does not occur (Blundell et al, 2003; Martins et al, 2008). Studies have shown that appetite does not increase in response to single bouts of exercise (Thompson et al, 1988; King et al, 1994; King and Blundell, 1995; King et al 1996; 1997; Hubert et al, 1998; Martins et al, 2007). Paradoxically, a consistent finding is that high intensity exercise (> 60 % of $\dot{V}O_2$ max) induces a transient suppression of

appetite (Thompson et al, 1988; Kissileff et al, 1990; King et al, 1994, King and Blundell, 1995; Martins et al, 2007), an effect which has been termed 'exercise induced anorexia' (King et al, 1994).

King et al (1994) examined appetite responses of 11 healthy males to acute bouts of high (27 min at 72% of $\dot{V}O_2$ max) and low (63 min at 36% of $\dot{V}O_2$ max) intensity cycling and made comparisons with responses on a sedentary control trial. The investigators established a suppressive effect of exercise on hunger during high, but not low intensity exercise. This effect was transient however as hunger ratings were not significantly different from values in the low intensity exercise and control trials 15 min after exercise.

Exercise induced anorexia has since been reported during exercise of varying modalities including running (King and Blundell, 1995; Broom et al, 2007), cycling (Kissileff et al, 1990; King et al, 1994; Martins et al, 2007) and resistance exercise (Broom et al, 2009). Interestingly, exercise may not suppress appetite in females to the same degree as males (King et al, 1996). Specifically, in response to 50 min of high intensity cycling (70% of $\dot{V}O_2$ max) female participants did not exhibit a reduction in appetite. Instead, compared with responses during a sedentary control trial a post-exercise meal was rated as more palatable after completing exercise. Its been suggested that this sex differential in appetite response may partly explain why exercise appears to be a less effective method for weight loss in females as compared with males (Hagobian et al, 2009).

Although intense exercise may suppress appetite, findings suggest this effect is brief and does not subsequently influence energy intake (King et al, 1994; King and Blundell, 1995). Instead, a resistance to begin eating i.e. a delay until participants voluntarily request to eat after completing exercise, appears to be a more typical response.

Although valuable information has been gathered from the studies cited in this section, a limitation of many of these studies is the somewhat brief period of observation. Typically, appetite ratings have been examined during exercise and then in a brief

period (i.e. one or two hours) leading up to a meal. Evidence from two recent studies suggest that changes in appetite may occur after an extended period. Malkova et al (2008) reported elevated subjective perceptions of hunger and desire to eat after a test meal that was consumed 2 h after a 60 min bout of moderate intensity cycling. Similarly, Broom et al (2007) observed that the AUC for hunger tended to be elevated over a 6 h period after a meal served 2 h post-exercise. Consequently, it has been suggested that additional work is needed to examine the more prolonged effects of exercise on appetite (Bilski et al, 2009).

2.4.3 Acute energy intake responses to exercise

Consistent with the majority of reports which have shown that single bouts of exercise do not increase appetite, the consensus of the available evidence suggests that exercise does not significantly alter energy intake (Jankowski and Foss, 1972; Reger et al, 1984; Thompson et al, 1988; King et al, 1994; King and Blundell, 1995; King et al, 1996; 1997; Hubert et al, 1998; George and Morganstein, 2003; Tsofliou et al, 2003; Maraki et al, 2005). Exceptions to this have been reported however, where energy intake has been found to be augmented (Verger et al, 1992; 1994; Pomerleau et al, 2004; Martins et al, 2007) or reduced (Kissileff et al, 1990; Westerterp-Plantenga et al, 1997) in response to acute bouts of exercise.

Jankowski and Foss (1972) submitted 14 middle aged, overweight men to three treatments: running one mile at 6.2 mph, running 440 yards at 6.2 mph or control. Food intake was assessed over the course of 24 h. The researchers observed no difference in energy intake between conditions, perhaps an expected response given the limited amount of exercise performed. In 11 healthy females Reger et al (1984) compared energy intake responses to three exercise protocols with that exhibited during a control trial. Long duration (60 min at 50% of $\dot{V}O_2$ max), short duration (30 min at 50% of $\dot{V}O_2$ max) and mixed intensity exercise protocols were completed. There were no significant differences in energy intake at a buffet meal provided 15 min post-exercise. Thompson et al (1988) submitted 16 lean males to bouts of high intensity (68% of $\dot{V}O_2$ max for 29 min) and low intensity (35% of $\dot{V}O_2$ max for 58 min) cycling and compared responses with control. *Ad libitum* energy intake was not significantly different between trials at a meal provided one hour after exercise.

A series of studies examining food intake responses to exercise have been conducted at the biopsychology department at the university of Leeds. Collectively, the findings from these studies also suggest that energy intake is not influenced by acute bouts of exercise. King et al (1994) reported findings from two investigations which assessed the influence of exercise intensity and exercise duration on energy intake. Free choice meals were offered 15 min after exercise in each study where participants were permitted to begin eating when desired. The researchers reported no significant changes in energy intake in either experiment. In separate experiments King and Blundell (1995) examined energy intake responses to single bouts of running and cycling in healthy male participants. In each experiment participants completed 50 min of exercise at 70% of $\dot{V}O_2$ max. Energy intake was assessed from *ad libitum* meals that were available immediately after exercise and also from that reported in food diaries during the remainder of trial days. The researchers observed no significant effects of exercise on energy intake in either the cycling or running trials. King et al (1996) repeated the cycling arm of the previous investigation using 13 healthy females. Again, no significant differences in energy intake occurred between the trials.

As with many of the studies which have examined the effects of exercise on appetite, a limitation of many of the studies that have assessed the effects of exercise on energy intake is the brief duration of observation. Effects of exercise on energy intake have typically been assessed at a single meal provided after exercise. It is possible that any effects on energy intake may occur over a longer duration, in response to a second or third meal taken after exercise. King et (1997) explored this possibility in an investigation examining food intake responses to exercise over 48 h. In this study eight healthy males completed an exercise trial and a control trial in a random order. Participants performed 50 min of running (70% of maximum heart rate) in both the morning and afternoon during the first 24 h of the exercise trial and rested during the second 24 h. No exercise was performed during the control trial. Despite the length of observation and the substantial energy expenditure elicited (5020 kJ, 1200 kcal) there were no significant effects of exercise on energy intake. A limitation of this study however was the assessment of energy intake via self-reported diaries. These are notoriously unreliable and biased towards socially desirable foods (Mattes et al, 2005). Moreover, the act of collecting data in this way often distorts eating behaviour (Burke,

2006). Assessment of energy intake in more controlled conditions, over a greater period of time, with buffet meals of wide-ranging content, may help to overcome these issues.

Imbeault et al (1997) examined the influence of exercise intensity on energy intake. Eleven young men completed a control trial and two exercise trials (low and high intensity). Participants either walked or ran on a treadmill for 72 min at 35% of maximum oxygen uptake in the low intensity trial whilst they ran for 34 min at 72% of maximum oxygen uptake on the high intensity trial. An *ad libitum* buffet was offered 15 min post-exercise. The researchers found no significant difference in energy intake after exercise although energy intake tended to be lower after the high intensity run.

Hubert et al (1998) compared appetite and energy intake responses to energy deficits induced by diet verses exercise. In their investigation 11 healthy females completed four day-long experimental trials in a random order. A 2 x 2 design was implemented with manipulations of exercise (or no-exercise) and breakfast (low energy or high energy). On trial days participants arrived at the laboratory and either cycled for 40 min at 70% of $\dot{V}O_2$ max or rested. Afterwards a standard breakfast of either low (268 kJ, 64 kcal) or high (2092 kJ, 500 kcal) energy content was consumed. An *ad libitum* meal was provided at lunch. Exercise did not significantly alter energy intake, however consuming a low energy breakfast led to elevated perceptions of hunger and energy intake at lunch (mean of low energy breakfasts 720 kcal, mean of high energy breakfast 600 kcal). These findings indicate that restricting energy intake stimulates appetite and food intake later on, responses not exhibited when energy deficits are induced through exercise.

Two studies have assessed the effects of walking on energy intake (George and Morganstein, 2003; Tsofliou et al, 2003). In the study conducted by George and Morganstein (2003) 12 normal weight and 12 overweight females walked on a treadmill for 60 min at ~60% of their maximum heart rate. In the other investigation 20 obese women completed 20 min of brisk walking at approximately 72% of maximum heart rate (Tsofliou et al, 2003). In each experiment energy intake was examined at buffet meals provided after exercise. Neither study showed any significant effects of walking on energy intake. It is possible that the relatively low energy expenditure induced by

walking in these participants (120 – 200 kcal) may have provided an insufficient stimulus to influence energy intake.

Maraki et al (2005) examined how the time of day during which exercise is undertaken influences post-exercise energy intake. Twelve healthy females completed four experimental trials, namely, morning control, morning exercise, evening control and evening exercise. As the exercise stimulus participants completed 60 min of exercise which included aerobic and resistance exercise components, sufficient to expend 1233 kJ (295 kcal). Energy intake responses were examined using 24 h food diaries. The researchers observed no significant effects of exercise or time of day on energy intake.

The aforementioned studies suggest that exercise does not influence energy intake however exceptions have been reported (Kissileff et al, 1990; Verger et al, 1992; 1994; Westerterp-Plantenga et al, 1997; Pomerleau et al, 2004; Martins et al, 2007; Erdmann et al, 2007). In a group of college students Verger et al (1992) examined energy intake responses after 2 h of ‘non-stop, various athletic activities’ (reported energy expenditure 2092 kJ, 500 kcal) and observed a significant increase in energy intake. In this study participants completed a control trial and four exercise trials with the timing of meal presentation manipulated between trials (0, 30, 60 and 120 min post-exercise). Averaging the energy intake data across the exercise trials revealed a significantly higher energy intake after exercise as compared with control (1966 kJ, 470 kcal). Moreover, the researchers found a positive relationship between feeding latency and energy intake i.e. participants consumed more energy at meals provided later after completing exercise than at meals provided soon after exercise. These findings suggest that participants compensated energy intake for that expended during exercise. The extent of this is questionable though as energy expenditure was estimated using values taken from the French army handbook of ergonomy rather than being measured directly.

A limitation of the previous investigation, and of many of the studies previously cited in this section, is the provision of a limited number of buffet items from which to determine energy intake responses to exercise. In the study of Verger et al (1992) only five items were available (semolina, gelled fruit, eggs, ham and cheese). In an effort to overcome this, a second study was completed by the same researchers (Verger et al,

1994). Fifty-eight young males were randomly assigned into an exercise group and a control group. In the exercise group participants completed 2 h of 'non-stop, various athletic activities' sufficient to expend 3347 kJ (800 kcal). Participants remained sedentary during the control trials. In each condition a comprehensive buffet meal (more than 50 items of varied macronutrient composition) was provided 30 min after exercise or rest. The findings from this study confirmed the researchers' previous results, showing a significant increase in energy intake in the exercise trial as compared with control (1841 kJ, 440 kcal). The results of this latter study should be interpreted with caution however as an independent groups design was implemented. Large variations in energy intake occur between individuals therefore with this study design it is possible that the results may have been influenced by differences between individual participants.

Pomerleau et al (2004) also reported data indicating an increase in energy intake in response to exercise. In a randomised fashion, 13 healthy females completed a control trial and exercise trials of high (69% of $\dot{V}O_2$ peak) and low (41% of $\dot{V}O_2$ peak) intensity with the duration being adjusted so that participants expended 1460kJ (350 kcal). As compared with control, high intensity exercise stimulated an increase in energy intake (531 kJ, 127 kcal) at a buffet meal provided one hour after exercise however no changes occurred in response to low intensity exercise. A more recent investigation has also observed augmented energy intakes after exercise as compared with responses in a control trial (Martins et al, 2007). In this study participants cycled for 60 min at 66% of their maximum heart rate (energy expenditure 2059 kJ, 492 kcal) and food intake responses were examined at a buffet meal provided one hour later. Energy intake was significantly higher after exercise as compared with that consumed during a sedentary control trial (Δ 632 kJ, 151 kcal).

Erdmann et al (2007) observed a significant increase in energy intake after prolonged low intensity cycling. In this study participants completed a sedentary control trial and three exercise trials in a random order. Herein, participants cycled at a low intensity (50 watts) for 30, 60 or 120 min. Energy intake was assessed from a standard meal consisting of bread, butter and ham sandwiches - provided 15 min after exercise. As compared with the control trial, energy intake was significantly greater after the 120

bout of cycling (Δ 900 kJ, 215 kcal) but was no different after the 30 or 60 min exercise bouts. The energy expenditure elicited during the 120 min of cycling was not that extreme (1423 kJ, 340 kcal) therefore it is unlikely that the energy deficit induced was responsible for the augmented energy intake. Energy intake may have been elevated as a 'reward' for the prolonged effort.

It has been suggested that energy intake responses to exercise can only be interpreted in relation to energy expenditure, a concept which has been described as 'relative energy intake' (King et al, 1994). In the studies identified above which have reported stimulatory effects of exercise on energy intake, participants have only partially compensated energy intake for that expended, that is, after accounting for the energy expended during exertion participants have remained in energy deficit after exercise. Consequently, in those circumstances it is misleading to say that exercise stimulated energy intake. Instead, these studies actually show that exercise induces a partial compensation in energy intake.

Two investigations have observed decreases in energy intake in response to exercise. Kissileff et al (1990) reported a significant decrease in the amount of strawberry yoghurt consumed 15 min after a 40 min bout of moderate intensity cycling (control 708 g, exercise 621 g). Westerterp-Plantenga et al (1997) also found a significant decrease in energy intake at a buffet meal provided 10 min after two hours of moderate intensity cycling (60% of maximum power output). In this study energy intake was 3100 kJ (741 kcal) and 2300 kJ (550 kcal) in the control and exercise trials, respectively.

It can be seen that the research literature is scattered with studies which have used diverse methodologies and participant groups through which to examine the effects of exercise on appetite and energy intake. Although the studies described in this section provide a useful starting point through which to examine the effects of exercise on food intake there are notable limitations of this work. Food provision is a significant issue. Buffet meals provided to participants have ranged extensively and in some instances energy intake has been assessed from single items such as strawberry yoghurt (Kissileff et al, 1990). A range of food items familiar to the study population would hold greater ecological validity and would permit investigators to more thoroughly assess the effects

of exercise on macronutrient intake. The period of observation is a second issue. Energy intake responses have typically been examined over a brief period of time, most often delineating energy intake responses to single meals. Changes in energy intake may occur over a longer duration, in response to latent exercise-induced changes in circulating hormones and metabolites. Consequently, assessment of responses to multiple meals requires examination.

2.4.4 Energy intake responses to repeated bouts of exercise

The first studies examining the effects of repeated bouts of exercise on energy intake were conducted in the 1980s (Woo et al, 1982a; 1982b; Woo and Pi-Sunyer et al, 1985). Taken collectively these studies showed that overweight women do not alter their food intake over the course of 52 days after starting an aerobic exercise program. Conversely, over 19 days healthy weight females compensated entirely for both mild and moderate elevations in daily energy expenditure (14% and 29% higher than on a sedentary day) induced through exercise. The authors speculated that the extra fuel reserves available in the obese subjects may be implicated in the lack of compensation in energy intake.

More recent data indicates that over the course of seven days males do not appear to increase their energy intake in response to the initiation of an exercise program although females may begin to partially increase their energy intake over this time scale (Stubbs et al, 2002a; 2002b; 2004). Whybrow et al (2008) sought to examine energy intake and energy balance in response to imposed exercise over 14 days. Twelve healthy participants (six males and six females) were each studied three times during 14 day protocols corresponding to prescriptions of no exercise (control), medium exercise and high exercise. In the medium exercise condition participants completed two, 40 min exercise sessions daily (cycling or running) whereas in the high exercise condition three, 40 min bouts were performed. Males expended 2.8 and 4.8 MJ·day^{-1} in the medium and high exercise conditions whilst females expended 2.0 and 3.8 MJ·day^{-1}. A strength of this study was that energy intake was assessed accurately from meals consumed at the research facility (rather than from self-report diaries) and energy expenditure was measured using doubly labelled water. In both males and females, the researchers observed no significant changes in energy intake in response to exercise however a partial (non-significant) compensation in energy intake was apparent

(~30%). The researchers suggest that its possible that this reflects the initial stages of energy intake/energy expenditure matching in active individuals. This response is likely one of many behavioural and metabolic reactions that occur in order to restrict extensive changes in body mass and composition (King et al, 2007). This reaction may be more sensitive in females than males, possibly due to reproductive biology, and may explain why females find it harder to lose weight through exercise than males (Hagobian et al, 2009).

2.4.5 Effects of exercise on macronutrient intake

Energy intake is determined by the type of food consumed in addition to the absolute amount. Therefore, when considering the influence of exercise on energy intake the question of whether exercise alters macronutrient preference requires attention. A simple depletion hypothesis has been cited suggesting that individuals may be driven to replace the substrate predominantly oxidised during exercise (King, 1998). The consensus of evidence does not appear to support this notion however. Short-term intervention trials examining the acute effects of single bouts of exercise on macronutrient intake have shown mixed results yet most typically demonstrate a lack of change (Tremblay and Drapeau, 1999; Elder and Roberts, 2007). Healthy male participants did not demonstrate a significant difference in macronutrient selection after 90 min of cycling (Alméras et al, 1995) or after two, 50 min running sessions (King et al, 1997). Similarly, in females, a lack of change in macronutrient selection has been reported in response to single bouts of walking (George and Morganstein, 2003; Tsofliou et al, 2003). More recently, Martins et al (2007) found no significant changes in macronutrient selection in response to 60 min of moderate intensity cycling.

Other researchers have observed changes in macronutrient intake after exercise. After 2 h of 'non-stop, various athletic activities,' Verger et al (1992) reported a significant increase in the percentage of energy derived from carbohydrate. Conversely, in response to a similar exercise stimulus, the same researchers did not observe any changes in carbohydrate intake but witnessed a significant increase in protein (Verger et al, 1994). At a buffet meal presented 60 min after high intensity treadmill exercise Pomerleau et al (2004) reported a significant increase in the amount of energy derived from fat and protein. Despite this, across the remainder of the day carbohydrate intake was elevated with the intake of fat and protein remaining no different to responses on a

control trial. This latter finding may have been influenced by the provision of predominantly high carbohydrate containing snacks to participants after exercise.

Westerterp-Plantenga et al (1997) also found an increase in carbohydrate preference after exercise. As compared with responses exhibited during a sedentary control trial the researchers observed a significant increase in the percentage of energy derived from carbohydrate after 2 h of moderate intensity cycling. In this study a buffet meal was provided 10 min after exercise. At this meal the researchers observed a significant increase in the percentage of energy derived from liquid source calories at the expense of solid food items. It is possible that this may explain the increase in carbohydrate intake as the beverages provided were juices high in carbohydrate. Another possible explanation is that participants opted for easily digestible foods at a meal presented soon after exercise, with high carbohydrate containing foods typically being easier to digest than foods high in fat and protein.

Overall, the findings from short-term intervention studies do not demonstrate a consistent effect of exercise on macronutrient selection (Tremblay and Drapeau, 1999; Elder and Roberts, 2007). Cross-sectional studies show that carbohydrate intake may be elevated in athletic individuals (Burke, 2006) which raises the possibility that changes in dietary preferences may occur only in response to periods of exercise training. Notwithstanding, Elder and Roberts (2007) collated findings from studies which have examined this issue and concluded that there currently is no support for this suggestion.

2.5 Effects of exercise on energy regulating hormones

The first studies examining the influence of exercise on gastrointestinal peptides were conducted in the late 1970s and during the 1980s. Recently, advances in knowledge about the neuro-endocrine control of appetite and food intake has provided a stimulus for further research examining the specific influence of exercise on gastrointestinal peptides responsible for regulating appetite. The studies described in this thesis have included measurements of plasma acylated ghrelin and $PYY_{3\text{-}36}$ therefore the ensuing sections of this review will focus on studies which have examined the impact of exercise on these hormones. Before this, studies which have examined the effects of exercise on other appetite regulatory peptides will be highlighted briefly.

Attempts have been made to assess the effects of exercise on pancreatic polypeptide, CCK, GLP-1, obestatin and leptin. The evidence suggests that circulating concentrations of pancreatic polypeptide increase during exercise (Gingerich et al, 1979; Martins et al, 2007) and are elevated in both the fasting and postprandial state afterward (Hilstedt et al, 1980; Greenberg et al, 1986; Hurley et al, 1991). Similarly, exercise appears to stimulate increases in circulating concentrations of GLP-1 (O'Connor et al, 1995; 2006; Martins et al, 2007; Ueda et al, 2009) and CKK (Bailey et al, 2001; Sliwowski et al, 2001). Acute bouts of exercise do not appear to influence circulating concentrations of obestatin (Ghanbari-Niaki et al, 2008a; 2008b; Wang et al, 2008; Manshouri et al, 2008) or leptin (Kraemer et al, 2002) however some evidence suggests that circulating levels of leptin may be reduced in response to extreme exercise challenges that pose a severe threat to energy homeostasis (Kraemer et al, 2002).

2.5.1 Acute effects of aerobic exercise on ghrelin

Initial studies examining the influence of exercise on ghrelin were undertaken to determine the role of ghrelin in mediating increases in GH in response to exercise. Early findings indicated that neither cycling nor running influenced circulating total ghrelin concentrations (Kallio et al, 2001; Dall et al, 2002; Kraemer et al, 2004a; Schmidt et al, 2004: Zoladz et al, 2005). Unfortunately none of these studies implemented sedentary control groups therefore it is difficult to determine whether these results are due to exercise, diurnal variation or chance.

Recently there has been a resurgence of interest regarding the influence of exercise on ghrelin. This second phase has evolved in response to work showing an important role of ghrelin in energy homeostasis. A central hypothesis to this inquiry was the expectation that exercise would augment circulating ghrelin concentrations as a compensatory mechanism to restore energy balance. Although an intuitive hypothesis, the results from investigations which have examined this have yielded inconsistent findings with some studies reporting that acute bouts of aerobic exercise increase circulating ghrelin (Christ et al, 2006; Jürimäe et al, 2007b; Erdmann et al, 2007; Jürimäe et al, 2009) whereas others have reported no change (Burns et al, 2007; Martins et al, 2007; Jürimäe et al, 2007a; Ueda et al, 2009; Shorten et al, 2009) and even decreases (Broom et al, 2007; Toshinai et al, 2007; Vestergaard et al, 2007; Marzullo et al, 2008; Broom et al, 2009).

Christ and associates (2006) examined whether a short-term dietary intervention would affect ghrelin before and during a single bout of cycling. Eleven trained athletes completed two experimental trials in a balanced fashion. Before completing exercise participants consumed a low fat diet (1.5 g/kg) for 24 h and then either maintained this for a further 36 h (energy balance trial – 2850 kcal·d^{-1}) or increased the fat content of the diet to 3.5g/kg (overfeeding trial – 5000 kcal kcal·d^{-1}). On the day after the dietary intervention participants cycled for 3 h at 50% of their maximum power output. Despite changes in diet the researchers observed no difference in pre-exercise total ghrelin levels between trials. During exercise, circulating concentrations of ghrelin increased in each condition with the highest levels observed during the last hour of exercise. This increase in ghrelin was significantly greater in the low-fat condition with the authors suggesting that this be related to differences in energy balance before exercise. Again, no control trial was employed in this study, weakening the strength of the findings. A meal was consumed three hours before exercise therefore the rise in ghrelin may simply represent a typical postprandial ghrelin response.

Burns et al (2007) examined plasma total ghrelin responses to a continuous bout of treadmill running. Nine healthy men and women completed an exercise trial and a sedentary control trial in a randomised-balanced fashion. Participants ran for 60 min at 74% of $\dot{V}O_2$ max or rested for the equivalent time. Plasma total ghrelin was determined from samples collected before, during and within two hours after exercise. The investigators observed no changes in plasma total ghrelin during or after exercise. These findings were confirmed by Martins et al (2007) who observed no change in circulating concentrations of total ghrelin in response to 60 min of moderate intensity cycling (65% of maximum heart rate).

Jürimäe *et al* (2007a) examined total ghrelin responses to sculling in elite male rowers. The researchers thought that an exercise modality capable of recruiting a larger proportion of the musculature would induce a greater stimulus than either cycling or running. Nine male members of the Estonian national rowing team completed 30 min of sculling at an intensity marginally above, and on another occasion below, the individual anaerobic threshold (~79% of $\dot{V}O_2$ max). Plasma total ghrelin was determined before, immediately after exercise and then 30 min later. No significant changes in circulating

total ghrelin concentrations were found in either trial although in the higher intensity trial an increase (7.1% from pre-exercise levels) immediately after exercise approached significance ($P = 0.051$).

Subsequent to this, the same researchers sought to impose an even more demanding stimulus (Jürimäe et al, 2007b). Eight trained male rowers completed a maximal 6000m rowing ergometer test (81% of $\dot{V}O_2$ max). Plasma total ghrelin levels were significantly higher (24%) immediately after exercise as compared with pre-exercise values however no difference was apparent 30 min later. The authors suggest that this study provides evidence indicating that an exertional bout of exercise with a correspondingly high level of energy expenditure (~1674 kJ, 400 kcal) is necessary to provoke an increase in circulating total ghrelin. Based on this reasoning it is unclear why changes in total ghrelin were not found in the studies of Burns et al (2007) and Martins et al (2007) where the gross energy expenditure of exertion was 895 kcal and 492 kcal, respectively. The intensity of exercise was especially high in the study of Jürimäe et al (2007b) which may indicate that ghrelin concentrations increase only in response to exercise of such an intensity.

A limitation of the previous studies conducted by Jürimäe et al (2007a; 2007b) is the failure to include sedentary control groups. To rectify this a third study was completed (Jürimäe et al, 2009). Nine national level rowers completed a 120 min rowing training session (87% of maximum heart rate, estimated energy expenditure 1200-1500 kcal) or rested for the equivalent time period on another occasion. Circulating total ghrelin concentrations remained unchanged immediately after exercise but were 15% greater than control 30 min afterwards. Moreover, the total distance covered during the session was positively correlated with total ghrelin ($r = 0.75$). These findings suggest that a significant challenge to energy balance is needed to perturb total ghrelin concentrations and that the metabolic reaction is determined by the absolute amount of work performed.

Other investigators have reported findings indicating that exercise reduces circulating concentrations of total ghrelin (Toshinai et al, 2007; Vestergaard et al, 2007; Malkova et al, 2008). Toshinai et al (2007) submitted five healthy males to a 40 min bout of

graded intensity cycling. After an overnight fast participants cycled for 10 min at 50% of their lactate threshold, at the lactate threshold, at the onset of blood lactate accumulation (OBLA) and finally the OBLA peak (a midpoint between the OBLA and $\dot{V}O_2$ max). Compared with baseline, circulating concentrations of total ghrelin were significantly reduced in an intensity dependant fashion. Significant inverse correlations were reported between changes in plasma ghrelin during exercise and adrenaline (r = -0.533) and noradrenaline (r = -0.607). The authors speculate that sympathetically mediated reduction in gastric blood flow may be responsible for the suppression in circulating ghrelin.

An increase in circulating GH during exercise has also been cited as a possible mediator of suppressed ghrelin (Vestergaard et al, 2007). Vestergaard and associates (2007) examined the total ghrelin response to a maximal exercise test in 29 elite athletes. Total ghrelin was determined from blood samples collected before, immediately after and frequently during two hours of recovery. As compared with pre-exercise values, a decrease in serum total ghrelin was found 30 min into recovery. A significant increase in circulating GH was reported to precede this change by 15 min.

Malkova et al (2008) examined changes in ghrelin in response to exercise and a meal consumed afterwards. Eleven healthy men cycled at 90% of their lactate threshold for 57 min or rested during the equivalent period in a sedentary control trial. Blood samples were collected immediately after exercise and then two hours later. Participants then consumed a standardised meal and postprandial blood was collected 30, 120 and 180 min after meal consumption. The key finding reported was that the ghrelin AUC was significantly lower than control in the 180 min after meal consumption, indicating a greater meal related suppression of ghrelin after performing exercise.

Up to this point the studies described in this section have measured circulating concentrations of total ghrelin. Total ghrelin is composed of acylated and unacylated moieties yet it is the acylated from that is recognised as being directly implicated in the regulation of appetite and energy intake. It is possible that assessment of total ghrelin may have masked important changes in acylated ghrelin. Since assays specific to acylated ghrelin have become available more recent studies have measured acylated

ghrelin, either preferentially or in addition to total ghrelin (Broom et al, 2007; 2009; Marzullo et al, 2008; Shorten et al, 2009; Ueda et al, 2009).

Broom et al (2007) were the first to examine the effects of exercise on circulating concentrations of acylated ghrelin. In their investigation nine healthy males completed two experimental trials (exercise and control) in a randomised crossover design. After an overnight fast, participants ran on a treadmill for 60 min at 72% of $\dot{V}O_2$ max (or rested in the control trial) and then rested in the laboratory for eight hours. Participants consumed a standardised test meal three hours into trials. Plasma acylated ghrelin concentrations were significantly lower during exercise and immediately afterwards. Moreover, the acylated ghrelin AUC was 38% lower over the first three hours of the exercise trial and 35% lower over the full nine hours compared with control. Interestingly, hunger ratings were significantly reduced over the first three hours of the exercise trial and this was positively correlated with the acylated ghrelin AUC during the equivalent period ($r = 0.699$, $P = 0.036$). These findings suggest a possible role of acylated ghrelin in mediating suppressed hunger in response to high intensity running.

Marzullo et al (2008) sought to compare acylated and total ghrelin responses to high intensity exercise in lean verses obese individuals. In fasting conditions, eight obese and eight lean participants completed a graded cycling test to volitional fatigue. The test began at 20 watts and was increased by the same amount at the end of each four min stage. Serum acylated and total ghrelin concentrations were assessed at baseline, peak exercise and then 20 and 40 min into recovery. The researchers described significantly lower fasting concentrations of acylated and total ghrelin in the obese individuals compared with the lean. Moreover, in each participant subgroup acylated ghrelin was suppressed at the point of peak exercise as compared with pre-exercise levels, with a significantly greater reduction apparent in the obese group (21% versus 36%). This was despite the obese sample achieving a lower exercise performance (140 vs. 90 watts). Interestingly, serum total ghrelin remained unchanged in response to exercise in both lean and obese participants. These findings provide evidence that acute exercise suppresses acylated ghrelin independent of adiposity and highlight marked differences in the acylated and total ghrelin responses to exercise.

Ueda et al (2009) also compared acylated ghrelin responses to exercise in obese individuals and healthy weight controls. In this study seven obese and seven lean young males completed two experimental trials (exercise and control) in a randomised, balanced fashion. Participants consumed a test meal 70 min before exercising on a recumbent ergometer for 60 min (50% of $\dot{V}O_2$ max). Acylated ghrelin was determined from blood samples collected before, during and at 30 min intervals throughout one hour of recovery. The investigators observed no significant changes in circulating levels of acylated ghrelin. It is possible that consumption of a substantial meal (2343 kJ, 560 kcal) prior to exercise may have masked any changes in circulating acylated ghrelin. Furthermore, the intensity of exercise completed was lower than that of other studies which have shown changes in circulating acylated ghrelin in response to exercise, thus perhaps changes in acylated ghrelin occur only in response to high intensity exercise.

Shorten et al (2009) recently examined the combined effect of exercise and environmental temperature on appetite regulatory hormones. Eleven active males completed a sedentary control trial and two exercise trials in a random order. In the exercise trials participants ran on a treadmill for 40 min (70% of $\dot{V}O_2$ max) either in the heat (36°C) or in a thermoneutral environment (25°C). Plasma acylated ghrelin was assessed before and 30 min after exercise. The researchers observed no significant changes in circulating acylated ghrelin although there was a tendency for acylated ghrelin to be suppressed by exercise when performed in the heat (P = 0.072). It is possible that the greater need to dissipate heat and consequently, a larger redistribution of blood from the splanchnic regions, may explain why acylated ghrelin tended to be lower only after exercise completed in the heat.

In an investigation which the author was involved with, Broom et al (2009) confirmed that acylated ghrelin is suppressed by high intensity aerobic exercise and demonstrated that acylated ghrelin is also suppressed by resistance exercise. In this study eleven healthy males completed three, eight hour trials (aerobic exercise, resistance exercise and control) in a randomised crossover design. In the aerobic exercise trial participants ran on a treadmill for 60 min at 69% of $\dot{V}O_2$ max and rested in the laboratory for seven hours after. In the resistance exercise trial participants completed a 90 min free weight exercise session, performing 12 repetitions of 10 different whole body exercises at 80%

of their 12 repetition maximum, and then rested for 6.5 h. Plasma concentrations of acylated ghrelin were significantly reduced during both aerobic and resistance exercise. In addition, subjective hunger ratings were lower during aerobic exercise and for up to 30 min after whilst hunger was suppressed at the end of the resistance exercise session. These findings confirm a transient suppression of appetite during and after aerobic and resistance exercise and suggest a possible mediating role of acylated ghrelin. Unfortunately, by nature of the study design, these findings cannot tell us whether the changes in acylated ghrelin would have influenced food intake. The studies described within this thesis have sought to shed light on this issue.

2.5.2 Acute effects of resistance exercise on ghrelin

To date, five studies have examined the influence of resistance exercise on ghrelin (Kraemer et al, 2004b; Takano et al, 2005; Ghanbari-Niaki, 2006; Ballard et al, 2009; Broom et al, 2009). Findings from these studies tend to suggest that ghrelin is suppressed by resistance exercise (Kraemer et al, 2004b; Ghanbari-Niaki, 2006; Ballard et al, 2009; Broom et al, 2009) although one study reported no change in circulating total ghrelin (Takano et al, 2005) whilst another found a suppression prior to a delayed increase (Ghanbari-Niaki, 2006).

Kraemer et al (2004b) reported that a resistance exercise session composed solely of concentric muscle contractions suppressed circulating total ghrelin levels measured immediately and 30 min after exercise. In this study nine healthy males performed four sets of four exercises (bench press, leg extension, military press and leg curls), each set comprising of 12 repetitions at 80% of each participant's 10 repetition maximum. Interestingly, a similar session of eccentric exercise did not alter circulating total ghrelin.

Takano et al (2005) observed no significant changes in circulating total ghrelin in response to a low intensity resistance exercise protocol where blood flow was partially occluded. In this study 11 healthy males performed 30 leg extension exercises at 20% of their one repetition-maximum with muscle blood flow partially occluded. After a short rest participants exercised until fatigue prevented them from continuing. Total ghrelin levels did not change throughout.

Ghanbari-Niaki (2006) sought to impose a particularly strenuous resistance exercise stimulus through which to examine changes in circulating concentrations of total ghrelin. Fourteen healthy males completed three circuits of 10 exercises, performing 10-12 repetitions of each exercise at 60% of their one repetition maximum. Total ghrelin was suppressed immediately after exercise however plasma concentrations were significantly higher than baseline 24 h post-exercise. It is possible that resistance exercise induced a latent anabolic stimulus however not including a sedentary control trial makes it difficult to tell whether this effect was due to exercise *per se*.

Ballard et al (2009) examined the interactive effects of resistance exercise and carbohydrate beverage consumption on circulating total ghrelin concentrations and post-exercise energy intake and found that a strenuous 80 min protocol (four sets of eight whole body exercises) suppressed circulating levels independent of whether a carbohydrate beverage was consumed before exercise.

Broom and co-workers (2009) are the only researchers who have examined the effects of resistance exercise on circulating levels of acylated ghrelin. In response to a strenuous 90 min bout of resistance exercise circulating levels of acylated ghrelin were suppressed during and immediately after exercise but were no different from control in the 6.5 h after. It is possible that this decline in acylated ghrelin may be implicated in the reported suppression of hunger during exercise.

2.5.3 Effects of exercise training on ghrelin

Evidence regarding the influence of repeated bouts of exercise on circulating concentrations of ghrelin is available but the interpretation of findings from many of these studies is difficult due to changes in body weight confounding study outcomes (Leidy et al, 2004; Foster-Schubert et al, 2005; Garcia et al, 2006; Kelishadi et al, 2008). Specifically, it is known that circulating concentrations of ghrelin increase in response to weight loss and decrease with weight gain. Notwithstanding, the findings from these particular studies suggest that ghrelin responds in a compensatory manner to chronic deficits in energy, regardless of whether this is induced through exercise or dietary means.

Potentially more useful data are available from studies in which body weight has remained stable over the course of exercise interventions (Mackelvie et al, 2007; Jones et al, 2009; Hagobian et al, 2009). Mackelvie et al (2007) submitted 17 overweight and 17 healthy weight adolescent boys to meal tolerance tests 36 h before and after five consecutive days of exercise. Participants performed 60 min of aerobic exercise at an intensity between 65-75% of maximal heart rate reserve on each day of the intervention. Fasting and meal related changes in circulating total ghrelin were no different after the intervention, however fasting levels of acylated ghrelin were elevated after the intervention in normal weight and overweight participants. Moreover, after the intervention the acylated ghrelin AUC during the four hours after the meal tolerance test was significantly higher in each group. Interestingly, changes in acylated ghrelin were positively related to changes in appetite. These results suggest that consecutive bouts of exercise elicit an increase in fasting and meal related changes in acylated ghrelin, possibly representing a counter measure in response to regular periods of elevated energy expenditure. Unfortunately, failure to include a sedentary control group and a lack of dietary control throughout this intervention make it difficult to determine whether these reported outcomes are due solely to exercise.

In another study, Jones et al (2009) observed no significant changes in circulating acylated ghrelin concentrations in response to an eight month exercise intervention. In this study 12 overweight adolescents completed 60 min of supervised training three times per week. After accounting for a warm-up and cool down participants completed 45 min of aerobic exercise during each session at an intensity between 60-85% of peak oxygen uptake. As compared with baseline, the researchers observed no significant difference in fasting acylated ghrelin concentrations after the intervention.

Hagobian et al (2009) examined sex differences in energy regulating hormone and appetite responses to four consecutive days of exercise in previously sedentary, overweight/obese, men and women. In a counterbalanced order, on two occasions nine males and nine females completed four consecutive days of moderate intensity treadmill exercise (50-65% of $\dot{V}O_2$ peak) sufficient to expend 30% of total daily energy expenditure. On one occasion participants increased their energy intake to replace the energy expended during exercise whilst on the other occasion participants consumed a

diet sufficient for their pre-intervention energy requirements and therefore were in energy deficit. Before and after each intervention circulating concentrations of acylated ghrelin were measured in the fasting state and in response to a 120 min meal tolerance test. Fasting concentrations of acylated ghrelin remained unchanged in response to the intervention in both men and women. Acylated ghrelin responses to the meal tolerance test did not vary by condition in men. Conversely, in the females, within both exercise conditions an attenuated meal related suppression of acylated ghrelin was observed with subsequently higher values being apparent at the end of the meal tolerance test. Expressed as AUC, in the females acylated ghrelin was elevated from baseline by 32% after the energy deficit trial and 25% after the energy balance trial. These results indicate that in women, exercise perturbs acylated ghrelin in a direction expected to stimulate energy intake, regardless of energy status. These data suggest that the mechanisms controlling energy balance are more strictly regulated in females than males.

