Title: Effect of Aerobic Exercise in Different Environmental Temperatures on Gut Hormones, Appetite and Energy Intake

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Effect of Aerobic Exercise in Different Environmental Temperatures on Gut Hormones, Appetite and Energy Intake

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AUTHOR DECLARATION

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ABSTRACT

Introduction: A strategy that combines both increasing energy expenditure and reducing energy intake (EI) to induce a negative energy balance is key for preventing and managing obesity. Exercise has been shown to reduce EI in a subsequent meal, an increase in temperature has also been shown to decrease appetite stimulation. Exercise in a hot environment may augment the appetite suppressing effect of exercise. However, there is currently little evidence available regarding the effect of environmental temperature during exercise on appetite. This study focused on the effect of exercise in different environmental temperatures on gut hormones and EI.

Methods: A total of 8 healthy males completed four 5.5 hour conditions in a counterbalanced order. A preliminary visit consisting of a submaximal and maximal exercise test was conducted prior to experimental visits. For experimental visits, participants arrived in a fasted, euhydrated state at 08:30 and were fitted with a cannula, heart rate monitor, rectal and skin thermistors before completing one of four conditions: exercise in 10°C, 20°C or 30°C or resting control. Participants ran for 60 minutes on a treadmill at 70% of maximal oxygen uptake or rested for 60 minutes before resting for 4.5 hours. Blood samples were taken at 0 (fasted), 1, 1.5, 2, 3, 4 and 5 hr. Perceptions of hunger were assessed using visual analogue scales at 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 h. *Ad libitum* meals were provided at 1.5 hr and 5 hr.

Results: Although there was a significant reduction in relative energy intake in all exercise conditions ($p < 0.001$), this was not augmented or attenuated by any change in environmental temperature. This decrease was also not supported by any decrease in acylated ghrelin or increase in PYY. Furthermore, the only significant decrease in overall appetite was stimulated by the intake of food in meal 1 ($p < 0.001$). There was also no significant difference in total energy intake, lending to the notion that the decrease in relative energy intake can be partially, if not completely attributed to the increase in energy expenditure from exercise.

Conclusion: These results suggest that exercise produces an energy deficit through a reduction in relative energy intake, regardless of environmental temperature. Further research into the effects of exercise in different environmental temperatures in an overweight and obese population is warranted.

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LIST OF ABBREVIATIONS

AUC	Area under the curve
BMI	Body mass index
CNS	Central nervous system
EI	Energy intake
EE	Energy expenditure
HR	Heart rate
iAUC	Incremental area under the curve
PFC	Prospective food consumption
PYY	Peptide tyrosine-tyrosine
REI	Relative energy intake
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
TEE	Total energy expenditure
VAS	Visual analogue scale

INTRODUCTION

Obesity is now the fifth leading cause of mortality worldwide (World Health Organisation, 2009). According to the World Health Organization (WHO), in 2014 1.9 billion adults were overweight; 600 million of those were classified as obese. This represents a two-fold increase in overweight and obesity since 1980 (World Health Organisation, 2014). Obesity is defined as an excess of adipose tissue (Gallagher *et al.*, 1996). A person is classified as overweight when their body mass index (BMI) is above 25 $kg.m^{-2}$ and obese when BMI is greater than 30 $kg.m^{-2}$ (Seidell, 2000). This classification using BMI is not without issue, as although an indicator of body fat, BMI is not a direct measure (Gallagher *et al.*, 1996). Overweight or obesity is fundamentally caused by an imbalance between energy intake (EI) and energy expenditure (EE). Specifically, an EI greater than EE leads to a positive energy balance and, if sustained, weight gain. This increase in weight can be attributed to a number of factors, particularly that evolution has led to a subconscious drive to eat whenever food is available (Bilski *et al.*, 2009). Furthermore, high calorie foods have become more widely available (Charlot *et al.*, 2017). On the other side of the energy balance equation, levels of moderate to vigorous physical activity have reduced and been replaced with an increase in sedentary behaviour, reducing EE (Hamer *et al.*, 2014, Bilski *et al.*, 2009). Furthermore, Tucker *et al.* (2011) suggests that as few as 10% of the population comply with physical activity guidelines. The substantial increase in the incidence of obesity has led to an increase in efforts to prevent it. In turn, this will reduce the incidence of the associated comorbidities of hypertension, type 2 diabetes, stroke and cancer (Greenway, 2015). It also reduces the cost to health and care services, predicted to increase £2 billion per year by 2030 (Wang *et al.*, 2011). Just as a chronic positive energy balance will increase body weight, a chronic negative energy balance will reduce it. Therefore, maintaining a negative energy balance is key for reducing body weight to manage obesity. A negative energy balance is achieved when total energy expenditure (TEE) is greater than EI (Hill *et al.*, 2012). Total energy expenditure is the sum of resting metabolic rate, the thermic effect of food and energy expended during physical activity (Hill *et al.*, 2012).

Reducing EI through diet restriction is the most common intervention for the management of obesity (Ross *et al.*, 2000). Known commonly as ‘dieting’, this often results in transient weight loss, especially in those utilising very-low-energy diets (Greenway, 2015, Anderson *et al.*, 2001). Weight regain may occur through weight loss-induced changes in appetite

regulatory hormones, increases in ghrelin and decreases in PYY and GLP-1 sustained for a minimum of a one year period (Greenway, 2015, Sumithran *et al.*, 2011). Therefore, maintaining weight loss is just as vital as the initial weight loss.

Energy expenditure from physical activity is the most practical component of EE to manipulate, and is commonly achieved by increasing levels of physical activity through exercise (Curioni & Lourenco, 2005). Independent of weight loss, exercise also aids the prevention of cardiovascular disease (Fletcher *et al.*, 1992), type 2 diabetes, cancer, osteoporosis (Warburton *et al.*, 2006) and has psychological benefits (Martinsen, 1990). Previous studies have indicated that individuals do not offset the amount of energy expended by increasing EI in the subsequent hours following exercise (Faure *et al.*, 2016, Schubert *et al.*, 2013). This phenomenon of exercise related acute reduction in hunger has been termed 'exercise induced anorexia', and coincides with increases in PYY and a decrease in acylated ghrelin (Larson-Meyer *et al.*, 2012, Bilski *et al.*, 2009, Ueda *et al.*, 2009a). These individuals would be at an energy deficit which, if maintained, would lead to weight loss. A strategy that combines both increasing EE and reducing EI has the potential to be a key tool in preventing and managing obesity; this combination has also been shown to aid in the maintenance of weight loss (Curioni & Lourenco, 2005).

In addition to exercise, it is also possible to alter appetite, therefore potentially EI, through the manipulation of environmental temperature. In the cold, Westerterp-Plantenga *et al.* (2002) detected a significantly greater EE in participants resting for 60 hours in 16°C compared to resting in 22°C, attributed to an increase in resting metabolic rate. This increase was not compensated for by an increase in EI, resulting in an energy deficit. Regarding the effect of heat on appetite, Faure *et al.* (2016) detected lower plasma acylated ghrelin following 40 minutes rest in the heat (31°C) compared to resting in a neutral temperature (22°C), suggesting a reduction in appetite stimulation, therefore, lower EI. Anecdotally, individuals do also report having a reduced appetite in the heat. Clearly, there is very little literature available on the independent effects of temperature on appetite. Despite this lack of literature, the manipulation of environmental temperature clearly has the potential to alter appetite.

The appetite suppressing effects of exercise and a manipulation of environmental temperature have the potential, if combined, to provide guidelines for weight control. This combination of an exercise intervention and manipulation of environmental temperature has been previously

investigated to a limited extent (Kojima *et al.*, 2016, Faure *et al.*, 2016, Crabtree & Blannin, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). However, the few available studies have produced contradictory findings and included considerable limitations. Faure *et al.* (2016), Kojima *et al.* (2015), Wasse *et al.* (2013) and Shorten *et al.* (2009) investigated the effect of exercise in the heat compared to exercise in a neutral environmental temperature. Wasse *et al.* (2013) detected a significant decrease in EI in an *ad libitum* meal after exercise in the heat compared to a neutral temperature, this was accompanied by lower perceptions of hunger but not a decrease in acylated ghrelin. Faure *et al.* (2016) and Shorten *et al.* (2009) also detected lower, but not significant, energy intake after exercise in the heat compared to exercise in a neutral temperature. Kojima *et al.* (2015) detected a significant decrease in perceived hunger following exercise in the heat compared to exercise in a neutral environmental temperature, but did not measure energy intake.

Crabtree & Blannin (2015), Kojima *et al.* (2015) and Wasse *et al.* (2013) also examined the effect of exercise in the cold. Crabtree & Blannin (2015) detected a significant increase in EI and a significant increase in acylated ghrelin after exercise in the cold compared to exercise in a neutral environmental temperature. Wasse *et al.* (2013) showed a higher, but not significant, EI after exercise in the cold and also detected lower perceived appetite but did not find any differences in gut hormones. The findings in the Wasse *et al.* (2013) study are conflicting, as it is expected that lower perceptions of appetite coincide with lower EI, however this was not the case.

However, these studies are limited by not comparing experimental conditions to a resting control trial (Kojima *et al.*, 2016, Faure *et al.*, 2016, Crabtree & Blannin, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). Therefore, the aim of the current study was to investigate the effect of exercise in different environmental temperatures on gut hormones and EI, and compare those effects to a resting control.

AIMS AND HYPOTHESES

The aims of this study were to:

a) Investigate the effect of an acute bout of running exercise in different environmental temperatures on perceived appetite and gut hormones.

b) Investigate the effect of an acute bout of running exercise in different environmental temperatures on *ad libitum* EI.

The hypotheses are as follows:

a) Exercise in the heat will augment the appetite-suppressing effect of exercise, while exercise in a cold environment will attenuate the appetite-suppressing effect of exercise when compared to exercise in a neutral temperature.

b) If exercise does reduce energy intake, this effect will be greatest when combined with an increase environmental temperature compared to exercise in a neutral environmental temperature. A decrease in environmental temperature will reduce the effect of exercise on energy intake compared to exercise in a neutral environmental temperature.

LITERATURE REVIEW

This section aims to give a brief overview of factors affecting appetite control before conducting a review of the current literature regarding the influence of gut hormones in appetite regulation. Furthermore, this section will discuss the independent effects of aerobic exercise and environmental temperature on appetite and appetite control, and will end with an examination of the combined effect of environmental temperature and aerobic exercise on appetite and its regulation.

Appetite Control

Appetite control is an intricate homeostatic process that at a physiological level includes gut hormones, the brain and other organs (Klok *et al.*, 2007). It should also be acknowledged that there is an environmental, social and psychological aspect to appetite control and it is possible that these factors can override the physiological mechanisms (Cruwys *et al.*, 2015, Martins *et al.*, 2008, Saper *et al.*, 2002). For example, it has been shown that when exposed to their peers eating larger portions, individuals will eat more than if eating alone, thus demonstrating a so-called 'modelling' effect (Nisbett & Storms, 1974). Furthermore, the process of evolution has resulted in an innate need to eat whenever food is available, and even the mere thought, sight or smell of food can begin the body's preparation for food intake (Bilski *et al.*, 2009). Despite this plethora of factors affecting appetite control, it has been shown that changes in those gut hormones can reduce energy intake (Druce *et al.*, 2006, Degen *et al.*, 2005).

