

THE EFFECTS OF EXERCISE ON APPETITE REGULATION

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Abstract

The effects of exercise on appetite and feeding responses can be influenced by several factors. Research has demonstrated that exercise-induced changes in appetite can be affected by ambient temperature. Furthermore, exercise intensity has also been shown to affect appetite and post-exercise caloric intake. The aim of this thesis was to investigate the impact of exercise at different ambient temperatures on appetite and energy intake (EI) in overweight and obese individuals. Furthermore, this thesis also aimed to examine the effects of high intensity exercise on both peripheral and central appetite regulation in lean healthy males.

The findings from this thesis demonstrated that exercise in a cold environment (8°C) stimulated post-exercise EI in overweight and obese men and women compared with exercise in a neutral environment (20°C). Exercise in the heat (32°C) caused an increase in desire to eat 5 hours post-exercise compared with rest in the heat in overweight and obese individuals, however no further differences in appetite sensations were observed between trials. Findings from this thesis have also demonstrated that an acute bout of intense running suppressed neural activation within the orbitofrontal cortex and hippocampus in response to images of high-calorie foods compared with rest. Furthermore, pictures of low-calorie foods enhanced activation within the insula and putamen post-exercise compared with rest. These central regions are associated with regulating the rewarding properties of food, therefore these findings showed that high intensity exercise is capable of suppressing the rewarding properties of high-calorie foods whilst enhancing the rewarding properties of low-calorie foods immediately post-exercise. However, an acute bout of intense running enhanced central reward system activation in response to food cues compared with rest several hours after exercise. Therefore, the appetite suppressing effects of an acute bout of high intensity exercise could be short-lived.

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CHAPTER 1 - GENERAL INTRODUCTION

1.1 The Increasing Prevalence and Cost of Obesity

Obesity is recognised as a global epidemic. The treatment of obesity-related diseases is rapidly becoming the greatest economic burden faced by national health care services across the world (Withrow and Alter, 2010). Forecasts conducted by the UK based Foresight Project reported that if the current obesity trends continue, by 2025 approximately 47% of men and 36% of women in the UK will be obese (Jebb *et al.* 2007). In 2005 the World Health Organisation (WHO) reported that worldwide obesity had almost doubled since 1980, and if obesity trends continue unchecked then by 2015 approximately 230 billion adults worldwide will be overweight and more than 700 million will be obese (WHO Report, 2006). Research has demonstrated that a BMI of 30-35 kg/m² can reduce median survival by 2-4 years, and a BMI of 40-45 kg/m² can shorten median survival by 8-10 years (Prospective Studies Collaboration, 2009). Obesity is associated with a number of health related disorders such as diabetes mellitus, hypertension, and coronary heart disease (Thompson *et al.* 1999; Mokdad *et al.* 2003). The Foresight project estimates that obesity-related diseases cost the Nation approximately £7 billion in 2007, and Foresight forecasts that this figure will rise to £49.9 billion in 2050 (Jebb *et al.* 2007). It is evident that the effects of obesity on public health and the economy are considerable, therefore interventions designed to prevent or suppress the impact of obesity may be of great benefit (Thompson *et al.* 1999).

1.2 The Effects of Exercise on Appetite and Feeding Behaviour

Exercise is often recommended to individuals who are attempting to reduce their body weight, as exercise increases energy expenditure (Donnelly *et al.* 2009). However, increasing energy expenditure through exercise creates an energy deficit, which may be compensated through an increase in energy intake (EI) thereby restoring energy balance (EB), or potentially creating a positive EB (Blundell and King, 2000). The effect of exercise on appetite and feeding responses can be influenced by several factors. Research has demonstrated that exercise-induced changes in appetite can be affected by the ambient temperature in lean active participants (Dressendorfer, 1993; see Table 1). However, no research has been conducted investigating the effects of exercise in different ambient temperatures on the appetite responses of overweight individuals. Furthermore, intense bouts of exercise have also been shown to affect sensations of appetite (Broom *et al.* 2007, 2009; see Table 2a). Whereas, acute bouts of low-moderate intensity exercise have little or no effect on feeding behaviour (Imbeault *et al.* 1997; Unick *et al.* 2010; see Tables 2b and 2c). Despite inducing large energy deficits, appetite and EI may not respond in a compensatory manner following acute bouts of high intensity exercise (King *et al.* 2011b). Paradoxically, previous research has shown that high intensity exercise is capable of suppressing appetite (Broom *et al.* 2007, 2009; Burns *et al.* 2007; see Table 2a), and modulating peripheral appetite hormones in such a way as to enhance satiety during and after exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a, 2011a; see Table 2a). This phenomenon is referred to as exercise-induced anorexia (King *et al.* 1994).

The impact of exercise intensity and environmental temperature on appetite and EI could be related to changes in blood flow during exercise, and the effects this has on the circulation of short-acting appetite regulating hormones located in the gut. Previous research has demonstrated that when thermoregulation is challenged by exercising at a high intensity

or in a hot environment, to defend core body temperature blood flow to the skin is enhanced to dissipate excess heat (Kenney and Johnson, 1992). As blood flow to the skin is increased blood flow to the splanchnic region is reduced, and this is most evident during high intensity exercise (Rowell *et al.* 1964). Redistribution in blood flow to the skin during strenuous exercise and reductions in splanchnic blood flow could potentially regulate the effects of exercise on appetite, as the splanchnic region is the predominant site for the production and secretion of peripheral appetite regulating hormones (Wren and Bloom, 2007). Several hormones secreted from the gut have been shown to mediate appetite and EI responses following exercise (for a review see Stensel *et al.* 2010). This thesis will focus on two appetite regulating hormones which have been shown to be affected by acute bouts of exercise, namely the orexigenic hormone ghrelin and the anorexigenic hormone PYY (Broom *et al.* 2009; King *et al.* 2011b; see Table 2a). The aim of this thesis was to investigate the impact of exercise at different ambient temperatures on appetite regulating gut hormones, appetite sensations and EI in overweight and obese individuals. Furthermore, this thesis also aimed to examine the effects of high intensity exercise on peripheral and central appetite regulation.

1.3 Exercise and Appetite Regulation in Different Ambient Temperatures

Gwinup, (1987) was the first to postulate that exercise in a cold environment may increase caloric intake in human participants. Gwinup, (1987) compared the impact of walking, stationary cycling, and swimming on weight loss over a 6 month period in overweight and obese young women. The findings from this study demonstrated that participants in the walking and cycling groups lost weight over the intervention period, whereas participants in the swimming group experienced weight gain. Gwinup, (1987)

suggested that the reason why walkers and cyclists lost weight but swimmers did not was due to the different environments in which participants exercised. The author speculated that swimming in cold water may somehow stimulate post-exercise EI and negate the energy expended when swimming, resulting in a positive energy balance and weight gain.

Overweight individuals are often advised to participate in non-weight bearing activities, such as swimming, as they can often experience orthopaedic problems while exercising. However, anecdotal evidence postulates that swimming may enhance the drive to eat (O'Connor and Caterson, 2006). Furthermore, swimmers have been reported to have greater body fat stores when compared with runners, and it has been speculated that a greater caloric intake following swimming may be responsible (Flynn *et al.* 1990). King *et al.* (2011a) recently investigated the effects of swimming on hunger and food intake in lean males. The authors demonstrated that 1 hour of swimming did stimulate hunger, but post-exercise food intake was not affected (see Table 2b). The potential effects of swimming on appetite could be influenced by several factors including water immersion, as previous research has shown that immersion in cold and warm water stimulates food intake with no differences between temperatures (Halse *et al.* 2011). Furthermore, exercise posture may influence appetite responses to swimming, as supine exercise has been shown to increase skin vasodilation (Roberts and Wenger, 1980) and reduce splanchnic blood flow (Bradley *et al.* 1956) when compared with upright exercise. Dressendorfer, (1993) removed the supine element of exercise in cold water and investigated the effect of high-intensity cycling (70% $\text{VO}_{2\text{max}}$) in cold water (22°C), warm water (34°C), and cycling on land in a neutral environment (24°C), on post-exercise EI (see Table 1). The author observed that exercising in cold water significantly increased post-exercise EI compared with exercising on land, whilst exercising in warm water significantly suppressed post-exercise EI compared with exercising in cold water and exercising on land. The study also found that rectal temperature

(T_{re}) during exercise was inversely related to post-exercise EI. Exercising in cold water reduced T_{re} and increased EI, whereas exercising in warm water increased T_{re} and reduced EI. More recent research conducted by White *et al.* (2005) confirms previous observations regarding cold water exercise and appetite stimulation (see Table 1). Healthy young males cycled at 60% $\text{VO}_{2\text{max}}$ for 45 min in cold (20°C) and neutral water (33°C), and rested on land. Participants consumed 44% more calories following exercise in cold water compared with exercise in neutral water, and 41% more calories compared with control. Furthermore, cold water cycling resulted in significantly greater fat intake post-exercise compared with neutral water cycling and control. Tympanic temperature (T_t) was also measured during exercise, and findings demonstrated that T_t was significantly lower during cold water cycling compared with neutral water cycling. However, the actual temperature difference between the two trials was 0.3°C, therefore this is unlikely to have been the main physiological factor responsible for the differences in EI (White *et al.* 2005). Based on the findings of Dressendorfer, (1993) and White *et al.* (2005) it appears that exercise in cold water stimulates appetite, whilst exercise in warm water suppresses appetite. However, the mechanisms responsible for these responses are unclear. In agreement with White *et al.* (2005), it is this author's opinion that a redistribution of blood flow could be one of the mechanisms by which circulating gut hormones mediate appetite responses to different ambient temperatures. Blood flow to the splanchnic region is reduced during exercise in thermoneutral conditions (Froelich *et al.* 1988), and a large proportion of splanchnic blood flow is shunted to the skin surface to dissipate the metabolic heat generated during exercise (Rowell *et al.* 1964; Froelich *et al.* 1988). Exercise in a hot environment causes a reduction in splanchnic blood flow that is significantly greater than the splanchnic blood flow reductions observed in a thermoneutral environment (Rowell *et al.* 1965). Blood flow redistribution away from the splanchnic region during exercise in the heat could cause a reduction in the volume of blood available

for the circulation of appetite regulating gut hormones, and thus affect EI. Furthermore, during exercise in the cold, blood flow to the splanchnic region may not be reduced as much as during exercise in the heat, as cold exposure stimulates cutaneous vasoconstriction (Kellogg, 2006). Therefore, more blood may be available for the circulation of appetite regulating gut hormones, such as ghrelin.

Ghrelin is a short-acting appetite regulating hormone which is produced within the stomach (Asakawa *et al.* 2001; Date *et al.* 2002). Studies which have investigated the effect of ghrelin on appetite response have found that ghrelin significantly increases energy intake compared to a placebo in a dose dependent manner in males and females (Wren *et al.* 2001, Schmid *et al.* 2005), and in lean and obese individuals (Druce *et al.* 2005). Tomasik *et al.* (2005) examined the effects of different ambient temperatures on the circulation of ghrelin (see Table 1). Participants rested in a warm (30°C), neutral (20°C), and cold (2°C) environment for 30 min, after which blood samples were taken for the determination of total plasma ghrelin concentrations. Exposure to the cold environment resulted in significantly elevated concentrations of ghrelin compared with the neutral environment, conversely exposure to the warm environment caused a significant reduction in ghrelin concentrations compared with the neutral environment. Elevated concentrations of ghrelin in human circulation are associated with increased hunger and EI, whereas ghrelin concentrations are suppressed when satiated (Cummings *et al.* 2001). Tomasik *et al.* (2005) did not measure EI post-exposure, however based on the observed ghrelin responses it could be postulated that EI may have been increased after cold exposure, and decreased after warm exposure. These findings partially support the White *et al.* (2005) theory that appetite hormones could respond differently when exercising at different ambient temperatures, however participants did not exercise during the Tomasik *et al.* (2005) study.

Shorten *et al.* (2009) was the first study to investigate the effects of exercising in the heat on appetite regulating hormones and post-exercise *ad libitum* EI (see Table 1). The authors reported that 40 min of high intensity exercise at 36°C significantly reduced relative energy intake (energy intake relative to energy expended during exercise; REI) compared with the resting control trial (25°C). The effects of exercising in the heat on REI could be related to the significantly greater concentrations of peptide YY (PYY) observed prior to the *ad libitum* meal (Shorten *et al.* 2009). Peptide YY is a short-acting anorexigenic gut hormone, which has been shown to reduce EI when infused into human circulation (Batterham *et al.* 2002). Furthermore, Shorten *et al.* (2009) found that T_i was significantly higher prior to the meal following exercise in the heat compared with exercise in the neutral environment. This finding is in agreement with previous research which has investigated the effects of exercise in the cold on EI and suggested that a fall in core temperature may be associated with an increase in EI (Dressendorfer, 1993; White *et al.* 2005). Furthermore, White *et al.* (2005) speculated that changes in the redistribution of blood flow to the skin following exercise in the cold may modify the circulation of gut derived appetite hormones, and thus affect post-exercise EI. This could be one of the potential mechanisms responsible for the different levels of EI observed after exercise in cold and warm environments. However, only Shorten *et al.* (2009) has investigated the effects of exercise at different ambient temperatures on appetite hormone responses and food intake (see Table 1). Furthermore, no studies have examined the effects of exercise at different temperatures on hunger and appetite hormone circulation in overweight and obese individuals (see Table 1). As previously mentioned, swimming in cold water may stimulate EI (Gwinup, 1987), however several mechanisms may influence the effects of swimming on EI, such as water temperature (Halse *et al.* 2011) and posture (Roberts and Wenger, 1980). **Chapter 2** of this thesis will describe a study which has isolated temperature and examined the effects of brisk

walking in the cold on post-exercise EI, thermoregulatory responses, and the circulation of ghrelin and PYY in overweight and obese men and women. Furthermore, **chapter 3** examines the effects of exercise in the heat on subjective appetite sensations, thermoregulatory responses, and the circulation of ghrelin and PYY in overweight and obese men and women.

Table 1: The Effects of Exercise and Rest at Different Ambient Temperatures on Appetite Regulation

Authors	Participants	Trials	Measurements	Findings
Dressendorfer, (1993)	6 trained males	30 min cycle (70% $\dot{V}O_{2max}$) in CW (22°C), WW (34°C) + on land (24°C) 30 min rest on land	EI T_{re}	CW EI \uparrow vs rest + 24 °C WW EI \downarrow vs 24°C + CW -ve correlation EI + T_{re}
Tomasik <i>et al.</i> (2005)	17 healthy males	30min rest at 2°C, 20°C + 30°C	Plasma total ghrelin T_a	Total ghrelin \uparrow after 2°C vs 20°C Total ghrelin \downarrow after 30°C vs 20°C
White <i>et al.</i> (2005)	11 healthy males	45 min cycle (60% $\dot{V}O_{2max}$) in CW (20°C) + NW (33°C) 45 min rest on land	EI T_t	EI after CW was \uparrow vs NW T_t \uparrow during NW vs CW
Shorten <i>et al.</i> (2009)	11 active males	40 min run (70% $\dot{V}O_{2peak}$) at 25°C + 36°C 40 min rest at 25°C	Plasma Ac-ghrelin and total PYY EI T_t	No differences in Ac-ghrelin PYY \uparrow pre- + post-meal in 36°C trials vs rest REI \downarrow after 36°C vs rest T_t \uparrow pre-meal in 36°C vs 25°C trials
Halse <i>et al.</i> (2011)	10 active males	40 min run (70% $\dot{V}O_{2peak}$) + 20 min in CW (15°C) + NW (33°C) Post-exercise 20 min rest	Plasma Ac-ghrelin and total PYY EI	EI \uparrow after CW and NW vs rest

Ac = acylated ghrelin; CW = cold water; EI = energy intake; NW = neutral water; T_a = axillary temperature; T_{re} = rectal temperature; T_t = tympanic temperature; WW = warm water

1.4 Exercise Intensity and Appetite Regulation

For the purposes of this thesis I have defined low-moderate intensity exercise as cycling or walking performed at an intensity of 35-69% $\text{VO}_{2\text{max}}$ /40-69% Hr max.

Furthermore, high intensity exercise was defined as cycling or running performed at an intensity of 70% $\text{VO}_{2\text{max}}$ /70% Hr max or above.

1.4.1 High intensity exercise

Early research conducted by Larue-Achagiotis and Louis-Sylvestre, (1987) on rats demonstrated that acute bouts of high intensity exercise reduced post-exercise caloric intake (see Table 2a). More recent research conducted by Ueda *et al.* (2009a) found that 30 min of high intensity cycling (75% $\text{VO}_{2\text{max}}$) suppressed *ad libitum* EI 60 min post-exercise in human participants. Furthermore, the authors also reported that subjective sensations of hunger were suppressed during exercise. This finding is in agreement with Burns *et al.* (2007) who examined the effects of a 60 min high intensity run (75% $\text{VO}_{2\text{max}}$) on subjective sensations of hunger during, and for 2 hours after exercise, compared with rest. The authors demonstrated that hunger was significantly suppressed during exercise and during the first hour of the recovery period compared with rest. Lann *et al.* (2010) also observed a reduction in feelings of hunger immediately following 35 min of high intensity cycling (70% Hr reserve); however hunger increased above baseline levels 30 min post-exercise. Therefore, the findings reported by Laan *et al.* (2010) indicate that following initial exercise-induced hunger suppression, hunger may ‘rebound’ post-exercise. However, this study only examined the impact of high intensity exercise on hunger for a short period of time. Broom *et al.* (2007) examined exercise-induced appetite responses for 8 hours after a 60 min bout of high intensity running (75% $\text{VO}_{2\text{max}}$; see Table 2a). The authors also observed a significant reduction in hunger ratings during and immediately after exercise compared with rest.

However, after a standardized meal provided 1 hour post-exercise/rest, this trend was reversed and hunger ratings tended to be greater following exercise. These findings indicate that high intensity exercise suppresses feelings of hunger during and immediately after exercise (Burns *et al.* 2007; Broom *et al.* 2007; Lann *et al.* 2010; see Table 2a), but this effect may be transient and hunger can increase several hours post-exercise (Broom *et al.* 2007). However, despite evidence indicating that high intensity exercise stimulates hunger during the hours following exercise, research has shown that this does not necessarily correspond to an increase in EI (King *et al.* 1997; King *et al.* 2010a; see Table 2a). Studies which have examined the effects of acute high intensity bouts of exercise on appetite and food intake over an extended period of time (24-48 hours) have found that increased exercise-induced energy expenditure does not cause a compensatory feeding response (King *et al.* 1997; King *et al.* 2010a; see Table 2a). Furthermore, research which has investigated the impact of daily acute bouts of high intensity exercise on energy intake over several days and weeks have demonstrated that there is a weak coupling between exercise-induced energy expenditure and energy intake (Stubbs *et al.* 2002ab; Whybrow *et al.* 2008; Martins *et al.* 2010). Consequently, bouts of high intensity exercise may substantially increase energy expenditure without causing a compensatory increase in EI immediately following exercise, or during several weeks of exercise training. However, caution should be observed when comparing studies which have observed the effects of acute high intensity exercise bouts on appetite regulation for several hours and studies which have examined the impact of chronic exercise training on appetite regulation. Regular bouts of exercise performed over several days and weeks can cause alterations in body composition and resting metabolic rate (Leidy *et al.* 2004), and changes in these factors can mediate physiological changes in the secretion of appetite hormones (Leidy *et al.* 2004, 2007, Foster-Schubert *et al.* 2005). Therefore, acute exercise and chronic exercise may affect appetite regulation via different mechanisms.

1.4.2 Low-Moderate intensity exercise

In contrast to high-intensity exercise findings indicate that moderate intensity exercise does not affect appetite regulation following exercise (George and Morganstein, 2003; Tsofliou *et al.* 2003; King *et al.* 2010b; Unick *et al.* 2010; see Table 2b). Unick *et al.* (2010) reported that in overweight and obese women, an acute bout of walking (70-75% $\text{VO}_{2\text{max}}$) did not affect feelings of hunger or post-exercise EI compared with rest, however brisk walking did reduce REI. Reductions in REI were also reported by King *et al.* (2010b) who examined the influence of a 60 min brisk walk (~45% $\text{VO}_{2\text{max}}$) on EI and sensations of appetite in lean males. The authors continued to monitor appetite sensations and post-exercise EI during a 7 hour recovery period, but they did not observe any significant differences between exercise and rest. Furthermore, a very unique and interesting study conducted by Cornier *et al.* (2011) examined the impact of an acute bout of moderate intensity exercise, performed prior to and following 6 months of exercise training, on subjective feelings of appetite and central appetite regulation (see Table 2b). In order to assess the effects of exercise on central appetite regulation the authors used functional magnetic resonance imaging (fMRI) techniques to examine the neural responses to images of high-calorie foods. Results from the study found that exercise training suppressed neural responses to food, however appetite sensations and neural responses to food cues measured after acute exercise at baseline did not differ 6 months later. Observations from this study indicate that an acute bout of moderate intensity exercise does not modulate central appetite regulation. Therefore, it would appear that moderate intensity exercise has little or no effect on peripheral or central appetite regulation.

1.4.3 High intensity exercise vs. low-moderate intensity exercise

Kissileff *et al.* (1990) examined the effects of 40 min of high (90 watts) and moderate (30 watts) intensity cycling on post-exercise *ad libitum* food consumption and post-meal

appetite sensations in obese and non-obese women. The authors observed a reduction in the food intake of non-obese women following high intensity exercise compared with moderate intensity exercise. Exercise intensity did not affect the post-exercise EI of obese women; however obese women did report an increase in hunger following the test meal during the moderate intensity trials compared with the high intensity trials. The Kissileff *et al.* (1990) study does have two limitations though, the intensity of the exercise bouts were absolute and not calculated relative to each participants individual fitness level, and the intensity of the high intensity exercise bout (90W) could be considered quite mild for most individuals (see Table 2c). Imbeault *et al.* (1997) had no such limitations and compared the effects of high intensity (75% $\text{VO}_{2\text{max}}$) and moderate intensity (35% $\text{VO}_{2\text{max}}$) treadmill exercise in young males on appetite and reported no differences in feelings of hunger and fullness. Despite observing no differences in post-exercise EI between trials, post-exercise EI tended to be lower after high intensity exercise compared with low intensity exercise. Furthermore, REI was lower during high versus low intensity exercise trials. Conversely, Pomerleau *et al.* (2004) found that both moderate (40% $\text{VO}_{2\text{max}}$) and high intensity (70% $\text{VO}_{2\text{max}}$) walking stimulated food intake 1 hour post-exercise in active women. However, daily EI was not different between trials over the subsequent 3 days, indicating that the exercise-induced appetite stimulation was short-lived. These findings conflict with those of Ueda *et al.* (2009a) who observed a transient suppression in feelings of hunger and a reduced EI 1 hour after a 30 min bout of moderate (50% $\text{VO}_{2\text{max}}$) and high intensity (70% $\text{VO}_{2\text{max}}$) cycling compared with rest in healthy males. Despite discrepancies in the literature, a majority of studies have shown that high intensity exercise consistently suppresses appetite and EI (Kissileff *et al.* 1990; Imbeault *et al.* 1997; King *et al.* 1997; Broom *et al.* 2007, 2009; Burns *et al.* 2007; King *et al.* 2010a; see Table 2c). Whereas, a majority of research has reported that acute bouts of moderate intensity exercise either increase (Pomerleau *et al.* 2004; see

Table 2c), or have no effect on appetite and feeding behaviour (Imbeault *et al.* 1997; George and Morganstein, 2003; Tsofliou *et al.* 2003; King *et al.* 2010b; Unick *et al.* 2010; see Table 2c). Furthermore, high intensity exercise has been shown to influence peripheral appetite regulating hormones (Broom *et al.* 2007, 2009; King *et al.* 2011b; see Table 2a), whereas moderate intensity exercise has not (King *et al.* 2010b; Unick *et al.* 2010; see Table 2b). In addition, novel findings reported by Cornier *et al.* (2011) suggest that acute bouts of moderate intensity exercise may not affect central appetite regulation either. Research has shown that changes in the circulation of peripheral appetite hormones can modulate central appetite regulation (Batterham *et al.* 2007; Malik *et al.* 2008); therefore it would appear reasonable that if moderate intensity exercise does not influence appetite hormone circulation then it is unlikely to influence the activity of central regions which mediate appetite responses. However, as high intensity exercise has the capacity to modify peripheral appetite regulating hormones in such a way as to enhance satiety, one might speculate that high intensity exercise has the ability to suppress activation in regions of the brain which regulate hunger. Nevertheless, the effects of high intensity exercise on central appetite regulation currently remain unknown. **Chapters 4 and 5** of this thesis describe two novel studies which have examined the modulating effects of high intensity exercise on neural responses to pictures of food.

Table 2a: The Effects of High Intensity Exercise on Appetite, Energy Intake and, Appetite Regulating Hormones

Authors	Participants	Trials	Measurements	Findings
Larue-Achagiotis & Louis-Sylvestre, (1987)	15 male rats	Swim (4 x 15 min bouts) Rest	Test meal EI	EI ↓ 1,3 + 12-h post-exercise vs rest
King <i>et al.</i> (1997)	9 healthy males	50 min run (70% Hr max) 50 min rest	Self-reported EI for 2 days Appetite VAS	No differences in EI AUC hunger values ↓ on day 1 of the exercise trial vs day 2
Broom <i>et al.</i> (2007)	9 healthy males	1-h run (75% VO_{2max}) + 8-h rest 9-h rest	Plasma Ac-ghrelin Appetite VAS	Ac-ghrelin ↓ at 30 min of exercise vs rest Ac-ghrelin AUC ↓ during first 3-h + full 9-h of exercise vs rest trials
Burns <i>et al.</i> (2007)	9 males + 9 females	1-h run (75% VO_{2max}) + 2-h rest 3-h rest	Plasma total ghrelin Appetite VAS	Total ghrelin unaffected by exercise Hunger ↓ during run + 1-h post-run vs rest No sex differences
Marzullo <i>et al.</i> (2008)	8 OB males, 8 lean males	Cycle test to exhaustion	Plasma Ac-ghrelin + serum total ghrelin	Ac-ghrelin ↓ at exercise peak vs baseline in both groups No effects of exercise on total ghrelin
Broom <i>et al.</i> (2009)	11 active males	1-h run (70% VO_{2max}) + 7-h rest 90 min resistance exercise (80% 12RM) + 6.5-h rest 8-hr rest	Plasma Ac-ghrelin + total PYY Appetite VAS	Ac-ghrelin ↓ during run and resistance exercise vs rest PYY ↑ during run vs rest Hunger ↓ during run and 30 min post-run vs rest

Ac = acylated ghrelin; AUC = area under the curve; EI = energy intake; OB = obese; PFC = prospective food consumption; RM = Repetition maximum; VAS = visual analogue scales

Table 2a cont...

Authors	Participants	Trials	Measurements	Findings
Laan <i>et al.</i> (2010)	9 males + 10 females	35 min cycle (70% Hr reserve) 35 min resistance exercise (70% 1RM) 35 min rest	<i>Ad libitum</i> EI Appetite VAS	EI ↑ post-cycle and resistance exercise vs rest Hunger ↓ during cycle vs rest Hunger ↑ above baseline levels pre-meal
King <i>et al.</i> (2010a)	9 healthy males	90 min run (70% VO _{2max}) + 8.5-h rest 10-h rest	Plasma Ac-ghrelin <i>Ad libitum</i> EI Appetite VAS	Ac-ghrelin, hunger + PFC ↓ during run vs rest No differences in EI on day of trial or 24-h after
King <i>et al.</i> (2011b)	12 active males	90 min run (70% VO _{2max}) + 7.5-h rest (Ex-def) 9.5-h energy deficit equal to run (Food-def) 9.5-h rest	Plasma Ac-ghrelin + PYY ₃₋₃₆ <i>Ad libitum</i> EI Appetite VAS	Ac-ghrelin, EI, hunger, + PFC ↑ during Food-def vs Ex-def + rest trials PYY ₃₋₃₆ ↓ during Food-def vs Ex-def + rest trials

Table 2b: The Effects of Low-Moderate Intensity Exercise on Appetite, Energy Intake and, Appetite Regulating Hormones

Authors	Participants	Trials		Measurements	Findings
George and Morganstein, (2003)	24 females (12 OW, 12 lean)	1-h walk (60% Hr max) 1-h rest		<i>Ad libitum</i> EI	Exercise had no effect on EI
Tsofliou <i>et al.</i> (2003)	10 OB females	20 min brisk walk 20 min snack 30 min rest		<i>Ad libitum</i> EI Appetite VAS	No differences in EI Satiety and fullness ↑ post-walk vs rest
Ueda <i>et al.</i> (2009b)	7 OB + 7 lean males	1-h cycle (50% VO _{2max}) 1-h rest		Plasma Ac-ghrelin + total PYY <i>Ad libitum</i> EI Appetite VAS	No differences in Ac-ghrelin or appetite PYY ↑ following exercise vs rest EI ↓ following exercise vs rest
Martins <i>et al.</i> (2007)	6 males + 6 females	1-h cycle (65% Hr max) 1-h rest		<i>Ad libitum</i> EI Appetite VAS	EI ↑ post-exercise vs rest REI ↓ during exercise vs rest Hunger ↓ during exercise vs rest
King <i>et al.</i> (2010b)	14 healthy males	60 min walk (45% VO _{2max}) + 7-h rest 8-h rest		Plasma Ac-ghrelin <i>Ad libitum</i> EI Appetite VAS	No differences in Ac-ghrelin, EI, or appetite REI ↓ during exercise vs rest trials
Unick <i>et al.</i> (2010)	19 OW females	~45 min walk (70-75% Hr max) + 2-h rest ~35 min rest		Plasma Ac-ghrelin <i>Ad libitum</i> EI Appetite VAS	No differences in Ac-ghrelin, EI, or appetite REI ↓ during exercise trial vs rest
King <i>et al.</i> (2011a)	14 healthy males	1-h swim + 6-h rest 7-h rest		Plasma Ac-ghrelin <i>Ad libitum</i> EI Appetite VAS	Ac-ghrelin, hunger + PFC ↓ during swimming vs rest No differences in EI Hunger ↑ several hours after swim vs rest
Cornier <i>et al.</i> (2011)	12 OW men + women	40-60 min walk (60-75% VO _{2max}) before + after 6 month exercise training regime		Neural responses to food cues Appetite VAS	No differences in neural responses or appetite sensations

Ac = acylated ghrelin; AUC = area under the curve; EI = energy intake; OB = obese; OW = overweight; PFC = prospective food consumption; REI = relative energy intake; VAS = visual analogue scales

Table 2c: Direct Comparison of the Effects of Low-Moderate Intensity Exercise vs High Intensity Exercise on Appetite, Energy Intake and, Appetite Regulating ..

Authors	Participants	Trials	Measurements	Findings
Kissileff <i>et al.</i> (1990)	18 female (9 lean, 9 OB)	40 min cycle at 90W + 30W 40 min rest	<i>Ad libitum</i> EI Appetite VAS	EI ↓ after high vs moderate intensity cycling in lean group Hunger ↑ after moderate vs high intensity cycling in OB group
Imbeault <i>et al.</i> (1997)	11 young males	~34 min run (75% VO _{2max}) ~72 min walk (35% VO _{2max}) ~72 min rest	<i>Ad libitum</i> EI Appetite VAS	No effect of exercise on EI, hunger ,or fullness REI ↓ after run vs walk and rest
Pomerleau <i>et al.</i> (2004)	13 active females	~40 min walk (70% VO _{2peak}) ~65 min walk (40% VO _{2peak}) 75 min rest	<i>Ad libitum</i> EI Appetite VAS	EI ↑ after high intensity walk vs rest REI ↓ post-high + low intensity exercise vs rest
Erdmann <i>et al.</i> (2007)	6 males + 8 females	Cycling intensity (30 min at 100W + 50W) Cycling duration (30, 60 +120min at 50W) 45 min rest	Plasma total ghrelin	Total ghrelin AUC ↑ during 50W cycle vs rest Total ghrelin unaffected by duration
Jürimäe <i>et al.</i> (2007)	9 male rowers	6.5 km row (above AT) 6.5 km row (below AT)	Plasma total ghrelin	Total ghrelin unaffected by exercise
Ueda <i>et al.</i> (2009a)	10 healthy males	30 min cycle (75% VO _{2max}) 30 min cycle (50% VO _{2max}) 30 min rest	Plasma PYY ₃₋₃₆ <i>Ad libitum</i> EI Appetite VAS	PYY ₃₋₃₆ ↑ after high vs moderate intensity exercise EI and hunger ↓ after high and moderate intensity exercise vs rest

AT = anaerobic threshold; EI = energy intake; OB = obese; REI = relative energy intake; VAS = visual analogue scales

1.5 Exercise Intensity and Appetite Regulating Hormones

1.5.1 Ghrelin

High intensity exercise appears to consistently suppress short-term feelings of appetite (King *et al.* 1997; Broom *et al.* 2007, 2009; Burns *et al.* 2007; King *et al.* 2010a; King *et al.* 2011b; see Table 2a). Therefore, one may expect to observe a reduction in circulating concentrations of ghrelin during, and for a short period of time after, exercise. Burns *et al.* (2007) observed suppressed feelings of hunger during and immediately after high intensity running, however ghrelin concentrations were not different between exercise and control trials. Similar hunger responses were reported by Broom *et al.* (2007), however this study did observe a significant reduction in ghrelin concentrations during exercise compared with control. The different ghrelin responses observed by Burns *et al.* (2007) and Broom *et al.* (2007) could be related to the different forms of ghrelin that were measured. Burns *et al.* (2007) measured total plasma ghrelin whereas Broom *et al.* (2007) measured the active form of ghrelin, known as acylated ghrelin, indicating that perhaps only the active form of ghrelin is capable of mediating exercise-induced appetite responses. Studies which have only measured total ghrelin during and after exercise have reported that exercise does not significantly affect total ghrelin concentrations (Erdmann *et al.* 2007; Jurimae *et al.* 2007; see Table 2c). Furthermore, Marzullo *et al.* (2008) demonstrated that acylated ghrelin levels were significantly suppressed during an exhaustive cycling test compared with baseline levels, whereas total ghrelin levels were unaffected by exercise. Several studies have shown that acylated ghrelin concentrations are significantly suppressed during and immediately after exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a, 2011b; see Table 2a). Furthermore, research examining the effects of exercise on ghrelin responses for several hours post-exercise have found that acylated ghrelin area under the curve (AUC) values are significantly

reduced compared with control values over a 9-10 hour period (Broom *et al.* 2007; King *et al.* 2010a). In addition, acylated ghrelin AUC values have been shown to correlate positively with appetite sensations after high intensity exercise (Broom *et al.* 2007). Therefore, exercise-induced ghrelin suppression may play a role in reducing appetite during and immediately after acute bouts of high intensity exercise.

1.5.2 Peptide YY

The majority of research has focused on the short-term mediating effects of ghrelin on exercise-induced appetite and EI responses; however the satiety enhancing hormone PYY has also received attention. Ueda *et al.* (2009b) observed a significant increase in total PYY concentrations compared with control following moderate intensity cycling, which was also accompanied by a reduction in post-exercise EI. When compared with rest, moderate and high intensity cycling both increased concentrations of PYY₃₋₃₆ (the active form of PYY) and reduced post-exercise food intake (Ueda *et al.* 2009a). Furthermore, the same study demonstrated that high intensity cycling increased levels of PYY₃₋₃₆ to a greater extent than moderate intensity cycling (Ueda *et al.* 2009a). Broom *et al.* (2009) investigated the impact of both high intensity running and resistance exercise on feelings of hunger and total PYY concentrations during exercise and for several hours after. Findings from the study demonstrated that a significant reduction in feelings of hunger observed during the run compared with control, were accompanied by a significant increase in PYY concentrations compared with resistance exercise and control. Furthermore, King *et al.* (2011b) observed suppressed hunger ratings and an increase in PYY₃₋₃₆ concentrations immediately following an intense bout of running compared with a food restriction trial. The authors also reported that postprandial PYY levels were elevated to a greater extent during exercise trials compared with control trials, indicating that exercise enhanced the satiating effects of a meal (King *et*

al. 2011b). Therefore, findings from previous research demonstrate that acute bouts of high intensity exercise stimulate PYY secretion, and that this may contribute to enhanced satiety following exercise (see Tables 2a and 2c).

1.6 Future Research Perspectives

The mechanisms by which different ambient temperatures and exercise intensities influence feeding behaviour require further research. Studies which have examined the effects of exercise in the cold on appetite responses, EI, and gut hormone circulation have thus far failed to isolate the impact of cold temperature from confounding factors such as posture and water immersion. Therefore, future research should attempt to separate the effects of cold temperature on appetite hormone circulation and post-exercise food intake from other factors which may influence appetite regulation. The impact that exercise in a warm environment can have on appetite and gut hormones has thus far only been examined in lean healthy individuals. The recruitment of overweight and obese individuals is essential for future research in order to improve our understanding of the effects of exercise on appetite regulation, and to assist with the development of effective exercise interventions for weight management. Therefore, future studies which aim to investigate the relationship between exercise in a warm environment and appetite should recruit overweight and obese men and women. Finally, extensive research has been conducted into the effects of acute bouts of high intensity exercise on peripheral appetite hormone circulation during exercise, immediately following exercise, and over an extended period of time post-exercise. However, no research has examined the impact of acute high intensity exercise on both peripheral and central appetite regulating mechanisms. In future, the effects of high intensity exercise on both peripheral and central appetite regulation needs to be examined immediately after exercise and over a prolonged period of time post-exercise.

1.7 Scope of the Thesis

This thesis contains 4 chapters which examine the effects of acute exercise on appetite and EI regulation. **Chapters 2 and 3** of this thesis describe the effects of acute exercise bouts in warm and cold environments on subjective appetite sensations, post-exercise EI, and appetite hormone circulation in middle-aged, overweight/obese men and women. **Chapter 2** isolated the effects of a cold environment on food intake and compared the effects of brisk walking in cold (8°C) versus neutral (20°C) conditions on post-exercise EI, and concentrations of ghrelin and PYY. Furthermore, this chapter investigated the effects of thermoregulatory responses (T_{re} and skin temperature) to exercise in the cold, and their relationship with EI and appetite hormone concentrations. **Chapter 3** examined the effects of brisk walking in a warm environment (32°C), compared with a cool environment (14°C), on appetite sensations, ghrelin concentrations, and PYY levels during exercise and for an extended period of time following exercise. **Chapters 4 and 5** of this thesis describe two studies which have for the first time examined the effects of high intensity exercise on neural responses to images of food, using fMRI techniques. **Chapter 4** investigated the effects of an acute bout of intense running on peripheral and central appetite regulation immediately following exercise in healthy males. **Chapter 5** is a pilot study that extends the study described in **Chapter 4** by reporting the effects of intense exercise on blood flow to appetite regulating regions of the brain when viewing pictures of food immediately post-exercise and several hours after exercise. Finally, **Chapter 6** discusses the findings from **Chapters 2-5** and the implications of these findings, as well as proposing future research directions.

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**CHAPTER 2 - THE EFFECTS OF
EXERCISING IN A COLD
ENVIRONMENT ON GHRELIN, PYY,
AND ENERGY INTAKE IN
OVERWEIGHT MEN AND WOMEN**

2.1 Abstract

Exercise whilst immersed in cold water has been shown to stimulate post-exercise energy intake (EI). To isolate the effects of cold temperature on post-exercise EI, this study investigated the impact of brisk walking in a cold (8°C) and neutral (20°C) environment on post-exercise EI and appetite hormone responses. Sixteen middle aged overweight men and women (age 50.1 ± 11.6 yr, body mass index 28.9 ± 4.2 kg·m⁻²) completed a 45 min treadmill walk at \square 60% VO_{2max} in both the cold and neutral conditions in a randomized, counterbalanced design. Participants were presented with an *ad libitum* buffet meal 45 min post-exercise and EI was covertly measured. Skin temperature (T_{sk}) and rectal temperature (T_{re}) were monitored throughout exercise and for 30 min post-exercise, and concentrations of the appetite hormones total ghrelin, acylated ghrelin, and total PYY were assessed pre- and post-exercise, and pre- and post-meal. Energy intake was significantly greater following exercise in the cold (1299 ± 657 kcal; mean \pm SD) compared with exercise in the neutral environment (1172 ± 537 kcal; mean \pm SD) ($P < 0.05$). Acylated ghrelin concentrations, relative to pre-exercise concentrations, were significantly greater after walking in the cold versus neutral condition ($P < 0.05$). Furthermore, total PYY AUC values were significantly lower during exercise in the cold ($P < 0.05$). These findings indicate that exercise in the cold stimulates post-exercise EI in overweight men and women, and that this may be modulated by changes in the circulation of acylated ghrelin and PYY during cold exercise.

Keywords: Exercise, cold, energy intake, ghrelin, PYY

2.2 Introduction

Obesity has been shown to contribute to a number of health related disorders such as diabetes mellitus, ischaemic heart disease, and certain forms of cancer (Prospective Studies Collaboration, 2009). In the UK approximately a quarter of adults are now obese, and by 2050 it is predicted that approximately 60% of men, 50% of women and 25% of children in the UK will be classified as obese (Jebb *et al.* 2007). The WHO predicts that by 2015 approximately 230 billion adults worldwide will be overweight and more than 700 million will be obese (WHO Report, 2006).

These obesity trends can potentially be attributed to an increase in energy intake (EI) and a reduction in energy expenditure, resulting in a positive energy balance (Blundell and King, 2000; Whybrow *et al.* 2008). Although energy balance is relatively simple, EI minus energy expenditure, the factors that govern these variables are multifaceted. Exercise is often recommended as an effective weight loss strategy as it increases energy expenditure (ACSM, 2001), which if unaccompanied by an increase in compensatory EI, will result in a negative energy balance. Studies which have examined the effects of exercise on EI and appetite sensations have demonstrated that acute high intensity exercise appears to transiently reduce hunger (Burns *et al.* 2007; Broom *et al.* 2007, 2009; Ueda *et al.* 2009a; King *et al.* 2010a) whilst also reducing post-exercise EI (Kissileff *et al.* 1990; Imbeault *et al.* 1997; Ueda *et al.* 2009a). Conversely, several studies have shown that acute moderate intensity exercise either does not affect, or causes an increase in, short-term appetite and EI (George and Morganstein, 2003; Finlayson *et al.* 2009; King *et al.* 2010b; Unick *et al.* 2010).

Swimming is often recommended to overweight individuals as they can often experience orthopaedic problems while exercising, and are therefore advised to participate in non-weight bearing activities. However, anecdotal evidence suggests that swimming in cold water stimulates post-exercise appetite (O'Connor and Caterson, 2006). Furthermore, Flynn

et al. (1990) speculated that increased EI after swimming could be a potential reason as to why swimmers typically have greater body fat stores when compared with runners. Therefore, if exercising in the cold results in appetite stimulation then the energy expended during swimming may be compensated for by an increase in EI (Gwinup, 1987). King *et al.* (2011) recently demonstrated that 1 hour of swimming did stimulate hunger sensations, but post-exercise food intake was unchanged. The potential effects of swimming on appetite could be influenced by a number of factors, including the temperature of the water, and/or exercise posture, as supine exercise has been shown to increase skin vasodilation (Roberts and Wenger, 1980) and reduce splanchnic blood flow (Bradley *et al.* 1956) when compared with upright exercise. Studies which have removed the supine element of exercise in cold water have demonstrated that upright exercise (cycling) in cold water stimulates post-exercise EI (Dressendorfer, 1993; White *et al.* 2005). Halse *et al.* 2011 found that immersion in water which is warm or cold stimulates post-immersion EI to a similar degree. However, exposure to warm and cold air temperatures has been shown to influence markers of appetite in different ways (Tomasik *et al.* 2005). Tomasik *et al.* (2005) demonstrated that concentrations of the appetite stimulating hormone ghrelin were elevated when resting in cold conditions and reduced in warm conditions. These findings indicate that the appetite stimulating effects of a cold environment may be mediated by ghrelin secretion.

Changes in EI and appetite are regulated by gastrointestinal satiety signals which act centrally to either suppress or stimulate appetite and EI (Simpson and Bloom, 2010; Zac-Varghese *et al.* 2010). Short-acting gastrointestinal signals include the gut peptides peptide YY (PYY) and ghrelin (Murphy and Bloom, 2006). PYY is released from the gastrointestinal tract following the ingestion of nutrients (Adrian *et al.* 1985), and has been shown to suppress feelings of hunger, and EI (Batterham *et al.* 2002, 2003). However, studies which have administered doses of ghrelin to rodents and humans have shown that

ghrelin has the opposite effect to PYY, stimulating hunger whilst also increasing EI (Wren *et al.* 2001*ab*; Druce *et al.* 2005). Research investigating the effects of an acute bout of exercise on post-exercise satiety hormones has produced a range of findings (Blundell and King, 1999). These could be attributed to differences in post-exercise blood timings, the form that the hormone was measured in, and the different exercise intensities, durations and types selected. In addition, exercising at different ambient temperatures may also have an effect on appetite hormone responses, which may be caused by blood flow redistribution during exercise to deal with thermal stress (Shorten *et al.* 2009). As many short-acting appetite regulating hormones, such as PYY and ghrelin, are derived by the gastrointestinal tract, anything that alters gastrointestinal tract blood flow, such as the intensity of exercise (Rowell *et al.* 1964), or the need to thermoregulate during exercise at different environmental temperatures (Rowell *et al.* 1965) could alter the concentration of such hormones and as such alter EI.