2.5.4 Acute effects of exercise on PYY

Martins et al (2007) were the first to report findings concerning the effects of exercise on circulating concentrations of PYY. As compared with responses on a sedentary control trial, the researchers observed significantly higher concentrations of total PYY during a 60 min bout of moderate intensity cycling (66% of maximum heart rate). Values were not significantly different from the control trial 30 min after though, indicating that the stimulatory effect of exercise is transient. Energy intake at a buffet meal one hour after exercise was significantly higher on the exercise trial (Δ 632 kJ, 151 kcal), indicating that the acute changes in PYY did not influence energy intake. Ueda and co-workers (2009) confirmed the findings of Martins et al (2007). In a sample of lean and obese young males, the researchers demonstrated that circulating concentrations of total PYY were increased during a 60 min bout of recumbent cycling at 50% of $\dot{V}O_2$ max. Again, total PYY concentrations were not different from values exhibited in a control trial 30 min afterwards.

Broom et al (2009) also observed heightened circulating concentrations of total PYY in response to aerobic, but not resistance exercise. In this study plasma total PYY concentrations increased significantly during continuous treadmill running (69% of

$\dot{V}O_2$ max) and remained higher than values on a sedentary control trial, for 30 min after. Furthermore, the total PYY response to feeding was accentuated after participants had completed the 60 min of running. In this study it is possible that the more prolonged increase in total PYY was due to the greater exercise intensity. Moreover, the failure of resistance exercise to alter circulating total PYY may be related to the lower energy expenditure elicited and/or the lesser gastrointestinal distress imposed.

Shorten et al (2009) measured total PYY responses to exercise in hot (36°C) and thermoneutral conditions (25°C) and made comparisons with a sedentary control trial. Running in the heat (70% of $\dot{V}O_2$ peak) significantly increased levels of total PYY 30 min post-exercise however no changes were apparent when exercise was performed in a thermo-neutral environment. It is possible that the greater stress imposed during exercise in the heat may be implicated in the PYY response as it is known that stress increases circulating levels of PYY (Chandarana et al, 2009).

A limitation of the studies cited in this section is that concentrations of total PYY have been measured, that is, assays have been used which detect concentrations of both PYY_{1-36} and PYY_{3-36}. Effects of PYY on appetite are mediated specifically by PYY_{3-36} therefore previous assays for total PYY will have been less sensitive to the physiologically relevant form of PYY.

To date, only one investigation has examined the PYY_{3-36} response to acute exercise (Cheng et al, 2009). In this study 12 participants completed a control trial and two exercise trials. In the control trial participants consumed a high fat liquid meal and were then observed for several hours postprandially. Identical meals were consumed during exercise trials, however 50 min of moderate intensity cycling (60% of $\dot{V}O_2$ max) were completed either one hour before (exercise-meal trial) or two hours after the meal (meal-exercise trial). Two findings were notable in this study. Firstly, in the exercise-meal trial, circulating PYY_{3-36} concentrations did not change during exercise (pre to post-exercise). Secondly, PYY_{3-36} tended to be lower after the meal in the control trial as compared with levels exhibited during trials where exercise was performed. These findings suggest that PYY_{3-36} does not respond to exercise *per se*, but exercise may potentiate the PYY_{3-36} response to feeding.

2.5.5 Effects of exercise training on PYY

Jones et al (2009) reported findings from an investigation examining the effects of exercise training on hormones related to appetite control and insulin sensitivity. Twelve overweight adolescents completed 32 weeks of exercise training, performing one hour of aerobic based activity (~60-85% of $\dot{V}O_2$ max) on three days each week. Fasting concentrations of total PYY were measured before and after the intervention. Total PYY was significantly higher (23%) after the intervention, a response which would imply a beneficial effect on appetite in this population. This study does have notable limitations however. Only fasting concentrations of PYY were assessed however PYY is a postprandial satiety signal therefore examination of postprandial responses would provide more useful information than fasting values. Furthermore, no control group was used in this study therefore it cannot be established whether changes in PYY were due solely to the exercise intervention.

In a recent investigation, Kelly et al (2009) randomly assigned 19 older aged obese men and women to an exercise intervention or a combined exercise and diet intervention, each lasting 12 weeks. In the former, participants completed 50-60 min of walking or cycling (75% of $\dot{V}O_2$ max) on five days each week. In the latter, participants also reduced their energy intake by ~700 kcal per day with a five percent decrease in fat contributing to the energy deficit. Circulating concentrations of PYY_{3-36} were assessed in the fasted state and during the course of an oral glucose tolerance test. In both conditions fasting PYY_{3-36} concentrations remained unchanged although responses to the oral glucose challenge (Δ 0-30 min) increased two-fold in the exercise group and 1.5 times in the exercise-diet group. These findings suggest an improvement in PYY_{3-36} sensitivity in response to these interventions. The implications of these findings regarding appetite and energy intake are not known.

2.6 Summary

The ever increasing global prevalence of overweight and obesity has prompted a need to better understand energy homeostasis and its associated mechanisms. In recent years the search for effective pharmacological targets has catalysed research examining the body's own appetite regulating signals within the gastrointestinal tract (Neary and Batterham, 2009a). Exercise is an important component of daily energy balance and an

increasing body of research has began to characterise the influence of exercise on appetite regulating hormones. This work sits alongside an existing body of data regarding the influence of exercise on appetite and energy intake. The work presented in this thesis has sought to contribute to existing knowledge within each of these topical and related areas of research.

CHAPTER III
General Methods

This chapter describes the experimental methods used in the studies presented within this thesis as certain aspects of the methodology were common between studies. Loughborough University's Ethical Advisory Committee approved each of the studies described in this thesis and written informed consent was gained from study participants before participating in these research investigations.

3.1 Participants

For the studies reported in this thesis participants were recruited from Loughborough University and the surrounding area by word of mouth, poster and email advertisement. Volunteers were given a participant information sheet describing the demands of the study and the associated risks and discomforts. Volunteers provided written informed consent (Appendix A) and completed a health screen questionnaire (Appendix B) before any experimental procedures began. Participants also completed questionnaires assessing physical activity (Appendix C) and dietary habits (Stunkard and Messick, 1985) (Appendix D), the latter being used to ensure the absence of any individuals with atypical eating tendencies which could potentially confound study outcomes. Most of the participants recruited were students completing their studies at Loughborough University and were physically active. Prior training was not a pre-requisite for participation in these studies however the physical demands of the protocols ensured that all of the participants were reasonably fit.

The inclusion criteria for the recruitment of study participants were as follows:

- male
- non-smokers
- recreationally active
- not taking any medication known to influence lipid or carbohydrate metabolism
- not dieting and did not have any extreme dietary habits
- weight stable within the last three months i.e. < 2.3 kg change in body weight (St Jeor et al, 1997)
- sufficient ability to complete the study demands i.e. the exercise protocols

- no history of cardiovascular disease, metabolic disease or dyslipidaemia
- resting arterial blood pressure < 140/90 mmHg
- BMI < 30 kg· m^{-2}
- Tolerance for the food items presented at the *ad libitum* buffet meals

3.2 Anthropometry

Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca Ltd, Germany) and body mass was measured to the nearest 0.1 kg using a digital scale (Seca Ltd, Germany). For measurements of height and body mass participants were bare foot and wearing light clothing. Participants' body mass index was calculated as weight in kilograms divided by the square of their height in metres. Waist circumference was measured with an inelastic polyfibre tape measure (Hokanson, Washington, USA). The measurement was taken at the end of expiration at the narrowest part of the torso (above the umbilicus and below the xiphoid process).

Measurements of subcutaneous fat were taken to estimate total body fatness. Skinfold thickness was measured using skinfold callipers (John Bull, British Indicators, West Sussex, UK) at four anatomical locations (biceps, triceps, subscapula, suprailiac) and body density was calculated using the predictive equations of Durnin and Womersley (1974). Percentage body fat was then estimated using the Siri equation (Siri, 1956). All measurements were made in duplicate on the right side of the body with the participant standing. If skinfold measurements were not within 1-2 mm the site was measured a third time. Measurements were made by rotating through the anatomical sites to allow the skin time to regain normal texture and thickness.

3.3 Heart rate measurement

Heart rate was measured during preliminary exercise tests and main trials using short-range telemetry (Polar F4, Polar Electro, Kempele, Finland).

3.4 Rating of perceived exertion

The Borg scale was used to ascertain subjective perceptions of exercise intensity during preliminary exercise testing and main trials (Borg, 1973). This scale ranges from six (no exertion) to 20 (maximal exertion).

3.5 Arterial blood pressure measurement

During preliminary screening arterial blood pressure was measured by auscultation using a sphygmomanometer (Hawksley MK. II, Hawksley and Sons Ltd, Sussex, UK) according to standard guidelines (Williams et al, 2009). Measurements were taken in duplicate after participants had been seated for five min. The mean of two measurements was the value used.

3.6 Exercise tests

3.6.1 Submaximal treadmill running test

In studies four, five and six (Chapters seven, eight and nine) a 16 min submaximal treadmill running test was used to determine the relationship between running speed and oxygen consumption. The test was designed to exercise the participants through a range of intensities ranging from moderate to vigorous, but not maximum. The test was continuous in nature but was composed of four, four min stages. The test began at a suitable speed for the participant (typically between 7-8.5 $km{\cdot}h^{-1}$) and was increased by 1-1.5 $km.h^{-1}$ following each stage. In the final min of each stage a 60 second sample of expired air was collected into Douglass bags (Plysu Protection Systems, Milton Keynes, UK) for the determination of oxygen consumption and carbon dioxide production. Heart rate was monitored continuously during the test and ratings of perceived exertion were ascertained during the expired air collection time. Oxygen consumption was plotted against running speed at each stage to identify the relationship between submaximal running speed and oxygen consumption.

3.6.2 Maximum oxygen uptake test

Participants were given 15-20 min to recover from the submaximal treadmill running test before undertaking the maximum oxygen uptake test. Maximum oxygen uptake was assessed using an incremental treadmill run to exhaustion and was designed so that participants reached volitional fatigue within 10-12 min (Taylor et al, 1955). The treadmill speed remained constant and was determined by each participant’s performance in the submaximal test. The treadmill gradient began at 3.5^{o} and was increased by 2.5^{o} at three min intervals until participants reached exhaustion. Samples of expired air were collected for one min, 1.75 min into each three min stage i.e. 1:45-2:45, 4:45-5:45 etc. A final expired air sample was collected when participants indicted

they were able to continue for one min only. Strong verbal encouragement was given to ensure that subjects completed this final collection.

After the maximum oxygen uptake test, oxygen consumption and carbon dioxide production were determined from each expired air sample and the highest value attained was accepted as the maximum oxygen uptake. The following criteria were used to confirm attainment of a true maximal value: 1) a plateau in oxygen consumption, 2) heart rate within 10 beats$\cdot$min^{-1} of age-predicted maximum heart rate, 3) a respiratory exchange ratio $\geq$ 1.15, 4) rating of perceived exertion of 19 or 20 (Cooke, 2001).

Once maximum oxygen uptake had been determined this was used in combination with data regarding the individual relationship between submaximal running speed and oxygen consumption to determine the treadmill speed that was necessary to elicit the desired percentage of maximum oxygen uptake during main trials. In main trials participants began exercising at this speed however adjustments were made during exercise if necessary i.e. for cardiovascular drift.

3.7 Expired air analysis

Expired air samples were collected into Douglas Bags (Plysu Protection Systems, Milton Keynes, UK). Oxygen consumption and carbon dioxide production were determined using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser (Series 1400, Servomex, Crowborough, East Sussex, UK). Prior to sample analysis the analysers were calibrated with certified reference gases. Expired gas volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and the expired air temperature was determined using a thermistor during evacuation (Edale, type 2984, Model C, Cambridge, UK). Barometric pressure was measured using a Fortin barometer (F.D. and company, Watford, UK). Expired air samples were corrected to standard temperature and pressure (dry). The dry gas meter was calibrated regularly using a three litre syringe (Series 5530, Hans Rudolph Inc, Kansas City, Missouri, USA).

3.8 Calculation of energy expenditure

For expired air samples collected at rest and during exercise oxygen consumption and carbon dioxide production values were used to determine energy expenditure and

substrate oxidation using the equations described by Frayn (1983). The intermittent nature of weight lifting invalidates the typical assumptions of these equations as the respiratory exchange ratio is consistently equal to or great than 1.0. Consequently, for expired air samples collected during study one (Chapter four) energy expenditure was estimated as 21.1 kJ (5.05 kcal) per litre of oxygen consumed (McArdle et al, 1991). This reflects the assumption that energy was derived from carbohydrate rather than fat and assumes no contribution of protein during exercise. No attempt was made to quantify the energy contribution from anaerobic sources therefore this may have led to underestimations of the energy expended during resistance exercise.

3.9 Physical activity and dietary control

The energy and nutrient content of meals consumed on the evening prior to experimental trials affects appetite perceptions and gut hormone levels on the subsequent morning (Chandarana et al, 2009). Therefore, in the studies presented in this thesis, during the 24 h before main trials participants standardised their food intake, consuming identical food items at identical times during this period. To do this, before undertaking the first experimental trial of a study, participants completed a weighed food record of all items consumed during this period. Participants then replicated their intake during the 24 h before subsequent trials. In this period participants abstained from alcohol, caffeine and structured sessions of physical activity. After 23:00 on evenings prior to main trials participants refrained from eating. During this time water was permitted *ad libitum* and was encouraged to avoid dehydration.

3.10 Assessment of appetite

In each of the studies described in this thesis perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption) were assessed periodically using previously validated visual analogue scales (Flint et al, 2000) (Appendix E). Participants rated their appetite perceptions by placing a mark on a 100 mm continuum with descriptors positioned at either end. Participants could not refer to their previous ratings when completing the appetite scales. The scales were analysed by measuring the horizontal distance from the left hand side to the point on the line indicated by the participant.

3.11 Breakfast snacks

In studies one, two and five (Chapters four, five and eight) main trials commenced after consumption of a breakfast snack. The breakfast provided was standardised to body weight and consisted of a commercial cereal bar (Kellogg's Nutri-grain®). Participants received 1.06 g per kilogram of body weight measured on the first trial visit. Identical amounts were consumed prior to subsequent main trials. For a 70 kg individual this provided 1092 kJ (260 kcal) of energy, 6 g of fat, 4 g of protein and 48 g of carbohydrate. The breakfast was consumed within 5 min in all trials.

3.12 Ad libitum buffet meals

During the main trials of each study participants were given access to buffet meals from which they were free to consume food *ad libitum*. Prior to main trials acceptability of the buffet food items was ensured by the completion of a food preference questionnaire (Appendix F). The questionnaire required participants to rate pre-selected food items on a Likert scale ranging from one (dislike extremely) to ten (like extremely). Questionnaires were examined to ensure that the foods presented at meals would be to the taste of each individual. Distaste for the buffet items (rating four items less than or equal to four) resulted in participant non-inclusion.

In all studies a cold buffet meal was made available to participants at distinct time points during trials (Appendix G). The buffet meal provided diversity in protein, fat and carbohydrate content in order to facilitate the detection of macronutrient preferences. At these meals food was presented in excess of expected consumption and participants were told that additional food was available if desired. Participants were given 30 min to eat at buffet meals and were told to eat until satisfied. Participants consumed meals in isolation so that social influence did not constrain food selection. Food consumption was ascertained by examining the weighted difference in food items remaining compared with that initially presented. The energy and macronutrient content of the items consumed was ascertained using manufacturer values. In study four (Chapter seven) energy intake was examined over the course of 24 h therefore a hot buffet lunch (Appendix H) was also provided during main trials.

3.13 Time cues, environmental temperature and humidity

In each of the studies described environmental temperature and relative humidity were assessed periodically throughout main trials using a hand-held hygrometer (Omega RH85, Manchester, UK). Participants were not devoid of time cues in the laboratory in each of the studies except for study five (Chapter eight) where a key outcome was to determine if exercise influenced feeding latency. In study five clocks were removed from the laboratory and participants were not allowed to bring mobile telephones into the laboratory.

3.14 Blood sample collection

Approximately 30 min before commencing main trials participants rested in a semi-supine position whilst a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein (Chapters six, seven, eight and nine). A cannula cannot be in place during immersion in water, hence in the swimming study (Chapter five) a cannula was inserted 30 min after exercise had been completed and therefore the baseline, pre and post-exercise blood samples were taken via venepuncture of an antecubital vein. Samples collected after this were obtained via a cannula. Also, in study four (Chapter seven) to minimise participant discomfort on the second day of main trials a venepuncture was used to collect a single fasting blood sample.

Venous blood samples were collected into pre-cooled 4.9 or 9 mL potassium-ethlenediamine tetra-acetic acid (EDTA)-coated monovettes (Sarstedt, Leicester, UK) via a multi-adapter (Sarstedt, Leicester, UK). Patency of the cannula was maintained by flushing with 10 mL of non-heparinised saline (0.9% w/v sodium chloride, Baxter Healthcare Ltd, Norfolk, UK) after each sample collection. To avoid dilution of subsequent samples residual saline was drawn off immediately prior to collection using a 2 mL syringe. To control for postural changes in plasma volume participants lay in a semi-supine position approximately five min prior to each blood sample and remained in this position during the collection. Exceptions to this occurred when blood samples were taken during exercise (treadmill running). In this situation participants straddled the treadmill whilst blood samples were obtained and this procedure took no longer than one min.

Blood samples collected into 9 mL EDTA monovettes were centrifuged immediately at 1681 *g* for 10 min in a refrigerated centrifuge (Heraeus Labofuge 400 R, Thermo Fisher Scientific Inc, Loughborough, UK) at four degrees Celsius. The plasma supernatant was then aliquoted into Eppendorf tubes (Sarstedt, Leicester, UK). Samples were stored at -20°C for the analysis of glucose, triacylglycerol, insulin (Chapters six and seven) at a later date.

Separate venous blood samples were collected into 4.9 mL monovettes for the determination of plasma acylated ghrelin concentrations. To prevent the degradation of acylated ghrelin by protease enzymes these monovettes contained EDTA and a 50 µL solution containing potassium phosphate buffer, *P*-hydroxymercuribenzoic acid and sodium hydroxide. These monovettes were spun immediately after sample collection at 1287 *g* for 10 min in a refrigerated centrifuge (GS-15R Centrifuge, Beckman Coulter, Fullerton, USA) at four degrees Celsius. The supernatant was then aliquoted into plain storage tubes where 100 µL of hydrochloric acid (1 M) was added per mL of plasma. Samples were then spun again for 5 min at 1287 *g* to ensure thorough mixing. Samples were then stored at -20°C prior to analysis later.

In study six (Chapter 9) addition 2 mL blood samples were taken periodically to measure circulating concentrations of $PYY_{3\text{-}36}$. To maintain peptide integrity, samples were collected into pre-chilled syringes containing dipeptidyl-peptidase-4 inhibitor (10 $\mu L.mL^{-1}$) (Millipore Ltd, Watford, UK). After mixing by gentle inversion samples were then dispensed into pre-chilled EDTA tubes containing aprotinin (Nordic Pharma Ltd, Reading, UK) at a final concentration of 500 $KIU.mL^{-1}$. These samples were spun at 1681*g* for 10 mins in a refrigerated centrifuge at 4 °C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage (at -20 °C until frozen and then transferred to -80°C). In all studies frozen plasma samples were analysed within 3 months of initial collection.

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

3.15 Blood sample analysis

3.15.1 Estimation of changes in plasma volume

Blood concentrations of haemoglobin and haematocrit were used to estimate plasma volume and determine changes over time (Dill and Costill, 1974). Haematocrit was determined in triplicate using a microlitre-haematocrit centrifuge (MIKRO, 20, Andreas Hettich GmbH and Co.KG, Tuttlingen, Germany). Haemoglobin was determined in duplicate using the cyanmethaemoglobin method with the aid of an ultra-violet spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, UK).

3.15.2 Glucose and triacylglycerol

Plasma glucose and triacylglycerol concentrations were determined by enzymatic colorimetric methods using an automated bench top analyser (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). To ensure precision of analysis internal quality controls exhibiting normal and pathological values were run prior to sample analysis.

3.15.3 Insulin

Plasma insulin concentrations were determined using a commercially available enzyme-linked immuno sorbent assay kit (Mercodia, Sylveniusgatan, Uppsala, Sweden) with the aid of a plate reader to measure absorbance (Expert Plus, ASYS, Eugendorf, Austria). To ensure precision of analysis internal quality controls (Mercodia diabetic antigen control) exhibiting low and high values were assayed.

3.15.4 PYY_{3-36}

Plasma PYY_{3-36} concentrations were determined using a commercially available radioimmunoassay kit (LINCO Research, Missouri, USA). Precision of analysis was ensured by the quantification of internal quality controls exhibiting high and low values.

3.15.5 Acylated ghrelin

Plasma acylated ghrelin concentrations were determined using a commercially available enzyme-linked immuno sorbent assay kit (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader to measure absorbance (Expert Plus, ASYS, Eugendorf, Austria). To ensure precision of analysis an internal quality control (included within the kit) was assayed with each assay plate.

3.15.6 Precision of analysis

To eliminate inter-assay variation, samples from each participant were analysed in the same run. The within batch coefficient of variation for each assay were calculated by repeated measurement of a single plasma sample 10 times. Values for each assay are displayed within the methods section of each experimental chapter.

3.16 Statistical analysis

Data were analysed using the Statistical Package for the Social Science (SPSS) software for Windows (SPSS Inc, Chicago, Il, USA) – version 16.0. All area under the curve values were calculated using the trapezoidal rule. Paired t-tests and one-way ANOVA (Chapter nine) were used to examine differences between fasting and AUC values. Repeated measured two-factor ANOVA was used to examine differences between trials over time for circulating acylated ghrelin, PYY_{3-36}, insulin, glucose, triacylglycerol and appetite perceptions. Where appropriate, post-hoc pair wise comparisons were performed using the Bonferroni method. Adjustment of the alpha criterion value for multiple comparisons (Bonferroni adjustment) cannot be performed in SPSS when there are less than three comparisons. Consequently, for studies in this thesis composed of two main trials (all except Chapter nine), alpha was adjusted manually by dividing alpha (0.05) by the number of comparisons being made across the main trials. For the post-hoc analyses in study six which made comparisons between three main trials (Chapter nine) the automatic Bonferroni adjustment function within SPSS was used. The Pearson product moment correlation coefficient was used to examine relationships between variables. Results are presented as mean ± SEM.

Power analysis can be used to calculate the minimal sample size required to accept the outcome of a statistical test with a particular level of confidence. However, for the studies presented in this thesis the exact sample size for each study was determined based upon practical reasons (e.g. to ensure efficient use of ELISA assays) after ensuring that the sample size was adequate to detect important differences. Specifically, in repeated measures designs eight participants are sufficient to detect significant appetite AUC differences ≥ 10% (Flint et al, 2000) and energy intake differences ≥ 240 kcal (Gregersen et al, 2008). Moreover, significant differences in acylated ghrelin can be detected with a sample size of nine (Broom et al, 2007).

CHAPTER IV

The influence of resistance exercise on appetite and energy/macronutrient intake

4.1 Introduction

Over the last three decades a significant body of research has accumulated regarding the influence of exercise on appetite, energy and macronutrient intake (Martins et al, 2008; Bilski et al, 2009). Although this work has provided valuable data there are some limitations of this work and additional areas of enquiry that require further attention.

One limitation of this research is that studies have predominantly examined the effects of aerobic exercise on appetite and food intake. Resistance exercise is now recognised as important for public health and weight control (Haskell et al, 2007; Donnelley et al, 2009) yet data concerning the effects of resistance exercise on appetite, energy and macronutrient intake are lacking. Information about how resistance exercise influences consumptive behaviour therefore remains rudimentary and as a result, knowledge regarding how resistance exercise influences energy balance remains incomplete. The characteristics of resistance exercise differ to those of aerobic exercise making it possible that appetite and food intake responses may differ. Specifically, aerobic activities are continuous and typically elicit a substantial rate of energy expenditure. Conversely, resistance exercise is intermittent and anaerobic and therefore induces a relatively minor level of energy expenditure. Hence, distinct metabolic and hormonal responses are apparent and this may have consequences for appetite and food intake.

In an investigation which the author was involved in the effects of resistance exercise on appetite and plasma concentrations of the appetite regulatory hormones, acylated ghrelin and total PYY were examined (Broom et al, 2009). As compared with responses during a control trial we observed a significant reduction in hunger during and immediately after a strenuous 90 min bout of resistance exercise. Circulating concentrations of the appetite stimulating hormone, acylated ghrelin were concomitantly reduced, highlighting a possible mediating role. Unfortunately the nature of this particular study did not allow us to examine the influence of resistance

exercise on food intake. It remains unknown how, or if, these reported changes in ghrelin influence food intake.

A recently published study has since provided some preliminary data on this question (Ballard et al, 2009). Ballard and co-workers (2009) reported that perceptions of hunger and satiety, along with *ad libitum* energy intake, remained unchanged in response to an 80 min bout of resistance exercise (four sets of eight exercises selected to stress the major muscle groups). These preliminary findings therefore suggest that resistance exercise does not influence energy intake. Further work is needed though to confirm and extend this work. Specifically, the researchers only examined appetite responses at three points within 2.5 h after exercise. It is possible that changes in appetite may have been detected if assessments had been made over a longer period of time. Similarly, energy intake was examined at one feeding opportunity provided two hours after exercise. Effects of exercise on energy intake may not occur at the first meal taken after exercise, but in response to a second or third meal. Further work is therefore needed to examine appetite and energy intake responses over a longer duration.

In the study conducted by Ballard et al (2009) a homogenous pasta meal was provided from which energy intake was deduced. A limitation with using a single item meal in this situation is that it precludes the ability to determine effects of exercise on macronutrient preferences (Arvaniti et al, 2000). The macronutrient content of foods consumed at a meal are an important determinant of energy intake and therefore understanding how exercise influences this variable is important.

To detect differences in macronutrient selection it is necessary that a buffet meal containing a variety of foods is provided for study participants (Arvaniti et al, 2000). Because one of the key aims of the studies presented in this thesis was to examine how exercise influences *ad libitum* energy and macronutrient intake, a buffet meal was developed containing a variety of foods and was provided to participants in this study. Therefore, an aim of the present study was to assess the feasibility of providing this particular meal to participants and to examine the validity of energy and macronutrient intake responses. It has been noted that the types of foods offered and the way these are presented to participants can potentially affect how much is

consumed at buffet meals provided in the laboratory (Benelam, 2009). Thus, particular attention was given to examining energy and macronutrient intake responses from the meals consumed in this study, with comparisons being made with estimated energy requirements and macronutrient intakes from this specific group of participants. Furthermore, specific attention was given to the relationship between subjective appetite ratings before meals and subsequent energy intake in order to confirm that participants were eating to their appetite with this particular meal, rather than overeating in response to the diversity of items presented (Larsen et al, 1995).

The purpose of the present investigation was two-fold. The first aim was to examine appetite, energy and macronutrient intake responses for an extended period of time after an acute bout of resistance exercise. The second aim was to assess the feasibility of assessing *ad libitum* energy and macronutrient intake from a buffet meal containing a variety of food items familiar to the study participants.

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4.2 Methods

4.2.1 Participants

After gaining Loughborough University ethical advisory committee approval 10 healthy males (21–28 y) gave their written informed consent to participate. Table 4.1 describes the participant characteristics.

Table 4.1 Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	23.8 ± 0.8
BMI ($kg \cdot m^{-2}$)	23.6 ± 0.7
Body Mass (kg)	76.9 ± 3.0
Body Fat (%)	16.2 ± 0.8

(*n* = 10)

4.2.2 Study design

Prior to taking part in main trials participants completed two preliminary exercise sessions. In the first session each participant's 12-repetition maximum for 10 resistance exercises were determined. The second session was a familiarisation session whereby participants completed a 90 min resistance exercise protocol, identical to that performed in the resistance exercise main trial. In subsequent weeks participants completed two main trials (resistance exercise and control) in a randomised, counterbalanced order. Each main trial was separated by at least one-week.

To standardise diet and physical activity before main trials participants completed a weighed food record of all items consumed within the 24 h preceding their first main trial and this feeding pattern was replicated prior to their second main trial. Alcohol, caffeine and structured physical activity were not permitted in the 24 h before main trials or in the hours after leaving the laboratory on the first trial day, before returning to the laboratory on the second day of each main trial (for the 24 h appetite assessment). To minimise physical exertion on the morning of main trials participants were asked to walk slowly to the laboratory if they lived within 0.5 km of the research

laboratory. Participants that lived further away arrived by motorised transport. Participants arrived at the laboratory in the fasted state.

4.2.3 12-repetition maximum test

A 12-repetition maximum test was completed for each of the 10 resistance exercises employed in the study. The 12-repetion maximum values were determined by adding and removing weight as necessary with participants having as much recovery time as required in between attempts. The order in which each exercise was performed was squat, dumbbell lateral raise, bench press, upright row, lunges, bicep curl, barbell pullover, seated shoulder press, triceps extension, and bent over row.

4.2.4 Resistance exercise familiarisation session

On a separate visit participants completed a 90 min familiarisation session in which they completed the full weight lifting session which was to be performed during the resistance exercise main trial: three sets of 12-repetions of 10 different exercises at 80% of 12 repetition maximum. The purpose of this session was to verify that participants could complete the protocol and to confirm fatigue from overload by the end.

4.2.5 Main trials

An interval of at least one-week separated the familiarisation session and each participant's first main trial. Each main trial began in the morning between 08:30 and 09:00 and lasted 24 h. Within the 24 h participants remained within the laboratory from 0 - 8 h and returned the next morning to provide information on overnight food consumption and to complete a final visual analogue scale (24 h assessment). Figure 4.1 provides a schematic illustration of the main trial protocol.

The resistance exercise trial began when participants were provided with a breakfast snack. This was consumed within five min. Participants then rested for the remainder of the first trial hour. At the beginning of the second hour participants began a 90 min free weights session. This session was identical to that performed during the familiarisation session (3 sets of 12 repetitions of 10 different exercises at 80% of 12 repetition maximum). Participants were given three min to complete each set. On completion of the 12 repetitions, participants rested for the remainder of the three

mins. Exercises were completed in the order described for the preliminary tests. All sets for each exercise were completed before moving onto the next exercise. To estimate energy expenditure an expired air sample was taken for three min during the third set of each exercise. After the resistance exercise session participants rested within the laboratory for a further 5.5 h. At the end of this period (16:30 – 17:00) participants left the laboratory and returned the next morning to complete final appetite assessments.

Identical procedures were completed in the control trial with the exception that no exercise was performed. Participants therefore rested throughout the entire trial. To permit the estimation of net energy expenditure during resistance exercise (1 - 2.5 h) (gross energy expenditure of exercise – resting energy expenditure) resting samples of expired air were collected in the semi-supine position throughout the equivalent period in the control trial.

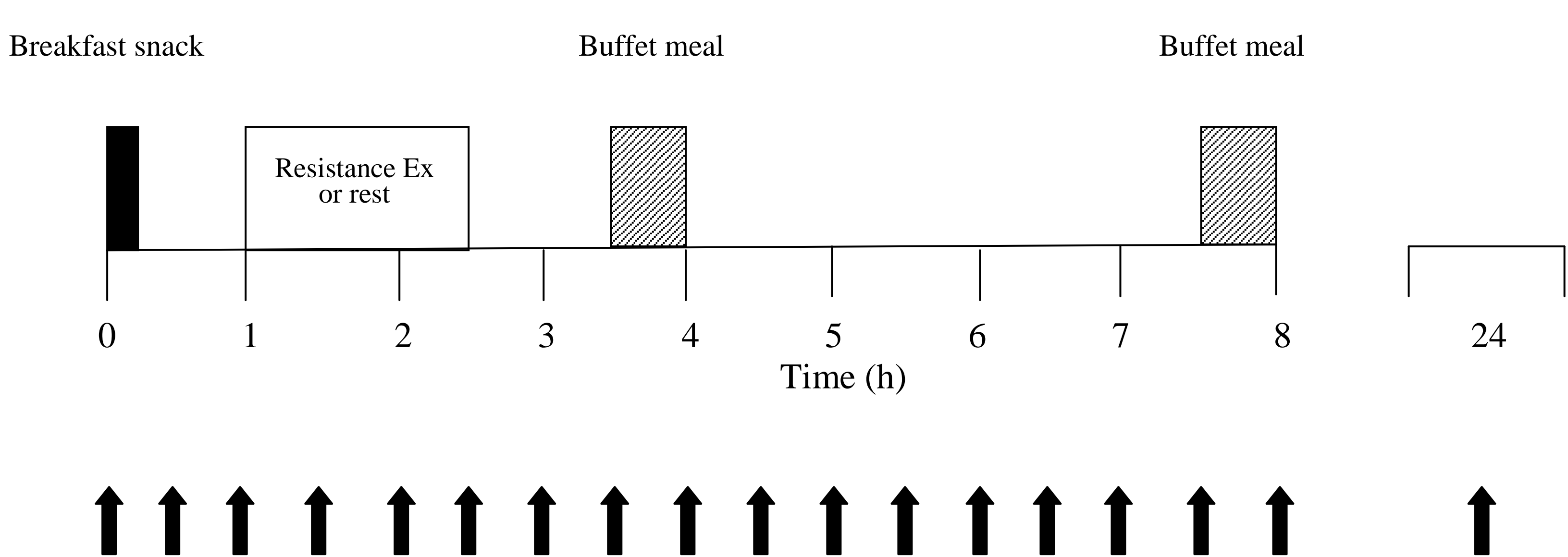

Figure 4.1: Schematic representation of the main trial protocol

4.2.6 Appetite assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption) (Flint et al, 2000). Scales were completed at baseline and then at 30 min intervals throughout the laboratory phase of trials. A single appetite questionnaire was also completed on the morning of the second trial day (24 h assessment).

4.2.7 Breakfast and ad libitum buffet meals

The breakfast provided was standardised to body weight and consisted of a commercial cereal bar (Kellogg's Nutri-grain®). Participants received 1.06 g per kilogram of body weight measured on the first trial visit. Identical amounts were consumed across trials. For a 70 kg individual this provided 1092 kJ (260 kcal) of energy, 6 g of fat, 4 g of protein and 48 g of carbohydrate.

At two points during the laboratory phase of main trials (3.5 – 4 h & 7.5 – 8 h) participants were provided with a buffet meal from which they could consume food *ad libitum*. Additionally, at the end of the first trial day participants were able to select items from the buffet to take away from the laboratory and consume *ad libitum* that evening prior to fasting at 23:00. The buffet meal provided diversity in protein, fat and carbohydrate content in order to facilitate the detection of macronutrient preferences (Appendix G). The food items provided were selected so that they would be familiar to study participants. Food was presented in excess of expected consumption. Participants were told to eat until satisfied and that additional food was available if desired. Participants consumed meals in isolation so that social influence did not constrain food selection. Food consumption was ascertained by examining the weighted difference in food items remaining compared with that initially presented. The energy and macronutrient content of the items consumed was ascertained using manufacturer values.

4.2.8 Calculation of energy expenditure

The short duration intermittent nature of weight lifting invalidates the assumptions of indirect calorimetry as the respiratory exchange ratio is consistently equal to or greater than 1.0. Energy expenditure was therefore calculated as 21.1 kJ (5.047 kcal) per litre of oxygen consumed. This reflects the assumption that energy was derived

from carbohydrate rather than fat and assumes no protein contribution to energy provision during exercise (McArdle et al, 1991).

4.2.9 Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows (SPSS Inc, Chicago, IL, USA). Area under the concentration verses time curve calculations were performed using the trapezoidal method. Student's *t*-tests for correlated data were used to assess differences between fasting and AUC values for appetite perceptions between the control and resistance exercise trials. Repeated measures, two-factor ANOVA was used to examine differences between the resistance exercise and control trials over time for appetite perceptions and energy/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 presented as mean ± SEM.

4.3 Results

4.3.1 Exercise responses

The total weight lifted during the 90 min resistance exercise session was 10,758 ± 621 kg. The net energy expenditure (exercise minus resting) induced by exercise was 1007 ± 92 kJ (241 ± 22 kcal).

4.3.2 Appetite responses

Baseline appetite ratings were not significantly different in the resistance exercise and control trials (Table 4.2).

Table 4.2: Baseline appetite perceptions in the resistance exercise and control trials

	Control	Exercise	*P*
Hunger (0-100)	67 ± 4	58 ± 7	0.270
Satisfaction (0-100)	27 ± 7	19 ± 3	0.419
Fullness (0-100)	16 ± 3	15 ± 3	0.833
PFC (0-100)	73 ± 2	75 ± 5	0.646

(n = 10). PFC = prospective food consumption.

Figure 4.2 shows the appetite responses (hunger, fullness, satisfaction and prospective food consumption) in the resistance exercise and control trials. Two-factor ANOVA revealed a main effect of time for each appetite perception assessed (all $P < 0.001$) signifying changes in appetite in response to the buffet meals. Two-factor ANOVA did not show any significant trial or interaction (trial x time) main effects (all $P > 0.05$) indicating that changes over time were not significantly different between the resistance exercise and control trials.