Appetite-Regulating Hormones

Several hormones are involved in appetite regulation in humans; some are anorectic such as peptide tyrosine-tyrosine (PYY). In contrast, acylated ghrelin is, to date, the only orexigenic (appetite-stimulating) gut hormone to be discovered (Kojima *et al.*, 1999). Previous literature suggests it is possible to alter the balance of the gut hormones PYY and acylated ghrelin to provide a significant decrease in appetite through manipulation of environmental temperature or exercise (Schubert *et al.*, 2014, Schubert *et al.*, 2013).

Acylated Ghrelin

Ghrelin is present in the body in both acylated and nonacylated forms, with 80-90% being nonacylated, and only about 10% being acylated (Broom *et al.*, 2007, Al Awar *et al.*, 2005). However, only the active form of ghrelin, acylated ghrelin, can bind to growth hormone secretagogue receptor (GHS-R1a) and cross the blood-brain barrier to stimulate appetite (Albarrn-Zeckler & Smith, 2013, Neary & Batterham, 2009, Broom *et al.*, 2007, Banks *et al.*, 2002). Acylated ghrelin is a 28-amino acid peptide hormone and is currently the only hormone that is known to stimulate hunger in humans (Neary & Batterham, 2009, Broom *et al.*, 2007). Acylated ghrelin was not widely measured, due to its instability and relatively short half-life of 9-13 minutes (Akamizu *et al.*, 2004), until Hosoda *et al.* (2004) developed a method of stabilising and preserving acylated ghrelin beyond this half-life.

Acylation is the process of adding an acyl group to the third serine residue of ghrelin, forming acylated ghrelin (Kojima *et al.*, 1999). This reaction is catalysed by the enzyme ghrelin-*O*-acyltransferase (GOAT), which has been detected in the circulatory system of humans, suggesting ghrelin is synthesised and acylated in different locations in the body (Prinz & Stengel, 2017, Yang *et al.*, 2008). Ghrelin is released predominantly from the gastric oxyntic glands in the stomach and to a lesser extent, the small intestine, pancreas and hypothalamus (Neary & Batterham, 2009, Broom *et al.*, 2007). The stomach is the main source of ghrelin synthesis, with gastrectomy in rats showing a large reduction in circulating ghrelin (Ariyasu *et al.*, 2001). In humans, Cummings *et al.* (2002) investigated differences in total ghrelin secretion among obese individuals undergoing weight loss through dieting and those post-gastric bypass surgeries. The elevated meal response was present in all individuals; however, the total 24-hour ghrelin profile in the gastric bypass group was 77% lower than the dieting group. This study not only suggests that a reduction in circulating total ghrelin may contribute to weight loss, but also that bypassing the stomach severely limits the body's ability to produce and secrete ghrelin. This is further evidenced as acylated ghrelin acts on the gastric afferent vagal nerve to stimulate feeding in rats, with Date *et al.* (2002) showing no increase in feeding following intravenous administration of total ghrelin after a blockade of the vagal afferent.

Supporting evidence for acylated ghrelin being orexigenic comes from findings in several studies that plasma acylated ghrelin concentrations rise in the pre-prandial phase and fall in the postprandial phase (Cummings *et al.*, 2002). Al Awar *et al.* (2005) showed that fasting plasma acylated ghrelin levels decrease within 15 minutes of EI, reaching the lowest

concentrations approximately 30 minutes postprandial; concentrations then begin to rise after 60 minutes and surpass fasting levels after 180 minutes. Nonacylated ghrelin may play a role in stimulating adipogenesis (Heppner *et al.*, 2014, Thompson *et al.*, 2004) and has been shown to enhance gastric motility in humans (Wren *et al.*, 2001a). Furthermore, Tong *et al.* (2013) suggest a role for nonacylated ghrelin in glucose homeostasis, detecting a significant decrease in insulin secretion with no change to insulin sensitivity in response to nonacylated ghrelin infusions. Nonacylated ghrelin has also been shown to stimulate gastric acid secretion in rats (Masuda *et al.*, 2000). There is also a suggestion that nonacylated ghrelin aids regulation of body temperature; Inoue *et al.* (2013) showed a significant decrease in the back temperature and vasodilation in the tails of rats following a central infusion of nonacylated ghrelin, suggesting an increase in nonacylated ghrelin may limit the body's ability to effectively reduce temperature. Therefore, studies involving a manipulation of environmental temperature should consider the functions of non-acylated ghrelin.

Studies into rats and humans that have supplied an acylated ghrelin infusion to participants provide evidence that acylated ghrelin is orexigenic. Firstly, in rats, Nakazato *et al.* (2001) showed that an intracerebroventricular (ICV) injection of total ghrelin to 8 hour fasted rats promoted a four-fold increase in food intake over a two-hour period compared to a saline injection. Wren *et al.* (2001b) found that a similar ICV injection of total ghrelin once daily stimulated a 15 g increase in body weight over seven days in rats compared to a 2 g increase over seven days of ICV saline injections. Also in rats, Tschöp *et al.* (2000) showed that total ghrelin administration promoted a significant increase in EI and a subsequent weight increase. In humans, Druce *et al.* (2006) found a 27% increase in EI 60-minutes after a subcutaneous injection of total ghrelin compared to the same dose of saline in healthy men and women. Wren *et al.* (2001a) also showed a 28% increase in EI when nine participants received a subcutaneous total ghrelin infusion compared to saline infusion. From this evidence, it seems reasonable to conclude that acylated ghrelin stimulates appetite in humans.

The response of acylated ghrelin to EI differs according to the macronutrient composition of the food ingested. Carbohydrate intake has a greater suppressive effect on acylated ghrelin than protein or fat (El Khoury *et al.*, 2006). Furthermore, acylated ghrelin has been shown to respond more quickly to carbohydrate intake than total ghrelin (Hosoda *et al.*, 2004). In contrast, it has been reported that fat intake does not significantly decrease plasma acylated ghrelin concentrations (Al Awar *et al.*, 2005, Tentolouris *et al.*, 2004). However, Broom *et al.* (2007) reported a decrease in acylated ghrelin following a meal consisting of 38%

carbohydrate, 10% protein and 52% fat. It is possible that the carbohydrate in this meal exerted much of the suppressive effect on acylated ghrelin. Furthermore, this study involved an exercise intervention that may have suppressed acylated ghrelin prior to the meal (Broom *et al.*, 2007). This difference in macronutrient response indicated that it is vital for studies that are measuring acylated ghrelin and *ad libitum* EI following an exercise intervention to consider the macronutrient composition of the test meals provided.

Peptide YY

Named because of the presence of the amino acid tyrosine at each terminus of the polypeptide, peptide tyrosine-tyrosine (PYY) is an anorectic hormone responsible for stimulating satiety in humans (Karra & Batterham, 2010). PYY is predominantly synthesised and secreted from specialised L-cells in the small intestine and colon (Prinz & Stengel, 2017, Karra & Batterham, 2010). Two forms of PYY, PYY_{1-36} and PYY_{3-36}, are present in the body in both the fasted and fed states. PYY_{3-36} forms most circulating PYY in both states (Grandt *et al.*, 1994).

Evidence proposing that PYY is anorectic in nature has come from studies showing that plasma PYY concentrations rise in response to EI. Levels initially rise 15 minutes after nutrient ingestion and peak after 1 to 2 hours, followed by a plateau lasting several hours (Adrian *et al.*, 1985). Further support for the satiating effects of PYY is the reduction in appetite following PYY administration; Degen *et al.* (2005) showed a 32% reduction in EI following subcutaneous PYY infusion compared to saline. Furthermore, Gantz *et al.* (2007) showed a significant decrease in body weight over a 12-week period of intranasal PYY administration 20 minutes before every meal. It has been shown that PYY_{3-36} acts as a satiety signal by binding the neuropeptide Y Y2 receptor (Y2R) in the arcuate nucleus of the hypothalamus (Batterham *et al.*, 2002). Evidence for this suggestion is supplied by studies showing that PYY administration reduced EI in normal mice but not in Y2R-null mice (Batterham *et al.*, 2002). Due to this evidence, it seems clear that PYY acts on the CNS as a satiety signal in humans.

Meals of different macronutrient composition have been shown to have varying postprandial effects on plasma PYY (Helou *et al.*, 2008). Fat intake appears to increase PYY within an hour and to the greatest extent, while protein intake delays the increase until after the first hour (Helou *et al.*, 2008). Helou *et al.* (2008) showed that a meal high in carbohydrate (60% carbohydrate, 20% fat, 20% protein) sustained the increase in PYY above baseline over a test

period of three hours. The combined effects show that a balanced meal (50% carbohydrate, 30% fat, 20% protein) has the potential to stimulate an increase in PYY over an extended period. These findings make it clear that since fat produces the most pronounced PYY response, meal composition should be carefully considered when comparing studies measuring changes in PYY for differences in composition of test meals that may alter the postprandial response.

Effect of acute aerobic exercise on gut hormones, appetite and energy intake

The effect of aerobic exercise on appetite has been widely studied, with the consensus from experimental research (Kojima *et al.*, 2016, Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Ueda *et al.*, 2009a, Broom *et al.*, 2007, Burns *et al.*, 2007, Jurimae *et al.*, 2007, Martins *et al.*, 2007, Dall *et al.*, 2002) and meta-analyses (Schubert *et al.*, 2014, Schubert *et al.*, 2013) being that appetite is acutely reduced by aerobic exercise.

Acylated Ghrelin

The effect of exercise on acylated ghrelin concentrations is an area of debate, with some studies showing a significant decrease in concentrations (Kojima *et al.*, 2016, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Broom *et al.*, 2007) and others showing no difference from a control trial (Douglas *et al.*, 2015). However, many studies are now of reduced significance due to not measuring the active, acylated form of ghrelin (Larson-Meyer *et al.*, 2012, Burns *et al.*, 2007, Jurimae *et al.*, 2007, Martins *et al.*, 2007, Dall *et al.*, 2002). Information on the participants, conditions and results of these studies is presented in table 1.

Several studies focusing on the effect of a single bout of aerobic exercise on plasma acylated ghrelin have produced comparable findings. Kojima *et al.* (2016), Larson-Meyer *et al.* (2012), King *et al.* (2010) and Broom *et al.* (2007) all detected a transient, yet significant decrease in plasma acylated ghrelin following exercise interventions ranging from 40-90 minutes in duration and at an intensity greater than 60% of $\dot{V}O_{2max}$. Conversely, Douglas *et al.* (2015) did not show a significant difference on day one of a two-day trial, measuring acylated ghrelin at 0 (baseline) and 7 hours (end of trial day 1) despite using an exercise protocol similar in intensity and duration. The first post-exercise Douglas *et al.* (2015) blood sample was at hour 7, following two *ad libitum* meals, had this study analysed plasma acylated ghrelin within the 30-minutes post-exercise, a significant difference may have been detected. These findings support the theory that any exercise induced decrease in acylated

ghrelin is transient. In the studies that detected a significant decrease in plasma acylated ghrelin, concentrations were only significantly different for up to 30 minutes after the end of exercise (Kojima *et al.*, 2016, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Broom *et al.*, 2007). Significant suppression of acylated ghrelin was not sustained beyond this time in any study to date. The majority of these findings support a suggestion from Bilski *et al.* (2009) that intense exercise, greater than 60% of $\dot{V}O_{2max}$, causes a short-term suppression of appetite through an decrease in secretion of acylated ghrelin.