Several limitations are apparent in the current literature. Appetite hormone responses and EI after exercising in the cold have not been measured together, therefore a relationship between the concentration of satiety hormones following exercise in the cold and subsequent EI remains unknown. Furthermore, studies which have examined the effect of exercising in the cold on thermoregulatory responses and post-exercise EI (Dressendorfer, 1993; White *et al.* 2005) have selected temperatures which are exceptionally cold and unlikely to represent actual exercising conditions. In addition, previous research (Dressendorfer, 1993; White *et al.* 2005) has not investigated changes in skin temperature, which acts as an indirect measurement of skin perfusion. During exercise in thermoneutral conditions splanchnic blood flow is reduced and blood flow is redistributed to the working muscles, and to the skin to dissipate heat (Takala, 1996). However, during exercise in a cold environment blood flow redistribution to the skin is not as substantial as during exercise in neutral and warm

environments (Galloway and Maughan, 1997). Therefore, during exercise in the cold, splanchnic blood flow may not be reduced as much as during exercise in thermoneutral or warm conditions. This could potentially alter the circulation of gut derived appetite hormones during exercise in the cold, and hence influence EI.

In order to isolate the effect of cold from other potential mechanisms during exercise this study aimed to investigate the acute effects of brisk walking in cold (8°C) and thermoneutral (20°C) air temperatures on post-exercise EI in overweight men and women. The temperature of the cold trial was set at an air temperature of 8°C as this has the same cooling effect as water at 28°C (Smith and Hanna, 1975), which is the typical temperature of a public swimming pool. Furthermore, plasma acylated and total ghrelin, and total PYY responses were also measured. Measuring both total and acylated ghrelin allows for the observation of the post-secretion process of acylation. It was hypothesized that exercise in the cold would result in a greater post-exercise EI compared with exercise in the thermoneutral environment, and that this would be related to appetite hormone responses. Furthermore, it was hypothesized that EI and appetite hormone responses would be related to changes in T_{sk} and T_{re} during and after exercise.

2.3 Method

2.3.1 Participants

Following ethical approval from the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, 10 overweight men and 6 overweight women were recruited from the local community. All participants were nonsmokers, free from cardiovascular and metabolic disorders, and participated in less than 1 hour of organized physical activity per week. Prior to their participation in the study, all volunteers provided written informed consent (Appendix A) and completed a general health questionnaire (Appendix B). In order to identify which participants were restrained eaters and which weren't, participants completed the Three-Factor Eating Questionnaire once they had completed their trials (Stunkard and Messick, 1985). Participants scoring ≤ 12 on the TFEQ restraint subscale were considered non-dietary restrained (Stunkard and Messick, 1985). The average score for the TFEQ restraint subscale was 9.38 ± 6.42 . However, 3 of the male participants and 1 of the female participants scored >12 on the TFEQ restraint subscale, therefore these participants were classed as dietary restrained. Table 1 details the participants' characteristics.

Table 1. Participant characteristics.

Characteristics	Men (n=10)	Women (n=6)
Age (yr)	50.0 ± 10.3	55.5 ± 4.9
BMI ($\text{kg}\cdot\text{m}^{-2}$)	29.9 ± 5.2	27.1 ± 1.6
Height (m)	1.73 ± 0.09	1.62 ± 0.06
Weight (kg)	90.3 ± 21.4	71.0 ± 3.3
Body fat (%)	26.4 ± 6.1	32.6 ± 7.3
TFEQ Score	9.20 ± 6.56	11.40 ± 5.90
Predicted $\text{VO}_{2\text{max}}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	32.1 ± 6.5	28.8 ± 4.8

Values are mean \pm SD

2.3.2 Experimental Design

Prior to the experimental trials each participant attended a preliminary visit during which anthropometric measurements (height, weight, BMI and body fat) were taken, and a submaximal incremental exercise test was performed. Following the preliminary visit, the participants were given a week to recover from the submaximal exercise test before the main experimental trials began. The main experimental trials were randomized and separated by one week (four weeks for pre-menopausal women), during which time the participants were asked to maintain their typical diet and physical activity habits. The trials involved an initial 45min baseline period followed by a 45 min brisk walk at 60% predicted $\text{VO}_{2\text{max}}$ in either a cold (8°C) or thermoneutral (20°C) environment. Participants would then rest for 45 mins before having free access to a buffet-style meal for 30 mins. Blood samples were taken during the trials for later analyses of plasma total PYY, and total and acylated plasma ghrelin concentrations. See Figure 1 for the experimental trials timeline.

2.3.3 Anthropometry

Height was measured using a stadiometer (Seca LTD, Birmingham, England) to the nearest 0.1 cm and weight was measured using electronic scales (Ohaus, Champ II, Germany) to the nearest 0.01 kg. BMI was calculated as weight in kilograms divided by the square of height in metres. Subcutaneous fat was measured using skinfold callipers (Harpenden, HKK-BI, Baty International, England). In accordance with Jackson and Pollock, (1978) male body density was calculated as the sum of seven skinfolds (pectoral, axillia (midaxillary), abdomen, suprailium, subscapular, triceps, midthigh). Female body density, in accordance with Jackson *et al.* (1980), was calculated using the same sites as for male body density. Body density was then converted into body fat percentage using the Siri, (1956) equation:

$$\%Fat = [(4.95 / \text{body density}) - 4.5] \times 100$$

2.3.4 Submaximal Incremental Exercise Test

Upon arrival at the laboratory participants completed a 10min seated rest period. During the final minute of the rest period Douglas bags (Cranlea, Birmingham, UK) were used to collect an expired air sample and resting heart rate was recorded every 15 seconds using short-range telemetry (Polar Vantage NV, Kempele, Finland). Participants were then familiarized with the treadmill (HP Cosmos, Quaser, Germany) prior to beginning the submaximal incremental exercise test. The aim of the test was for the participants to achieve 85% of their predicted maximum heart rate. Maximum heart rate was predicted according to Tanaka *et al.* (2001). The test was continuous in nature but was subdivided into 5 min stages. The speed of the treadmill was selected by the participant and remained constant throughout the test. The treadmill gradient was initially set at 0% and was increased by 2-3% at the end of each 5 min stage. Expired air samples were collected into Douglas bags during the final minute of each 5 min stage for the determination of oxygen consumption and carbon dioxide production. Heart rate was measured throughout the test and recorded every 15 seconds during the final minute of each 5 min stage. Ratings of perceived exertion (Borg, 1973) were assessed at the completion of each 5 min stage. The test was terminated once the participant had reached 85% of their predicted maximum heart rate. Oxygen consumption and carbon dioxide production were determined from expired air samples using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Analyser Series 1440, Servomex, Crowborough, East Sussex, UK) calibrated with gas mixtures of known concentrations. Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to standard temperature and pressure (dry). The heart rate recorded

during the final minute of each 5 min stage was plotted against oxygen consumption at each stage to calculate the heart rate-oxygen consumption relationship. From this relationship a trend line equation could be used to predict $\text{VO}_{2\text{max}}$. Furthermore, the oxygen consumption at each 5 min stage was plotted against the gradient at each stage to calculate the gradient-oxygen consumption relationship. From this relationship, a trend line equation could be used to predict a gradient that would evoke 60% $\text{VO}_{2\text{max}}$.

2.3.5 Dietary Control

Prior to the first experimental trial participants completed a three day breakfast diary. The information from the breakfast diary was entered into the dietary analysis software CompEatPro (Nutrition Systems, Banbury, England) in order to calculate the amount of calories consumed for breakfast on each day. The average caloric intake was used to prescribe an individualised breakfast for each participant. On the morning of each trial the participants were instructed to consume only the prescribed breakfast at 0800 hours consisting of cereal and milk (305 ± 116 kcals), and a midmorning snack (a cereal bar) consumed at 1100 hours. Participants were then requested to fast, and to only consume water prior to the main experimental trials. Prior to the submaximal incremental exercise test, and the experimental trials, participants were asked to refrain from consuming caffeine 12 hours prior to testing, and were asked to refrain from performing strenuous physical activity, and from consuming alcohol 24 hours prior to testing. Water was available *ad libitum* during all trials.

2.3.6 Main Experimental Trials

Participants arrived at the laboratory at 14:00. Participants inserted a rectal thermocouple probe (YSI 400 Series, Harvard Apparatus, MA, US) in privacy, 12cm beyond the external anal sphincter in order to monitor rectal core temperature (T_{re}). The rectal thermocouple probe was attached to a data receiver (Squirrel 2020 Series, Grant, Cambridge, UK), which was programmed to log T_{re} once every minute. In order to measure skin temperature (T_{sk}) skin thermistors (Grant EUS-U, Grant Instruments LTD, Cambridge, UK) were attached to participants using adhesive, water-resistant tape (Hypafix, BSN Medical GmbH, Hamburg, Germany). In accordance with Nielsen and Nielsen, (1984), mean T_{sk} was measured at 4 weighted body locations, as represented by the following equation:

$$Mean\ T_{sk} = 9.429 + (0.137 \times T_{forehead}) + (0.102 \times T_{hand}) + (0.290 \times T_{lower\ back}) + (0.173 \times T_{lower\ leg}).$$

The thermistors were connected to the data logger, which was programmed to log T_{sk} once every minute. Heart rate was monitored throughout each trial using short range telemetry.

Following their preparation, participants then completed a 45 min baseline period in a laboratory maintained at room temperature. During the baseline period participants sat upright for 20 min whilst T_{re} and T_{sk} were recorded. Participants then lay semi-supine for 25 min whilst resting energy expenditure was estimated using a ventilated hood (Datex Ohmeda, Helsinki, Finland) attached to an Oxycon gas mixing chamber (Oxycon-pro, Jaeger, Mannheim, Germany). The final 8 min of each 25 min measurement was used to estimate resting energy expenditure. At the completion of the baseline period participants were then asked to rate their thermal comfort using the McGinnis 13-point scale (Hollies and Goldman,

1977; Appendix C). The exercise bout consisted of a 45 min brisk walk on a treadmill (HP Cosmos, Mercury, Germany) at a speed and gradient predicted to elicit 60% of predicted $\text{VO}_{2\text{max}}$. Exercise was performed in an environmental chamber (Design Environment Ltd, Gwent, UK) maintained at either 8°C (cold trial) or 20°C (thermoneutral trial) depending on the trial, relative humidity was maintained at 40% for both trials. One minute expired air samples were collected into Douglas bags at 14-15 min, 29-30 min and 44-45 min during the walk. At the completion of each expired air sample participants were asked to rate their thermal comfort. Following the completion of the walk participants returned to the laboratory and sat recovering for 45 min. Thirty minutes into the recovery period the participants were asked to rate their thermal comfort, and were then instructed to remove the skin thermistors, heart rate monitor and the rectal thermocouple, in private. Participants then returned to the lab to complete the remainder of the post-exercise recovery period. Participants were then allowed free access to a buffet-style test meal for 30 min and instructed to eat *ad libitum*. Immediately after the meal the participants rested for 30 min, after which they were allowed to leave the laboratory. See Figure 1 for an experimental trial timeline.

2.3.7 Buffet-Style Test Meal

Forty five minutes after the completion of the brisk walk participants were provided with an *ad libitum* buffet-style test meal. Participants ate alone and were encouraged to eat until they were satisfied during the 30 min test meal period. Participants were also provided with a DVD to watch while they ate. This acted as a distraction from the unfamiliar environment, which could have potentially discouraged the participants from feeding as they normally would (Stubbs *et al.* 1998). The buffet-style test meal consisted of a variety of foods and drink which included: bread, margarine, cheese, ham, mayonnaise, pickle, jam,

snack size sausage rolls, crisps, salted peanuts, snack size Mars bars, Kit-Kats, flap jack bars, yoghurts, apples, bananas, orange juice, milk, cordial, lemonade, and water, all of which were provided in excess. The participants received the same buffet-style test meal for both trials. The buffet food was weighed before and after the meal, to allow for the calculation of absolute energy and macronutrient intake. Prior to taking part in the study, the participants were not informed that their *ad libitum* EI during the buffet-style test meal would be measured, as this information may have influenced their eating habits.

2.3.8 Blood Sampling and Analysis Procedure

An intravenous cannula (BD Venflon, Oxford, UK) was inserted into an antecubital vein for the collection of venous blood samples. Blood samples were drawn from the intravenous line following the completion of the initial 45 min baseline period, immediately after exercise, 30 min after exercise and 1 hour 45 min after exercise (30 min postprandial). During the trials the cannula was kept patent with 2ml flushes of 0.9% NaCl_(aq) isotonic saline solution (Baxter Healthcare, Northampton, UK) following each blood-letting. Blood samples were collected using 5 ml syringes, 3 ml of whole blood was added immediately to a pre-chilled EDTA vacutainer (BD Vacutainers, Oxford, UK) which had been pre-treated with 0.06 ml of the serine protease inhibitor 4-(2-Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF) (Alexis Biochemicals, Lausen, Switzerland). After gentle inversion, the vacutainers were then immediately spun in a centrifuge at 3000 g for 10 min at 4°C. Following whole blood separation, 0.6 ml aliquots of the AEBSF treated plasma were added to pre-treated eppendorfs containing 120 µl 1N HCL and gently inverted. Plasma was stored at -80°C for later analysis of plasma acylated and total ghrelin, and total PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations.

Commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA) were used to specifically measure total plasma ghrelin (acylated human ghrelin and des-acyl human ghrelin), plasma acylated ghrelin, and total plasma PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations. Absorbance was read using a microplate reader (ELx800, BioTek, Bedfordshire, UK) and standard curves were created to calculate unknown sample concentrations. The minimum limits of detection of total ghrelin, acylated ghrelin, and total PYY were 100 pg/ml, 25 pg/ml, and 16.1 pg/ml respectively when using a 20 µL sample. The intra-assay CVs for total ghrelin, acylated ghrelin, and total PYY were 4.57%, 5.55% and 4.58% respectively. All sample measurements were performed in duplicate.

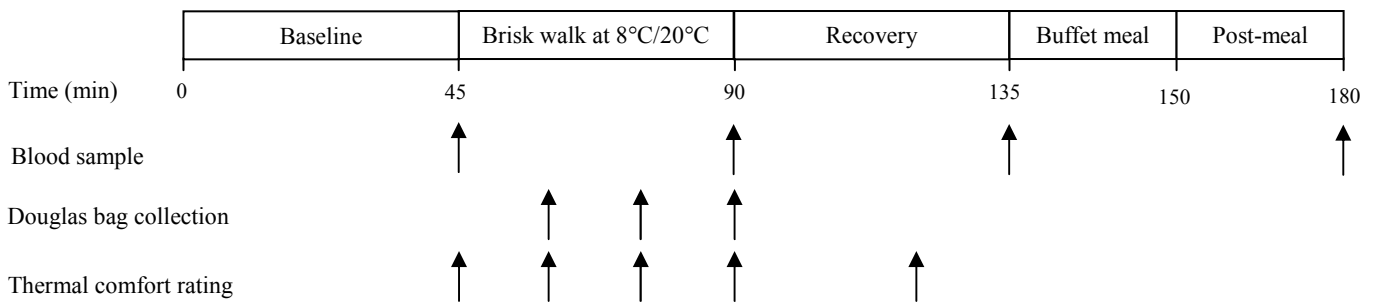


Fig 1. Experimental trial timeline.

2.3.9 Statistical Analysis

Data are expressed as mean \pm standard deviation (SD) in the text and in tables, and data are expressed as mean \pm standard error (SE) in figures. Significant differences were accepted at $P < 0.05$. Data was checked for normal distribution using the Shapiro-Wilk test of normality. Two-way repeated measures ANOVAs were used to assess differences between trials across time for heart rate, T_{re} , T_{sk} , acylated and total plasma ghrelin concentrations, and plasma PYY concentrations. ANOVA data was split into baseline, exercise, and recovery

periods. Data was split into sections to avoid changes during exercise being diluted and lost by stability at baseline and, to a lesser extent recovery. Main effects were analysed using Bonferroni *post-hoc* tests. Raw PYY, and total and acylated ghrelin data were converted to area under the curve (AUC) using the trapezoidal rule and differences were analysed by paired *t* tests. Paired *t* tests were used to determine the effects of the two trials on EI, relative EI (REI), and absolute and relative macronutrient intake (carbohydrate, fat, protein and fibre). Pearson correlation coefficient was used to examine relationships between variables. Gender differences were compared using independent samples *t*-tests. Statistical analysis was carried out with SPSS for Windows 16.0.1 (SPSS Inc., Chicago, US).

2.4 Results

2.4.1 Participants

Two male participants were not included in the resting energy expenditure data analyses due to a fault with the ventilated hood system during their trials. Subsequently, these participants were not included in the REI data analyses.

2.4.2 Baseline Measurements

Resting energy expenditure prior to exercise in the cold ($1476 \pm 379 \text{ kcal}\cdot\text{day}^{-1}$) and neutral environment ($1346 \pm 337 \text{ kcal}\cdot\text{day}^{-1}$) was not significantly different between trials ($P > 0.05$). Thermal comfort ratings immediately prior to exercise were not significantly different between the cold (6.93 ± 0.59) and neutral trials (7.13 ± 0.52) ($P > 0.05$). Furthermore, heart rate did not differ significantly between trials at baseline ($P > 0.05$; Fig 2).

2.4.3 Responses to Exercise and Temperature

The average treadmill speed selected by the participants to complete the brisk walk was $5.46 \pm 0.69 \text{ km}\cdot\text{h}^{-1}$. The average treadmill gradient was $5.44 \pm 0.82\%$. Average exercise intensity ($\%\text{VO}_{2\text{max}}$) was significantly higher during exercise in the neutral environment compared with the cold environment ($P < 0.05$; Table 2). Mean RER during exercise was not significantly different between trials ($P > 0.05$; Table 2). Average thermal comfort ratings were significantly greater during exercise in the neutral environment compared with the cold environment ($P > 0.001$; Table 2). Furthermore, thermal comfort ratings did not differ significantly between cold (7.07 ± 0.26) and neutral (6.93 ± 0.26) trials 30 min post-exercise ($P > 0.05$). There was a significant main effect of trial ($P < 0.05$), a significant main effect of time ($P < 0.001$) and a significant trial x time interaction ($P < 0.05$) for heart rate values

during exercise. *Post hoc* analysis revealed that mean heart rate was significantly higher during exercise in the neutral environment compared with the cold environment at 15, 20, 25, 30, 40 and 45 min ($P < 0.05$; Figure 2). In addition, there was a significant main effect of time ($P < 0.001$) for heart rate during recovery from exercise in both trials, but no significant time x trial interaction was observed ($P > 0.05$).

Table 2. Responses to exercise in the cold and neutral trials.

	Cold Exercise	Neutral Exercise
Exercise intensity (%VO _{2max})	55.0 ± 11.4*	60.9 ± 7.1
VO ₂ (L·min ⁻¹)	1.34 ± 0.40*	1.51 ± 0.43
Total Energy expenditure (kcal)	306 ± 8*	340 ± 6
RER	0.88 ± 0.05	0.87 ± 0.06
Thermal comfort	6.56 ± 0.68*	8.47 ± 0.85

Values are presented as means ± SD; $N = 16$ for all measurements, except thermal comfort ($n = 15$). Significant differences between trials, * $P < 0.05$.

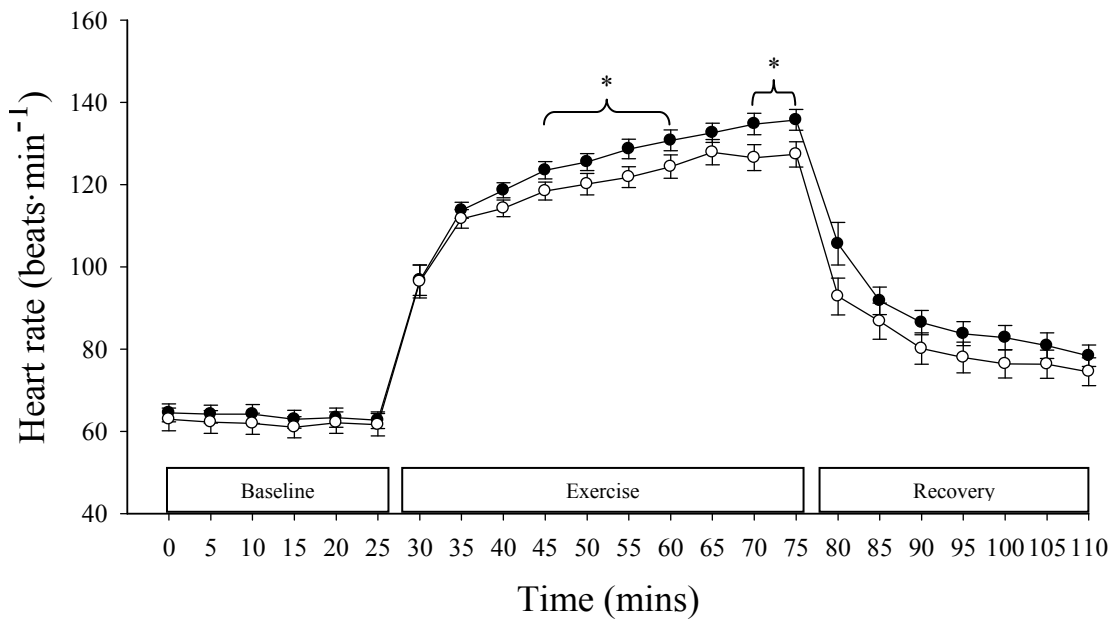


Fig 2. Heart rate values at baseline, and during exercise and recovery in the cold and neutral trials. ○ cold trial, ● neutral trial. Values presented as means ± SE; $N = 15$. Significant differences between trials * $P < 0.05$.

2.4.4 Rectal temperature

There were no significant differences in baseline T_{re} between trials ($P > 0.05$; Figure 3). There was a significant ($P < 0.001$) main effect of time on T_{re} during exercise; however no significant trial x time interaction was observed ($P < 0.05$). Rectal temperature decreased significantly during recovery from exercise in both trials ($P < 0.001$; Figure 3). Furthermore, *post hoc* analysis demonstrated that T_{re} was significantly lower in the cold trials compared with the neutral trials at 25 and 30 min post-exercise ($P < 0.05$; Figure 3).

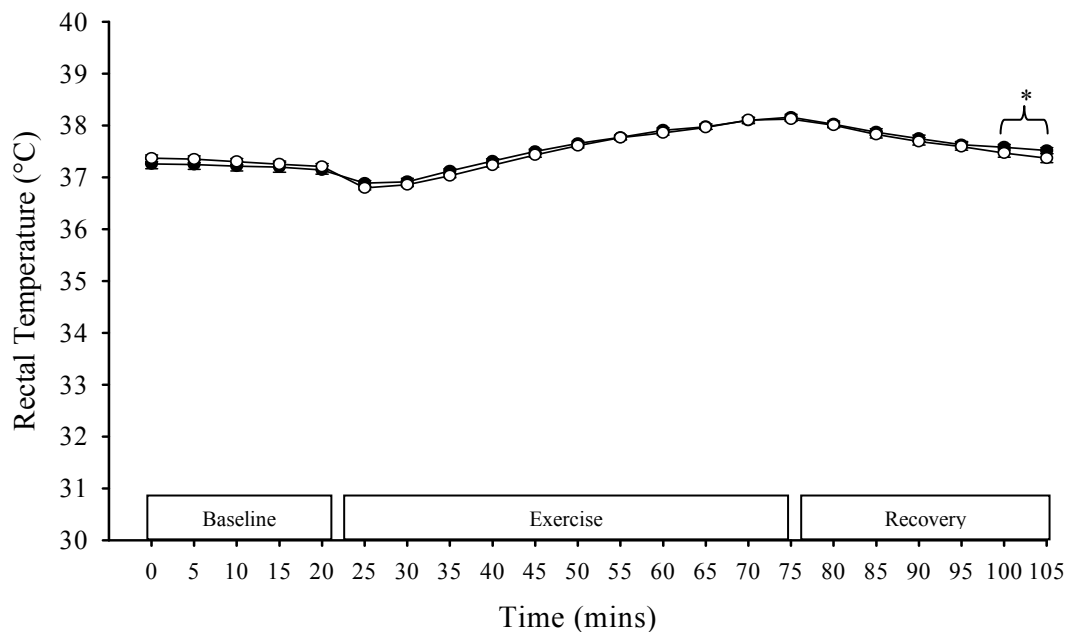


Fig 3. Rectal temperature at baseline, and during exercise and recovery in the cold and neutral trials. ○ cold trial, ● neutral trial. Values presented as means \pm SE; $N = 15$. Significant differences between trials * $P < 0.05$.

2.4.5 Skin temperature

Skin temperature did not differ significantly between trials prior to exercise ($P > 0.05$; Figure 4). There was a significant main effect of trial ($P < 0.01$), time ($P < 0.001$) and a significant trial x time interaction ($P < 0.001$) for T_{sk} during exercise. *Post hoc* analysis

revealed that T_{sk} was significantly lower throughout exercise in the cold environment compared with the neutral environment ($P < 0.001$; Figure 4). Furthermore, during recovery there was a significant main effect of trial ($P < 0.001$), time ($P < 0.001$) and a significant trial x time interaction ($P < 0.001$) for T_{sk} post-exercise. *Post hoc* analysis indicated that T_{sk} was significantly lower during the 30 min recovery period following exercise in the cold environment compared with the neutral environment ($P < 0.01$; Figure 4).

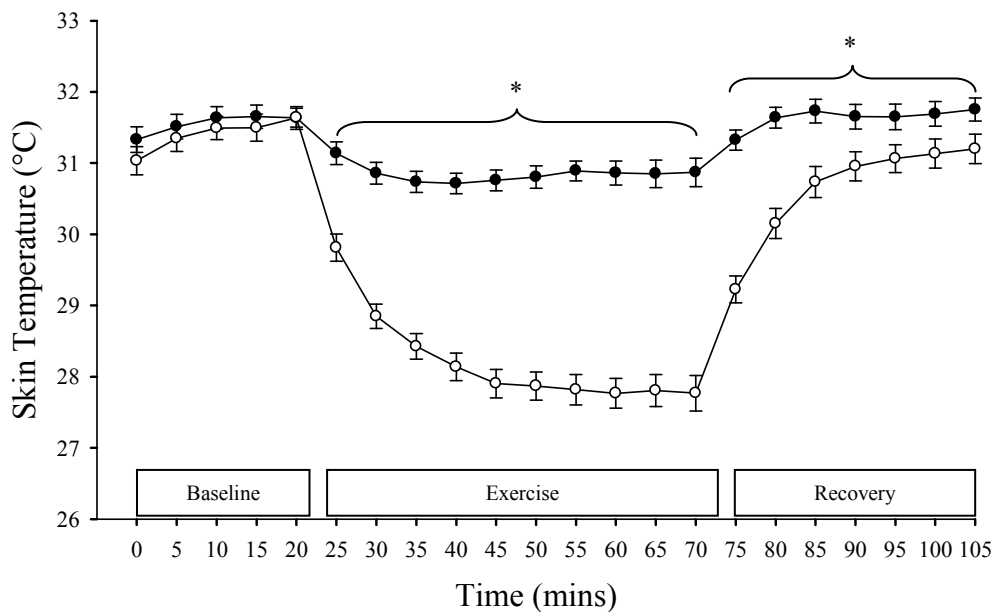


Fig 4. Skin temperature at baseline, and during exercise, and recovery in the cold and neutral trials. ○ cold trial, ● neutral trial. Values presented as means \pm SE; $N = 15$. Significant differences between trials * $P < 0.05$.

2.4.6 Energy and Macronutrient Intake

Total EI was significantly higher following exercise in the cold compared with the neutral environment ($P < 0.05$; Figure 5). Differences in relative EI between trials approached significance ($P = 0.065$). Absolute carbohydrate intake was significantly greater following exercise in the cold compared with the neutral environment ($P < 0.05$), however when expressed as a percentage of total EI there were no significant differences between

trials ($P > 0.05$; Table 3). There were no significant between trial differences in fat, protein or fibre intake when expressed as absolute values, or as a percentage of total EI ($P > 0.05$; Table 3).

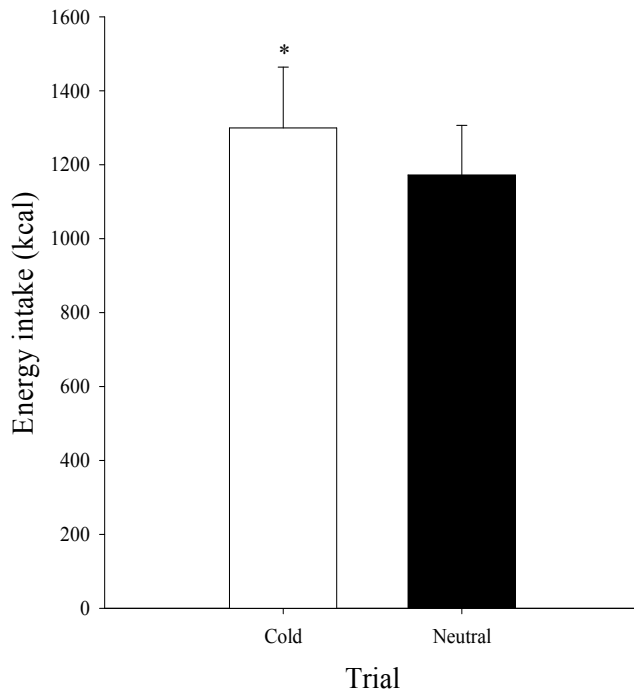


Fig 5. *Ad libitum* EI during 30 min buffet meal following exercise in the cold and neutral environment. Values presented as means \pm SE; Significant differences between trials $*P < 0.05$.

Table 3. Absolute and relative (%) macronutrient intake following exercise in the cold and neutral trials.

	Cold Trial	Neutral Trial
Carbohydrate (kcal)	596 \pm 335*	536 \pm 292*
Carbohydrate (%)	46.2 \pm 11.5	45.6 \pm 12.1
Fat (kcal)	521 \pm 282	469 \pm 227
Fat (%)	39.2 \pm 8.62	39.5 \pm 9.12
Protein (kcal)	159 \pm 66	144 \pm 51
Protein (%)	12.7 \pm 3.3	13.0 \pm 3.8
Fibre (kcal)	23.1 \pm 11.8	22.4 \pm 11.1
Fibre (%)	1.83 \pm 0.48	1.93 \pm 0.49

Values are presented as means \pm SD; Significant differences between trials, $*P < 0.05$.

2.4.7 Appetite Hormones

One female participant was removed from the acylated ghrelin analyses as their inclusion influenced the outcomes of the analyses. With this participant included, acylated ghrelin concentrations were significantly greater immediately following exercise in the cold environment compared with the neutral environment ($P < 0.05$). This participant's mean acylated ghrelin concentration was 3.2 standard deviations above the group mean for the cold trials, and 2.4 standard deviations above the group mean for the neutral trials. Furthermore, two female participants were removed from the total ghrelin analyses as their mean plasma concentrations were 2 standard deviations higher than the group mean. However, their removal did not change the outcomes of the total ghrelin analyses.

2.4.7.1 Acylated ghrelin

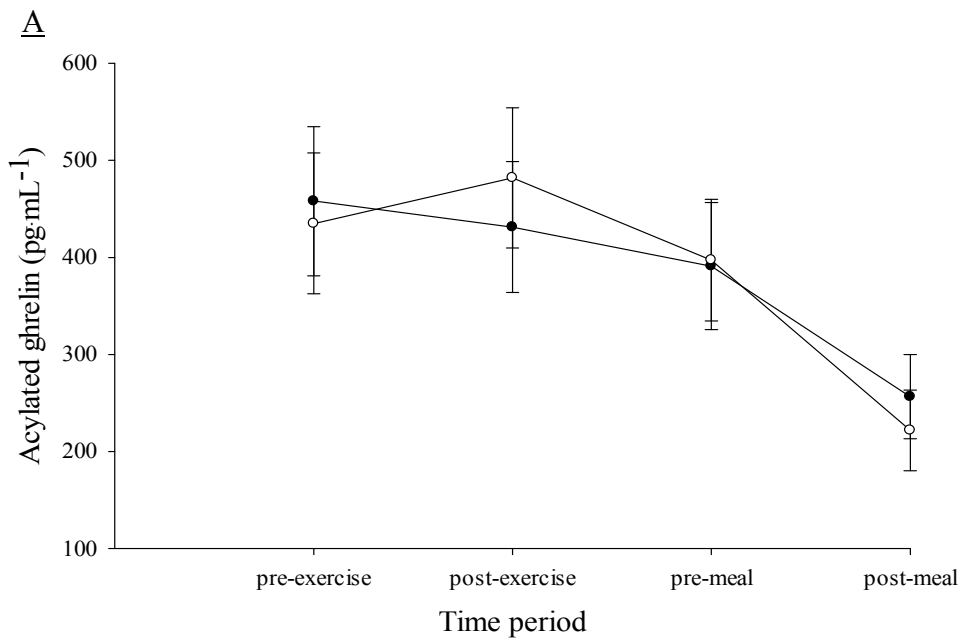
There was a significant interaction between time and condition when examining differences in plasma acylated ghrelin concentrations between trials ($P < 0.05$; Figure 7A), though only differences in post-exercise acylated ghrelin concentrations between trials approached significance ($P = 0.066$). However, when data were expressed as relative to pre-exercise values, acylated ghrelin was significantly higher post-exercise during the cold trials compared with the neutral trials (1.10 ± 0.31 vs 0.93 ± 0.24 ; $P < 0.05$). Acylated ghrelin AUC values did not differ between trials ($P > 0.05$; data not shown). Furthermore, there were no significant differences in the percentage of total ghrelin which was acylated following exercise between trials ($P > 0.05$).

2.4.7.2 Total ghrelin

Total plasma ghrelin concentrations did not differ between trials ($P > 0.05$; Figure 7B). Furthermore, Total ghrelin AUC values did not differ between trials ($P > 0.05$; data not shown).

2.4.7.3 Total PYY

Total plasma PYY concentrations did not differ significantly between trials ($P > 0.05$; Figure 7C). Total PYY AUC values for the baseline to post-exercise period were significantly lower during the cold trial compared with the neutral trial ($P < 0.05$; data not shown).



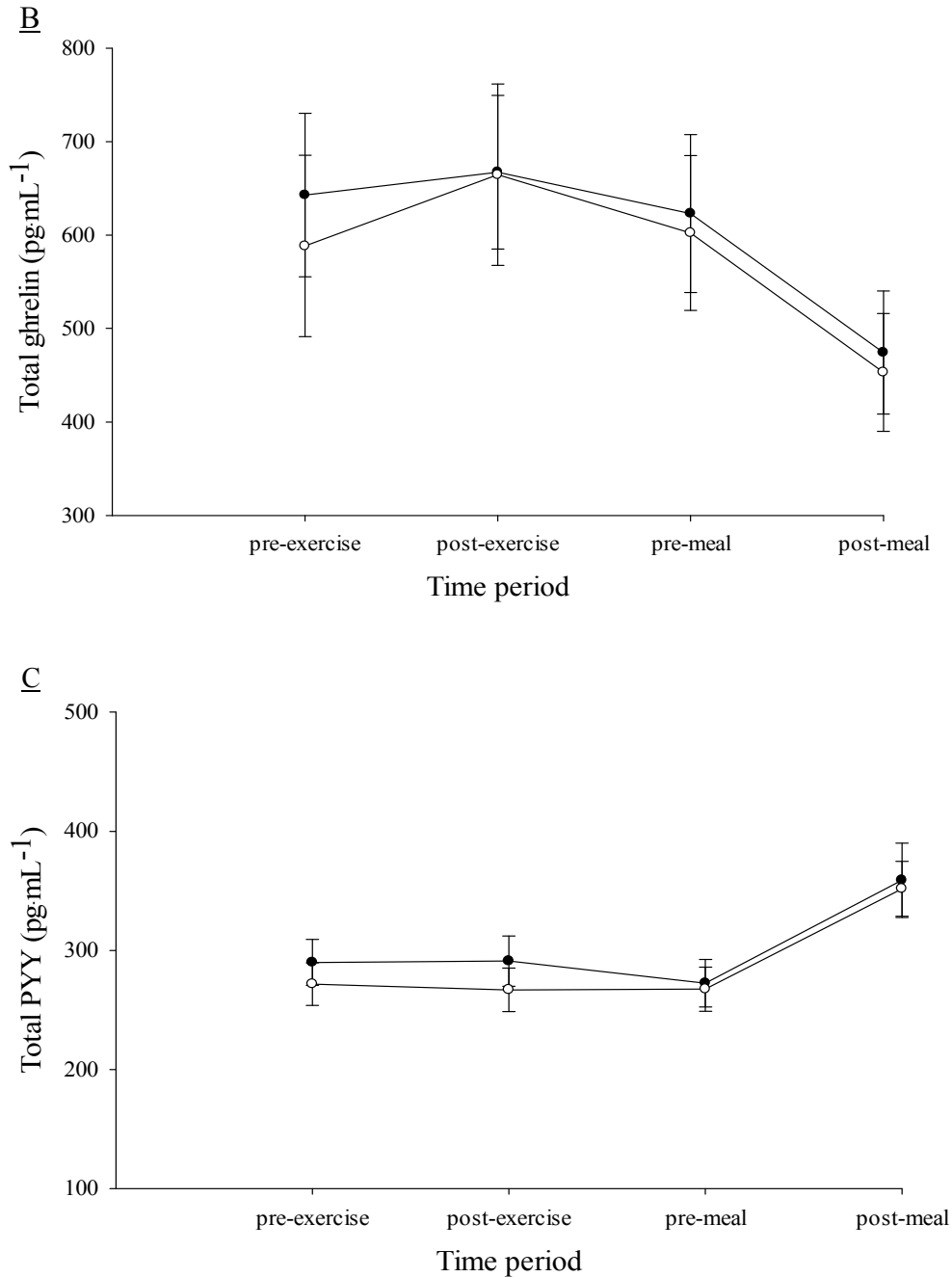


Fig 7. Plasma concentrations of acylated ghrelin (A), total ghrelin (B) and total PYY (C). ○cold trial, ● neutral trial. Values presented as means \pm SE. Acylated ghrelin $n = 15$, total ghrelin $n = 14$, total PYY $n = 16$. Significant differences between trials * $P < 0.05$.

2.4.8 Correlations

Fasting total ghrelin concentrations were inversely correlated with body weight ($P < 0.05$; $r = -0.503$), and correlations between total ghrelin and BMI approached significance ($P = 0.081$; $r = -0.449$). Correlations between acylated ghrelin and body weight ($P = 0.053$; $r = -0.491$), and acylated ghrelin and BMI ($P = 0.094$; $r = -0.433$) approached significance. No

relationship between T_{sk} or T_{re} AUC values and appetite hormone AUC values during exercise or recovery was observed. Furthermore, there were no significant correlations between EI and appetite hormone AUC values during exercise and recovery ($P > 0.05$).

2.4.9 Gender Differences

Males absolute *ad libitum* energy consumption was significantly greater than females after exercising in the cold environment ($P < 0.001$; males: 1632 ± 596 kcal, females: 745 ± 252 kcal), and the neutral environment ($P < 0.01$; males: 1459 ± 455 kcal, females: 693 ± 231 kcal). There were no further gender differences.

2.5 Discussion

The aim of this study was to investigate the transient effects of a brisk walk in a cold (8°C) and neutral (20°C) environment on EI, appetite hormone responses, T_{sk} , and T_{re} in overweight men and women. The primary finding from this study was that exercising in a cold environment stimulated post-exercise EI in overweight men and women when compared with exercising in a neutral environment (20°C). Secondly, relative to baseline values, acylated ghrelin concentrations were greater immediately after exercise in the cold environment compared with the neutral environment. Furthermore, PYY AUC values were lower following exercise in the cold environment. Therefore, increases in EI following exercise in the cold may be mediated by circulating acylated ghrelin and total PYY concentrations. Furthermore, despite observing a lower T_{sk} during and after exercise in the cold compared with neutral environment, this study did not identify a relationship between changes in T_{sk} and appetite hormone responses between trials.

Previous research suggests that changes in the concentrations of ghrelin and PYY post-exercise may mediate feelings of hunger and food intake following an acute bout of exercise (Broom *et al.* 2009; Shorten *et al.* 2009; Ueda *et al.* 2009 *ab*; King *et al.* 2010a). For the purposes of this study total and acylated plasma ghrelin were measured, and the appetite suppressing gut hormone plasma PYY. This study measured total plasma PYY and not the active form of PYY, plasma PYY₃₋₃₆. The active form of PYY has been shown to be the predominant form of PYY in human circulation (Batterham *et al.* 2006). However, evidence has demonstrated that concentrations of circulating total PYY and PYY₃₋₃₆ exhibit a strong positive correlation (Tsilchorozidou *et al.* 2008). In the present study total PYY AUC values were lower during exercise in the cold trial. Lower PYY AUC values during exercise in the cold environment suggests that participants may have been more hungry following exercise, causing them to consume more energy at the buffet meal. Furthermore, acylated

ghrelin increased following exercise in the cold, however there was no difference in total ghrelin concentrations between the trials. In addition, there was no difference in the percentage of total ghrelin which was acylated between trials, indicating that the temperature at which the exercise was performed did not affect the ghrelin acylation process post-secretion. Ghrelin exists in the circulation in two forms, the inactive form des-acyl ghrelin and the active form acylated ghrelin (Asakawa *et al.* 2004). Acylated ghrelin has been shown to stimulate feeding behaviour, whereas des-acyl ghrelin has been shown to have no effect (Neary *et al.* 2006). Therefore, based on the mediating effects of acylated ghrelin on EI, the findings from this study indicate that the increase in acylated ghrelin secretion following exercise in the cold may potentially act as a mechanism for the increase in post-exercise EI.

Previous research has found that the temperature at which exercise is performed can influence post-exercise food intake (Dressendorfer *et al.* 1993; White *et al.* 2005; Shorten *et al.* 2009). The primary finding from the current study which demonstrates that brisk walking in a cold environment stimulates EI for a short period of time post-exercise in overweight individuals is a novel finding, yet also concurs with previous studies conducted using lean active individuals cycling while immersed in cold water, which have shown that exercise in the cold stimulates EI immediately post-exercise (Dressendorfer *et al.* 1993; White *et al.* 2005). The present study also demonstrated that absolute carbohydrate consumption was greater following exercise in the cold environment compared with the neutral environment. This finding is in contrast to White *et al.* (2005) who found that relative fat intake was greater following exercise in cold water. It is possible that the effects of exercising in the cold prompted participants in the current study to consume a greater amount of carbohydrates, as carbohydrate consumption has been shown to produce a greater thermic response than fat consumption (Karst *et al.* 1994). However, if the participants food preferences were driven by the need to enhance thermogenesis following exercise in the cold, then you may expect to

observe a greater carbohydrate intake following exercise in cold water as well. One possible reason for the contrasting findings between the present study and that of White *et al.* (2005) is the environments in which the exercise bouts were performed, water versus land. The food preferences of overweight individuals following exercising in cold water, i.e. following swimming, warrants further investigation, as enhanced EI and fat consumption following cold water exercise may negate the effects of exercise on weight loss.

Increases in EI following exercise in the cold could be mediated by changes in the circulation of peripheral appetite hormones, but it could also be mediated by central processes, for example, the hypothalamus is involved in the regulation of appetite (Suzuki *et al.* 2010) and core body temperature (Hammel *et al.* 1963). Therefore, peripheral hormone secretion may not be the only mechanism responsible for mediating increases in EI following exercise in the cold. It has been suggested that the stimulation of food intake immediately following exercise in the cold may be related to a reduction in core temperature during such exercise (Dressendorfer *et al.* 1993; White *et al.* 2005). However, despite observing a significant reduction in core temperature when cycling in cold water, White *et al.* (2005) stated that the actual reduction of 0.3°C was unlikely to have been the physiological stimulus which triggered the observed increase in EI immediately post-exercise. Instead the authors suggested that the enhanced feeding behaviour following cold water exercise may have been related to changes in blood flow, which may in turn influence the circulation of short acting appetite hormones located in the gut. The present study did not observe a difference in core temperature responses when exercising in the cold or neutral environment, though core temperature was lower prior to the meal following exercise in the cold. However, the actual difference in core temperature prior to the meal between the two trials was only 0.16°C. In agreement with White *et al.* (2005), this small difference in temperature between trials is unlikely to have influenced EI. Therefore, perhaps interactions between regions of the

hypothalamus which regulate appetite and those which regulate core body temperature are less important than peripheral hormone responses when exercising in a cold environment.