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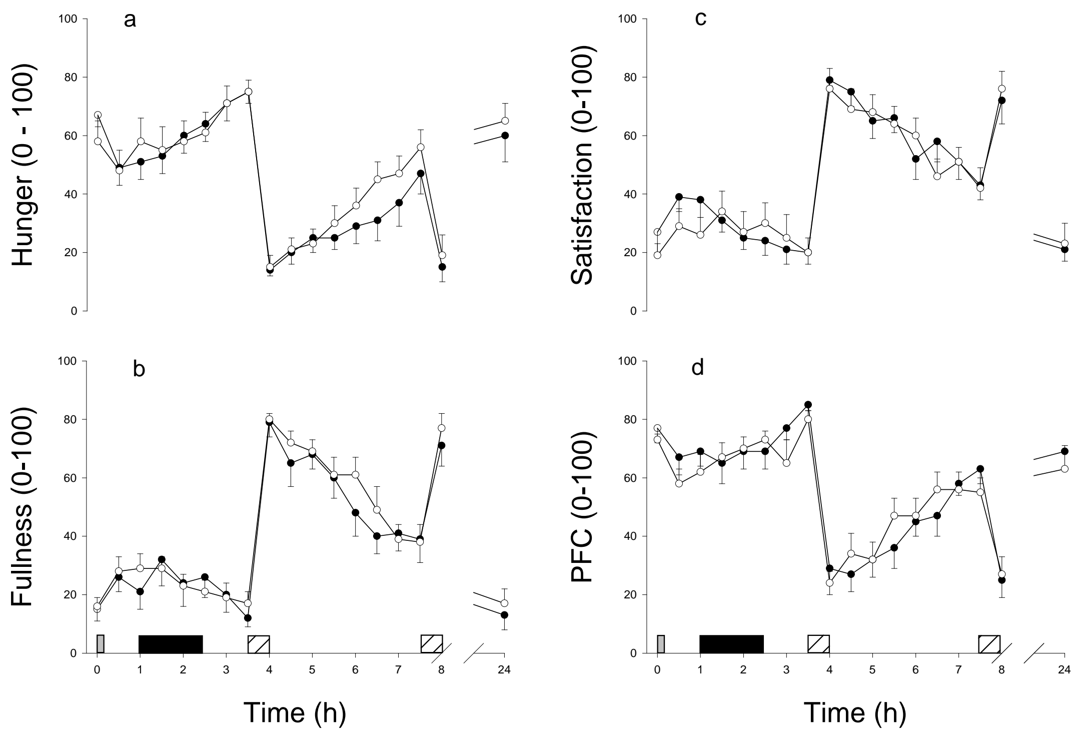


Figure 4.2: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the resistance exercise (○) and control (●) trials. Values are mean ± SEM *(n* = 10). Grey rectangle indicates a breakfast snack. Black rectangle indicates exercise. Diagonal rectangles indicate buffet meals.

Between trial differences in appetite ratings were also evaluated using AUC values for the 3.5 h before the morning buffet meal (0 - 3.5 h) and for the remaining 4 h of the laboratory phase of trials (4 - 8 h). These analyses confirmed that there were no significant differences in appetite between the resistance exercise and control trials (student's *t*-test, all $P > 0.05$).

4.3.3 Energy and macronutrient intake

Two-factor ANOVA showed significant differences in energy intake between the individual meals consumed during main trials (main effect of time, $P = 0.001$) with higher intakes being apparent at the morning buffet meal (3.5 - 4 h) compared with the afternoon meal (7.5 - 8 h) and that consumed overnight (8 - 14 h) (Table 4.3). Two-factor ANOVA did not show any trial ($P = 0.228$) or interaction (trial x time) ($P = 0.541$) main effects, indicating that no significant differences existed in energy intake between the resistance exercise and control trials. Examination of the relative energy intake (energy intake – (resistance exercise energy expenditure – resting

energy expenditure)) showed that there were no significant differences between the resistance exercise (12535 ± 652 kJ, 2996 ± 156 kcal) and control trials (12418 ± 1047 kJ, 2968 ± 250 kcal) (P = 0.913).

Table 4.3: Energy intake in the resistance exercise and control trials

	Control	Resistance exercise
Morning meal (3.5 – 4 h)	5323 ± 405 (1272 ± 97)	5762 ± 362 (1377 ± 86)
Afternoon meal (7.5 – 8 h)	3880 ± 805 (927 ± 192)	3738 ± 386 (894 ± 92)
Overnight (8 – 14 h)	3215 ± 407 (768 ± 97)	4043 ± 341 (966 ± 81)
Total trial (0 – 24 h)	12418 ± 1147 (2967 ± 274)	13543 ± 738 (3237 ± 176)

Values are kJ (kcal) (n = 10).

Two-factor ANOVA was used to examine macronutrient intake (absolute and percent) across the morning and afternoon meals during the resistance exercise and control trials (Table 4.4). There was a significant main effect of time for the absolute intake (grams) of each of the macronutrients, indicating differences in intake at the individual meals during main trials. No significant trial or interaction (trial x time) main effects were apparent therefore there were no significant differences in the absolute intake of the macronutrients between the resistance exercise and control trials.

For the percentage of energy derived from the macronutrients two-factor ANOVA revealed a significant interaction effect (trial x time) for the percentage intake of carbohydrate (P = 0.024) and protein (P = 0.022). Post hoc analysis showed that in comparison with the control trial, the percentage intake of carbohydrate was significantly higher at the afternoon buffet meal in the exercise trial (Student's t-test, P = 0.032) whilst the percentage intake of protein was significantly reduced (Student's t-test, P = 0.020).

Table 4.4: Macronutrient intake in the resistance exercise and control trials

Control Trial	**Fat**	**Carbohydrate**	**Protein**
Morning meal (3.5 – 4 h)	47 ± 4 (33.3)	159 ± 17 (49.3)	55 ± 4 (17.4)
Afternoon meal (7.5 – 8 h)	33 ± 7 (32.1)	118 ± 28 (51.3)	40 ± 10 (16.6)
Overnight (8 – 14 h)	25 ± 5 (26.7)	105 ± 11 (57.5)	36 ± 6 (15.8)
Total Trial (0 – 24 h)	105 ± 11 (31.6)	382 ± 41 (51.3)	131 ± 16 (17.1)
Exercise Trial	**Fat**	**Carbohydrate**	**Protein**
Morning meal (3.5 – 4 h)	50 ± 5 (32.7)	167 ± 10 (49.1)	64 ± 7 (18.2)
Afternoon meal (7.5 – 8 h)	31 ± 7 (28.4)	131 ± 13 (60.4)	26 ± 4 (11.2)
Overnight (8 – 14 h)	34 ± 4 (31.7)	120 ± 12 (50.0)	46 ± 5 (18.3)
Total Trial (0 – 24 h)	115 ± 13 (31.3)	418 ± 22 (52.2)	136 ± 10 (16.5)

Values are gram and (%) (n = 10)

4.3.4 Correlations between appetite and energy intake

Examination of the relationships between ratings of appetite prior to the buffet meals provided within the laboratory and subsequent energy intake at the meals revealed many significant correlations (Tables 4.5 and 4.6).

4.3.5 Water intake and environmental conditions

There were no significant differences in water intake (control 1255 ± 307, resistance exercise 1580 ± 276 mL; P = 0.265), laboratory temperature (control 22.3 ± 0.2, resistance exercise 21.9 ± 0.2 ^{o}C; P = 0.098) or relative humidity (control 53 ± 3, resistance exercise 54 ± 2%; P = 0.768) between the resistance exercise and control trials.

Table 4.5: Correlations between appetite ratings immediately prior to *ad libitum* buffet meals and energy intake at the subsequent meal

	Morning meal (3.5 – 4 h)				**Afternoon meal (7.5 – 8 h)**			
	Control		**Resistance exercise**		**Control**		**Resistance exercise**	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Hunger	0.744	0.014*	0.538	0.105	0.672	0.033*	0.920	<0.001**
Fullness	-0.568	0.075	-0.590	0.072	-0.498	0.143	-0.928	<0.001**
Satisfaction	-0.646	0.044*	-0.763	0.010*	-0.604	0.065	-0.910	<0.001**
PFC	0.580	0.079	0.651	0.042*	0.401	0.251	0.759	0.011*

* = $P < 0.05$, ** = $P < 0.001$, PFC = prospective food consumption

Table 4.6: Correlations between appetite area under the curve one hour prior to *ad libitum* buffet meals and energy intake at the subsequent meal

	Morning meal (3.5 – 4 h)				**Afternoon meal (7.5 – 8 h)**			
	Control		**Resistance exercise**		**Control**		**Resistance exercise**	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Hunger	0.716	0.020*	0.460	0.181	0.401	0.246	0.766	0.010*
Fullness	-0.708	0.022*	-0.262	0.464	-0.293	0.411	-0.868	<0.001**
Satisfaction	-0.729	0.017*	-0.657	0.039*	-0.453	0.183	-0.961	<0.001**
PFC	0.543	0.105	0.306	0.390	0.488	0.153	0.777	0.008*

* = $P < 0.05$, ** = $P < 0.001$, PFC = prospective food consumption

4.4 Discussion

The present study is the first to examine appetite, energy and macronutrient intake responses to resistance exercise over an extended 24 h period. The novel findings arising from this study were that a single session of resistance exercise did not perturb subjective appetite perceptions or *ad libitum* energy intake during this period although resistance exercise did stimulate a preference for foods high in carbohydrate.

In a previous investigation which the author was involved in subjective ratings of hunger were significantly reduced during and at the end of a 90 min bout of resistance exercise (Broom et al, 2009). This particular investigation was the first to demonstrate a transient suppression of appetite during resistance exercise, consistent with the presence of exercise induced anorexia which is a phenomenon that has repeatably been observed in response to high intensity bouts of aerobic exercise (King et al, 1994; King and Blundell, 1995). In the present study four markers of appetite (hunger, fullness, satisfaction and prospective food consumption) were examined in response to the same resistance exercise protocol as that employed by Broom et al (2009). It is therefore surprising that none of the appetite markers examined were significantly influenced by exercise, either during or in the hours afterwards. Differences in pre-exercise feeding status are the most likely explanation for this discrepancy. Specifically, in the study conducted by Broom et al (2009) participants completed exercise having not eaten since the prior evening whereas in the present study participants consumed a breakfast snack one hour before exercise. Consequently, in the present study appetite ratings would have been less extreme to begin and therefore less amenable to change in response to the exercise stimulus. This notion is supported by recent data which also reported a lack of change in subjective appetite ratings in response to a strenuous 80 min bout of resistance exercise. In this latter study the participants consumed a somewhat larger breakfast (25% of daily energy intake) 2.5 h before exercise (Ballard et al, 2009).

In the present investigation resistance exercise did not alter subjective ratings of appetite in the hours of observation after exercise. That is, resistance exercise did not stimulate any latent changes in appetite. Some recent work has been published suggesting that appetite may be stimulated by exercise in the later hours of recovery (Broom et al, 2007; Malkova et al, 2008). In these studies aerobic exercise was performed for a prolonged duration and therefore the amount of energy expended was

considerable (500 – 930 kcal). Due to the anaerobic nature of resistance exercise the amount of energy expended in the present study was considerably lower (comparable gross expenditure 373 kcal) than in the studies of Broom et al (2007) and Malkova et al (2008). Resistance exercise may therefore have provided an insufficient stimulus to evoke any latent changes in appetite.

Consistent with no change in appetite, the present study found no significant effects of resistance exercise on energy intake. When the present study was conducted there were no other data available regarding the effects of resistance exercise on energy intake although one recently published study has since provided some preliminary data (Ballard et al, 2009). In this latter study 20 healthy young males completed three experimental trials. On two occasions an 80 min bout of resistance exercise (four sets of eight exercises designed to stress the major muscle groups) was completed with participants consuming 150 mL of either a carbohydrate or placebo beverage immediately before and after exercise. On another occasion participants rested and consumed the carbohydrate beverage at equivalent time points (control). Energy intake was determined from a homogenous pasta meal provided two hours after exercise. The researchers observed no significant difference in energy intake between the experimental trials (control 5234 kJ, exercise carbohydrate beverage 5427 kJ, exercise placebo beverage 5720 kJ).

The findings from the present study support those of Ballard et al (2009) and extend them by showing that there are no latent changes in energy intake at the subsequent meals after. In the present study energy intake was higher at the morning buffet meals compared with the afternoon meals and that consumed overnight however this was consistent between trials. Although there were no significant differences in energy intake between the resistance exercise and control trials energy intake was 1130 kJ (270 kcal) higher across the 24 h in the resistance exercise trial. Large variation in responses were apparent however, making it difficult to delineate any clear effect. Specifically, as compared with that consumed on the control trial, seven participants increased their energy intake on the exercise trial whilst three participants reduced their energy intake. Despite this, these results indicate that within the 24 h after an acute bout of resistance exercise participants may begin to compensate their energy intake for the relatively minor amount of energy expended during an acute bout of resistance exercise. Further

research with a larger number of study participants is needed to provide further clarity on this issue.

An interesting finding in the present study was that resistance exercise significantly altered macronutrient intake. Specifically, in the resistance exercise trial the percentage of energy derived from carbohydrate increased at the afternoon buffet meal whilst the intake of protein decreased. This finding is in contrast to consensus of research on this issue which has failed to demonstrate any consistent effect of exercise on macronutrient preferences (Besile, 1999; Tremblay and Drapeau, 1999; Elder and Roberts, 2007). The present findings suggest that exercise stimulated a latent preference for food items high in carbohydrate however the mechanism responsible for this is not clear. It has been suggested that the substrate oxidised during exercise has a role in determining food choices thereafter (Besile, 1999). Carbohydrate is the primary fuel source for high intensity resistance exercise therefore it is possible that an increase in carbohydrate oxidation may have stimulated a preference for carbohydrate rich foods. This is only speculation however and there is no way to examine this issue further from the data collected during this study.

One of the principal aims of this study was to develop an *ad libitum* buffet meal which was feasible to provide to participants during studies presented in this thesis. The buffet meal developed was extensive and contained a variety of items of varied macronutrient content in order to enable the examination of macronutrient preferences, in addition to permitting the assessment of energy intake. Appendix G lists the food items presented at the buffet meals. One of the risks that comes with providing a diverse range of food items from which to assess energy/macronutrient intake is that this may lead to atypically high intakes (Larsen et al, 1995). Moreover, it is possible that macronutrient selection may change as participants consume items that are novel rather than those reflecting their habitual food intake patterns. To examine these issues, in the present investigation a comparison was made between energy and macronutrient intakes during the control trial (no intervention) with estimated energy requirements for each individual – determined using validated equations (Mifflin et al, 1990). The percentage of energy derived from the macronutrients was also compared to that typically consumed in a Western diet i.e. ~35% fat, ~15% protein and ~50% carbohydrate (Cordain et al, 2005). The findings revealed no significant differences in 24 h energy

intake in the control trial as compared with predicted daily energy requirements for each study participant (student's *t*-test $P = 0.432$). Furthermore, the distribution of energy from the macronutrients closely resembled a typical Western diet (see Table 4.4). These outcomes indicate two important points regarding *ad libitum* energy and macronutrient intake from the buffet meal. First, participants did not increase their energy intake over their daily needs i.e. the array of items available did not lead to overconsumption. Second, the diversity of items available did not skew macronutrient intake.

Flint et al (2000) correlated energy intake at an *ad libitum* buffet meal with subjective ratings of appetite as a means of assessing the validity of the data derived from the appetite scales. If this is reversed, a corollary is that we can look at the relationship between appetite ratings and energy intake to get an indication of the validity of the documented energy intakes. Tables 4.5 and 4.6 show that in the present study many highly significant correlations were found between energy intake at the morning and afternoon buffet meals and ratings of appetite, both immediately before meals and also within the preceding hour (assessed using AUC values). These findings suggest that participants were eating to their appetite, rather than their intakes being driven by external factors. This finding, along with the data regarding energy and macronutrient intake, suggest that this particular buffet meal can be used as a valid method for assessing energy and macronutrient intake responses to interventions.

This present study has notable strengths and limitations. A key strength is that energy intake was assessed accurately under controlled conditions with a buffet meal of wide ranging content. Moreover, energy intake was assessed at three points across a 24 h period of observation, rather than in response to a single meal. A limitation of this work is that the participants were young, healthy, males therefore it is unclear whether these findings can be generalised to other populations including females and older adults. Additionally, responses to a single bout of resistance exercise were examined yet it is possible that changes in energy or macronutrient intake may occur over a longer duration, in response to a period of resistance exercise training. Finally, a greater number of study participants may have been needed to detect significant differences in various study outcomes.

In conclusion, this study has shown that an acute bout of resistance exercise does not influence appetite during exercise or in the 21.5 h after. Furthermore, resistance exercise does not alter *ad libitum* energy intake at meals consumed throughout the day on which exercise is performed. Resistance exercise stimulated a delayed preference for foods high in carbohydrate however further research is needed to confirm this and to identify the responsible mechanisms. This data adds to knowledge regarding the specific influence of resistance exercise on energy homeostasis.

CHAPTER V

The acute effects of swimming on appetite, energy/macronutrient intake and plasma acylated ghrelin

5.1 Introduction

The benefits of aerobic exercise for promoting good health and successful weight control are well documented (Haskell et al, 2007; Donnelley et al, 2009). Swimming is a popular form of aerobic exercise, partly due to the reduced musculo-skeletal stresses imposed as compared with land-based activities such as running and cycling. Such benefits make swimming an especially attractive exercise modality for those who are overweight and/or obese (Sheldahl et al, 1982), individuals who may have taken up swimming in an effort to more successfully regulate their body weight.

Despite the attractiveness of swimming as a form of physical activity, whether swimming favourably influences body weight and body composition is contentious. Studies which have compared the influence of swimming interventions on indices of body weight and composition have found swimming to be less effective than other land-based exercise modalities (Gwinup et al, 1987; Tanaka et al, 1997). It has been suggested that the most logical explanation for these findings is that swimming stimulates a compensatory increase in energy intake (White et al, 2005). This notion is consistent with anecdotal reports of swimming stimulating appetite. Specifically, it has been stated that individuals often feel like 'eating a horse' after an acute bout of swimming (Burke et al, 2006). This suggestion is consistent with the findings from a limited base of empirical research which has described elevations in energy intake after immersed cycling performed on a modified ergometer in cold water (Dressendorfer, 1993; White et al, 2005). Unfortunately there remains a lack of empirical research which has examined the precise effects of swimming on appetite and food intake and further research is needed to examine this.

The mechanisms by which exercise influences appetite have recently begun to receive explicit interest with direct attention being given to gut peptides implicated in the neuroendocrine regulation of feeding (Martins et al, 2008; Bilski et al, 2009). Ghrelin is an acylated peptide secreted primarily from the stomach and remains unique as the only known circulating hormone that stimulates appetite (Karra and Batterham, 2010).

Defined roles of ghrelin in both the short and long-term regulation of feeding have been uncovered and more recently investigators have sought to determine how exercise influences circulating concentrations of acylated ghrelin (Broom et al, 2007; Marzullo et al, 2008; Broom et al, 2009; Ueda et al, 2009). These studies suggest that intense exercise induces a transient suppression in circulating acylated ghrelin concentrations. Concomitant suppressions in hunger have been reported by Broom and colleagues (2007) raising the possibility that acylated ghrelin may be important in determining changes in appetite in response to exercise.

This study had two primary objectives. The first was to examine the influence of an acute bout of swimming on appetite, energy and macronutrient intake – in order to empirically test anecdotal suggestions that swimming increases appetite and food intake. The second aim of this study was to explore the potential role of acylated ghrelin as a mediator of appetite and food intake, during and after a typical bout of moderate intensity, recreational swimming. In this study the buffet meal piloted in the previous chapter were employed to examine the effects of swimming on *ad libitum* energy/macronutrient intake.

5.2 Methods

5.2.1 Participants

After gaining Loughborough University Ethical Advisory Committee approval 14 healthy male volunteers (18 - 26 y) gave their written informed consent to participate. Table 5.1 describes the participant characteristics.

Table 5.1: Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	21.7 ± 0.6
BMI ($kg·m^{-2}$)	23.2 ± 0.6
Body Mass (kg)	76.6 ± 2.1
Body Fat (%)	17.2 ± 1.2

(n = 14)

5.2.2 Study design

Before taking part in main trials participants visited the laboratory in order to familiarise them with the environment. At this visit participants completed the necessary screening questionnaires and anthropometric measurements were taken. Participants were also taken to the university swimming pool to confirm swimming competence. At the pool the participants completed a 60 min intermittent swimming set of moderate intensity, the same protocol which was to be performed during main trials at a later date. In this session participants were made accustomed to wearing heart rate monitors in the pool and taking recordings periodically and were familiarised with the RPE scale.

In subsequent weeks participants completed two main trials (swimming and control) in a randomised, counterbalanced order. Each main trial was separated by at least one-week. To standardise diet and physical activity before main trials participants completed a weighed food record of all items consumed within the 24 h preceding their first main trial and this feeding pattern was replicated prior to their second main trial. Alcohol, caffeine and physical activity were not permitted in the 24 h before main trials. To minimise physical exertion on the morning of main trials participants were asked to

walk slowly to the laboratory if they lived within 0.5 km of the research laboratory. Participants living further away arrived by motorised transport. Participants arrived at the laboratory in the fasted state.

5.2.3 Main trials

An interval of at least one-week separated the familiarisation session and each participant's first main trial. Each main trial began in the morning between 08:30 and 09:00 and lasted 8 h. Main trials commenced when participants began eating a breakfast snack. This was consumed within 5 min. On the swimming trial participants rested within the laboratory for the first 40 min. They were then escorted to the University swimming pool by motorised transport, in time to commence swimming at the beginning of the second trial hour. At the start of the second trial hour participants began a 60 min intermittent swimming set. The set was composed of six, 10 min blocks. In each block participants swam continuously for the first seven min using their preferred stroke and then rested for the final three min. The speed of swimming was ultimately determined by the participant although they were instructed to swim at a moderate intensity, defined as a rating of perceived exertion between 12 and 14. During exercise the stroke used and distance completed was recorded in order to estimate energy expenditure during exercise using equations based on metabolic equivalents (Ainsworth et al, 2000). Heart rate was assessed continuously throughout each swimming block. Upon completion of each block participants rested on the pool side with their legs immersed in the water. Ratings of perceived exertion were then assessed. After completing the swimming protocol participants were escorted back to the research laboratory where they rested for a further six hours (sitting reading, writing, working at a computer or watching television).

Identical procedures were completed in the control trial except no exercise was performed. Participants therefore rested in the research laboratory for the entire duration of the trial. During the second trial hour resting expired air samples were collected in the semi-supine position in order to estimate resting oxygen consumption. This permitted the calculation of net energy expenditure (gross energy expenditure of exercise minus resting energy expenditure) during exercise. Figure 5.1 provides a schematic illustration of the study protocol.

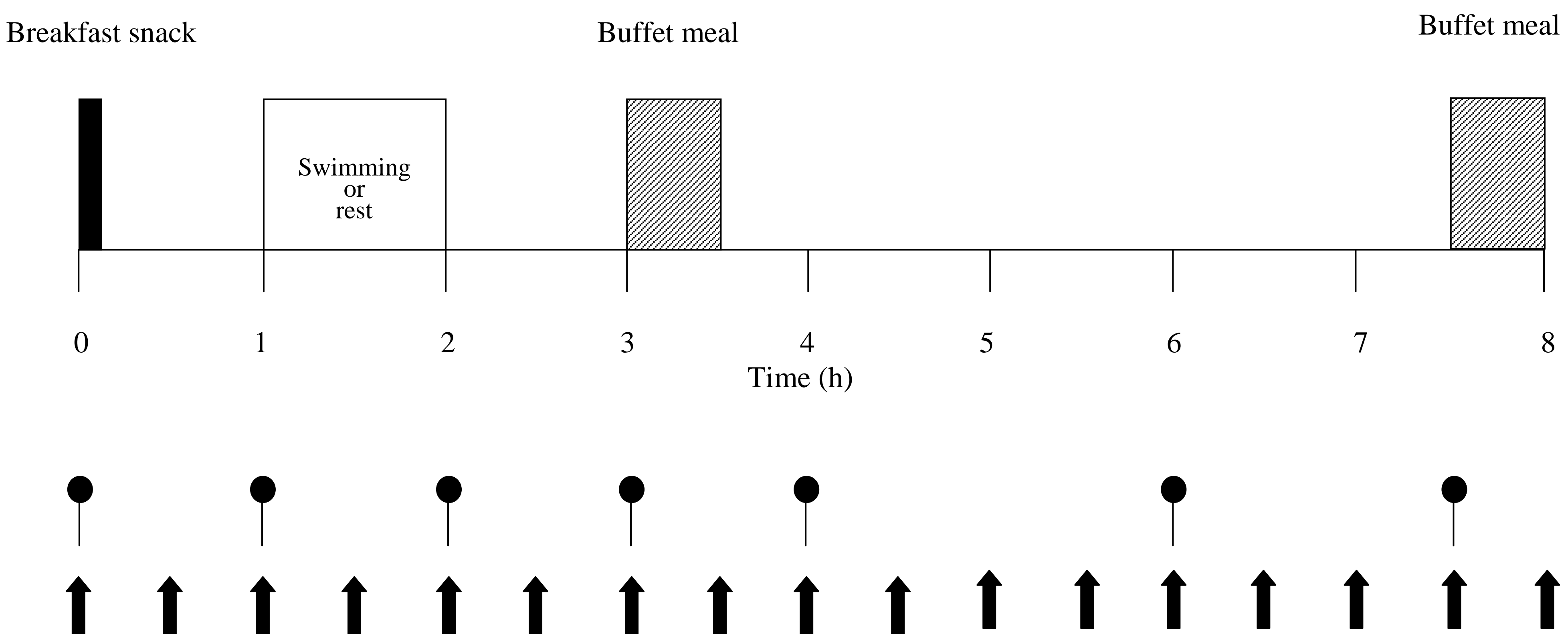


Figure 5.1: Schematic representation of the main trial protocol

5.2.4 Appetite assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at baseline and then at 30 min intervals throughout.

5.2.5 Breakfast and ad libitum buffet meals

The breakfast provided to participants at the beginning of main trials was standardised to body weight and consisted of a commercial cereal bar (Kellogg's Nutri-grain®). Participants received 1.06 g per kilogram of body weight measured on the first trial visit. Identical amounts were consumed across trials. For a 70 kg individual this provided 1092 kJ (260 kcal) of energy, 6 g of fat, 4 g of protein and 48 g of carbohydrate. The breakfast snack was consumed within 5 min on all trials.

At two points during main trials (3 – 3.5 h & 7.5 – 8 h) participants were provided with a buffet meal for 30 min from which they could consume food *ad libitum* (Appendix G). Section 3.12 provides details on the format of the buffet meal.

5.2.6 Environmental conditions

The environmental temperature and relative humidity of the laboratory and swimming pool were monitored throughout main trials using a handheld hygrometer. The temperature of the water in the swimming pool was assessed using a glass thermometer.

5.2.7 Blood sampling

Due to the expense of measuring acylated ghrelin, blood was analysed for 10 of the 14 participants. In both the swimming and control trials baseline blood samples and the equivalent pre- and post-exercise blood samples were taken via venepuncture of an antecubital vein. Thereafter, the remaining samples (3, 4, 6, and 7.5 h) were collected via a cannula inserted into an antecubital vein. Patency of the cannula was maintained by flushing with non-heparinised saline (0.9 % w/v sodium chloride) after sample collection. Dilution of subsequent samples was prevented by discarding the first 2 mL of sample prior to collection.

Venous samples were collected into pre-chilled 4.9 mL EDTA monovettes for the determination of plasma acylated ghrelin (see section 3.14 for details on acylated

ghrelin sample processing). Additional samples were collected into pre-chilled 9 mL EDTA monovettes for the determination of plasma glucose and triacylglycerol. These samples were spun at 1,681*g* for 10 min in a refrigerated centrifuge at 4 ^{o}C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage for analysis later.

To estimates plasma volume changes, at each blood sampling point duplicate 20 µL blood samples were collected into micropipettes and triplicate 20 µL samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations.

5.2.8 Biochemical analysis

An enzyme immunoassay was used to determine concentrations of plasma acylated ghrelin. Plasma glucose and triacylglycerol concentrations were determined spectrophotometrically using an automated bench top analyzer. To eliminate inter-assay variation samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.8%, glucose 0.4% and triacylglycerol 1.63%.

5.2.9 Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows. Area under the concentration verses time curve calculations were performed using the trapezoidal method. Student's *t*-tests for correlated data were used to assess differences between fasting and AUC values for acylated ghrelin, glucose, triacylglycerol, temperature, humidity and appetite between the swimming and control trials. Repeated measures, two-factor ANOVA was used to examine differences between the swimming and control trials over time for appetite, energy and macronutrient intake, acylated ghrelin, glucose and triacylglycerol. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

5.3 Results

5.3.1 Exercise responses

During the 42 min of swimming (6 x 7 min intervals) the mean distance completed was 1875 ± 156 m. The mean swimming speed performed was 0.74 ± 0.1 $m.s^{-1}$ and this elicited an estimated net energy expenditure (exercise minus resting) of 1921 ± 83 kJ (459 ± 20 kcal). The corresponding mean heart rate and RPE values during the swimming sessions were 155 ± 5 $beats.min^{-1}$ (78 ± 2 % of age predicted maximum heart rate) and 14 ± 0. To complete the swimming session four participants swam breaststroke for all of the intervals whilst three participants used only front crawl and two participants used only backstroke. Three participants used a combination of front crawl and breast stroke whilst two participants alternated between breaststroke and backstroke.

5.3.2 Appetite responses

Baseline appetite ratings were not significantly different in the swimming and control trials (Table 5.2).

Table 5.2: Baseline appetite perceptions in the swimming and control trials

	Control	Swimming	*P*
Hunger (0-100)	66 ± 4	63 ± 5	0.547
Satisfaction (0-100)	24 ± 4	26 ± 5	0.723
Fullness (0-100)	21 ± 5	23 ± 4	0.783
PFC (0-100)	71 ± 4	76 ± 3	0.881

(n = 14). PFC = prospective food consumption

Figure 5.2 shows the appetite responses in the swimming and control trials. Two-factor ANOVA revealed significant time (all $P < 0.001$) and interaction (trial x time) main effects for hunger, satisfaction, fullness and prospective food consumption (all $P < 0.014$) indicating that appetite responses differed significantly over time between the swimming and control trials. Post hoc analysis identified significantly higher ratings of hunger in the swimming trial compared with the control trial at 5, 5.5 and 6.5 h. Perceived rating of fullness were significantly lower on the swimming trial at 5 and 7 h whilst ratings of satisfaction were lower on the swimming trial at 6.5 h. Ratings of

prospective food consumption were significantly lower in the swimming trial at 1.5 h yet were higher than values on the control trial at 5 and 7 h. After correcting for multiple comparisons using the Bonferroni method not all of these differences remained (see Figure 5.2).

Between trial differences in appetite ratings were also evaluated using AUC values calculated from baseline to the morning buffet meal (0 - 3 h), for the 4.5 h after the morning buffet meal (3.5 – 8 h) and over the total trial (0 – 8 h). Analysis of the hunger AUC data confirmed that ratings of hunger were significantly higher in the swimming trial than the control trial after the morning buffet meal (3.5 – 8 h) (swimming 177.7 ± 20.0, control 152.3 ± 18.5; $P = 0.028$). From baseline to consumption of the morning buffet meal (0 – 3 h) the fullness AUC was significantly higher in the swimming trial as compared with control (swimming 74.0 ± 11.7, control 54.4 ± 8; $P = 0.025$). Conversely, after consuming the morning buffet meal ratings of fullness tended to be reduced in the swimming trial as compared with control (swimming 227.3 ± 21.1, control 243.2 ± 17.0; $P = 0.052$). Before the morning buffet meal the prospective food consumption AUC was lower on the swimming trial as compared with the control trial (swimming 220.7 ± 12.0, control 230.5 ± 11.1; $P = 0.049$).

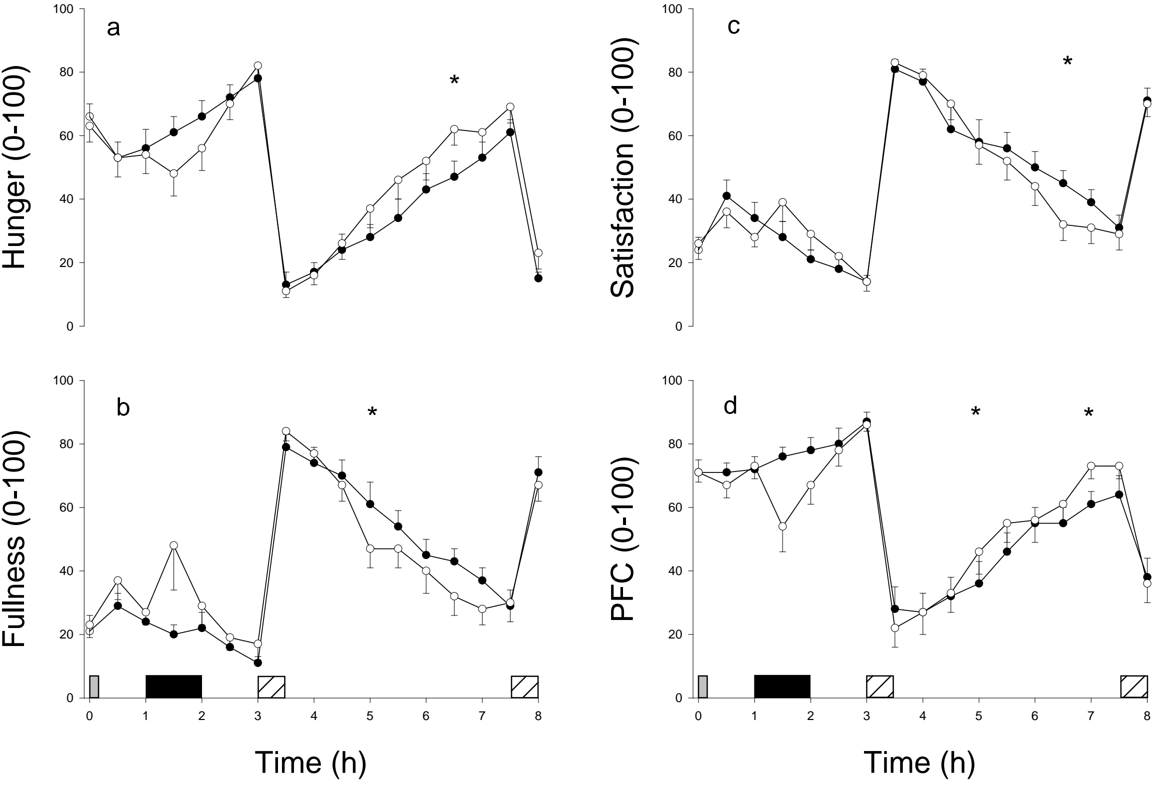


Figure 5.2: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the swimming (○) and control (●) trials. Values are mean ± SEM *(n* = 14). Grey rectangle indicates a breakfast snack. Black rectangle indicates swimming. Diagonal rectangles indicate buffet meals. * = significantly different from control after correcting for multiple comparisons using the Bonferroni method.

5.3.3 Energy and macronutrient intake

For energy intake two-factor ANOVA revealed a main effect of time (P = 0.003) indicating that energy intake at the morning buffet meals was significantly higher than at the afternoon meals (Table 5.3). No significant trial (P = 0.208) or interaction (trial x time, P = 0.811) main effects were found therefore there were no significant differences in energy intake between the swimming and control trials. Examination of the relative energy intake (energy intake – net exercise energy expenditure) showed that the swimming trial (7825 ± 776 kJ, 1871 ± 185 kcal) induced an energy deficit relative to control (9161 ± 719, 2190 ± 172) (P = 0.008).

Table 5.3: Energy intake in the swimming and control trials

	Control	Swimming
Morning meal	5517 ± 434	5856 ± 403
(3-3.5 h)	(1319 ± 104)	(1400 ± 96)
Afternoon meal	3644 ± 459	3893 ± 577
(7.5-8 h)	(871 ± 138)	(931 ± 138)
Total Trial	9161 ± 719	9749 ± 809
(0 – 8 h)	(2190 ± 203)	(2331 ± 229)

Values are kJ (kcal) (n = 14)

Two-factor ANOVA was used to examine macronutrient intake (absolute and percent) across the morning and afternoon meals during the swimming and control trials (Table 5.4). For absolute intake (grams) there was a significant main effect of time for each macronutrient (all $P < 0.015$) however no significant trial or interaction (trial x time) main effects were apparent (all $P > 0.05$). This indicates that the absolute intake of each macronutrient varied between the morning and afternoon buffet meals yet was not significantly different between the swimming and control trials. Analyses of the percentage of energy derived from the macronutrients did not show any significant main effects.

Table 5.4: Macronutrient intake in the swimming and control trials

Control Trial	**Fat**	**Carbohydrate**	**Protein**
Morning meal (3-3.5 h)	54 ± 5 (34.1)	156 ± 11 (49.1)	59 ± 9 (16.8)
Afternoon meal (7.5 – 8 h)	33 ± 5 (33.8)	107 ± 15 (49.9)	38 ± 8 (16.3)
Total Trial (0 – 8 h)	87 ± 8 (34.9)	263 ± 21 (49.1)	97 ± 16 (16.0)
Swimming Trial	**Fat**	**Carbohydrate**	**Protein**
Morning meal (3-3.5 h)	55 ± 5 (34.0)	164 ± 12 (49.3)	60 ± 8 (16.7)
Afternoon meal (7.5 – 8 h)	35 ± 5 (33.1)	117 ± 20 (50.2)	38 ± 7 (16.7)
Total Trial (0 – 8 h)	90 ± 9 (34.2)	281 ± 26 (49.4)	98 ± 14 (16.4)

Values are gram and (%) (n = 14)

5.3.4 Acylated ghrelin

Acylated ghrelin was analysed with data from 10 participants however upon closer inspection of the data one participant was a clear outlier exhibiting fasting values on both trials which were approximately nine times (26 standard deviations) higher than the mean fasting values of the other nine participants (949 ± 30 pg·mL^{-1} for the outlier verses 108 ± 10 pg·mL^{-1} for the mean of the other nine participants). Data from this participant was therefore removed and the analyses repeated with data from the other nine participants.

Fasting plasma acylated ghrelin concentrations did not differ (P = 0.348) between the swimming and control trials (112 ± 13 verses 105 ± 10 pg·mL^{-1}). For circulating concentrations of acylated ghrelin two factor ANOVA yielded significant time (P < 0.001) and interaction (trial x time) (P < 0.001) main effects, indicating that acylated ghrelin responses differed significantly over time between the swimming and control trials (Figure 5.3). After correcting for multiple comparisons using the Bonferroni method post hoc analysis showed that circulating acylated ghrelin concentrations were

significantly lower in the swimming trial than the control trial at the end of exercise (2 h) ($P < 0.001$).

Between trial differences in acylated ghrelin were also evaluated using AUC values calculated for the hours before the morning buffet meal (0 – 3 h), over the remainder of the trial (3 – 8 h) and across the entire trial duration (0 – 8 h). This analyses confirmed suppressed concentrations of acylated ghrelin prior to the first buffet meal (0 – 3 h) on the swimming trial (swimming 476 ± 232, control 505 ± 217 $pg \cdot mL^{-1} \cdot 3h$) ($P < 0.001$) however no other differences were apparent.