In addition to measuring plasma acylated ghrelin, numerous studies have assessed the response of total ghrelin to an acute bout of aerobic exercise, however none of these studies detected a significant difference in total ghrelin following acute aerobic exercise (Larson-Meyer *et al.*, 2012, Burns *et al.*, 2007, Jurimae *et al.*, 2007, Martins *et al.*, 2007, Kraemer *et al.*, 2004). The difference in response to exercise between acylated and total ghrelin may be explained by the acylation of nonacylated ghrelin already present in the body at the time of exercise. This would have the effect of maintaining plasma total ghrelin while the ratio of acylated to nonacylated ghrelin increased. This theory is supported by Larson-Meyer *et al.* (2012), detecting a significant decrease in acylated ghrelin but no change in total ghrelin, suggesting the acylation of ghrelin is inhibited during exercise. Furthermore, it has been postulated that a low, rather than high intensity exercise has the greater effect on total ghrelin response. This suggestion is due to studies conducted by Erdmann *et al.* (2007) and Dall *et al.* (2002), Erdmann *et al.* (2007) showed a significant change in plasma total ghrelin using low intensity cycling (50 W), while Dall *et al.* (2002) exercised participants at a high intensity (175 W). However, Erdmann *et al.* (2007) utilised an exercise duration of 120 minutes, while Dall *et al.* (2002) exercised participants for only 45 minutes. This raises the question if exercise intensity or duration has the greater effect on plasma total ghrelin. The consensus from the previous literature is that despite having no effect on total ghrelin, an acute bout of aerobic exercise of 60 minutes in duration and an intensity of 60% of $\dot{V}O_{2max}$ or greater has a significant suppressive effect on plasma acylated ghrelin (Schubert *et al.*, 2014).

Peptide YY

Current literature suggests that aerobic exercise acutely increases plasma PYY concentrations. Research conducted by Ueda *et al.* (2009a) and Martins *et al.* (2007) shows a significant increase in plasma PYY compared to a control condition following exercise at a minimum of 60% of $\dot{V}O_{2max}$ lasting at least 30 minutes. Following 30 minutes exercise at

75% of $\dot{V}O_{2max}$ PYY AUC was 16 times greater than PYY AUC after 30 minutes rest (Ueda *et al.* (2009a). PYY levels increased during exercise and remained elevated for the following 30 minutes in both studies (Ueda *et al.*, 2009a). Monitoring was ceased 30 minutes post exercise in both studies; thus, it may have been possible to observe a prolonged effect of exercise had monitoring been continued. In contrast, Kojima *et al.* (2016) showed no significant difference in plasma PYY concentrations following a 20-km run, despite this being of a greater intensity and duration than the afore mentioned studies. The discrepancy in results between these studies may be explained by the fact that PYY concentrations were already significantly lower at baseline in the exercise condition of the Kojima *et al.* (2016) study than the control condition. This may have had the effect that despite a rise in plasma PYY concentrations no significance was detected. Furthermore, it has been shown that aerobic, but not resistance exercise, stimulates an increase in plasma PYY concentrations (Broom *et al.*, 2009). Broom *et al.* (2009) investigated the effects of 90-minutes resistance training and 60-minutes aerobic exercise on PYY concentrations. PYY was significantly increased in the aerobic exercise condition, but was not different in the resistance exercise condition from the resting control. However, EE in the aerobic condition was 2.6 times greater than that of the resistance condition, raising questions of whether the increased EE or the mode of exercise increased PYY concentrations. Nevertheless, it is clear that aerobic exercise acutely increases plasma PYY concentrations.

Perceptions of appetite

Human appetite can be broken down into four facets; hunger - the feeling of starvation signalling that an individual should eat, satiation - the process that leads to the termination of eating, fullness - the feeling of the stomach being full, and prospective food consumption (PFC) - the amount of food an individual expects to consume (Sørensen *et al.*, 2003). Subjective perceptions of hunger, satiation, fullness and prospective food consumption are commonly assessed by visual analogue scales (VAS); an "overall appetite" rating is also often calculated as the mean after inverting the values of satiation and fullness (Blundell *et al.*, 2010, Stubbs *et al.*, 2000). Visual analogue scales are lines of 10 cm in length with an extreme at each end ('Not at all full' and 'Totally full') and participants make a single mark along the line. The distance from the left end of the line to the mark is measured and recorded as a percentage (Flint *et al.*, 2000). Visual analogue scales have been used extensively to assess changes in appetite in response to exercise (Kojima *et al.*, 2016, Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Ueda *et al.*, 2009a, Martins *et al.*, 2007, Jurimae

et al., 2007, Burns *et al.*, 2007, Broom *et al.*, 2007, Dall *et al.*, 2002). Previous literature suggests that hunger and PFC are suppressed, satiety and fullness increased during and immediately after exercise (Kojima *et al.*, 2016, Ueda *et al.*, 2009a, Martins *et al.*, 2007, Burns *et al.*, 2007) but these effects are rarely sustained longer than 30 minutes (Kojima *et al.*, 2016).

Energy intake

Energy intake is a key variable in appetite research, as regardless of changes in gut hormones and perceptions of hunger, if the actual amount of energy consumed is not altered these changes are meaningless. The effect of exercise on total EI is an area of dispute with some studies reporting a significant decrease in EI following exercise when compared with a resting control trial (Kojima *et al.*, 2016, Ueda *et al.*, 2009a), others report no difference (Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, King *et al.*, 2010) and some even report an increase following exercise (Shorten *et al.*, 2009, Martins *et al.*, 2007).

Of the studies that report EI, those observing a significant decrease as a result of exercise used the greatest exercise intensities, a 20 km run reduced EI 13% (Kojima *et al.*, 2016), and Ueda *et al.* (2009a) also detected a significant decrease in EI after a 30 minute treadmill run at 75% of $\dot{V}O_{2max}$, but did not report exact figures for EI. These findings complement the reduction in subjective appetite often found during and immediately post-exercise (Bilski *et al.*, 2009). However, subjective ratings of appetite do not always correlate with EI. For example, Douglas *et al.* (2015) detected a significant decrease in ratings of hunger, but this did not relate to any decrease in EI following exercise. Although, these variables do link a reduction in EI and subjective feelings of appetite in other research articles (Kojima *et al.*, 2016, Ueda *et al.*, 2009a, Martins *et al.*, 2007), this may suggest that the use of VAS to assess appetite is a poor predictor of EI.

However, when EI is offset against energy expended during exercise, a decrease after exercise compared to a control is often observed (Kojima *et al.*, 2016, Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Martins *et al.*, 2007). The offset EI is known as relative energy intake (REI). This would lead to a negative energy balance in the short term (Schubert *et al.*, 2013).

Table 1: Effect of acute aerobic exercise on gut hormones, appetite and energy intake.

Author	Participants	Conditions	Energy Intake		Ghrelin		PYY	Hunger
			Absolute	Relative	Total	Acylated		
Kojima *et al.* (2016)	23 male college endurance runners	EX - 20 km outdoor run CON - 120 min rest	EX = 1325 ± 55 CON = 1529 ± 55 (kcal) *	13% fewer kcal consumed in EX *	NR	No difference between EX and CON	No difference between EX and CON	EX = 60 ± 5 CON = 71 ± 3 (mm) *
Douglas *et al.* (2015)	15 physically active males	EX - 60 min, 70% $\dot{V}O_{2max}$ treadmill run CON - 60 min rest	EX = 2802 ± 780 CON = 2893 ± 756 (kcal)	EX = 760 ± 140 CON = 986 ± 158 (kcal) *	NR	No difference between EX and CON	No difference between EX and CON	Lower in EX than CON *
Larson-Meyer *et al.* (2012)	9 trained females	EX - 60 min, 70% $\dot{V}O_{2max}$ treadmill run CON - 60 min rest	EX = 485.8 ± 183.4 CON = 480.4 ± 126.4 (kcal)	EX = -193.9 ± 205.8 CON = 283.8 ± 120.6 (kcal) *	EX = 144.7 ± 52.6 CON = 167.8 ± 37.0 (pmol/L)	EX = 10.6 ± 8.6 CON = 25.4 ± 15.7 (pmol/L) *	EX = 45.0 ± 8.9 CON = 43.6 ± 10.9 (pmol/L)	EX = 8.9 ± 9.4 CON = 13.6 ± 16.4
King *et al.* (2010)	9 healthy males	EX - 90 min, 70% $\dot{V}O_{2max}$ treadmill run CON - 90 min rest	EX = 3898 ± 484 CON = 4854 ± 416 (kcal)	EX = 2935 ± 299 CON = 4109 ± 273 (kcal) *	NR	EX = 130.3 ± 15.1 CON = 147.1 ± 19.7 (pg/ml) *	NR	EX = 74 ± 5 CON = 65 ± 8 (mm)

Broom *et al.* (2009)	11 healthy males	EX - 60 min, 70% $\dot{V}O_{2max}$ treadmill run CON - 60 min rest	NR	NR	NR	EX = 188 ± 68 CON = 228 ± 62 (pg/ml)	EX = 324 ± 54 CON = 229 ± 29 (pg/ml) *	Lower in EX than CON *
Shorten *et al.* (2009)	11 healthy males	EX - 40 min, 70% $\dot{V}O_{2max}$ treadmill run CON - 40 min rest	EX = 954 ± 377 CON = 678 ± 244 (kcal) *	No difference between EX and CON	NR	No difference between EX and CON	No difference between EX and CON	NR
Ueda *et al.* (2009a)	10 young males	EX50 - 30 min, 50% $\dot{V}O_{2max}$ treadmill run EX75 - 30 min, 75% $\dot{V}O_{2max}$ treadmill run CON - 30 min rest	Lower in EX50 and EX75 than CON *	NR	NR	NR	EX75 = 446.8 ± 187.0 EX50 = 293.4 ± 171.9 CON = 26.7 ± 16.2 (pmol/ml×60 min) *	Lower in EX50 and EX75 compared to CON *
Broom *et al.* (2007)	9 healthy males	EX - 60 min, 75% $\dot{V}O_{2max}$ treadmill run CON - 60 min rest	NR	NR	NR	38% lower in EX than CON *	NR	No difference between EX and CON

Burns *et al.* (2007)	18 healthy males and females	EX - 60 min, 75% $\dot{V}O_{2max}$ treadmill run CON - 60 min rest	NR	NR	EX = 1240.7 ± 179.8 CON = 1374.9 ± 231.7 (pmol/L)	NR	NR	Reduced during and after exercise in EX and CON *
Martins *et al.* (2007)	12 healthy males and females	EX - 60 min, 65% maxHR cycle CON - 60 min rest	EX = 913 ± 363 CON = 762 ± 252 (kcal) *	EX = 421 ± 302 CON = 565 ± 226 (kcal) *	No difference between EX and CON	NR	Increased in EX *	Decreased in EX *

* indicates statistical significance ($p < 0.05$) exact figures stated when reported, relative energy intake calculated as energy intake minus energy expenditure, NR – not reported

Effect of temperature at rest on gut hormones, appetite and energy intake

The effect of environmental temperature on appetite is an area that has not been widely examined with only three research studies examining the effect of environmental temperature at rest on appetite (table 2) (Faure *et al.*, 2016, Langeveld *et al.*, 2016, Westerterp-Plantenga *et al.*, 2002). This said, anecdotal evidence suggests appetite decreases in the heat and increases in the cold when compared to a thermoneutral condition (Charlot *et al.*, 2017).