Differences in T_{sk} between the trials during exercise and recovery however may offer a potential explanation for the observed differences in *ad libitum* EI. During exercise in normal conditions blood flow to the splanchnic region is reduced and blood is redistributed to the working muscles and to the skin to dissipate heat generated during exercise (Takala, 1996). Throughout exercise and recovery during the cold trials a reduction in skin temperature was observed, this may indicate a reduction in skin blood flow, and therefore an increase in the volume of blood within the splanchnic region. Increased splanchnic blood flow during exercise in the cold may provide more blood for the circulation of the appetite stimulating hormone ghrelin, and hence potentially increase EI. However, this theory warrants further investigation as skin blood flow was not directly measured, nor was blood flow to the splanchnic region.

The present study is one of the very few studies which have examined the transient effects of an acute bout of exercise on EI in both males and females (Erdmann *et al.* 2007). Previous research which has compared the impact of acute exercise on gut hormones, appetite, and EI in men and women has not shown any gender differences (Burns *et al.* 2007; Erdmann *et al.* 2007). However, studies which have compared appetite hormone responses to exercise training in men and women have demonstrated that a period of prolonged exercise can cause marked increases in acylated ghrelin levels (Hagobain *et al.* 2009) and reductions in leptin levels (Hickey *et al.* 1997) in women, whereas men do not demonstrate changes to the same extent. These studies suggest that women may experience greater changes in appetite hormone concentrations as a result of exercise compared with men, and that these changes may stimulate the drive to eat more in women than in men (Hagobain and Braun, 2009). The present study observed that males demonstrated a greater absolute EI following

exercise compared with females. However, there were no differences between males and females regarding the effects of exercise temperature on food consumption or appetite hormone concentrations. However, it should be noted that only 6 women participated in the present study. Therefore, the gender comparisons conducted in this study should be interpreted with caution. Further research is required to investigate how the effects of acute exercise on EI and appetite hormone responses differ between males and females.

Despite being one of the few studies to examine exercise-induced appetite responses in both male and female participants, the uneven number of males and females participating in this study is one of the limitations associated with it. Also, this study did not include a resting control condition therefore, one cannot be sure if it is the exercise or the cold environment that stimulates EI. Furthermore, it is unknown whether the effects of exercising in the cold on EI are different in overweight versus lean individuals, as this study did not include a lean control group. Finally, appetite was only measured once in the present study by offering a buffet meal 45 min post-exercise. Future studies should extend this observation period, as the feeding behaviour of the participants may have been altered for a prolonged period of time as a result of the intervention. In order to achieve this, future studies could use visual analogue scales to monitor subjective appetite sensations, such as hunger and fullness, in addition to measuring actual EI.

In conclusion, this study has shown that brisk walking in a cold environment stimulates post-exercise *ad libitum* EI in overweight men and women. Furthermore, acylated ghrelin concentrations were increased following exercise in the cold, thus potentially increasing the drive to eat. These findings are of particular significance to individuals who are looking to lose weight and have been recommended swimming as a form of non-weight bearing exercise to help facilitate weight loss. The present study has removed the elements of swimming which might influence appetite, such as water pressure and a supine body position,

and isolated the effects of cooling. Despite previous research demonstrating that swimming does not significantly alter post-exercise EI in lean active participants (King *et al.* 2011), the present study has demonstrated that overweight individuals do consume more calories following exercise in a cold environment. There has to date been no research conducted investigating the influence of swimming on EI in overweight individuals. Therefore, perhaps future research could investigate the direct impact of swimming in cold water on EI in overweight and obese men and women.

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**CHAPTER 3 - THE EFFECTS OF
EXERCISING IN A WARM
ENVIRONMENT ON GHRELIN, PYY,
AND APPETITE IN OVERWEIGHT MEN
AND WOMEN**

3.1 Abstract

The aim of this study was to investigate the effects of brisk walking in a warm environment (32°C) compared with a cool environment (14°C) on appetite sensations and appetite hormone responses in 13 middle-aged overweight men and women (age 49.7 ± 8.8 yr, body mass index $33.5 \pm 7.4 \text{ kg}\cdot\text{m}^{-2}$). Participants completed four randomized, partially counterbalanced trials (32°C exercise, 32°C rest, 14°C exercise, and 14°C rest). The trials consisted of 45 min of exercise/rest at 14°C/32°C, a standardized meal was then consumed 90 min post-exercise/rest, after which participants rested for 3 and a half hours. Subjective appetite sensations (hunger, desire to eat, fullness, and thirst) were measured throughout the trials and blood samples were taken at regular intervals for the determination of plasma acylated and total ghrelin, and total PYY concentrations. Skin temperature and rectal temperature were measured throughout exercise and rest, and during recovery. Results demonstrated that the only significant difference in appetite sensations between trials was a greater desire to eat at the end of the 32°C exercise trial compared with the 32°C rest trial ($P < 0.05$). As regards appetite hormone responses, PYY AUC values were significantly greater when resting compared with exercising at 32°C ($P < 0.05$). Furthermore, total ghrelin AUC values were significantly lower during the 32°C exercise trials compared with the 14°C exercise trials ($P < 0.05$). This finding indicates that exercise in the heat may have suppressed the hormonal drive to eat compared with exercise in the cool environment, however hunger and desire to eat ratings did not significantly change. Overall, this study was underpowered and therefore the findings therein should be interpreted with caution.

Keywords: Exercise, warm environment, appetite sensations, ghrelin, PYY

3.2 Introduction

A long term imbalance between the intake of high calorie foods and energy expenditure is often associated with an increase in body weight and the development of obesity (WHO Report, 2006). Increasing energy expenditure through increased physical activity is widely recommended as a means of promoting a negative energy balance, and thus weight loss, in overweight and obese individuals (Donnelly *et al.* 2009). Short term energy balance can be greatly influenced by acute bouts of exercise (Blundell *et al.* 2003), because acute bouts of exercise have been shown to influence energy intake and sensations of appetite, such as hunger and satiety (Pomerleau *et al.* 1997; Burns *et al.* 2007; Broom *et al.* 2007, 2009; Ueda *et al.* 2009ab; King *et al.* 2010).

The effects of acute exercise on short-term appetite appears to be partly regulated by exercise intensity, moderate intensity exercise appears to have little affect on appetite (Imbeault *et al.* 1997; King *et al.* 2010b; Unick *et al.* 2010), whereas high intensity exercise, have been shown to suppress appetite during and after exercise (Burns *et al.* 2007; Broom *et al.* 2007, 2009; Ueda *et al.* 2009ab; King *et al.* 2010a). The suppressive effects of high intensity exercise on appetite could be due to a redistribution of blood flow during exercise (Blundell *et al.* 2003). Research demonstrates that as exercise intensity increases, blood flow to the skin also increases in order to dissipate heat (Kenney and Johnson, 1992).

Furthermore, Rowell *et al.* (1964) observed large reductions in blood flow to the splanchnic region during moderate to high intensity upright exercise. Redistribution in blood flow to the skin during exercise, and reductions in splanchnic blood flow could potentially regulate the effects of exercise on appetite, as the splanchnic region is the predominant site for peripheral appetite hormone production (Wren and Bloom, 2007). Several studies have attempted to investigate the mechanisms which regulate appetite responses to acute bouts of exercise. The response of the short-acting appetite hormone ghrelin has been associated with appetite

responses to acute bouts of exercise (Erdmann *et al.* 2007; Broom *et al.* 2007, 2009, King *et al.* 2010). Ghrelin is the main appetite stimulating hormone in the circulation (Kojima *et al.* 1999), and has been shown to transiently stimulate food intake when infused into humans (Wren *et al.* 2001). Ghrelin is derived from the gastrointestinal tract (Date *et al.* 2000); therefore anything that alters blood flow to and from the gastrointestinal tract could alter the concentration of ghrelin in the circulation, and hence potentially alter short-term appetite sensations and energy intake.

In the same way that exercise intensity affects thermoregulation (and blood redistribution) the same is also true for exercise at different temperatures. During exercise in hot conditions splanchnic blood flow is substantially reduced, which is partly due to an increase in the volume of blood which is redistributed to the skin in order to dissipate heat (Rowell *et al.* 1965). Studies investigating the relationship between thermoregulation and appetite responses during and after a bout of acute exercise suggest that post-exercise EI can be influenced by the environmental temperature at which the individuals perform exercise. Dressendorfer *et al.* (1993) demonstrated that cycling (30 min at 70% $\text{VO}_{2\text{max}}$) in warm water (34°C) significantly suppressed post-exercise *ad libitum* energy intake compared with exercise in cold water (22°C) and control. Whereas, cycling in cold water significantly increased post-exercise energy intake in comparison with control. White *et al.* (2005) reported similar findings demonstrating that cycling (45 min at 60% $\text{VO}_{2\text{max}}$) in cold water (22°C) significantly increased *ad libitum* energy intake compared with cycling in warm water (33°C) and control. Recent research conducted by Shorten *et al.* (2009) has investigated the effects of high intensity exercise in the heat on post-exercise *ad libitum* energy intake. The study found that running (40 min at 70% $\text{VO}_{2\text{max}}$) in the heat (36°C) significantly suppressed relative energy intake (energy intake relative to energy expenditure during exercise) compared with resting in a neutral (25°C) environment. These findings would suggest that

acute post-exercise energy intake could be influenced by environmental temperature (Dressendorfer, 1993; White *et al.* 2005; Shorten *et al.* 2009). Furthermore, Tomasik *et al.* (2005) demonstrated that ghrelin concentrations were significantly suppressed after resting in a warm environment (30°C) compared with resting in a neutral environment (20°C). Therefore, the influence of environmental temperature on appetite could be mediated by changes in ghrelin circulation (Tomasik *et al.* 2005). However, a limitation of the Tomasik *et al.* (2005) study is that subjective appetite responses to the different temperatures were not measured, therefore it is not clear if changes in the concentrations of circulating ghrelin translated into changes in appetite during or after exposure.

Several limitations are apparent in the current research literature. The ambient temperatures selected by Shorten *et al.* (2009) for both the neutral (25°C) and hot (36°C) exercise trials are somewhat high, and perhaps do not represent typical exercising conditions. In particular, the temperature selected for the neutral exercise trials is high in comparison with what is typically considered as a neutral temperature (~20°C). Furthermore, previous research has selected healthy, physically active participants and as yet no study has examined an overweight population. No studies have investigated the effects of ambient temperature on appetite sensations during an exercise bout, therefore it is currently unknown if appetite is affected by ambient temperature during exercise. Furthermore, it is unknown as to how prolonged these effects may be, as no study has monitored post-exercise appetite response over an extended period of time. The mechanisms which mediate the effects of ambient temperature on post-exercise appetite responses are currently unclear. White *et al.* (2005) suggested that changes in the regulation of skin blood flow during exercise in different ambient temperatures could modify appetite hormone release thereby potentially influencing appetite. During exercise in the heat blood flow to the skin is markedly elevated compared with exercise in a thermoneutral environment, at the expense of blood flow to the splanchnic

region which is greatly reduced (Rowell *et al.* 1965). Exaggerated redistribution of blood flow away from the splanchnic region to the skin during exercise in the heat could potentially reduce the volume of blood available for the circulation of ghrelin, and hence lead to a suppression in appetite.

In view of this theory, and the current limitations in the research literature, the aim of this study was to investigate the effects of an acute bout of brisk walking in cool (14°C) and warm (32°C) conditions on appetite sensations during exercise and for an extended period of time after exercise in overweight middle-aged men and women. Furthermore, this study aimed to examine the effects of exercise and temperature on the responses of plasma total PYY, and plasma total and acylated ghrelin. Measuring both the total and acylated forms of ghrelin allowed us to examine the effects of temperature on the post-secretion process of acylation. An air temperature of 14°C was chosen for the cool trial as it was felt that this represented a comfortable environment in which to exercise. Furthermore, the warm trial temperature was set at 32°C as this study aimed for participants to experience thermal stress without feeling the need to stop exercising. It was hypothesized that exercise in the heat would transiently suppress appetite during and immediately after exercise compared with exercise in a cool environment, and that this would be related to a reduction in plasma ghrelin, and an increase in plasma PYY concentrations following exercise in the warm condition. Furthermore, it was hypothesized that appetite sensations and appetite hormone responses would be influenced by changes in core temperature and skin temperature (a surrogate for skin blood flow) during and after exercise.

3.3 Method

3.3.1 Participants

Following ethical approval from the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, 7 overweight men and 6 overweight women were recruited for participation in the study. Participant characteristics are shown in table 1. Prior to their participation, all volunteers provided written informed consent (Appendix A) and completed a general health questionnaire (Appendix B). At the completion of their trials each participant completed the Three-Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985). All of the male participants scored ≤ 12 on the TFEQ restraint subscale indicating that all of the male participants were non-dietary-restrained (Stunkard and Mesick, 1985), however 3 out of the 6 female participants scored >12 on the restraint subscale indicating that half of the female participants were dietary restrained (Stunkard and Mesick, 1985). All participants were non-smokers, were not taking any medication which may have affected their metabolism, were not taking any medication which may have influenced their appetite, and participated in less than 1 hour of organised physical activity per week.

Table 1. Participant characteristics.

Characteristics	Men (n=7)	Women (n=6)
Age (yr)	49.0 \pm 10.8	50.5 \pm 6.5
Height (m)	1.75 \pm 0.06	1.65 \pm 0.04
Weight (kg)	98.0 \pm 24.5	96.2 \pm 16.8
BMI (kg·m ⁻²)	32.0 \pm 8.1	35.3 \pm 6.7
Body fat (%)	23.1 \pm 3.6	34.6 \pm 5.6
TFEQ	7.14 \pm 6.59	10.50 \pm 5.17
Predicted VO _{2max} (mL·kg ⁻¹ ·min ⁻¹)	32.8 \pm 6.8	23.1 \pm 4.0

Values are mean \pm SD

3.3.2 Experimental Design

Four experimental trials were performed in a partially counterbalanced crossover design. Prior to the experimental trials, each participant visited the laboratory for a preliminary session during which, height, weight, BMI and body fat were measured and a submaximal incremental treadmill test was performed. The four main experimental trials were separated by one week (four weeks for pre-menopausal women), during which time the participants were asked to maintain their typical diet and physical activity habits. During the experimental trials participants completed either a 45 min brisk walk (exercise trial) or 45 min of rest (control trial), in either a cool (14°C) or warm (32°C) environment. Participants were given a standardized meal 90 min post-exercise, and then proceeded to rest for 3 hours 30 min. Subjective appetite sensations (desire to eat, hunger, fullness and thirst) were measured throughout the trials using visual analogue scales (VAS; See Appendix D for an example). Blood samples were taken during the trials for later analyses of plasma total PYY, and total and acylated plasma ghrelin concentrations.

3.3.3 Anthropometric Measurements

Height was measured using a stadiometer (Seca LTD, Birmingham, England) to the nearest 0.1 cm and weight was measured using electronic scales (Ohaus, Champ II, Germany) to the nearest 0.01 kg. BMI was calculated as weight in kilograms divided by the square of height in metres. Subcutaneous fat was measured at seven sites using skinfold calipers, and the sum of the seven skinfolds was used to calculate body density (Harpender, HKK-BI, Baty International, England). Male body density was calculated in accordance with Jackson and Pollock, (1978), and female body density was calculated in accordance with Jackson *et al.* (1980). Body density was converted into body fat percentage using the Siri, (1956) equation.

3.3.4 Submaximal Incremental Treadmill Test

Participants were asked to refrain from performing strenuous physical activity 24 hours prior to the submaximal treadmill test. The aim of the test was for the participants to achieve 85% of their predicted maximum heart rate. Maximum heart rate was predicted according to Tanaka *et al.* (2001). The test was continuous in nature but was subdivided into 5 min stages. The speed of the treadmill (HP Cosmos, Quaser, Germany) remained constant throughout the test and was selected by the participant. Participants were encouraged to select a speed which they considered a brisk walk. The treadmill gradient was initially set at 0% for the first 5 min and was increased by 2-3% at the end of each 5 min stage. Expired air samples were collected into Douglas bags (Cranlea, Birmingham, UK) during the final minute of each 5 min stage for the determination of oxygen consumption and carbon dioxide production. Ratings of perceived exertion (Borg, 1973) were assessed at the completion of each 5 min stage. Heart rate (Polar Vantage NV, Kempele, Finland) was measured throughout the test and recorded every 15 seconds during the final minute of each stage. The test was terminated once the participant had reached 85% of their predicted maximum heart rate. Carbon dioxide production and oxygen consumption were determined from Douglas bag samples using an infrared carbon dioxide analyser and a paramagnetic oxygen analyser (Analyser Series 1440, Servomex, Crowborough, East Sussex, UK), which were calibrated using gas mixtures of known concentrations. Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to standard temperature and pressure (dry). Maximal oxygen consumption was predicted using the participant's predicted maximum heart rate and a trend line equation created from plotting the relationship between heart rate and oxygen consumption at each stage. The treadmill gradient predicted to elicit 60% of predicted $\text{VO}_{2\text{max}}$ during the experimental trials was calculated using a trend line created by plotting the relationship between the oxygen

consumption in the final minute of each stage against the gradient at each stage. The treadmill speed selected by the participants remained the same for the experimental trials.

3.3.5 Main Experimental Trials

Participants ate a prescribed breakfast (see Dietary Control) at 0800 before arriving at the laboratory at 0930. Core body temperature (T_{re}) was measured using a rectal thermocouple probe (YSI 400 Series, Harvard Apparatus, MA, US) which participants inserted 12cm beyond the anal sphincter. Skin temperature (T_{sk}) was measured using skin thermistors (Grant EUS-U, Grant Instruments LTD, Cambridge, UK) which were attached to participants using adhesive water-resistant tape (Hypafix, BSN Medical GmbH, Hamburg, Germany). In accordance with Nielsen and Nielsen (1987), mean T_{sk} was measured at 4 weighted body locations (forehead, left hand, left lower back and right shin). The same amount of adhesive tape was applied to each site for each trial. The rectal thermocouple probe and the skin thermistors were attached to a data receiver (Squirrel 2020 Series, Grant, Cambridge, UK), which was programmed to record T_{re} and T_{sk} once every minute. Participants then sat upright for 20 min whilst baseline T_{re} and T_{sk} were recorded. For a further 25 min participants lay semi-supine underneath a ventilated hood (Datex Ohmeda, Helsinki, Finland) attached to a mixing chamber (Oxycon-Pro, Jaeger, Mannheim, Germany), whilst resting energy expenditure was measured using an open-circuit indirect calorimeter (Oxycon-Pro, Jaeger, Mannheim, Germany). During the same 25 min period heart rate was recorded every 5 min. At the completion of the rest period participants were then asked to rate their thermal comfort, using the 13-point (1 = “so cold I am helpless”, 13 = “so hot I am sick and nauseated”) McGinnis thermal comfort scale (Hollies & Goldman 1977; Appendix C). The baseline venous blood sample was then taken using an intravenous cannula (BD Venflon, Oxford, UK), inserted into an antecubital vein. Subsequent blood samples were

collected immediately after exercise, 30 min after exercise, immediately prior to the standardized meal, 30 min after the meal and 3 hours 30 min after the meal. The intravenous cannula was kept patent with 2ml flushes of 0.9% NaCl_(aq) isotonic saline solution (Baxter Healthcare, Northampton, UK) following each blood-letting and at regular intervals throughout each trial.

Participants then completed either a 45 min bout of brisk walking or rested for 45 min (control) in an environmental chamber (Design Environment Ltd, Gwent, UK) maintained at either 14°C (cool trial) or 32°C (warm trial) depending on the trial. Relative humidity was maintained at 40% for all trials. The target intensity of the brisk walk was 60% of predicted $\text{VO}_{2\text{max}}$. During the brisk walk one minute expired air samples were collected into Douglas bags at 14-15 min, 29-30 min and 44-45 min to determine energy expenditure, and to ensure that the participants were exercising at the correct intensity. If the participant's VO_2 was above or below 60% of their predicted $\text{VO}_{2\text{max}}$ adjustments to the treadmill gradient were made during the first exercise bout and these adjustments were replicated in the second exercise bout. Thermal comfort was measured following each expired air sample and at 35 and 40 min. Following the completion of the walk participants returned to the laboratory and sat resting for 90 min. Participants were not permitted to shower post-exercise. After 30 min of rest participants rated their thermal comfort and were instructed to remove the skin thermistors, heart rate monitor, and the rectal thermocouple in private. Participants then rested for a further 60 min, after which thermal comfort was measured. Participants were then provided with a standardized meal. Immediately after the meal participants rested for a further 3 hours and 30 min during which time they completed a VAS every 30 min for the first 90 min, and then one every hour thereafter. Following the completion of the post-meal period the participants were allowed to leave the laboratory. See Figure 1 for experimental trial timeline.

3.3.6 Standardised Meal

Participants consumed a standardized meal consisting of white bread, cheese, and margarine, with either, pickle, tomatoes, or mayonnaise; a chocolate bar; crisps; and either milkshake powder with milk or just milk. The average macronutrient content of the meals was $55.4 \pm 1.6\%$ carbohydrate, $19.8 \pm 0.6\%$ protein and $24.8 \pm 2.1\%$ fat. The energy content of the meal was set at 10 kcal per kilogram of body mass. The content of each participants meal and the amount of food provided was kept constant for each trial. During the meal participants were monitored by a member of the research team to ensure that they consumed all of their meal. Participants were encouraged to consume their meal within 15 min.

3.3.7 Subjective Appetite Sensations

Ratings of subjective appetite sensations were measured using 100-mm VAS anchored at each end by the statements “not at all” and “extremely”. Validated VAS were used to assess hunger, thirst, fullness and desire to eat (Flint *et al.* 2000). Participants were presented with the VAS at the beginning of the experimental trials, at the end of the baseline period, every 15 min during exercise/rest in the 14°C/32°C environment, every 30 min thereafter prior to the standardized meal, immediately after the meal, every 30 min after the meal for 90 min, and once every hour for the final 2 hours of the postprandial rest period. Instructions regarding the completion of the VAS were provided at the beginning of each trial. Participants were not allowed to refer to their previous VAS ratings when completing a VAS.

3.3.8 Dietary Control

Prior to the first experimental trial participants completed a three day breakfast diary. The average caloric intake was calculated using nutrition analysis software (CompEatPro, Nutrition Systems, Banbury, England), and this information was used to prescribe an individualised breakfast for each participant. On the morning of each trial the participants were instructed to consume only the prescribed breakfast, consisting of cereal and milk (323 ± 40 kcal), at 0800. Participants were also instructed to consume 5 ml per kilogram body mass of water with their breakfast. Prior to the submaximal incremental exercise test, and the experimental trials, participants were asked to refrain from consuming caffeine 12 hours prior to testing and were asked to refrain from consuming alcohol 24 hours prior to testing. During the experimental trials water was available *ad libitum* during the resting periods, however during the exercise/rest in the 14°C/32°C environment water intake was controlled and participants were provided with 150ml of water every 15 min (a requirement of ethical approval).

3.3.9 Blood Sampling and Analysis

Blood was immediately added to pre-chilled treated EDTA vacutainers (BD Vacutainers, Oxford, UK). The vacutainers had been pre-treated with 0.06 ml of the serine protease inhibitor 4-(2-Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF) (Alexis Biochemicals, Lausen, Switzerland). After gentle inversion, the vacutainers were immediately spun in a centrifuge at 3000 g for 10 min at 4°C. Following whole blood separation, 0.6 ml aliquots of the AEBSF treated plasma were added to pre-treated eppendorfs containing 120 µl 1N HCL and gently inverted. Blood samples were later analysed for plasma total ghrelin, acylated ghrelin, and total PYY concentrations. Plasma was stored at -80°C for later analysis.

Total plasma ghrelin (acylated human ghrelin and des-acyl human ghrelin), plasma acylated ghrelin, and total plasma PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA). Absorbance was read at 450 and 630 nm using a microplate reader (ELx800, BioTek, Bedfordshire, UK), and standard curves were created to calculate the plasma concentration of each hormone. The sensitivities (minimum limits of detection) of total ghrelin, acylated ghrelin, and total PYY were 100 pg/ml, 25 pg/ml, and 16.1 pg/ml respectively when using a 20 μ L sample. The intra-assay CVs for total ghrelin, acylated ghrelin, and total PYY were 2.68%, 3.37%, and 4.69% respectively. All sample measurements were performed in duplicate.

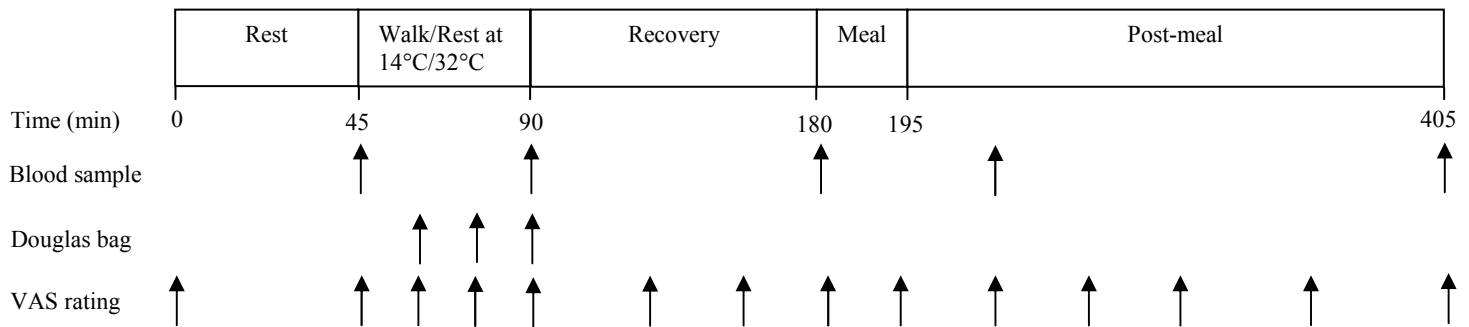


Fig 1. Experimental trial timeline.

3.3.10 Statistical Analysis

Data are expressed as mean \pm standard deviation (SD) in the text and in tables, and data are expressed as mean \pm standard error (SE) in figures. Significant differences were accepted at $P < 0.05$. Normal distribution was checked using the Shapiro-Wilk test of normality. Differences in subjective appetite sensations, T_{re} and T_{sk} across time during the 14°C and 32°C exercise and rest trials were analysed using three-way repeated measures

ANOVAs, with significant differences between trials analysed using Bonferroni *post-hoc* pair-wise comparisons. ANOVA data was split into baseline, exercise/rest, recovery, and post-meal periods. Data was split into segments to avoid changes during exercise/rest being diluted and lost by stability at baseline and, to a lesser extent recovery. Furthermore, a meal induces substantial changes in appetite measures; therefore by not separating whole time into segments the changes caused by the meal may overpower other more subtle changes in appetite. Area under the curve (AUC) values for subjective appetite sensations, PYY and total and acylated ghrelin data were calculated using the trapezoidal method. Area under the curve data were analysed using two-way repeated measures ANOVAs, with significant differences between trials analysed using Bonferroni *post-hoc* pair-wise comparisons. Statistical analysis was carried out with SPSS version 17.0 for Windows (SPSS Inc., Chicago, US).

3.4 Results

3.4.1 Baseline Measurements

During the baseline period there were no significant differences in mean energy expenditure, heart rate, or thermal comfort ratings between trials ($P > 0.05$; data not shown).

3.4.2 Responses to Exercise and Rest at 14°C and 32°C

During the exercise trials participants walked at an average treadmill speed of $5.25 \pm 0.7 \text{ km}\cdot\text{h}^{-1}$, and an average treadmill gradient of $4.23 \pm 0.96\%$. This elicited a significantly greater mean exercise intensity during the 32°C exercise trials ($61 \pm 5\% \text{ VO}_{2\text{max}}$) compared with the 14°C exercise trials ($58 \pm 5\% \text{ VO}_{2\text{max}}$) ($P < 0.05$). However, no differences in average VO_2 were observed between exercise trials ($P > 0.05$; Table 2). Average VO_2 was significantly higher when resting at 14°C compared with resting at 32°C ($P < 0.05$; Table 2). There was no main effect of condition (exercise/rest) or temperature for mean RER values ($P > 0.05$; Table 2). Total energy expenditure was significantly higher when resting in the 14°C environment compared with the 32°C environment ($P < 0.05$; Table 2). Significant increases in average heart rate ($P < 0.01$; Table 2) and thermal comfort ratings ($P < 0.001$; Table 2) were observed in the 32°C vs 14°C trials.

Table 2. Responses to rest and exercise at 14°C and 32°C.

	14°C Rest	14°C Exercise	32°C Rest	32°C Exercise
$\text{VO}_2 (\text{L}\cdot\text{min}^{-1})$	0.21 ± 0.10^{bc}	1.56 ± 0.37^a	0.18 ± 0.07^{ad}	1.58 ± 0.36^c
Total energy expenditure (kcal)	47.7 ± 24.0^{bc}	348.3 ± 86.3^a	39.2 ± 15.6^{ad}	362.1 ± 88.7^c
RER	0.94 ± 0.03	0.90 ± 0.03	0.86 ± 0.01	0.88 ± 0.01
Heart rate ($\text{beats}\cdot\text{min}^{-1}$)	59.8 ± 9.6^{bc}	115.8 ± 9.0^{ad}	67.6 ± 11.7^{ad}	124.3 ± 8.5^{bc}
Thermal comfort	4.19 ± 1.08^{bc}	7.94 ± 1.19^{ad}	7.65 ± 0.49^{ad}	9.83 ± 1.20^{bc}

Values are presented as means \pm SD. Significant differences between trials, $P < 0.05$. *a, b, c, d* indicate significant differences between 14°C rest (*a*), 14°C exercise (*b*), 32°C rest (*c*) and 32°C exercise (*d*) trials respectively. Comparisons between trials which differed in condition (exercise/rest) and temperature were not conducted.

3.3.3 Recovery from Exercise and Rest at 14°C and 32°C

Average heart rate was significantly higher in recovery following exercise compared with rest in both the 14°C (76 ± 10 vs. 58 ± 9 beats·min⁻¹; $P < 0.001$) and 32°C (84 ± 11 vs. 65 ± 14 beats·min⁻¹; $P < 0.001$) environments. Average recovery heart rate was also significantly higher following exercise at 32°C compared with exercise at 14°C (84 ± 11 vs. 76 ± 10 beats·min⁻¹; $P < 0.01$), and significantly higher following rest at 32°C compared with rest at 14°C (65 ± 14 vs. 58 ± 9 beats·min⁻¹; $P < 0.05$). There were no differences in thermal comfort ratings immediately prior to the test meal between trials ($P > 0.05$; data not shown).

3.3.4 Thermoregulatory Responses

No differences in baseline T_{re} were observed between trials ($P > 0.05$; Figure 2). Rectal temperature was significantly higher during the exercise trials compared with the rest trials from 10 minutes onwards in both conditions ($P < 0.001$), an effect that was still apparent during recovery ($P < 0.001$; Figure 2). There was no significant main effect of temperature for T_{re} ($P > 0.05$; Figure 2).

Differences in baseline T_{sk} between trials were not significant ($P > 0.05$; Figure 3). Skin temperature was significantly higher from 20 minutes onwards during exercise compared with rest at 14°C ($P < 0.01$), remaining significantly higher during recovery ($P < 0.001$; Figure 3). No significant differences were found between the 32°C trials ($P > 0.05$; Figure 3). Skin temperature was significantly higher during the 32°C trials compared with the 14°C trials during exercise/rest and recovery ($P < 0.001$; Figure 3).

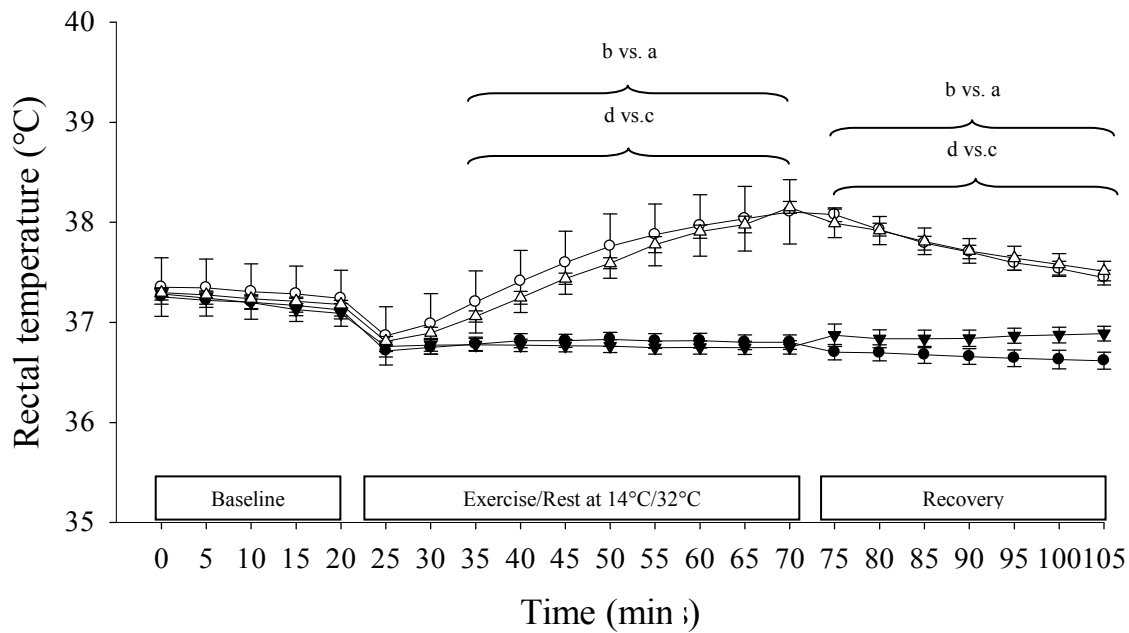


Fig 2. Rectal temperature during baseline measurements, exercise and rest at 14°C and 32°C, and recovery. Values presented as means \pm SE. Significant differences between trials $P < 0.05$. ● 14°C rest, ○ 14°C exercise, ▼ 32°C rest, △ 32°C exercise. *a, b, c, d* indicate significant differences between 14°C rest (*a*), 14°C exercise (*b*), 32°C rest (*c*), and 32°C exercise (*d*) trials respectively. Comparisons between trials which differed in condition (exercise/rest) and temperature were not conducted.

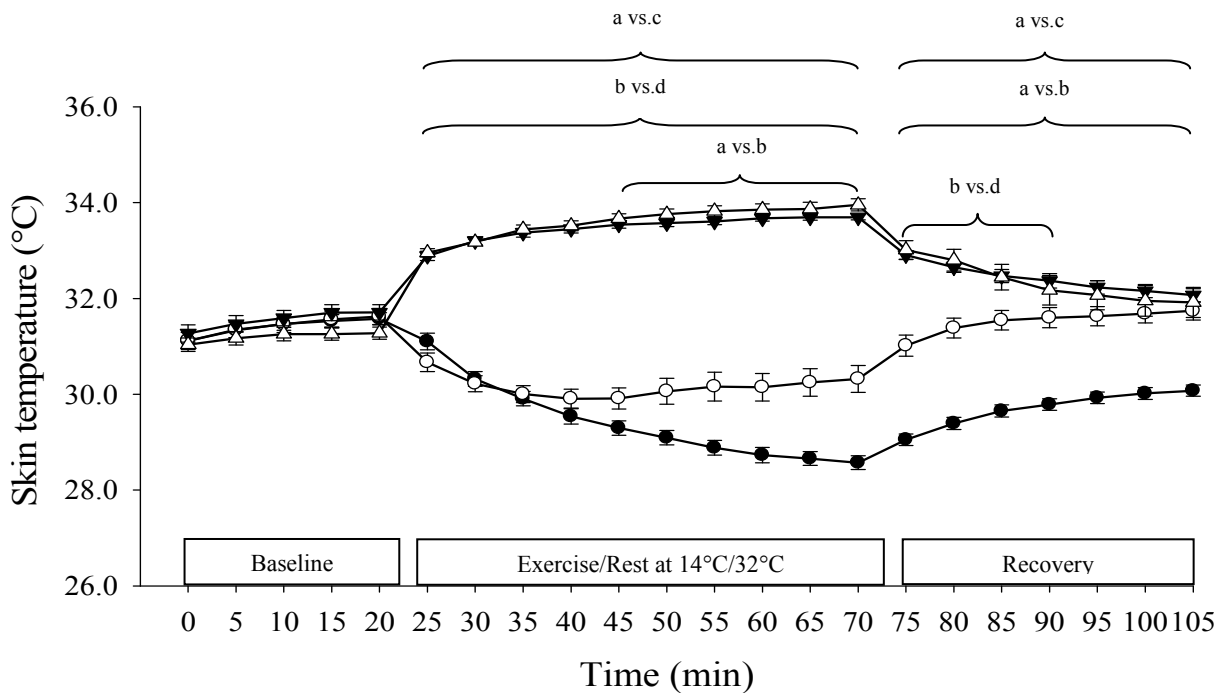
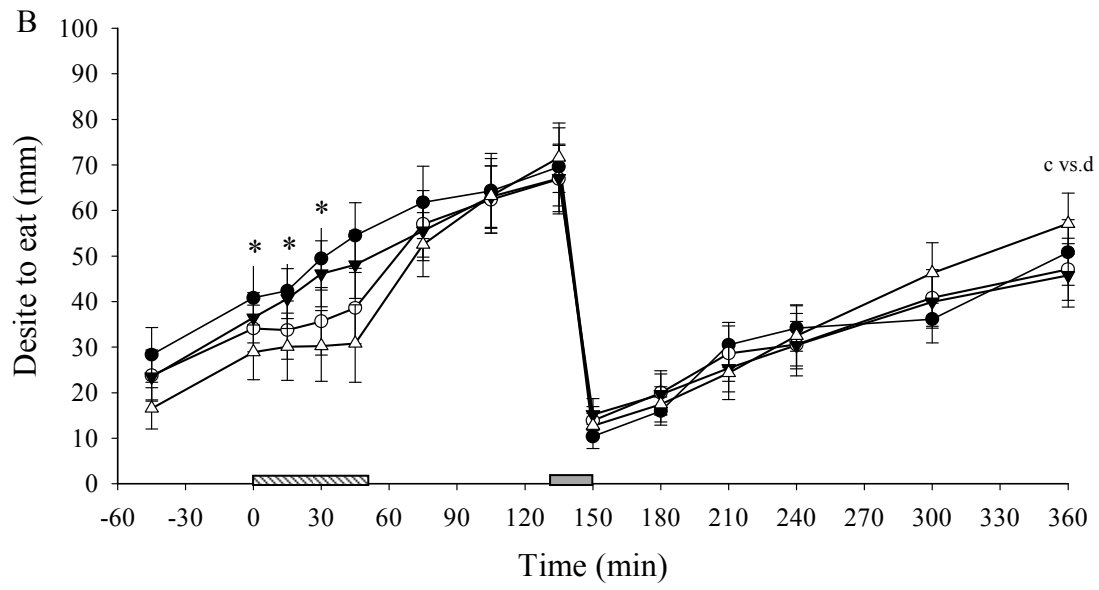
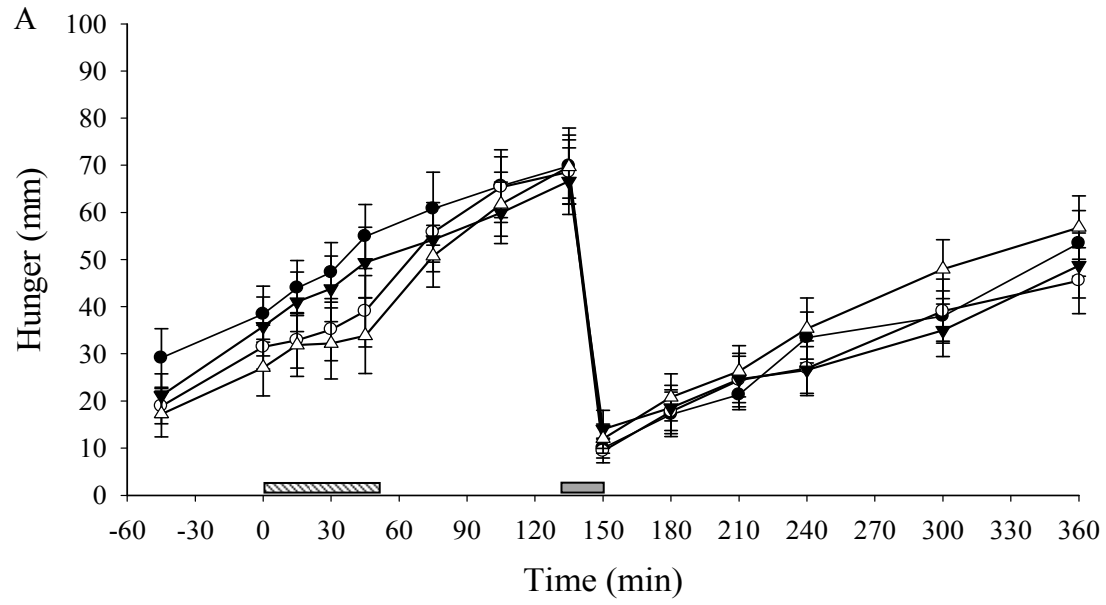


Fig 3. Skin temperature during baseline measurements, exercise and rest at 14°C and 32°C, and recovery. Values presented as means \pm SE. Significant differences between trials $P < 0.05$. ● 14°C rest, ○ 14°C exercise, ▼ 32°C rest, △ 32°C exercise. *a, b, c, d* indicate significant differences between 14°C rest (*a*), 14°C exercise (*b*), 32°C rest (*c*), and 32°C exercise (*d*) trials respectively. Comparisons between trials which differed in condition (exercise/rest) and temperature were not conducted.

3.3.5 Subjective Appetite Sensations

Hunger, desire to eat, fullness and thirst were not significantly different between trials during the baseline period ($P > 0.05$; Figure 4). There was no main effect of condition or temperature for hunger sensations ($P > 0.05$; Figure 4A). There was a significant main effect of time for hunger ratings during recovery prior to the meal ($P < 0.01$), however no between trial differences were observed ($P > 0.05$; Figure 4A). Desire to eat was significantly higher during rest compared with exercise at 30 and 45 min ($P < 0.05$), however there was no main effect of temperature ($P > 0.05$; Figure 4B). At the end of the trial (3 hours 30 min post-meal) desire to eat was significantly higher during the 32°C exercise trials compared with the 32°C rest trials ($P < 0.05$; Figure 4B). There was no main effect of condition or temperature for feelings of fullness or thirst between trials ($P > 0.05$; Figure 4C and Figure 4D). There were no differences in subjective appetite sensations AUC values between trials ($P > 0.05$).