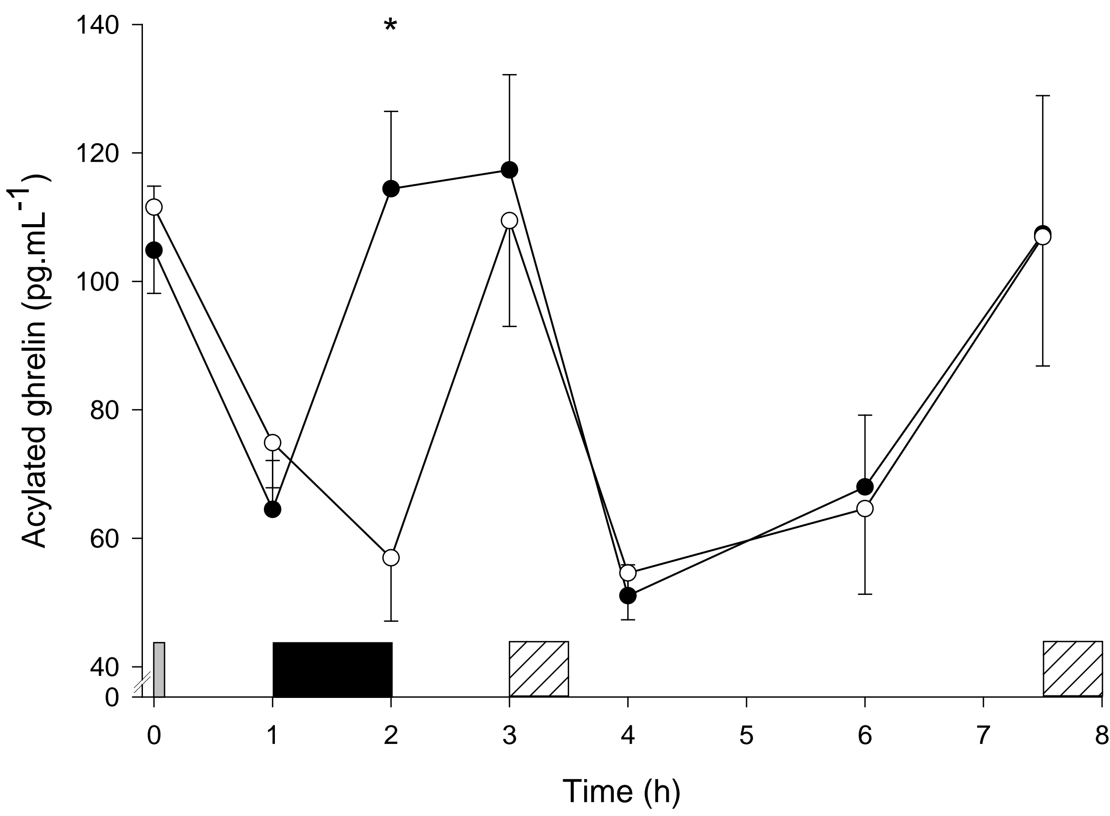


Figure 5.3: Plasma concentrations of acylated ghrelin in the swimming (○) and control (●) trials. Values are mean ± SEM (n = 9). Grey rectangle indicates a breakfast snack. Black rectangle indicates swimming. Diagonal rectangles indicate buffet meals. * = significantly different from control trial after adjusting for multiple comparisons using the Bonferroni method.

3.3.5 Glucose and triacylglycerol

Fasting plasma glucose concentrations did not differ ($P = 0.133$) between the swimming and control trials (4.93 ± 0.1 verses 4.79 ± 0.1 mmol·L^{-1}). Two-factor ANOVA revealed a main effect of time for plasma glucose ($P < 0.001$) however no trial or interaction (trial x time) main effects were found.

Fasting plasma triacylglycerol concentrations did not differ ($P = 0.782$) between the swimming and control trials (1.13 ± 0.1 verses 1.11 ± 0.1 mmol·L^{-1}). For plasma triacylglycerol concentrations two-factor ANOVA yielded a significant main effect of time ($P = 0.013$) but no trial or interaction (trial x time) effects were found (Figure 5.4).

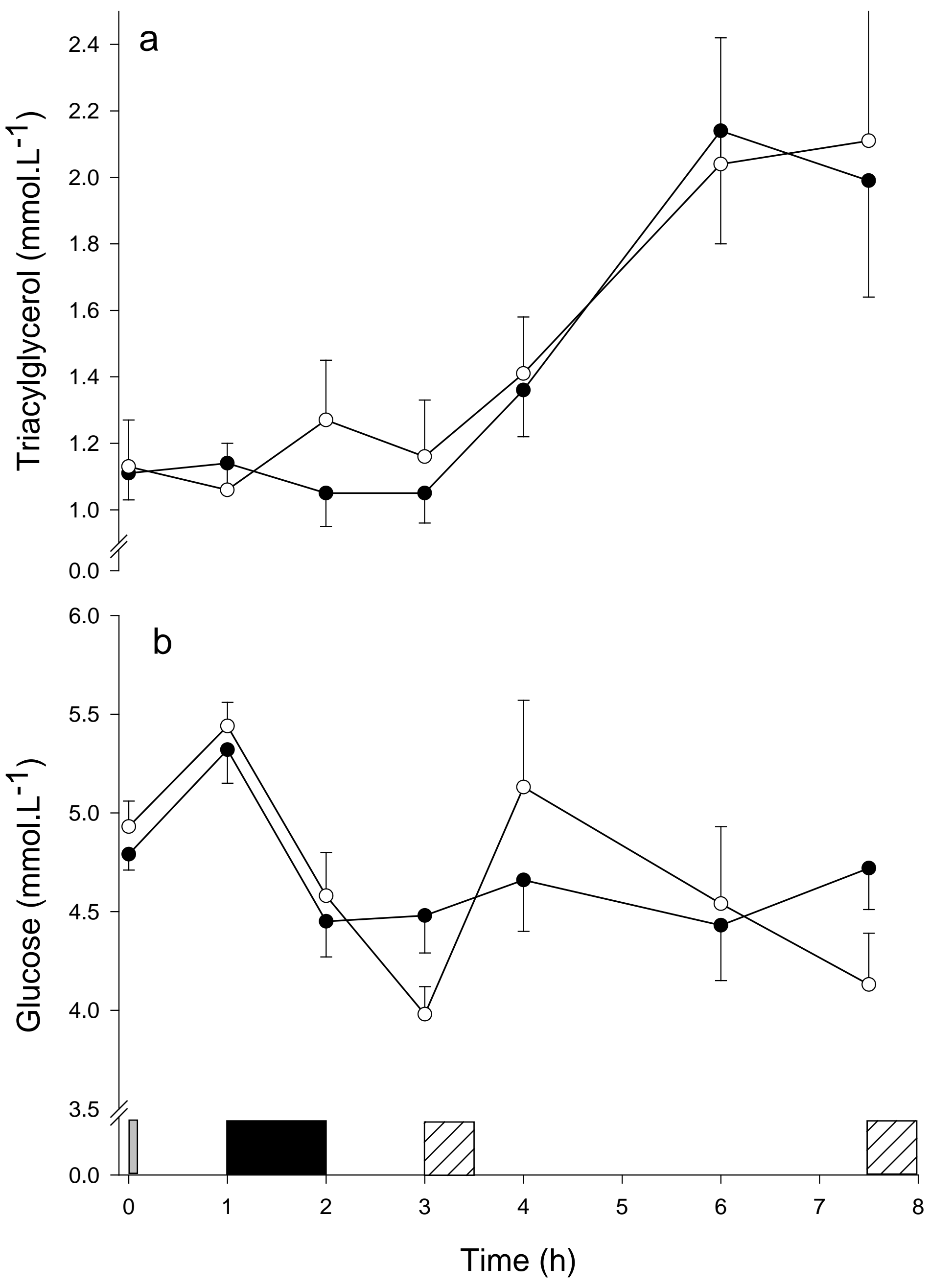


Figure 5.4: Plasma concentrations of triacylglycerol (a) and glucose (b) in the swimming (○) and control (●) trials. Values are mean ± SEM (n = 10). Grey rectangle indicates a breakfast snack. Black rectangle indicates swimming. Diagonal rectangles indicate buffet meals.

5.3.6 Correlations between plasma metabolites, appetite and energy intake

During the control trial plasma acylated ghrelin was inversely related to plasma glucose at 2 h (r = -0.667, P = 0.05). Moreover, after consumption of the morning buffet meal

the acylated ghrelin and glucose AUC were inversely correlated (r = - 0.673, P =0.047). In the swimming trial plasma acylated ghrelin was inversely correlated with plasma glucose immediately after exercise (2 h) (r = - 0.936, $P < 0.001$). Furthermore, acylated ghrelin and glucose AUC values were inversely related both after the morning meal (r = -0.718, P = 0.029) and over the course of the entire trial (r = - 0.786, P = 0.012). In the swimming trial ratings of hunger immediately before the afternoon meal were positively associated with subsequent energy intake (r = 0.533, P = 0.049).

5.3.7 Water intake and environmental conditions

There were no significant differences between the swimming and control trials in water intake (control 1402 ± 219, swimming 1302 ± 226 mL, P = 0.398), laboratory atmospheric temperature (control 21.6 ± 0.3, swimming 21.4 ± 0.3 ^{o}C, P = 0.350) and relative humidity (control 37.8 ± 4.1, swimming 37.8 ± 4.1%, P = 0.537). The atmospheric temperature and relative humidity at the swimming pool were 26.4 ± 0.8 ^{o}C and 50.9 ± 1.7%, respectively. The temperature of the swimming pool water was 28.1 ± 0.1 ^{o}C.

5.4 Discussion

The main findings arising from this investigation are three-fold. Firstly, moderate intensity swimming exhibited a bi-phasic influence on appetite with an inhibition during exercise and a later stimulation in the hours thereafter. Secondly, swimming did not influence *ad libitum* energy or macronutrient intake. Finally, swimming transiently suppressed circulating concentrations of acylated ghrelin however no effects were apparent after exercise. This outcome indicates that acylated ghrelin does not mediate the reported stimulation of appetite after swimming.

The suppression of appetite (decreased hunger and prospective food consumption/elevated satisfaction and fullness) observed during swimming is a novel finding yet is consistent with previous research showing a transient inhibition of appetite resulting from land-based exercise modalities such as running and cycling (King and Blundell, 1995; Blundell and King, 2000). This phenomena has been termed exercise induced anorexia (King et al, 1994) and has been consistently observed during land-based activities performed at moderate intensities or higher (> 60% of $\dot{V}O_2$ max). Broom et al (2007) reported suppressed hunger and plasma acylated ghrelin during treadmill running and suggested a potential role of acylated ghrelin in determining suppressed appetite during exercise. Supporting this notion, the findings from the present study confirm that acylated ghrelin and appetite are concomitantly suppressed during swimming however the absence of any significant correlations between these variables immediately after swimming questions the strength of this relationship.

In the hours after consumption of the morning buffet meal, ratings of hunger and prospective food consumption were higher in the swimming trial than the control trial, whilst ratings of fullness were reduced. These findings indicate that swimming stimulated a delayed increase in appetite. This response is contrary to research which has examined appetite responses to land-based activities which have typically shown no acute compensation in appetite after performing exercise, even when significant amounts of energy are expended (King and Blundell, 1995; King et al, 1997). The mechanism responsible for these discrepant findings is not immediately clear. It has been suggested that changes in body temperature may be important (White et al, 2005; Burke, 2006) however this is unlikely in the present study as appetite was not

stimulated until more than two hours after swimming. By this time core temperature would almost certainly have normalised. White et al (2005) speculate that the cooling and then subsequent reheating of the body may be associated with the release of ‘certain hormones’ which stimulate the appetite. In the present study we measured circulating concentrations of acylated ghrelin, a peptide responsible for stimulating appetite and food intake (Wren et al, 2001; Kojima and Kangawa, 2005). The present findings suggest that acylated ghrelin is not responsible for the augmented appetite response after swimming as circulating concentrations were no different from control values after the morning buffet meal. Appetite is regulated on an acute basis by many gut peptides including peptide YY, pancreatic polypeptide and glucagon-like peptide-1 (Karra and Batterham, 2010) therefore it is possible that changes in these peptides may have influenced the appetite response to swimming.

Some research indicates that swimming may be less effective than land-based activities for inducing weight loss or reductions in body fat (Gwinup et al, 1987; Tanaka et al, 1997). Furthermore, it has been observed that levels of adiposity are typically higher in swimmers than equal calibre runners (Novak et al, 1977; Jang et al, 1987). It has been suggested that an unparalleled stimulation of appetite and energy intake after swimming may explain these findings (Burke, 2006). Despite the changes in appetite observed, the present investigation did not find any significant differences in energy or macronutrient intake between the swimming and control trials, either during the morning or afternoon meals. These findings are difficult to reconcile. It is known that food intake is influenced by a host of physiological, environmental, psychological and social factors, some of which are learned over time and are resistant to change (Besile, 1999). In this study it seems that the factors influencing appetite were insufficient to overcome other competing forces governing food intake. Nonetheless, as a consequence of the lack of change in energy intake, participants therefore failed to compensate for the energy expended during exercise and relative energy intake was subsequently lower on the swimming trial than the control trial (swimming 7828 ± 774 kJ, control 9163 ± 720, P = 0.008). This outcome contradicts the suggestion that energy intake is augmented by swimming and therefore does not support the notion that swimming is an ineffective exercise modality for successful body weight control.

When comparing the present findings to previous data water temperature emerges as an important variable influencing food intake responses to exercise performed in water. White and Colleagues (2005) examined energy intake responses in healthy participants who performed cycling exercise while immersed in either cold water (20 ^{o}C) or neutral water (33°C) and compared these responses to control responses (i.e. while resting in a dry environment). Energy intake was significantly higher after exercise in cold water (873 kcal) as compared with the neutral water (608 kcal) and the resting trial (618 kcal), indicating that exercise in cold water stimulates energy intake. In similar fashion, Dressendorfer (1993) submitted six trained males to 30 min of modified cycling in cold water (22 ^{o}C), warm water (34 ^{o}C), cycling on land and a resting control trial. Participants consumed significantly more energy in the cold water trial than all other trials at a buffet meal provided immediately after exercise. Furthermore, energy intake was significantly reduced in the warm water trial. Collectively, these findings suggest that water temperature, and possibly subsequent core body temperature, are important determinants of feeding responses after exercise. Despite these established findings, no study has previously examined the specific effects of swimming (rather than modified cycling) on appetite and food intake. Our findings appear to support the notion that exercise only in cold water stimulates food intake as in the present study the water temperature was modest (28-28.5 ^{o}C) and no change in energy intake was observed. Unfortunately core temperature was not assessed in the present study, therefore the exact relationship between this variable and energy intake cannot be explored. Further work is therefore needed to examine this issue.

This investigation has two notable limitations. Firstly, an immersed, resting control trial was not included therefore making it difficult to determine whether the reported increase in appetite was due to immersion in water or the physical work completed. Secondly, participants were young, healthy males and it is impossible to tell whether these findings would generalise to other populations such as females, older adults and the overweight/obese. The structure of the swimming session used in this study was selected to resemble a typical recreational session and the freedom for participants to select the stroke and speed of swimming (within guidelines) was thought to improve the validity of this. It is possible that the sessions completed by other populations would not be the same in terms of intensity and duration. Additional work is therefore required to

examine these issues, particularly in overweight individuals as it is within this population that findings hold the most clinical importance.

In conclusion, this investigation has shown that an acute bout of moderate intensity swimming suppresses appetite during exercise before leading to an increase later on in the day. Despite this, energy intake and macronutrient selection appear resistant to change over the duration of time examined. Circulating concentrations of acylated ghrelin were suppressed during swimming and this may possibly have contributed to the reduction in appetite observed. Nonetheless, acylated ghrelin does not appear to mediate the reported increase in appetite in the hours after exercise. These findings provide novel information regarding the influence of swimming on the acute regulation of energy homeostasis.

Chapter VI

Influence of brisk walking on appetite, energy intake and plasma acylated ghrelin

6.1 Introduction

The objectives of the studies described in the two previous chapters (Chapters four and five) were to gain a better understanding of the effects of popular modes of physical activity (resistance exercise and swimming) on appetite, energy intake and in Chapter five, circulating concentrations of acylated ghrelin. Continuing on this theme, across the population walking remains the commonest mode of physical activity undertaken (Simpson et al, 2003; NHS Information Centre, 2008). A significant amount of research has examined the effects of walking on numerous health related outcomes (for a review see Morris and Hardman, 1997). This work has shown that walking can yield many health benefits, particularly in relation to lessening the risk of developing cardiovascular disease and type 2 diabetes mellitus (Caspersen and Fulton, 2008; Lee and Buchner, 2008). Unfortunately less attention has been directed at examining the effects of walking on energy balance and weight control. This is a concern given the rising prevalence of overweight and obesity. Further work is therefore necessary to provide a better understanding of the effects of walking on energy homeostasis (Morris and Hardman, 1997).

As with all forms of physical exertion walking expends energy. The extent of this is directly related to the intensity performed and the body weight of the individual. Thus, if performed regularly, walking should make an important contribution to successful energy balance. This account may be too simplistic however as the consensus of evidence does not demonstrate a consistent effect of walking on indices of weight control. It has been suggested that this may be related to changes in appetite and energy intake (Morris and Hardman, 1997).

Studies that have examined the effects of exercise on appetite and energy intake have typically observed a lack of influence in the short-term (Martins et al, 2008; Bilski et al, 2009). With specific regards to walking, no change in appetite (Imbeault et al, 1997) or energy intake (Imbeault et al, 1997; George and Morganstein, 2003; Pomerleau et al, 2004) are also common findings, although one report has described a suppression of hunger after 20 min of brisk walking in a sample of obese women (Tsofliou et al, 2003).

The regulation of appetite and energy intake is under complex neuroendocrine control involving both centrally and peripherally mediated systems (Murphy and Bloom, 2006). Gut peptides within the enteric endocrine system are integral to this process and efforts seeking to define how these peptides respond to exercise have recently begun (Martins et al, 2008; Bilski et al, 2009). Ghrelin is an acylated peptide released from the stomach and stimulates appetite and feeding (Kojima et al, 1999; Wren et al, 2001). Recent work has sought to characterise the effect of exercise on acylated ghrelin (Broom et al, 2007; Broom et al, 2009; Ueda et al, 2009). Unfortunately this research has primarily examined the influence of high intensity bouts of exercise. Whether low intensity exercise, such as walking, influences acylated ghrelin is not known.

The purpose of this study was to examine appetite, energy intake and plasma acylated ghrelin responses over an extended period of time after an acute bout of brisk walking. The aim was to assess both the immediate and prolonged effects of walking on acylated ghrelin, appetite and energy intake. These findings may have implications concerning the promotion of walking for successful weight management.

6.2 Methods

6.2.1 Participants

After gaining Loughborough University Ethical Advisory Committee approval 14 healthy males (18 – 26 y) gave their written informed consent to participate. Table 6.1 describes the participant characteristics.

Table 6.1: Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	21.9 ± 0.5
BMI ($kg \cdot m^{-2}$)	23.4 ± 0.6
Body Mass (kg)	76.8 ± 2.5
Body Fat (%)	19.2 ± 1.2
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	55.9 ± 1.8

(n = 14)

6.2.2 Study design

Before taking part in main trials participants visited the laboratory in order to familiarise themselves with the environment and to enable the collection of the necessary anthropometric and preliminary exercise test data. After being made aware of the protocol, participants were health screened and then gave their written informed consent to participate. Anthropometric data was then collected after which participants completed two preliminary exercise tests: 1) a five min submaximal treadmill walking test, 2) a maximum oxygen uptake ($\dot{V}O_2$ max) treadmill running test. There was a 15 to 20 min interval between tests.

The submaximal treadmill walking test was completed to ascertain the brisk walking speed that participants would walk at during the brisk walking main trial. Participants were told that brisk walking was defined as an exercise intensity yielding a mild shortening of breath yet still enabling the individual to converse (Miyashita et al, 2008). During the test the treadmill speed was initially adjusted until a suitable pace was

determined. Participants then maintained this speed for five min. In the final min of the test heart rate and RPE were recorded.

In subsequent weeks participants completed two main trials (brisk walking and control) in a randomised, counterbalanced order. Each main trial was separated by at least one-week. In order to standardise diet and physical activity before these trials participants completed a weighed food record of all items consumed within the 24 h preceding their first main trial and this feeding pattern was replicated before their second main trial. Alcohol, caffeine and physical activity were not permitted during this period. On the morning of trial days participants arrived at the laboratory having fasted overnight. To minimise physical exertion on the morning of trials participants were asked to walk slowly to the laboratory if they lived within 0.5 km of the research laboratory. Participants living further away arrived by motorised transport.

6.2.3 Main trials

An interval of at least one-week separated the preliminary session and each participant's first main trial. Trials began in the morning between 08:30 and 09:00 and lasted eight hours. The brisk walking trial commenced when participants began a 60 min subjectively paced brisk walk on a level motorised treadmill. The initial walking pace was that ascertained in the preliminary laboratory visit although adjustments were made if discomfort was experienced. Samples of expired air were collected at 15 min intervals throughout to estimate energy expenditure and substrate oxidation. Heart rate and RPE were also assessed at these times. After completing the walk participants rested for 7 h within the laboratory (sitting reading, writing, working at a computer or watching television).

Identical procedures were completed in the control trial except no exercise was performed. Participants therefore rested for the entire duration of the trial. In order to estimate the net energy expenditure of brisk walking (gross energy expenditure of exercise minus resting energy expenditure), during the first hour of the control trial samples of expired air were collected in the semi-supine position to estimate resting oxygen consumption. Figure 6.1 provides a schematic illustration of the main trial protocol.

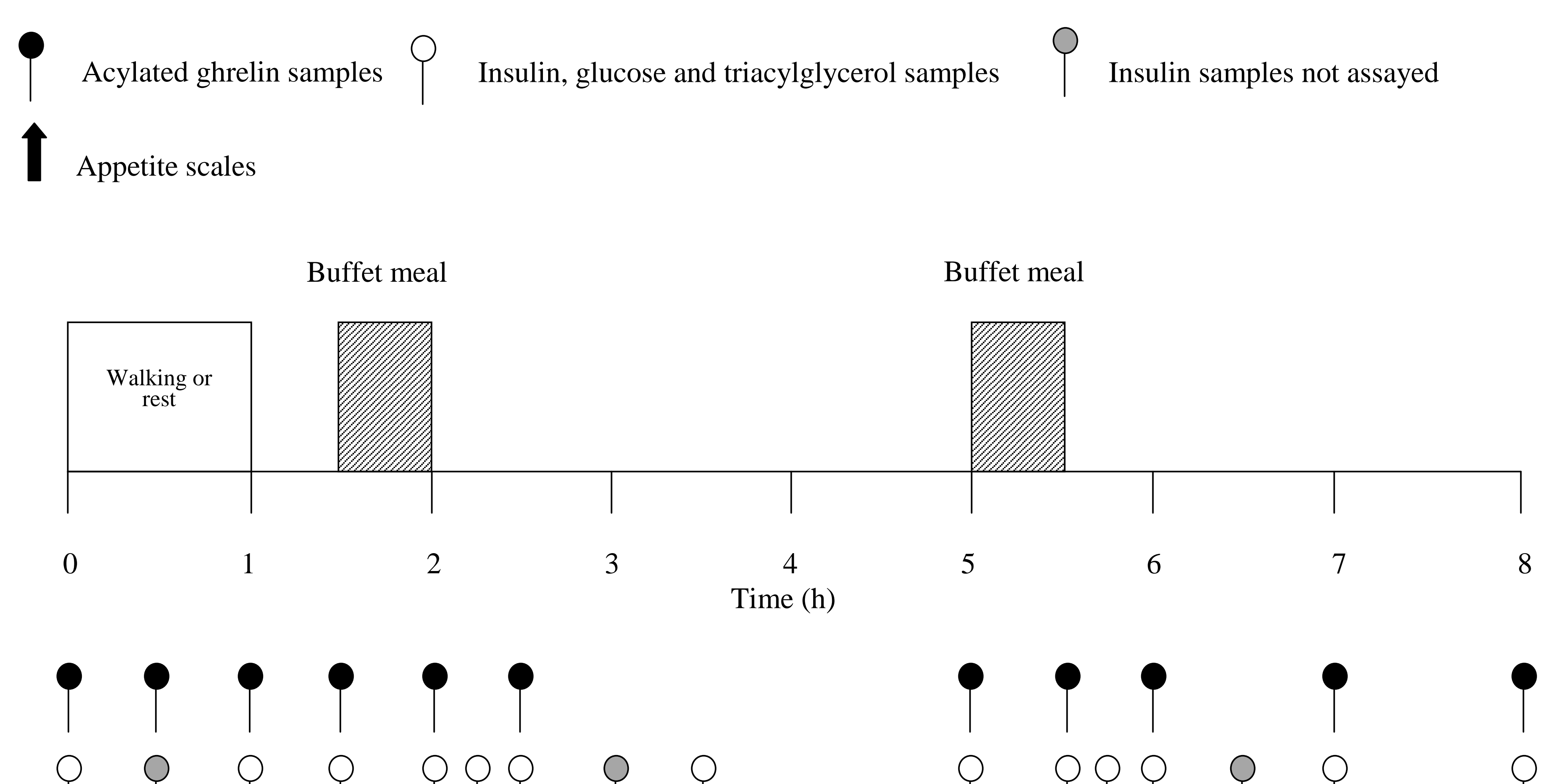


Figure 6.1: Schematic representation of the main trial protocol

6.2.4 Appetite and energy intake assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at baseline and then at 30 min intervals throughout.

At two points during main trials (1.5 – 2 h & 5 – 5.5 h) participants were provided with a buffet meal (Appendix G) for 30 min from which they could consume food *ad libitum*. The buffet meal was identical to that provided in the previous studies described in this thesis. Section 3.12 provides details on the format of the buffet meal.

6.2.5 Blood sampling

A cannula was inserted into an antecubital vein while participants lay in a semi-supine position ~30 min before main trials commenced. Venous blood samples were taken into pre-chilled 4.9 mL monovettes at baseline, 0.5, 1, 1.5, 2, 2.5, 5, 5.5, 6, 7, and 8 h to measure plasma acylated ghrelin (see section 3.14 for details on acylated ghrelin sample processing). Additional blood samples were collected into pre-chilled 9 mL EDTA monovettes at baseline, 0.5, 1, 1.5, 2, 2.25, 2.5, 3, 3.5, 5, 5.5, 5.75, 6, 6.5, 7 and 8 h for the determination of plasma glucose and triacylglycerol. Plasma insulin was determined from collections at 0, 1, 1.5, 2, 2.25, 2.5, 3.5, 5, 5.5, 5.75, 6, 7 and 8 h. These monovettes were spun at 1,681*g* for 10 min in a refrigerated centrifuge at 4 ^{o}C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage for analysis later.

All blood samples were taken in the semi-supine position except for 0.5 h collections during the brisk walking trial where participants straddled the treadmill. During trials patency of the cannula was maintained by flushing with non-heparinised saline (0.9 % w/v sodium chloride) after each sample collection. Dilution of subsequent samples was avoided by discarding residual saline before collections.

To estimates changes in plasma volume, at each blood sampling point duplicate 20 μL blood samples were collected into micropipettes and triplicate 20 μL samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations.

6.2.6 Biochemical analysis

Enzyme immunoassays were used to determine concentrations of plasma acylated ghrelin and insulin. Plasma glucose and triacylglycerol concentrations were determined spectrophotometrically using an automated bench top analyzer. To eliminate inter-assay variation samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.8%, insulin 6.3%, glucose 0.4% and triacylglycerol 2.7%.

6.2.7 Statistical analysis

Data was analyzed using the Statistical Package for the social Sciences (SPSS) software version 16.0 for Windows. Area under the concentration verses time curve calculations were performed using the trapezoidal method. Student's *t*-tests for correlated data were used to assess differences between fasting and AUC values for acylated ghrelin, glucose, insulin, triacylglycerol, environmental temperature, humidity and appetite between the brisk walking and control trials. Repeated measures, two-factor ANOVA was used to examine differences between the brisk walking and control trials over time for appetite, energy and macronutrient intake, acylated ghrelin, glucose, insulin and triacylglycerol. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

6.3 Results

6.3.1 Exercise responses

Participants completed the 60 min brisk walk at 7.0 ± 0.1 km.h^{-1}. This elicited a mean oxygen consumption equivalent to 45.2 ± 2% of $\dot{V}O_2$ max and generated an average heart rate and net (exercise minus resting) energy expenditure of 137 ± 6 beats.min^{-1} and 2008 ± 134 kJ (480 ± 32 kcal), respectively. A mean non-protein respiratory quotient of 0.89 ± 0.01 reflected the proportional contributions of carbohydrate and fat (61± 3% and 39 ± 3%) to energy provision. A median RPE value of 11 indicated that the intensity of the walk was perceived as 'fairly light'.

6.3.2 Appetite responses

There were no significant differences in baseline ratings of appetite (hunger, fullness, satisfaction and prospective food consumption) between the brisk walking and control trials (Table 6.2).

Table 6.2: Baseline appetite perceptions in the brisk walking and control trials

	Control	Brisk walking	*P*
Hunger (0-100)	67 ± 4	58 ± 7	0.270
Satisfaction (0-100)	27 ± 7	19 ± 3	0.419
Fullness (0-100)	16 ± 3	15 ± 3	0.833
PFC (0-100)	73 ± 2	75 ± 5	0.646

Values are mean ± SEM (n = 14). PFC = Prospective food consumption

Two-factor ANOVA revealed significant main effects of time for each appetite perception assessed (all $P < 0.001$) indicating changes in appetite in response to the buffet meals consumed during main trials. No significant trial or interaction (trial x time) effects were found therefore appetite responses did not differ between the brisk walking and control trials (Figure 6.2).

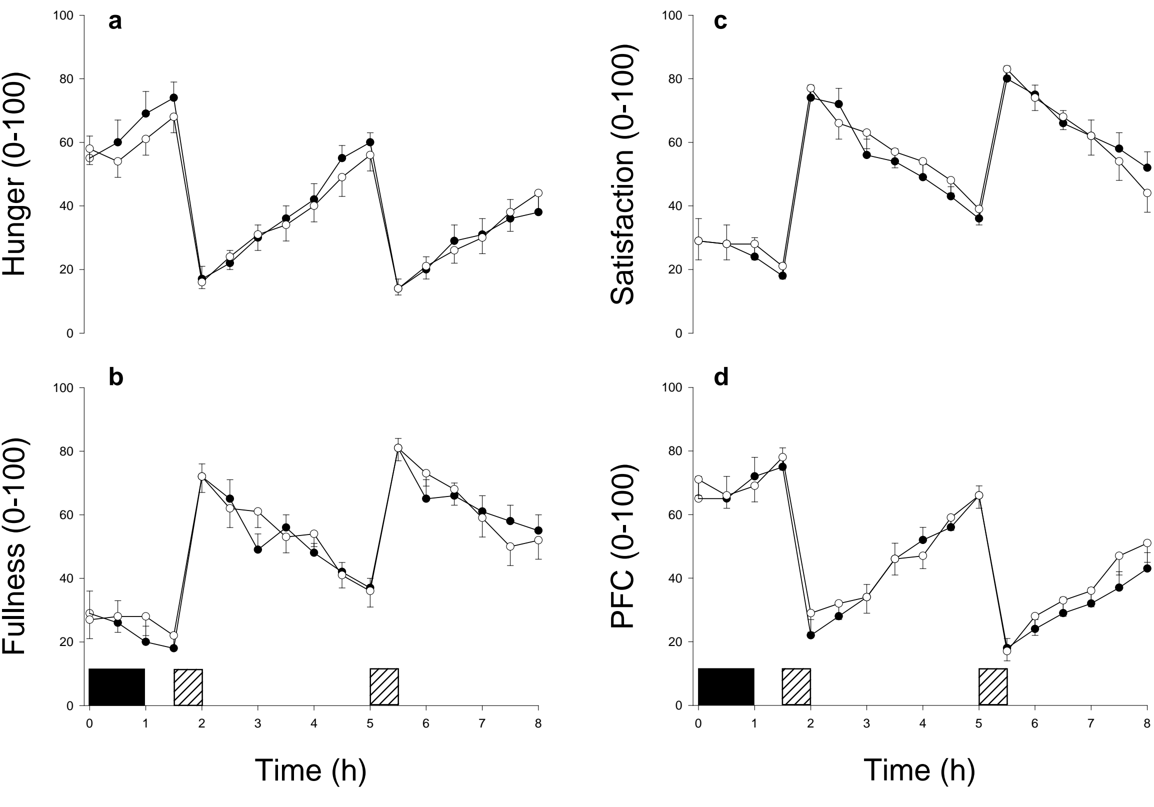


Figure 6.2: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the brisk walking (○) and control (●) trials. Values are mean ± SEM *(n =* 14). Black rectangle indicates brisk walking. Diagonal rectangles indicate buffet meals.

6.3.3 Energy and macronutrient intake

Two-factor ANOVA showed no significant trial, time or interaction (trial x time) main effects for energy intake (all $P > 0.05$) indicating that energy intake was not significantly different between the brisk walking and control trials (Table 6.3). Examination of the relative energy intake (energy intake – net energy expenditure of exercise) showed that brisk walking (7376 ± 657 kJ, 1763 ± 157 kcal) induced a relative energy deficit as compared with the control trial (9213 ± 590 kJ, 2202 ± 141 kcal) ($P = 0.001$).

Table 6.3: Energy intake in the brisk walking and control trials

	Control	Brisk walking
Morning meal	4483 ± 378	4520 ± 436
(1.5 – 2 h)	(1072 ± 90)	(1080 ± 104)
Afternoon meal	4729 ± 348	4864 ± 490
(5 – 5.5 h)	(1130 ± 83)	(1163 ± 117)
Total Trial	9212 ± 588	9384 ± 659
(0 – 8 h)	(2202 ± 141)	(2243 ± 157)

Values are kJ (kcal) (n = 14)

Two-factor ANOVA was used to examine macronutrient intake (absolute and percent) across the morning and afternoon meals during the brisk walking and control trials (Table 6.4). There were no significant trial, time or interaction (trial x time) main effects for the absolute intake of fat, protein or carbohydrate (all $P > 0.05$). Analyses of the percentage intake of the macronutrients revealed significant main effects of time for fat ($P = 0.009$) and carbohydrate ($P = 0.010$) indicating that the intake of fat was higher at the afternoon meals than in the morning meals whereas the percentage intake of carbohydrate was lower at the afternoon meals than in the morning meals. No significant trial or interaction (trial x time) main effects were apparent therefore these changes over time were not significantly different between the brisk walking and control trials.

Table 6.4: Macronutrient intake in the brisk walking and control trials

Control	**Fat**	**Carbohydrate**	**Protein**
Morning meal (1.5 – 2 h)	38 ± 5 (30.5)	144 ± 8 (56.0)	40 ± 9 (13.5)
Afternoon meal (5 – 5.5 h)	46 ± 5 (36.7)	137 ± 16 (48.6)	43 ± 7 (14.7)
Total Trial (0 – 8 h)	84 ± 8 (34.0)	281 ± 20 (51.7)	83 ± 15 (14.3)
Brisk walking	**Fat**	**Carbohydrate**	**Protein**
Morning meal (1.5-2 h)	35 ± 4 (29.0)	149 ± 14 (56.4)	42 ± 9 (14.6)
Afternoon meal (5 – 5.5 h)	44 ± 6 (34.2)	141 ± 14 (50.1)	50 ± 10 (15.7)
Total Trial (0 – 8 h)	79 ± 8 (32.6)	290 ± 19 (51.9)	92 ± 16 (15.5)

Values are gram and (%) (*n* = 14)

6.3.4 Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ (P = 0.507) between the brisk walking and control trials (89 ± 9 verses 93 ± 22 pg·mL $^{-1}$). For plasma concentrations of acylated ghrelin two-factor ANOVA revealed a significant main effect of time (P = 0.003) indicating that circulating concentrations of acylated ghrelin changed across time in response to the meals consumed during main trials (Figure 6.3). No significant trial or interaction main effects were found indicating that acylated ghrelin responses were not significantly different between the brisk walking and control trials.

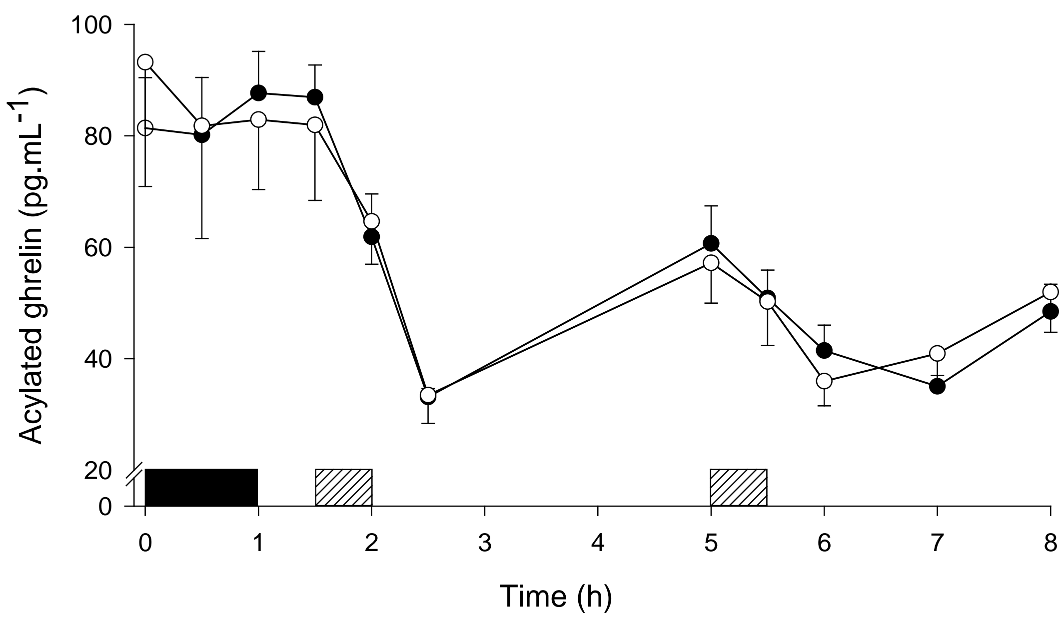


Figure 6.3: Plasma concentrations of acylated ghrelin in the brisk walking (○) and control (●) trials. Values are mean ± SEM (n = 14). Black rectangle indicates brisk walking. Diagonal rectangles indicate buffet meals.