Effect of heat on gut hormones, appetite and energy intake

To date, only one study has investigated the effect of heat during rest on appetite (Faure *et al.*, 2016). The general findings of this study indicate that there is no significant effect of heat (31°C) during rest for 100 minutes on EI or relative energy intake (REI). However, there was a trend for lower perceptions of hunger in the heat compared to a neutral control (22°C).

Effect of cold on gut hormones, appetite and energy intake

There are currently two studies that have examined the response of appetite to a cold environment at rest (Langeveld *et al.*, 2016, Westerterp-Plantenga *et al.*, 2002). These two studies observed similar findings despite using slightly different temperatures that may be considered too high to be "cold" (16 and 18°C) (Charlot *et al.*, 2017). The two studies by Langeveld *et al.* (2016) and Westerterp-Plantenga *et al.* (2002) also exposed participants to the experimental environmental temperature for drastically different periods of time, the Langeveld *et al.* (2016) study lasted for 150 minutes while Westerterp-Plantenga *et al.* (2002) exposed participants for 60 hours. However, both studies reported an increase in EE when resting in the cold through a greater resting metabolic rate. Also, both studies reported no increase in EI when compared to a resting control trial. These findings show that individuals do not compensate for the increase in EE in the cold by increasing EI. Furthermore, due to the difference in duration, the mechanisms involved in short term appetite control in the cold may differ from those involved in long term appetite control.

Table 2: Effect of environmental temperature on gut hormones, appetite and energy intake.

Author	Participants	Conditions	Energy Intake		Ghrelin		PYY	Hunger
			Absolute	Relative	Total	Acylated		
Faure *et al.* (2016)	10 healthy men	HEAT - 100 min rest in 31°C CON - 100 min rest in 22°C	HEAT = 1039 ± 217 CON = 1042 ± 330 (kcal)	No difference between HEAT and CON	No difference between HEAT and CON	NR	NR	No difference between HEAT and CON
Langeveld *et al.* (2016)	10 healthy males and females	COLD - 150 min rest in 18°C CON - 150 min rest in 24°C	COLD = 688 ± 118 CON = 655 ± 136 (kcal)	NR	NR	NR	NR	No difference between COLD and CON
Westerterp-Plantenga *et al.* (2002)	9 healthy males	COLD - 60 hours rest in 16°C CON - 60 hours rest in 22°C	COLD = 7588 ± 1195 CON = 7110 ± 1315	NR	NR	NR	NR	No difference between COLD and CON

* indicates statistical significance ($p < 0.05$) exact figures stated when reported, relative energy intake calculated as energy intake minus energy expenditure, NR – not reported

Combined effect of environmental temperature and exercise on gut hormones, appetite and energy intake

The effect of environmental temperature during exercise is an area that has been investigated by a small number of studies previously (table 3) (Faure *et al.*, 2016, Crabtree & Blannin, 2015, Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). Some studies have observed the effects of exercise in the cold, neutral and the heat. However, none of these studies have compared the effect of exercise in heat, cold and neutral temperatures to a resting control.

Effect of heat and exercise on gut hormones, appetite and energy intake

The effect of heat during exercise has been investigated in several recent research articles (Faure *et al.*, 2016, Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). These studies generally conclude that exercising in the heat does not augment the appetite suppressing effect of exercise and are summarised in Table 3.

Before commenting on the effects of exercise in the heat it is necessary to note the differing temperatures that are referred to as 'heat' exposure. Temperatures ranging from 30°C (Wasse *et al.*, 2013) to 36°C (Kojima *et al.*, 2015, Shorten *et al.*, 2009) are utilised to expose individuals to the heat. Therefore, care must be taken when comparing studies investigating exercise in the heat. Furthermore, a range of exercise intensities and modes are also used. Most commonly used are cycling and running at intensities between 60 and 70% of $\dot{V}O_{2max}$ (Faure *et al.*, 2016, Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009), however walking has also been used when investigating an overweight population (Crabtree & Blannin, 2015).

Gut Hormones

None of the studies to investigate the effect of exercise in the heat on appetite detected a significant difference in acylated ghrelin or PYY during or after exercise in the heat compared to a neutral temperature (Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). These studies also did not detect any difference in total ghrelin. It is not possible to examine the magnitude of change caused by either the exercise or the heat in these studies, as none included a control trial.

Energy intake

The effect of exercise in the heat on absolute EI is assessed in three studies (Faure *et al.*, 2016, Wasse *et al.*, 2013, Shorten *et al.*, 2009). Faure *et al.* (2016) and Shorten *et al.* (2009)

did not observe a difference in absolute EI, however Wasse *et al.* (2013) detected a decrease after exercise in the heat compared to exercise in a neutral temperature. The difference detected in this study may be due to the greater duration of exercise (Shorten *et al.*, 2009) or greater intensity and different mode of exercise, cycling in the Faure *et al.* (2016) study and treadmill running in the Wasse *et al.* (2013) study. The response of appetite to running and cycling has not widely been investigated, the effect of these two modes of exercise may differ, possibly due to a difference in active muscle mass. None of these studies detected a significant difference in REI (Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009).

Effect of cold and exercise on gut hormones, appetite and energy intake

The effect of exercise in the cold has not been widely examined (Crabtree & Blannin, 2015, Kojima *et al.*, 2015, Wasse *et al.*, 2013). As with exercise in the heat, these studies generally conclude that exercise in the cold does not affect appetite.

Acylated Ghrelin

Crabtree & Blannin (2015), Kojima *et al.* (2015) and Wasse *et al.* (2013) investigated the effect of exercise in the cold on plasma acylated ghrelin. Crabtree & Blannin (2015) was the only study to detect a change, however this was a significant increase after exercise. This could be due to the exercise being the lowest intensity of the studies, this may affect changes in acylated ghrelin as studies detecting changes all used exercise intensities of at least 70% $\dot{V}O_{2max}$. However, these changes have previously been detected after exercise independently of manipulation of environmental temperature (King *et al.*, 2010, Broom *et al.*, 2009, Broom *et al.*, 2007).

Peptide Tyrosine-Tyrosine

Crabtree & Blannin (2015) and Kojima *et al.* (2015) also investigated the effect of exercise in the cold on plasma PYY. However, neither Crabtree & Blannin (2015) or Kojima *et al.* (2015) detected a significant difference in PYY after exercise in the cold compared to exercise in a neutral environment. Once again, these studies cannot be compared to a resting trial as Crabtree & Blannin (2015) nor Kojima *et al.* (2015) included a control trial.

Perceptions of hunger

Wasse *et al.* (2013) and Kojima *et al.* (2015) investigated the effect of exercise in the cold on perceived hunger. These studies produced conflicting findings; Kojima *et al.* (2015) detected a significant increase in perceived hunger, whereas Wasse *et al.* (2013) detected a decrease. This may be due to differences in exercise duration and mode, Wasse *et al.* (2013) used the whole body exercise of running for 60 minutes, 15 minutes greater than the cycling exercise

used in the Kojima *et al.* (2015) study. Again, the differences in active muscle mass may have affected participants perceptions of appetite.

Energy intake

Crabtree & Blannin (2015) and Wasse *et al.* (2013) investigated the effect of exercise in the cold on energy intake. Both studies detected a significant increase in EI following exercise in the cold compared to exercise in a neutral environmental temperature, this increase was only significant in the Crabtree & Blannin (2015) study. This may be due to differences in the populations observed in each study, Crabtree & Blannin (2015) examined overweight men and women, while Wasse *et al.* (2013) studied normal weight males. There were also some slight differences in study design, Crabtree & Blannin (2015) used a cold temperature of 8°C and a 45-minute treadmill walk at 60% $\dot{V}O_{2max}$, while Wasse *et al.* (2013) used a similar design with a 10°C cold temperature and a 60-minute treadmill run at 65% $\dot{V}O_{2max}$. The lower temperature used by Crabtree & Blannin (2015) may have increased individuals EE beyond the increase caused by exercise, through a further increase in basal metabolic rate, causing an increase in EI.

Table 3: Combined effect of environmental temperature and exercise on gut hormones, appetite and energy intake.

Author	Participants	Conditions	Energy Intake		Ghrelin		PYY	Hunger
			Absolute	Relative	Total	Acylated		
Faure *et al.* (2016)	10 healthy males	HEAT - 40 min cycling at 60% $\dot{V}O_{2max}$ in 31°C CON - 40 min cycling at 60% $\dot{V}O_{2max}$ in 22°C	HEAT = 1090 ± 296 CON = 1156 ± 236 (kcal)	No difference between HEAT and CON	No difference between HEAT and CON	NR	NR	No difference between HEAT and CON
Crabtree & Blannin (2015)	16 overweight men and women	COLD - 45 min treadmill walk at 60% $\dot{V}O_{2max}$ in 8°C CON - 45 min treadmill walk at 60% $\dot{V}O_{2max}$ in 20°C	COLD = 1299 ± 657 * CON = 1172 ± 537 (kcal)	COLD = 1001 ± 664 CON = 846 ± 518	No difference between COLD and CON	COLD = 1.1 ± 3 CON = 0.9 ± 2 (pg/ml) *	No difference between COLD and CON	NR

Kojima *et al.* (2015)	11 healthy males	COLD - 45 min cycling at 65% $\dot{V}O_{2max}$ in 12°C CON - 45 min cycling at 65% $\dot{V}O_{2max}$ in 24°C HEAT - 45 min cycling at 65% $\dot{V}O_{2max}$ in 36°C	NR	NR	NR	No difference between COLD, CON and HEAT	No difference between COLD, CON and HEAT	COLD = 67 ± 5 CON = 45 ± 6 HEAT = 49 ± 45 ± 9 (mm) *
Wasse *et al.* (2013)	11 healthy males 10 healthy males	COLD - 60 min treadmill run at 65% $\dot{V}O_{2max}$ in 10°C CON - 60 min treadmill run at 65% $\dot{V}O_{2max}$ in 20°C HEAT - 60 min treadmill run at 65% $\dot{V}O_{2max}$ in 30°C	COLD = + 347 ± 560 from CON (kcal) HEAT = - 335 ± 574 from CON (kcal)	NR	NR	No difference between COLD, CON and HEAT	NR	Lower in COLD and HEAT than CON

Shorten *et al.* (2009)	11 healthy males	CON - 40 min treadmill run at 70% $\dot{V}O_{2max}$ in 25°C HEAT - 40 min treadmill run at 70% $\dot{V}O_{2max}$ in 36°C	CON = 1194 ± 493 HEAT = 994 ± 459 (kcal)	No difference between CON and HEAT	NR	No difference between CON and HEAT	No difference between CON and HEAT	NR

* indicates statistical significance ($p < 0.05$) exact figures stated when reported, relative energy intake calculated as energy intake minus energy expenditure, NR – not reported

Summary

A clear understanding of the role of exercise and environmental temperature on appetite could enhance current physical activity programmes and recommendations for weight control. The general consensus of the current literature is that although exercise reduces appetite and EI, and individuals resting in the cold do not increase EI to compensate for an increase in EE, the manipulation of environmental temperature does not seem to reduce EI to a greater extent than exercise in a neutral temperature. However, no study to date has compared the effect of exercise under heat stress, cold and thermoneutral conditions to a resting control. This lack of a control trial could explain why some studies did not report differences in perceived appetite, EI and gut hormones. Comparing trials back to a control trial may prove that exercise reduces appetite, and provide new recommendations for weight control if manipulating environmental temperature does augment the appetite reducing effect of exercise.

METHODS

Participants

Eleven participants volunteered to take part in this study, three participants did not complete the study, the final sample was eight male participants. Participants ethnicities were not recorded. All participants were regularly involved in physical activity (competitive sport, running, resistance exercise) (table 4).