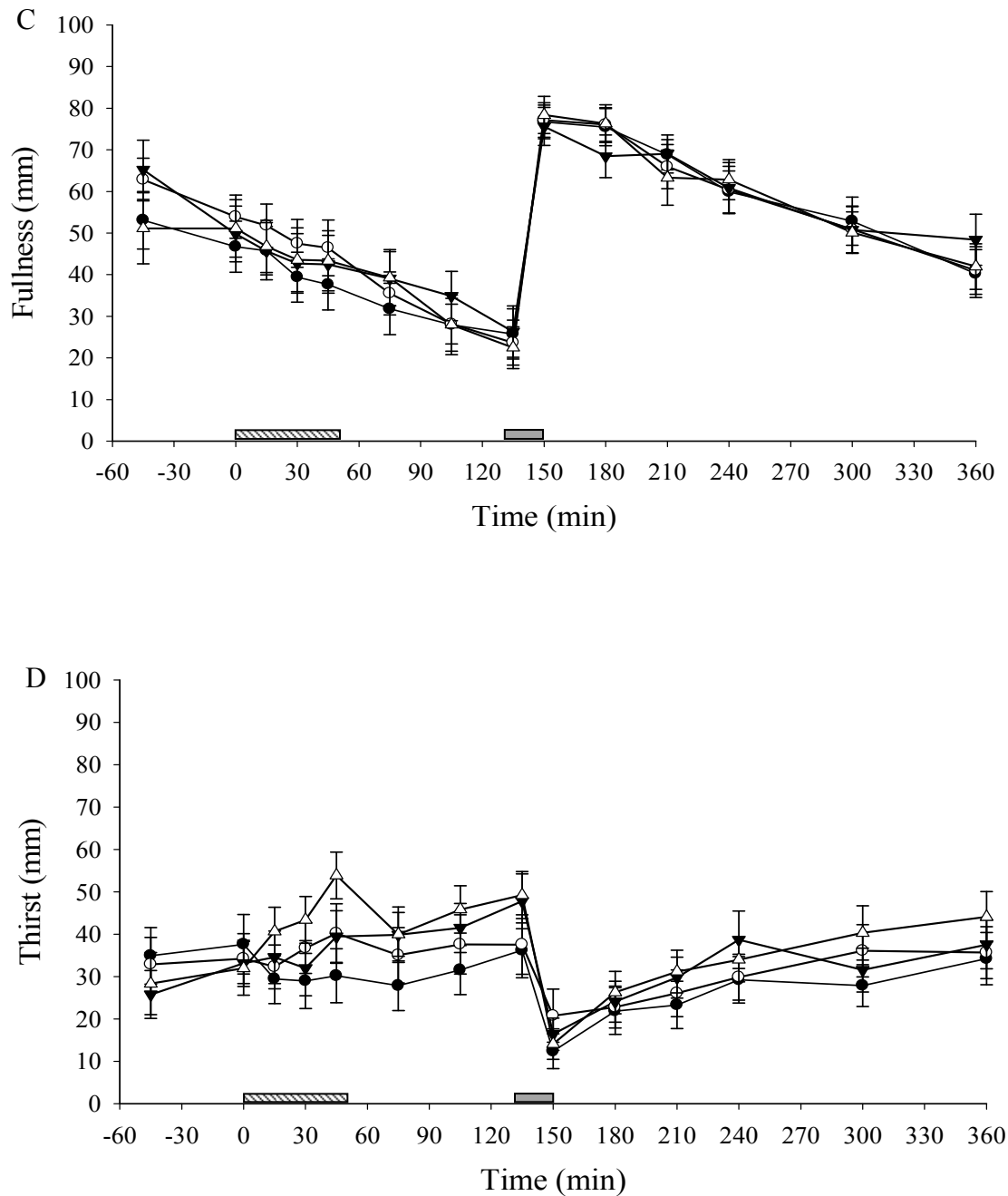
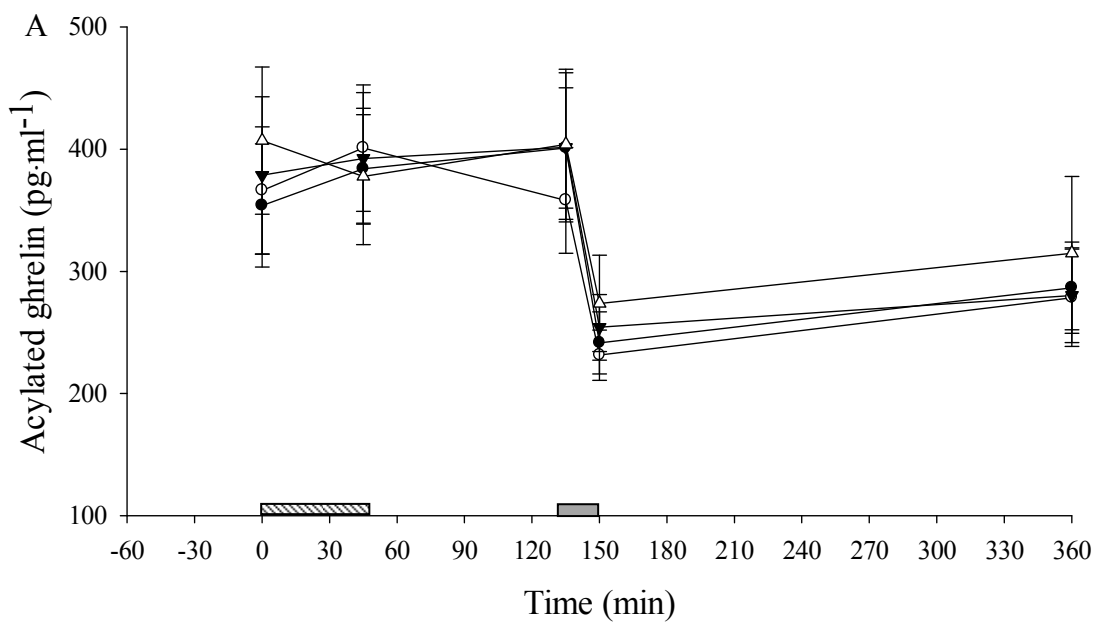


Fig 4. Subjective sensations of hunger (A), desire to eat (B), fullness (C), and thirst (D) throughout each trial. Values presented as means \pm SE. Significant differences between trials $P < 0.05$. ● 14°C rest, ○ 14°C exercise, ▼ 32°C rest, △ 32°C exercise. ▨ exercise/rest at 14°C/32°C, ■ standardized meal. *c, d* indicate significant differences between 32°C rest (*c*) and 32°C exercise (*d*) trials. * Significant differences between rest and exercise (no main effect of temperature) ($P < 0.05$). Comparisons between trials which differed in condition (exercise/rest) and temperature were not conducted.

3.3.6 Appetite Hormones

There was no main effect of condition or temperature for total ghrelin (Figure 5A), acylated ghrelin (Figure 5B), or total PYY (Figure 5C) concentrations ($P > 0.05$). There were no significant differences in the percentage of total ghrelin which was acylated between trials ($P > 0.05$; data not shown). However, total ghrelin total AUC values were significantly lower during the 32°C exercise trials compared with the 14°C exercise trials ($P < 0.05$; data not shown). Furthermore, total PYY AUC values during the intervention period were significantly greater during rest compared with exercise at 32°C ($P < 0.05$; data not shown). There was no main effect of condition or temperature for acylated ghrelin AUC values ($P > 0.05$; data not shown).



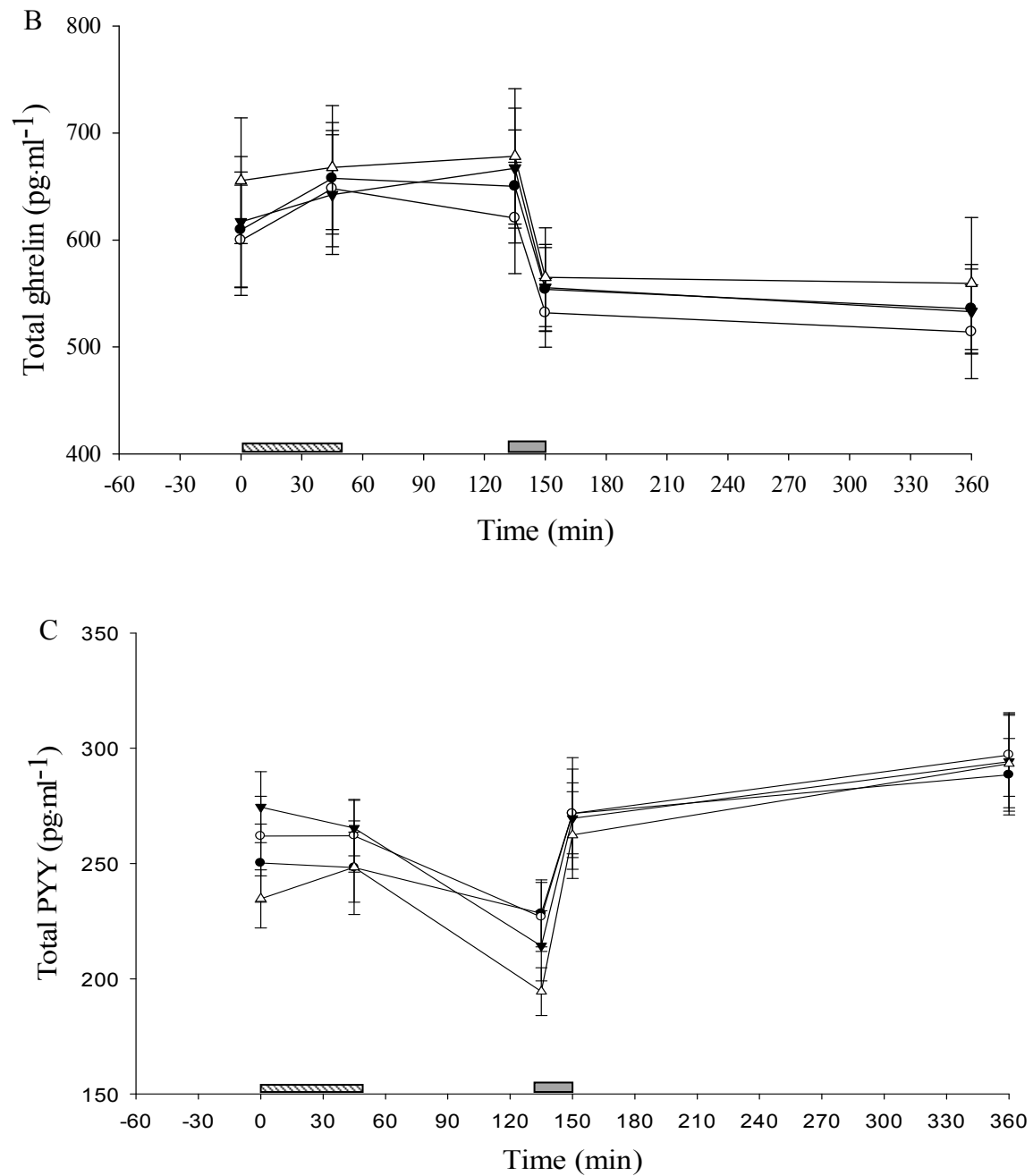




Fig 5. Plasma concentrations of acylated ghrelin (A), total ghrelin (B), total PYY (C) throughout each trial. Values presented as means \pm SE. \bullet 14°C rest, \circ 14°C exercise, \blacktriangledown 32°C rest, \triangle 32°C exercise.  exercise/rest at 14°C/32°C,  standardized meal.

3.4 Discussion

The present study aimed to investigate the effects of brisk walking in a cool (14°C) and warm (32°C) environment on the subjective appetite sensations and appetite hormone responses of middle-aged overweight men and women. The study also aimed to examine the effects of changes in T_{sk} (a surrogate of skin blood flow) and T_{re} on appetite responses and appetite hormone circulation. Despite observing marked differences in T_{sk} and T_{re} between trials, findings from the present study demonstrated that the only significant difference in appetite sensations observed between trials was an increase in the desire to eat at the end of the 32°C exercise trial compared with the 32°C rest trial. No further differences in appetite sensations were observed between trials. However, this study found that total ghrelin AUC values were suppressed during the warm exercise trials compared with the cool exercise trials. Furthermore, this study found that total PYY AUC values were lower when exercising at 32°C compared with resting at 32°C.

The findings from the present study are in slight contrast to the findings of Shorten *et al.* (2009) who found that running in the heat reduced REI compared with resting in a neutral environment. The authors also found that running in the heat increased circulating concentrations of PYY prior to the buffet meal which may have contributed towards a lower REI. There are however several differences between the present study and the Shorten *et al.* (2009) study. Shorten *et al.* (2009) assessed the impact of exercise on appetite by measuring EI following exercise, whereas the present study measured sensations of appetite and did not measure actual EI. The present study aimed to compare appetite responses between a warm (32°C) and cool (14°C) environment, whereas Shorten *et al.* (2009) compared responses between a hot (36°C) and ‘neutral’ (25°C) environment. The present study aimed to minimise the thermal stress of exercising in the control condition, therefore a cool comfortable ambient temperature was selected. However, the temperature selected by

Shorten *et al.* (2009) to represent the neutral environment could be considered quite warm and potentially uncomfortable when exercising. Furthermore, Shorten *et al.* (2009) exercised participants at a high intensity (70% $\text{VO}_{2\text{peak}}$), whereas the present study exercised participants at a moderate intensity (60% $\text{VO}_{2\text{max}}$), this could partly explain the differences between the two studies. High intensity exercise has been shown to suppress sensations of hunger and EI following exercise (Burns *et al.* 2007; Broom *et al.* 2007, 2009; Ueda *et al.* 2009ab; King *et al.* 2010a), whereas studies have shown that moderate intensity exercise has very little effect on appetite (Imbeault *et al.* 1997; King *et al.* 2010b; Unick *et al.* 2010).

Previous research has demonstrated that fluctuations in core body temperature during and after exercise could potentially affect appetite hormone responses (Shorten *et al.* 2009) and post-exercise EI (Dressendorfer *et al.* 1997; White *et al.* 2005; Shorten *et al.* 2009). White *et al.* (2005) found that tympanic temperature was lower following exercise in the cold compared with exercise in a neutral environment. This may have been one of the potential mechanisms which stimulated a greater EI following exercise in the cold. However, White *et al.* (2005) stated that the actual tympanic temperature difference of 0.3°C was unlikely to have influenced post-exercise EI. In contrast to exercise in a cold environment, exercise in a hot environment has been found to suppress REI (Shorten *et al.* 2009). Shorten *et al.* (2009) demonstrated that exercise in the heat elevated both tympanic temperature and PYY concentrations prior to feeding. Consequently, it could be speculated that a rise in core body temperature may be associated with a rise in PYY and hence an increase in satiety. However, it is worth noting that both White *et al.* (2005) and Shorten *et al.* (2009) used tympanic temperature as a measurement of core temperature. This method of core temperature measurement has previously been shown to be unreliable during exercise when compared with T_{re} (Ganio *et al.* 2009). In the present study there were no differences in T_{re} measurements between the cool and warm exercise conditions during or after exercise.

Exercise in the heat produces a substantial elevation in blood flow to the skin in order to enhance heat dissipation, this results in a reduction in blood flow to the splanchnic region (Rowell *et al.* 1965). Previous research has speculated that exercise-induced changes in skin blood flow may influence the circulation of appetite hormones (White *et al.* 2005). Redistribution of blood flow away from the splanchnic region towards the skin may reduce the volume of blood available for the circulation of appetite regulating hormones secreted from regions within the gut. Therefore, this could theoretically reduce the volume of blood available for the circulation of appetite regulating hormones, such as the appetite stimulating hormone ghrelin, and hence potentially cause a reduction in the drive to eat. One could also speculate that the opposite effect may occur during exercise in a cool environment, as skin blood flow may be reduced, thus potentially increasing the volume of blood available in the gut for the circulation of ghrelin. The present study found that T_{sk} (a surrogate of skin blood flow) was greater during exercise at 32°C compared with exercise at 14°C. No differences in circulating concentrations of PYY or acylated ghrelin were observed between trials. In addition, there was no difference in the percentage of total ghrelin which was acylated, indicating that neither exercise, temperature, nor feeding influenced the acylation process in this study. Total ghrelin AUC levels were suppressed during the warm exercise trial compared with the cool exercise trial. This finding indicates that the hormonal drive to eat may have been reduced during the warm exercise trials. However, this finding was not supported by desire to eat or hunger ratings. The only significant difference in appetite sensations observed between trials was an increase in the desire to eat at the end of the 32°C exercise trials compared with the 32°C rest trials. This result indicates that exercise in the heat stimulates appetite several hours post-exercise compared with rest in the same environment, this has been shown previously following exercise and rest in neutral conditions

(Broom *et al.* 2007). However, as actual food intake was not measured, one cannot be certain that this translates into an increased EI.

The present study has several limitations, the most important of which was the small sample size. A power calculation conducted using the G*Power power analysis software (G*Power 2, Heinrich-Heine-Universität, Düsseldorf, Germany), revealed that 24 participants were required in order to provide adequate power (80%) to achieve a significant ($P < 0.05$) difference between groups. Furthermore, this study did not measure EI and instead only measured subjective appetite sensations using VAS. Despite the appetite sensations measured as part of this study having been previously validated against EI (Flint *et al.* 2000), perhaps future research should opt for a combined approach and measure both appetite sensations and EI in order to fully ascertain the impact of an intervention on appetite. In addition, none of the participants involved in this study had previously experience of using VAS to rate their appetite and thus the process was very novel to them. In order to familiarize the participants with the VAS procedure, participants could have been requested to the scales at regular intervals during the week prior to taking part in the study. Finally, total PYY was measured in the current study as opposed to the active form of PYY (PYY₃₋₃₆), however it has been demonstrated that total PYY and PYY₃₋₃₆ exhibit a strong positive correlation (Tsilchorozidou *et al.* 2008).

In conclusion, under the conditions of this study, an acute bout of brisk walking in the heat did not significantly influence sensations of appetite or circulating concentrations of appetite regulating hormones during exercise. However, exercise at 32°C did suppress total ghrelin total AUC values compared with exercise at 14°C, indicating that perhaps exercise in the heat reduced the hormonal drive to eat. This finding however was not supported by ratings of hunger or desire to eat. Overall, this study suffered from a lack of power due to its

small sample size. Therefore, the results presented in this study should be interpreted with caution.

3.5 References

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CHAPTER 4 - ACUTE HIGH INTENSITY EXERCISE SUPPRESSES NEURAL RESPONSES TO FOOD CUES

4.1 Abstract

The impact of high intensity exercise on sensations of appetite and appetite regulating hormone concentrations has attracted a lot of recent attention. However, the effects of exercise on regions of the brain which regulate appetite and reward are yet to be investigated. The present study aimed to examine the effects of a 60 min bout of high intensity running (70% $\text{VO}_{2\text{max}}$) on sensations of appetite, total PYY, total and acylated ghrelin concentrations, and neural responses when viewing pictures of high- and low-calorie foods using functional magnetic resonance (fMRI) techniques. Sixteen lean healthy males (age 22.3 ± 3.1 yr, body mass index $24.0 \pm 2.5 \text{ kg}\cdot\text{m}^2$) completed two trials, exercise and control, in a counterbalanced order. Running significantly suppressed hunger, desire to eat and desire to eat savoury food, whilst increasing thirst. Furthermore, exercise significantly suppressed ghrelin concentrations and significantly enhanced PYY concentrations. Neural responses to images of high-calorie foods significantly increased dorsolateral prefrontal cortex (DLPFC) activation, and suppressed left orbitofrontal cortex (OFC) and left hippocampus activation following exercise compared with rest. In addition, during exercise trials low calorie foods increased left insula, and left and right putamen activation, and reduced left OFC activation compared with rest. Furthermore, left pallidum activity was significantly elevated when viewing low-calorie vs high-calorie food images following exercise compared with rest. These findings demonstrate that high intensity exercise suppresses appetite, and influences appetite regulating hormones in such a way as to reduce hunger. Furthermore, it was observed that neural activity in reward regulating regions of the brain increased in response to images of low-calorie foods, and was suppressed when viewing images of high-calorie foods post-exercise. This is the first study to demonstrate that an acute bout of intense running modulates both peripheral and central appetite regulation.

Keywords: Exercise, fMRI, appetite, insula, OFC, pallidum, ghrelin, PYY.

4.2 Introduction

Appetite and energy intake are regulated through complex interactions between peripheral gut hormones and their central receptors (Simpson and Bloom, 2010). Within the central nervous system (CNS), the hypothalamus plays a key role in energy homeostasis (Hetherington & Ranson, 1942). The hypothalamus contains several areas which have been shown to regulate appetite, including the arcuate (ARC), paraventricular (PVN), ventromedial (VMH), and lateral (LHA) hypothalamus (See Schwartz *et al.* 2000 for a review). The ARC is considered the primary hypothalamic site for the integration of hormonal and neural appetite signals (Lopez *et al.* 2007). The signals produced by neurons within the ARC contribute towards the regulation of homeostatic feeding behaviour by projecting to other appetite regulating areas within the brain (See Konturek *et al.* 2004 for a review).

However, the homeostatic control of feeding behaviour can be over-ridden by the hedonic nature of foods, which can trigger implicit wanting and non-homeostatic feeding behaviour (Finlayson *et al.* 2008). Non-homeostatic feeding is mediated by neurons located within the orbitofrontal cortex (OFC), the insula cortex, the hippocampus, and the striatum, all of which form part of the mesolimbic reward system (Lenard & Berthoud, 2008; Berridge, 2009). The reward system demonstrates a biased response to food cues which have a high hedonic value, such as high-calorie fattening foods, which may as a result lead to a disproportionate motivation toward non-homeostatic consumption of high fat energy dense foods (Stoeckel *et al.* 2008; Chechlacz *et al.* 2009; Schur *et al.* 2009; Goldstone *et al.* 2009; Frank *et al.* 2010; Ng *et al.* 2011). Peripheral appetite regulating hormones which project signals regarding energy status to the hypothalamus, have been shown to influence the central reward system (Abizaid *et al.* 2006; Batterham *et al.* 2007; Farooqi *et al.* 2007; Malik *et al.* 2008).

Ghrelin is a peripheral appetite regulating hormone secreted from the gastric mucosa (Kojima *et al.* 1999; Ariyasu *et al.* 2001; Date *et al.* 2002), which has been shown to stimulate central reward system activity (Malik *et al.* 2008). Intravenous injections of ghrelin prior to feeding have been shown to transiently stimulate appetite in a dose dependent manner in males and females (Wren *et al.* 2001; Schmid *et al.* 2005), and in lean and obese individuals (Druce *et al.* 2005). Ghrelin exists in two forms, acylated (active) and des-acyl (inactive) ghrelin (Hosoda *et al.* 2000). Acylated ghrelin influences feeding behaviour by crossing the blood brain barrier and stimulating appetite by binding to ghrelin receptors located on NPY/AgRP neurons (Nakazato *et al.* 2001; Banks *et al.* 2002). In addition, ghrelin has been shown to modulate hedonic responses to food stimuli by enhancing the neural activity of regions within the brain associated with reward and motivation, including the OFC, anterior insula, and the striatum (Malik *et al.* 2008).

The gut-derived satiety signal PYY also acts centrally to modulate feeding behaviour, however in contrast to ghrelin, PYY suppresses caloric intake (Batterham *et al.* 2002, 2003; Challis *et al.* 2003). PYY is secreted from endocrine L cells in response to energy intake (Adrian *et al.* 1985), and exists as both PYY₁₋₃₆ and PYY₃₋₃₆ (Grandt *et al.* 1994). Sloth *et al.* (2006) demonstrated that PYY₃₋₃₆ infusions suppressed energy intake in humans, whereas PYY₁₋₃₆ infusions had no effect on energy intake. When administered centrally, PYY₃₋₃₆ has been reported to mediate homeostatic feeding by coordinating the suppression of NPY mRNA expression, whilst increasing the neural activity of POMC (Challis *et al.* 2003; Batterham *et al.* 2007). In addition to acting as a metabolic signal for appetite control, PYY has been shown to mediate the activation of reward processing centres, including the OFC and ventral striatum (Batterham *et al.* 2007). Furthermore, in comparison to hypothalamic activity, OFC activity has been shown to predict a majority (77%) of food intake variance when postprandial concentrations of PYY are present (Batterham *et al.* 2007). These

findings demonstrate that, like ghrelin, PYY can also influence areas of the brain which process the rewarding aspects of food (Batterham *et al.* 2007).

The circulation of appetite regulating gut hormones, and appetite responses are influenced by a number of factors, including physical activity (King *et al.* 2011). Acute bouts of low to moderate intensity exercise do not appear to greatly affect appetite (King *et al.* 2010a; Unick *et al.* 2010), however high intensity has been shown to consistently suppress appetite for a brief period of time (Broom *et al.* 2007, 2009; King *et al.* 2010b; Laan *et al.* 2010). Previous research suggests that the appetite regulating hormones PYY and ghrelin contribute towards exercise induced appetite suppression during and after acute bouts of high intensity exercise (Broom *et al.* 2007, 2009; Ueda *et al.* 2009a; King *et al.* 2010b). Broom *et al.* (2009) demonstrated that 1-hr of high intensity running (70% $\text{VO}_{2\text{max}}$) suppressed acylated ghrelin concentrations and increased PYY levels during the run and for 1-hr after. Changes in ghrelin and PYY concentrations coincided with a transient reduction in sensations of hunger during and after exercise (Broom *et al.* 2009). Previous research has speculated that high intensity exercise suppresses appetite by disturbing blood flow to the splanchnic region and causing a redistribution of blood from the splanchnic region to the working muscles and the skin, thereby reducing the volume of blood available for the circulation of ghrelin (Burns *et al.* 2007). Furthermore, a recent investigation conducted by Montenegro *et al.* (2011) found that transcranial direct current stimulation (tDCS) within the dorsolateral prefrontal cortex (an area of the brain which promotes satiation) combined with aerobic exercise (70% VO_2 reserve) transiently induced greater appetite suppression compared with tDCS at rest. In addition, Cornier *et al.* (2011) examined the impact of chronic exercise training with and without an acute bout of moderate intensity exercise post-training, on neural responses to hedonic food cues in overweight participants using functional magnetic resonance imaging (fMRI) techniques. The authors observed that exercise training reduced neural activation

when viewing pictures of food, particularly within the insula cortex. However, Cornier *et al.* (2011) reported that an acute bout of moderate intensity exercise performed after chronic exercise training did not influence neural responses to food cues. Therefore, these findings suggest that acute moderate intensity exercise may not influence central appetite regulation. As previously mentioned, research has found that acute moderate intensity exercise does not greatly affect subjective appetite responses (King *et al.* 2010a; Unick *et al.* 2010) or peripheral appetite hormone circulation (King *et al.* 2010a). However, acute bouts of high intensity exercise have been shown to modulate markers of appetite during and after exercise (Broom *et al.* 2007, 2009).

Thus far, research which has investigated the effects of acute bouts of high intensity exercise on appetite has focused entirely upon subjective appetite sensations and the responses of peripheral satiety regulating hormones; however no study has investigated the acute effects of high intensity exercise on central appetite regulation using fMRI techniques. Therefore, the primary aim of this study was to determine the effects of an acute bout of high intensity exercise on the central neural responses to viewing pictures of food using fMRI. Furthermore, this study aimed to investigate the effect of high intensity exercise on ratings of appetite, plasma total and acylated ghrelin concentrations, and total plasma PYY concentrations. Total and acylated ghrelin was measured so that it was possible to assess the effects of exercise on the post-secretion acylation process. It was hypothesized that, due to the known effects of high-intensity exercise on appetite regulatory hormones and subjective appetite ratings, the activation of reward-related brain regions to visual food cues would be modulated following exercise.

4.3 Method

4.3.1 Participants

Sixteen physically active males volunteered to participate in the study which had ethical approval from the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham and the Birmingham University Imaging Centre Ethics Committee. All volunteers provided written informed consent (Appendix A) and completed a general health questionnaire (Appendix B) prior to their participation in the study. Upon completion of the study participants were assessed for dietary restraint using the Three-Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985). Fourteen participants scored <12 on the restraint subscale indicating that these participants were non-dietary-restrained, however 2 of the participants scored >12 on the restraint subscale indicating that these participants were dietary restrained (Stunkard and Messick, 1985). All participants were non-smokers, free from cardiovascular and metabolic disorders, were not taking any medication which may have affected their appetite, were not following a special diet, and regularly participated in moderate/vigorous physical activity. Table 1 details the participants' characteristics.

Table 1. Participant characteristics.

Characteristics	
Age (yr)	23.3 ± 3.1
Height (m)	1.80 ± 0.06
Weight (kg)	77.6 ± 11.7
BMI ($\text{kg}\cdot\text{m}^2$)	24.0 ± 2.5
$\text{VO}_{2\text{max}}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	54.5 ± 5.9
TFEQ score	7.13 ± 4.27
Values are mean \pm SD ($n = 16$)	

4.3.2 Experimental Design

Participants performed two counterbalanced trials, a resting control trial and an exercise trial. Prior to the experimental trials each participant visited the laboratory for a preliminary session during which anthropometric measurements (height, weight, and BMI) were obtained and an exhaustive incremental exercise test was performed. Following the preliminary visit the participants were given a week to recover before the experimental trials began. For both experimental trials participants reported to the laboratory in the morning following an overnight fast. During the experimental trials participants completed either 60 min of high intensity running (exercise trial), or 60 min of rest (control trial). Participants then completed an fMRI assessment, during which their regional brain activation to food and non-food stimuli was measured. The food stimuli were presented in a random order, and included pictures of high- and low-calorie foods which were visually matched with the non-food control pictures. The images were presented to the participants briefly so that neural responses were not deep cognitive responses. Following the fMRI scan participants were asked to rate how pleasant and arousing they found the food and non-food pictures. Subjective appetite sensations were measured throughout the trial using visual analogue scales (VAS; See Appendix D for an example). Blood samples were taken during the trials for later analyses of total plasma PYY, and total and acylated plasma ghrelin concentrations.

4.3.3 Exhaustive Incremental Treadmill Test

Participants were requested not to perform any physical activity 24 hours prior to the exhaustive treadmill test. Upon arrival at the laboratory height was measured using a stadiometer (Seca LTD, Birmingham, England) to the nearest 0.1 cm and weight was measured using electronic scales (Ohous, Champ II, Germany) to the nearest 0.01 kg. BMI was calculated as weight in kilograms divided by the square of height in metres. Participants

then completed a 10 min seated rest period. During the final minute of the rest period, resting heart rate was recorded every 15 seconds using short-range telemetry (Polar Vantage NV, Kempele, Finland). The exercise test was continuous in nature but was subdivided into 5 min stages. The speed of the treadmill (HP Cosmos, Quaser, Germany) was initially set at 10 km/h and was increased by 2 km/h every 5 min until volitional exhaustion. The treadmill gradient was set at 0% throughout the test. Expired air samples were collected into Douglas bags (Cranlea, Birmingham, UK) during the final minute of each 5 min stage for the determination of oxygen consumption and carbon dioxide production. Heart rate was measured throughout the test and recorded every 15 seconds during the final minute of each 5 min stage. Ratings of perceived exertion (Borg, 1973) were assessed at the completion of each 5 min stage. The participants ran until exhaustion and indicated when they could only continue exercising for one more minute. At this point the final expired air sample was collected. Throughout the test participants received verbal encouragement. Maximal oxygen uptake was adjudged to have been attained when participants met two of the following criteria: heart rate was within $\pm 10 \text{ beats} \cdot \text{min}^{-1}$ of age predicted maximum heart rate ($220 \text{ beats} \cdot \text{min}^{-1} - \text{age}$), a respiratory exchange ratio ≥ 1.15 , and a plateau in oxygen consumption (Howley *et al.* 1995). Using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Analyser Series 1440, Servomex, Crowborough, East Sussex, UK), calibrated using a mixture of known gas concentrations, oxygen consumption and carbon dioxide production were determined from expired air samples. Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to standard temperature and pressure (dry). The oxygen consumption at each 5 min stage was plotted against the speed at each stage to calculate the speed-oxygen consumption relationship. From this relationship, a trend line equation could be used to estimate the speed which would elicit 70% of the participants $\text{VO}_{2\text{max}}$.

4.3.4 Experimental Trials

Exercise trial. Participants arrived at the Birmingham University Imaging Centre in a fasted state at 0745. Upon arrival the participants completed the Positive Affect Negative Affect Scale (PANAS) (Watson *et al.* 1988; Appendix E). The PANAS was used to ensure that the participants' mood was similar for both trials, as mood has been shown to influence feeding behaviour (Macht, 1999). Blood samples were taken through an intravenous cannula (BD Venflon, Oxford, UK) which was inserted into an antecubital vein. During the trial the cannula was kept patent with 2ml flushes of 0.9% NaCl_(aq) isotonic saline solution (Baxter Healthcare, Northampton, UK) following each blood-letting. Venous blood samples were drawn from the intravenous line at the beginning of the experimental trials, at the end of exercise/rest period, at the end of the post-exercise/rest recovery period, and immediately after the fMRI assessment. Core body temperature was monitored using a rectal thermocouple probe (YSI 400 Series, Harvard Apparatus, MA, US) inserted 12cm beyond the external anal sphincter, which participants applied in private. The rectal thermocouple probe was attached to a data receiver (Squirrel 2020 Series, Grant, Cambridge, UK), which was programmed to record mean rectal temperature (T_{re}) every minute. Heart rate was monitored during exercise using short range telemetry. Participants completed a 60 min run at a speed predicted to elicit 70% of their VO_{2max} . If during the run the participants could not maintain the required speed, then the speed was reduced slightly (3 participants had their speed reduced). The gradient of the treadmill remained at 0% throughout the run. Following the completion of the run the participants rested for 10 min to allow heart rate and cerebral blood flow to return to resting values (Ogoh *et al.* 2007), during which time they completed the PANAS and removed the rectal thermocouple, and the heart rate monitor. After resting fMRI scans were conducted. Following the fMRI scans, the participants completed two post-scan tasks. The participants were shown the images which were presented to them during the

fMRI scan, intermixed with 10 novel images, and were then asked to identify which images were shown to them during their fMRI scan. This task was performed to ensure that the participants were paying attention during their fMRI scan (Malik *et al.* 2008). Finally, the participants were asked to rate how ‘arousing’ and how ‘pleasant’ they found the images presented to them during the scan on a scale of 1 to 9 (1 = “very calming/very unpleasant” and 9 = “very exciting/very pleasant”) (Bradley *et al.* 1994; appendix F).

Control trials. The control trial was identical to the exercise trial; however participants rested for 60 min rather than exercised. Participants were instructed to wear the same clothing for both trials.

4.5.5 Visual Stimuli and Experimental Procedure

During each fMRI scan, food and non-food pictures (see Figure 1 for examples) were presented to the participant on a screen in the scanner and viewed through a mirror attached to a head coil (SENSE Head coil 8 elements, Phillips Electronics, Best, Netherlands). Participants were instructed to look carefully at each picture during the scanning. In total, 24 food and 24 non-food visually matched (in terms of shape, complexity, brightness and colour) control pictures were presented. Most of these images had previously been used in an investigation comparing the brain responses to food cues in type 2 diabetics and healthy controls (Chechlacz *et al.* 2009). The food pictures consisted of high fat sweet, high fat savoury, low fat sweet and low fat savoury foods. The total caloric density, and carbohydrate and fat composition of the foods were as follows – high fat sweet foods: 350 ± 90 kcal/100g, $51 \pm 8\%$ carbohydrate, $45 \pm 7\%$ fat: high fat savoury foods: 310 ± 120 kcal/100g, $37 \pm 16\%$ carbohydrate, $47 \pm 12\%$ fat: low fat sweet foods: 50 ± 10 kcal/100g, $93 \pm 4\%$ carbohydrate, $4 \pm 3\%$ fat: low fat savoury: 70 ± 80 kcal/100g, $64 \pm 16\%$ carbohydrate, $16 \pm 9\%$ fat.

During scanning, the food and non-food stimuli were presented in a random-order using an event-related design. Each stimulus was presented for 2 s followed by an inter-stimulus interval of 8 s, during which time a central fixation cross was presented. Each picture was presented once, therefore, the total time of each scanning assessment was approximately 16 min. Halfway through each scan the picture presentation was halted and the participants were reminded to look carefully at each picture before the sequence recommenced. The total time taken to complete a scan, including the time required to prepare the participant, was approximately 30 min.

A



B

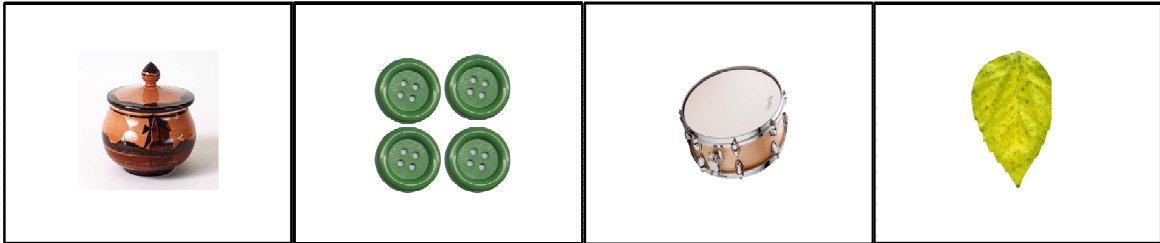


Fig 1: Examples of visually matched food (A) and non-food (B) pictures presented to participants during the fMRI experimental procedure. The food pictures represent, from left to right, the four selected food categories; high fat savoury, low fat savoury, high fat sweet, and low fat sweet.

4.3.6 Functional MRI Data Acquisition

Neuroimaging was conducted on a 3T Philips Achieva whole body scanner (Phillips Electronics, Best, Netherlands) equipped with an 8-channel SENSE head coil. Five hundred and seventy six functional whole brain T2*-weighted images were acquired from each

participant using a 2D single-shot EPI sequence (34 axial slices, whole brain coverage, echo time = 35 ms, repetition time = 2 s, flip angle = 65°, field of view = 240 mm × 102 mm × 240 mm, 3 mm × 3 mm × 3 mm resolution). Prior to the baseline fMRI assessment, a high resolution T1-weighted structural image (1 mm × 1 mm × 1 mm resolution) was also acquired for co-registration and display of the functional data. At the beginning of every run the scanning time and stimulus presentation were synchronized by a trigger signal transmitted from the fMRI scanner.

4.3.7 Functional MRI Data Analysis

Functional MRI data analysis was conducted using FSL software library version 4.1 written by the Oxford Centre for Functional MRI of the Brain (FMRIB; Smith *et al.* 2004; Woolrich *et al.* 2009). Motion artefacts were corrected prior to analysis using FMRIB's Linear Image Registration Tool (MCFLIRT; Jenkinson *et al.* 2002). Non-brain structures were removed from each participant's structural image by using the FSL Brain Extraction Tool (BET; Smith, 2002). Functional images were spatially smoothed using a 9mm Gaussian kernel with a high-pass filter of sigma = 20 s. A statistical parametric map was created for each participant using a general linear model to calculate the contrast of food>object, high-calorie food>object (HC>object), low-calorie food>object (LC>object), high-calorie food>low-calorie food (HC>LC), and low-calorie food>high-calorie food (LC>HC) at each voxel for exercise and rest. All statistical parametric maps were registered to structural images and to the Montreal Neurological Institute 152 standard image using FLIRT (FMRIB's Linear Image Registration Tool).

The effect of condition (exercise>rest/rest>exercise) on each contrast was determined using higher level (mixed effects: FLAME 1+2) paired group analyses. Z statistic images for each contrast were thresholded at $Z > 3.1$ (equivalent to an uncorrected one-tailed P of

0.001). In order to minimize random effects, only clusters with ≥ 20 continuous voxels were retained. MRIcro was used to display higher level statistical maps on a standard brain template and the AAL atlas was used to identify the anatomical location of clusters of significant activation. Only clusters of activity within the orbitofrontal cortex, anterior insula, dorsolateral prefrontal cortex, hippocampus and striatum will be discussed. These regions of interest have previously been shown to respond to food stimuli and be influenced by subjective appetite responses (Tataranni *et al.* 1999; Porubská *et al.* 2006). In each cluster of significant activation, the peak voxel was identified and the percentage change in BOLD signal extracted using the FEATquery tool. The BOLD signal was then statistically compared in the exercise and rest trials using a paired Student's t test performed in SPSS.

4.3.8 Blood Sampling and Analysis

For the measurement of plasma total and acylated ghrelin, and total PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations, 3 ml of whole blood was immediately added to a pre-chilled EDTA vacutainer (BD Vacutainers, Oxford, UK) which had been pre-treated with 0.06 ml of the serine protease inhibitor, 4-(2-Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF) (Alexis Biochemicals, Lausen, Switzerland). After gentle inversion, the vacutainers were then immediately spun at 3000 g for 10 min in a centrifuge at 4°C. Following whole blood separation, 0.6 ml aliquots of the AEBSF treated plasma was added to eppendorf tubes containing 120 µl 1N HCL and gently inverted. Treated plasma was stored at -80°C for later analysis.

Total plasma ghrelin (acylated human ghrelin and des-acyl human ghrelin), plasma acylated ghrelin, and total plasma PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA). A microplate reader (ELx800, BioTek,

Bedfordshire, UK) was used to measure absorbance, and standard curves were generated by plotting the absorbance unit against the standard concentrations of each ELISA to calculate the plasma concentration of each hormone. The appropriate ranges of the total ghrelin, acylated ghrelin, and total PYY ELISAs were 100 pg/ml to 5000 pg/ml, 25 pg/ml to 2000 pg/ml, and 14 pg/ml to 1800 pg/ml respectively. The intra-assay CVs were 2.42%, 2.96%, and 7.88% for total ghrelin, acylated ghrelin, and total PYY respectively. All sample measurements were performed in duplicate.

4.3.9 Subjective Appetite Sensations

Ratings of subjective appetite sensations were measured using 100-mm VAS, with extreme statements anchored at each end (e.g. not at all hungry – extremely hungry). Visual analogue scales were used to assess hunger, thirst, fullness, desire to eat, and desire to eat something sweet, savoury, salty and fatty, as previously validated by Flint *et al.* (2000). Participants were presented with the VAS at the beginning of the experimental trials, after 30 min of exercise/rest, at the end of exercise/rest period, and immediately after the fMRI assessment. Instructions relating to the completion of the VAS were provided at the beginning of each trial. Participants were not allowed to refer to their previous VAS ratings when completing a VAS.

4.3.10 Statistical Analysis

Data are expressed as mean \pm standard deviation (SD) in the text and in tables, and data are expressed as mean \pm standard error (SE) in figures. Significant differences were accepted at $P < 0.05$. Mean heart rate data were analysed between trials using paired samples t-test. Rectal temperature data, mean positive and negative PANAS ratings, total PYY, and

total and acylated ghrelin data were analysed over time between trials using two-factor (trial x time) repeated measures ANOVA. Pleasantness and arousal ratings for each trial were separated into scores for pictures of foods which were high fat and sweet (HF/SW), high fat and savoury (HFSA), low fat and sweet (LF/SW), and low fat and savoury (LF/SA), and analysed using two-factor repeated measures ANOVA. Interactions were analysed using Bonferroni *post-hoc* tests. Raw total PYY, and total and acylated ghrelin data were converted to area under the curve (AUC) using the trapezoidal rule and differences were analysed by paired *t* tests. Pearson correlation coefficient was used to examine relationships between variables. Statistical analysis was carried out with SPSS for Windows version 17.0 (SPSS Inc., Chicago, US).

4.4 Results

4.4.1 Participants

One participant was unable to complete all of the necessary fMRI scans due to a technical fault during an fMRI assessment. Therefore, they were excluded from the fMRI data analyses. Consequently, the participant was also excluded from the post-fMRI scan tasks data analyses and the blood sampling analyses.

4.4.2 Physiological and Emotional Responses to Exercise and Rest

Participants exercised at a treadmill speed of $11.7 \pm 1.8 \text{ km} \cdot \text{h}^{-1}$ during the high intensity run. Average heart rate was significantly higher during exercise compared with rest (159 ± 8 vs. $60 \pm 9 \text{ beats} \cdot \text{min}^{-1}$; $P < 0.05$). Exercising T_{re} was significantly higher from 10 min onwards compared with resting T_{re} ($P < 0.05$; Figure 2). Mean PANAS positive (exercise: 3 ± 1 ; rest: 3 ± 1) and negative (exercise: 1 ± 0 ; rest: 1 ± 0) affect ratings measured prior to exercise and rest were not significantly different between trials ($n = 14$; $P > 0.05$). Furthermore, mean PANAS positive (exercise: 3 ± 1 ; rest 3 ± 1) and negative (exercise: 1 ± 0 ; rest: 1 ± 0) affect ratings measured immediately after exercise and rest were not significantly different between trials ($n = 14$; $P > 0.05$).

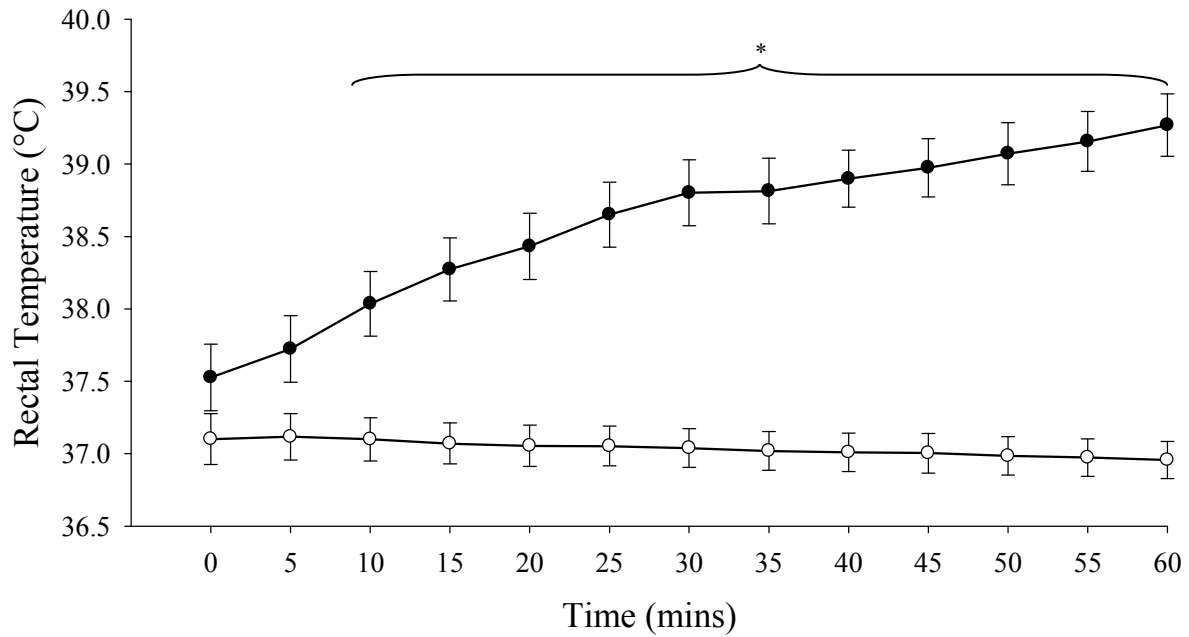


Fig 2: Rectal temperature values during exercise and rest ($n = 14$). ● exercise, ○ rest. Values presented as means \pm SE. *Significant differences between trials $P < 0.05$.

4.4.3 Functional MRI Data

4.4.3.1 Food > object contrast

When viewing pictures of food, right insula activation was significantly greater following exercise compared with rest ($P < 0.05$; Table 2; Figure 3). Furthermore, exercise suppressed neural activation within the left OFC ($P < 0.01$; Table 2; Figure 3) and the left hippocampus ($P < 0.01$; Table 2; Figure 3) compared with rest.

Table 2. Region of activation, Talaraich coordinates, Z score, and significance level of exercise vs rest comparisons for the food > object contrast.