6.3.5 Insulin, glucose and triacylglycerol

Fasting plasma concentrations of insulin (brisk walking 30.1 ± 5.0, control 27.5 ± 5.2 pmol·L^{-1}, P = 0.671), glucose (brisk walking 4.41 ± 0.20, control 4.49 ± 0.24 mmol·L^{-1}, P = 0.520) and triacylglycerol (brisk walking 0.82 ± 0.1, control 0.78 ± 0.1 mmol·L^{-1}, P = 0.671) did not differ significantly between the brisk walking and control trials. Plasma insulin, glucose and triacylglycerol concentrations changed significantly over time (main effect of time, $P < 0.001$ for each) however no trial or interaction main effects were found (all $P > 0.05$). Figure 6.4 shows the plasma insulin, glucose and triacylglycerol responses in the brisk walking and control trials.

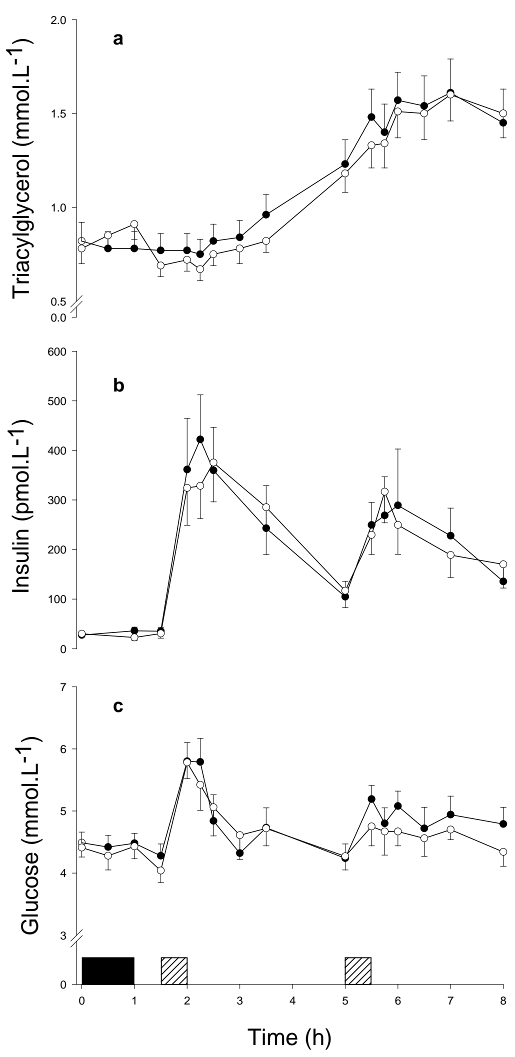


Figure 6.4: Plasma concentrations of triacylglycerol (a), insulin (b) and glucose (c) in the brisk walking (○) and control (●) trials. Values are mean ± SEM (n = 14). Black rectangle indicates brisk walking. Diagonal rectangles indicate buffet meals.

6.3.6 Correlations between acylated ghrelin and other variables

In the brisk walking trial acylated ghrelin and prospective food consumption AUC were positively correlated during the intermeal interval (1.5 - 5 h) (r = 0.723, P = 0.028) whilst acylated ghrelin and plasma triacylglycerol AUC were negatively correlated during the intermeal interval (1.5 - 5 h) (r = -0.827, P = 0.006) and across the total trial (0 - 8 h) (r = -0.694, P = 0.038). The acylated ghrelin and insulin AUC values tended to be inversely correlated across the total trial (0 - 8 h) (r = -0.660, P = 0.053).

When examining correlations at individual time points many significant relationships emerged ($P < 0.05$). At 1 and 2 h during the control trial acylated ghrelin was positively correlated with hunger and prospective food consumption. Correlation coefficients ranged from 0.672 to 0.809. During the control trial acylated ghrelin was negatively correlated with fullness at 1 and 2 h and with satisfaction at 1, 2 and 5.5 h. Correlation coefficients ranged from -0.716 to -0.898. Inverse correlations were observed between acylated ghrelin and plasma triacylglycerol at 2.5 h in the brisk walking trial (r = -0.714, P = 0.031) and with plasma insulin at 5 h in the brisk walking trial (r = -0.744, P = 0.022).

In both the brisk walking and control trials no significant correlations were found between circulating acylated ghrelin concentrations immediately before buffet meals and *ad libitum* energy intake at the meals. Additionally, no significant correlations were found between energy intake at the buffet meals and the percentage change in circulating acylated ghrelin 30 min after eating.

6.3.7 Water intake, temperature and humidity

There were no significant differences in water intake (brisk walking 1582 ± 249, control 1247 ± 217 mL, P = 0.095), laboratory environmental temperature (brisk walking 22.8 ± 0.2, control 22.7 ± 0.2 ^{o}C, P = 0.590) or relative humidity (brisk walking 34.4 ± 1.5, control, 32.1 ± 1.2 %, P = 0.224) between the brisk walking and control trials.

6.4 Discussion

The purpose of this investigation was to examine appetite, energy intake and plasma acylated ghrelin responses during and for several hours after a 60 min bout of brisk walking. The main finding arising from this study is that despite inducing a moderate energy deficit, an acute bout of brisk walking did not modify appetite, energy intake or circulating concentrations of acylated ghrelin. These findings can be looked upon favourably in terms of the potential for brisk walking to facilitate successful weight control.

The finding of no difference in appetite (hunger, satisfaction, fullness and prospective food consumption) between the brisk walking and control trials can perhaps be explained by the relatively moderate intensity of exertion and subsequent energy expenditure elicited through walking. Previous work has consistently observed a suppression of appetite during and briefly after intense bouts of activity (> 60% of $\dot{V}O_2$ max) (King et al, 1994; King and Blundell, 1995; Martins et al, 2008). This response may therefore have been unanticipated in the present study as brisk walking provided a much lesser physiological challenge to the relatively fit sample of participants examined.

Consistent with no change in appetite, brisk walking also failed to influence energy intake. At both the morning and afternoon buffet meals energy intake was highly congruent between trials. This observation confirms previous findings which have typically shown no difference in energy intake in the short term (1 – 2 days) once individuals have completed an acute bout of exercise (King et al, 1994; 1996; 1997; King and Blundell, 1995). Consequently, in the present investigation the participants failed to compensate for the exercise-induced energy expenditure. King and co-workers (1994) have suggested that the relative post-exercise energy intake response (absolute energy intake adjusted for the net exercise induced energy expenditure) is of greater importance than the absolute amount of energy consumed. Using this formula, brisk walking induced a relative deficit in energy in comparison with control (1836 kJ, 439 kcal). This finding suggests that brisk walking does not elicit an automatic compensation in energy intake in the immediate hours after completing a single bout of

brisk walking. Although a more delayed response remains a possibility, this initial finding lends support for the utility of brisk walking in successful body weight control.
In the present study brisk walking did not perturb food preferences i.e. there were no differences in the macronutrient composition of the food items consumed between the brisk walking and control trials. In both the morning and afternoon meals the distribution of energy was typical of a Western diet (Cordain et al, 2005). This observation confirms results from the majority of previous laboratory interventions which have failed to show any consistent effect of exercise on food preferences (Tremblay and Drapeau, 1999; Elder and Roberts, 2007). In the present study, within each trial the percentage of energy derived from carbohydrate was higher at the morning buffet meals whilst that of fat was higher in the afternoon meals. At the morning meals the selection of high carbohydrate, breakfast type items such as cereals and milk most likely explains the greater proportion of energy derived from carbohydrate. Moreover, at the afternoon meals, consumption of typical lunch items familiar to the study participants (sandwiches, crisps, chocolate, cookies etc) most likely accounts for the higher percentage intake of fat. In this investigation no change in macronutrient selection contributed to the lack of difference in energy intake observed between trials. Previous work has shown that switching from low-fat to high-fat food options completely reverses the energy deficit induced by prior exercise (King and Blundell, 1995; King et al 1996). It is therefore appealing that brisk walking did not stimulate an appetite for foods with a higher content of fat and therefore energy.

This study is the first to examine the acylated ghrelin response to low intensity exercise. Therefore, a novel finding is that plasma acylated ghrelin concentrations are not affected during or for several hours after an acute bout of brisk walking. This finding is consistent with the lack of difference in appetite and energy intake observed between trials. Previously, a concomitant suppression of plasma acylated ghrelin and hunger has been observed during and briefly after an intense bout of treadmill running (Broom et al, 2007). Similarly, in Chapter five of this thesis (study two) a concomitant suppression of acylated ghrelin and hunger were found during a single bout of swimming. Brisk walking did not affect hunger in the present study therefore given the role of ghrelin in appetite regulation no change in acylated ghrelin is a logical outcome. The reduced physiological challenge imposed by walking compared with running may account for the difference in findings between studies. Specifically, the lower energy expenditure

elicited, gastrointestinal disturbance and/or redistribution of splanchnic blood volume may be implicative.

This study has two notable limitations. Firstly, the sample of participants was composed of a relatively homogenous population of young, healthy males therefore these findings may not generalize to clinical populations where brisk walking may provide a greater physiological challenge. Secondly, appetite, energy intake and acylated ghrelin responses were observed merely for several hours after walking. Assessment of these variables over an even greater period of time may be necessary in order to detect any possible compensation.

In conclusion, this study demonstrates that an acute bout of brisk walking does not increase appetite, energy intake or plasma acylated ghrelin concentrations, despite inducing a moderate deficit in energy. These findings lend support for a role of brisk walking in successful weight management.

Chapter VII

Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin

7.1 Introduction

Ghrelin is an appetite stimulating hormone with a defined role in the acute regulation of energy homeostasis (Cummings et al, 2006). Circulating concentrations of ghrelin rise before meals and fall thereafter, evidence which has been interpreted as indicating a role of ghrelin in meal initiation (Cummings et al, 2001; 2004). In both the short and long-term, the diurnal profile of ghrelin is sensitive to changes in energy flux. A positive relationship exists between the energy content of meals and the subsequent suppression of ghrelin postprandially (Callahan et al, 2004). Moreover, an inverse relationship exists between the energy content of meals and the subsequent rise in ghrelin prior to the next meal (Leidy and Williams, 2006). These findings imply that ghrelin is sensitive to dietary manipulations in energy balance.

Less is known regarding how exercise-induced changes in energy flux impact on circulating levels of ghrelin. In the study presented in the previous chapter (Chapter 6) circulating acylated ghrelin concentrations remained unchanged during and for several hours after an acute bout of brisk walking. It is possible that the perturbation to energy balance induced by 60 min of brisk walking was insufficient to elicit changes in circulating acylated ghrelin. The limited evidence available, including the findings presented in Chapter five of this thesis, suggest that bouts of exercise completed at moderate intensities or higher induce a transient suppression in circulating concentrations of acylated ghrelin (Broom et al, 2007; Marzullo et al, 2008; Broom et al, 2009). Despite this, in none of these previous studies have circulating acylated ghrelin concentrations been stimulated after exercise, a response that may be anticipated if acylated ghrelin were sensitive to changes in energy expenditure resulting from exercise. It remains possible that the energy deficit induced in these previous interventions was not severe enough to elicit a compensatory increase in circulating levels of acylated ghrelin.

Over the last two decades research that has examined the influence of exercise on appetite and energy intake has tended to show a lack of influence in the short-tem

(Martins et al, 2008; Bilski et al, 2009). A limitation of this previous work is the brief period over which appetite and energy intake have tended to be examined, typically during exercise and then leading up to a meal provided shortly after. It is possible that changes may occur over a longer duration, at a second, third or fourth meal taken after completing exercise (Broom et al, 2007; Malkova et al, 2008). Thus, a longer duration of observation, under strictly controlled conditions, may be necessary to detect changes in appetite and/or energy intake after completing exercise (Bilski et al, 2009). Changes may also occur, only after significant challenges to energy homeostasis.

This study sought to examine prolonged appetite, energy intake and acylated ghrelin responses to an acute bout of exercise which was sufficient to induce a substantial energy deficit. Its known that ghrelin is sensitive to acute changes in energy balance in response to dietary manipulation however it is not known whether such a coupling exists between exercise induced changes in energy balance and acylated ghrelin. It was anticipated that a large perturbation to energy balance would be associated with a compensatory increase in circulating concentrations of acylated ghrelin as a signal to augment appetite and subsequent energy intake.

7.2 Methods

7.2.1 Participants

After gaining Loughborough University Ethical Advisory Committee approval nine healthy male volunteers (18 – 27 y) gave their written informed consent to participate. Table 7.1 describes the characteristics of the study participants.

Table 7.1: Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	22.2 ± 0.8
BMI ($kg \cdot m^{-2}$)	23.6 ± 0.4
Body Mass (kg)	77.5 ± 2.3
Body Fat (%)	17.8 ± 1.7
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	60.5 ± 1.5

($n = 9$)

7.2.2 Study design

Before taking part in main trials participants visited the laboratory in order to familiarise themselves with the environment and to enable the collection of the necessary anthropometric and preliminary exercise test data. After being made aware of the protocol, participants were health screened and gave their written informed consent to participate. Anthropometric data was then collected after which participants completed two preliminary exercise tests: 1) a submaximal-incremental treadmill running test, 2) a maximum oxygen uptake ($\dot{V}O_2$ max) treadmill running test. There was a 15 to 20 min interval between tests.

7.2.3 Main trials

In subsequent weeks participants completed two, 24 h trials (exercise and control) in a randomised, counterbalanced fashion with trials being separated by at least one-week. Main trials began in the morning of day one between 08:30 - 09:00 and participants were confined to the laboratory for the subsequent 10 h. Participants left the laboratory at this point and returned the next morning (day two) to provide a fasting blood sample

and to complete a final visual analogue scale (24 h measurements). To standardise diet and physical activity before main trials participants completed a weighed food record of all items consumed within the 24 h preceding their first main trial and this feeding pattern was replicated before their second main trial. Alcohol, caffeine and physical activity were not permitted during this period (or in the time spent away from the laboratory between trial days).

On the morning of main trials participants arrived at the laboratory having fasted overnight. To minimise physical exertion on the morning of trials participants were asked to walk slowly to the laboratory if they lived within 0.5 km of the research laboratory. Participants living further away arrived by motorised transport. The exercise trial commenced when participants began a 90 min run on a level treadmill at a speed predicted to elicit 70% of maximum oxygen uptake. During the run samples of expired air were collected at 15 min intervals to monitor the intensity and adjustments were made to the speed of the treadmill if necessary. On completion of the run participants rested within the laboratory for 8.5 h (sitting reading, working at a computer or watching television). Identical procedures were completed during the control trial except participants rested within the laboratory for the entire duration. In the first 90 min of the control trial samples of expired air were collected in the semi-supine position in order to estimate resting oxygen consumption. This permitted the estimation of net energy expenditure during exercise (exercise energy expenditure minus resting energy expenditure). Figure 7.1 provides a schematic illustration of the main trial protocol.

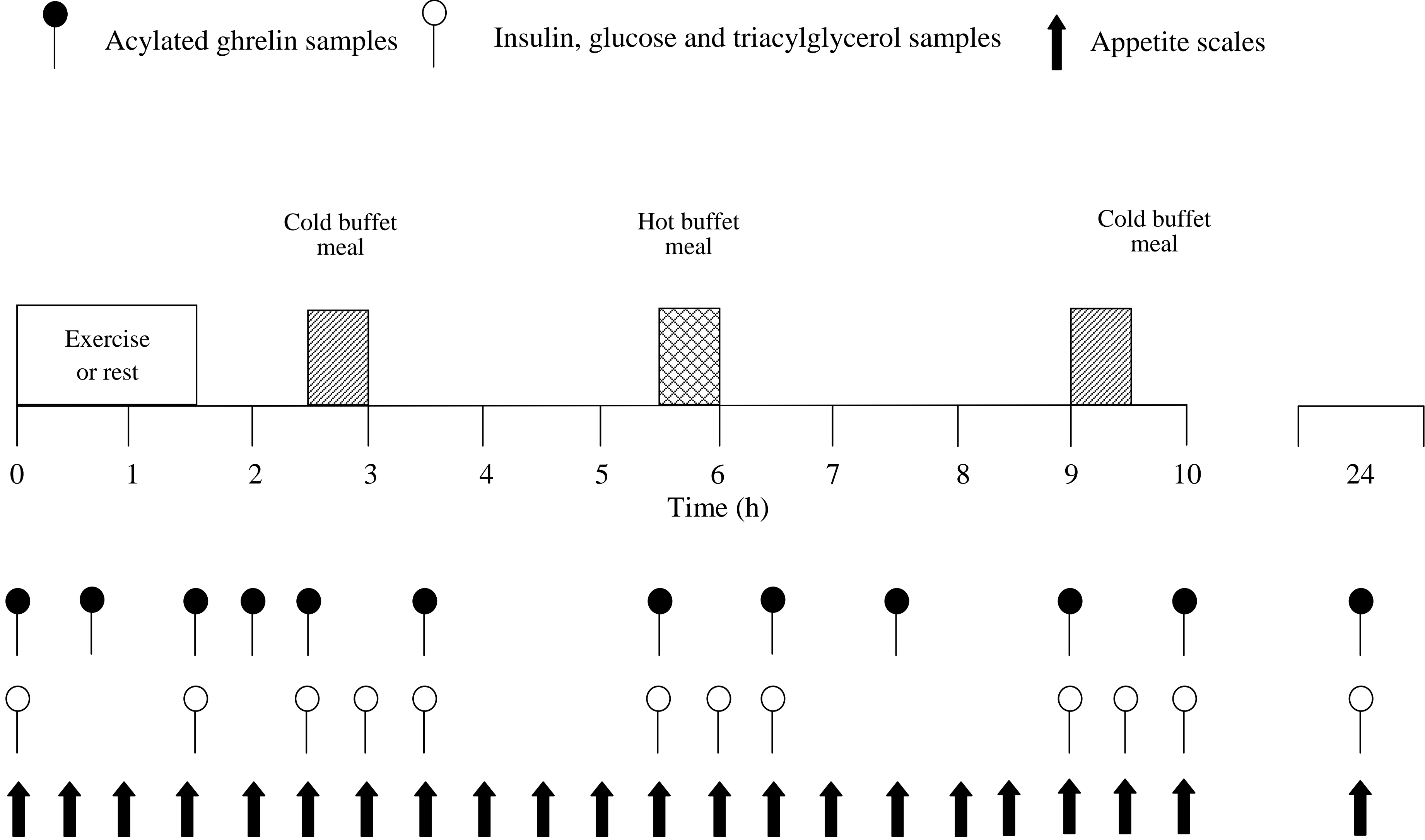


Figure 7.1: Schematic representation of the main trial protocol

7.2.4 Appetite assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at baseline and then at 30 min intervals throughout the laboratory phase of trials. Final visual analogue scales were completed on the morning of the second trial day (24 h measurement).

7.2.5 Ad libitum buffet meals

During the laboratory phase on the first day of trials participants consumed food from *ad libitum* buffet meals provided at three time points throughout. The cold buffet meal, which was provided in Chapters four, five and six of this thesis, was provided at 2.5 and 9 h (Appendix G). Due to the length of the trial days a hot buffet meal was also made available at 5.5 h (Appendix H). Food was available for 30 min at each meal. In the time spent away from the laboratory in-between laboratory visits on days one and two participants were free to select, and subsequently consume if desired, any items presented at the cold buffet meal. Participants were permitted to consume these items after leaving the laboratory on day one until 23:00 prior to fasting. Section 3.12 provides details on the format of the buffet meals.

7.2.6 Blood sampling

On the first day of each main trial venous blood was collected via a cannula inserted into an antecubital vein. Venepuncture was used to collect the 24 h blood sample on the second trial day. Blood samples were taken into pre-chilled 4.9 mL monovettes at baseline, 0.75, 1.5, 2, 2.5, 3.5, 5.5, 6.5, 7.5, 9, 10 and 24 h to measure plasma acylated ghrelin (see section 3.14 for details on acylated ghrelin sample processing).

For the determination of plasma insulin, glucose and triacylglycerol additional samples were collected into pre-chilled 9 mL EDTA monovettes at baseline, 0, 1.5, 2.5, 3, 3.5, 5.5, 6, 6.5, 9, 9.5, 10 and 24 h. The EDTA monovettes were spun at 1681*g* for 10 mins in a refrigerated centrifuge at 4 ^{o}C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage for analysis later. All samples were collected in the semi-supine position except for the 0.75 h sample during exercise whereby participants straddled the treadmill. For samples collected using a cannula, patency was maintained by flushing with non-heparinised saline (0.9 % w/v sodium chloride) after each

collection. To avoid the dilution of subsequent samples residual saline was discarded using a 2 mL syringe before sample collection.

To estimates changes in plasma volume, at each blood sampling point duplicate 20 μL blood samples were collected into micropipettes and triplicate 20 μL samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations.

7.2.7 Biochemical analysis

Enzyme immunoassays were used to determine plasma concentrations of acylated ghrelin and insulin. Plasma glucose and triacylglycerol concentrations were determined spectrophotometrically using an automated bench top analyzer. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.8%, insulin 2.5%, glucose 0.4% and triacylglycerol 2.7%.

7.2.8 Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows. All area under the concentration verses time curve calculations were performed using the trapezoidal method. Student's *t*-tests for correlated data were used to assess differences between fasting and AUC values for acylated ghrelin, glucose, insulin, triacylglycerol and appetite perceptions between the control and exercise trials. Repeated measures, two-factor ANOVA was used to examine differences between the exercise and control trials over time for appetite, energy and macronutrient intake, acylated ghrelin, glucose, insulin and triacylglycerol. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

7.3 Results

7.3.1 Exercise responses

Participants completed the 90 min run at 10.3 ± 0.3 km·h^{-1}. This elicited a mean oxygen consumption equivalent to 68.8 ± 0.8% of maximum oxygen uptake and generated a mean heart rate and net (exercise minus resting) energy expenditure of 173 ± 3 beats·min^{-1} and 5326 ± 186 kJ (1273 ± 45 kcal), respectively. A mean non-protein respiratory quotient of 0.89 ± 0.01 reflected the proportional contributions of carbohydrate and fat (64 ± 5% and 36 ± 5%) to energy provision. A median RPE value of 15 indicated that the participants perceived the intensity of the run to be 'hard.'

7.3.2 Appetite responses

There were no significant differences in baseline ratings of appetite (hunger, fullness, satisfaction and prospective food consumption) between the exercise and control trials (Table 7.2).

Table 7.2: Baseline appetite perceptions in the exercise and control trials

	Control	Exercise	*P*
Hunger (0-100)	65 ± 8	74 ± 5	0.169
Satisfaction (0-100)	22 ± 5	19 ± 5	0.405
Fullness (0-100)	17 ± 6	16 ± 4	0.765
PFC (0-100)	77 ± 7	78 ± 5	0.858

(n = 9). PFC = prospective food consumption

Two-factor ANOVA revealed a significant main effect of time (all $P < 0.001$) and a significant trial x time interaction effect (all $P < 0.023$) for each appetite perception assessed (hunger, fullness, satisfaction and prospective food consumption) indicating that responses differed over time between the exercise and control trials (Figure 7.2). Post-hoc analysis revealed significant differences in hunger and prospective food consumption at 0.5, 1 and 1.5 h indicating suppressed hunger and prospective food consumption during exercise. Significant differences in ratings of fullness and satisfaction were apparent at 0.5 and 1 h demonstrating elevated perceptions during exercise. After adjusting for multiple comparisons using the Bonferroni method none of these differences remained significant.

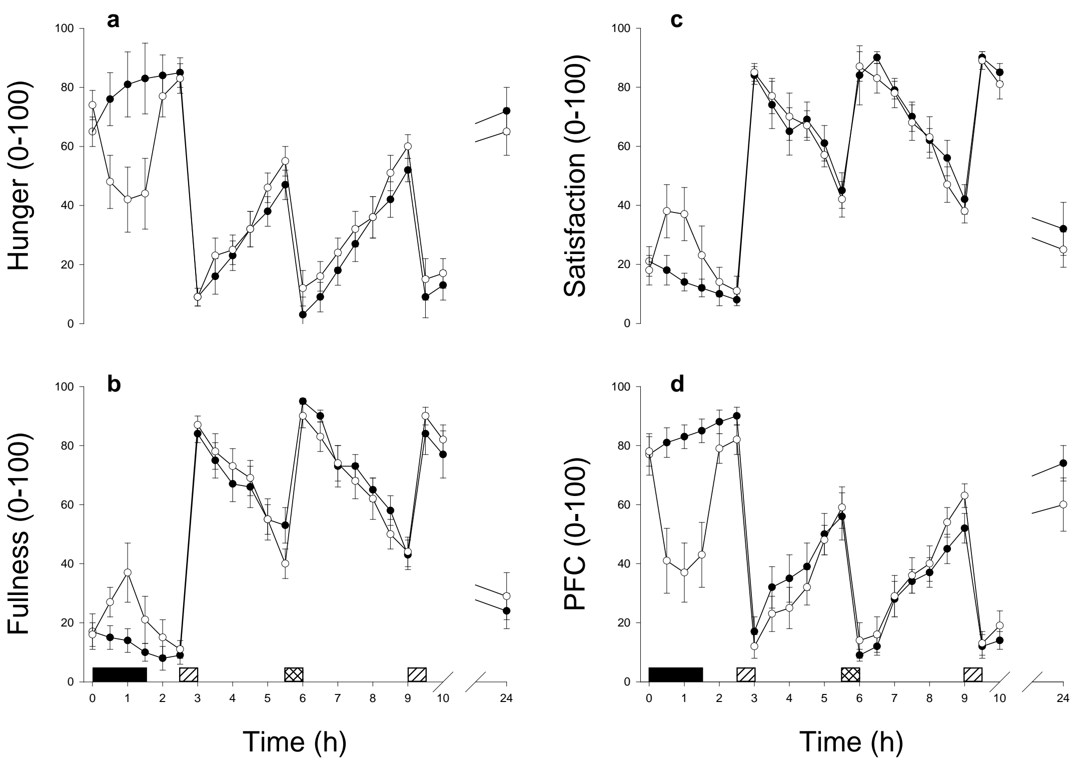


Figure 7.2: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the exercise (○) and control (●) trials. Values are mean ± SEM *(n* = 9). Black rectangles indicate exercise. Diagonal rectangles indicate cold buffet meals. Crossed rectangles indicate hot buffet meals.

7.3.3 Energy and macronutrient intake

For energy intake two-factor ANOVA revealed a significant main effect of time ($P < 0.001$) signifying that energy intake was higher at the morning cold buffet meals than at the meals consumed later in the day. No significant trial ($P = 0.532$) or interaction (trial x time, $P = 0.450$) main effects were apparent, therefore energy intake did not differ significantly between the exercise and control trials (Table 7.3). Analyses of the relative energy intake (energy intake – net exercise energy expenditure) showed that exercise (12282 ± 1252 kJ, 2935 ± 299 kcal) induced an energy deficit relative to the control trial (17191 ± 1144 kJ, 4109 ± 273 kcal) ($P < 0.001$).

Table 7.3: Energy intake in the exercise and control trials

	Control	Exercise
Cold meal (morning) (2.5 – 3 h)	6879 ± 700 (1644 ± 167)	6165 ± 700 (1474 ± 167)
Hot meal (5.5 – 6 h)	4553 ± 621 (1088 ± 148)	4928 ± 618 (1178 ± 148)
Cold meal (evening) (9 – 9.5 h)	4694 ± 341 (1122 ± 82)	5214 ± 623 (1246 ± 149)
Overnight (10 – 14 h)	1065 ± 275 (255 ± 66)	1299 ± 299 (310 ± 71)
Total trial (0 – 24 h)	17191 ± 1144 (4109 ± 273)	17606 ± 1384 (4208 ± 331)

Values are kJ and (kcal) ($n = 9$)

Two-factor ANOVA was used to examine macronutrient intake (absolute and percent) across the buffet meals during the exercise and control trials (Table 7.4). For the absolute intake (grams) two-factor ANOVA revealed significant main effects of time ($P < 0.001$) for each of the macronutrients (protein, fat and carbohydrate) indicating differences between the individual meals consumed during main trials. A significant main effect of trial was observed for protein ($P = 0.007$) demonstrating a consistently higher intake of protein in the exercise trial than the control trial.

Two-factor ANOVA revealed significant main effects of time (all $P < 0.001$) for the percentage intake of each of the macronutrients however no trial or interaction (trial x time) main effects were apparent (all $P > 0.05$). Thus, there were no significant differences in the percentage of energy derived from fat, carbohydrate or protein between the exercise and control trials.

Table 7.4: Macronutrient intake in the exercise and control trials

Control trial	**Fat**	**Carbohydrate**	**Protein**
Cold meal (morning) (2.5 – 3 h)	56 ± 7 (30.7)	220 ± 30 (53.6)	65 ± 12 (15.7)
Hot meal (5.5 – 6 h)	12 ± 2 (9.9)	221 ± 31 (81.2)	24 ± 4 (8.9)
Cold meal (evening) (9 – 9.5 h)	40 ± 5 (31.5)	140 ± 12 (50.0)	50 ± 5 (18.5)
Overnight (10 – 14 h)	5 ± 2 (17.0)	49 ± 12 (78.5)	3 ± 1 (4.5)
Total Trial (0 – 24 h)	113 ± 10 (24.7)	630 ± 49 (61.3)	142 ± 16 (14.0)
Exercise trial	**Fat**	**Carbohydrate**	**Protein**
Cold meal (morning) (2.5 – 3 h)	46 ± 7 (28.1)	205 ± 27 (55.3)	59 ± 7 (16.6)
Hot meal (5.5 – 6 h)	15 ± 3 (11.1)	232 ± 29 (78.9)	29 ± 4 (10.0)
Cold meal (evening) (9 – 9.5 h)	45 ± 6 (32.6)	150 ± 22 (48.1)	60 ± 10 (19.3)
Overnight (10 – 14 h)	8 ± 2 (22.4)	56 ± 13 (72.7)	4 ± 1 (4.9)
Total Trial (0 – 24 h)	114 ± 12 (24.1)	643 ± 56 (61.2)	152 ± 14 (14.7)

Values are gram and (%) ($n = 9$)

7.3.4 Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ ($P = 0.103$) between the exercise and control trials (130 ± 15 verses 147 ± 20 pg mL^{-1}). Two-factor ANOVA revealed a significant main effect of trial ($P = 0.009$), time ($P < 0.001$) and a significant interaction effect (trial x time, $P < 0.001$) indicating that acylated ghrelin responses differed over time between the exercise and control trials.

Post-hoc analysis using the Bonferroni method demonstrated between trial differences at 0.75 and 1.5 h indicating suppressed acylated ghrelin during and immediately after exercise. Between trial differences in acylated ghrelin were also evaluated using AUC

values calculated for the time before morning buffet meal (0 – 2.5 h), the time after the first buffet meal (3.5 – 10 h) and for the total duration within the laboratory on day one (0 – 10 h). The acylated ghrelin AUC was significantly lower over the first 2.5 h of the exercise trial relative to control (control 347 ± 47, exercise 209 ± 35 pg·mL^{-1}· 2.5 h) (P = 0.002) and for the total 10 h (control 934 ± 129, exercise 697 ± 115 pg·mL^{-1}·10 h) (P = 0.011). The acylated ghrelin AUC was not significantly different (P = 0.114) between trials after consumption of the first meal (3.5 – 10 h) (control 490 ± 75, exercise 418 ± 74 pg·mL^{-1}·6.5 h).

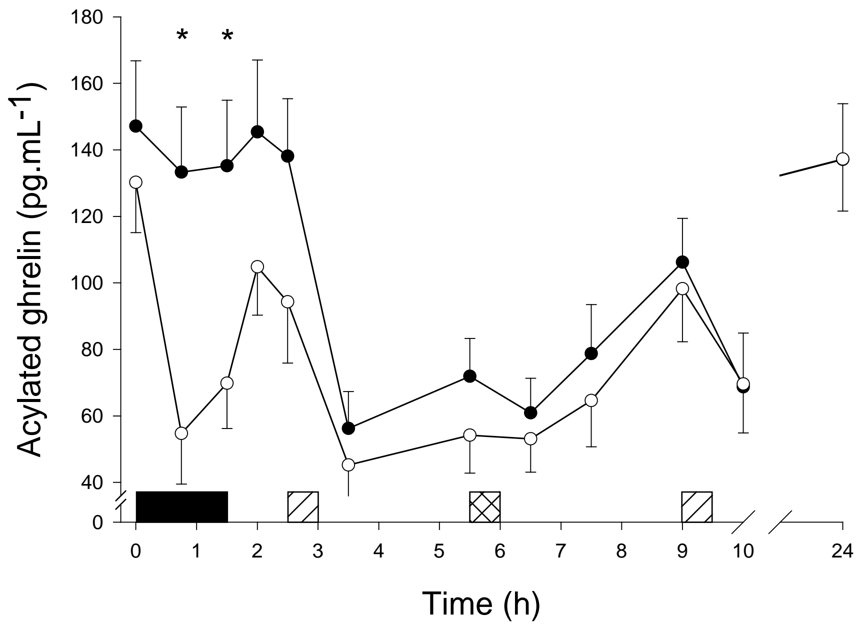


Figure 7.3: Plasma concentration of acylated ghrelin in the exercise (○) and control (●) trials. Values are mean ± SEM (n = 9). Black rectangle indicates exercise, diagonally shaded rectangles indicate cold meals, hatched shaded rectangle indicates the hot meal. *Significantly different from control after correcting for multiple comparisons using the Bonferroni method.

7.3.5 Insulin, glucose and triacylglycerol

Fasting plasma insulin concentrations did not differ (P = 0.403) between the exercise and control trials (29.5 ± 5.4 verses 24.3 ± 2.5 pmol·L^{-1}). Two-factor ANOVA identified a significant main effect of time (P < 0.001) however no significant trial (P = 0.103) or interaction (trial x time, P = 0.102) main effects were found.

Fasting plasma glucose concentrations did not differ (P = 0.403) between the exercise and control trials (5.07 ± 0.12 verses 5.10 ± 0.05 pmol·L^{-1}). Two-factor ANOVA

revealed a significant main effect of time ($P < 0.001$) and a significant interaction effect (trial x time, $P < 0.001$) indicating that glucose responses differed over time between the exercise and control trials. Post-hoc analysis identified between trial differences at 2.5 and 6 h highlighting lower and then subsequently higher circulating concentrations of glucose in the exercise trial than in the control trial. These differences did not remain after Bonferroni adjustment.

Fasting plasma triacylglycerol concentrations did not differ ($P = 0.200$) between the exercise and control trials (0.79 ± 0.08 verses 0.90 ± 0.11 $mmol \cdot L^{-1}$). Two-factor ANOVA yielded significant main effects of trial ($P = 0.036$), time ($P < 0.001$) and a significant interaction (trial x time, $P < 0.001$) effect. Thus, circulating triacylglycerol responses were significantly different over time in the exercise and control trials. Post-hoc analysis indicated between trial differences at 1.5, 5.5, 6, 6.5, 9 and 9.5 h, denoting an increase in circulating triacylglycerol during exercise before falling and remaining below control values in the time thereafter. After correcting for multiple comparisons the only difference to remain significant was at 1.5 h. Figure 7.4 shows the plasma insulin, glucose and triacylglycerol responses in the exercise and control trials.

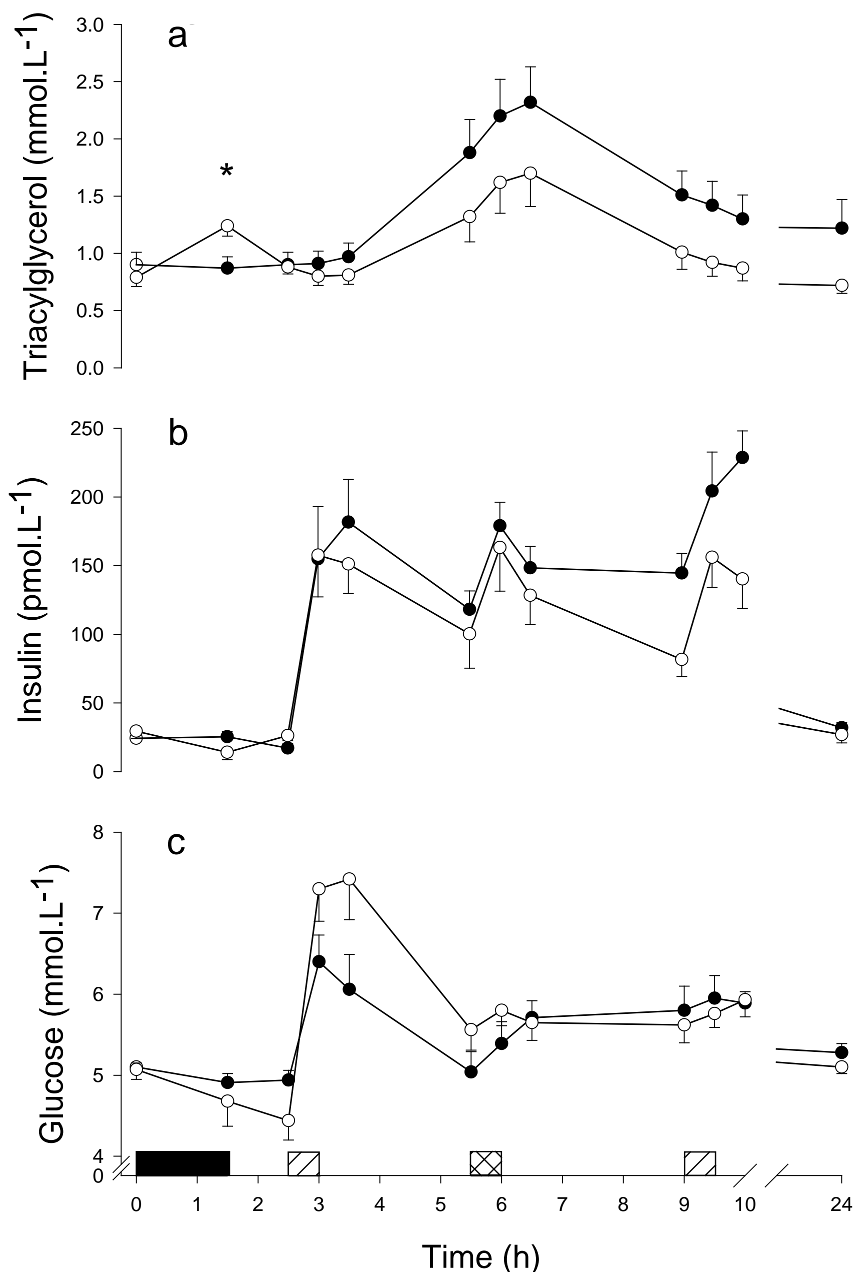


Figure 7.4: Plasma concentrations of triacylglycerol (a), insulin (b) and glucose (c) in the exercise (○) and control (●) trials. Values are mean ± SEM *(n* = 9). * Significantly different from control after Bonferroni adjustment. Black rectangle indicates running, diagonally shaded rectangles indicate cold meals, hatched shaded rectangle indicates the hot meal.