Table 4: Participant characteristics

Characteristic	Mean ± SD
Age (years)	23.1 ± 4.4
Height (m)	1.79 ± 0.07
Body mass (kg)	80.9 ± 11.0
Body fat (%)	18.2 ± 4.8
Body mass index ($kg.m^2$)	25.4 ± 3.4
$\dot{V}O_{2max}$ ($mL.kg^{-1}.min^{-1}$)	51 ± 9

$\dot{V}O_{2max}$ = maximum rate of oxygen consumption

Prior to participation, participants completed an Informed Consent Form (Appendix A), Physical Activity Readiness Questionnaire (PAR-Q) (Appendix B), a Pre-test Medical Questionnaire (Appendix C), Blood Screening Form (Appendix D), Breakfast Habits Questionnaire (Appendix E) and a Three-Factor Eating Questionnaire to assess for abnormal eating habits (Appendix F) (Karlsson *et al.*, 2000). 18 questions were rated on a four point scale from definitely true (4) to definitively false (1), with six questions examining cognitive restraint, nine examining unrestrained eating and three examining emotional eating. Participants scoring greater than 11 were considered to be exhibiting signs of abnormal eating behaviours and were excluded from the study (Stunkard & Messick, 1985). Participants also completed a 1-9 rating scale for foods included in each meal used for the experimental conditions (Appendix G), participants that did not rate at least one bread or cereal as a 5 or above (meal 1) or did not rate the pasta meal as 5 or above (meal 2) were excluded from the study to avoid differences resulting from participants disliking a large proportion of the provided foods. Participants were informed of their right to withdraw from the study without question at any time. Ethical approval for this study was gained from the University of Bedfordshire Ethics Committee (approval number: 2016ISPAR010, Appendix J).

Preliminary measurements

The participants' standing height to the nearest 0.01 m (Stadiometer, Holtain Ltd., Pembrokeshire, United Kingdom), body mass to the nearest 0.1 kg (BWB0800, Allied Weighing, Amsterdam, Netherlands) and body fat percentage to the nearest 0.1% (Bod Pod 2000A, Life Measurement, Concord, CA., USA) were measured. Participants then completed a sub-maximal exercise test on a motorised treadmill (PPS55 Med-I, Woodway, Waukesha, WI., USA). This protocol consisted of four, 4-minute stages of increasing intensity ranging from 30-80% of maximal oxygen uptake ($\dot{V}O_{2max}$) at a 1% gradient to reflect energetic costs of outdoor running (Jones & Doust, 1996). After a 10-minute rest, participants completed a graded maximal exercise test. The test was performed on a motorised treadmill, beginning at a speed equating to a heart rate (HR) of ~160 beats·min^{-1} in the submaximal test and a 0% gradient. Speed remained constant throughout the protocol while gradient increased by 1% every minute until volitional exhaustion. Expired gas was analysed continuously using breath-by-breath online gas analysis for both exercise tests (MetaLyzer 3B, Cortex, Leipzig, Germany). Values of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were collected and averaged with HR and rating of perceived exertion (RPE) (Borg, 1970) in the final 60 seconds of each stage in the submaximal test and in the final 30 seconds of each stage of the graded maximal exercise test. $\dot{V}O_{2max}$ was determined using a 30 second rolling average and was deemed to have been achieved when $\dot{V}O_2$ (mL.kg^{-1}.min^{-1}) increased by less than 2 mL.kg^{-1}.min^{-1} in any stage, or when 2 of the following 3 criteria were met; HR within 10 bpm of predicted HR_{max}, RER above 1.05 or RPE greater than 19 (ACSM, 2013). Linear regression was used to estimate the treadmill speed corresponding to 60% $\dot{V}O_{2max}$ for the experimental conditions.

Experimental design

For the experimental conditions, participants arrived at the University of Bedfordshire Sport and Exercise Science Laboratories at 08:30 after a 12 h fast. All experimental conditions were completed in a custom built environmental chamber (T.I.S. Services, Hampshire, UK) in 50% relative humidity (RH). The experimental conditions were: exercise in the heat (30°C, EX30), exercise in a thermoneutral environment (20°C, EX20), exercise in the cold (10°C, EX10), and resting control (20°C, CON) (Figure 1). Participants were instructed to refrain from consuming caffeine or alcohol and participating in strenuous physical activity for 24 hours prior to arrival for

experimental conditions. Participants completed a weighed food diary (Appendix H) for the 24 hours preceding the initial experimental condition and were asked to replicate their diet in the 24 h before all subsequent visits. Participants wore the same clothing (t-shirt and shorts) for all conditions.

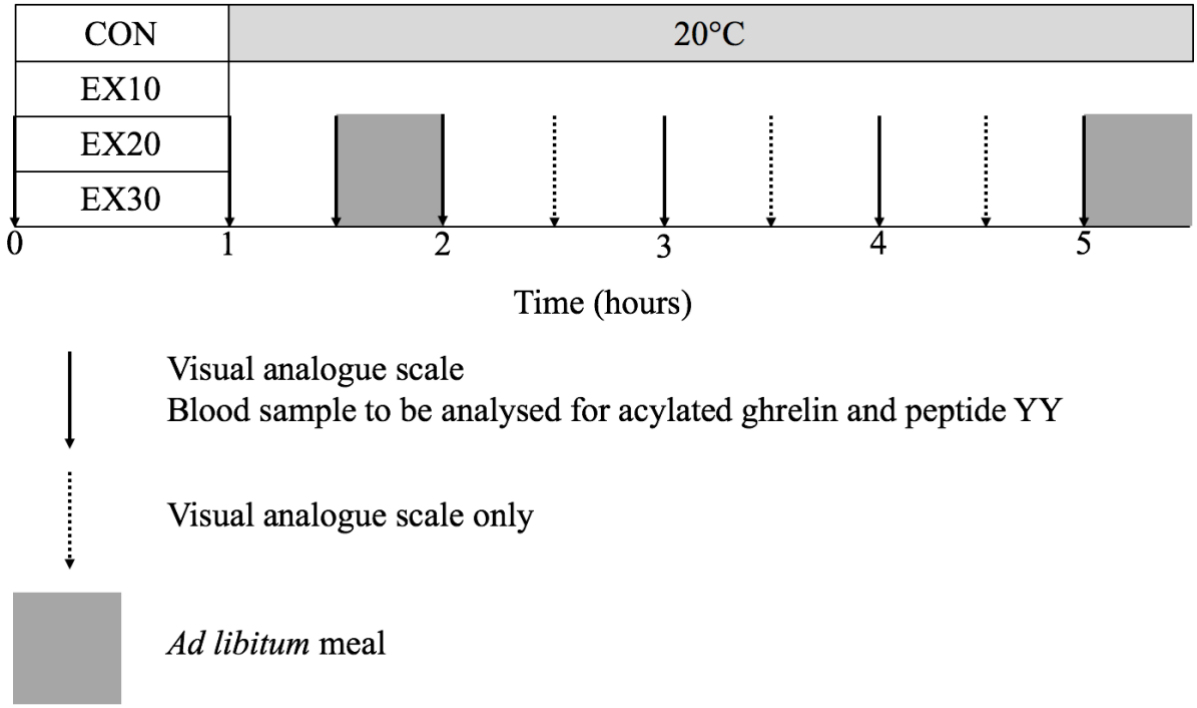

Figure 1: Schematic representation of the study design.

The participant's urine osmolality was measured (Osmocheck, Vitech Scientific, West Sussex, United Kingdom) on arrival to ensure a euhydrated state (< 700 mOsmol/kg H_2O) (ACSM, 1996) Once a euhydrated state was confirmed, participants inserted a rectal thermistor (400H, Yellow Springs Instruments, Yellow Springs, OH., USA) 10 cm past the anal sphincter for measurement of core temperature (T_{core}); HR was monitored during the insertion to ensure participants safety. Skin thermistors (Eltek Squirrel Data, Eltek, Cambridge, UK) were placed at four sites on the left of the body (tricep – T_{arm}, pectoralis major - T_{chest}, rectus femoris - T_{thigh}, gastrocnemius - T_{calf}) and secured with hypafix tape for measurement of skin temperature (T_{skin}). Weighted mean skin temperature (MST) was calculated using the following equation (Ramanathan, 1964):

$$\mathbf{MST = 0.3 \times (T_{arm} + T_{chest}) + 0.2 \times (T_{thigh} + T_{calf})}$$

Mean body temperature (MBT) was calculated at rest using the equation (Livingstone, 1968):

$$\mathbf{MBT = 0.65 \times T_{core} + 0.35 \times MST}$$

MBT was calculated during exercise using the equation:

$$\mathbf{MBT = 0.8 \times T_{core} + 0.2 \times MST}$$

The participants HR, thermal sensation, skin and core temperatures were measured every 10 minutes throughout each condition.

A cannula was inserted into an antecubital vein, blood samples of 10 ml were collected at 0, 1, 1.5, 2, 3, 4 and 5 hr into vacutainers pre-treated with Ethylene Di-amine Tetra Acetic acid (EDTA) (BD, Oxford, UK). Perceptions of hunger, satiation, fullness and prospective food consumption were assessed using 100 mm visual analogue scales (VAS) at 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 hr. These perceptions of appetite were also averaged to give an overall appetite rating after inverting the values for satiation and hunger (Stubbs *et al.*, 2000).

Following fasted measures (0 hr), participants entered the environmental chamber and completed one of four conditions. In the exercise conditions (EX10, EX20, EX30) participants completed a 60-minute run on a motorised treadmill (PPS55 Med-I, Woodway, Waukesha, WI., USA) at 60% $\dot{V}O_{2max}$; in the control condition (CON) participants were seated for 60 minutes. Energy expenditure and substrate oxidation were estimated using breath-by-breath online gas analysis (MetaLyzer 3B, Cortex, Leipzig, Germany) and indirect calorimetry (Frayn, 1983) at 5-10 minutes, 30-35 minutes and 55-60 minutes of each 60 min condition. During this initial hour, participants' HR, RPE, thermal sensation, skin and core temperatures were measured every five minutes. Resting measures were collected with participants in a seated position.

Ad libitum meals were provided at 1.5 and 5 hours. Food quantities were presented in excess of expected consumption and were of a precisely known quantity and macronutrient composition. Participants had 30 minutes to consume each meal. Meal 1 was a buffet-type breakfast meal consisting of two types of breakfast cereal, white and brown bread, jam, butter, apples, bananas, orange juice and semi-skimmed milk. Meal 2 consisted of 500g of pasta (uncooked) and tomato sauce (500g) cooked and prepared per manufacturer's specifications. Meals were presented in an identical manner for each condition. Energy intake was offset against EE to give a relative

energy intake (REI). Participants were able to consume water *ad libitum* throughout each condition; total water consumption was recorded.

Blood collection and analysis

Two 60 µL blood samples were collected from coated EDTA vacutainers into heparinised microhematocrit capillary tubes (Heinz Herenz, Hamburg, Germany) and spun in a micro centrifuge (Haematospin 1300, Hawksley, Staines, UK) for 2 minutes before haematocrit was determined (Reader, Hawksley, Staines, UK). One 10 µL sample was collected into a microcuvette (Hb 201, HemoCue, Ängelholm, Sweden) for determination of haemoglobin concentration (Hb 201 DM System, HemoCue, Ängelholm, Sweden).