Region	Hem	x	y	z	Z score	P
Insula	R	-46	24	36	8.51	< 0.05
OFC	L	-8	36	-10	4.12	< 0.01
Hippo	L	-25	-38	-2	5.89	< 0.01

Hem = hemisphere. L = left hemisphere. R = right hemisphere. OFC = orbitofrontal cortex. Hippo = hippocampus

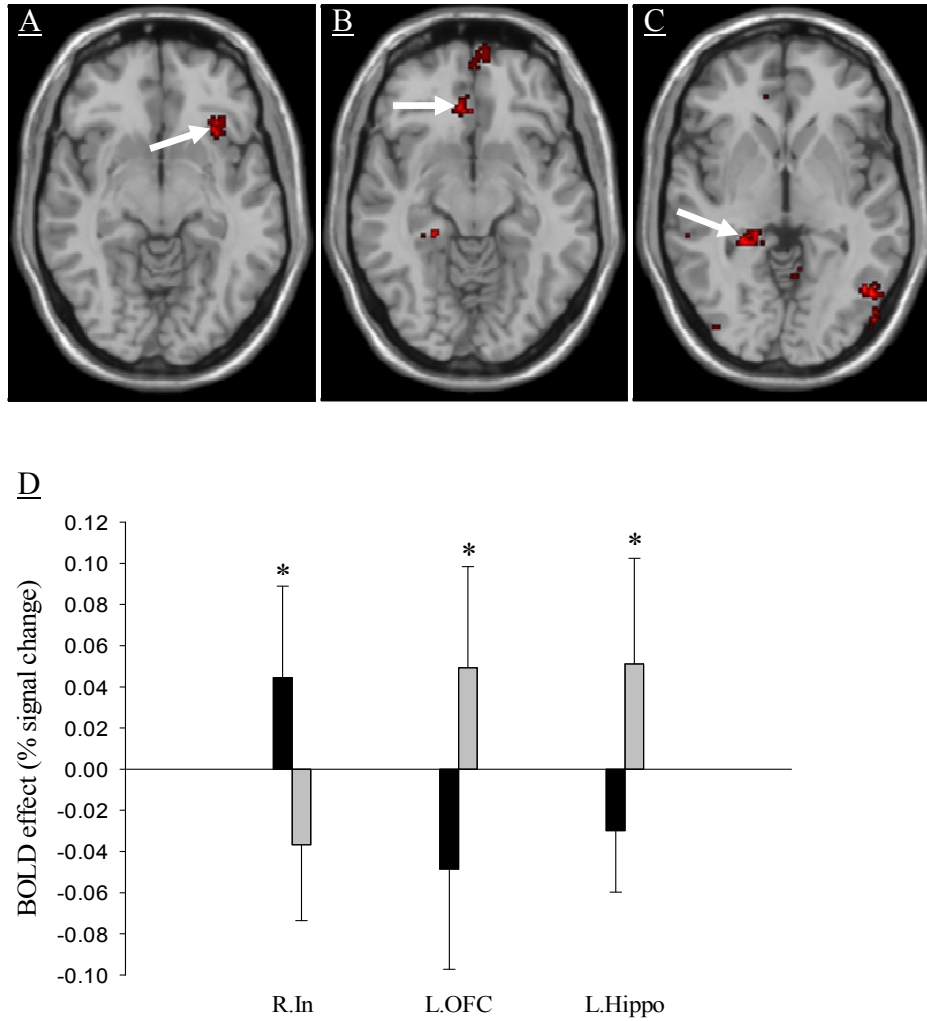


Fig 3: Image A is taken from the food>object, exercise>rest contrast, and images B and C are taken from the food>object, rest>exercise contrast. Arrows indicate the peak voxel activation in the right insula (A), the left OFC (B), and the left hippocampus (C). D is a bar graph showing the food>object BOLD effect (% signal change) in the exercise (black bars) and rest (grey bars) conditions for the right insula (R.In), the left OFC (L.OFC), and the left hippocampus (L.Hippo). Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

4.4.3.2 HC>object contrast

Pictures of high calorie foods enhanced BOLD response in the dorsolateral prefrontal cortex following the exercise trials compared with the rest trials ($P < 0.05$; Table 3; Figure 4). Whereas exercise suppressed activation within the left OFC ($P < 0.05$; Table 3; Figure 4) and left hippocampus ($P < 0.05$; Table 3; Figure 4) compared with rest.

4.4.3.3 LC > object contrast

Low calorie food images significantly increased left insula ($P < 0.01$; Table 3; Figure 5), left putamen ($P < 0.01$; Table 3; Figure 5), and right putamen ($P < 0.01$; Table 3; Figure 5) activity following exercise in comparison to rest. Furthermore, exercise significantly reduced left OFC activity when viewing images of low calorie foods compared with rest ($P < 0.01$; Table 3; Figure 5).

Table 3. Region of activation, Talaraich coordinates, Z score, and significance level of exercise vs rest comparisons for the HC > object, and the LC > object contrasts.

Contrast	Region	Hem	x	y	z	Z score	P
HC > object	DLPFC	L	-46	24	36	8.51	< 0.05
	OFC	L	-4	58	-10	7.48	< 0.05
	Hippo	L	-32	-30	-16	22.1	< 0.05
LC > object	Insula	L	-34	24	8	11.6	< 0.01
	Putamen	L	-22	16	2	11.2	< 0.01
		R	22	-14	2	11.2	< 0.01
	OFC	L	-8	38	-14	11.7	< 0.01

Hem = hemisphere. L = left hemisphere. R = right hemisphere. DLPFC = dorsolateral prefrontal cortex. OFC = orbitofrontal cortex. Hippo = hippocampus.

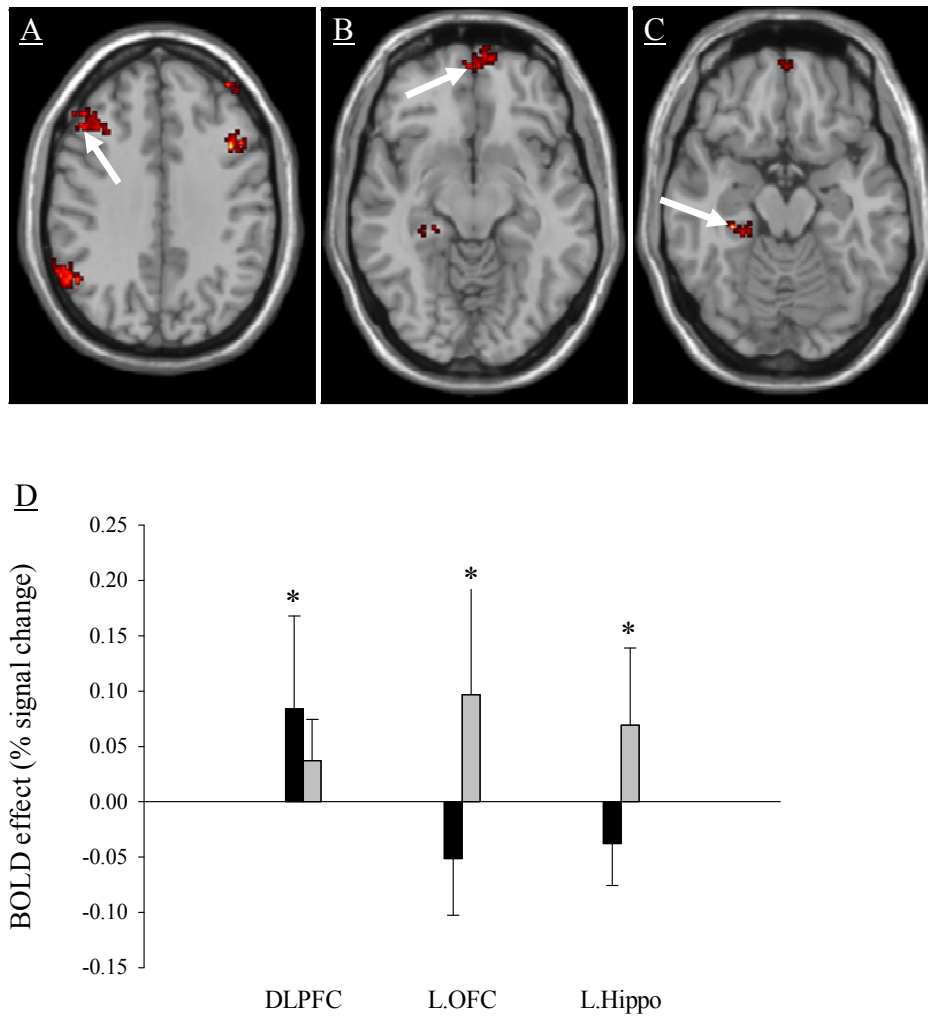


Fig 4: Image A is taken from the HC food>object, exercise>rest contrast, and images B and C are taken from the HC>object, rest>exercise contrast. Arrows indicate the peak voxel activation in the DLPFC (A), the left OFC (B), and the left hippocampus (C). D is a bar graph showing the HC>object BOLD effect (% signal change) in the exercise (black bars) and rest (grey bars) conditions for the DLPFC, the left OFC (L.OFC), and the left hippocampus (L.Hippo). Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

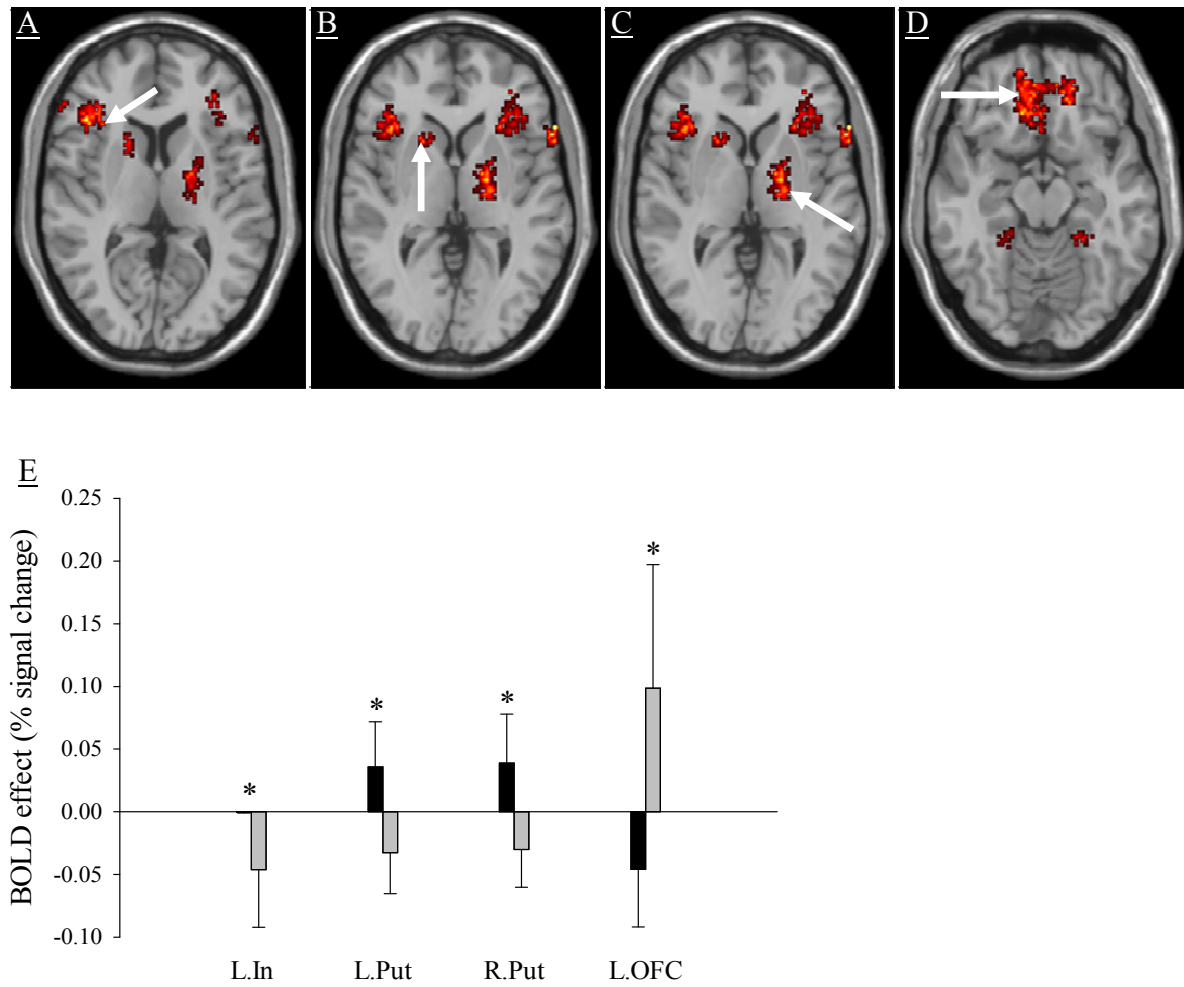


Fig 5: Image A, B, and C are taken from the LC food>object, exercise>rest contrast, and image D is taken from the LC>object, rest>exercise contrast. Arrows indicate the peak voxel activation in the left insula (A), the left (B) and right (C) putamen, and the left OFC (D). E is a bar graph showing the LC>object BOLD effect (% signal change) in the exercise (black bars) and rest (grey bars) conditions for the left insula (L.In), the left putamen (L.Put), the right putamen (R.Put), and the left OFC (L.OFC). Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

4.4.3.4 HC>LC contrast

Following exercise there was significantly less neural activation in response to high calorie vs low calorie foods within the left pallidum compared with rest ($P < 0.01$; Table 4; Figure 6).

4.4.3.5 LC>HC contrast

There was significantly greater neural activation within the left pallidum in response to low calorie vs high calorie foods following exercise compared with rest ($P < 0.01$; Table 4; Figure 6).

Table 4. Region of activation, Talaraich coordinates, Z score, and significance level of exercise vs rest comparisons for the HC > LC, and the LC > HC contrasts.

Contrast	Region	Hem	x	y	z	Z score	P
HC > LC	Pallidum	L	-22	-4	-2	20.0	< 0.01
LC > HC	Pallidum	L	-18	-8	-6	11.2	< 0.01

Hem = hemisphere. L = left hemisphere. R = right hemisphere.

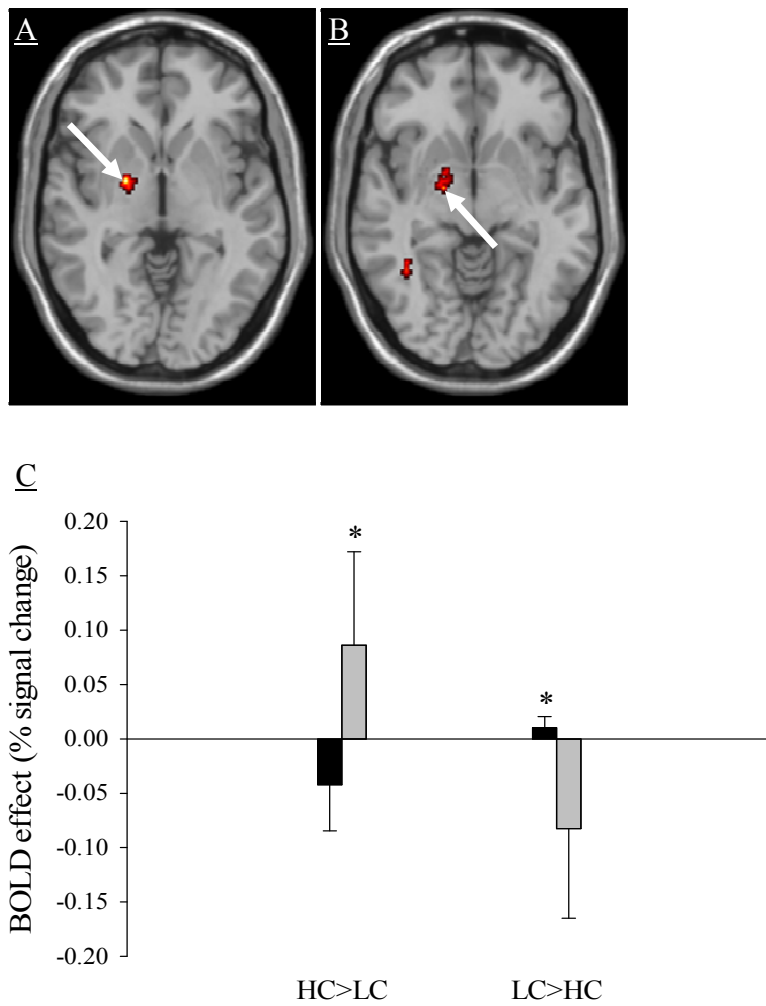


Fig 6: Images A and B are taken from the HC>LC and LC>HC contrasts. Arrows indicate the peak voxel activation in the left pallidum, HC>LC, exercise>rest contrast (A) and the left pallidum, LC>HC, rest>exercise contrast (B). C is a bar graph showing the left pallidum BOLD effect (% signal change) for the HC>LC and the LC>HC in the exercise (black bars) and rest (grey bars) conditions. Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

4.4.4 Post-fMRI Scan Tasks

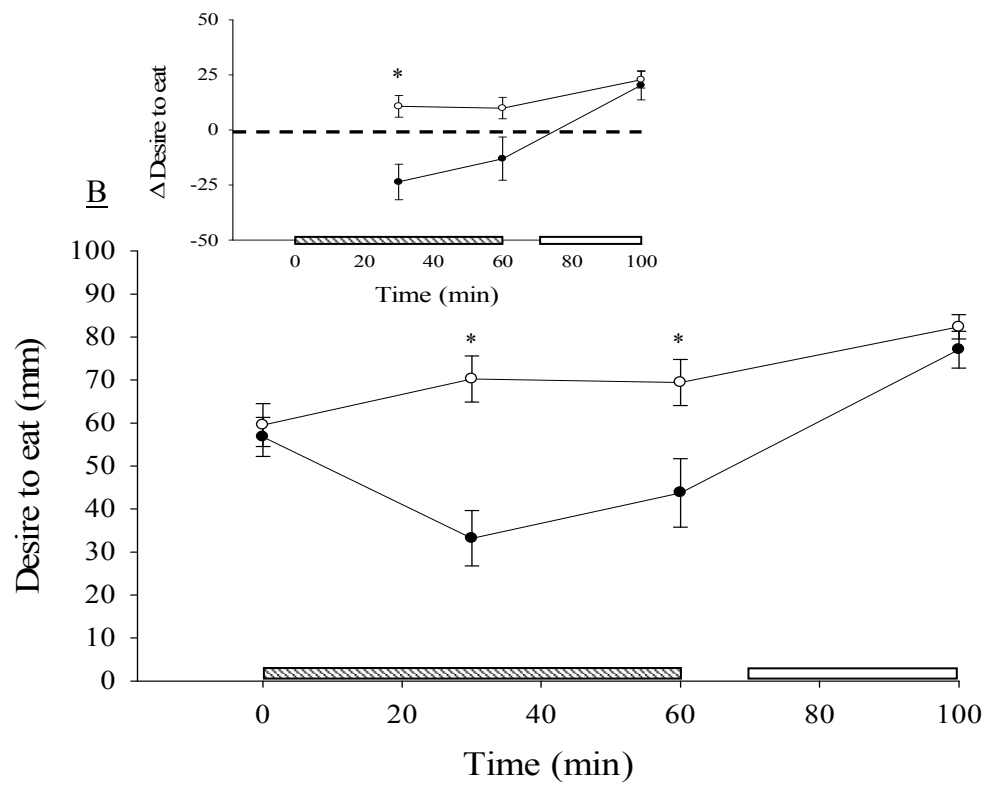
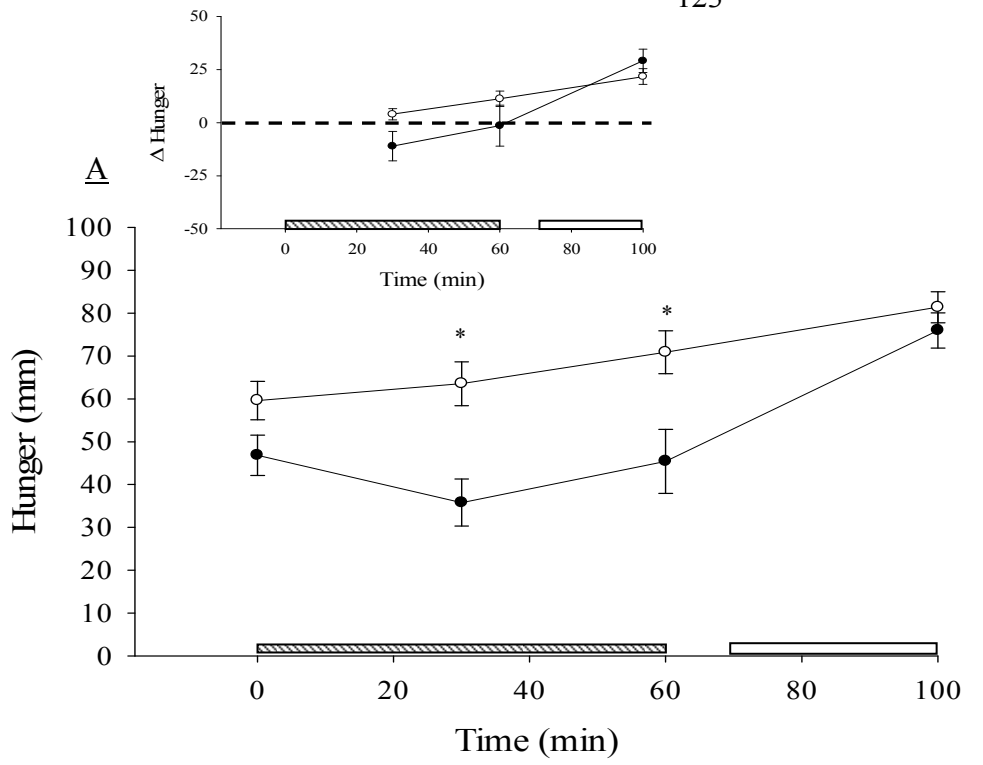
There were no differences in picture recognition scores (exercise: $97.2 \pm 3.8\%$; rest: $94.5 \pm 5.1\%$) between trials ($P > 0.05$). For the food picture pleasantness ratings no significant differences were observed between trials ($P > 0.05$) for HF/SW (exercise: 7 ± 1 ; rest: 7 ± 1), HF/SA (exercise: 7 ± 1 ; rest: 7 ± 1), LF/SW (exercise: 7 ± 1 ; rest: 6 ± 1), and LF/SA (exercise: 6 ± 1 ; rest: 6 ± 1). Furthermore, for the food picture arousal ratings there were no significant differences between trials ($P > 0.05$) for HF/SW (exercise: 7 ± 2 ; rest: 7 ± 1), HF/SA (exercise: 7 ± 1 ; rest: 7 ± 1), LF/SW (exercise: 6 ± 1 ; rest: 6 ± 1), and LF/SA (exercise: 5 ± 1 ; rest: 5 ± 1).

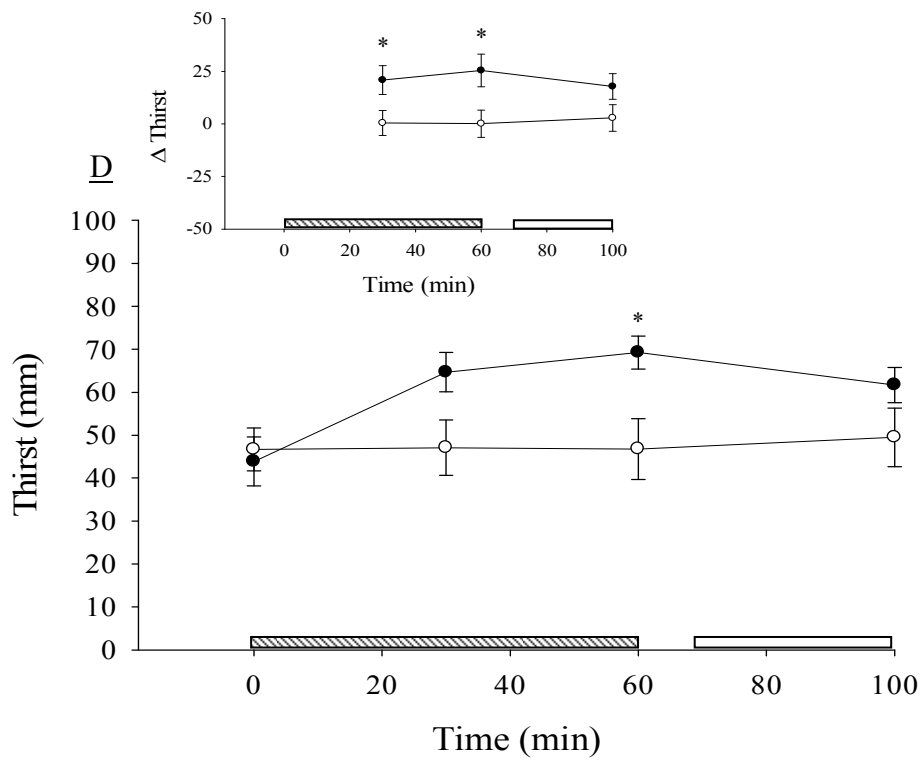
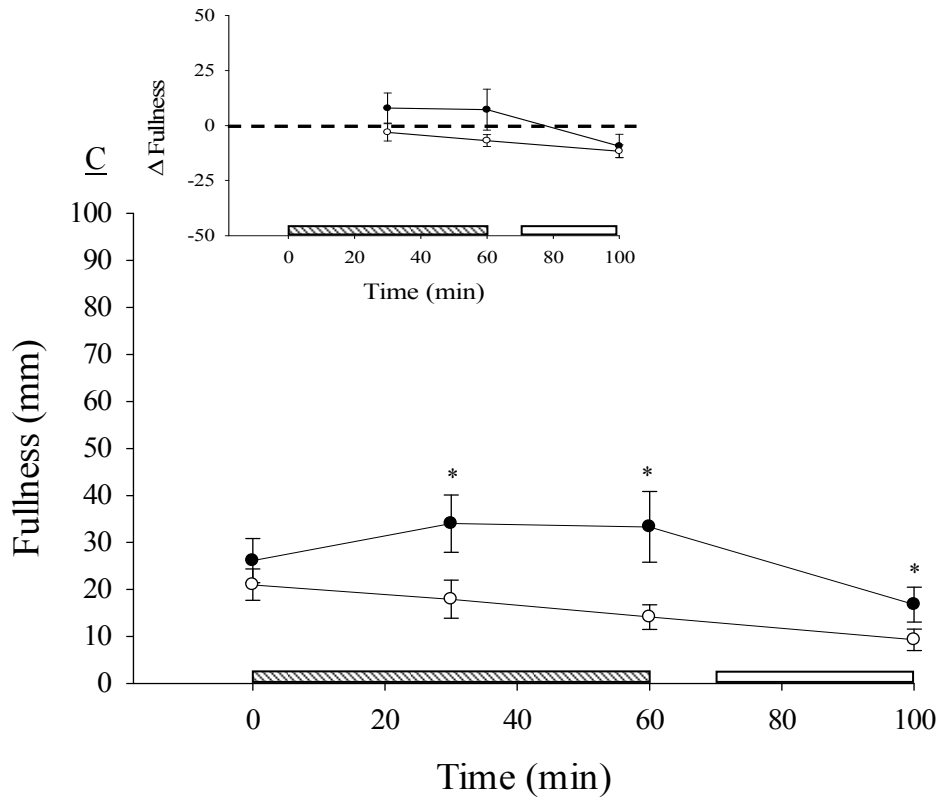
4.4.5 Appetite Sensations

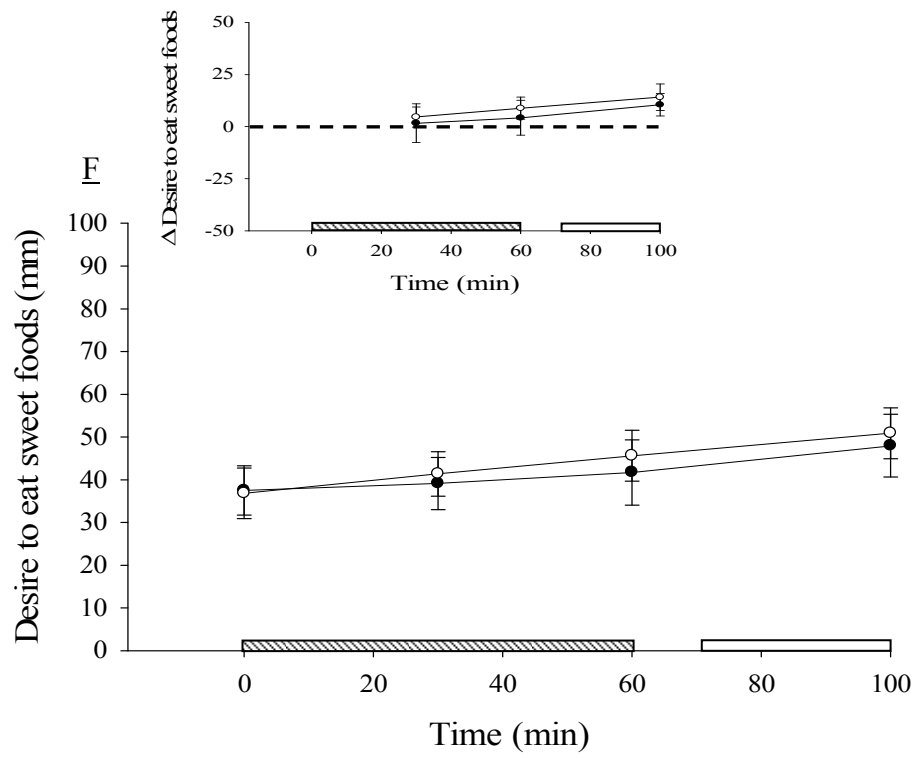
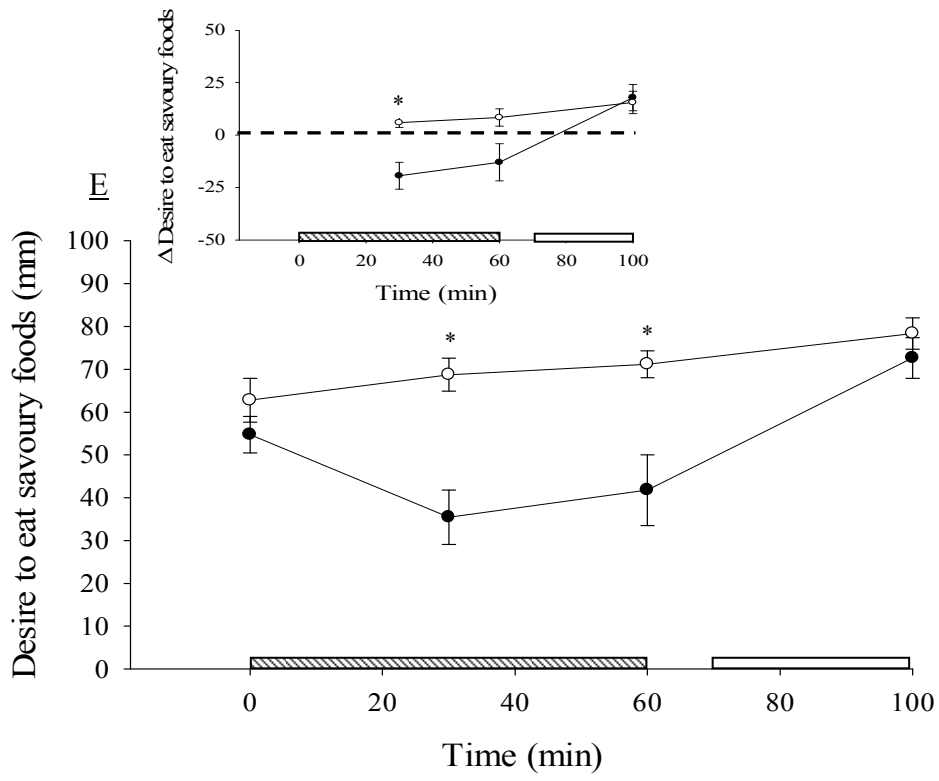
Raw hunger scores were significantly lower during exercise and immediately post-exercise compared with during rest and immediately post-rest ($P < 0.01$; Figure 7A). Hunger delta scores (difference from baseline) were not significantly different between trials ($P > 0.05$; Figure 7A). Desire to eat was significantly lower during exercise and immediately post-exercise compared with during rest ($P < 0.001$) and immediately after rest ($P < 0.01$; Figure 7B). Desire to eat delta scores were significantly lower during exercise compared with during rest ($P < 0.01$; Figure 7B). Fullness ratings were significantly greater during exercise, immediately post-exercise, and post-scan compared with rest (all $P < 0.05$; Figure 7C). There were no differences in fullness ratings when expressed as delta scores ($P > 0.05$; Figure 7C). Sensations of thirst were significantly higher immediately post-exercise compared with immediately post-rest ($P < 0.05$; Figure 7D). Furthermore, differences in thirst sensations between trials approached significance during exercise/rest ($P = 0.053$) and after the fMRI scan ($P = 0.055$; Figure 7D). Thirst delta scores were significantly greater

during exercise ($P < 0.05$) and immediately post-exercise ($P < 0.01$) compared with rest (Figure 7D).

Savoury foods were rated as significantly less desirable during exercise and immediately post-exercise compared with during rest ($P < 0.001$) and immediately post-rest ($P < 0.01$; Figure 7E). Desire to eat savoury foods delta scores were significantly lower during exercise compared with rest ($P < 0.01$; Figure 7E). Desire to eat sweet foods (Figure 7F) and desire to eat salty foods (Figure 7G) ratings did not differ between trials when expressed as either raw scores or delta scores ($P > 0.05$). The difference between trials in desire to eat fatty foods approached significance ($P = 0.055$; Figure 7H). There were no significant differences between trials when desire to eat fatty foods data were expressed as delta scores ($P > 0.05$; Figure 7H).







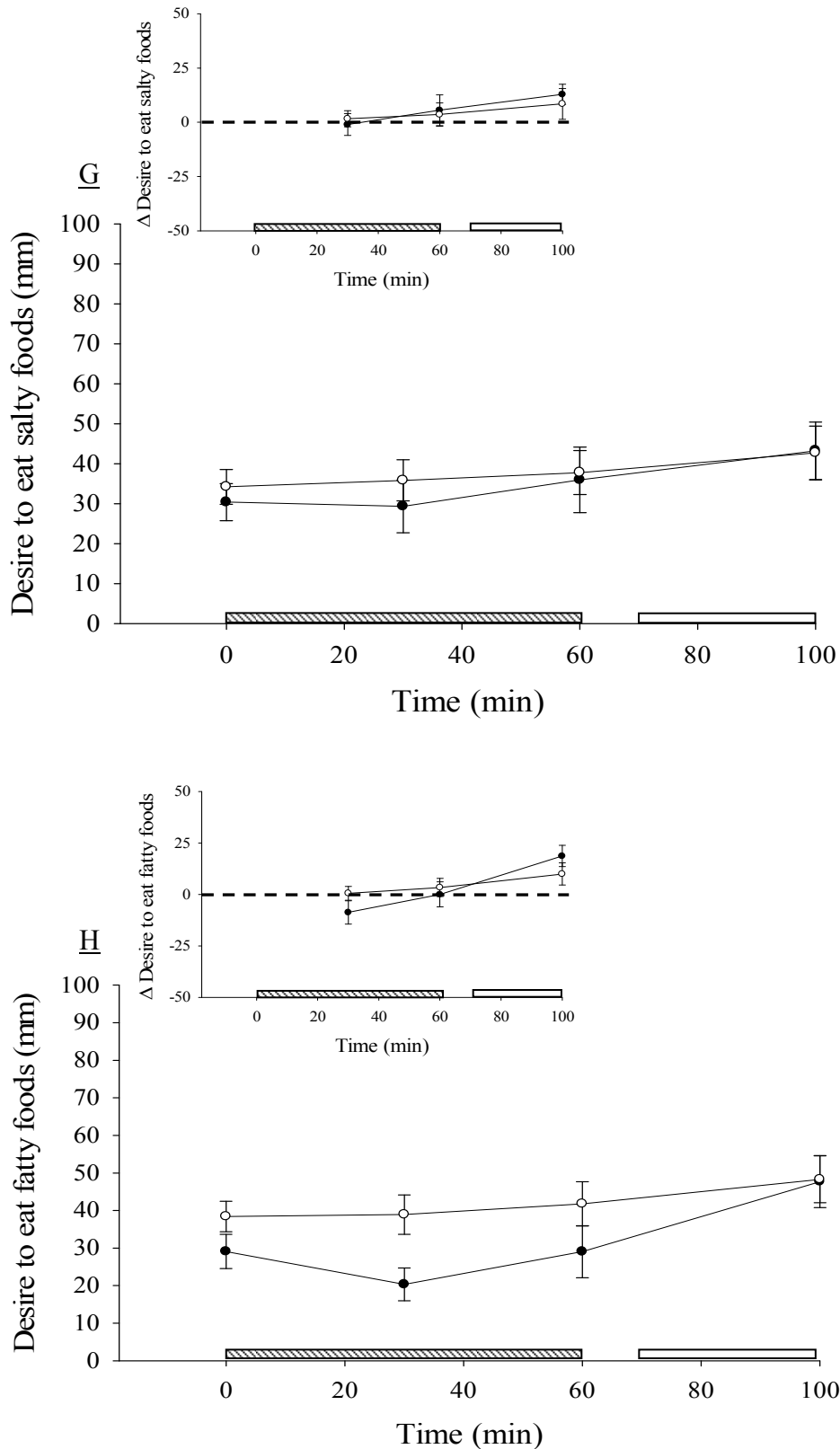
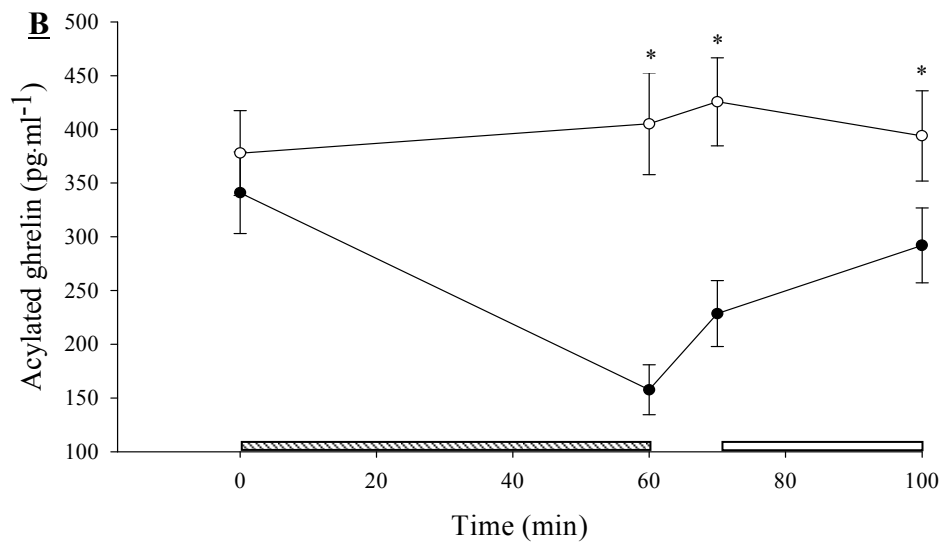
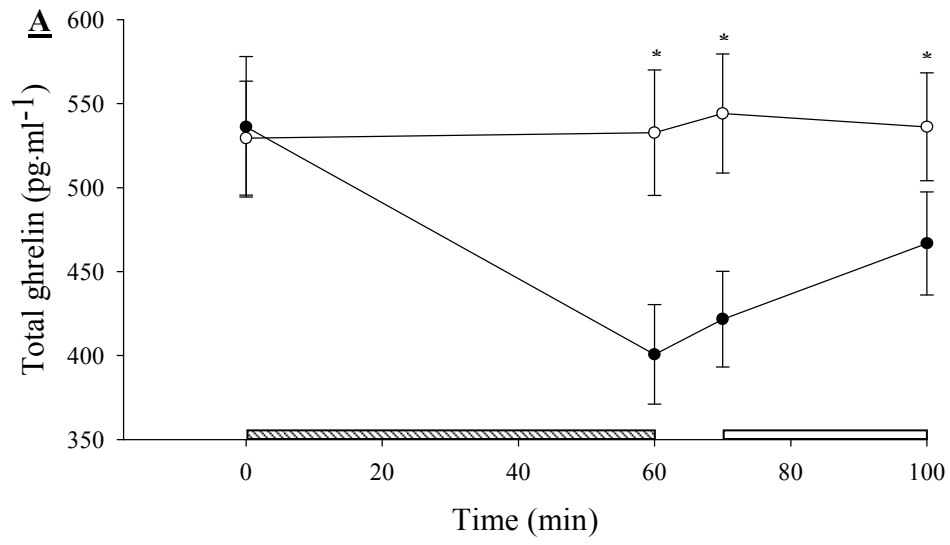


Fig 7: Raw scores for hunger (A), desire to eat (B), fullness (C), thirst (D), desire to eat savoury foods (E), desire to eat sweet foods (F), desire to eat salty foods (G), and desire to eat fatty foods (H) at the start of each trial, after 30 min of exercise and rest, at the end of exercise and rest, and after the fMRI scan. Inset, delta scores for corresponding data after 30 min of exercise and rest, at the end of exercise and rest, and after the fMRI scan. ● exercise, ○ rest. ▨ exercise/rest, ▭ fMRI scan. Values presented as means \pm SE. *Significant differences between trials $P < 0.05$.

4.4.6 Appetite Hormones

Two participants were removed from the total PYY statistical analyses, however their removal did not significantly affect the outcome of the analyses. These participants were removed because their mean plasma concentrations were $\pm 2SD$ from the group mean. One participant was removed from the acylated ghrelin statistical analyses, and two participants were removed from the total ghrelin statistical analyses, however their removal did not significantly affect the outcome of either analyses. These participants were removed because their mean plasma concentrations were $\pm 2SD$ from the group mean.

There were no differences in plasma total ghrelin, acylated ghrelin, or total PYY concentrations at baseline between trials ($P > 0.05$). Total plasma ghrelin concentrations were significantly lower immediately post-exercise ($P < 0.001$), prior to the fMRI scan ($P < 0.001$), and following the fMRI scan ($P < 0.01$) during the exercise trials compared with the rest trials (Figure 8A). Plasma acylated ghrelin concentrations were also significantly suppressed during the exercise trials compared with the rest trials immediately post-exercise, pre-fMRI scan, and post-fMRI scan (all $P < 0.001$; Figure 8B). Furthermore, the percentage of total ghrelin which was acylated post-exercise/rest was significantly greater post-exercise compared with rest ($P < 0.001$; exercise: $65.2 \pm 11.9\%$ vs rest: $31.1 \pm 12.6\%$). Total plasma PYY levels were significantly elevated following exercise compared with rest, and PYY levels remained significantly elevated prior to the fMRI scan, and after the scan during the exercise trials compared with the rest trials (all $P < 0.001$; Figure 8C).



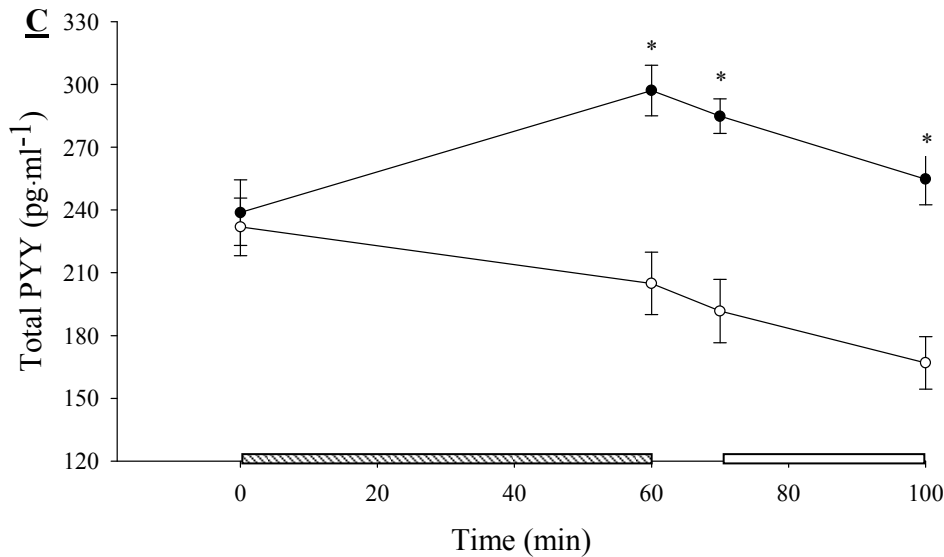


Fig 8: Total plasma ghrelin (A; $n = 13$), plasma acylated ghrelin (B; $n = 14$), and total plasma PYY (C; $n = 13$) concentrations at the start of each trial, at the end of the exercise and rest period, prior to the fMRI scan, and after the fMRI scan. ● exercise ○ rest. ▨ exercise/rest, ▭ fMRI scan. Values presented as means \pm SE. *Significant differences between trials $P < 0.05$.