7.3.6 Correlations between acylated ghrelin and other variables

Fasting concentrations of plasma acylated ghrelin were inversely correlated with body weight (r = -0.720, P = 0.029) and tended to be negatively correlated with BMI (r = -0.611, P = 0.081). A significant inverse association was observed between insulin and acylated ghrelin AUC on the exercise trial in between the first and second meals (2.5 – 5.5 h) (r = -0.851, P = 0.031). A tendency towards a significant inverse relationship was observed between plasma triacylglycerol and acylated ghrelin values in the exercise trial in-between the second and third meal (5.5 – 9 h) (r = -0.663, P = 0.052). At individual time points during the exercise trial acylated ghrelin was inversely related with triacylglycerol at 6.5 h (r = -0.675, P = 0.046) and tended to be inversely related with insulin at 1.5 h (r = -0.808, P = 0.052). On both the control and exercise trials no significant correlations were observed between plasma acylated ghrelin concentrations immediately prior to *ad libitum* meals and subsequent energy consumption. Moreover, no relationships were found between energy intake at each meal and the percentage change in plasma acylated ghrelin.

7.3.7 Water intake, temperature and humidity

Water intake was significantly higher during the exercise trial compared with the control trial (2795 ± 258 mL vs. 1553 ± 303 mL, P = 0.003). There were no significant differences in the mean environmental temperature (control 24.1 ± 0.2, exercise 25.0 ± 0.3^{o}C, P = 0.792) or relative humidity (control 41.4 ± 2.3, exercise 40.6 ± 1.9%, P = 0.209) within the laboratory during the exercise and control trials.

7.4 Discussion

The purpose of this investigation was to examine appetite, energy intake and plasma acylated ghrelin concentrations during and for an extended period after a prolonged bout of treadmill running which was sufficient to induce a substantial energy deficit. The primary findings are that exercise induced a brief suppression of appetite and plasma acylated ghrelin yet did not influence appetite, acylated ghrelin or *ad libitum* energy/macronutrient intake in the 22.5 h after.

During exercise perceptions of hunger and prospective food consumption were transiently suppressed while ratings of satisfaction and fullness were increased. These responses indicate an inhibition of appetite during exercise and this outcome is consistent with previous reports of exercise induced anorexia resulting from bouts of activity performed at moderate intensities or higher (> 60% of $\dot{V}O_2$ max) (King et al, 1994; King & Blundell, 1995). The mechanisms responsible for changes in appetite as a consequence of exercise are not well defined however the role of circulating concentrations of gut hormones have began to receive attention (Broom et al, 2007; Martins et al, 2007; Martins et al, 2008). Broom et al (2007) reported data supporting a role of acylated ghrelin in determining suppressed ratings of hunger during one hour of intense treadmill running. The data from the present investigation support this notion as circulating concentrations of acylated ghrelin were significantly lower during and at the end of exercise although the absence of any significant correlations between acylated ghrelin and markers of appetite during exercise questions this hypothesis.

Changes in appetite diminished soon after exercise, remaining no different from control values over the course of the trial. This outcome was unexpected given the extreme energy deficit induced during exercise. It was thought that ratings of appetite would be higher at some point within the hours after exercise in an effort to stimulate a compensatory increase in energy intake. Heightened perceptions of hunger and desire to eat have been reported in two previous investigations which have examined the appetite response to exercise over an extended duration (Broom et al, 2007; Malkova et al, 2008). The energy expenditure induced by exercise was greater in the present study therefore it remains unclear why a compensatory appetite response was not observed.

Ghrelin is an appetite stimulating hormone with an important role in the acute regulation of energy homeostasis (Cummings, 2006). Circulating concentrations ghrelin rise before meals and fall thereafter, evidence suggesting a role in determining meal initiation (Cummings et al, 2001; Cummings et al, 2004). In both the short and long-term the diurnal profile of ghrelin is sensitive to changes in energy flux. An inverse relationship exists between the energy content of meals and the subsequent pre-prandial rise in ghrelin (Leidy & Williams, 2006). Moreover, chronic energy restriction through diet and exercise induces heightened circulating concentrations of ghrelin during the nocturnal period and at meal related ghrelin peaks (Leidy et al, 2007). Based on this, in the present study it was postulated that the large energy deficit induced by exercise would stimulate a compensatory increase in acylated ghrelin in the hours after as a stimulus to increase appetite and energy intake. It is unclear why circulating acylated ghrelin concentrations were not elevated in the hours after exercise. Surprisingly, although not statistically different, values actually appeared lower after exercise compared with those observed during control and it is possible that this may have been implicated in the lack of compensation in appetite and energy intake. Differences in feeding responses are unlikely to be implicated as both energy and macronutrient intakes were very similar between the exercise and control trials. It is possible that some exercise related factor may interfere in the metabolism of acylated ghrelin however the associated mechanisms are not known.

Despite exercise inducing a transient suppression of appetite and acylated ghrelin energy intake at the first buffet meal after exercise was not significantly different between the exercise and control trials. Moreover, energy intake was also no different at any of the three other feeding opportunities provided during this investigation. This response confirms previous findings which have observed no change in energy intake during meals consumed within the hours after exercise (King et al, 1994; King & Blundell, 1995) or on the day afterwards (King et al, 1997). Despite this, no previous investigation has induced such an energy deficit during a single bout of exercise. It was thought that this stimulus may invoke a response in energy intake that had not been observed by previous researchers. It is possible that a delayed response may occur (King, 1998) however a longer period of observation would be needed to test this.

It has been shown that missing a meal or consuming a meal of reduced energy content results in elevated hunger and energy intake at the next opportunity (Hubert et al, 1998). For the participants in this study the energy expended during exercise would have been greater than the energy content of a typical meal therefore it appears that a different homeostatic response is elicited when energy deficits are induced by dietary means as compared with exercise i.e. energy leaving the system verses a restriction on energy entering the system. This disparity highlights the potential usefulness of exercise in weight loss programs.

This study has two notable limitations. Firstly, the exercise protocol used in this study was designed to be physically challenging to invoke a high level of energy expenditure. Consequently, the outcomes reported may not transfer to situations where typical volumes of exercise are performed. Secondly, as previously mentioned, the 24 h observation period may not have been long enough to detect more delayed responses in the variables assessed. Further work is therefore required to scrutinise these responses over a longer duration of time.

In conclusion, this study has shown that a 90 min bout of treadmill running induces a transient suppression of appetite and plasma acylated ghrelin, yet does not influence short-term energy/macronutrient intake – despite inducing a substantial energy deficit. These outcomes contribute knowledge regarding the role of exercise in energy homeostasis. These findings indicate that exercise can induce substantial deficits in energy without eliciting compensatory responses in acylated ghrelin, appetite and energy intake.

Chapter VIII

The influence of treadmill running on feeding latency, plasma acylated ghrelin and ad libitum energy/macronutrient intake

8.1 Introduction

It has repeatedly been observed that exercise performed at moderate intensities or higher (> 60% of $\dot{V}O_2\text{max}$) suppresses appetite (King et al, 1994; King and Blundell, 1995). This effect has been termed exercise-induced anorexia (King et al, 1994). The appetite suppressive effect of exercise appears to be a transient event however and does not appear to subsequently influence food intake. Instead, it has been suggested that exercise-induced anorexia may manifest as a resistance to commence feeding after undertaking exercise (King et al, 1994; 1996; King and Blundell, 1995).

King et al (1994) examined appetite and energy intake responses to bouts of short (26 min) and long duration (56 min), high intensity cycling (~75% of $\dot{V}O_2\text{max}$) and reported that participants requested an *ad libitum* lunch approximately 5 min later in each trial, as compared with responses on a sedentary control trial. Similar findings have been observed by the same researchers in other investigations with running as the exercise stimulus (King and Blundell, 1995) and also in female participants (King et al, 1996). In spite of these findings the mechanisms responsible for mediating a reduction in appetite and a resistance to begin eating after exercise are not well understood. It is possible that appetite regulatory peptides such as acylated ghrelin may be important (Broom et al, 2007).

Broom et al (2007) reported that subjective ratings of hunger and circulating concentrations of acylated ghrelin were concomitantly reduced in response to a high intensity bout of treadmill running. These responses were positively correlated leading the researchers to suggest a possible role of acylated ghrelin in determining suppressed hunger during exercise. It is known that circulating concentrations of ghrelin exhibit a diurnal rhythm across the day, with levels rising prior to meals and falling thereafter in close proximity to changes in appetite (Cummings et al, 2001; 2004). This evidence has been interpreted as identifying ghrelin as a meal initiation signal. Considering these findings collectively it is possible that ghrelin may have a role in determining changes

in appetite in response to exercise and the subsequent decision to eat afterwards. At least three investigations have shown that high intensity exercise suppresses levels of ghrelin within the circulation (Broom et al, 2007; Marzullo et al, 2008; Broom et al, 2009) therefore it is possible that this suppression and subsequent recovery may be significant.

Another consistent finding within the research literature is that energy intake remains unchanged at meals consumed shortly after performing exercise (Blundell et al, 2003; Martins et al, 2008; Bilski et al, 2009). A limitation of the majority of studies which have examined the acute influence of exercise on food intake is that meals have been provided to participants on a predetermined schedule (Thompson et al, 1988; Kissileff et al, 1990; Verger et al, 1994; Imbeault et al, 1997; Westerterp-Plantenga et al, 1997; Hubert et al, 1998; George and Morganstein, 2003; Tsofliou et al, 2003; Pomerleau et al, 2004; Martins et al, 2007). This type of protocol restricts participants' eating behaviour to discrete intervals and may have influenced amounts eaten in past studies as people eat in expectation of appetite rather than eating to their appetite at that particular moment in time. An alternative to this protocol is to allow participants complete *ad libitum* access to foods after a bout of exercise so that participants are free to determine the timing, frequency, duration and content of meals and this may provide a more valid assessment of the effect of exercise on food intake.

The objective of this investigation was two-fold. The first aim was to assess the influence of an acute bout of exercise on feeding latency and to explore the potential role of acylated ghrelin in determining this. The second aim was to examine the influence of an acute bout of exercise on food intake when participants were provided with complete free access to food during main trials.

8.2 Methods

8.2.1 Participants

After gaining Loughborough University Ethical Advisory Committee approval 10 healthy males (19 – 22 years) gave their written informed consent to participate. Table 8.1 describes the characteristics of the study participants.

Table 8.1: Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	21.3 ± 0.7
BMI ($kg \cdot m^{-2}$)	23.9 ± 0.7
Body Mass (kg)	78.7 ± 2.7
Body Fat (%)	14.9 ± 1.0
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	65.1 ± 1.5

(*n* = 10)

8.2.2 Study design

Before taking part in main trials participants visited the laboratory in order to familiarise themselves with the environment and to enable the collection of the necessary anthropometric and preliminary exercise test data. After being made aware of the protocol, participants were health screened and gave their written informed consent to participate. Anthropometric data was then collected after which participants completed two preliminary exercise tests: 1) a submaximal-incremental treadmill running test, 2) a maximum oxygen uptake ($\dot{V}O_2$ max) treadmill running test. There was a 15 to 20 min interval between tests.

8.2.3 Main trials

In subsequent weeks each participant completed two, eight hour trials (exercise and control) in a randomised, counterbalanced fashion with trials being separated by at least one-week. To standardise diet and physical activity prior to main trials participants completed a weighed food record of all items consumed within the 24 h preceding their

first main trial and this feeding pattern was replicated prior to their second main trial. Alcohol, caffeine and physical activity were not permitted during this period.

Main trials began in the morning between 08:30 - 09:00. On the morning of trials participants arrived at the laboratory having fasted overnight. To minimise physical exertion on the morning of trials participants were asked to walk slowly to the laboratory if they lived within 0.5 km of the research laboratory. Participants living further away arrived by motorised transport. The exercise trial commenced when participants were provided with a breakfast snack. This was consumed within five min. Participants then rested for the remainder of the first trial hour. In the second trial hour participants ran on a treadmill at a speed predicted to elicit 70% of maximum oxygen uptake. During the run samples of expired air were collected at 15 min intervals to monitor the intensity and adjustments were made to the speed of the treadmill if necessary. After completing the run participants rested within the laboratory for a further six hours (sitting reading, working at a computer or watching television). Upon completion of the run participants were told that an *ad libitum* lunch was available on request and that after lunch food would remain available throughout the remainder of the trial. When lunch was voluntarily requested a blood sample was immediately collected before participants selected their lunch from the buffet. When participants had finished their meal a clock was started and blood samples were collected 30 and 60 min after. Within this hour participants were not permitted to eat. After the 60 min sample the cannula was removed and participants rested within the laboratory until the end of the trial. During this time *ad libitum* food intake was monitored. To avoid participants becoming aware that the purpose of the remainder of the trial was to monitor food intake an expired air sample was taken during the final 5 min of the trial and participants were told that this was to examine latent changes in metabolism after exercise.

Identical procedures were completed during the control trial except participants rested within the laboratory for the entire duration. During the second trial hour samples of expired air were collected in the semi-supine position in order to estimate resting oxygen consumption. This permitted the estimation of net energy expenditure during exercise (exercise energy expenditure minus resting energy expenditure). Two hours into the trial (synonymous with the end of exercise in the exercise trial) participants

were told that an *ad libitum* lunch was available on request and that food would also remain available for the rest of the trial.

8.2.4 Appetite assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at 30 min intervals throughout the first two hours of trials, at the point when participants requested lunch and then immediately prior to blood sample collections 30 and 60 min after lunch.

8.2.5 Breakfast and ad libitum buffet meals

Main trials commenced with the consumption of a breakfast snack. The breakfast provided was standardised to body weight and consisted of a commercial cereal bar (Kellogg's Nutri-grain®). Participants received 1.06 g per kilogram of body weight measured on the first trial visit. Identical amounts were consumed across trials. For a 70 kg individual this provided 1092 kJ (260 kcal) of energy, 6 g of fat, 4 g of protein and 48 g of carbohydrate. The breakfast snack was consumed within 5 min on all trials.

During main trials a buffet meal (Appendix G) was made available to the participants immediately after the run on the exercise trial and after the respective time point on the control trial (see section 3.12 for details on the buffet meal). At this point participants were told that a buffet lunch was available upon their request and that the buffet would remain available throughout the remainder of each trial. An exception to this was during the 60 min immediately after consuming lunch which permitted the collection of blood samples taken 30 and 60 min after eating. The buffet was presented in the research kitchen and participants were free to select items from the buffet when desired. Food consumption was ascertained by weighing the food items before and after each eating episode.

8.2.6 Blood sampling

A cannula was inserted into an antecubital vein while participants lay in a semi-supine position approximately 30 min before main trials commenced. To determine plasma acylated ghrelin concentrations venous blood samples were collected into pre-chilled 4.9 mL monovettes at baseline, 0.5, 1, 1.5, 2 h, upon lunch request and at 30 and 60 min

after lunch (see section 3.14 for details on acylated ghrelin sample processing). Additional 9 mL samples were collected at these time points into pre-chilled EDTA monovettes to determine plasma glucose and triacylglycerol concentrations. These monovettes were spun at 1,681 *g* for 10 min in a refrigerated centrifuge at 4°C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage for analysis later. All blood samples were collected in the semi-supine position except for the 1.5 h collection during the exercise trial where participants straddled the treadmill. Patency of the cannula was maintained during trials by flushing with non-heparinised saline (0.9% w/v sodium chloride) after sample collection. To avoid dilution of samples 2 mL of residual saline was discarded prior to each collection.

To estimate changes in plasma volume, at each blood sampling point duplicate 20 μL blood samples were collected into micropipettes and triplicate 20 μL samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations.

8.2.7 Biochemical analysis

An enzyme immunoassay was used to determine plasma concentrations of plasma acylated ghrelin with the aid of a plate reader. Plasma glucose and triacylglycerol concentrations were determined spectrophotometrically using a bench top analyser. 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: acylated ghrelin: 9.9%, glucose: 0.42% and triacylglycerol: 1.63%.

8.2.8 Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows. Area under the concentration verses time curve calculations were performed using the trapezoidal method. Repeated measures, two-factor ANOVA was used to examine differences between the exercise and control trials over time for appetite perceptions, energy and macronutrient intake, acylated ghrelin, glucose and triacylglycerol. Student's *t*-tests were used to assess differences between fasting and AUC values for acylated ghrelin, glucose, triacylglycerol, temperature, humidity and appetite perceptions between the exercise and control trials. The Pearson product moment correlation coefficient was used to examine relationships between

variables. For analysis of the energy/macronutrient intake, data was grouped as that consumed at the freely requested lunch and then as subsequent intake throughout the remainder of each trial. Significant decreases in plasma volume were apparent at 1.5 and 2 h in the exercise trial therefore plasma acylated ghrelin, glucose and triacylglycerol concentrations were corrected accordingly at these points. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

8.3 Results

8.3.1 Exercise responses

Participants completed the 60 min run at 10.6 ± 0.3 $km.h^{-1}$. This elicited a mean oxygen consumption equivalent to 71.8 ± 1.3% of $\dot{V}O_2$ max and generated an average heart rate and net (exercise minus resting) energy expenditure of 165 ± 3 $beats{\cdot}min^{-1}$ and 4117 ± 117 kJ (984 ± 28 kcal), respectively. A mean non-protein respiratory quotient of 0.92 ± 0.01 reflected the proportional contributions of carbohydrate and fat (74 ± 2% and 26 ± 2%) to energy provision. A median RPE value of 13 indicated that the participants perceived the intensity of the run to be 'fairly hard.'

8.3.2 Appetite responses

There were no significant differences in baseline ratings of appetite (hunger, fullness, satisfaction and prospective food consumption) between the exercise and control trials (Table 8.2).

Table 8.2: Baseline appetite perceptions in the exercise and control trials

	Control	Exercise	*P*
Hunger (0-100)	59 ± 9	52 ± 9	0.198
Satisfaction (0-100)	24 ± 5	25 ± 6	0.802
Fullness (0-100)	22 ± 8	25 ± 7	0.793
PFC (0-100)	66 ± 8	65 ± 7	0.814

(n = 10). PFC = prospective food consumption

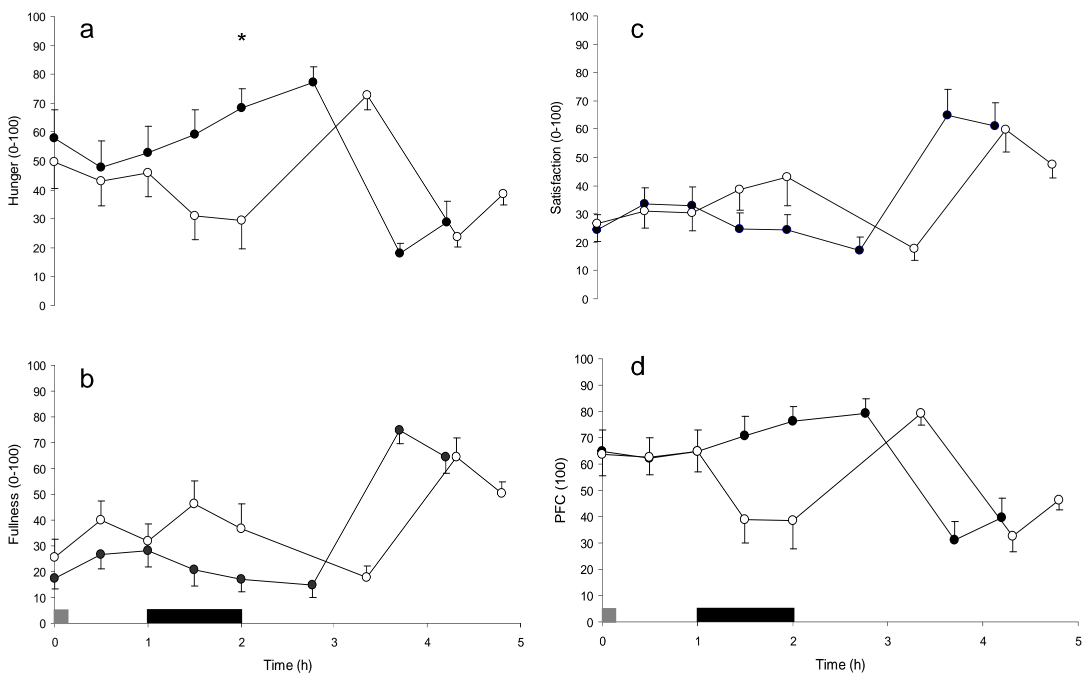


Figure 8.1: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the exercise (○) and control (●) trials. Values are mean ± SEM *(n* = 10). Grey rectangle indicates a breakfast snack, black rectangle indicates exercise. Samples after exercise (2 h) represent pre-lunch, 30 and 60 min post-lunch. N.B: samples upon lunch request were collected at different times between trials (exercise 3.35 ± 0.22 h, control 2.77 ± 0.27 h). *Significantly different from control after correcting for multiple comparisons using the Bonferroni method.

For subjective ratings of hunger two-factor ANOVA revealed a significant main effect of trial (P = 0.024), time (P = 0.002) and a significant interaction effect (trial x time, P =0.001). Post-hoc analysis identified between trial differences at 1.5 and 2 h however after correcting for multiple comparisons the only difference to remain was at 2 h. A significant main effect of time (P < 0.001 for each) and a significant interaction effect (trial x time, P < 0.003 for each) were apparent for ratings of fullness and prospective food consumption. Post hoc analysis revealed significant differences between trials at 1.5 h for ratings of fullness and at 1.5 and 2 h for prospective food consumption (P < 0.014 for each) however these differences did not remain significant after correcting for multiple comparisons using the Bonferroni method. For subjective ratings of satisfaction two-factor ANOVA revealed a significant main effects of time (P = 0.001) yet no trial (P = 0.663) or interaction effects (P = 0.106) were apparent (Figure 8.1).

8.3.3 Energy and macronutrient intake

There was a significant difference in the timing of the requested lunch between the control and exercise trials (P = 0.009). In the exercise trial participants requested to eat 81 ± 14 min after exercise completion. This was a 35 ± 10 min delay in the spontaneous request of lunch compared with control. Therefore, the lunch request in the exercise trial was at 3.35 h (3 h 21 min) and in the control trial at 2.77 h (2 h 46 min).

Two-factor ANOVA revealed a significant main effect of time (P < 0.001), indicating that energy intake was significantly higher at the freely requested lunch than that consumed over the remainder of trials. No significant trial (P = 0.993) or interaction (trial x time, P = 0.138) main effects were found, thus energy intake was not significantly different between the exercise and control trials (Table 8.3).

Table 8.3: Energy intake in the exercise and control trials

	Control	**Exercise**
Requested Lunch	4778 ± 464	5385 ± 537
	(1142 ± 111)	(1287 ± 128)
Subsequent intake	2648 ± 761	2033 ± 540
	(633 ± 182)	(486 ± 129)
Total Trial	7426 ± 1004	7418 ± 905
	(1775 ± 240)	(1773 ± 684)

Values are kJ and (kcal) (n = 10)

Two-factor ANOVA was used to examine macronutrient intake (absolute and percent) across the buffet meals during the exercise and control trials (Table 8.4). For the absolute intake (grams) there were no significant trial or interaction main effects (all P > 0.05), indicating that there were no between trial differences in macronutrient consumption within the exercise and control trials. A significant main effect of time were found for fat, protein and carbohydrate, (all P < 0.003), indicating that more grams of these macronutrients were consumed at the freely requested lunch than the subsequent intake over the remainder of each trial.

For the percentage intake of fat and carbohydrate no significant main effects were found (all $P > 0.05$). For protein, two-factor ANOVA revealed a significant main effect of time ($P = 0.004$) however no trial ($P = 0.320$) or interaction ($P = 0.164$) effects were found. Thus, the percentage intake of protein was higher at the freely requested lunch than over the remainder of each trial.

Table 8.4: Macronutrient intake in the exercise and control trials

Control Trial	**Fat**	**Carbohydrate**	**Protein**
Requested Lunch	51 ± 6 (39.8)	125 ± 15 (43.7)	48 ± 5 (16.5)
Subsequent intake	28 ± 9 (39.9)	79 ± 22 (49.9)	16 ± 7 (10.2)
Total trial	79 ± 11 (40.0)	204 ± 32 (45.7)	64 ± 8 (14.3)
Exercise	**Fat**	**Carbohydrate**	**Protein**
Requested Lunch	60 ± 7 (41.9)	134 ± 14 (41.6)	53 ± 5 (16.5)
Subsequent intake	20 ± 6 (37.0)	60 ± 16 (49.2)	17 ± 6 (13.8)
Total Trial	80 ± 11 (40.4)	194 ± 26 (43.6)	70 ± 9 (16.0)

Values are gram and (%) ($n = 10$)

No significant correlations were found between any of the appetite measurements at the point of lunch request with subsequent energy intake at the *ad libitum* buffet lunch. In the exercise trial a positive association was found between energy intake at the *ad libitum* lunch and the percentage change in hunger 30 min after the meal ($r = 0.661$, $P = 0.037$).

8.3.4 Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ ($P = 0.755$) between the exercise and control trials (98 ± 16 verses 102 ± 13 pg mL $^{-1}$). For circulating concentrations of acylated ghrelin two-factor ANOVA revealed a significant main effect of time ($P < 0.001$) and main effect of trial approached significance ($P = 0.056$)

(Figure 8.2). Post hoc analysis showed that plasma acylated ghrelin concentrations were significantly lower on the exercise trial than the control trial at 1.5 and 2 h ($P < 0.05$ for each) however after correcting for multiple comparisons these difference did not remain significant. Analysis of the acylated ghrelin AUC during exercise (1-2 h) demonstrated suppressed plasma acylated ghrelin concentrations as compared with control (exercise 53 ± 18, control 85 ± 8 pg.mL· 1h) ($P = 0.014$).

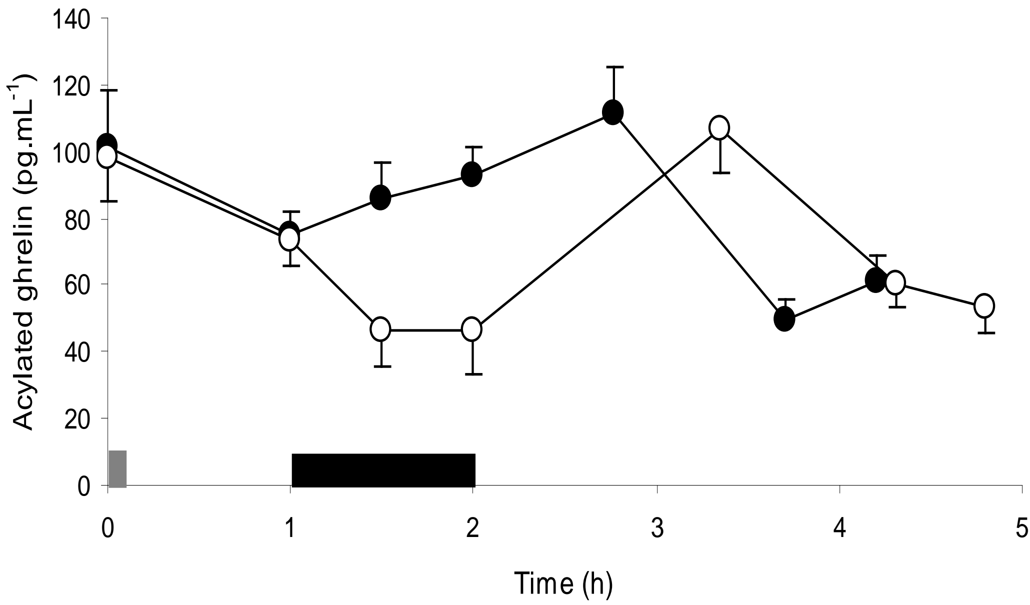


Figure 8.2: Plasma concentrations of acylated ghrelin in the control (●) and exercise (○) trials. Values are mean ± SEM ($n = 10$). Grey rectangle indicate a breakfast snack, black rectangle indicates exercise. Samples after exercise (2 h) represent pre-lunch, 30 and 60 min post-lunch. N.B: samples at lunch request were collected at different times between trials (exercise 3.35 ± 0.22 h, control 2.77 ± 0.27 h).

From baseline concentrations of plasma acylated ghrelin decreased ~25% in both the exercise and control trials one hour after consuming the breakfast snack. Thereafter, in the control trial circulating acylated ghrelin concentrations steadily increased over the next two hours prior to the spontaneous lunch request. At this point acylated ghrelin concentrations were 10% higher than fasting values. In the exercise trial plasma acylated ghrelin was suppressed during exercise with values approximating 47% of fasting at the end of exercise. At the spontaneous meal request acylated ghrelin values were 9% higher than those observed during fasting. At the time of the lunch request there was no significant difference between trials ($P = 0.697$) in circulating acylated ghrelin concentrations (exercise 106 ± 14, control 111 ± 13 pg.mL^{-1}). In both trials

consumption of the *ad libitum* meal led to a rapid decline in circulating acylated ghrelin 30 min after the meal and values remained suppressed one hour after consumption.

8.3.5 Glucose and triacylglycerol

Figure 8.3 shows the plasma glucose and triacylglycerol responses during the exercise and control trials. For plasma glucose two-factor ANOVA revealed a significant main effect of trial (P = 0.004) and a main effect of time (P = 0.001) indicating higher glucose concentrations on the exercise trial. For plasma triacylglycerol two factor ANOVA revealed a significant main effect of time ($P < 0.001$) however no trial (P = 0.180) or interaction (trial x time, P = 0.240) main effects were found.

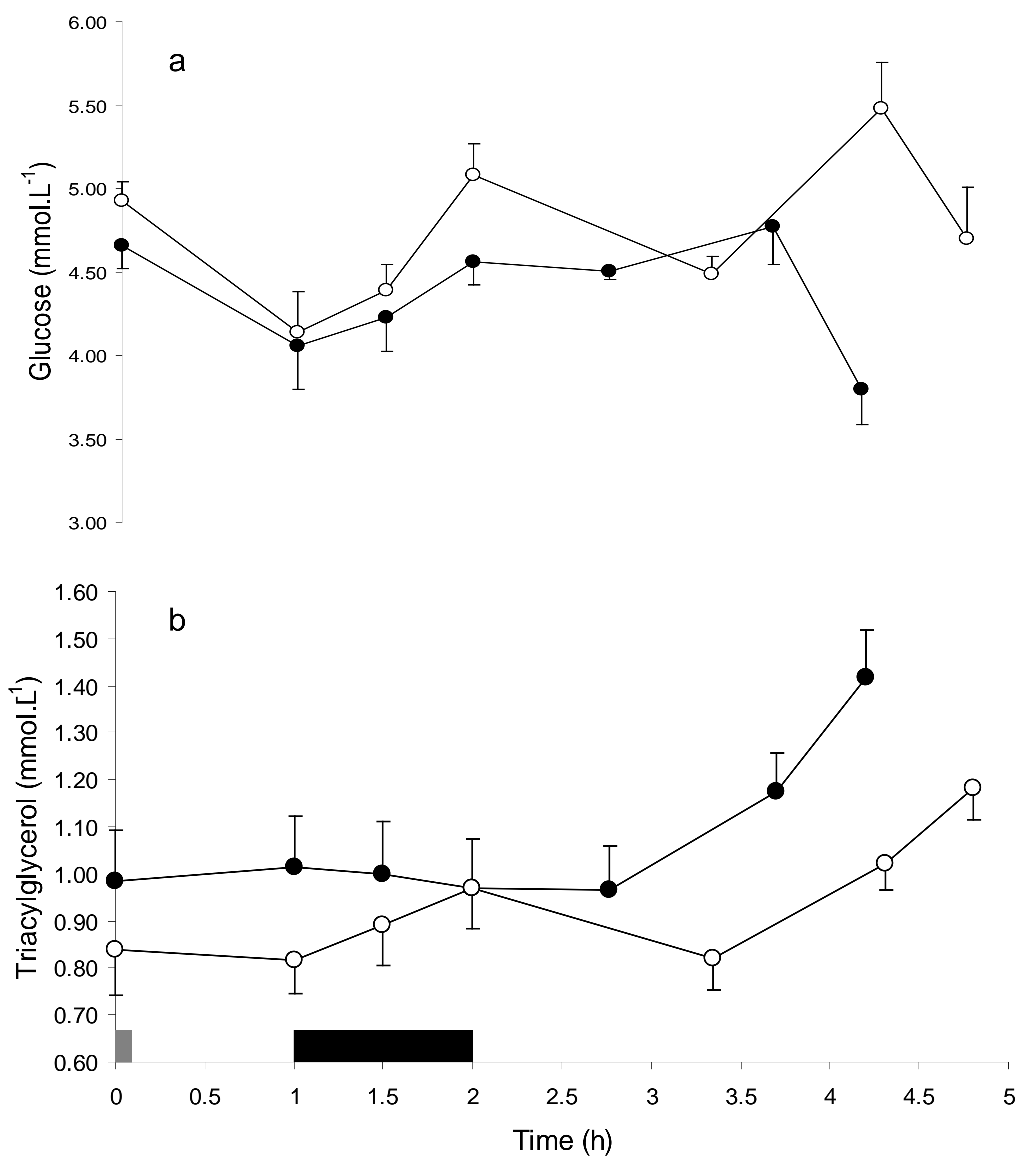


Figure 8.3: Plasma glucose (a) and triacylglycerol (b) responses in the exercise (○) and control (●) trials. Grey rectangle indicate a breakfast snack, black rectangle indicates exercise. Samples after exercise (2 h) represent pre-lunch, 30 and 60 min post-lunch. N.B: samples at lunch request were collected at different times between trials (exercise 3.35 ± 0.22 h, control 2.77 ± 0.27 h).

8.3.6 Correlations between acylated ghrelin and other variables

Fasting plasma concentrations of acylated ghrelin were inversely correlated with body fat percentage (r = - 0.693, P = 0.025). During the exercise trial acylated ghrelin was inversely correlated with plasma triacylglycerol concentrations at 1.5 h (r = -0.737, P = 0.015), 2 h (r = - 0.755, P = 0.012) and 60 min after consumption of the voluntarily requested lunch (r = - 0.611, P = 0.037). At this latter point acylated ghrelin was

positively associated with ratings of hunger (*r* = 0.699, *P* = 0.025) and prospective food consumption (*r* = 0.588, *P* = 0.074). During the control trial acylated ghrelin was positively associated with hunger (*r* = 0.785, *P* = 0.007) and prospective food consumption (*r* = 0.587, *P* = 0.074) 60 min after the voluntarily requested lunch and inversely related at this point with ratings of satisfaction (*r* = - 0.646, *P* = 0.043). Thirty min after the voluntarily requested lunch acylated ghrelin was inversely related with plasma glucose concentrations (*r* = - 0.680, *P* = 0.030). Acylated ghrelin and triacylglycerol AUC were inversely related during exercise (*r* = - 0.745, *P* = 0.013).

In the exercise trial there was a positive association which approached significance between acylated ghrelin at the point of voluntary lunch request and subsequent energy intake at the buffet lunch (*r* = 0.558, *P* = 0.094). No such relationship was found in the control trial. In the control trial energy intake was significantly related with the percentage change in acylated ghrelin 30 min after lunch (*r* = 0.853, *P* = 0.002). In the exercise trial the percentage change in acylated ghrelin and the percentage change in hunger 30 min after lunch were significantly related (*r* = 0.774, *P*= 0.009). Sixty min after lunch in the control trial there was a positive relationship between the percentage change in acylated ghrelin and the percentage change in hunger (*r* = 0.669, *P* = 0.034). In the exercise trial there was a positive relationship between percentage changes in acylated ghrelin and prospective food consumption 60 min after lunch (*r* = 0.664, *P* = 0.036).

8.3.7 Water intake, temperature and humidity

Water intake was significantly higher on the exercise trial than the control trial (exercise 1627 ± 210, control 1005 ± 202, *P* = 0.042). There were no significant differences in laboratory temperature (exercise 21.2 ± 0.3, control 20.8 ± 0.1 ^{o}C, *P* = 0.078) or relative humidity (exercise 30.4 ± 2.2, control 31.4 ± 2.8%, *P* = 0.614) between the exercise and control trials.

Discussion

The main findings arising from this investigation are that a high intensity bout of treadmill running delayed the decision to eat after exercise. Moreover, when participants voluntarily requested a meal after completing exercise, circulating acylated ghrelin concentrations were 10% higher than fasting values and this was no different from values exhibited on the control trial. This investigation has also shown no difference in post-exercise energy or macronutrient intake when participants were given complete free access to food.

Previous research has shown the existence of a short delay to the onset of feeding after an acute bout of high intensity exercise (King et al, 1994; 1996; King and Blundell, 1995). King et al (1994) found that participants requested an *ad libitum* lunch approximately five min later after completing a high intensity bout of cycling as compared with responses on a control trial. This brief resistance to commence feeding after exercise has subsequently been confirmed by the same research group with running as an exercise stimulus (King and Blundell, 1995) and in female participants (King et al, 1996). The results from the present investigation confirm these findings although a greater delay to the onset of feeding was apparent. In the present study participants chose to eat approximately 35 min later after exercise as compared with control. It is not entirely clear why a greater delay was found in the present study as the exercise duration and relative intensity completed were similar to that performed in the previous studies. It is possible that the greater absolute intensity of exercise completed by the comparatively fitter subjects in this investigation may have been influential due to the greater absolute level of stress imposed.

The mechanisms responsible for inducing a delay to feeding after exercise remain unknown. Ghrelin is a hormone that stimulates appetite and the initiation of meals (Cummings et al, 2001; Wren et al, 2001). Across the day circulating concentrations of ghrelin rise prior to meals and decline postprandially, a sequence that relates closely to subjective appetite perceptions (Cummings et al, 2004). It is known that high intensity exercise suppresses acylated ghrelin and appetite (Broom et al, 2007; 2009; Marzullo et al, 2008) therefore it was thought that circulating concentrations of acylated ghrelin may be important in determining the latency to feeding after an acute bout of exercise. In this study exercise suppressed acylated ghrelin to approximately 50% of fasting

levels yet at the point of spontaneous meal request values had returned to those exhibited in the control trial. At this point circulating acylated ghrelin concentrations were approximately 10% higher than fasting values within both the control and exercise trials. These findings provide preliminary evidence indicating a possible role of acylated ghrelin in mediating the spontaneous decision to eat after exercise. This idea is supported by research which has shown increases in circulating ghrelin prior to both scheduled (Cummings et al, 2001) and freely requested meals (Cummings et al, 2004; Blom et al, 2009). Specifically, Blom et al (2009) reported mean circulating ghrelin values 5% higher than fasting immediately prior to a voluntarily requested meal and this outcome is consistent with the values reported here.