Following determination of haematocrit and haemoglobin concentrations, one vacutainer was spun immediately at 1500 *g* for 10 minutes at 4°C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant was separated and placed into two cryovials and stored at -80°C until analysis of total PYY. To prevent degradation of acylated ghrelin, the remaining filled vacutainer was treated with a solution of 50% P-hydroxymercuribenzoic acid (PHMB), 49.4% potassium phosphate buffer (PBS) and 0.6% sodium hydroxide (NaOH) in a ratio of 10 µL per 1 ml of blood and spun at 1500 *g* for 10 minutes at 4°C. The plasma supernatant was separated and treated with 100 µL of hydrochloric acid (HCl) per 1 ml of plasma before being spun at 1500 *g* for 5 minutes at 4°C (Hosoda *et al.*, 2004). The plasma was then placed into two separate cryovials and stored at -80°C until analysis of plasma acylated ghrelin. Gut hormone concentrations were analysed using commercially available enzyme-linked immunosorbent assay (ELISA), samples from each participant were analysed in the same run to avoid intra-assay variation. Inter-assay variation for PYY was 6.1% - 6.9% (Human PYY (Total) ELISA, Merck, Darmstadt, Germany) and was 5.9% - 10.9% for acylated ghrelin (Acylated Ghrelin (human) Express ELISA kit, Bertin Pharma, Paris, France). Due to problems with blood sampling, acylated ghrelin and plasma YY concentrations are presented as n = 6. Specifically, participants felt nervous and anxious with regard to the insertion of the cannula, the decision was therefore taken in those instances not to continue with blood sampling in the consideration for the well-being of the participant and to avoid confounding other outcome measures. Intra-assay coefficient of variation was 6.62%

and 3.59% for PYY Plates 1 and 2 respectively, and 3.48% and 6.98% for acylated ghrelin plates 1 and 2 respectively.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL., United States). Data are presented as mean ± SD. Significance was accepted at an alpha level (type 1 error rate) of 0.05. Normality was assessed using a Shapiro-Wilk test. Environmental temperature was separated into hours 0-1 (exercise) and hours 1-5 (rest) for analysis. Two-way repeated measures analysis of variance (ANOVA) were used to detect significant effects of condition (4), time (11 for mean body temperature, 7 & 9 for environmental temperature, 2 for EI, 6 for gut hormones or 11 for perceived appetite) and condition × time interactions, with Greenhouse-Geisser correction applied where sphericity was violated. Area under the curve (AUC) was calculated for perceptions of hunger, satiation, fullness, PFC and overall appetite. Incremental area under the curve (iAUC) was calculated for plasma PYY and plasma acylated ghrelin. One-way analysis of variance was used to identify significant effects of condition on AUC and iAUC variables, $\dot{V}O_2$, % of $\dot{V}O_{2max}$, EE and water consumed. The location of main effects was followed up using Bonferroni correction. Where a significant effect of condition and a significant condition × time interaction was detected, paired-samples t-tests were used to identify differences between the individual conditions at each individual time point. Where a significant effect of time and a significant condition × time interaction was detected, paired-samples t-tests were used to identify specific changes over time within each individual condition. Pearson's correlation was used to examine correlations between gut hormones and overall appetite.

RESULTS

Environmental temperature

Environmental temperature during the exercise bout (0-1 h) in each trial differed significantly ($p < 0.05$) between CON (20.3 ± 0.4°C), EX10 (11.1 ± 1.8°C), EX20 (20.4 ± 1.0°C) and EX30 (29.2 ± 0.9°C). Post-hoc analyses using Bonferroni correction confirmed that environmental temperature was higher in EX30 compared with EX20 ($p < 0.001$), EX10 ($p < 0.001$) and CON ($p < 0.001$) and lower in EX10 compared with EX20 ($p < 0.001$) and CON ($p < 0.001$). Environmental temperature at rest during hours 1-5 did not differ significantly ($p > 0.065$) between the conditions (CON: 20.3 ± 0.4°C, EX10: 20.4 ± 0.4°C, EX20: 20.5 ± 0.6°C, EX30: 21.0 ± 0.4°C).

Exercise characteristics

The participants mean responses to exercise are presented in Table 5.

Table 5: Mean response to exercise in each condition.

	CON	EX10	EX20	EX30
$\dot{V}O_2$ (L.min^{-1})	0.28 ± 0.07 *	2.53 ± 0.39	2.41 ± 0.44	2.46 ± 0.45
% of $\dot{V}O_{2max}$	7 ± 2 *	63 ± 11	60 ± 11	61 ± 09
EE (kJ.hour^{-1})	356 ± 88 *	3427 ± 527	3381 ± 634	3259 ± 498
Water consumed (mL)	638 ± 308	824 ± 412	689 ± 488	855 ± 341

* = significantly lower than EX10, EX20 and EX30 ($p < 0.001$)

Thermoregulation

Participants' MBT was significantly affected by condition ($F_{3,18} = 15.133$, $p < 0.001$), time ($F_{10,60} = 33.866$, $p < 0.001$) and there was a condition × time interaction ($F_{30,180} = 14.048$, $p < 0.001$). Mean body temperature was significantly greater in EX30 (35.45 ± 0.11°C) than both CON (34.51 ± 0.19°C) ($p = 0.007$) and EX10 (34.92 ± 0.13°C) ($p = 0.01$). Participants' MBT was significantly greater in EX30 than CON ($p < 0.019$) from hours 0-2. MBT was also significantly greater in EX20 than CON ($p < 0.025$) between hours 0 and 1.5. MBT was greater in EX30 than EX20 ($p < 0.024$) in hours 0.5-1 and greater in EX30 than EX10 ($p < 0.035$) in hours 1-2.

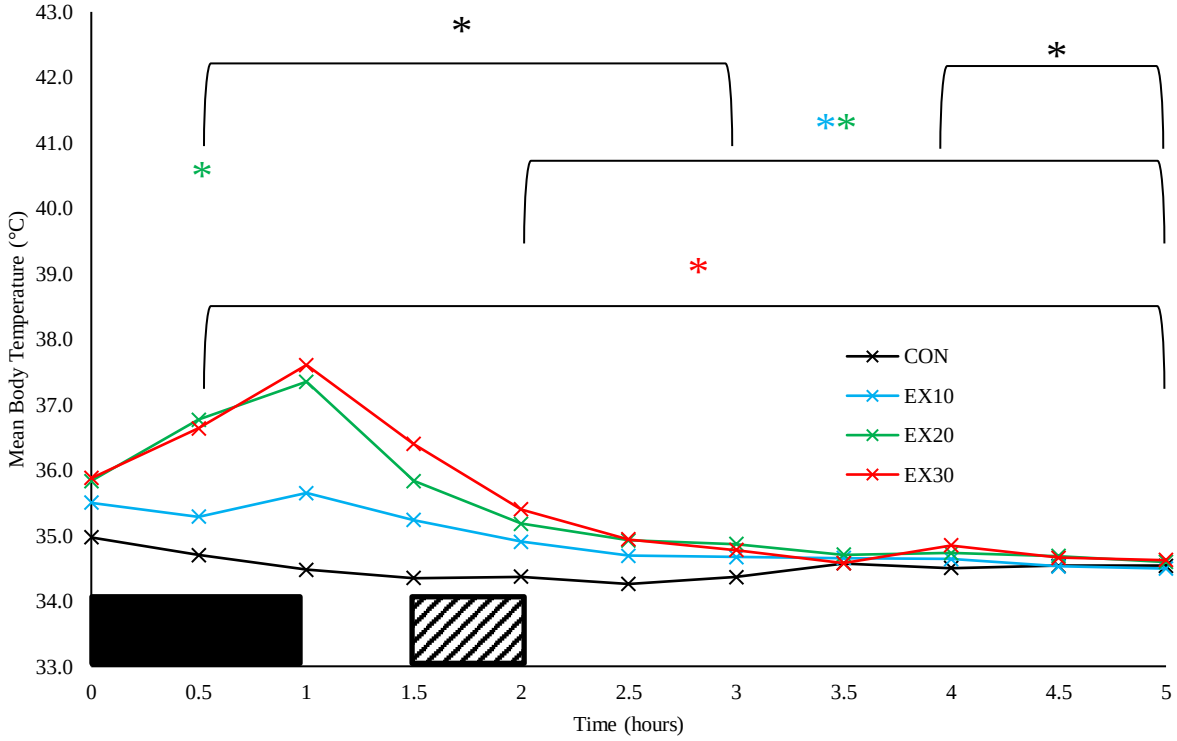

Figure 2: Mean Body Temperature measured over all conditions. Black box = exercise bout and hashed box = test meal. * = statistical significant difference from baseline over time in CON ($p < 0.033$). * = statistical significant difference from baseline over time in CON ($p < 0.004$). * = statistical significant difference from baseline over time in CON ($p < 0.043$). * = statistical significant difference from baseline over time in CON ($p < 0.007$).

Energy intake

There was no significant main effect for condition ($F_{3,21} = 1.734$, $p = 0.191$) on EI. There was a significant main effect for time ($F_{1,7} = 7.574$, $p = 0.028$), but no significant condition × time interaction ($F_{3,21} = 0.742$, $p = 0.539$). Energy intake was significantly lower ($p = 0.028$) in meal 1 (4368 ± 448 kJ) than in meal 2 (5347 ± 628 kJ). Relative energy intake was affected significantly by condition ($F_{3,21} = 14.462$, $p < 0.001$). Relative energy intake was significantly lower in EX10 (5795 ± 2289 kJ) ($p = 0.005$), EX20 (6635 ± 3138 kJ) ($p = 0.028$) and EX30 (6648 ± 3151 kJ) ($p = 0.027$) when compared with CON (9870 ± 3280 kJ). There were no significant differences in macronutrient selection between conditions in meal 1 ($p > 0.055$).

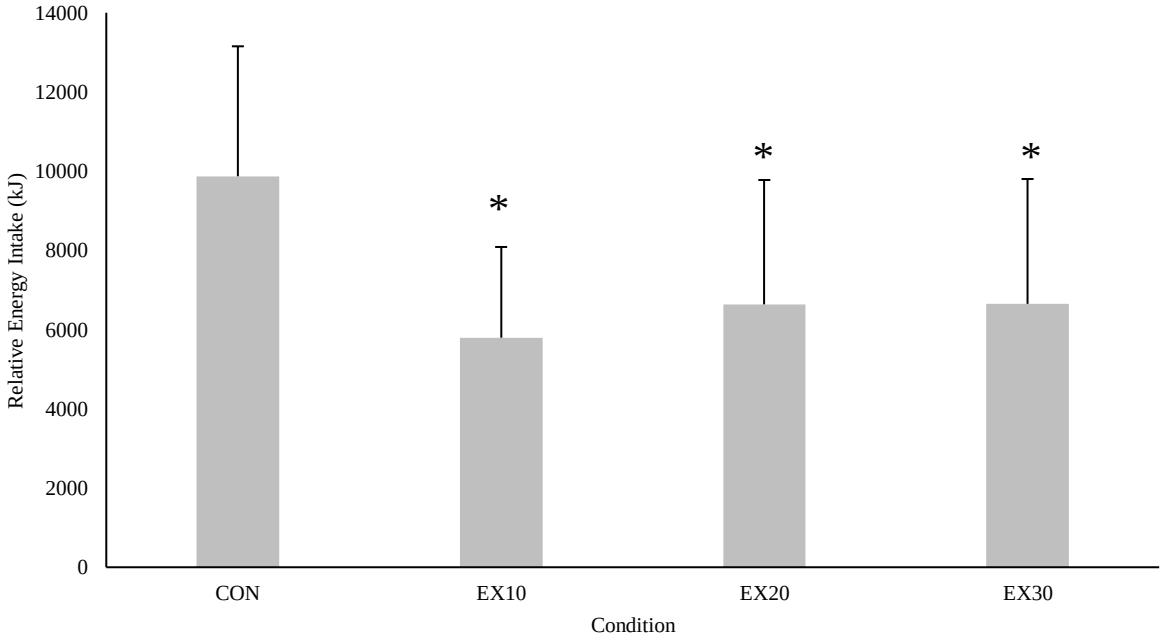

Figure 3: Relative Energy Intake between conditions for both meals. * = statistical significance from CON.