4.4.7 Correlations

Reduced left pallidum activation when viewing pictures of high-calorie vs low-calorie foods during the exercise trials compared with the rest trials was correlated with lower high-calorie vs low-calorie food picture pleasantness ratings following exercise compared with rest ($P < 0.01$; $r = -0.696$). Greater increases in core temperature during exercise compared with rest were correlated with a greater desire to consume sweet foods during exercise trials compared with rest trials ($P < 0.01$; $r = 0.848$). Appetite hormone concentrations did not correlate with any of the subjective appetite sensations measured ($P > 0.05$).

4.5 Discussion

The primary aim of this study was to investigate the effects of an acute bout of intense running (70% $\text{VO}_{2\text{max}}$) on the central neural responses of lean active males when viewing pictures of food, using fMRI techniques. The secondary aims of this study were to examine the impact of high intensity exercise on appetite regulating hormone concentrations and subjective appetite sensations. The findings from the present study demonstrate that following an intense bout of running, pictures of food reduced neural activation within the left OFC and the right hippocampus and increased activation within the anterior insula. Furthermore, high calorie food cues increased activation within the DLPFC, and low calorie food cues increased activation within the insula and the left and right putamen following intense exercise. Interestingly, when viewing pictures of low-calorie compared with high-calorie foods left pallidum activation was greater following exercise, however exercise reduced left pallidum activation when viewing pictures of high-calorie foods compared with low-calorie foods. Furthermore, running transiently suppressed total and acylated plasma ghrelin concentrations, and stimulated the secretion of total plasma PYY post-exercise. As regards subjective appetite responses, running suppressed hunger, the desire to eat, and the desire to eat savoury foods, as well as increasing sensations of thirst.

4.5.1 Neural responses to stimuli

The foods vs objects contrast revealed that images of food reduced activity within the OFC and hippocampus following exercise. It has been previously demonstrated that these areas of the brain regulate the drive to eat and process the rewarding aspects of foods (Morris and Dolan, 2001; Small *et al.* 2001; Tataranni *et al.* 1999). The primary role of the hippocampus is memory retrieval (Fortin *et al.* 2002), however this area of the brain also

contributes towards appetite regulation (Tataranni *et al.* 1999; Stoeckel *et al.* 2008), and has been implicated in reward system interaction when viewing pictures of high-calorie foods (Goldstone *et al.* 2009). The OFC assigns reward values to stimuli such as high-calorie foods by communicating with the amygdala and the anterior cingulate cortex (Kringelbach, 2005). Goal orientated behaviour in response to highly desirable foods is mediated through amygdala and OFC activation (Hinton *et al.* 2004). Furthermore, enhanced OFC activity in obese individuals in response to images of high-calorie foods may contribute greatly to hyperphagia (Stoeckel *et al.* 2008). In the present study activity within the OFC and hippocampus was lower following exercise when viewing pictures of high-calorie foods and low-calorie foods vs objects.

When examining neural responses to pictures of foods vs objects following exercise, the present findings indicate that following a period of intense exercise images of low-calorie foods activate regions within the brain which regulate reward based eating behaviour, namely the putamen and the left insula. The putamen forms part of the striatal region within the basal ganglia, an area of the brain which is associated with the initiation of feeding (Hinton *et al.* 2004). Putamen activation has been observed when viewing pictures of foods which are perceived as rewarding (Stoeckel *et al.* 2008; Schur *et al.* 2009), therefore it has been proposed that the putamen may form part of the central network which processes the motivational value of foods (Porubská *et al.* 2006). The insula is recognized as the primary taste cortex (Kringelbach *et al.* 2004), and previous research has shown that anterior insula activity is increased in a state of hunger (Tataranni *et al.* 1999; Hinton *et al.* 2004), in anticipation of rewarding foods (Stice *et al.* 2008), and when consuming foods which are considered very pleasant (Small *et al.* 2001; Schienle *et al.* 2009). In the present study, insula activation when viewing pictures of low-calorie foods following exercise was correlated with reductions in acylated ghrelin concentrations during exercise. This finding indicates that

increased insula activity in response to low-calorie food images following exercise may not be related to an increase in the drive to eat. Due to the role that the left insula plays in the regulation of thirst (Egan *et al.* 2003), it could be speculated that one possible reason why low-calorie foods were perceived as rewarding following exercise was because participants may have perceived the low-calorie foods as having a high water content (e.g. grapes, cucumber) and therefore able to satisfy their exercise-induced thirst. Modified insula activation following exercise has been reported previously (Cornier *et al.* 2011). Cornier *et al.* (2011) demonstrated that chronic exercise training suppressed insula activity in response to pictures of high-calorie foods in overweight men and women, and that suppressed insula activity correlated with reductions in body fat. Consequently, based on the findings from the present study and those of Cornier *et al.* (2011), the insula cortex may potentially play an important role in mediating the exercise-induced neural responses to food.

An intense bout of running suppressed appetite ratings in the present study, therefore one might expect to observe an increase in blood flow to central regions which signal satiety and meal termination. Following the bout of exercise, DLPFC was increased in response to images of high-calorie foods compared with objects. The DLPFC has previously been shown to regulate inappropriate behaviour (Le *et al.* 2006), and promote satiation by transmitting inhibitory signals to appetite stimulating areas of the brain, such as the insular cortex and the OFC (Tataranni *et al.* 1999; Gautier *et al.* 2001; Small *et al.* 2001). Indeed, in the present study neural activity within the OFC, and the hippocampus, was suppressed in response to images of high-calorie foods vs objects following exercise. Le *et al.* (2006, 2007) demonstrated that postprandial DLPFC activation was suppressed in overweight compared with lean men and women, indicating that blunted DLPFC activation may be associated with overconsumption and weight gain. Montenegro *et al.* (2011) demonstrated that direct current stimulation of the DLPFC suppressed desire to eat in overweight participants at rest, and that

the suppressing effect of DLPFC stimulation on appetite was enhanced when stimulation was combined with exercise. Furthermore, in the present study exercise also suppressed the subjective desire to consume high-calorie foods, such as savoury and fatty foods. Therefore, this study demonstrates that an acute bout of high intensity exercise suppresses the subjective desire to consume fattening foods, and stimulates neural activation within a satiation promoting region of the brain when viewing images of high-calorie foods. In addition, exercise suppresses neural activity within regions of the brain which form part of the central reward system in response to high-calorie food images. These findings differ from those reported by Cornier *et al.* (2011) who demonstrated that performing an acute bout of moderate intensity exercise following 6 months of exercise training did not affect neural responses to pictures of high-calorie foods compared with baseline measures in overweight/obese participants. Differences between the studies may be explained by the different participant populations selected (lean vs overweight/obese) and different intensities of the exercise bouts (high vs moderate). Previous research indicates that acute bouts of moderate intensity exercise do not influence the appetite responses of overweight individuals (Unick *et al.* 2010), however high intensity exercise suppresses the appetite of lean healthy participants (Broom *et al.* 2007, 2009; Burns *et al.* 2007; King *et al.* 2011b). The findings from the present study and those of Cornier *et al.* (2011) indicate that exercise intensity may modulate central appetite responses in a similar fashion.

When directly comparing BOLD responses to high-calorie vs low-calorie food images, left pallidum activation was observed in both trials. Pallidum activation was reduced in response to pictures of high-calorie foods and increased in response to low-calorie foods pictures following exercise. In addition, suppressed left pallidum activation in response to high-calorie vs low-calorie food images following exercise was correlated with reduced subjective high-calorie food vs low-calorie food pleasantness ratings measured following

exercise compared with rest. The pallidum is reported to be the final pathway for limbic ‘liking’ and ‘wanting’ signals and receives projections from regions of the brain which motivate reward-related feeding (Smith *et al.* 2009). Tindell *et al.* (2006) suggests that the pallidum is ideally located to process and cause the hedonic reactions to foods which are perceived as pleasant. In support of this, Stoeckel *et al.* (2008) found that obese individuals demonstrated increased pallidum reactivity in response to high-calorie foods compared to low-calorie foods. Therefore, as pallidum activation is reported to impact on the hedonic reward values of foods, the results of the present study suggest that following exercise the hedonic reward of low-calorie foods is perceived as being greater than the hedonic reward of high-calorie foods. However, in the no exercise condition high-calorie foods seemed more pleasant and rewarding. Previous research has found that exercise training reduces neural activation within central appetite regulating regions in overweight/obese individuals when viewing high-calorie food images (Cornier *et al.* 2011). However, responses to high-calorie food images have not been compared with responses to low-calorie food images following exercise in overweight/obese men and women. It would be of interest to determine if the responses to high- and low-calorie food pictures observed in the present study are still evident when examining the impact of exercise on the neural responses of overweight individuals viewing images of high- and low-calorie foods.

4.5.2 Appetite regulating hormones

The present study demonstrated that high intensity running suppressed post-exercise levels of total and acylated plasma ghrelin, and increased levels of total plasma PYY. These findings are in agreement with previous studies which have investigated the impact of an acute bout of high intensity exercise on appetite hormone responses (Broom *et al.* 2007, 2009; Ueda *et al.* 2009a; King *et al.* 2010a, 2011). Furthermore, it was observed that the

percentage of total ghrelin which was acylated immediately post-exercise/rest was greater following exercise. This finding indicates that high intensity exercise may affect the secretion and clearance of ghrelin, and the post-secretion acylation process. The present study is one of the few studies which have measured the acute effects of high intensity exercise on both ghrelin and PYY responses (Broom *et al.* 2009; Ueda *et al.* 2009b). Acute bouts of high intensity exercise appear to transiently elevate both the total form of PYY (Broom *et al.* 2009; Ueda *et al.* 2009b), as measured in this study, and the active form (PYY₃₋₃₆) (Ueda *et al.* 2009a; King *et al.* 2011). Furthermore, high intensity running appears to consistently suppress the active form of ghrelin (acylated ghrelin) during and immediately after exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a, 2011), however total ghrelin appears to be unaffected by exercise (Burns *et al.* 2007; Jürimäe *et al.* 2007; Marzullo *et al.* 2008). Total ghrelin consists of both acylated ghrelin and des-acyl ghrelin, the physiological roles of which are not fully understood (Cummings, 2006). It could be speculated that des-acyl ghrelin does not respond to exercise-induced energy balance disturbances, and this may explain why studies have failed to observe an effect of exercise on total ghrelin. However, contrary to previous findings, the present study found that both acylated and total ghrelin concentrations were suppressed immediately following an acute bout of running. Previous research which has examined the effects of exercise on total ghrelin has used both male and female participants (Burns *et al.* 2007), measured total serum ghrelin, as opposed to total plasma ghrelin (Marzullo *et al.* 2008), and used rowing and cycling as the modes of exercise (Jürimäe *et al.* 2007; Marzullo *et al.* 2008). These differences between previous research and the present study may explain the discrepancies in total ghrelin results. This study clearly demonstrates that high intensity running has a potent effect on total plasma PYY, and both total and acylated plasma ghrelin, and influences these appetite-regulating hormones in such a way as to suppress appetite.

4.5.3 Subjective appetite sensations

The present study observed suppression in sensations of hunger and desire to eat during and immediately after the run. This finding is in agreement with several studies which have shown a transient decline in appetite during and following high intensity exercise (King *et al.* 1997; Broom *et al.* 2007, 2009; King *et al.* 2010a; Laan *et al.* 2010), a phenomenon termed exercise-induced anorexia (King *et al.* 1994). Furthermore, the present study found that treadmill running suppressed the desire for savoury foods during and immediately after exercise. In addition, running also suppressed the desire to consume fatty foods compared with rest, however this difference was not significant. The current findings indicate that high intensity exercise not only transiently suppresses hunger and the desire to eat, but that it also suppresses the desire to consume foods which may be considered unhealthy. Interestingly, T_{re} was correlated with the desire to eat sweet foods during the exercise trials, perhaps indicating that as core temperature increased during exercise participants' craved foods which have a high water content, such as low-calorie sweet foods, that would satisfy their elevated thirst. Previous findings demonstrate that the body's drive to maintain fluid balance can be more powerful than the drive to maintain energy balance (Stricker & Verbalis, 1999). In addition, research suggests that compensating for the energy expended during exercise may be delayed by slowing the feeding response, and instead priority is given to maintaining fluid balance (Stubbs *et al.* 2004). Therefore, the present study speculates that appetite suppression following high intensity exercise may be related to increased fluid loss and the body's preference for maintaining fluid balance ahead of maintaining energy balance.

4.5.4 Limitations and conclusions

One of the limitations of this study was that an overweight/obese group comparison was not included. Examining the effects of exercise on neural responses to food would be far more clinically important in the overweight population, as previous research has shown that images of high-calorie foods evoke greater reward system activation in obese compared with lean participants (Rothmund *et al.* 2007; Stoeckel *et al.* 2008). This effect has also been seen in overweight individuals with type 2 diabetes (Chechlacz *et al.* 2009). However, as the first study investigating the effects of high-intensity exercise on brain responses to pictures of food, individuals that were capable of performing such a demanding exercise task were recruited. During the treadmill run oxygen consumption could not be measured as the study was conducted within an fMRI facility and not an exercise laboratory. However, the average heart rate during exercise was 159 ± 8 beats·min⁻¹ indicating that the run was performed at a suitably high intensity. Furthermore, similar changes were observed in appetite sensations and appetite hormone concentrations as have been observed previously during and after an acute bout of treadmill running performed at 70%VO_{2max} (Broom *et al.* 2007, 2009). Another limitation of the present study was that it did not directly measure energy intake at any point during the study, therefore it is not possible to relate the exercise-induced changes in markers of appetite to actual food intake. Furthermore, this study only measured changes in appetite immediately following exercise. Research has demonstrated that reductions in sensations of appetite following high intensity exercise are short-lived (Laan *et al.* 2010), and hunger may ‘rebound’ over several hours post-exercise (Broom *et al.* 2009). Therefore, future research should continue to observe changes in appetite sensations and appetite regulating hormones over an extended time period following exercise. Furthermore, future studies which choose to assess the impact of exercise on neural responses to food images could conduct an fMRI scan several hours post-exercise, as well as conducting an fMRI scan immediately post-

exercise. This type of study would contribute greatly to the research of exercise and appetite regulation.

In conclusion, this study supports previous findings by demonstrating that high intensity exercise suppresses appetite sensations, and reduces ghrelin levels whilst enhancing PYY concentrations. In addition, this is the first study to show that an acute bout of intense exercise modulates activation in central regions which contribute towards the motivational control of food intake. The findings from the present study have demonstrated that following a bout of high intensity running, pictures of low-calorie foods caused increased reward system activation. Whereas, high-calorie food pictures resulted in increased activation within the DLPFC, an area of the brain associated with satiation, following exercise. This study speculates that motivation to consume low-calorie foods may be related to an increased drive to maintain fluid balance following exercise, as low-calorie foods may be perceived as having a high water content and thus more likely to satisfy exercise-induced thirst. The current findings require further investigation. Future studies could attempt to examine the impact of exercise on neural responses to food stimuli in both lean and overweight populations using different modes, intensities and durations of exercise.

4.6 References

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CHAPTER 5 - HIGH INTENSITY EXERCISE STIMULATES NEURAL

RESPONSES TO FOOD CUES SEVERAL HOURS POST-EXERCISE

5.1 Abstract

The aim of the present study was to investigate the effects of an acute bout of high intensity running on the neural responses of appetite-regulating regions of the brain when viewing images of food immediately following exercise, and several hours post-exercise, compared with rest. Subjective appetite sensations and circulating concentrations of plasma total and acylated ghrelin, and total PYY were also measured throughout each trial. Six lean active men (age 19.2 ± 0.8 yr, body mass index 24.4 ± 2.8 kg·m²) each completed two trials in a counterbalanced design. The trials consisted of a 60 min run (70% VO_{2max})/rest followed by a functional magnetic resonance imaging (fMRI) scan during which participants viewed matched images of food and objects. A standardized meal was provided 1 hour and 45 min post-exercise and a second fMRI scan was conducted 3 hours post-meal. Results demonstrated that central reward system activation during the second fMRI scan was greater compared with the first in both conditions; however reward system activation was more widespread during the exercise trials. When comparing neural responses between exercise and rest, exercise suppressed anterior cingulate cortex (ACC), amygdala, and insula cortex activity when viewing pictures of food during the first fMRI scan. However, during the second fMRI scan ACC and insula activation were increased in response to food cues during the exercise trials compared with the rest trials. In addition, compared with rest, exercise transiently suppressed concentrations of total (exercise: 447.9 ± 147.6 pg·ml⁻¹; rest: 633.5 ± 246.7 pg·ml⁻¹) and acylated (exercise: 173.6 ± 105.9 pg·ml⁻¹; rest: 444.9 ± 259.7 pg·ml⁻¹) ghrelin. Feelings of hunger were also suppressed post-exercise, however hunger increased postprandially during the exercise trials. Findings from the present study show that an acute bout of intense running suppresses central reward system activation immediately post-exercise in response to food cues, but these effects are short-lived and the rewarding properties of food are enhanced several hours after exercise.

Keywords: Exercise, fMRI, amygdala, insula, ACC, hunger, ghrelin, PYY.

5.2 Introduction

Food reward behaviour is driven by physiological and psychological factors that interact with areas of the brain which process the rewarding properties of food (Blundell, 2006). Areas of the brain which are associated with motivational and emotional responses to food include the amygdala, the insula cortex, the anterior cingulate cortex (ACC), and the orbitofrontal cortex (OFC) (Small *et al.* 2001; Killgore *et al.* 2003; Fühler *et al.* 2008). Studies which have measured the neural activity of participants presented with pictures of foods which are perceived as pleasant and rewarding, have demonstrated enhanced neural activation in the aforementioned reward processing areas (Stoeckel *et al.* 2008; Schienle *et al.* 2009; Schur *et al.* 2009; Frank *et al.* 2010; Ng *et al.* 2011).

Areas within the central nervous system which control food reward processing have been shown to interact with peripheral appetite regulating hormones including ghrelin and peptide YY (PYY) (Batterham *et al.* 2007; Malik *et al.* 2008). Ghrelin is a 28 amino acid endogenous ligand produced predominantly within the stomach (Kojima *et al.* 1999). Studies which have infused ghrelin into the circulation have demonstrated that ghrelin stimulates feeding behaviour in a dose dependent manner (Wren *et al.* 2001; Druce *et al.* 2005; Schmid *et al.* 2005). Furthermore, rodents infused with ghrelin have been shown to increase their preference for high fat foods (Perello *et al.* 2010). When injected centrally, ghrelin enhances neural activation within regions of the brain associated with food intake and reward (Naleid *et al.* 2005). Malik *et al.* (2008) demonstrated that ghrelin infusion enhanced activation within the anterior insula, amygdala, and OFC when viewing pictures of food, furthermore, the responses of the amygdala and OFC were positively correlated with sensations of hunger. In contrast to ghrelin's orexigenic action, other appetite regulating hormones like PYY are anorexigenic hormones, as they suppress appetite (Batterham *et al.* 2002, 2003; Degen *et al.* 2005; le Roux *et al.* 2006). Peptide YY infusion reduces sensations of hunger, and

suppresses food intake in humans through communications between the gastrointestinal tract and the brain via the vagus nerve (Batterham *et al.* 2002; Abbott *et al.* 2005). Peripherally injected PYY has also been shown to penetrate the blood brain barrier (Nonaka *et al.* 2003) and influence feeding behaviour by interacting with receptors in the arcuate nucleus (Challis *et al.* 2003). Batterham *et al.* (2007) used functional magnetic resonance imaging (fMRI) techniques to investigate the effects of intravenous PYY infusions on central responses whilst participants viewed pictures of food. The authors discovered that activation within the ACC and OFC increased during PYY infusion, and that following PYY infusion participants consumed 25% less calories during an *ad libitum* buffet meal. Batterham *et al.* (2007) found that the reduction in *ad libitum* calorie intake was correlated with the observed increase in OFC activation following PYY infusion, indicating that food may have been perceived as less rewarding due to the mediating effect of PYY on the OFC.

It would appear that circulating ghrelin and PYY are both capable of interacting with regions within the central reward system (Batterham *et al.* 2007; Malik *et al.* 2008). Therefore, factors which influence circulating concentrations of ghrelin and PYY may in turn influence the activation of central regions which regulate hedonic behaviour. It has been observed that circulating concentrations of both ghrelin and PYY are influenced by bouts of acute high intensity exercise (Broom *et al.* 2007, 2009; Ueda *et al.* 2009; King *et al.* 2010a, 2011a). Research has shown that acute bouts of intense running affect the responses of ghrelin and PYY during exercise, immediately post-exercise, and several hours post-exercise (Broom *et al.* 2007, 2009). Short bouts of high intensity running (~1 hour) appear to transiently suppress ghrelin concentrations whilst increasing concentrations of PYY, indicating that high intensity running suppresses appetite and increases satiety (Broom *et al.* 2009; King *et al.* 2011a). These findings are supported by evidence which demonstrates that subjective ratings of hunger are reduced during and after high intensity exercise (Broom *et al.*

2007, 2009; Ueda *et al.* 2009, King *et al.* 2010a). However, studies have demonstrated that post-exercise ratings of hunger increase over an extended period of time, indicating that exercise-induced appetite suppression is transient and exercise may induce a compensatory increase in the drive to eat several hours post-exercise (Broom *et al.* 2007; Malkova *et al.* 2008; King *et al.* 2011b). The ‘rebound’ in appetite observed following exercise could be related to the energy expended during exercise, resulting in an energy deficit which may be compensated for in the hours after exercise (Broom *et al.* 2007). Furthermore, exercise, particularly high intensity running, greatly reduces blood flow to the splanchnic region (Rowell *et al.* 1964), thus potentially reducing the concentrations of ghrelin and as such hunger. However, splanchnic blood flow returns post-exercise (Perko *et al.* 1998), and this could potentially increase the levels of ghrelin in the circulation thereby stimulating hunger. Due to the effect of circulating ghrelin and PYY on central appetite regulating areas (Batterham *et al.* 2007; Malik *et al.* 2008), it could be speculated that exercise-induced alterations in concentrations of circulating ghrelin and PYY could potentially influence the response of reward regulating areas within the brain post-exercise.

Two recent studies have demonstrated that exercise can modulate neural responses post-exercise (Cornier *et al.* 2011; Montenegro *et al.* 2011). Montenegro *et al.* (2011) investigated the effects of transcranial direct current stimulation (tDCS) on the dorsolateral prefrontal cortex (an area of the brain associated with appetite suppression), combined with an acute bout of cycling, on desire to eat and hunger sensations during and after exercise. The authors observed a greater reduction in appetite when exercise was combined with tDCS compared with tDCS at rest, and despite observing an increase in appetite post-exercise, tDCS attenuated the increase. Furthermore, Cornier *et al.* (2011) demonstrated that chronic exercise training suppressed insula activation when viewing pictures of high-calorie foods compared with baseline activation using fMRI techniques. However, Cornier *et al.* (2011)

reported that neural responses to high-calorie food images were unaffected by an acute bout of moderate intensity exercise performed post-exercise training. Therefore, the findings reported by Cornier *et al.* (2011) suggest that an acute bout of moderate intensity exercise may not influence neural responses to food stimuli. Previous research has found that appetite sensations and appetite hormone responses are not greatly affected by acute moderate intensity exercise (King *et al.* 2010b; Unick *et al.* 2010). However, as previously mentioned, high intensity exercise has been shown to suppress appetite during and after exercise (Broom *et al.* 2007, 2009).

At present no studies have investigated the effects of acute high intensity exercise on neural responses to pictures of food, immediately post-exercise and several hours post-exercise. Therefore, the primary aim of this study was to examine the impact of high intensity exercise on neural activation within central regions of the brain which regulate appetite, when viewing pictures of food immediately post-exercise and several hours post-exercise. Furthermore, this study aimed to investigate the impact of exercise on total and acylated ghrelin, and total PYY concentrations. The total and acylated forms of ghrelin were measured so that the effects of exercise on the post-secretion acylation process could be observed. In addition, subjective appetite sensations were measured, immediately post-exercise and several hours post-exercise. It was hypothesized that a reduction in sensations of hunger during and immediately after high intensity exercise would be associated with a reduction in central reward system activity. However, it was hypothesized that there would be a 'rebound' in hunger following a post-exercise standardized meal, as demonstrated by Broom *et al.* (2009), which would in turn be related to an increase in central reward system activation.

5.3 Method

5.3.1 Participants

The study was ethically approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham and the Birmingham University Imaging Centre Ethics Committee. Six physically active male students were recruited to participate in the study. Participants provided written informed consent (Appendix A) to take part in the study and completed a general health questionnaire (Appendix B). Following their participation, all volunteers were assessed for dietary restraint using the Three-Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985). Five participants scored ≤ 12 on the restraint subscale indicating that these participants were non-dietary-restrained, however one participant was classed as dietary restrained (score > 12) (Stunkard and Messick, 1985). All participants were non-smokers, not following a special diet, free from metabolic disorders, not taking any medication which may have affected their appetite, and regularly participated in moderate/vigorous exercise. Table 1 details the participants' characteristics.

Table 1. Participant characteristics.

Characteristics	
Age (yr)	19.2 ± 0.8
Height (m)	1.77 ± 0.08
Weight (kg)	77.3 ± 15.2
BMI ($\text{kg} \cdot \text{m}^2$)	24.4 ± 2.8
$\text{VO}_{2\text{max}}$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	56.5 ± 6.6
TFEQ score	10.2 ± 3.4
Values are mean \pm SD ($n = 16$)	

5.3.2 Study Protocol

Participants performed two counterbalanced experimental trials, a resting control trial and an exercise trial. Prior to the experimental trials each participant visited the laboratory for a preliminary session during which their height (stadiometer, Seca LTD, Birmingham, England), weight (electronic scales, Ohaus, Champ II, Germany), and BMI (weight divided by the square of height) were obtained and an exhaustive incremental exercise test was performed. During the experimental trials participants completed either 60 min of high intensity running (exercise trial), or 60 min of rest (control trial). Participants then completed the first of two fMRI assessments, during which their neurological responses to food and non-food stimuli were measured. Food stimuli included images of high-calorie foods (i.e. ice cream, pizza) and low-calorie foods (i.e. carrots, apple), which were visually matched with non-food images. Each image was presented to the participant once during the scan, and the image was presented for only 2 s to ensure that neural responses to the images were not deep cognitive responses. Following the scan participants rated the images for pleasantness and arousal, and completed a memory task to ensure that they had paid attention to the images during the scan. Participants were then provided with a standardized meal 1 hour and 45 min post-exercise/rest (1 hour post-scan), before completing a second fMRI scan 3 hours and 15 min post-meal. Subjective appetite sensations were measured throughout the trial using visual analogue scales (VAS; See Appendix D for an example). Blood samples were taken during the trials for later analyses of total plasma PYY, and acylated and total plasma ghrelin concentrations. See Figure 1 for the experimental trials timeline.

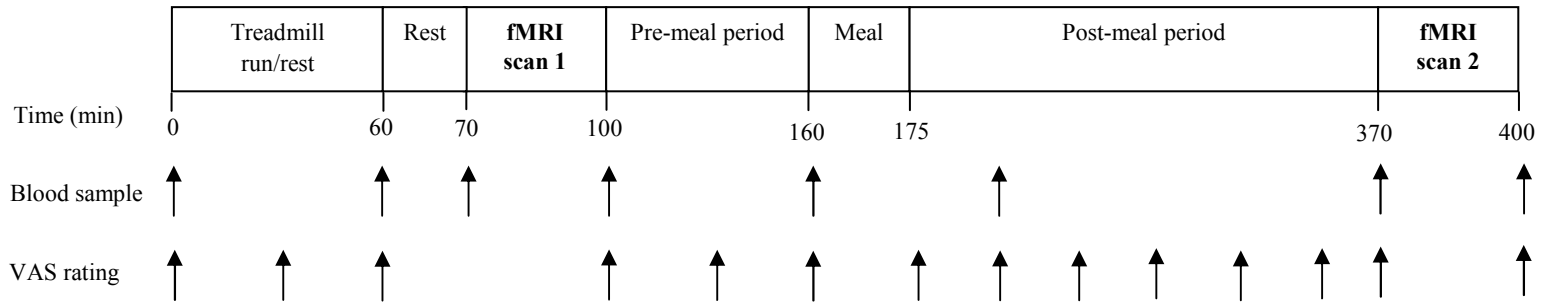


Fig 1. Experimental trial timeline.

5.3.3 Maximal Incremental Treadmill Test

Participants rested for 10 min upon their arrival at the laboratory, during which resting heart rate (Polar Vantage NV, Kempele, Finland) was recorded. The maximal incremental treadmill test was continuous in nature but was subdivided into 5 min stages. The speed of the treadmill (HP Cosmos, Quaser, Germany) was initially set at 10 km/h and was increased by 2 km/h every 5 min until volitional exhaustion. The treadmill gradient was set at 0% throughout the test. Expired air samples were collected into Douglas bags (Cranlea, Birmingham, UK) during the final minute of each 5 min stage for the determination of oxygen consumption and carbon dioxide production. Heart rate was monitored throughout the test and recorded every 15 seconds during the final minute of each 5 min stage. Ratings of perceived exertion (Borg, 1973) were assessed at the completion of each 5 min stage. Participants were encouraged to continue exercising for as long as possible and received verbal encouragement throughout the test. The participants ran until exhaustion and indicated when they could only continue exercising for one more minute. At this point the final expired air sample was collected. Maximal oxygen uptake was adjudged to have been attained when participants met two of the following criteria: a respiratory exchange ratio ≥ 1.15 , heart rate was within $\pm 10 \text{ beats} \cdot \text{min}^{-1}$ of age predicted maximum heart rate (220

beats \cdot min⁻¹ - age), and a plateau in oxygen consumption (Howley *et al.* 1995). Oxygen consumption and carbon dioxide production were determined from expired air samples using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Analyser Series 1440, Servomex, Crowborough, East Sussex, UK), which were calibrated using a mixture of known gas concentrations. Volumes of expired air were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to standard temperature and pressure (dry). The oxygen consumption at each 5 min stage was plotted against the speed at each stage to calculate the speed-oxygen consumption relationship. From this relationship, a trend line equation could be used to estimate the speed which would elicit 70% of the participants $\text{VO}_{2\text{max}}$ during the exercise trial.

5.3.4 Exercise and Control Trials

Participants arrived at BUIC at 07:45 in a fasted state, having refrained from physical activity for 24 hours. Participants were requested to wear the same clothing (shorts and t-shirt) for each trial. Mood has been shown to affect appetite (Hill *et al.* 1991), therefore participants completed the Positive Affect Negative Affect Scale (PANAS; Watson *et al.* 1988; Appendix E) upon arrival to ensure that mood was similar at the beginning of both trials. Core body temperature was monitored using a rectal thermocouple probe (YSI 400 Series, Harvard Apparatus, MA, US) which participants inserted 12cm beyond the external anal sphincter. The rectal thermocouple probe was attached to a data receiver (Squirrel 2020-1F8 Series, Grant, Cambridge, UK), which was programmed to record T_{re} once every minute from the beginning of the 60 min bout of exercise/rest. Heart rate was monitored during exercise using short range telemetry. During both trials blood was collected using an intravenous cannula (BD Venflon, Oxford, UK) inserted into an antecubital vein. Intravenous blood samples were drawn from the intravenous line at the beginning of the

experimental trials, at the end of exercise/rest period, immediately before and after the first scan, immediately prior to the standardized meal, 30 min post-meal, 3 hours post-meal (pre-scan 2), and immediately after the second scan. The intravenous cannula was kept patent with 2ml flushes of 0.9% NaCl_(aq) isotonic saline solution (Baxter Healthcare, Northampton, UK) following each blood-letting, and at regular intervals during each trial.

Following their preparation, participants then completed a 60 min run at a speed predicted to elicit 70% of their $\text{VO}_{2\text{max}}$. Participants were informed that if during the run they could not complete the run at their estimated speed, then the speed of the treadmill could be slightly reduced (none of the participants had their speed reduced). The gradient of the treadmill remained at 0% throughout the run. During the control trials the participants rested for 60 min. Following the completion of the exercise/rest the participants rested for 10 min to allow heart rate and cerebral blood flow to return to resting values (Ogoh *et al.* 2007), during which time they completed the PANAS, and removed the rectal thermocouple and the heart rate monitor. After resting for 15 min, the first fMRI scan was conducted (~09:45). Following the fMRI scan, the participants completed two post-scan tasks. The participants completed a recognition task during which they were shown the 48 food and non-food images which were presented to them during the fMRI scan, intermixed with 10 novel images, and were asked to identify which images were shown to them during their fMRI scan. This task was performed to ensure that the participants were concentrating on the images presented to them during the fMRI scan (Malik *et al.*, 2008). Finally, the participants were asked to rate how ‘arousing’ and how ‘pleasant’ they found the images presented to them during the scan on a scale of 1 to 9 (1 = “very calming/very unpleasant” and 9 = “very exciting/very pleasant”) (Bradley and Lang, 1994; Appendix F).

After the completion of these tasks participants were escorted from BUIC to The Sport and Exercise Science Department on foot (~5 min walk). At 1 hour 45 min post-

exercise/rest (~11:15) a standardized meal was provided (see later for details). Participants were instructed to consume all of the meal in 15 min. Following the meal, participants rested for 3 hours before being escorted back to BUIC for the second fMRI scan (~14:30). Upon arrival at BUIC participants rested for 10 min prior to the second fMRI scan (~14:45). Following the second fMRI scan, the participants completed the same two post-scan tasks that they completed after the first fMRI scan. Participants were then free to leave.

5.3.5 Stimuli and fMRI Experimental Procedure

During scanning food and non-food pictures (see Figure 2 for examples) were presented to the participant on a screen in the scanner and viewed through a mirror attached to a head coil (SENSE Head coil 8 elements, Phillips Electronics, Best, Netherlands). Participants were instructed to look carefully at each picture during the scanning, and to communicate with the scanner operator via the intercom if they experienced any problems. In total, 24 food and 24 non-food visually matched (in terms of shape, complexity, brightness and colour) control pictures were presented. These images had previously been used in an investigation comparing the brain responses to food cues in type 2 diabetics and healthy controls (Chechlacz *et al.*, 2009). The food pictures consisted of high fat sweet (HF/SW), high fat savoury (HF/SA), low fat sweet (LF/SW), and low fat savoury (LF/SA) foods. The total caloric density, and fat and carbohydrate composition of the foods were as follows – HF/SW: 350 ± 90 kcal/100g, $45.3 \pm 7.0\%$ fat, $51.1 \pm 7.5\%$ carbohydrate: HF/SA: 310 ± 120 kcal/100g, $46.9 \pm 11.9\%$ fat, $36.5 \pm 16.2\%$ carbohydrate: LF/SW: 48.2 ± 13.1 kcal/100g, $3.87 \pm 2.53\%$ fat, $92.9 \pm 4.0\%$ carbohydrate: LF/SA: 65.0 ± 79.4 kcal/100g, $16.4 \pm 9.0\%$ fat, $63.6 \pm 15.7\%$ carbohydrate.

During scanning, the food and non-food stimuli were presented in a random-order using an event-related design. Each stimulus was presented for 2 s followed by an inter-stimulus interval of 8 s, during which time a central fixation cross was presented. Each picture was presented once, therefore, the total time of each scanning assessment was approximately 16 min. Halfway through each scanning assessment the picture presentation was halted and the scanner operator reminded participants to look carefully at each picture before the sequence recommenced.

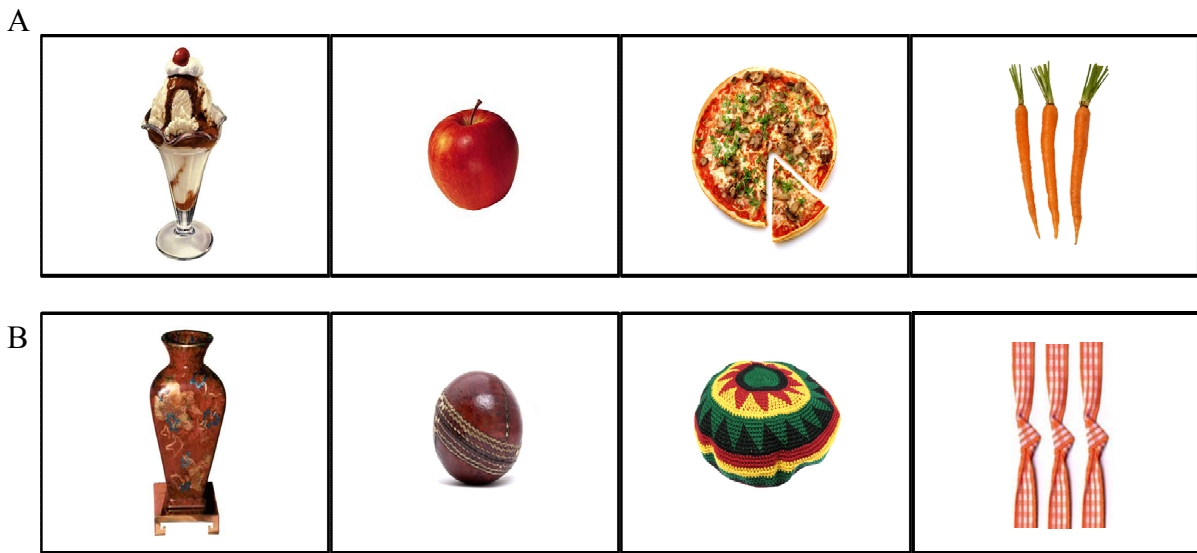


Fig 2: Examples of visually matched food (A) and non-food (B) pictures presented to participants during the fMRI experimental procedure. The food pictures represent, from left to right, the four selected food categories; high fat sweet, low fat sweet, high fat savoury, and low fat savoury.

5.3.6 Functional MRI Data Acquisition

Neuroimaging was conducted on a 3T Philips Achieva whole body scanner (Phillips Electronics, Best, The Netherlands) equipped with an 8-channel SENSE head coil. Five hundred and seventy six functional whole brain T2*-weighted images were acquired from each participant using a 2D single-shot EPI sequence (34 axial slices, whole brain coverage, echo time = 35 ms, repetition time = 2 s, flip angle = 65°, field of view = 240 mm × 102 mm

$\times 240$ mm, $3\text{ mm} \times 3\text{ mm} \times 3\text{ mm}$ resolution). Prior to the baseline fMRI assessment, a high resolution T1-weighted structural image ($1\text{ mm} \times 1\text{ mm} \times 1\text{ mm}$ resolution) was also acquired for co-registration and display of the functional data. At the beginning of every run the scanning time and stimulus presentation were synchronized by a trigger signal transmitted from the fMRI scanner.

5.3.7 Functional MRI Data Processing

Functional MRI data analysis was conducted using FSL software library version 4.1 written by the Oxford Centre for Functional MRI of the Brain (FMRIB; Smith *et al.* 2004; Woolrich *et al.* 2009). Motion artefacts were corrected prior to analysis using FMRIB's Linear Image Registration Tool (MCFLIRT; Jenkinson *et al.* 2002). Non-brain structures were removed from each participant's structural image by using the FSL Brain Extraction Tool (BET; Smith, 2002). Functional images were spatially smoothed using a 9mm Gaussian kernel with a high-pass filter of $\text{sigma} = 20$ s. A statistical parametric map was created for each participant using a general linear model to calculate the contrast of food>object at each voxel for exercise and rest. All statistical parametric maps were registered to structural images and to the Montreal Neurological Institute 152 standard image using FLIRT (FMRIB's Linear Image Registration Tool).

The effect of time post-exercise (fMRI scan 1>scan 2/fMRI scan 2>scan1), time post-rest (fMRI scan 1>scan 2/fMRI scan 2>scan1), exercise vs rest fMRI scan 1 (exercise>rest/rest>exercise), and exercise vs rest fMRI scan 2 (exercise>rest/rest>exercise) for the food>object contrast was determined using higher level (mixed effects: FLAME 1+2) paired group analyses. Z statistic images for each contrast were thresholded at $Z > 3.1$ (equivalent to an uncorrected one-tailed P of 0.001). In order to minimize random effects, only clusters with ≥ 20 continuous voxels were retained. MRICro was used to display higher

level statistical maps on a standard brain template and the AAL atlas was used to identify the anatomical location of clusters of significant activation. Only clusters of activity within the left and right anterior insula, the left and right amygdala, and the left and right ACC will be discussed. These regions have previously been shown to respond to food stimuli and be influenced by subjective appetite responses (Tataranni *et al.* 1999, Hinton *et al.* 2004). In each cluster of significant activation, the peak voxel was identified and the percentage change in BOLD signal extracted using the FEATquery tool. The BOLD signal was then statistically compared in the exercise and rest trials using a paired Student's t test performed in SPSS.

5.3.8 Blood Processing

A 5ml syringe was used to collect intravenous blood samples. For the measurement of plasma total and acylated ghrelin, and total PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations, 3 ml of whole blood was immediately added to a pre-chilled EDTA vacutainer (BD Vacutainers, Oxford, UK) which had been pre-treated with 0.06 ml of the serine protease inhibitor, 4-(2-Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF) (Alexis Biochemicals, Lausen, Switzerland). After gentle inversion, the vacutainers were then immediately spun at 3000 g for 10 min in a centrifuge at 4°C. Following whole blood separation, 0.6 ml aliquots of the AEBSF treated plasma was added to eppendorf tubes containing 120 µl 1N HCL and gently inverted. Treated plasma samples were stored at -80°C for later analysis.

5.3.9 Blood Analysis

Total plasma ghrelin (acylated human ghrelin and des-acyl human ghrelin) and plasma acylated ghrelin concentrations were measured using commercially available

sandwich enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA). Absorbance was read at 450 and 630 nm using a plate reader (Absorbance Microplate Reader ELx800, BioTek, Bedfordshire, UK), and standard curves were created to calculate the plasma concentrations of each hormone. The appropriate ranges of the total and acylated ghrelin ELISAs were 100 pg/ml to 5000 pg/ml and 25 pg/ml to 2000 pg/ml respectively. Total plasma PYY (PYY₁₋₃₆ and PYY₃₋₃₆) concentrations were determined using a commercially available ELISA kit (Millipore Corporation, Billerica, MA, USA). The appropriate range of the total PYY ELISA was 14 pg/ml to 1800 pg/ml. The intra-assay CVs were 2.32%, 3.01%, and 7.85% for total ghrelin, acylated ghrelin, and total PYY respectively. All sample measurements were performed in duplicate.

5.3.10 Subjective Appetite Sensations

Ratings of subjective appetite sensations were measured using 100-mm validated VAS (Flint *et al.* 2000), with anchors at each end (e.g. not at all hungry – extremely hungry). Hunger, thirst, fullness, desire to eat, and desire to eat something sweet, salty, savoury, and fatty were assessed. Participants were presented with the VAS at the beginning of the experimental trials, after 30 min of exercise/rest, at the end of exercise/rest period, immediately after the first fMRI scan, 30 min after the first scan, immediately before and after the standardized meal, every 30 min thereafter, and immediately after the second scan. Participants were not allowed to refer to their previous VAS ratings when completing a VAS. Instructions relating to the completion of the VAS were provided at the beginning of each trial, and participants received reminders throughout the trials.

5.3.11 Standardized Meal

Participants consumed a standardized meal consisting of white bread, cheese, and margarine with either pickle, tomatoes, or mayonnaise; a chocolate bar; crisps; and either milkshake powder with semi-skimmed milk or just semi-skimmed milk. The average macronutrient content of the meals was $55.4 \pm 1.6\%$ carbohydrates, $19.8 \pm 0.6\%$ protein and $24.8 \pm 2.1\%$ fat. The energy content of the meal for each participant was 10 kcal per kilogram of body mass. The content of each participant's meal, and the amount of food provided was kept constant for each trial. Participants were monitored throughout the 15 min standardized meal period to ensure that they consumed the entire meal.

5.3.12 Dietary Control

Prior to the exhaustive incremental treadmill test, and the experimental trials, participants were asked to refrain from caffeine 12 hours prior to testing, and were asked to refrain from consuming alcohol 24 hours prior to testing. During the experimental trials, participants were not permitted to consume any food other than that which was provided during the standardized meal. Water was available *ad libitum* throughout each experimental trial.

5.3.13 Statistical Analysis

Subjective appetite sensations, rectal temperature data, mean positive and negative PANAS ratings, and raw PYY, and acylated ghrelin data were analysed over time between trials using two-way (trial x time) repeated measures ANOVA. Pleasantness and arousal ratings for each trial were separated into scores for pictures of foods which were high HF/SW, HFSA, LF/SW, and LF/SA, and analysed using a two-way (trial x time) repeated

measures ANOVA. Interactions were analysed using Bonferroni *post-hoc* tests. ANOVA data was split into baseline, exercise, recovery, and post-meal periods. Data was split into sections to avoid changes during exercise being masked by stability at baseline and, to a lesser extent recovery. Mean heart rate data at baseline and during exercise/rest were analysed between trials using paired *t* test. Subjective appetite sensations, and raw PYY, and acylated ghrelin data were converted to area under the curve (AUC) using the trapezoidal rule and differences in exercise/rest, recovery, post-meal, and total AUC values between trials were analysed by paired samples *t* tests. Data are expressed as mean \pm standard deviation (SD) in the text and in tables, and data are expressed as mean \pm standard error (SE) in figures. Significant differences were accepted at $P < 0.05$. Statistical analysis was carried out with SPSS for Windows version 17.0 (SPSS Inc., Chicago, US).