A limitation of this study is that singular assessment of acylated ghrelin before lunch constrains the ability to determine whether changes in acylated ghrelin were directly influential in determining the decision to request lunch after exercise. A greater sampling frequency in the time after exercise until the voluntary requested meal would have been needed to examine this more closely. Nonetheless, the present findings can be judged in reference to fasting acylated ghrelin concentrations and those reported at the voluntary requested meal on the control trial. Specifically, a threshold level in relation to fasting has been proposed as important in determining the decision to initiate feeding rather than a preprandial rise in ghrelin levels *per se* (Cummings et al, 2004; Blom et al, 2009). Moreover, when participants voluntarily requested lunch acylated ghrelin values were very similar between the exercise and control trials despite dissimilar sampling times. Collectively, these findings offer preliminary evidence suggesting that changes in acylated ghrelin may influence the decision to eat after performing exercise.

A second aim of this investigation was to examine the influence of exercise on energy and macronutrient intake. The majority of previous research which has assessed the influence of exercise on food intake has provided meals to participants on a predetermined schedule (Thompson et al, 1988; Kissileff et al, 1990; Verger et al, 1994; Imbeault et al, 1997; Westerterp-Plantenga et al, 1997; Hubert et al, 1998; George and Morganstein, 2003; Tsofliou et al, 2003; Pomerleau et al, 2004; Martins et al, 2007). This protocol places a restriction on participants' eating behaviour and it is possible that this may have influenced the research findings in past studies. The present investigation

sought to examine the influence of exercise on food intake when participants are given complete free access to food for an extended period of time after exercise. In this situation participants are able to consume food whenever desired without a time limit on each eating episode or a restriction on the number of eating episodes across trials. Despite the difference in protocol the results from this investigation support those of previous studies which have shown no change in energy or macronutrient intake in response to an acute bout of exercise (Blundell and King, 2000; Martins et al, 2008). At the voluntarily requested meal participants consumed 611 kJ (146 kcal) more on the exercise trial than the control trial however during the remainder of trials participants compensated for this and the total trial energy intake was within 6 kJ (1.4 kcal) on the exercise and control trials. Furthermore, no difference in the percentage of energy derived from the macronutrients was found. These outcomes therefore confirm the findings from previous studies which have shown no difference in energy/macronutrient intake after single bouts of exercise.

In this investigation a number of significant associations were found. Of particular interest, in the control trial energy intake at the voluntarily requested lunch was significantly related to the percentage change in acylated ghrelin 30 minutes after the meal. This finding confirms previous data which has shown associations between the energy content of meals and postprandial changes in circulating ghrelin (Callahan et al, 2004; Le Roux et al, 2005; Leidy and Williams, 2006). Moreover, in the exercise trial a positive association between premeal acylated ghrelin values and subsequent *ad libitum* energy intake approached significance. These outcomes supports the role of acylated ghrelin as a regulator of acute energy homeostasis.

This study has two notable limitations. Firstly, the participants were young healthy males therefore these findings may not generalise to other populations including females, overweight individuals and older adults. Secondly, singular assessment of acylated ghrelin immediately prior to the voluntarily requested lunch limits the ability to precisely determine the exact importance of acylated ghrelin in regards to post-exercise feeding latency. Further work is needed with more frequent blood sampling in order to shed more light on this issue.

In conclusion, this investigation has shown that a high intensity bout of running induces a resistance to commence eating after exercise and provides preliminary evidence suggesting a role of acylated ghrelin in determining this feeding latency. Findings have also confirmed that a high intensity bout of exercise does not affect energy intake in the hours after even when participants are provided with complete *ad libitum* access to food.

Chapter IX

Differential acylated ghrelin, peptide $YY_{3\text{-}36}$, appetite and food intake responses to equivalent energy deficits induced by exercise and food restriction

9.1 Introduction

The findings reported in studies four and five within this thesis (Chapters seven and eight) suggest that acylated ghrelin, appetite and energy intake are not stimulated by acute energy deficits induced through exercise. This lack of response is exhibited even when the deficits in energy are severe and when participants are permitted to feed *ad libitum* across trial days. Contrary to this, missing a meal or consuming a meal of reduced energy content leads to an increase in hunger and subsequent energy intake at the next eating opportunity (Lawton et al, 1993; Green et al, 1994; Hubert et al, 1998). Thus, it appears that two methods of inducing a short-term energy deficit have markedly different effects on appetite and food intake i.e. a different response is elicited when there is a restriction on energy entering the body (mouth and gastrointestinal tract) as compared with the situation where there is an increase in energy leaving the body (through muscular work). The associated mechanisms mediating these disparate appetite and food intake responses have not been fully determined yet it is possible that circulating gut hormones may be implicated (Borer et al, 2005).

The studies reported within this thesis have included measurements of plasma acylated ghrelin. Besides the transient suppression that occurs during exercise, these studies have shown that acylated ghrelin is not influenced by exercise in the hours after. Conversely, it has been shown that ghrelin is sensitive to acute perturbations in energy intake resulting from dietary manipulation (Callahan et al, 2004; Leidy and Williams, 2006). It is therefore possible that disparate acylated ghrelin responses may contribute to the paradoxical differences in the reaction of appetite and food intake to energy deficits induced through diet as compared with exercise. A direct comparison of acylated ghrelin responses to equivalent energy deficits induced by diet and exercise would be needed to test this hypothesis.

Many gut peptides are implicated in the acute regulation of energy homeostasis (Murphy and Bloom, 2006). At present, ghrelin is the only known circulating gut peptide which stimulates appetite and food intake. Conversely, several circulating gut

peptides exist which suppress appetite and food intake. Peptide YY is an appetite inhibiting peptide with a defined role in the acute regulation of energy homeostasis (Neary and Batterham, 2009b). Circulating concentrations of PYY increase in direct proportion to ingested nutrients and heightened levels inhibit appetite and feeding (Batterham et al, 2002; 2003). Peptide YY is therefore an important mediator of within meal satiation, and perhaps even more so, intermeal satiety. Two forms of PYY exist within the circulation, namely PYY_{1-36} and PYY_{3-36}. The latter is a truncated 34 amino acid peptide produced by cleavage of the N-terminal tyrosine and proline amino acid residues from PYY_{1-36} and is the major circulating form (Batterham et al, 2006).

The influence of exercise on PYY has been examined in a handful of studies with data suggesting that single bouts of aerobic exercise stimulate an increase in circulating PYY (Martins et al, 2007; Broom et al, 2009; Ueda et al, 2009). Unfortunately in these investigations measurements of circulating total PYY were made, that is the assays used have not been able to distinguish PYY_{1-36} and PYY_{3-36}. The inhibitory effect of PYY on appetite and food intake is thought to be specific to PYY_{3-36} therefore measurements taken in previous studies may have suffered from a lack of sensitivity. To date, only one study has examined the PYY_{3-36} response to exercise with findings suggesting that PYY_{3-36} does not respond to exercise *per se*, yet exercise may potentiate the PYY_{3-36} response to feeding (Cheng et al, 2009). Further work is required to confirm and extend these preliminary findings.

The present study had two primary objectives. The first aim was to compare gut hormone (acylated ghrelin and PYY_{3-36}), appetite and energy intake responses to equivalent energy deficits induced through acute dietary restriction as compared with exercise. The second aim was to examine PYY_{3-36} responses to exercise and feeding in order to extend knowledge regarding how exercise influences circulating levels of $PYY_{3-36.}$

9.2 Methods

9.2.1 Participants

After gaining Loughborough University Ethical Advisory Committee approval 12 healthy male volunteers (20 – 30 y) gave their written informed consent to participate. Table 1 describes the characteristics of the study participants.

Table 9.1: Characteristics of the study participants

Characteristic	Mean ± SEM
Age (y)	23.4 ± 1.0
BMI ($kg \cdot m^{-2}$)	22.8 ± 0.4
Body Mass (kg)	71.6 ± 1.7
Body Fat (%)	15.4 ± 0.7
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	57.3 ± 1.2

(n = 12)

9.2.2 Study design

Before taking part in main trials participants visited the laboratory twice in order to familiarise themselves with the environment and to enable the collection of the necessary anthropometric and preliminary exercise test data. On their first laboratory visit participants were made aware of the protocol before giving their written informed consent to participate. After this, participants were health screened and anthropometric measurements were taken. Participants then completed two preliminary exercise tests: 1) a submaximal-incremental treadmill running test, 2) a maximum oxygen uptake ($\dot{V}O_2$ max) treadmill running test. There was a 15 to 20 min interval between tests.

On the second laboratory visit participants ran on treadmill for 90 min at a speed predicted to elicit 70% of their maximum oxygen uptake. During the run expired air samples were collected at 15 min intervals to determine energy expenditure and substrate oxidation. This session served two purposes. Firstly, it confirmed participants could complete the 90 min run which would later be undertaken during main trials. Secondly, it permitted an accurate estimation of energy expenditure from the run which

was necessary in order to calculate the amount of food to be provided at the test meals during main trials (section 9.2.5).

9.2.3 Main trials

In subsequent weeks participants completed three main trials in a randomised-counterbalanced fashion with each trial being separated by at least one-week:

1. control trial – participants rested throughout the trial and were provided with sufficient food across the day to meet their estimated individual energy requirements.
2. exercise-induced energy deficit (Ex-Def) – at the start of the trial participants completed a 90 min run (at 70% of $\dot{V}O_2$ max). Throughout the remainder of the trial sufficient food was provided across the day to meet participants' daily energy requirements (same amount as provided in the control trial).
3. food-restriction induced energy deficit (Food-Def) – participants remained sedentary during the trial but were provided with a restricted amount of food across the day in order to evoke an energy deficit through dietary means equivalent to the deficit invoked through exercise in the Ex-Def trial.

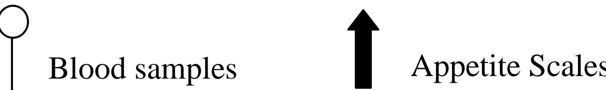
Each main trial began at 08:00 and lasted nine hours. To standardise diet and physical activity before main trials participants completed a weighed food record of all items consumed within the 24 h preceding their first main trial and this feeding pattern was replicated prior to subsequent trials. Alcohol, caffeine and structured physical activity were not permitted during this period. To minimise physical exertion on the morning of main trials participants were asked to walk slowly to the research laboratory if they lived within 0.5 km. Participants living further away arrived by motorised transport. Participants arrived at the laboratory in the fasted state having consumed only water since 23:00 on the prior evening.

Figure 9.1 provides a schematic representation of the main trial protocol. On the control trial participants rested for the entire duration (sitting reading, writing, working at a computer). At two points (2 and 4.75 h) participants were provided with test meals which were of sufficient energy content for each participant's individually estimated

energy requirements. At 8 h a buffet meal was offered to participants from which they were free to consume food *ad libitum*.

The Ex-Def trial commenced when participants began a 90 min run on a level treadmill. The speed of the treadmill was identical to that completed during preliminary testing and was set to elicit 70% of maximum oxygen uptake. Expired air samples were collected at 15 min intervals throughout the run to monitor the intensity and adjustments were made to the speed of the treadmill if necessary. After the run participants rested within the laboratory for 7.5 h. At 2 h and 4.75 h participants consumed test meals which were identical to those provided in the control trial i.e. were of sufficient energy content for each participant's individual energy needs. At 8 h a buffet meal was offered to participants from which they were free to consume food *ad libitum*

On the Food-Def trial participants remained sedentary throughout. Test meals were provided at 2 h and 4.75 h however the amount provided was restricted so that an energy deficit was induced relative to control. The energy deficit was identical to that elicited by exercise in the Ex-Def trial. This permitted a comparison of responses to identical energy deficits induced through diet as compared with exercise. At 8 h a buffet meal was offered to participants from which they were free to consume food *ad libitum*.



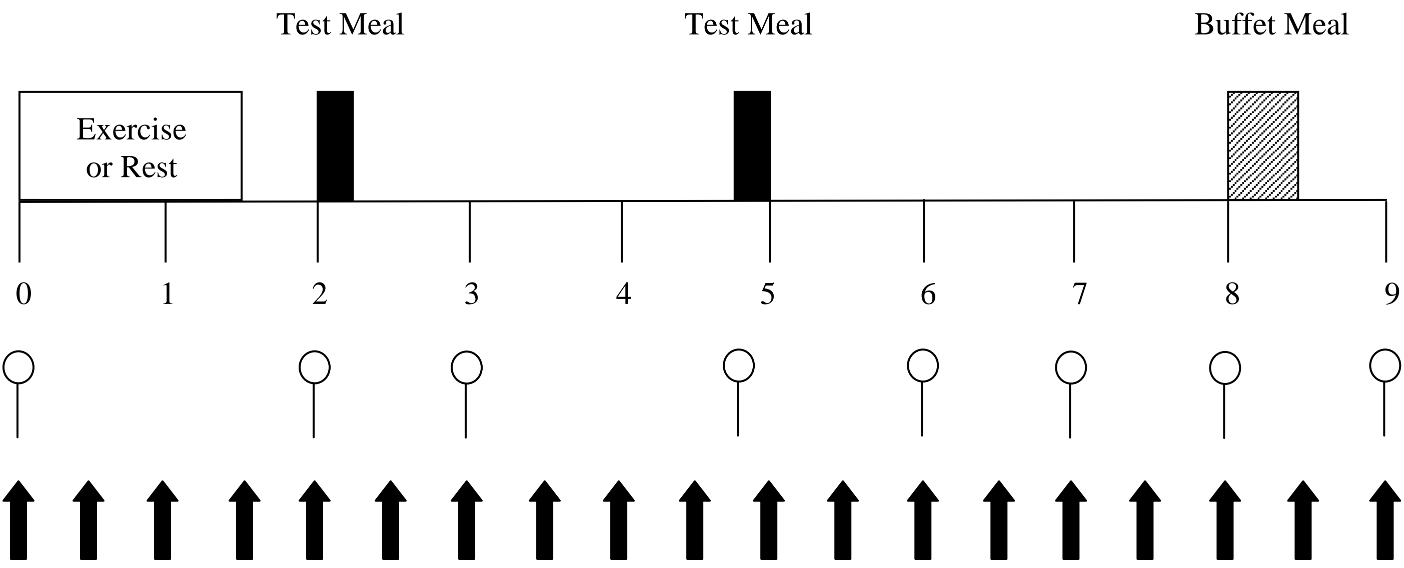


Figure 9.1: Schematic representation of the main trial protocol

9.2.4 Appetite assessment

During main trials 100 mm visual analogue scales were completed to assess perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption). Scales were completed at baseline and then at 30 min intervals throughout.

9.2.5 Test meals

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

The energy content of the test meals were identical in the control and Ex-Def trials and was calculated to be sufficient to meet each participant's individual energy requirements. To calculate the amount to be provided for each participant resting daily energy requirements were estimated using validated predictive equations (Mifflin et al, 1990). This amount was then multiplied by a physical activity level of 1.4 (an amount deemed sufficient to meet the energy needs of individuals across a resting day). Participants received 70% of this amount divided equally across two identical test meals. The rationale for providing this amount was based on pilot work which showed that provision of this amount during a resting day was sufficient to induce a comfortable level of satiation.

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

9.2.6 Ad libitum buffet meals

Eight hours into main trials participants were given access to a buffet meal from which they were free to consume food *ad libitum*. The buffet meal was identical to that described in the previous studies in this thesis (Appendix G). Participants were given 30 min to select and consume food items from the buffet. At each meal food was presented in excess of expected consumption. Participants were told to eat until satisfied and that additional food was available if desired. Meals were consumed in isolation so that social influence did not affect food selection. Food consumption was ascertained by examining the weighted difference in food items remaining compared to that initially presented. The energy and macronutrient content of the items consumed was ascertained using manufacturer values.

9.2.7 Blood sampling

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

Blood samples were collected into 4.9 mL EDTA monovettes at baseline, 2, 3, 4.75, 6, 7, 8 and 9 h to measure circulating concentrations of acylated ghrelin (see section 3.14 for details on acylated ghrelin sample processing) and into 9 mL EDTA monovettes to measure circulating glucose and triacylglycerol concentrations. Additional 2 mL samples were also collected at these times to measure circulating concentrations of PYY_{3-36}. To maintain the integrity of the PYY_{3-36} samples, blood was collected into pre-chilled syringes containing dipeptidyl-peptidase-4 inhibitor (10 $\mu L.mL^{-1}$). After mixing by gentle inversion samples were then dispensed into pre-chilled EDTA tubes containing aprotinin at a final concentration of 500 $KIU.mL^{-1}$. These samples were spun at 1681*g* for 10 min in a refrigerated centrifuge at 4 ^{o}C. The plasma supernatant was then aliquoted into 2 mL Eppendorf tubes prior to storage at -80 ^{o}C.

All blood samples were collected in the semi-supine position. For samples collected using a cannula patency was maintained by flushing with non-heparinised saline (0.9 % w/v sodium chloride). To avoid subsequent sample dilution residual saline was discarded using a 2 mL syringe before sample collection. To estimate changes in

plasma volume, at each blood sampling point duplicate 20 μL blood samples were collected into micropipettes and triplicate 20 μL blood samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentrations, respectively.

9.2.8 Biochemical analysis

An enzyme immunoassay was used to determine plasma concentrations of acylated ghrelin. Plasma concentrations of PYY_{3-36} were determined using a radio-immunoassay kit. Plasma glucose and triacylglycerol concentrations were determined spectrophotometrically using an automated bench top analyzer. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 7.8%, PYY_{3-36} 8.7%, glucose 0.59%, triacylglycerol 2.7%.

9.2.9 Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows. All area under the concentration verses time curve calculations were performed using the trapezoidal method. One-way repeated measures ANOVA was used to assess differences between trials in fasting and AUC values for acylated ghrelin, PYY_{3-36}, glucose, triacylglycerol, appetite perceptions and buffet meal energy and macronutrient intake. Repeated measures, two-factor ANOVA was used to examine differences between trials over time for appetite perceptions, acylated ghrelin, PYY_{3-36}, glucose and triacylglycerol. Where significant main effects were found post-hoc analysis was performed using the Bonferroni correction for multiple comparisons. The Pearson product moment correlation coefficient was used to examine relationships between variables. Correction of values for changes in plasma volume did not alter the statistical significance of findings therefore for simplicity the unadjusted values are presented. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

9.3 Results

9.3.1 Exercise responses

Participants completed the 90 min run at 9.6 ± 0.2 $km \cdot h^{-1}$. This elicited a mean oxygen consumption equivalent to 69.8 ± 0.9% of maximum oxygen uptake and generated a mean heart rate and net (exercise minus resting) energy expenditure of 173 ± 3 $beats \cdot min^{-1}$ and 4715 ± 113 kJ (1127 ± 27 kcal), respectively. A mean non-protein respiratory quotient of 0.92 ± 0.01 reflected the proportional contributions of carbohydrate and fat (72 ± 3% and 28 ± 3%) to energy provision. A mean RPE value of 14 ± 1 indicated that the participants perceived the intensity of the run to be 'somewhat hard.' The mean difference between estimated energy expenditure during the familiarisation session and the run in the Ex-Def main trial was 46 kJ (11 kcal).

9.3.2 Appetite responses

Fasting appetite ratings (hunger, fullness, satisfaction and prospective food consumption) did not differ significantly between the control, Ex-Def and Food-Def trials (Table 9.2).

Table 9.2: Baseline appetite perceptions in the control, Ex-Def and Food-Def trials

	Control	Ex-Def	Food-Def	*P*
Hunger (0-100)	61 ± 6	59 ± 5	52 ± 6	0.490
Satisfaction (0-100)	31 ± 6	27 ± 4	29 ± 6	0.796
Fullness (0-100)	26 ± 7	22 ± 4	25 ± 6	0.769
PFC (0-100)	64 ± 5	66 ± 4	58 ± 4	0.351

Values are mean ± SEM (n = 12). PFC = prospective food consumption

For each appetite perception examined (hunger, fullness, satisfaction and prospective food consumption) two-factor ANOVA revealed significant trial, time and interaction (trial x time) main effects (all P <0.001) indicating that appetite responses differed over time between the main experimental trials (Figure 9.2). For each appetite marker

examined, post hoc analysis revealed trial differences between the Food-Def and control trial (P <0.001) and the Food-Def and Ex-Def trial (P <0.001) demonstrating significantly higher ratings of hunger and prospective food consumption and significantly reduced ratings of satisfaction and fullness in the Food-Def trial. At individual time points post-hoc analysis identified differences between the Food-Def and control trial (all P < 0.004) and the Food-Def and Ex-Def trial (all P < 0.006) at 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 h.

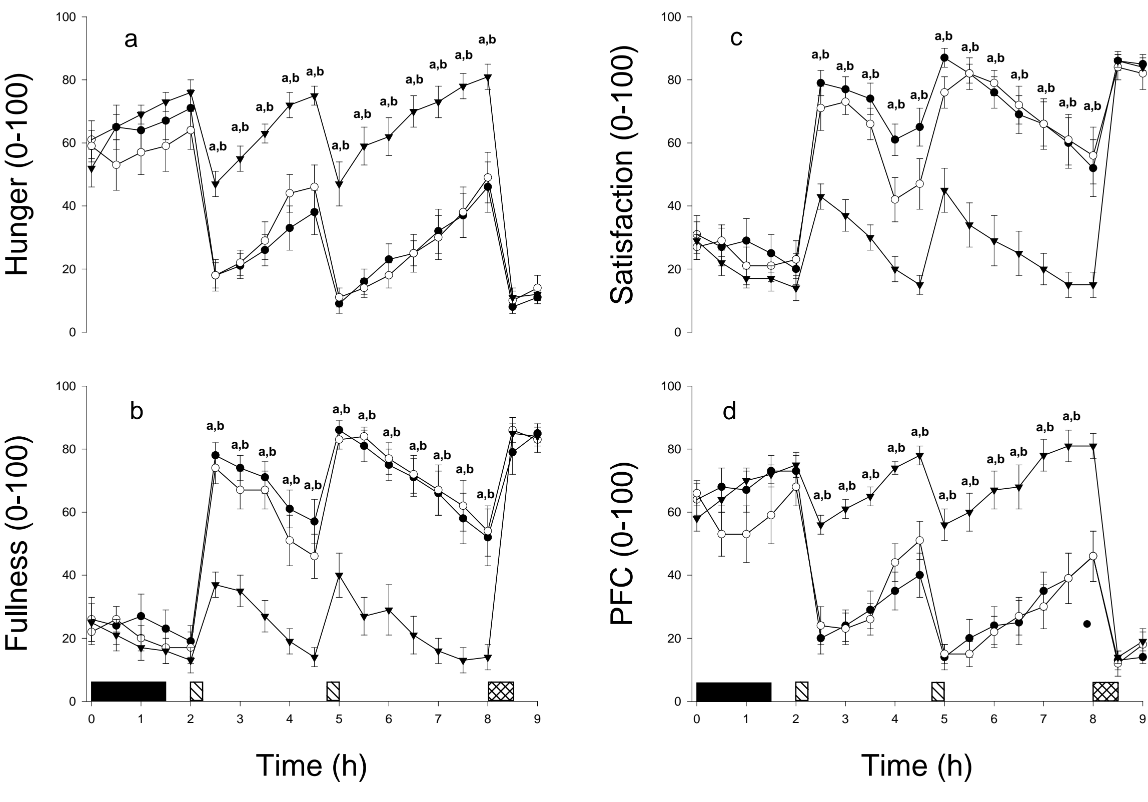


Figure 9.2: Ratings of hunger (a), fullness (b), satisfaction (c) and prospective food consumption (d) in the control (●), Ex -Def (○) and Food-Def (▼) trials. Values are mean ± SEM (n = 12). Black rectangle indicates exercise, diagonal rectangles indicate test meals, hatched rectangle represents the buffet meal. [a]Food-Def different from control P < 0.05; [b]Food-Def different from Ex-Def P < 0.05.

Appetite data were also examined by calculating the AUC for the total trial (0 – 9 h), preprandially (0 – 2 h), between the test-meals (2.5 – 4.5 h) and after the test meals (4.5 – 9 h) (Table 9.3). This analysis revealed significant differences in ratings of appetite between the Food-Def and control trials and between the Food-Def and Ex-Def trials (all P < 0.001) for the total trial, inter test-meals, post-test meals but not preprandially.

Table 9.3: Appetite area under the curve in the control, Ex-Def and Food-Def trials

	Preprandial (0-2 h) units 2 h	Inter-test meal (2.5-4.5 h) units 2 h	Post-test meals (4.5-9 h) units 4.5 h	Total trial (0-9 h) units 9 h
Hunger				
Control	131 ± 12	76 ± 12	110 ± 20	317 ± 40
Ex-Def	115 ± 12	84 ± 12	112 ± 18	312 ± 33
Food-Def	135 ± 6	156 ± 7 [a,b]	262 ± 17 [a,b]	553 ± 25 [a,b]
Satisfaction				
Control	53 ± 12	167 ± 11	326 ± 22	546 ± 39
Ex-Def	48 ± 8	143 ± 11	321 ± 21	512 ± 31
Food-Def	39 ± 7	72 ± 9 [a,b]	158 ± 19 [a,b]	269 ± 32 [a,b]
Fullness	±			
Control	48 ± 12	162 ± 12	319 ± 24	529 ± 43
Ex-Def	41 ± 7	145 ± 10	324 ± 19	509 ± 26
Food-Def	36 ± 8	66 ± 10 [a,b]	146 ± 18 [a,b]	248 ± 34 [a,b]
PFC				
Control	138 ± 10	82 ± 13	121 ± 22	342 ± 40
Ex-Def	116 ± 12	88 ± 12	120 ± 20	324 ± 31
Food-Def	136 ± 6	166 ± 5 [a,b]	276 ± 16 [a,b]	579 ± 22 [a,b]

Values are mean ± SEM (n = 12)
[a]different from control ($P < 0.001$)
[b]different from Ex-Def ($P < 0.001$)

9.3.3 Energy and macronutrient intake

At the test meals (combined intake of the 1st and 2nd meal) participants consumed 7021 ± 92 kJ (1678 ± 22 kcal) in the control and Ex-Def trials and 2200 ± 142 kJ (526 ± 34 kcal) in the Food-Def trial. Consequently, the energy deficit induced by restricting food intake in the Food-Def trial was 4820 ± 151 kJ (1152 ± 36 kcal). This was comparable with the energy deficit induced through exercise in the Ex-Def trial where the net expenditure was 4715 ± 113 kJ (1127 ± 27 kcal).

Table 9.4 displays the energy and macronutrient intake data at the *ad libitum* buffet meal. For energy intake one-factor ANOVA revealed a significant main effect of trial ($P < 0.002$). Post hoc analysis showed that energy intake was significantly higher on the Food-Def trial than the control trial ($P < 0.001$) whilst energy intake tended to be higher on the Food-Def trial than the Ex-Def trial ($P = 0.058$).

Table 9.4: Ad libitum energy and macronutrient intake in the control, Ex-Def and Food-Def trials

	Control	Ex-Def	Food-Def
Energy	4004 ± 427	4343 ± 653	6167 ± 318[a]
kJ & (kcal)	(957 ± 102)	(1038 ± 156)	(1474 ± 76)[a]
Fat	34 ± 5	38 ± 5	63 ± 5[a,b]
grams & (%)	(30.7 ± 3.3)	(33.4 ± 2.0)	(38.3 ± 1.7)[a,b]
Protein	40 ± 10	47 ± 15	67 ± 9[a]
grams & (%)	(14.7 ± 2.1)	(15.0 ± 2.5)	(17.9 ± 1.8)
Carbohydrate	124 ± 12	129 ± 17	159 ± 10[a]
grams & (%)	(54.6 ± 4.4)	(51.6 ± 3.1)	(43.8 ± 2.5)[a,b]

Values are mean ± SEM (n = 12)
[a]different from control ($P < 0.05$)
[b]Food-Def different from Ex-Def ($P < 0.05$)

Both the absolute amount (grams) and percentage of energy derived from the macronutrients was compared across main trials. One-factor ANOVA showed a significant difference ($P < 0.001$) in the absolute amount of fat consumed with a significantly higher intake apparent in the Food-Def trial than the control and Ex-Def trials. Moreover, the percentage of energy derived from fat was significantly higher on the Food-Def trial than the control and Ex-Def trials. For protein intake, one-factor ANOVA revealed a significant difference in the absolute intake between trials (P = 0.016) with intakes being significantly higher on the Food-Def trial than the control trial. There was no significant difference in the percentage of energy derived from protein. For absolute carbohydrate intake, one-factor ANOVA revealed a significant main effect of trial (P = 0.045) with higher intakes apparent in the Food-Def trial than the control trial. Additionally, one-factor ANOVA revealed a significant difference between trials in the percentage of energy derived from carbohydrate (P = 0.006), with the percentage intake being significantly lower on the Food-Def trial than both the control and Ex-Def trials.

9.3.4 Acylated ghrelin

Figure 9.3 (top section) shows circulating acylated ghrelin responses during the main trials. Fasting plasma acylated ghrelin concentrations did not differ (P = 0.226) between

the control (162 ± 37 pg·mL^{-1}), Ex-Def (162 ± 35 pg·mL^{-1}) and Food-Def (175 ± 37 pg·mL^{-1}) trials. Two-factor ANOVA revealed significant trial, time and interaction (trial x time) main effects (all $P < 0.001$) indicating that acylated ghrelin responses differed over time between trials. Across trials, post-hoc analysis identified significantly higher circulating acylated ghrelin concentrations in the Food-Def trial as compared with the control ($P = 0.002$) and Ex-Def ($P < 0.001$) trials. At individual time points post hoc analysis identified significant differences between trials at 2, 4.75, 6, 7 and 8 h (all $P < 0.05$).

Between trial differences in acylated ghrelin were also evaluated using AUC (Table 9.5 upper panel). Preprandially (0 – 2 h), acylated ghrelin was significantly lower in the Ex-Def trial than the control ($P = 0.015$) and Food-Def ($P < 0.001$) trials, indicating a suppressive effect of exercise. Postprandially (2 – 9 h), the acylated ghrelin AUC was significantly higher in the Food-Def trial than the control ($P = 0.003$) and Ex-Def ($P < 0.001$) trials.

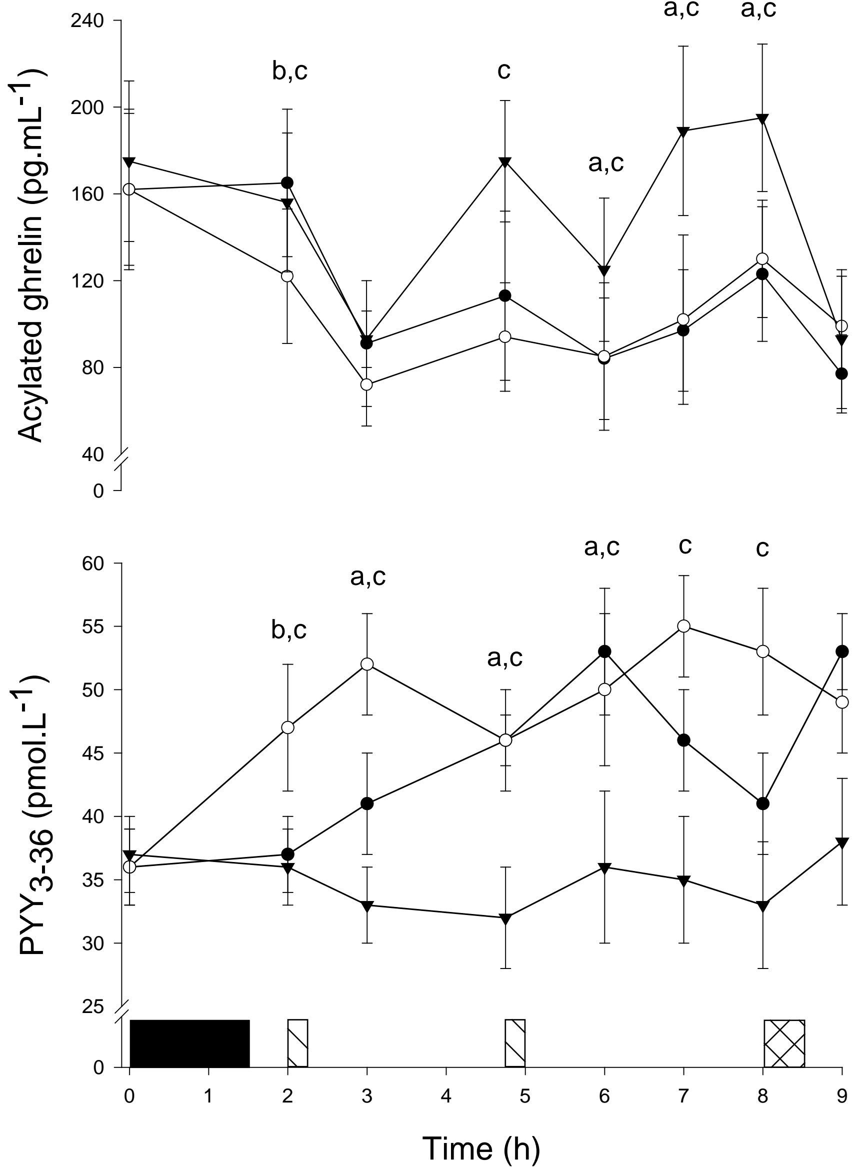


Figure 9.3: Plasma concentrations of acylated ghrelin (top) and $PYY_{3\text{-}36}$ (bottom) in the control (●), Ex-Def (○) and Food-Def (▼) trials. Values are mean ± SEM *(n* = 12). Black rectangle indicates exercise, diagonal rectangles indicate test meals, hatched rectangle represents the buffet meal. [a]Food-Def different from Control $P < 0.05$; [b]Ex-Def different from Control $P < 0.05$; [c]Food-Def different from Ex-Def $P < 0.05$.

Table 9.5: Acylated ghrelin and $PYY_{3\text{-}36}$ AUC in the control, Ex-Def and Food-Def trials

	Preprandial (0-2 h) *units 2 h*	**Postprandial (2-9 h)** *units 7 h*	**Total trial (0-9 h)** *units 9 h*
Acylated ghrelin			
Control	327 ± 70	729 ± 209	1055 ± 276
Ex-Def	284 ± 65[a]	677 ± 190	961 ± 254[a]
Food-Def	331 ± 69[b]	1040 ± 195[a,b]	1371 ± 262[b]
$PYY_{3\text{-}36}$			
Control	73 ± 6	318 ± 17	391 ± 22
Ex-Def	84 ± 7[a]	354 ± 25	438 ± 31[a]
Food-Def	73 ± 6[b]	237 ± 28[a,b]	310 ± 34[a,b]

Values are mean ± SEM ($n = 12$)
[a]Different from control $P < 0.05$; [b]different from Ex-Def $P < 0.05$

9.3.5 Peptide YY $_{3\text{-}36}$

Figure 9.3 (bottom section) shows circulating $PYY_{3\text{-}36}$ responses during the main trials. Fasting plasma $PYY_{3\text{-}36}$ concentrations did not differ ($P = 0.908$) between the control (36 ± 3 pmol.L^{-1}), Ex-Def (36 ± 3 pmol.L^{-1}) and Food-Def (37 ± 3 pmol.L^{-1}) trials. Two-factor ANOVA revealed significant trial, time and interaction (trial x time) main effects (all $P \leq 0.001$) indicating that $PYY_{3\text{-}36}$ responses differed over time between trials. Across trials, post-hoc analysis identified significantly lower circulating $PYY_{3\text{-}36}$ concentrations in the Food-Def trial as compared with the control trial ($P = 0.004$) and Ex-Def ($P < 0.001$) trials. At individual time points post hoc analysis identified significant differences between trials at 2, 4.75, 6, 7 and 8 h (all $P < 0.05$).

Preprandially, the $PYY_{3\text{-}36}$ AUC was significantly greater on the Ex-Def trial as compared with the control ($P = 0.029$) and Food-Def ($P = 0.028$) trials highlighting a stimulatory effect of exercise. Postprandially (2 – 9 h), the $PYY_{3\text{-}36}$ AUC was lower on the Food-Def trial than the control ($P = 0.001$) and Ex-Def ($P < 0.001$) trials.

9.3.6 Glucose and triacylglycerol

Figure 9.4 shows plasma glucose and triacylglycerol responses during the main trials. Fasting plasma glucose concentrations did not differ ($P = 0.221$) between the control (4.96 ± 0.10 mmol.L^{-1}), Ex-Def (5.05 ± 0.08 mmol.L^{-1}) and Food-Def (4.87 ± 0.09 mmol.L^{-1}) trials. For plasma glucose two-factor ANOVA revealed significant time ($P =$

0.003) and interaction (trial x time, $P = 0.011$) main effects, indicating differences in responses over time during the main trials. Post hoc analysis identified significantly higher plasma glucose concentrations in the Ex-Def trial than the Food-Def trial at 7 h ($P = 0.022$).

Fasting plasma triacylglycerol concentrations did not differ ($P = 0.264$) between the control (1.14 ± 0.14 $mmol.L^{-1}$), Ex-Def (1.15 ± 0.14 $mmol.L^{-1}$) and Food-Def (0.96 ± 0.10 $mmol.L^{-1}$) trials. For plasma triacylglycerol, two-factor ANOVA revealed significant trial ($P < 0.001$), time ($P < 0.001$) and interaction (trial x time, $P < 0.001$) main effects. Across trials, post hoc analysis identified significantly lower circulating triacylglycerol levels in the Food-Def trial than both the control ($P = 0.002$) and Ex-Def trials ($P = 0.001$). At individual time points post hoc analysis identified significant differences between trials at 4.75, 6, 7 and 8 h (all $P < 0.05$). The triacylglycerol AUC was significantly lower on the Food-Def trial (7.3 ± 0.7 units· 7 h) than the control (10.2 ± 0.8 units· 7 h, $P = 0.003$) and Ex-Def trials (10.5 ± 1.2 units· 7 h, $P = 0.001$) after the completion of exercise (2 – 9 h) but no differences occurred before (0 – 2 h): Control 2.1 ± 0.2, Ex-Def 2.2 ± 0.3, Food-Def 1.8 ± 0.2 units· 2 h, $P = 0.191$) .