Table 6: Total energy intake, relative energy intake and percentage of total macronutrient intake of carbohydrate, fat and protein in meal 1 across 4 conditions.

	CON	EX10	EX20	EX30
Total energy intake (kJ)	10226 ± 3343	8950 ± 2293	9937 ± 3259	9753 ± 3347
Relative energy intake (kJ)	9870 ± 3280	5795 ± 2289	6635 ± 3138	6648 ± 3151
Carbohydrate intake (%)	72 ± 3	73 ± 3	75 ± 5	78 ± 6
Fat intake (%)	12 ± 3	15 ± 3	13 ± 5	11 ± 4
Protein intake (%)	16 ± 2	12 ± 3	12 ± 2	11 ± 3

Gut hormones

Peptide YY

There was no significant effect of condition on Δ PYY ($F_{3,15} = 1.064$, $p = 0.394$). There was a significant main effect for time in Δ PYY ($F_{5,25} = 3.903$, $p = 0.009$). There was no significant condition × time effect for Δ PYY ($F_{15,75} = 1.205$, $p = 0.287$). Post-hoc analysis did not identify any specific changes over time. There was no significant main effect of condition for PYY iAUC ($F_{3,15} = 1.065$, $p = 0.393$).

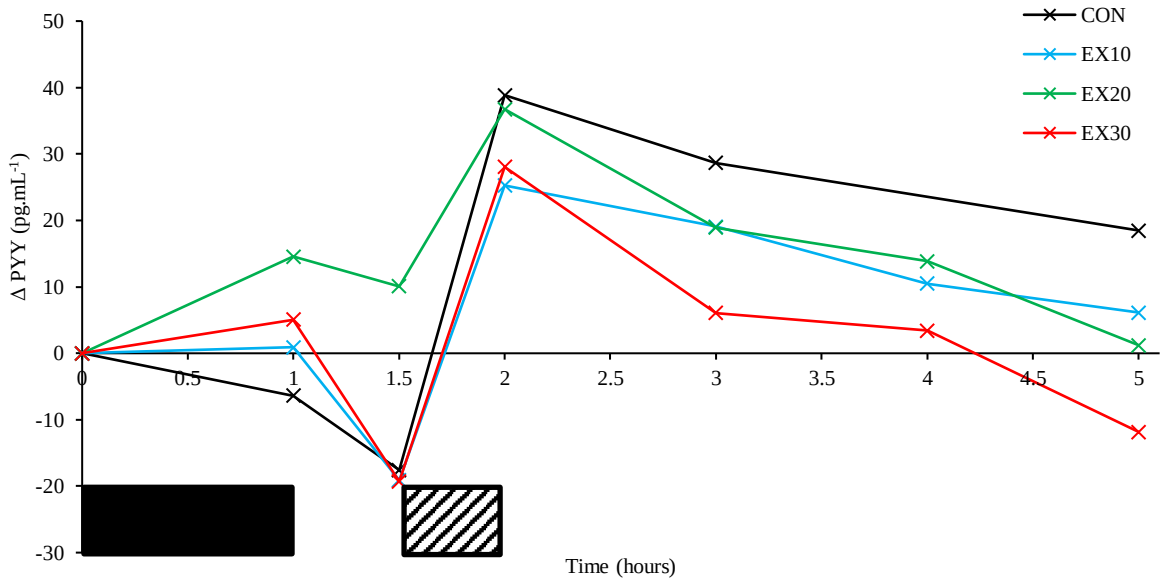

Figure 4: Δ PYY concentrations from baseline in all conditions. Values are mean ± SD. Black box = exercise bout and hashed box = test meal.

Acylated ghrelin

There was no significant effect of condition on Δ acylated ghrelin ($F_{3,15} = 1.179$, $p = 0.351$). There was a significant main effect for time ($F_{5,25} = 9.869$, $p < 0.001$) and a significant condition × time ($F_{15,75} = 2.104$, $p = 0.019$) effect for Δ acylated ghrelin. Differences in Δ acylated ghrelin over time from baseline for each individual condition were identified using paired-samples t-tests, and are shown in Figure 5 a-d. Specifically, although Δ acylated ghrelin changed over time from baseline during CON, EX10 and EX20, no significant differences were found for EX30. In CON, Δ acylated ghrelin was lower at 3 h compared to 0 h only, in EX10 and EX20 Δ acylated ghrelin was suppressed at hours 2 and 3 compared to baseline. There was no significant effect of condition for acylated ghrelin iAUC ($F_{3,15} = 0.731$, $p = 0.549$).

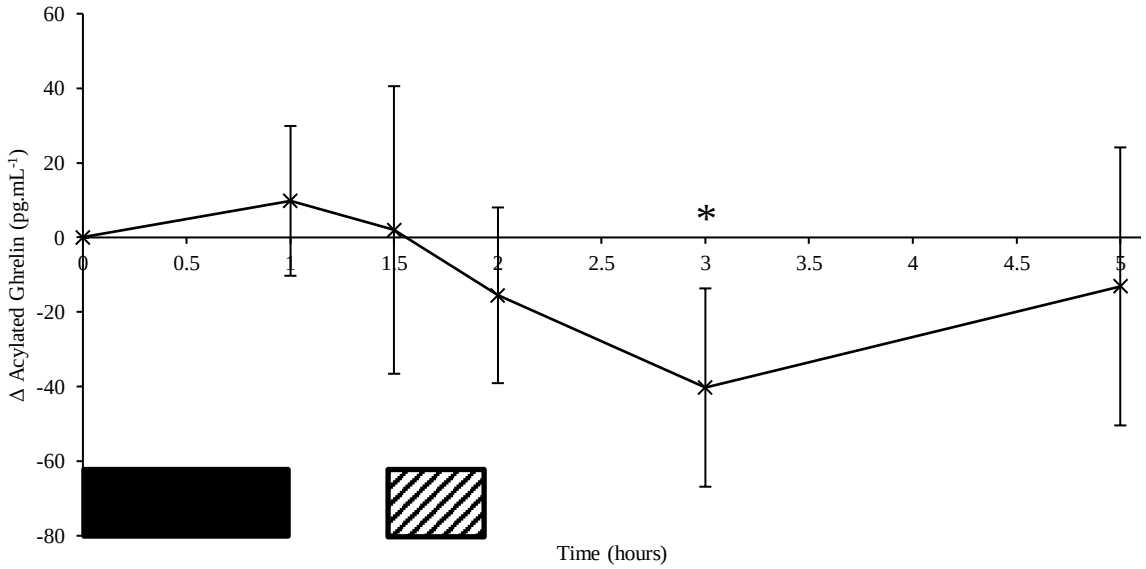

Figure 5a: Δ acylated ghrelin concentrations from baseline in CON**.** Black box = exercise bout and hashed box = test meal. * = statistically significant change from 0 ($p < 0.05$).

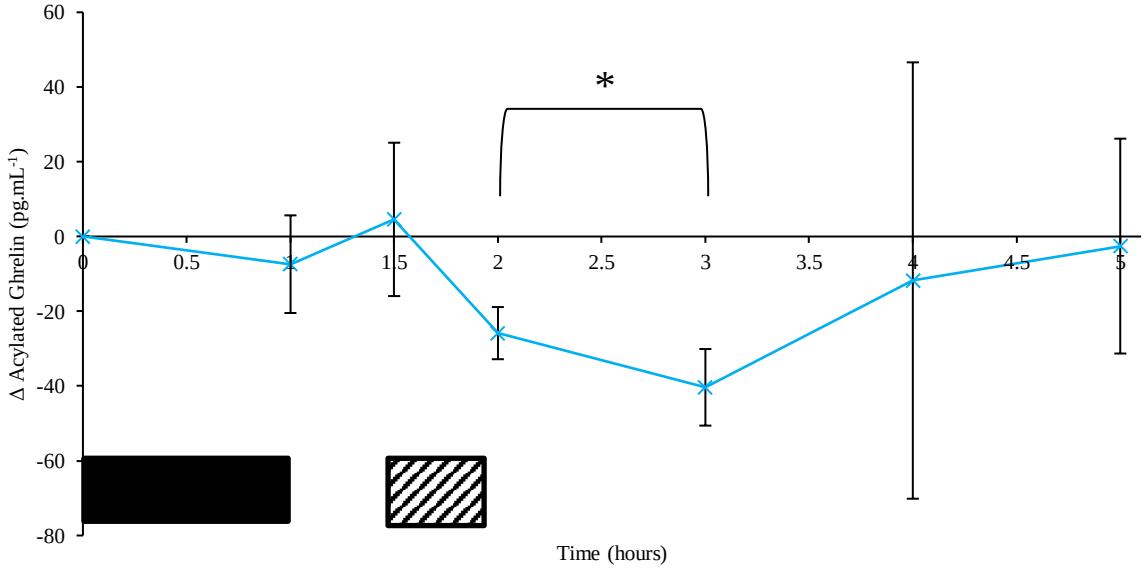

Figure 5b: Δ acylated ghrelin concentrations from baseline in EX10. Black box = exercise bout and hashed box = test meal. * = statistically significant change from 0 ($p < 0.05$).

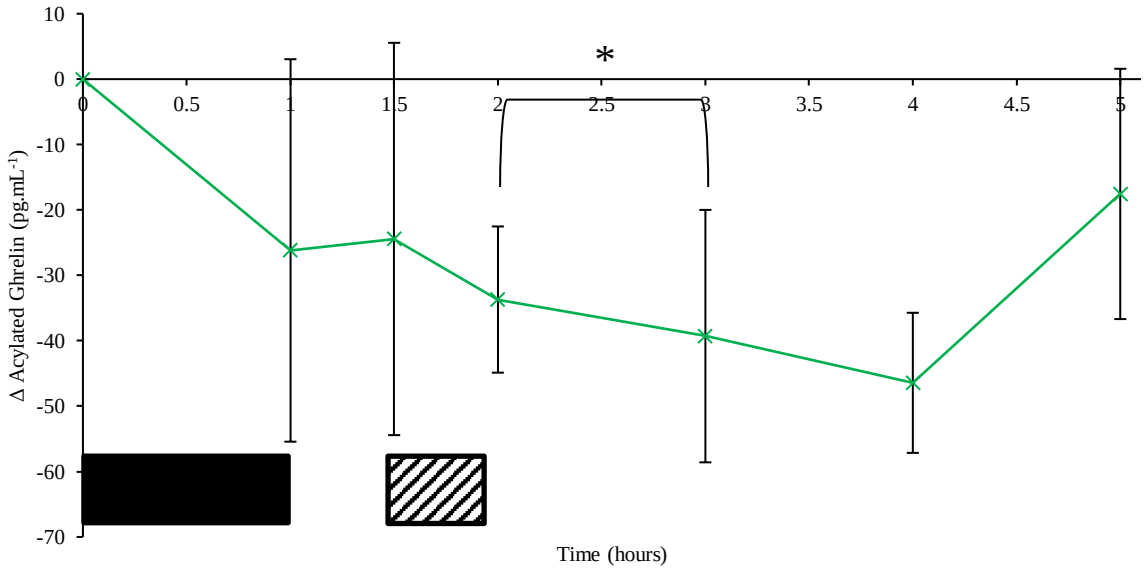

Figure 5c: Δ acylated ghrelin concentrations from baseline in EX20. Black box = exercise bout and hashed box = test meal. * = statistically significant change from 0 ($p < 0.05$).