5.4 Results

5.4.1 Participants

One participant was unable to complete all of the necessary fMRI scans due to a technical fault during an fMRI assessment. Therefore, the participant was excluded from the fMRI data analyses, and the post-fMRI scan tasks data analyses.

5.4.2 Exercise and Rest Responses

The mean \pm SD treadmill speed during the high intensity run was $12.5 \pm 2.4 \text{ km}\cdot\text{h}^{-1}$. Mean heart rate was significantly higher throughout exercise ($159 \pm 10 \text{ beats}\cdot\text{min}^{-1}$) compared with rest ($61.1 \pm 11.4 \text{ beats}\cdot\text{min}^{-1}$) ($P < 0.001$). Furthermore, rectal temperature was also significantly higher throughout exercise compared with rest ($P < 0.001$; Figure 3). Positive and negative PANAS ratings measured pre- and post-exercise/rest, were not significantly different between trials ($P > 0.05$) (data not shown).

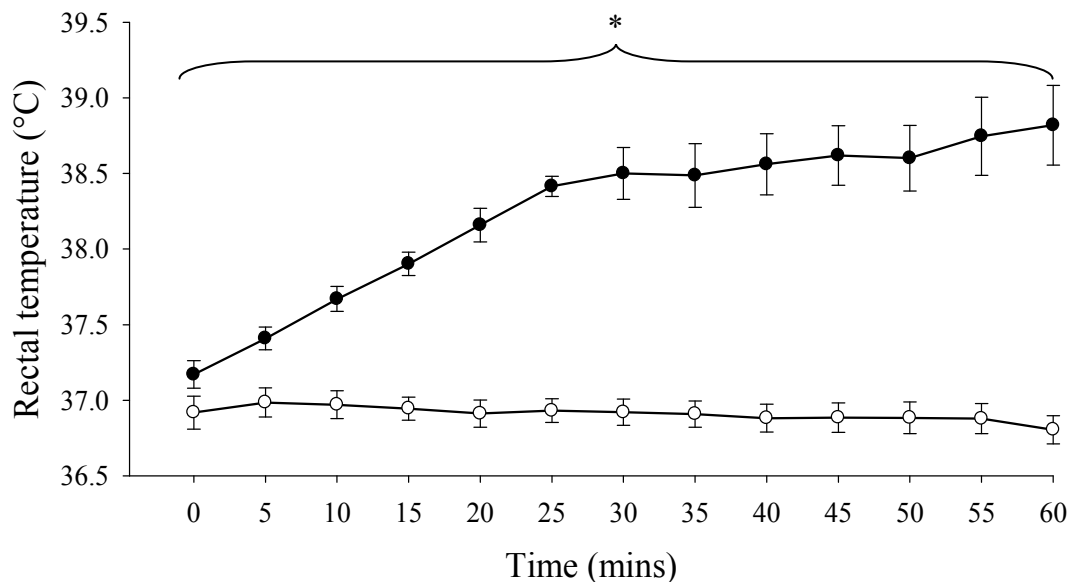


Fig 3: Rectal temperature measurements during 60 min of exercise and rest ($n = 5$). Values presented as means \pm SE. *Significant differences between trials $P < 0.05$. ● exercise ○ rest.

5.4.3 Functional MRI Data

5.4.3.1 Food>object contrast: Exercise, scan 1 vs scan 2 comparisons

During scan 2 of the exercise trials brain activity was significantly greater within the ACC, amygdala, insula, and the occipital lobe compared with scan 1 (Table 2, Figure 4).

Table 2. Region of activation, Talaraich coordinates, Z score, and significance level of exercise, scan 1 vs scan 2 comparisons for the food > object contrast.

Contrast	ROI	Hem	x	y	z	Z score	P
Exercise Scan2>scan1	ACC	L	-8	14	28	31.3	<.0001
		R	12	14	30	19.6	<.0001
	Amygdala	L	-26	-6	-14	15.5	<.05
	Insula	L	-36	14	4	14.5	<.0001
		R	44	0	6	11.9	<.0001
	Occipital lobe	L	-46	-76	8	16.5	<.01
		R	46	-82	12	28.8	<.0001

Hem = hemisphere. L = left hemisphere. R = right hemisphere. ACC = anterior cingulate cortex.

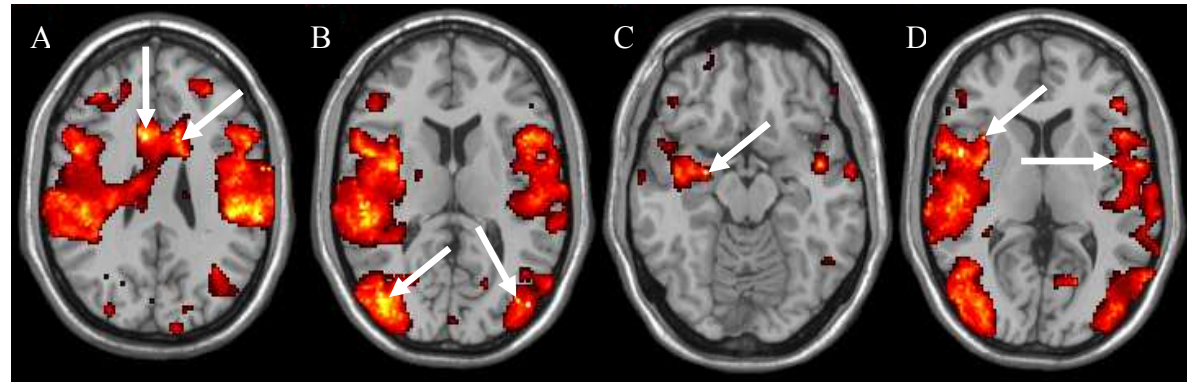


Fig 4: Images A, B, C, and D represent statistical parametric maps of the food>object, exercise fMRI scan 1 vs fMRI scan 2 contrast. Arrows indicate the peak voxel activation in the left and right ACC (A), the left and right occipital lobe (B), the left amygdala (C), and the left and right insula (D). E is a bar graph showing the food>object BOLD effect (% signal change) for scan 1 (black bars) and scan 2 (grey bars) within the left and right ACC (L.ACC, R.ACC), the left and right occipital lobe (L.Occip, R.Occip), the left amygdala (L.Amyg), and the left and right insula (L.Ins, R.Ins). Values presented as means \pm SE. Significant differences between scans $*P < 0.05$.

5.4.3.2 Food>object contrast: Rest, scan 1 vs scan 2 comparisons

During scan 2 of the rest trials neural activation was significantly greater within the insula, occipital lobe, and the cerebellum compared with scan 1 (Table 3, Figure 5).

Table 3. Region of activation, Talaraich coordinates, Z score, and significance level of rest, scan 1 vs scan 2 comparisons for the food > object contrast.

Contrast	ROI	Hem	x	y	z	Z score	P
Rest Scan2>scan1	Insula	R	32	-4	14	7.21	<.05
	Occipital lobe	L	-2	-100	16	8.45	<.05
	Cerebellum		-22	-44	-26	15.1	<.05

Hem = hemisphere. L = left hemisphere. R = right hemisphere.

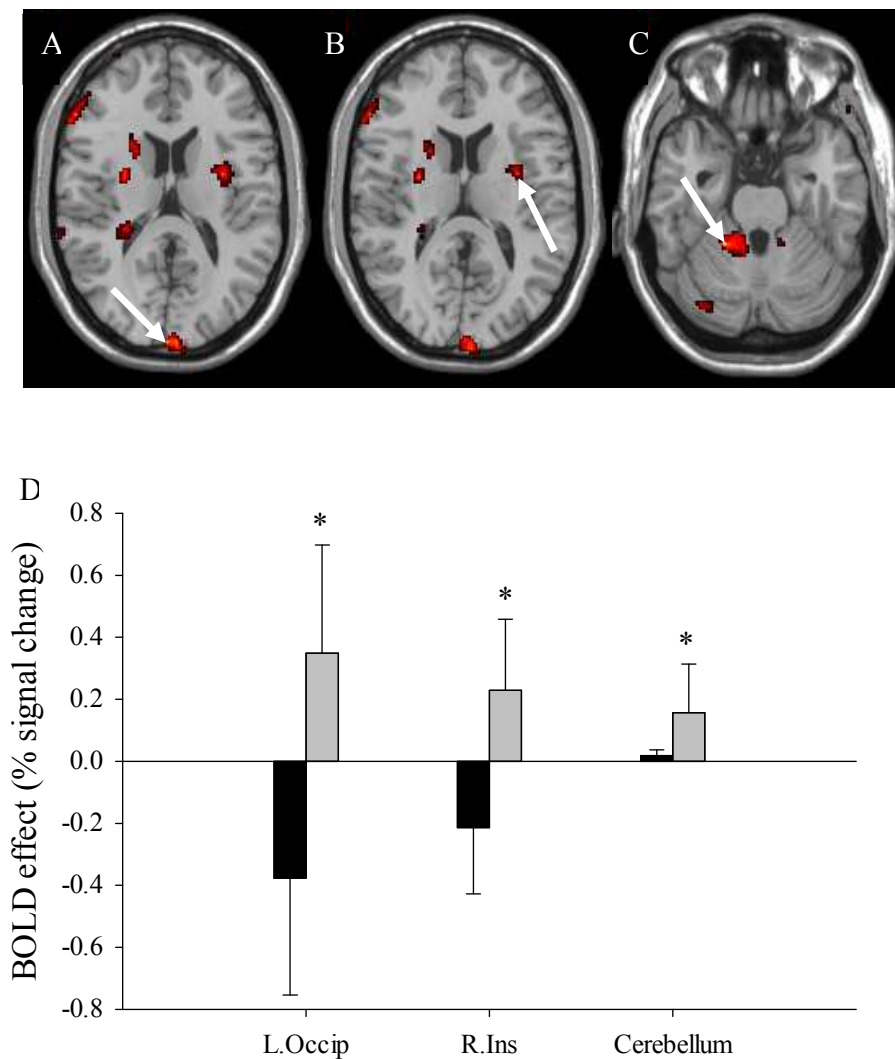


Fig 5: Images A, B, and C represent statistical parametric maps of the food>object, rest fMRI scan 1 vs fMRI scan 2 contrast. Arrows indicate the peak voxel activation in the left occipital lobe (A), the right insula (B), and the cerebellum (C). D is a bar graph showing the food>object BOLD effect (% signal change) for scan 1 (black bars) and scan 2 (grey bars) within the left occipital lobe (L.Occip), the right insula (R.Ins), and cerebellum. Values presented as means \pm SE. Significant differences between scans $*P < 0.05$.

5.4.3.3 Food>object contrast: Exercise vs rest comparisons

The first fMRI scan revealed that neural activation was significantly reduced in the ACC, amygdala, insula, and the occipital lobe following exercise compared with rest (Table 4, Figure 6). During the second fMRI scan, activation was significantly enhanced in the ACC and insula following exercise compared with rest (Table 4, Figure 7). Furthermore, it was observed that activation within the cerebellum was significantly lower during scan 2 of the exercise trial compared with the rest trial (Table 4, Figure 7).

Table 4. Region of activation, Talaraich coordinates, Z score, and significance level of exercise vs rest comparisons for the food > object contrast.

Contrast	Region	Hem	x	y	z	Z score	P
Scan1 Exercise<rest	ACC	R	10	2	34	15.2	<.01
	Amygdala	L	-14	0	-22	7.01	<.05
	Insula	L	-42	-6	12	7.10	<.05
	Occipital lobe	L	-10	-74	42	25.5	<.05
Scan 2 Exercise>rest	ACC	L	-8	30	18	10.2	<.05
		R	3	31	7	16.1	<.05
	Insula	L	-36	18	-4	7.01	<.05
Scan 2 Exercise<rest	Cerebellum		2	-56	-20	8.27	<.05

Hem = hemisphere. L = left hemisphere. R = right hemisphere. ACC = anterior cingulate cortex.

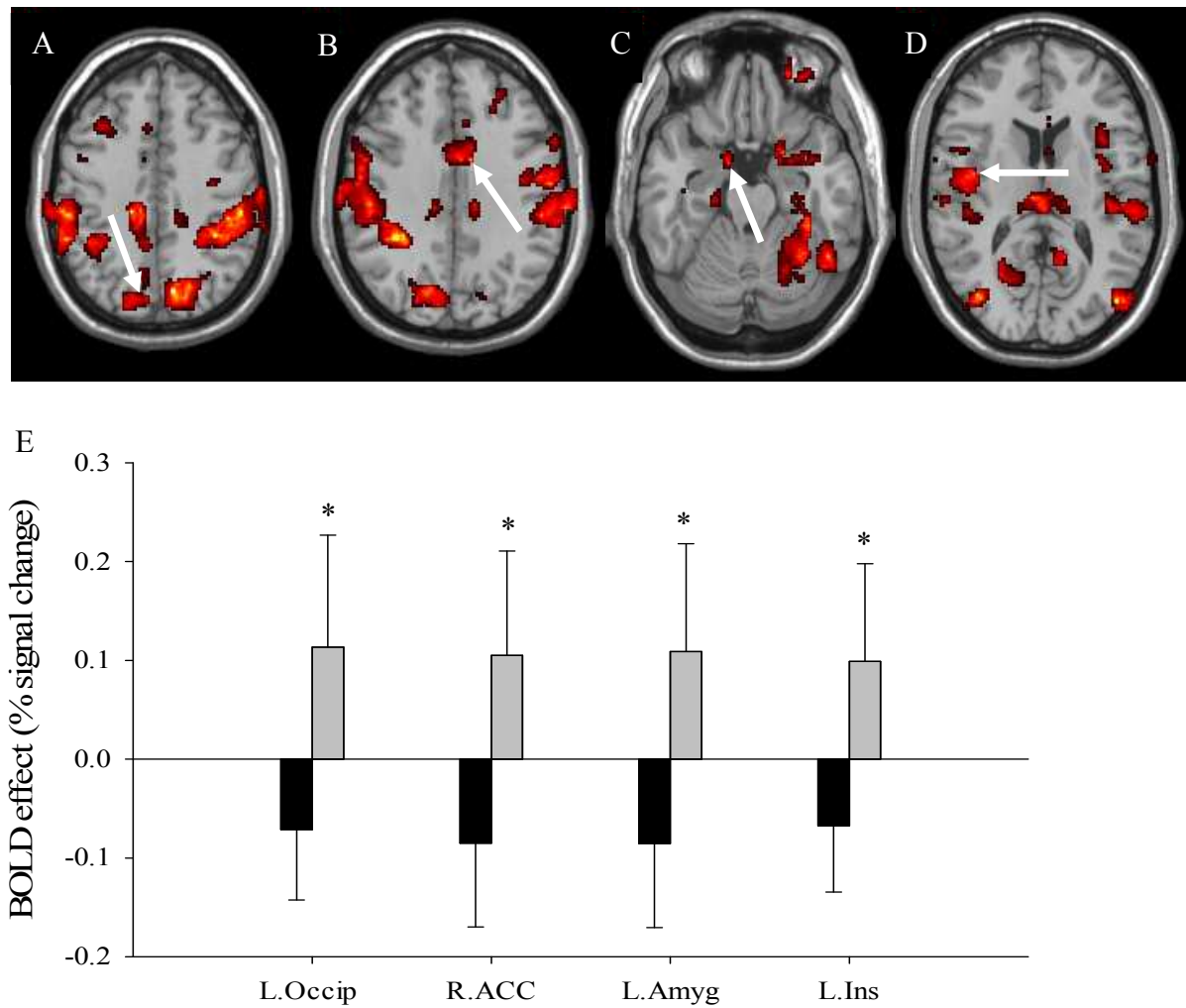


Fig 6: Images A, B, C, and D represent statistical parametric maps of the food>object, exercise vs rest, fMRI scan 1 contrast. Arrows indicate the peak voxel activation in the left occipital lobe (A), the right ACC (B), the left amygdala (C), and the left insula (D). E is a bar graph showing the food>object BOLD effect (% signal change) in the exercise (black bars) and rest (grey bars) conditions for fMRI scan 1 within the left occipital lobe (L.Occip), the right ACC (R.ACC), the left amygdala (L.Amyg), and the left insula (L.Ins). Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

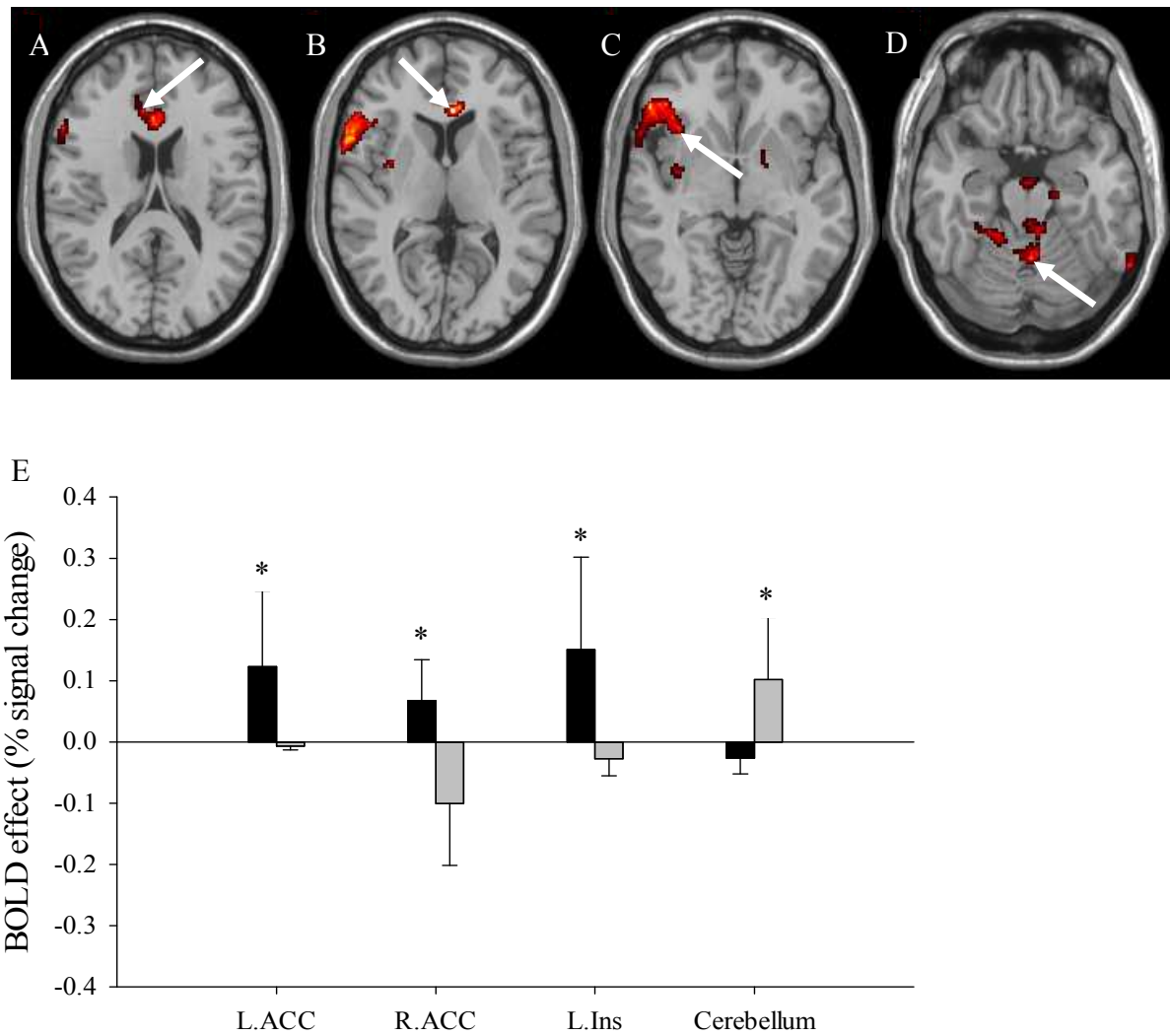


Fig 7: Images A, B, C, and D represent statistical parametric maps of the food>object, exercise vs rest, fMRI scan 2 contrast. Arrows indicate the peak voxel activation in the left ACC (A), the right ACC (B), the left insula (C), and the cerebellum (D). E is a bar graph showing the food>object BOLD effect (% signal change) in the exercise (black bars) and rest (grey bars) conditions for fMRI scan 2 within the left ACC (L.ACC), the right ACC (R.ACC), the left insula (L.Ins), and the cerebellum. Values presented as means \pm SE. Significant differences between trials $*P < 0.05$.

5.4.4 Post-fMRI Scan Tasks

There were no significant differences between trials in the percentage of pictures correctly identified by the participants as being presented in the fMRI scan picture sequence following the first (exercise: $98.3 \pm 1.5\%$; rest: $94.5 \pm 2.2\%$) and second (exercise: $97.1 \pm 3.2\%$; rest: $95.2 \pm 8.0\%$) fMRI scan ($P > 0.05$). Food picture pleasantness and arousal ratings

were not significantly different between trials following the first and second scan ($P > 0.05$; data not shown).

5.4.5 Appetite Hormones

5.4.5.1 Acylated ghrelin

Acylated ghrelin concentrations were significantly lower post-exercise ($P < 0.05$), pre-scan 1 ($P < 0.001$), post-scan 1 ($P < 0.05$), pre-meal ($P < 0.05$), and post-scan 2 ($P < 0.05$) compared with rest (Figure 8A). However, when data were expressed as normalised to baseline, acylated ghrelin was not significantly different between trials at any time point ($P < 0.05$), but differences between trials did approach significance post-exercise/rest ($P = 0.075$) and pre-scan 1 ($P = 0.056$; Figure 8A). There were no significant differences in acylated ghrelin AUC values between trials ($P > 0.05$; data not shown). Furthermore, no significant differences were observed between trials as regards the percentage of total ghrelin which was acylated post-exercise/rest ($P > 0.05$). Acylated ghrelin concentrations immediately prior to fMRI scan 1 and scan 2 were not significantly different during the exercise trial or the rest trial ($P > 0.05$; data not shown).

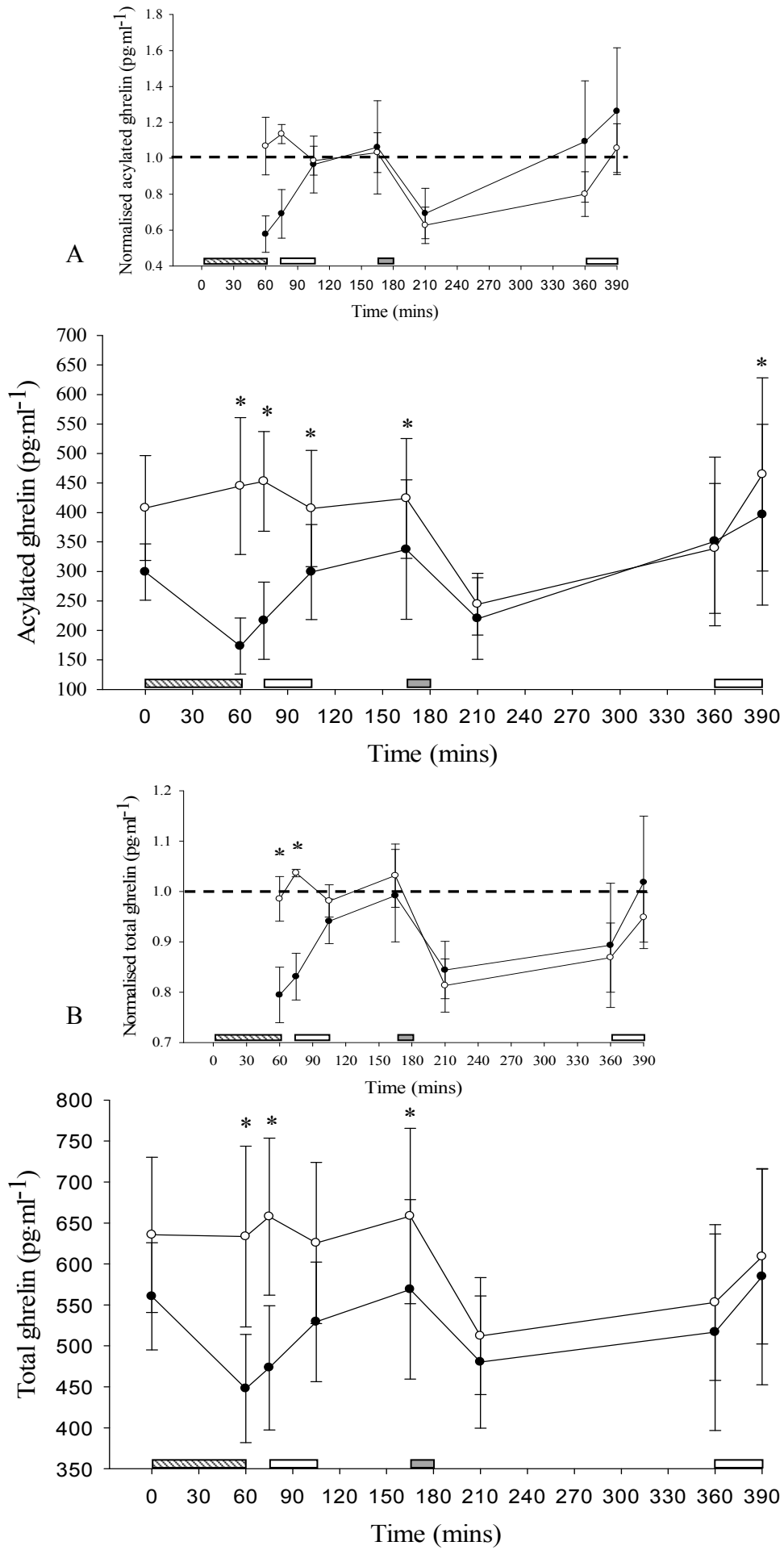
5.4.5.2 Total ghrelin

Exercise significantly suppressed total ghrelin levels post-exercise ($P < 0.05$), pre-scan 1 ($P < 0.01$), and pre-meal ($P < 0.05$) compared with rest (Figure 8B). When data were expressed as normalised to baseline, total ghrelin was significantly lower post-exercise and pre-scan 1 following exercise compared with rest (both $P < 0.05$; Figure 8B). There were no significant differences in total ghrelin AUC values between trials ($P > 0.05$; data not shown).

Total ghrelin concentrations immediately prior to fMRI scan 1 and scan 2 were not significantly different during the exercise trial or the rest trial ($P > 0.05$; data not shown).

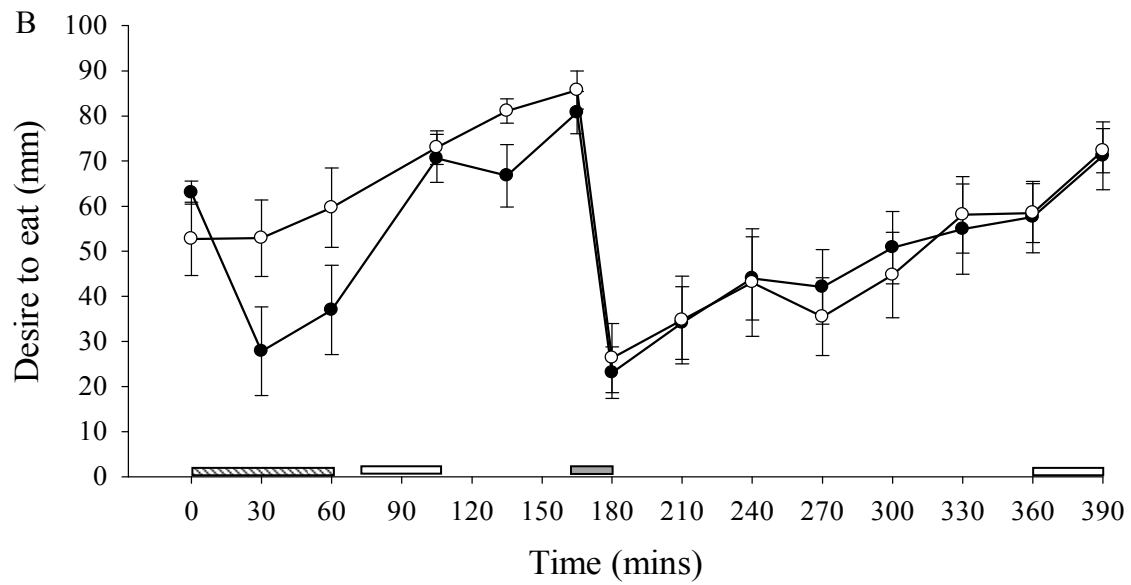
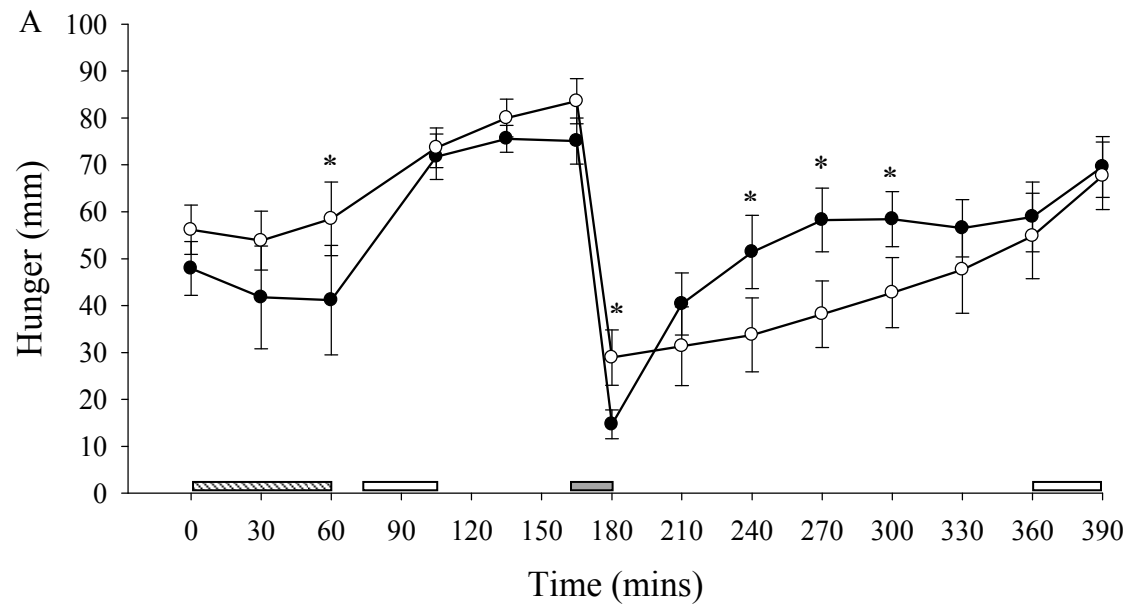
5.4.5.3 Total PYY

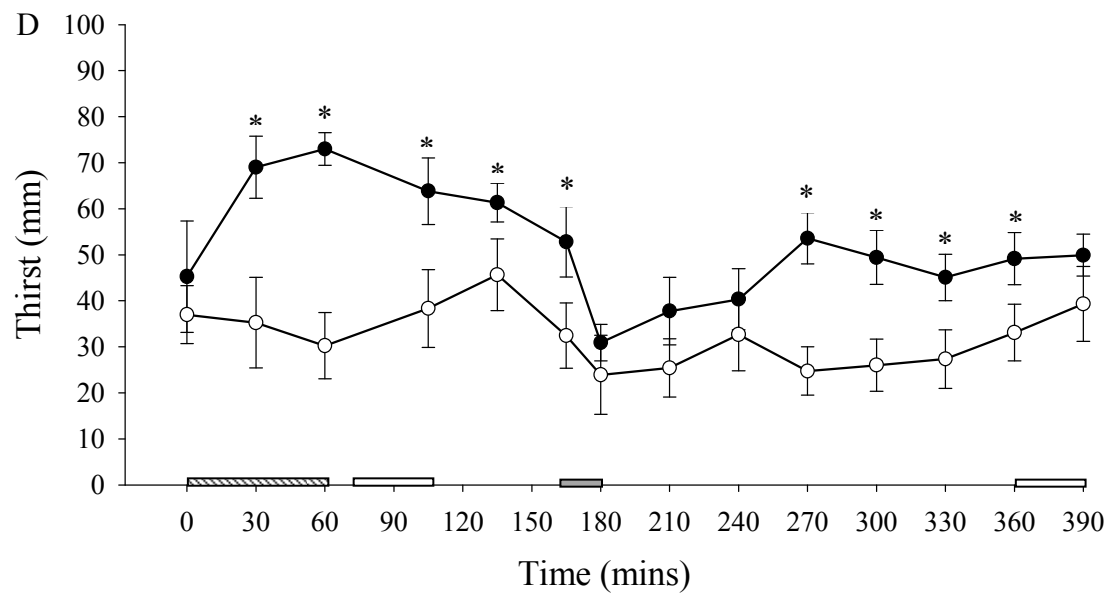
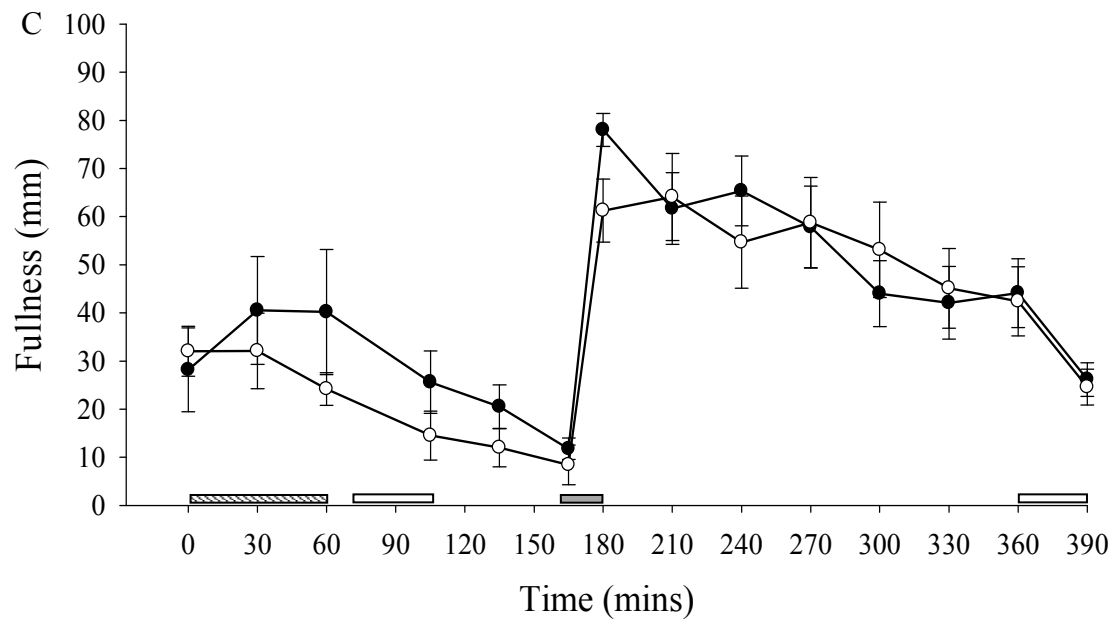
Total PYY concentrations were significantly lower at baseline during the exercise trials compared with the rest trials ($P < 0.05$; Figure 8C). In addition, concentrations of PYY were significantly higher following fMRI scan 1 during the exercise trial compared with the rest trial ($P < 0.05$), and differences between trials approached significance post-exercise/rest ($P = 0.075$) and prior to fMRI scan 1 ($P = 0.084$; Figure 8C). Furthermore, total PYY was significantly greater post-exercise, pre-scan 1, post-scan 1, and post-meal during the exercise trials compared with the rest trials when data were normalised to baseline (all $P < 0.05$; Figure 8C). There were no significant differences in total PYY AUC values between trials ($P > 0.05$; data not shown). Total PYY concentrations immediately prior to fMRI scan 1 and scan 2 were not significantly different during the exercise trial or the rest trial ($P > 0.05$; data not shown).

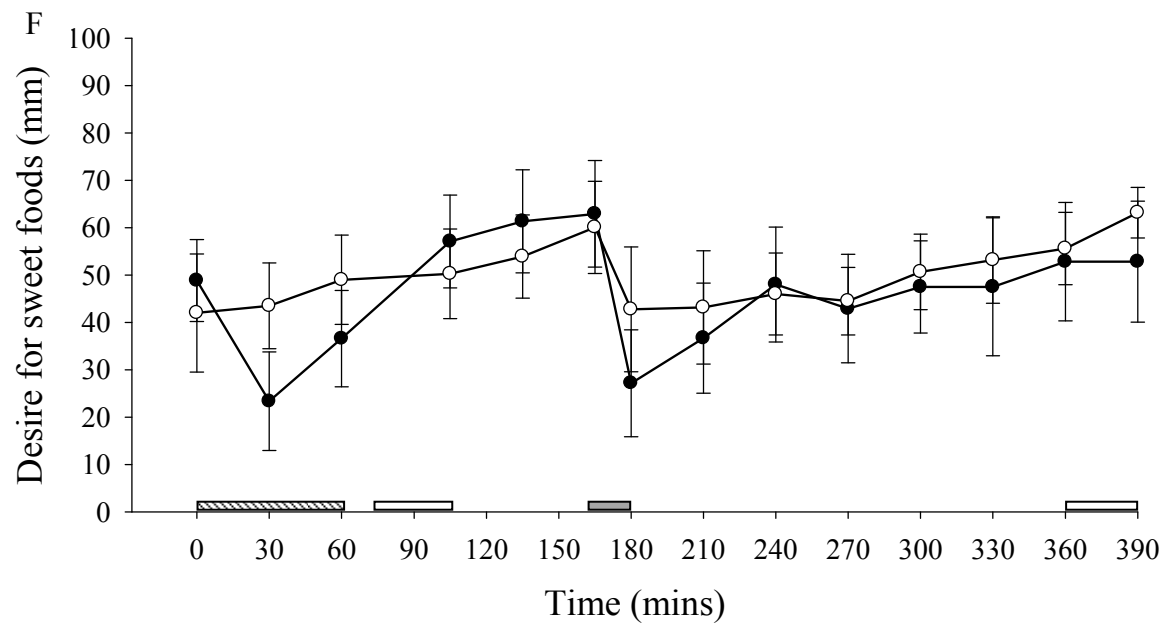
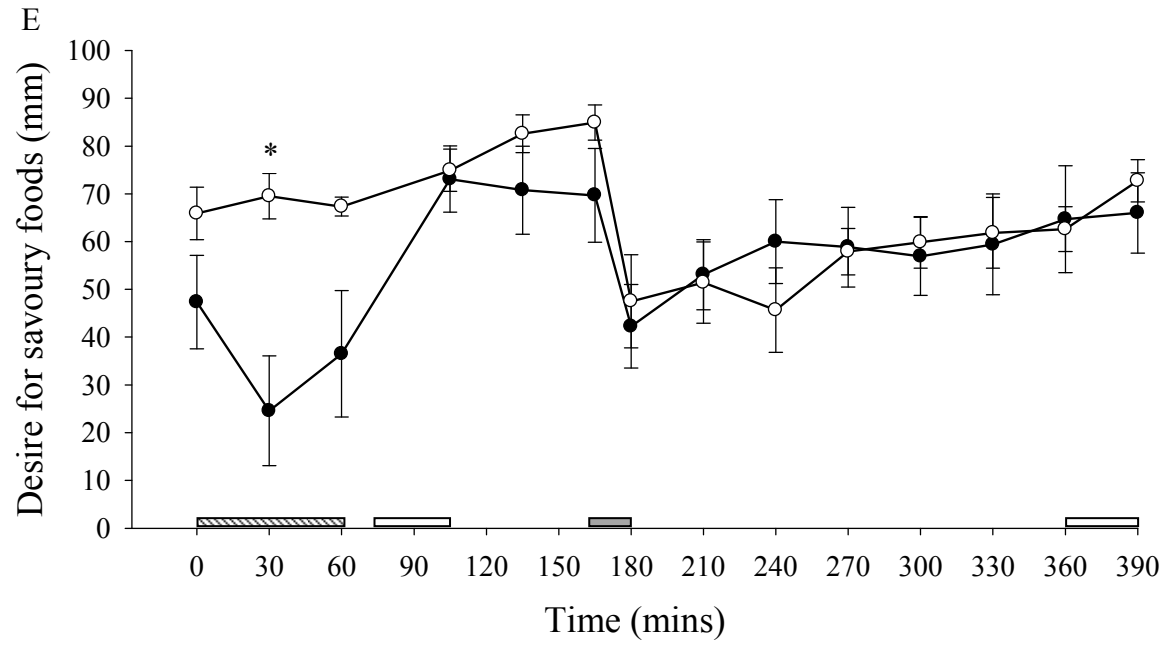


significantly higher from 30 min of exercise until 1 hour 45 min post-exercise compared with rest ($P < 0.05$; Figure 9D). Furthermore, thirst sensations were also significantly higher from 90 min post-meal until 3 hours post-meal during the exercise trials compared with the rest trials ($P < 0.05$; Figure 9D). Thirst AUC values were significantly higher during the exercise trials compared with the rest trials ($P < 0.05$; Table 5).

Desire for savoury foods ratings were significantly lower during exercise compared with during rest ($P < 0.01$; Figure 9E). Differences in desire for savoury foods ratings between trials immediately post-exercise/rest approached significance ($P = 0.055$; Figure 9E). Desire for sweet (Figure 9F), salty (Figure 9G), and fatty foods (Figure 9H) ratings were not significantly different between trials ($P > 0.05$). Desire to eat savoury and salty foods AUC values were significantly lower during exercise compared with rest (both $P < 0.05$; Table 5). During the pre-meal period desire to eat fatty foods AUC was significantly lower during the exercise trial compared with the rest trial ($P < 0.05$; Table 5). Subjective appetite sensations immediately prior to fMRI scan 1 and scan 2 were not significantly different during the exercise trials or the rest trials ($P > 0.05$; data not shown).







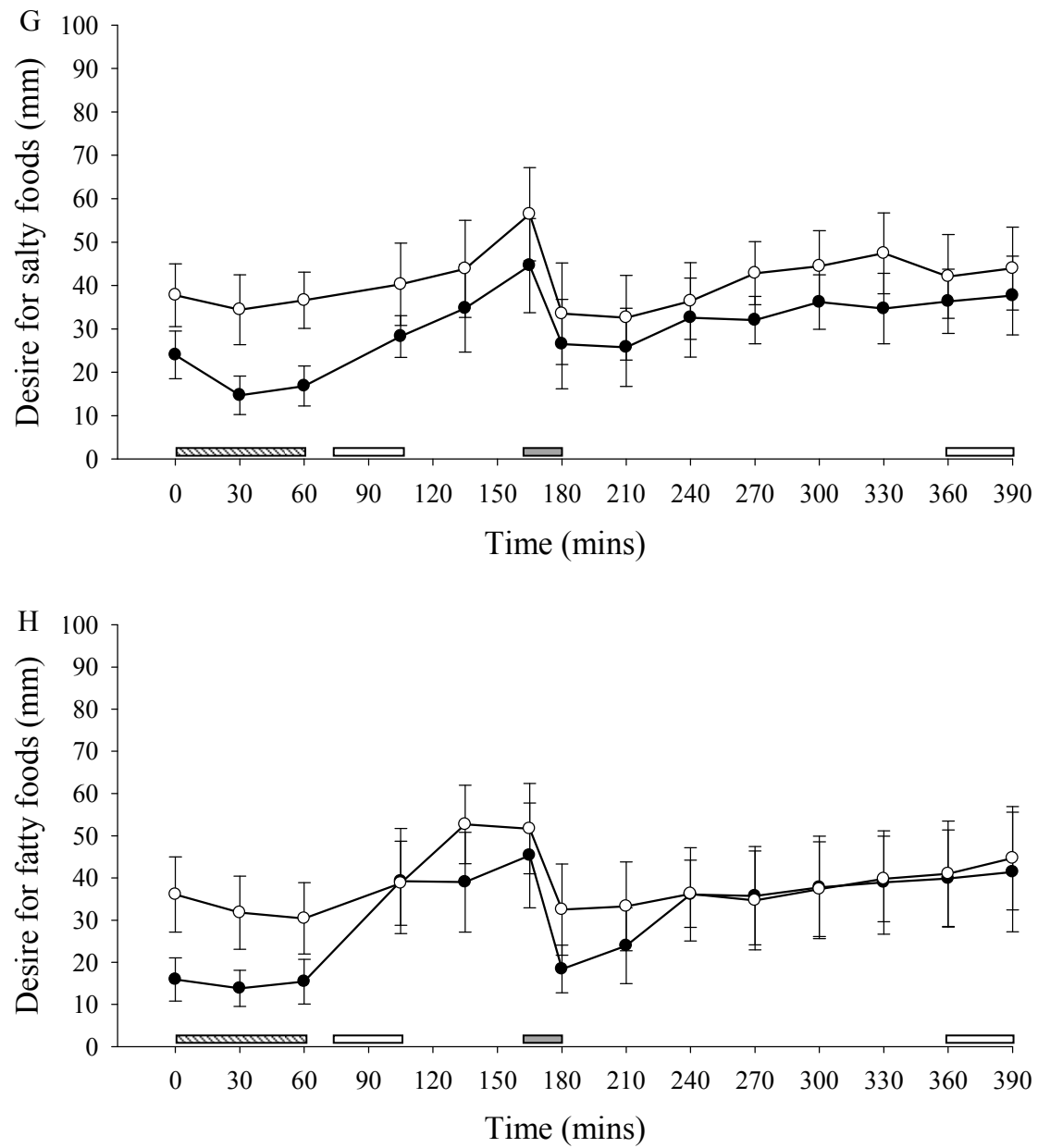


Fig 9: Subjective appetite ratings during exercise and rest trials. Values presented as means \pm SE. *Significant differences between trials $P < 0.05$. ▨ exercise/rest, □ fMRI scan, ■ standardized meal. ● exercise ○ rest.