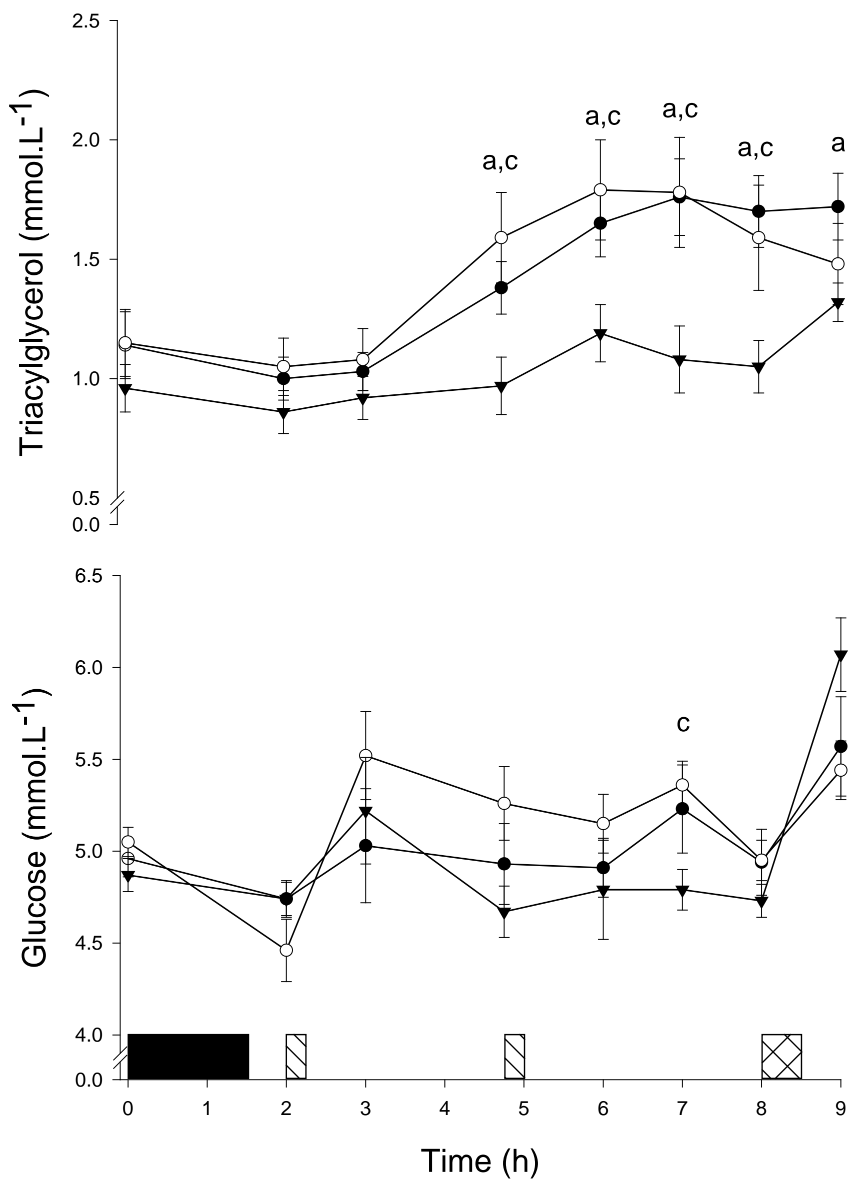


Figure 9.4: Plasma concentrations of triacylglycerol (top) and glucose *(*bottom) in the control (●), Ex-Def (○) and Food-Def (▼) trials. Values are mean ± SEM *(n* = 12). Black rectangle indicates exercise, diagonal rectangles indicate test meals, hatched rectangle represents the buffet meal. [a]Food-Def different from Control $P < 0.05$; [b]Ex-Def different from Control $P < 0.05$; [c]Food-Def different from Ex-Def $P < 0.05$.

9.3.7 Acylated ghrelin and peptide YY_{3-36} correlations

Circulating acylated ghrelin and PYY_{3-36} concentrations were not significantly correlated with subjective appetite ratings (hunger, fullness, satisfaction or prospective food consumption) or with circulating glucose or triacylglycerol levels. There were no significant correlations between acylated ghrelin values immediately before buffet

meals and *ad libitum* energy intake. Peptide $YY_{3\text{-}36}$ concentrations immediately before meals were inversely related to energy intake in the control ($P = 0.319$, $r = -0.315$) and Food-Def ($P = 0.530$, $r = -0.201$) trials however this was only statistically significant in the Ex-Def trial ($P = 0.046$, $r = -0.584$).

9.3.8 Water intake, temperature and humidity

Water intake was significantly higher in the Ex-Def trial than in both the control and Food-Def trials: Control 802 ± 145, Ex-Def 1804 ± 181, Food-Def 1007 ± 129 mL, one-factor ANOVA $P < 0.001$). The mean temperature of the laboratory was not significantly different ($P = 0.967$) between the experimental trials (control 22.4 ± 0.2, Ex-Def 22.5 ± 0.2, Food-Def 22.5 ± 0.1 ^{o}C). There were no differences ($P = 0.870$) between trials in the mean relative humidity within the laboratory (control 36.2 ± 5.1, Ex-Def 37.8 ± 4.1, Food-Def 38.3 ± 3.9 %).

9.4 Discussion

This study was designed to compare acylated ghrelin, PYY_{3-36}, appetite and energy intake responses to equivalent energy deficits induced through acute dietary restriction as compared with exercise. Two key findings have emerged from this work. Firstly, appetite and energy intake increase in response to an acute deficit in energy induced by food restriction but remain unchanged in response to an identical energy deficit induced by exercise. Secondly, acylated ghrelin and PYY_{3-36} responses to these interventions are consistent with the reported effects of food restriction and exercise on appetite and energy intake. These findings suggest a role for acylated ghrelin and PYY_{3-36} in mediating the divergent effects of food restriction and exercise on appetite and energy intake.

This study has shown that appetite and energy intake increase in a compensatory fashion to acute deficits in energy induced by restricting food intake i.e. the intake of smaller meals with reduced energy content. Conversely, when an equivalent energy deficit is induced through exercise, appetite and energy intake do not change. It is therefore apparent that two methods of inducing an acute energy deficit have markedly different influences on appetite and subsequent energy intake. These findings confirm those reported in study four (Chapter seven) and other previously established work which has shown that exercise does not stimulate appetite and energy intake, even when levels of energy expenditure are large (King et al, 1994; King and Blundell, 1995; King et al, 1997). The present findings are also consistent with other research which has shown that acute food restriction leads to an increase in appetite and subsequent food intake (Lawton et al, 1993; Green et al, 1994; Hubert et al, 1998). From a practical perspective these data provide an indication of why dieting is often so difficult and typically unsuccessful. Equally, this work underscores the potential for exercise to facilitate successful weight control.

In an investigation that was published more than a decade ago, Hubert et al (1998) made a direct comparison of appetite and *ad libitum* energy intake responses to acute food restriction as compared with exercise. In this study 11 healthy females completed four main trials in a crossover fashion with manipulations of exercise (40 min of cycling at 70% of $\dot{V}O_2$ max or rest) and diet (500 kcal breakfast or 64 kcal breakfast).

The researchers observed that exercise did not alter subjective ratings of hunger or subsequent *ad libitum* energy intake at a meal provided four hours after exercise. Conversely, food restriction led to a rapid increase in hunger and a significantly higher energy intake (~20%) at the *ad libitum* meal. The researchers proposed that the increase in appetite observed in response to food restriction may be related to weakened post-ingestive satiety signals although the researchers did not outline the precise mechanisms. The findings from the present study support this hypothesis and suggest an important mediating role of acylated ghrelin and PYY_{3-36}.

The studies within this thesis have made measurements of circulating acylated ghrelin. These studies have shown that exercise does not increase circulating levels of acylated ghrelin after exercise, even when the energy deficit induced is severe. This lack of change in acylated ghrelin in response to exercise may be one reason why exercise does not cause compensatory appetite and energy intake responses. In contrast to this, it has been shown that ghrelin is sensitive to acute perturbations in energy balance resulting from dietary manipulation (Callahan et al, 2004; Leidy and Williams, 2006; Borer et al, 2009). Consequently, it was thought that disparate appetite and energy intake responses to exercise compared with food restriction may be associated with disparate changes in circulating levels of acylated ghrelin.

In a recent study, Borer et al (2009) compared appetite, energy intake and circulating total ghrelin responses to energy deficits induced by food restriction and on another occasion, exercise. The researchers showed that circulating levels of total ghrelin exhibited a compensatory increase in response to food restriction and also to a lesser extent, exercise. A key limitation of this work however was that the energy deficit induced by exercise was approximately 40% larger as compared with that induced by food restriction, thus confounding the interpretation of the results. Moreover, circulating total ghrelin concentrations were measured. Acylation of ghrelin is thought to be necessary for ghrelin to exert effects on appetite therefore assessment of total ghrelin may have masked important effects of acylated ghrelin (Liu et al, 2008). Hence, the present study sought to specifically examine acylated ghrelin responses to equivalent energy deficits induced by exercise and diet. Within this, a central aspect was to precisely match the energy deficits induced by each intervention.

The findings of the present study demonstrate that exercise does not alter circulating levels of acylated ghrelin in the 7.5 h after. These findings confirm those reported in study four (Chapter seven) where plasma acylated ghrelin concentrations did not change in the 8.5 h after exercise (90 min of treadmill running) or on the morning after (22.5 h after exercise). Conversely, in the present study, food restriction which induced an energy deficit equal to that elicited by exercise was associated with significantly higher acylated ghrelin concentrations throughout the trial. After the first test meal provided two hours into trials the acylated ghrelin AUC was 42% higher on the Food-Def trial than the control trial and 54% higher than the Ex-Def trial. Thus, in the Food-Def trial circulating levels of acylated ghrelin were perturbed in a direction expected to stimulate appetite and feeding. These heightened levels of plasma acylated ghrelin are likely to have been related to the reported increase in appetite and energy intake.

In the present study circulating concentrations of PYY_{3-36} were also measured. Circulating concentrations of PYY increase in response to nutrient ingestion and serve to promote within-meal satiation and inter-meal satiety (Neary and Batterham, 2009b). In the circulation PYY exists in two forms, namely PYY_{1-36} and PYY_{3-36}. Peptide YY_{3-36} is the major circulating form and is chiefly responsible for determining the appetite suppressing action of PYY (Batterham et al, 2006; Karra et al, 2009). A handful of studies suggest that circulating levels of total PYY increase transiently in response to single bouts of exercise (Martins et al, 2007; Broom et al, 2009; Shorten et al, 2009; Ueda et al, 2009). At present, only one study has examined acute PYY_{3-36} responses to exercise (Cheng et al, 2009). The results from this investigation suggest that exercise (60 min of cycling at 50% of $\dot{V}O_2$ max) does not alter circulating levels of PYY_{3-36} *per se*, however exercise may potentiate the PYY_{3-36} response to feeding.

In the present investigation, circulating PYY_{3-36} concentrations were 27% higher than control when measured 30 min after the end of exercise. Thus, exercise stimulated circulating levels of PYY_{3-36} and therefore this finding contradicts that reported by Cheng et al (2009). In the present study the intensity and duration of exercise was greater therefore it is possible that only intense and/or prolonged exercise stimulates an increase in circulating PYY_{3-36}.

In the present study, PYY_{3-36} responses to feeding were also examined. Interestingly, although the differences were not quite statistically significant, levels of PYY_{3-36} were notably higher after the test meals on the Ex-Def trial compared with control. Thus, exercise appeared to potentiate increases in PYY_{3-36} after eating. These findings support the observations of others who have shown that both total PYY and PYY_{3-36} responses to meals are augmented after exercise (Broom et al, 2009; Cheng et al, 2009). These outcomes suggest a beneficial effect of exercise on appetite regulation. It is possible that an accentuated PYY_{3-36} response to exercise is implicated in the lack of change in appetite and energy intake observed afterwards.

Despite the induction of an equivalent energy deficit, changes in circulating levels of PYY_{3-36} were markedly different in response to food restriction as compared with exercise. On the Food-Def trial, after consumption of the first test meal, circulating levels of PYY_{3-36} were significantly lower than on the control trial and remained so throughout the remaining seven hours of the trial. This finding is consistent with the idea proposed by Hubert et al (1998) who suggested that the rapid compensatory increase in appetite witnessed after restricted meals is related to weakened post-ingestive satiety signals. In the present study, in addition to the aforementioned effects of food restriction on PYY_{3-36}, after the first test meal the postprandial suppression of plasma acylated ghrelin was less prolonged in the Food-Def trial compared with the control and Ex-Def trials. Moreover, circulating levels of acylated ghrelin became higher in the Food-Def trial prior to the second test meal and remained so throughout the final hours of the trials. Thus, compared with the control and Ex-Def trials, postprandial acylated ghrelin and PYY_{3-36} responses were attenuated in the Food-Def main trials. These changes may therefore have been implicated in the heightened energy intake response observed at the *ad libitum* meal in the Food-Def trial.

Within each main trial circulating concentrations of acylated ghrelin and PYY_{3-36} were not consistently correlated with any of the appetite markers assessed (hunger, fullness, satisfaction and prospective food consumption). It is possible that the relatively low number of homogenous participants included in this study may have prevented the detection of significant correlations however it is also possible that there may not have been a direct link between these gut peptides and appetite. For instance, gastric emptying has been intricately linked with subjective ratings of hunger and satiety

(Bergman et al, 1992). It is possible that acylated ghrelin and PYY_{3-36} may have influenced appetite indirectly, by modulating gastric emptying. Further work is needed however to explore this possibility. In the present study acylated ghrelin and PYY_{3-36} were also not significantly correlated with plasma glucose or triacylglycerol concentrations. These data indicate that the reported changes in acylated ghrelin and PYY_{3-36} were not mediated by these metabolites.

In summary, this study has shown that equivalent energy deficits induced by food restriction and exercise have markedly different effects on appetite and energy intake. Food restriction elicits a rapid increase in appetite and energy intake and these responses appear to be related to an attenuated postprandial PYY_{3-36} response and to a more transient postprandial suppression of circulating acylated ghrelin. In contrast to this, acute energy deficits induced by exercise do not alter appetite or energy intake and the results of this study suggest that this may be related to the failure of exercise to induce compensatory acylated ghrelin and PYY_{3-36} responses. Further research is needed to examine other appetite regulating peptides such as CCK and GLP-1 to see whether their reactions to the present interventions are consistent with those of acylated ghrelin and PYY_{3-36}. It may also be important to compare responses in overweight individuals as it is here that findings may hold the most practical significance.

Chapter X

General discussion

10.1 Introduction

Over the last decade there has been a rapid expansion in our understanding of the physiological regulation of energy homeostasis. One specific area where knowledge has developed substantially is the role that gut hormones occupy in the regulation of energy balance. Exercise is an important determinant of energy balance therefore recent work has sought to characterise the effects of exercise on gut hormones. This novel work sits alongside an established body of research which has examined the effects of exercise on appetite and food intake. The studies presented within this thesis have endeavoured to extend knowledge within each of these related areas of enquiry, by examining appetite, food intake and gut hormone (acylated ghrelin and $PYY_{3\text{-}36}$) responses to various forms of exercise. An integral feature of these studies was to examine the aforementioned responses over a prolonged duration of time so that both the immediate and latent effects of exercise could be examined. The purpose of this chapter is to reflect upon and collectively discuss the findings presented within the experimental chapters of this thesis. Table 10.1 provides a summary of the study protocols and variables examined within each experimental chapter.

Table 10.1: Summary of the study protocols presented within the experimental chapters of this thesis

Study (Chapter)	Trials	Exercise mode	Intensity	Exercise Duration	Net energy expenditure kJ & (kcal)	Measurements
1 (4)	Resistance Ex Control	Free weights Rest	79.9 ± 0.3 % of 12- RM	90 min -	1007 ± 92 (241± 22)	Appetite Energy/macronutrient intake
2 (5)	Swimming Control	Intermittent swim Rest	78 ± 2 % of heart rate max	60 min (intermittent) -	1921 ± 83 (459 ± 20)	Appetite Energy/macronutrient intake Acylated ghrelin Glucose and TAG
3 (6)	Brisk walking Control	Brisk Walking Rest	45.2 ± 2.0% of $\dot{V}O_2$ max	60 min -	2008 ± 134 (480 ± 32)	Appetite Energy/macronutrient intake Acylated ghrelin Insulin, glucose and TAG
4 (7)	Prolonged Run Control	Treadmill running Rest	68.8 ± 0.8% of $\dot{V}O_2$ max	90 min -	5326 ± 186 (1273 ± 45)	Appetite Energy/macronutrient intake Acylated ghrelin Insulin, glucose and TAG
5 (8)	Running Control	Treadmill running Rest	71.8 ± 1.3% of $\dot{V}O_2$ max	60 min -	4117 ± 117 (984 ± 28)	Appetite Energy/macronutrient intake Acylated ghrelin Glucose and TAG
6 (9)	Ex-Def Food-Def Control	Treadmill running - Rest	69.8 ± 0.9% of $\dot{V}O_2$ max	90 min - -	4715 ± 113 (1127 ± 27)	Appetite Energy/macronutrient intake Acylated ghrelin PYY_{3-36} Glucose and TAG

10.2 Exercise and appetite

One aim of the studies presented in this thesis was to characterise the effects of exercise on appetite. Before undertaking this work the author was aware of a significant body of research which has previously investigated appetite responses to exercise however the studies described here have sought to add to this established work by characterising the effects of novel modes of exercise (resistance exercise and swimming) and by also examining responses over a prolonged duration of time within the laboratory.

Several studies have shown that exercise suppresses appetite when performed at moderate intensities or higher, an effect that has been termed exercise-induced anorexia (King et al, 1994). Exercise induced anorexia has previously been described in response to acute bouts of running (King and Blundell 1995), cycling (Martins et al, 2007) and resistance exercise (Broom et al, 2009). Study two (Chapter five) has now shown that appetite may also be suppressed during a typical recreational bout of swimming. Similar to other modes of exercise, the appetite suppressing effect of swimming is brief and does not influence energy intake at meals consumed afterwards. It has previously been suggested that 'gut disturbance' may contribute to the inhibition of appetite during exercise (Broom et al, 2009) however the fact that swimming reduces appetite indicates that this may not be important as the non-weight bearing nature of swimming means there is a lack of gastrointestinal distress.

The findings within this thesis confirm that exercise intensity is an important determinant of exercise induced anorexia as 60 min of brisk walking (45% of $\dot{V}O_2$ max) in study three (Chapter six) did not influence appetite whereas high intensity running (70% of $\dot{V}O_2$ max) in studies four and five (Chapters seven and eight) markedly suppressed appetite. The findings from study four (Chapter seven) also confirm that the appetite inhibitory effect of exercise is transient as no differences in appetite were seen 30 min after exercise.

Previous research has shown that exercise induced anorexia does not influence energy intake at the next feeding opportunity after exercise, yet may induce a delay until the voluntary request of a meal (King et al, 1994; King and Blundell, 1995; King et al, 1996). The findings from studies two and four (Chapters five and seven) are consistent

with this as 60 min of swimming and 90 min of running did not alter energy intake at buffet meals made available 60 min after exercise, despite marked reductions in appetite being apparent. The notion that exercise induced anorexia manifests as a resistance to begin eating after exercise is supported by the findings in study five (Chapter eight). After 60 min of high intensity running (71.8 ± 1.3% of $\dot{V}O_2$ max) participants requested lunch 35 min later (1 h 21 min after exercise) than on a control trial. Energy intake was not significantly different between trials at this spontaneously requested meal.

Many studies that have assessed the effects of exercise on appetite have examined responses over a brief period of time, commonly during exercise and then leading up to a meal consumed shortly afterwards (Thompson et al, 1988; Verger et al, 1992; King et al, 1994; King and Blundell, 1995; King et al, 1996; Imbeault et al, 1997; Westerterp-Plantenga et al, 1997; Erdmann et al, 2007; Martins et al, 2007; Shorten et al, 2009). It was thought possible however that a more prolonged period of observation may be needed to detect changes in appetite after exercise. Thus, the studies in this thesis assessed appetite responses to exercise over a prolonged duration of time, in response to multiple meals, within a strictly controlled laboratory setting. The findings derived from the studies presented in this thesis show that appetite is not stimulated in the hours after completing exercise (on land), even when large amounts of energy are expended (up to 5324 kJ, 1273 kcal) and observations are made for up to 22.5 hours after exercise. Thus, it seems that energy deficits induced by exercise are not consciously detected. An exception to this occurred in study two where 60 min of swimming led to an increase in appetite in the later hours of recovery after exercise (60 – 90 min after a post-exercise meal). These changes did not influence energy intake however.

10.3 Energy intake responses to exercise

Before undertaking the work in this thesis the consensus of evidence suggested that energy intake does not change in response to acute bouts of exercise (Martins et al, 2008; Bilski et al, 2009). However, a limitation of many previous studies is that energy intake has been determined within the laboratory from food consumed at single meals provided shortly after exercise (Kissileff et al, 1990; Verger et al, 1992; 1994; Imbeault et al, 1997; Hubert et al, 1998; George and Morganstein, 2003; Erdmann et al, 2007;

Martins et al, 2007; Shorten et al, 2009). It was thought possible that a longer period of observation may have been needed to detect changes in energy intake after exercise as differences may be seen not at the first meal after exercise, but at a second or third meal. Hence, the studies presented in this thesis sought to examine energy intake responses over a prolonged duration of time i.e. at multiple meals taken after completing exercise. Furthermore, these studies examined the effects of resistance exercise and swimming on energy intake, each popular modes of exercise which had not previously received attention. The influence of high levels of energy expenditure on energy intake was also examined to see whether this would stimulate a compensatory increase in energy intake.

The findings presented in this thesis show that energy intake does not change after exercise, regardless of the modality and the extended period of observation. Specifically, energy intake did not change significantly in response to exercise (walking, running, swimming, resistance exercise) in any of the six studies reported in this thesis. This is despite participants expending amounts of energy equating to half of their daily requirements and with assessments of energy intake being made at up to four eating opportunities over the course of the day after exercise (and on the next morning in studies one and four). Conversely, in study six (Chapter nine), food restriction which induced an energy deficit in an identical amount to that expended during 90 min of high intensity running, significantly increased *ad libitum* energy intake by more than 50% of that consumed on the control trial. These findings highlight a marked difference in energy intake responses to acute deficits in energy when induced by exercise compared with diet i.e. when there is a restriction on energy entering the body compared with an increase in energy leaving the body. These findings demonstrate why weight loss strategies based solely on restricting food intake are so difficult and typically unsuccessful. Moreover, these findings underscore the potential for exercise to facilitate weight management.

10.4 Effects of exercise on macronutrient intake

Energy intake is determined by the type of food consumed in addition to the absolute amount. Therefore, when considering the influence of exercise on energy intake the question of whether exercise alters macronutrient selection requires attention. To examine macronutrient intake responses to exercise it is necessary that foods of

sufficient diversity are made available so that any preferences can be delineated (Arvaniti et al, 2000). To permit the assessment of this outcome, in the studies described in this thesis a buffet meal was developed in study one and was provided to participants at defined eating opportunities within each investigation. Although there are limitations of this methodology (e.g may promote increased consumption and/or delay satiation), this protocol permits the assessment of macronutrient intake even if energy intake remains the same between experimental treatments (Blundell et al, 2010).

Short-term intervention trials that have examined the influence of exercise on macronutrient intake have revealed mixed findings although the most consistent outcome has been for macronutrient intake to remain unchanged (Tremblay and Drapeau, 1999; Elder and Roberts, 2007). The studies in this thesis have contributed to these established findings by assessing macronutrient intake responses to previously unstudied modes of exercise (resistance exercise and swimming) and by characterising responses to multiple meals provided after exercise. Notwithstanding, the findings from five of the six studies presented in this thesis (studies two, three, four, five and six) are consistent with the established consensus which has described a lack of change in macronutrient intake in response to acute bouts of exercise. Based on these findings it would appear that exercise does not stimulate a drive to seek out any particular macronutrient. It remains possible however that any physiological drive induced by exercise may be masked by the stubbornness of an individual's food intake choices, ultimately a behavioural act based on learned preferences which have developed over years (Stubbs et al, 1998). Scrutiny of macronutrient intake responses to more provocative exercise stimuli, such as to repeated bouts, may be needed to tease out any explicit effect of exercise. In such studies it may be necessary to recruit participants who do not have significant knowledge regarding nutrition and/or exercise physiology so that such information does not influence food choices.

The aforementioned discussion focused on studies that have examined the effects of aerobic type exercise on macronutrient intake. In the first study presented in this thesis (Chapter four) the effects of resistance exercise on macronutrient intake was examined. Interestingly, in this study an increase in the percentage of energy derived from carbohydrate was observed at a buffet meal consumed five hours after completing a 90 min free weights session. The reason for this difference is not clear. Resistance exercise

is predominantly an anaerobic form of exercise that is fuelled primarily by the anaerobic metabolism of carbohydrate. It has been suggested that individuals may seek to replace the substrate principally utilised during exercise so that there is a matching between the respiratory quotient generated and the food quotient of the meal consumed afterwards (King, 1998). It is therefore possible that this increased preference for carbohydrate rich foods after resistance exercise may reflect a drive to seek out this macronutrient. This explanation is perhaps unlikely given that no changes in carbohydrate preference were seen at a meal consumed one hour after exercise when presumably the drive to consume carbohydrate would be strongest. Additional work is needed to test the reproducibility of this initial finding within a larger group of study participants.

10.5 Acylated ghrelin responses to exercise

Within the last decade many studies have examined the effects of exercise on ghrelin however in the majority of these investigations circulating total ghrelin was measured, that is, the assays which were used did not distinguish between acylated ghrelin and unacylated ghrelin (for a review see Kraemer and Castracane, 2007). It is now believed that acylated ghrelin is chiefly responsible for the appetite stimulating effects of ghrelin, therefore more recent investigations have began to evaluate the effect of exercise on acylated ghrelin (Broom et al, 2007; Marzullo et al, 2008; Hagobian et al 2009). The studies in this thesis sought to extend this work by characterising acylated ghrelin responses to various exercise challenges over extended periods of observation. Interactions, between acylated ghrelin, appetite and energy intake were also examined.

The findings in this thesis show that moderate-high intensity aerobic exercise transiently suppresses circulating levels of acylated ghrelin. These data therefore confirm the previous observations of others (Broom et al, 2007; Marzullo et al, 2008; Broom et al, 2009). The physiological significance of this decline in circulating levels of acylated ghrelin during exercise is still not entirely clear. The findings in study five suggest that the return of acylated ghrelin to pre-exercise levels may be necessary before individuals choose to eat afterwards. Broom et al (2007) previously suggested a role of acylated ghrelin as a determinant of exercise induced anorexia during high intensity running as they found a significant positive correlation between subjective ratings of hunger and acylated ghrelin during exercise. The studies in this thesis have

not reproduced this finding however, as no significant relationships between acylated ghrelin and any of the appetite markers examined were found during exercise (Table 10.2). A lack of statistical power does not appear to explain these outcomes either as no significant relationships were found between acylated ghrelin and appetite AUC during exercise when data was combined for the four studies identified in Table 10.2 (n = 40). Correlation between acylated ghrelin and; hunger P = 0.131, r = 0.419; satisfaction P = 0.317, r = -0.162; fullness P = 0.140, r = -0.238; prospective food consumption P = 0.982, r = 0.004. These findings may therefore suggest that the effect of acylated ghrelin on appetite is indirect.

At present the mechanisms responsible for suppressed circulating concentrations of acylated ghrelin during high intensity exercise remain unclear. Circulating levels of insulin and glucose have been implicated in the regulation of ghrelin however the data presented in this thesis suggest that insulin and glucose are not responsible for the suppression of acylated ghrelin during exercise as circulating levels of glucose and insulin were not elevated during exercise at times when marked reductions in acylated ghrelin were apparent. Instead it is possible that the reduction in acylated ghrelin is related to exercise related changes in gut perfusion (Burns et al, 2007) or to the activation of the sympathetic nervous system (Toshinai et al, 2001).

Table 10.2: Correlations between acylated ghrelin and appetite AUC calculated during exercise. No significant relationships were observed.

	Study 2 (Chapter 5) 60 min intermittent swim (78% of heart rate max) $n = 14$		**Study 4 (Chapter 7)** 90 min run (~69% $\dot{V}O_2$ max) $n = 9$		**Study 5 (Chapter 8)** 60 min run (~72% $\dot{V}O_2$ max) $n = 10$		**Study 6 (Chapter 9)** 90 min run (~70% $\dot{V}O_2$ max) $n = 12$	
	P	***r***	***P***	***r***	***P***	***r***	***P***	***r***
Hunger	0.396	0.323	0.254	0.425	0.870	0.06	0.461	-0.236
Fullness	0.867	-0.065	0.051	-0.527	0.961	0.018	0.572	-0.182
Satisfaction	0.252	0.427	0.061	-0.645	0.671	0.154	0.496	-0.218
PFC	0.784	-0.107	0.330	0.368	0.406	0.296	0.387	-0.275

N.B. acylated ghrelin not measured in study one (Chapter four) and no suppression was observed in study three (Chapter six)
PFC = prospective food consumption

An additional objective of the studies in this thesis was to examine the latent effects of exercise on acylated ghrelin. Previous studies have shown that high intensity exercise acutely suppresses circulating acylated ghrelin (Broom et al, 2007; 2009) however in these studies no changes in acylated ghrelin were evident in the hours of observation afterward, despite participants remaining in energy deficit after exercise. Given the role of ghrelin in the acute regulation of food intake, these findings were unexpected as it was thought that at some point after exercise acylated ghrelin levels would be higher to promote the restoration of energy balance.

In studies two and three (Chapters five and six) plasma acylated ghrelin concentrations remained no different to control in the six/seven hours of observation after completing acute bouts of swimming or brisk walking. It was initially thought that in each of these instances the disruption of energy balance may have been insufficient to provoke a compensatory increase in circulating levels of acylated ghrelin. Therefore, in study four (Chapter seven), to purposefully induce a high level of energy expenditure participants completed 90 min of treadmill running at 68.8 ± 0.8% of $\dot{V}O_2$ max (energy expenditure 5324 kJ, 1273 kcal). Despite this considerable challenge, circulating concentrations of acylated ghrelin were no different to control in the 8.5 h after exercise or on the morning after (24 h measurement). This finding was reproduced in study six where an identical bout of running did not alter circulating levels of acylated ghrelin in the 7.5 h after exercise. This latter finding was in stark contrast to acylated ghrelin responses to an equivalent energy deficit invoked by restricting food intake. In this instance circulating acylated ghrelin was consistently elevated throughout the extended period of observation as the postprandial suppression in response to each test meal was severely weakened after consuming meals of restricted energy/nutrient content.

The data reported within this thesis therefore show that acylated ghrelin is not sensitive to acute changes in energy expenditure resulting from exercise. Conversely, acylated ghrelin is sensitive to food/nutrients passing through the mouth and gastrointestinal tract. It remains possible that changes in circulating acylated ghrelin may have been seen if participants had completed repeated bouts of exercise. Hagobian et al (2009) recently showed that circulating acylated ghrelin responses to test meals were significantly attenuated i.e. the postprandial suppression was less marked, after four

consecutive days of exercise in a group of overweight females. This response was independent of the energy deficit induced by exercise as this response was still apparent when participants increased their energy intake to buffer that expended during exercise. Thus, exercise on its own perturbed acylated ghrelin in a direction expected to increase appetite and energy intake. Interestingly, this intervention did not alter levels of acylated ghrelin in a group of males, possibly representing a stronger biological imperative to defend body fat stores in females. It is therefore possible that in males, acylated ghrelin levels may only respond to more demanding exercise challenges when energy homeostasis is severely perturbed over several days.

10.6 Peptide YY_{3-36} responses to exercise and reduced food intake

In the final study presented within this thesis circulating concentrations of PYY_{3-36} were measured. The decision to measure PYY_{3-36} rather than other appetite regulating gut hormones was based on an expanding body of literature regarding the central role of PYY in appetite regulation (Ueno et al, 2008; Neary and Batterham, 2009b) and yet a dearth of research which has examined the influence of exercise on PYY. At present only a handful of studies have examined total PYY responses to exercise (Martins et al, 2007; Broom et al, 2009; Shorten et al, 2009; Ueda et al, 2009). Moreover, only one investigation has made measurements of circulating PYY_{3-36} (Cheng et al, 2009), the peptide variant chiefly responsible for determining the appetite suppressive action of PYY. In study six (Chapter nine), circulating PYY_{3-36} levels were 27% higher than control 30 min after completing 90 min of high intensity treadmill running (69.8 ± 0.9% of $\dot{V}O_2$ max). Circulating concentrations of PYY_{3-36} also remained visibly higher (2 – 9 h AUC, 11%) within the seven hours after exercise although this difference was not quite statistically significant. Enhanced circulating levels of PYY_{3-36} after exercise may represent a beneficial effect for appetite control. Circulating levels of PYY_{3-36} are lower in overweight than lean individuals and this has been linked to impaired perceptions of satiety (Le Roux et al, 2006). Future research may therefore seek to examine the effects of exercise on PYY_{3-36} in overweight individuals as the findings may be of clinical relevance.

10.7 Limitations and future directions

There are some notable limitations of the studies presented in this thesis and these have been identified within each experimental chapter. Two limitations which are common across studies are the low number of study participants and their homogeneity in terms of their physical characteristics and demographics. Assessment of a larger number of more diverse participants may have increased the ability to detect important relationships between variables and enhanced the ability to extrapolate findings to wider populations. Notwithstanding, future studies are needed to characterise gut hormone, appetite and food intake responses to exercise in other important participant groups such as overweight/obese, the elderly and those who are completely sedentary. Furthermore, recent work has unveiled differences in gut hormone and appetite responses to exercise in males versus females (Hagobian et al, 2009) therefore it would be interesting to investigate these initial findings further. Studies seeking to provide a mechanistic understanding for the findings presented here are also welcomed.

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APPENDIX A

INFORMED CONSENT FORM

(to be completed after the 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

HEALTH SCREEN QUESTIONNAIRE FOR STUDY VOLUNTEERS Name/Number

- 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.

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	☐
(q)	High cholesterol………………………………………...	Yes	☐	No	☐
(r)	High triacylgycerol or any other form of dyslipidaemia…………………………………………...	Yes	☐	No	☐
(s)	Food allergies of any kind…………………………….	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 ☐

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 answer true/false

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

   True □ False □

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

    True □ False □

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

    True □ False □

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

    True □ False □

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

    True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

30. I eat anything I want, anytime I want

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

Part 2:

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

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

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

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

39. How often do you feel hungry?

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

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

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

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

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

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

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

43. How frequently do you avoid ‘stocking up’ on tempting foods?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

0
Eat whatever you want, whenever you want it

1
Usually eat whatever you want, whenever you want it

2
Often eat whatever you want, whenever you want it

3
Often limit food intake, but often ‘give in’

4
Usually limit food intake, rarely ‘give in’

5
Constantly limiting food intake, never ‘give in’

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

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

Scoring

One point is given for each item in Part 1 and each item in part 2. The correct item for the true/false section is in brackets next to the respective question number within the section below. The direction of the questions in part 2 is determined by splitting responses at the middle. If the item is labelled '(+)', those responses above the middle are given zero. Vice versa occurs for those with a '(-)'.

Question numbers refer to the following factors:

Dietary restraint: **4**(T), **6**(T), **10**(F), **14**(T), **18**(T), **21**(F), **23**(T), **28**(T), **30**(F), **32**(T), **33**(T), **35**(T), **37**(+), **38**(+), **40**(+), **42**(+), **43**(+), **44**(+), **46**(+), **48**(+), **50**(+)

Disinhibited eating: **1**(T), **2**(T), **7**(T), **9**(T), **11**(T), **13**(T), **15**(T), **16**(F), **20**(T), **25**(F), **27**(T), **31**(F), **36**(T), **45**(+), **49**(+), **51**(+)

Hunger: **3**(T), **5**(T), **8**(T), **12**(T), **17**(T), **19**(T), **22**(T), **24**(T), **26**(T), **29**(T), **34**(T), **39**(+), **41**(+), **47**(-)

Suggested cut-points

Dietary restraint: 0-10 = low restraint, 11-13 = high restraint, 14-21 = clinical range

Disinhibited eating: 0-8 = low disinhibition, 9-11 = high disinhibition, 12-16 = clinical range

Hunger: 0-7 = low susceptibility to hunger, 8-10 = high susceptibility to hunger, 11-14 = clinical range

Source

Stunkard AJ, Messick SM. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of psychometric research*. 1985. 29(1): 71-83.

APPENDIX E

Subject Number:______ Trial__________ Date:_______

Visual Analogue Scale
Time:

Place a mark on the horizontal lines below after considering the

How hungry do you feel?

I am not hungry |———————————| I have never been

How satisfied do you feel?

I am completely |———————————| I cannot eat

How full do you feel?

Not at all |———————————| Totally

How much do you think you can eat?

Nothing at |———————————| A

Temperature (°C)	Humidity (%)

APPENDIX F

Food Preferences Questionnaire

To complete, please assign each food item a rating on each 10 point scale after considering the following anchors: 1= *dislike extremely;* 10 *like extremely*

Please indicate your selection by circling the relevant option

Coco-pops

1 2 3 4 5 6 7 8 9 10

Cornflakes

1 2 3 4 5 6 7 8 9 10

Frosties

1 2 3 4 5 6 7 8 9 10

Nutri-grain bars

1 2 3 4 5 6 7 8 9 10

White bread

1 2 3 4 5 6 7 8 9 10

Brown bread

1 2 3 4 5 6 7 8 9 10

Ham

1 2 3 4 5 6 7 8 9 10

Tuna

1 2 3 4 5 6 7 8 9 10

Cheese

1 2 3 4 5 6 7 8 9 10

Butter

1 2 3 4 5 6 7 8 9 10

Mayonnaise

1 2 3 4 5 6 7 8 9 10

Margarine

1 2 3 4 5 6 7 8 9 10

Crisps (ready salted)

1 2 3 4 5 6 7 8 9 10

Apple

1 2 3 4 5 6 7 8 9 10

Orange

1 2 3 4 5 6 7 8 9 10

Banana

1 2 3 4 5 6 7 8 9 10

Muffins

1 2 3 4 5 6 7 8 9 10

Cookies

1 2 3 4 5 6 7 8 9 10

Chocolate rolls

1 2 3 4 5 6 7 8 9 10

Milk

1 2 3 4 5 6 7 8 9 10

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

APPENDIX G

Buffet items available at the cold buffet meals.
(used in all studies)

Cereals (3 varieties)
Milk
White Bread
Brown Bread
Cheddar Cheese
Ham
Tuna
Salted Crisps
Mayonnaise
Butter
Margarine
Apple
Orange
Banana
Chocolate Rolls
Muffins
Cookies
Nutri-grain bars
Chocolate bar

APPENDIX H

Buffet items available at hot buffet meals
(used in study four/Chapter seven)

Rice or Pasta
Sweet & Sour Sauce – Tomato Sauce – Curry Sauce
Naan bread or Bread Rolls
Ice cream – Yoghurt – Tinned Mixed Fruit