Table 5. Subjective appetite ratings AUC values.

	Exercise Trial	Rest Trial
Hunger		
Exercise/Rest	2589 ± 319	3335 ± 124
Pre-meal	7011 ± 936	7733 ± 1088
Post-meal	11652 ± 486	9748 ± 427
Total	21251 ± 4532	20815 ± 3279
Desire to eat		
Exercise/Rest	2335 ± 276	3274 ± 201
Pre-meal	6693 ± 914	7799 ± 1074
Post-meal	10698 ± 451	10561 ± 482
Total	19726 ± 4182	21633 ± 3679
Fullness		
Exercise/Rest	2240 ± 283	1805 ± 129
Pre-meal	2654 ± 705	1574 ± 584
Post-meal	11686 ± 535	11353 ± 516
Total	16580 ± 5338	14731 ± 5580
Thirst		
Exercise/Rest	3844 ± 376 *	2066 ± 137
Pre-meal	6669 ± 1142 *	3974 ± 646
Post-meal	10101 ± 352 *	6449 ± 257
Total	20613 ± 3133 *	12489 ± 2197
Desire to eat savoury foods		
Exercise/Rest	1995 ± 221 *	4084 ± 88
Pre-meal	6729 ± 1000	8076 ± 1244
Post-meal	13051 ± 447	12974 ± 376
Total	21775 ± 5547	25134 ± 4453
Desire to eat sweet foods		
Exercise/Rest	1981 ± 130	2670 ± 117
Pre-meal	5748 ± 817	5506 ± 873
Post-meal	10138 ± 430	11153 ± 404
Total	17867 ± 4082	19328 ± 4318
Desire to eat salty foods		
Exercise/Rest	1053 ± 147 *	2148 ± 166
Pre-meal	3149 ± 468	4494 ± 736
Post-meal	7421 ± 292	9208 ± 306
Total	11623 ± 3245	15849 ± 3596
Desire to eat fatty foods		
Exercise/Rest	885 ± 83	1950 ± 88
Pre-meal	3669 ± 561 *	4493 ± 650
Post-meal	7743 ± 306	11890 ± 817
Total	12297 ± 3449	18333 ± 5164

Exercise/rest, pre-meal, post-meal, and total AUC values for each appetite sensation. Values presented as means ± SD. *Significant differences between trials $P < 0.05$.

5.5 Discussion

The primary aim of the present study was to examine the affects of an acute bout of high-intensity running on neural responses to pictures of food immediately post-exercise and several hours post-exercise. The findings from this study demonstrated that 1 hour of intense running suppressed ACC, amygdala, insula cortex, and occipital lobe activation in response to food cues immediately post-exercise compared with rest. However, several hours after exercise, ACC and insula activity was increased when viewing pictures of food compared with rest. Furthermore, when comparing neural responses to pictures of food between the first and second fMRI scans following exercise it was observed that the time post-exercise altered neural activity within the ACC, amygdala, insula, and the occipital lobes (activity was higher in the later scan). The present study also demonstrated that intense exercise suppressed acylated and total ghrelin concentrations immediately post-exercise, whilst transiently increasing post-exercise PYY levels. Furthermore, in agreement with previous findings (Broom *et al.* 2009), running suppressed hunger sensations immediately following exercise, and increased hunger during the post-meal period.

5.5.1 Neural responses to food cues

For both exercise and rest trials neural responses to images of food were greater during the second fMRI scan compared with the first fMRI scan. During the rest trials food stimuli enhanced occipital lobe and insula cortex activation during the second scan compared with the first. Furthermore, during the afternoon scan on the exercise trials activity was greater in several regions compared with the morning scan, including the left and right ACC, the left and right occipital lobes, the left amygdala, and the left and right insula. Exercise and rest both enhanced occipital lobe activation to food cues during the afternoon scan. Studies

have demonstrated that occipital lobe activation is increased in response to food vs non-food images (Füher *et al.* 2008, Schur *et al.* 2009), and in response to high-calorie vs low-calorie food images (Frank *et al.* 2010). Enhanced stimulation of the occipital lobes when viewing pictures of high-calorie vs low-calorie foods may indicate that occipital lobe activation is increased when food stimuli are perceived as more rewarding. Therefore, images of food vs non-food images in the present study may have been identified as more rewarding several hours after both exercise and rest. Enhanced insula activation was also found during afternoon scans in both trials when viewing pictures of food; however exercise also enhanced ACC and amygdala activation during the second fMRI scan. The ACC, amygdala, and insula are associated with the integration and processing of internal and external signals which encode for the rewarding properties of food, and interact to form part of the central reward system (Tataranni *et al.* 1999; Small *et al.* 2001; Killgore *et al.* 2003; Hinton *et al.* 2004). These interconnected areas process the motivational value of a stimulus and regulate the appropriate goal-directed behaviour (Devinsky *et al.* 1995). In the context of feeding behaviour, research has shown that these brain regions are activated in a state of hunger (Tataranni *et al.* 1999; Hinton *et al.* 2004; Porubská *et al.* 2006), and respond to food cues which are perceived as rewarding and high in calories (Schienle *et al.* 2008; Stoeckel *et al.* 2008; Schur *et al.* 2009; Grabenhorst *et al.* 2010). The results from the present study indicate that when viewing pictures of food several hours after a period of intense exercise and rest greater reward system activation is evident when compared with viewing pictures of food immediately post-exercise/rest. However, reward system activation is more widespread following exercise, indicating that the hedonic value of food may increase several hours after intense exercise. Greater reward system activation during the second fMRI scan compared with the first scan in both conditions could be due to an increase in appetite during the time between scans. However, there were no differences in appetite hormone levels or appetite

ratings immediately prior to scan 1 compared with scan 2 during either of the conditions. Therefore, it would appear that differences in neural responses to images of food between morning and afternoon fMRI scans were unrelated to appetite sensations and appetite hormone concentrations.

Research which has examined the impact of intense exercise on hunger over an extended period of time has found that hunger is initially suppressed immediately following exercise compared with rest, however the anorectic effects of exercise are transient and several hours post-exercise hunger increases above resting levels (Broom *et al.* 2007). In the present study, when directly comparing neural responses to food cues between rest and exercise it was observed that exercise suppressed right ACC, left amygdala, and left insula activity when viewing pictures of food immediately following exercise compared with rest. However, it was observed that when participants completed a second fMRI scan several hours after exercise, ACC and left insula activation was increased in response to food stimuli compared with rest. Therefore, despite initially suppressing the activity of central reward processing regions in response to food cues, exercise increased the rewarding properties of food several hours later. These findings show that high intensity exercise has the capacity to modulate neural responses to food cues.

The findings reported in the present study are in contrast to Cornier *et al.* (2011) who reported that 40-60 min of moderate intensity exercise (60-75% VO_2max), performed following 6 months of exercise training, did not affect neural responses to pictures of high-calorie foods immediately post-exercise compared with baseline responses in overweight individuals. Discrepancies between the findings of Cornier *et al.* (2011) and the current study may be partly explained by the intensity of the exercise bout. Recent research has shown that acute bouts of moderate intensity exercise have little or no effect on appetite and peripheral appetite regulating hormones in lean and obese individuals (King *et al.* 2010b; Unick *et al.*

2010), whereas acute bouts of high intensity exercise consistently suppresses appetite, and modulate peripheral appetite hormone circulation accordingly (Broom *et al.* 2007, 2009; King *et al.* 2010a). The contrasting results of the present study to those of Cornier *et al.* (2011), suggest that the differential effects of moderate and high intensity exercise on peripheral appetite regulation may also be evident at a central level following exercise. Differences between Cornier *et al.* (2011) and the present study may also be related to the study designs; the present study compared responses between exercise and rest, whereas Cornier *et al.* (2011) did not include a control trial and instead compared neural responses to food images at baseline with neural responses 6 months later. Furthermore, the types of participants selected for each study (lean vs overweight) may have contributed towards the contrasting findings. Therefore, it is difficult to compare the neural responses observed by Cornier *et al.* (2011) with those observed in the present study. Interestingly, neither the present study nor the Cornier *et al.* (2011) study included an overweight or lean group comparison, so the differences in exercise-induced neural responses to food images between lean and overweight individuals remain unknown. Therefore, it would be of interest in the future to compare neural responses to pictures of food between lean and obese groups following exercise.

5.5.2 Appetite Regulating Hormones

5.5.2.1 Ghrelin

The present study observed that 60 min of high intensity running transiently suppressed circulating concentrations of both total and acylated ghrelin. However, there was no difference in the percentage of total ghrelin which was acylated between trials; therefore it would appear that, under the condition of the present study, exercise affected the secretion of

ghrelin and not the acylation process post-secretion. It is well established that acute bouts of intense exercise have the capacity to suppress acylated ghrelin secretion (Broom *et al.* 2007, 2009; Marzullo *et al.* 2008; King *et al.* 2010a), however research has shown that total ghrelin secretion is unaffected by exercise (Burns *et al.* 2007; Jürimäe *et al.* 2007; Marzullo *et al.* 2008). Discrepancies between the exercise-induced total ghrelin responses reported in the present study and those reported by previous studies could be related to the type of exercise performed (Jürimäe *et al.* 2007; Marzullo *et al.* 2008), the form in which total ghrelin was analysed (i.e. serum vs plasma) (Marzullo *et al.* 2008), and the gender of the participants selected (Burns *et al.* 2007). In addition to observing suppressed total ghrelin concentrations immediately following exercise the current study also observed that total ghrelin was suppressed immediately prior to the standardized meal, indicating that the drive to eat may have been suppressed prior to feeding. Following the meal both total and acylated ghrelin concentrations were similar between trials; however acylated ghrelin concentrations were suppressed following the second fMRI scan during the exercise trials. This would suggest that perhaps exercise suppressed the drive to eat when viewing pictures of food several hours post-exercise. However, this result contradicts the neural responses to food stimuli detected during the second fMRI scan of the exercise trials. Furthermore, research shows that acylated ghrelin concentrations are similar several hours after a bout of exercise and rest (Broom *et al.* 2007, 2009; King *et al.* 2010a). This contradictory finding could perhaps be related to the small sample size of the study.

5.5.2.2 PYY

Concentrations of PYY, relative to baseline values, were briefly elevated following exercise in the current study. Transient enhancements in PYY concentrations as a result of high intensity exercise have been reported previously (Broom *et al.* 2009; Ueda *et al.* 2009;

King *et al.* 2011a). Ueda *et al.* (2009) found that high intensity exercise suppressed feelings of hunger, and stimulated greater elevations in PYY concentrations than moderate intensity exercise. Furthermore, King *et al.* (2011a) found that an acute bout of high intensity running did not influence food intake over an extended period of time despite inducing a large energy deficit, and suggested that exercise-induced enhancements in PYY concentrations may contribute towards the lack of change in food intake. Research which has examined appetite hormone responses to high intensity exercise over an extended period of time have reported that intense exercise can modulate PYY responses during the hours after exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a). The present study found that during the exercise trials concentrations of PYY were increased 30 min after the meal. These findings indicate that exercise may have increased post-meal satiety to a greater extent when compared with rest. This finding is in agreement with King *et al.* (2011a), who demonstrated that PYY levels were greater following a buffet meal on the high intensity exercise trials compared with the control trials. The authors stated that the postprandial PYY response observed during the exercise trials indicates that acute exercise may enhance the satiating effects of a meal. However, in the present study PYY levels were similar between trials 3 hours after the meal. Therefore, if elevated PYY levels do mediate an increase in post-meal satiety following exercise then this effect is short lived.

5.5.3 Subjective Appetite Sensations

This study is one of the few studies to investigate the impact of high intensity exercise not only on feelings of hunger, but also on the desire to eat certain food varieties (i.e. desire to eat savoury/fatty foods). Despite inducing large energy deficits high intensity exercise has been shown to reduce feelings of hunger (Broom *et al.* 2009; Ueda *et al.* 2009; King *et al.* 2010a; Laan *et al.* 2010), a phenomenon known as exercise-induced anorexia (King *et al.*

1994). However, research has shown that this hunger suppression is transient and hunger ratings can increase above resting values within 30 min post-exercise (Laan *et al.* 2010). The present study observed that ratings of hunger and the desire to eat savoury foods were suppressed immediately following exercise, however hunger ratings were similar between trials following the first fMRI scan (~40 min post-exercise). Interestingly, hunger ratings were reduced again on the exercise trials compared with the rest trials following the meal, indicating that a standardized meal was more effective at inducing satiety following exercise compared with rest. King *et al.* (2009) demonstrated that exercise training reduced hunger sensations following a fixed meal, and that the satiety-inducing effects of exercise remained during the 4 hour period between meals. However, despite observing a reduction in hunger ratings immediately following the meal on the exercise trials in the present study, it was also observed that hunger began to increase above resting values 30 min after the meal. Therefore, the capacity for acute exercise to increase the satiating effects of a meal may be brief.

5.5.4 Limitations

There are a number of limitations associated with this study. This study was unable to directly measure oxygen consumption during the run as the study was conducted within an fMRI facility and not an exercise laboratory. However, the average heart rate during the run was 159 ± 10 beats·min⁻¹ indicating that the exercise bout was intense. The present study measured sensations of appetite using VAS and did not measure actual food intake post-exercise. However, VAS were selected as they are a valid measure of appetite (Flint *et al.* 2000) and, unlike actual food intake measures, VAS can be used to repeatedly assess appetite over several hours. Despite this, perhaps future research should use a combined approach to appetite assessment and measure both subjective appetite sensations and actual food intake.

Furthermore, this study measured total plasma PYY and not the active form of PYY (PYY₃₋₃₆). The active form of PYY has been shown to be the predominant form of PYY in human circulation (Batterham *et al.* 2006), and exhibits a more powerful appetite-suppressing effect than the inactive form of PYY (PYY₁₋₃₆) (Chelikani *et al.* 2006). However, evidence has demonstrated that concentrations of circulating total PYY and PYY₃₋₃₆ exhibit a strong positive correlation (Tsilchorozidou *et al.* 2008). Ghrelin and PYY concentrations were lower at baseline during the exercise trials compared with the rest trials, and in the case of PYY significantly lower. Stress has been shown to suppress appetite and circulating ghrelin concentrations. In the present study participants may have experienced stress in anticipation of the intense bout of running they were about to perform, and this may explain why ghrelin concentrations were lower at baseline during the exercise trials. However, as stress reduces appetite one would expect to observe greater PYY concentrations prior to exercise, but PYY levels were suppressed prior to exercise in this study. Therefore, it is unclear as to why PYY levels were lower prior to exercise, however this could be related to the main limitation of this study, the small sample size. Due to the low number of participants recruited for this study, the initial findings presented here should be interpreted with caution.

5.5.5 Conclusions

The present study confirms previous findings which have reported that the anorectic effects of high intensity exercise are transient, and that hunger increases several hours post-exercise (Broom *et al.* 2007). Furthermore, this study demonstrates for the first time that within regions of the brain which form part of the central reward system the neural activation in response to images of food is reduced following an acute bout of high-intensity exercise. However, several hours after exercise pictures of food enhance central reward system

activation. Therefore, the exercise-induced responses of appetite regulating regions within the brain mirror those of subjective appetite responses, and peripheral appetite hormone responses, observed following intense exercise. Consequently, it would appear that an acute bout of high intensity exercise is capable of modulating both central and peripheral appetite regulators for a prolonged period of time. The use of fMRI techniques to assess the impact of exercise on the central regulation of food intake will provide further insight into the relationship between exercise and appetite. Future research needs to investigate the impact of different types, intensities, and durations of exercise on neural responses to images of food in both lean and overweight men and women.

5.6 References

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CHAPTER 6 - GENERAL DISCUSSION

6.1 General Discussion

With the number of obese individuals worldwide due to increase to 700 million by 2015 (WHO Report, 2006), research into the effectiveness of weight loss interventions, such as physical activity, is vital. The ways by which various different exercise interventions affect appetite regulation and food intake has received a great deal of scientific attention (for a review see Martins *et al.* 2008). Acute bouts of exercise have the capacity to modulate appetite and EI responses, however these responses can be influenced by several factors including the intensity of the exercise bout (Kissileff *et al.* 1990; Imbeault *et al.* 1997) and the ambient temperature in which the exercise is performed (Dressendorfer, 1993; Shorten *et al.* 2009). This thesis had two main aims:

- 1) Investigate the impact of brisk walking on appetite, food intake, and appetite hormone circulation in response to a cold and warm environment.
- 2) Examine the effects of high intensity exercise on neural responses to food cues immediately after exercise and several hours later.

Therefore, this general discussion will be subdivided into 2 sections. Section 1 will discuss the key findings from **chapters 2 and 3**. **Chapter 2** investigated the effects of brisk walking in a cold environment on post-exercise EI and appetite hormone responses in overweight men and women. **Chapter 3** examined the effects of exercise in the heat on subjective appetite sensations and appetite hormone circulation over an extended period of time. Section 2 will discuss the key findings from **chapters 4 and 5**. These chapters described two studies which have, for the first time, observed the modulating effects of high intensity exercise on neural responses to images of food, using functional magnetic resonance imaging (fMRI) techniques. **Chapter 4** demonstrates the effects of an acute bout of intense running on appetite sensations, appetite hormone concentrations, and the responses of central

appetite regulating regions when viewing pictures of food immediately following exercise.

Chapter 5 extends this study by observing the effects of an acute bout of running on appetite responses, gut hormone circulation, and neural activation in response to food stimuli immediately post-exercise, and several hours later.

6.2 Section 1: Exercise, Ambient Temperature, and Appetite

Chapters 2 and 3 investigated the effects of exercise in the cold (**chapter 2**) and the heat (**chapter 3**) on appetite hormone circulation, post-exercise EI, and appetite sensations. Furthermore, **chapter 2** examined the relationship between changes in thermoregulatory responses to exercise in the cold and appetite hormone circulation. **Chapter 2** found that an acute bout of brisk walking in a cold environment (8°C) stimulated EI 45 min post-exercise in overweight men and women compared with brisk walking in a neutral environment (20°C), and that this is likely to be related to an increase in acylated ghrelin concentrations observed immediately following exercise in the cold. Furthermore, despite observing a substantial reduction in skin temperature (T_{sk}) during exercise in the cold, a relationship between changes in T_{sk} and appetite hormone circulation was not observed. Findings from **Chapter 3** demonstrated that exercise in a warm environment (32°C) did not affect subjective appetite sensations during exercise. Furthermore, despite observing a greater desire to eat at the end of the warm exercise trials (5 hours post-exercise) compared with the warm resting trials, no further differences in appetite were observed over an extended period of time in overweight men and women. However, total ghrelin AUC was suppressed during the warm exercise trials compared with the cool exercise trials.

Exercise at different ambient temperatures has been shown to influence post-exercise food intake (Dressendorfer, 1993; White *et al.* 2005; Shorten *et al.* 2009). Exercise in a cold environment is reported to stimulate calorie consumption (Dressendorfer, 1993; White *et al.*

2005), which may explain why swimmers have been shown to have greater body fat stores compared with athletes who participate in land based activities (Flynn *et al.* 1990). As swimming is often recommended to overweight individuals as an alternative to weight bearing exercises it is important to investigate the potential effects that swimming may have on appetite and the motivation to eat. King *et al.* (2011a) reported that swimming in cold water did not affect post-exercise EI, and suppressed appetite during and immediately after exercise in lean healthy individuals. However, it is perhaps more important to understand the effects that exercise in a cold environment may have on the appetite of overweight and obese individuals. Furthermore, several factors may contribute towards the effects that swimming has on appetite and EI, including water temperature and body position. Previous research demonstrates that supine exercise can enhance skin vasodilation and reduce splanchnic blood flow in comparison with upright exercise (Rowell *et al.* 1964; Froelich *et al.* 1988).

Therefore, **chapter 2** attempted to isolate the effects of a cold environment on exercise-induced changes to food intake by having overweight and obese men and women complete an acute bout of brisk walking in a cold (8°C) and neutral environment (20°C), and then measuring *ad libitum* EI post-exercise (**chapter 2**). In agreement with previous research (Dressendorfer, 1993; White *et al.* 2005), it was found that exercise in the cold increased post-exercise food intake compared with exercise in the neutral environment. These findings may have important implications for overweight and obese individuals who have been recommended swimming as an effective weight loss approach.

In contrast to exercise in the cold, exercise in the heat appears to suppress calorie consumption (Dressendorfer *et al.* 1993; Shorten *et al.* 2009). Recent research has demonstrated that high intensity exercise performed in the heat suppressed post-exercise EI relative to the energy expended during exercise (REI) compared with rest in neutral conditions in lean healthy individuals (Shorten *et al.* 2009). Previous studies which have

examined the impact of exercise in the heat on calorie consumption have selected lean participants; however, as with **chapter 2**, **chapter 3** described a study which selected overweight and obese men and women, for whom this research is more clinically relevant. Furthermore, the effects of exercise in the heat on appetite sensations over an extended period of time have yet to be examined. Studies which have observed a reduction in hunger immediately following exercise have found that hunger can ‘rebound’ over an extended period of time (Broom *et al.* 2007). Therefore, if exercise in the heat has the capacity to transiently suppress food intake following exercise, one could speculate that individuals may experience a delayed compensatory response to the energy expended during exercise several hours later. The findings from this study demonstrated that exercise in the heat did not influence appetite during exercise or immediately following exercise, however desire to eat was greater 5 hours after exercising in the warm environment compared with resting in the warm environment. The scarcity of significant findings may in part be due to the small sample size of this study.

Research speculates that exercise-induced appetite modifications may be associated with changes in splanchnic blood flow during exercise, which may alter the volume of blood available for the circulation of appetite regulating gut hormones (Burns *et al.* 2007). Exercise in a neutral environment reduces blood flow to the splanchnic region and increases blood flow to the working muscles, and the skin in order to dissipate heat (Rowell *et al.* 1964). When exercise is performed in a hot environment blood flow to the splanchnic region is substantially reduced compared with exercise in a neutral environment, as more blood is shunted to the skin in order to alleviate the greater heat stress imposed on the body by the hot environment (Rowell *et al.* 1965). Therefore, one may assume that during exercise in a cold environment the reduction in blood flow to the splanchnic region, and the increase in blood flow to the skin, is less as the body attempts to reduce heat dissipation and protect core

temperature. Furthermore, research has demonstrated that resting exposure to cold temperatures can stimulate ghrelin secretion, whereas exposure to warm temperatures can suppress ghrelin secretion (Tomasik *et al.* 2005). Consequently, this study speculated that a reduction in splanchnic blood flow during exercise in the heat would reduce the volume of blood available for the circulation of ghrelin, thereby suppressing appetite. Whereas an increase in splanchnic blood flow during exercise in the cold would increase the volume of blood available for the circulation of ghrelin, thereby stimulating food intake. Skin temperature (a surrogate of skin blood flow) was substantially reduced during and after exercise in the cold (**chapter 2**), while exercise in the heat considerably increased T_{sk} (**chapter 3**). This observation indicates that blood flow to the skin may have been reduced during and after cold exercise and increased during and after warm exercise, causing redistribution of splanchnic blood flow and changes in appetite hormone circulation. However, in contrast to previous findings (Shorten *et al.* 2009), exercise in the heat did not affect PYY levels. Furthermore, there was no change in ghrelin concentrations during or immediately after exercise in the heat. Exercise in the cold did increase acylated ghrelin concentrations, which may have been related to reductions in skin blood flow, and could have been one of the mechanisms responsible for the increase in post-exercise EI. However, there were no significant correlations between acylated ghrelin concentrations and T_{sk} , or calorie consumption following exercise.

Peripheral appetite hormone secretion may not be the only mechanism responsible for mediating the increases in EI observed in **chapter 2**. Increases in EI following exercise in the cold could also be mediated by regions within the brain which regulate appetite. The hypothalamus is involved in the regulation of both appetite (Suzuki *et al.* 2010) and core body temperature (Hammel *et al.* 1963). Therefore, changes in core body temperature may impact upon central appetite regulation. Dressendorfer *et al.* (1993) speculated that the

stimulation of food intake immediately following exercise in the cold may be related to a reduction in core temperature during cold exercise. However, in **chapter 2** of this thesis core temperature responses were similar when exercising in both the cold and neutral environments. Therefore, this study suggests that perhaps interactions between regions of the hypothalamus which regulate appetite and those which regulate core body temperature are less important than peripheral gut hormone responses when exercising in a cold environment. As such, changes in blood flow to the gut may be the primary mechanism responsible for increases in EI following cold exercise.

To summarize, these findings support those which have reported an increase in *ad libitum* EI following exercise in the cold (Dressendorfer, 1993; White *et al.* 2005), however exercise in a warm environment did not affect appetite. Appetite may have been unaffected by exercise in the heat due to the lack of change in ghrelin and PYY concentrations following exercise. Exercise in the cold however increased concentrations of acylated ghrelin, and this may have contributed towards the increase in post-exercise EI. It could be speculated that modifications in blood flow to the skin and splanchnic region during exercise in hot and cold environments may cause changes in appetite hormone concentrations. However, despite observing enhanced ghrelin concentrations following exercise in the cold, and a substantial reduction in T_{sk} , there was no relationship between the two. Therefore, the exact mechanisms by which exercise in the cold stimulates food intake remain unknown. Both studies are the first to have examined the impact of exercise in different ambient temperatures on appetite and calorie consumption in overweight and obese middle-aged men and women.

Furthermore, the findings from **chapter 2** especially have important implications for overweight and obese individuals who are attempting to lose weight by increasing physical activity, as exercise in a cold environment (i.e. swimming) may increase EI and thereby negate the energy expended during exercise. Therefore, future research which investigates

the effects of exercise in different ambient temperatures on appetite and calorie consumption should select an overweight/obese population. Furthermore, future research should attempt to directly measure skin and splanchnic blood flow in order to understand the relationships between exercise, appetite hormone circulation, and feeding behaviour.

6.3 Section 2: High Intensity Exercise and Central Appetite Regulation

The effects of high intensity exercise on peripheral appetite regulation are fairly well established (Broom *et al.* 2007, 2009; King *et al.* 2010; King *et al.* 2011*b*); however the impact of high intensity exercise on central appetite regulation has yet to be examined. Therefore, **Chapters 4 and 5** of this thesis describe two studies which have examined the effects of an acute bout of high intensity exercise on peripheral and central appetite regulation, using fMRI techniques. **Chapter 4** found that when viewing images of food immediately following an intense bout of running, activation within regions of the brain which regulate the rewarding properties of food was suppressed. In addition, reward system activation was enhanced in response to pictures of low-calorie foods vs high-calorie foods. In order to extend these findings, the effects of intense running on neural responses to images of food immediately following exercise, and several hours after exercise were examined (**chapter 5**). Once again it was observed that high intensity exercise suppressed central reward system activation in response to images of food immediately post-exercise compared with rest. However, several hours following the exercise bout pictures of food stimulated reward system activation compared with rest. Furthermore, when comparing neural responses to food during the first and second fMRI scan on the exercise trials only, neural activation was greater when viewing images of food several hours after exercise compared with immediately post-exercise. In addition, both **chapters 4 and 5** report that running transiently suppressed feelings of hunger, and total and acylated ghrelin concentrations,

whilst enhancing total PYY levels. However, in agreement with previous research (Broom *et al.* 2007), hunger increased during the hours following exercise (**chapter 5**).

Research demonstrates that high intensity exercise consistently suppresses feelings of hunger (Burns *et al.* 2007; Broom *et al.* 2007, 2009; King *et al.* 2010; Laan *et al.* 2010), whilst modifying peripheral appetite hormone circulation to enhance satiety (Marzullo *et al.* 2008; Broom *et al.* 2009; Ueda *et al.* 2009; King *et al.* 2011*b*). Studies have shown that changes in subjective appetite sensations (Tataranni *et al.* 1999; Morris and Dolan, 2001; Porubska *et al.* 2006) and appetite hormone concentrations (Batterham *et al.* 2007; Malik *et al.* 2008) when viewing images of food are related to neural responses within regions of the brain which regulate the drive to eat. Therefore, if high intensity exercise has the capacity to influence appetite sensations and peripheral appetite regulating hormones, then one might speculate that high intensity exercise will also influence central appetite regulation.

However, the effects of intense exercise on central appetite regulation have yet to be examined. **Chapters 4 and 5** have demonstrated that an acute bout of high intensity exercise suppressed neural activity within the orbitofrontal cortex (OFC), insula, amygdala, and the anterior cingulate cortex when viewing images of foods vs objects immediately following exercise. Research has reported that these areas of the brain process the hedonic properties of food and play important roles within the central reward system (Small *et al.* 2001; Killgore *et al.* 2003; Stoeckel *et al.* 2008; Schur *et al.* 2009; Frank *et al.* 2010). High intensity exercise also enhanced the rewarding properties of low-calorie foods and suppressed the rewarding properties of high-calorie foods compared with rest (**chapter 4**). When viewing images of high-calorie foods vs objects activation within the dorsolateral prefrontal cortex (DLPFC) was increased post-exercise. This region of the brain is shown to regulate satiation (Le *et al.* 2006), and suppressed DLPFC in response to food consumption in obese individuals has been proposed as one of the potential mechanism responsible for hyperphagia (Le *et al.* 2006,

2007). In addition, Montenegro *et al.* (2011) reported that stimulation of the DLPFC enhanced satiety at rest in overweight individuals, however when DLPFC stimulation was combined with exercise the effect was further enhanced. Therefore, the DLPFC may play an important role in the regulation of appetite following exercise. Furthermore, previous research has identified the insula as a potential mediator of exercise-induced appetite responses (Cornier *et al.* 2011). As described in **chapter 4**, insula and putamen activity was enhanced when viewing pictures of low-calorie food vs objects. In addition to regulating the rewarding aspects of food, the insula cortex has been shown to respond to sensations of thirst (Egan *et al.* 2003). Therefore, as exercise increased sensations of thirst, it could be speculated that the drive to consume foods which have a high water content may be increased following exercise, thereby enhancing the motivation to consume low-calorie foods such as grapes and apples. When directly comparing neural responses to low-calorie foods with neural responses to high-calorie foods, exercise stimulated left pallidum activation when viewing low- vs high-calorie food images. As the left pallidum has previously been shown to respond to images which individuals perceive as pleasant and rewarding (Stoeckel *et al.* 2008), it would therefore appear that exercise enhances the hedonic aspects of low-calorie foods, whereas high-calorie foods are perceived as less rewarding. Overall, **chapters 4 and 5** demonstrate that an acute bout of high intensity exercise has the capacity to suppress the rewarding aspects of food, especially high-calorie food, whilst increasing the attractiveness of low calorie food immediately following exercise. Furthermore, the neural responses to images of food observed following exercise were supported by appetite ratings and appetite hormone concentrations (**chapters 4 and 5**). Running suppressed hunger, the desire to eat, and the desire to eat savoury foods during and immediately after exercise. Furthermore, running reduced concentrations of both total and acylated ghrelin, whilst increasing total PYY levels. In addition, **chapter 4** reports that the percentage of total ghrelin which was

acylated immediately following exercise/rest was greater following exercise compared with rest, indicating that high intensity exercise affects both the secretion of ghrelin and the post-secretion acylation process. Therefore, **chapter 4** show for the first time that high intensity exercise has the capacity to enhance satiety by influencing subjective appetite sensations, peripheral appetite hormone circulation, and neural responses to food immediately following exercise.

Chapter 5 examined the effects of high intensity exercise on markers of appetite over an extended period of time and found that hunger sensations were suppressed immediately prior to the standardized meal presented 1 hour and 45 min following exercise. Furthermore, PYY concentrations were greater following the meal during the exercise trial compared with the rest trial. This observation is in agreement with King *et al.* (2011b), who stated that an increase in postprandial PYY levels following an acute bout of running indicates that intense exercise may enhance the satiating effects of a meal. However, this study demonstrates that sensations of hunger were greater 1 hour after the meal following exercise compared with rest. This finding is supported by the neural responses to food cues observed several hours after exercise. During the second fMRI scan, exercise enhanced ACC and left insula activation compared with rest. Furthermore, central reward system activation when viewing pictures of food was greater during the second fMRI scan compared with the first scan in both the exercise and rest trials. Neural responses within the right insula were greater during the second fMRI scan following rest. However, reward system activation was more widespread during the exercise trials, with greater activation observed within the left and right ACC, the left amygdala, and the left and right insula cortex during the second fMRI scan compared with the first scan. Furthermore, visual cortex activation was greater in response to food cues several hours post-exercise compared with immediately post-exercise; enhanced visual cortex activity has been reported previously in response to images of food

which are perceived as rewarding (Frank *et al.* 2010). Therefore, despite a reduction in feelings of hunger and central rewarding system activation in response to food cues immediately following exercise, an acute bout of high intensity running enhanced hunger and neural responses to images of food several hours post-exercise.

As previously mentioned in section 1 of this discussion, changes in core body temperature could be one of the potential mechanisms responsible for mediating central appetite responses. In **chapters 4 and 5** of this thesis, high intensity exercise was shown to increase core body temperature above 39.5°C in certain participants. As core temperature rises during exercise, the central nervous system inhibits the ability to exercise in an attempt to prevent heat stress-induced cellular damage (González-Alonso *et al.* 1999; Nielsen *et al.* 2001). Therefore, as body temperature begins to rise during intense exercise neural activation within areas of the brain which regulate body temperature may increase and thus perhaps inhibit the neural activity of regions within the brain which regulate food intake. Furthermore, food intake can stimulate heat production through diet-induced thermogenesis (DIT; Westerterp, 2004). Consequently, when the body is experiencing thermal stress, the brain may inhibit food intake so as to avoid the additional heat production imposed by DIT. However, as thermal stress subsides during recovery, the function of the brain may shift from heat stress prevention to appetite stimulation in an attempt to compensate for the energy expended during exercise. As the hypothalamus contributes towards thermoregulatory control (Hammel *et al.* 1963) and appetite regulation (Suzuki *et al.* 2010), this area of the brain may signal a change in focus from body temperature protection to EI. Consequently, it could be speculated that the mechanisms responsible for appetite stimulation over an extended period of time following exercise may be two-fold; a redistribution of peripheral blood flow from the skin to the splanchnic region thereby enhancing gut hormone circulation,

and an increase in the neural stimulation of hunger caused by cross-talk between areas of the hypothalamus which regulate thermoregulation and appetite.

Overall, **chapters 4 and 5** have shown for the first time that an acute bout of high intensity exercise is capable of modulating activity within regions of the brain which mediate the drive to eat. These findings indicate that the effect of high intensity exercise on regions of the brain which regulate appetite is similar to the effects of high intensity exercise on hunger. When viewing pictures of food immediately following an intense bout of running the neural activity of central regions which encode for the hedonic properties of food was suppressed. Furthermore, exercise suppressed the rewarding aspects of high-calorie foods, and increased the rewarding aspects of low-calorie foods. However, several hours following exercise central reward system activation was increased in response to food cues. Therefore, high intensity exercise has the capacity to influence not only appetite sensations and peripheral appetite hormone circulation, but also neural activation of regions within the brain which regulate appetite. Examining exercise-induced changes in peripheral and central appetite regulation will greatly enhance our knowledge of the effects exercise has on appetite and feeding behaviour.

6.4 Conclusions

Chapters 2 and 3, which examine the effects of an acute bout of brisk walking at different ambient temperatures on appetite, post-exercise EI, and appetite hormone responses in overweight/obese middle-aged men and women found that:

1. Exercise in a cold environment stimulates post-exercise EI compared with exercise in a neutral environment.

2. This is likely to be mediated by an increase in acylated ghrelin secretion following exercise in the cold compared with exercise in a neutral environment.
3. Brisk walking in the heat did not affect appetite sensations, however desire to eat was greater 5 hours following exercise in the heat compared with rest.
4. Exercise in the heat suppressed total ghrelin AUC compared with exercise in a cool environment, however there were no differences in appetite sensations between trials.

Chapters 4 and 5 investigated the impact of an acute bout of high intensity running on sensations of appetite, peripheral appetite hormone circulation, and neural activation within regions of the brain which regulate appetite when viewing images of food immediately post-exercise and following an extended period of time in lean active males. The findings from these studies demonstrated that:

1. Exercise suppressed the hedonic aspects of high-calorie foods, and increased the rewarding aspects of low-calorie foods by modulating central reward system activation.
2. Hunger, desire to eat, desire to eat savoury foods, and ghrelin concentrations were suppressed, whilst PYY levels were enhanced immediately post-exercise.
3. Several hours following exercise central reward system activation was increased in response to food cues compared with rest, and this may be associated with increased feelings of hunger.
4. High intensity exercise is capable of enhancing satiety at both a peripheral and central level immediately following exercise, however neural responses to food increase several hours later.

6.5 Future Research Ideas

This thesis examined the effects of different exercise interventions on appetite and feeding behaviour. **Chapters 2 and 3** enhanced our knowledge regarding the effects of acute exercise on appetite, feeding behavior, and appetite hormone responses in overweight/obese men and women. Furthermore, chapters 4 and 5 demonstrated for the first time that an acute bout of high intensity exercise has the capacity to modulate neural responses to images of food immediately following exercise and following an extended period of time. The findings from this thesis have produced a number of future research ideas:

1. How long does the EI stimulating effect of exercising in a cold environment last for?
2. Does swimming in cold water enhance post-exercise food intake in overweight and obese men and women?
3. Can exercise in the heat suppress caloric intake in overweight and obese individuals?
4. Does splanchnic blood flow redistribution following exercise at different ambient temperatures mediate peripheral appetite hormone circulation?
5. How do various different exercise modes, intensities, and durations affect central reward system responses to images of high- and low-calorie food?
6. Do neural responses to food following acute bouts of exercise differ between lean and obese individuals?
7. What are the effects of chronic exercise training, of a moderate and high intensity, on central responses to low- and high-calorie food cues in obese and lean individuals?

Investigating these research ideas will contribute towards our knowledge of how exercise can influence appetite, energy intake, and appetite hormone circulation. This knowledge will

enable us to design effective exercise interventions aimed at inducing a negative energy balance and enhancing weight loss in overweight and obese individuals.

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APPENDICES

Appendix A**Consent Form***Study title:*

Research Centre:

Researchers:

Participant ID Number:

Please initial box

1. I confirm that I have read and understand the information sheet
for the above study and have had the opportunity to ask questions.

☐

2. I understand that my participation is voluntary and that I am free to withdraw at
any time. A decision to withdraw will not affect my medical care or legal rights.

☐

3. All samples left at the end of the study will be destroyed without further analysis.

☐

4. I agree to take part in the above study.

☐_____
Name of Participant_____
Date Signature_____
Researcher_____
Signature_____
Date

Appendix B

The University of Birmingham

School of Sport and Exercise Sciences

General Health Questionnaire

Name:

Address:

.....

.....

Phone:

Name of the responsible investigator for the study:

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.....	Male	Female
2.	<p>What is your exact date of birth?</p> <p>Day..... Month.....Year..19.....</p> <p>So your age is..... Years</p>		
3.	<p>When did you last see your doctor? In the:</p> <p>Last week..... Last month..... Last six months.....</p> <p>Year..... More than a year.....</p>		
4.	Are you currently taking any medication?	YES	NO
5.	Has your doctor ever advised you not to take vigorous exercise?	YES	NO
6.	Has your doctor ever said you have "heart trouble"?	YES	NO
7.	Has your doctor ever said you have high blood pressure?	YES	NO
8.	Have you ever taken medication for blood pressure or your heart?	YES	NO

9.	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
12.	Have you had a cold or feverish illness in the last month?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14.	a) Do you suffer from back pain b) if so, does it ever prevent you from exercising?	YES YES	NO NO
15.	Do you suffer from asthma?	YES	NO
16.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
17.	Has your doctor ever said you have diabetes?	YES	NO
18.	Have you ever had viral hepatitis?	YES	NO
19.	If you are female, to your knowledge, are you pregnant?	YES	NO

20.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
21.	Are you accustomed to vigorous exercise (an hour or so a week)?	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed:

Date:

Health Questionnaire:

Notes for the investigator

This questionnaire is for use in circumstances where you are intending to carry out a procedure which has been approved by the Ethics Subcommittee (Section 2 of the *Health and Safety Issues* document) but where a health screen is indicated. Questions 3 and 4 should be used to test, discretely, the veracity of the other answers.

If your subject is within the age group specified (usually 18 to 30 years) and has answered NO to questions 5-20 and YES to question 21, you may include him or her in your study.

If you are using this, or a similar, questionnaire for subjects outside this age range or with possible pathologies, you must have agreed with the Ethics Subcommittee the criteria for accepting subjects into the study and safeguarding their health.

Appendix C**McGiniss Thermal Comfort Scale**

- 1 – So cold I am helpless**
- 2 – Numb with cold**
- 3 – Very cold**
- 4 – Cold**
- 5 – Uncomfortably cold**
- 6 – Cool but fairly comfortable**
- 7 – Comfortable**
- 8 – Warm but fairly comfortable**
- 9 – Uncomfortably warm**
- 10 – Hot**
- 11 – Very hot**
- 12 – Almost as hot as I can stand**
- 13 – So hot I am sick and nauseated**

Appendix D**Date:**

Participant ID _____

Time _____

Trial _____

HOW TO COMPLETE A RATING SCALE

A rating scale consists of a line with two end-anchor points. The line represents a continuum of possibilities between these two statements. Above the line is a question.

When making a rating you should:

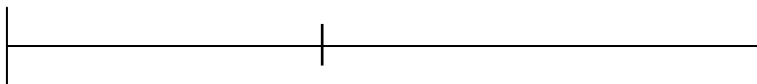
1. Read and think about the question.
2. Read and think about BOTH anchor points.
3. Think of the line as a continuum of possibilities between the anchor points.
4. Place a single vertical line on the rating scale. This should intersect the line at the point that relates your answer to the appropriate corresponding position on the line.

Example:

How **THIRSTY** do you feel right now?

Not at all

Very

Thirsty**Thirsty**

Thank you for your participation

Appendix E

Participant ID:

Trial:

Time

Positive and negative affect scale (PANAS) measuring current emotional state

Please read each item and indicate the extent you feel this way at the present moment in time.

	<i>Very slightly/ not at all</i>	<i>A little</i>	<i>Moderately</i>	<i>Quite a bit</i>	<i>Extremely</i>
<i>Interested</i>	1	2	3	4	5
<i>Distressed</i>	1	2	3	4	5
<i>Excited</i>	1	2	3	4	5
<i>Upset</i>	1	2	3	4	5
<i>Strong</i>	1	2	3	4	5
<i>Guilty</i>	1	2	3	4	5
<i>Scared</i>	1	2	3	4	5
<i>Hostile</i>	1	2	3	4	5
<i>Enthusiastic</i>	1	2	3	4	5
<i>Proud</i>	1	2	3	4	5
<i>Irritable</i>	1	2	3	4	5
<i>Alert</i>	1	2	3	4	5
<i>Ashamed</i>	1	2	3	4	5
<i>Inspired</i>	1	2	3	4	5
<i>Nervous</i>	1	2	3	4	5
<i>Determined</i>	1	2	3	4	5
<i>Attentive</i>	1	2	3	4	5
<i>Jittery</i>	1	2	3	4	5
<i>Active</i>	1	2	3	4	5
<i>Afraid</i>	1	2	3	4	5

Appendix F**PARTICIPANT ID:** _____**TRIAL:** _____**Post-Experiment Image Assessment**

Please rate your level of emotional involvement for each image on scales of pleasure and arousal using the 9-point scales by placing a cross in the relevant circle below.

Page 1

Image Number	Rating Score
1	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
2	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
3	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
4	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
5	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
6	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
7	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

8	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
9	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
10	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
11	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

Page 2

Image Number	Rating Score
12	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
13	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
14	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
15	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

16	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
17	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
18	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
19	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
20	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
21	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
22	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
23	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
24	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

Page 3

Image Number	Rating Score
25	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
26	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
27	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
28	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
29	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
30	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
31	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
32	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
33	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

34	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
35	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
36	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
37	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

Page 4

Image Number	Rating Score
38	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
39	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
40	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
41	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
42	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming

43	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
44	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
45	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
46	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
47	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming
48	Very Pleasant (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Unpleasant
	Very Exciting (9) (8) (7) (6) (5) (4) (3) (2) (1) Very Calming