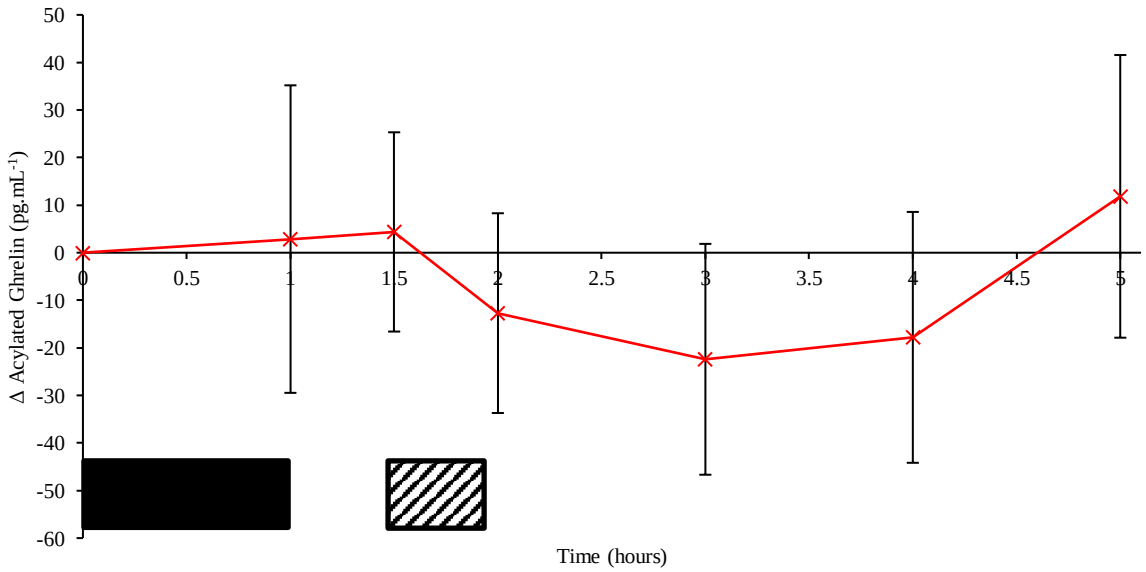

Figure 5d: Δ acylated ghrelin concentrations from baseline in EX30. Black box = exercise bout and hashed box = test meal.

Perceptions of appetite

There were no main effects of condition for hunger, satiation, fullness, PFC or overall appetite ($p > 0.254$). There was a significant effect of time on hunger, satiation, fullness, PFC and overall appetite ($p < 0.001$). There were no significant condition × time interactions in hunger, satiation, fullness, PFC or overall appetite ($p > 0.636$). Significant changes in overall appetite over time are detailed in Figure 9. Overall appetite increased significantly at hour 1.5 and decreased significantly at hour 2. Overall appetite remained suppressed significantly until hour 4. There were no

significant differences in AUC for hunger, satiation, fullness, PFC or overall appetite between conditions ($p > 0.281$).

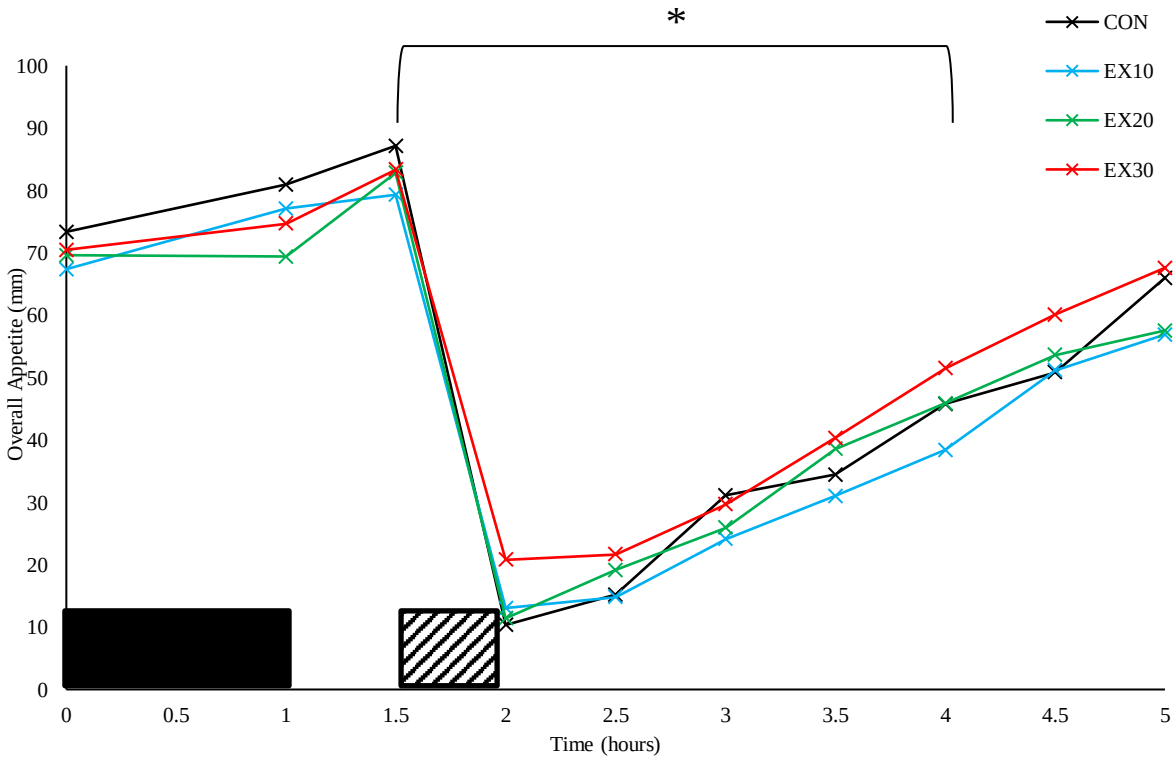

Figure 6: Overall appetite in all conditions. Black box = exercise bout and hashed box = test meal. * = significant difference for time from hour 0.

Correlations

There was a significant positive relationship between absolute PYY and overall appetite in EX20 at hours 3 ($r = 0.823$, $p = 0.044$) and 5 ($r = 0.897$, $p = 0.015$). There was also a significant positive relationship between absolute acylated ghrelin and overall appetite in EX20 at hour 1 ($r = 0.837$, $p = 0.038$) and a significant negative relationship between absolute acylated ghrelin and overall appetite in EX30 at hour 5 ($r = - 0.832$, $p = 0.4$).

DISCUSSION

This is the first study to compare exercise in the heat, cold and neutral conditions to a resting control. The aim was to investigate the effect of exercise in heat exposure and cold conditions on perceptions of appetite, gut hormones and energy intake. The main findings concur with the findings of previous research that exercise reduces REI. However, regarding the effects of environmental temperature, this study detected findings inconsistent with those of previous research. The addition of exercise and the manipulation of environmental temperature did not alter plasma PYY compared to rest. Plasma acylated ghrelin responded similarly in all conditions, however, the addition of heat produced a blunted response to exercise.

Energy intake

Total energy intake

This study did not detect any significant differences in total energy intake between conditions. This is in accordance with the finding of minimal meaningful changes in plasma acylated ghrelin, plasma PYY and feelings of appetite between the conditions. These findings differ from those of previous studies, which generally detected lower EI after exercise in the heat (Faure *et al.*, 2016, Wasse *et al.*, 2013, Shorten *et al.*, 2009) and greater EI after exercise in the cold (Crabtree & Blannin, 2015, Wasse *et al.*, 2013). The differences described may be due to differences in experimental temperature, exercise mode and intensity or participant characteristics. This study also detected a significant effect of time on EI, EI in meal 1 was significantly lower than EI in meal 2. This may reinforce the theory that exercise produces a transient suppression of appetite, although there were no differences when compared with the resting control condition. Other studies that did detect significant differences in EI offered a wider range of food for consumption at a buffet meal (Kojima *et al.*, 2016). This wider variety may have allowed participants to select different macronutrients in response to exercise in different environmental temperatures. However, a wider variety of available food may also have encouraged over-eating (Blundell *et al.*, 2010).

As there was no significant difference in EI between exercise and rest conditions in this study, it can be said that exercise had no significant effect on EI regardless of

environmental temperature. Studies by Kojima *et al.* (2016) and Ueda *et al.* (2009a) both detected significant decreases in EI after exercise compared to rest in thermoneutral conditions. Unlike the present study, Kojima *et al.* (2016) did detect a significant decrease in EI after aerobic exercise compared to a resting control, however the exercise protocol in that study (20 km outdoor run) was greater in time and distance than that used in the present study, proving a likely cause for the differences observed between the studies. Ueda *et al.* (2009a) detected significant decreases compared to a resting control following 30 minutes moderate (50% of $\dot{V}O_{2max}$) and high (75% of $\dot{V}O_{2max}$) intensity cycling exercise. However, Ueda *et al.* (2009a) also provided a test meal to participants prior to exercise, whereas participants in the present study exercised in a fasted state. Studies by Shorten *et al.* (2009) and Martins *et al.* (2007) however, detected increases in EI after exercise compared to control, which conflicts with the findings of Kojima *et al.* (2016) and Ueda *et al.* (2009a). The difference in the findings of Martins *et al.* (2007) and this study may be that EE in that study was not sufficient to supress appetite. Energy expenditure from a 1 hour treadmill run at 61% of $\dot{V}O_{2max}$ in this study was 3381 ± 634 kJ compared to 2059 ± 385 kJ from 1 hour cycling at 65% HR_{max} in the Martins *et al.* (2007) study. Shorten *et al.* (2009) also detected a significant increase in EI compared to a resting control after a 40-minute run at 72% of $\dot{V}O_{2max}$ expending 2375 ± 280 kJ. This raises the question of a possible EE, or exercise intensity threshold that must be achieved in order to supress appetite.

As with past research, this study did not find any difference in EI between exercise in the heat and exercise in a neutral temperature (Faure *et al.*, 2016, Wasse *et al.*, 2013, Shorten *et al.*, 2009). With regard to exercise in the cold, this study concurs with Wasse *et al.* (2013) in finding no significant difference in EI after exercise in the cold compared to exercise in a neutral environmental temperature. However, Crabtree & Blannin (2015) report a significant increase in EI after exercise in the cold compared to a neutral environmental temperature, although these differences may be explained by differences in population and study design. Firstly, Crabtree & Blannin (2015) studied an overweight population; as previously stated, overweight individuals have been shown to have higher circulating levels of plasma acylated ghrelin than their normal weight counterparts (Ueda *et al.*, 2009b). This may, in turn, increase appetite and EI.

Overall, the present study suggests that for exercise in the heat to have a significant effect on perceived appetite, the temperature may need to exceed 30°C and may need to be at least 36°C, as this temperature showed a significant decrease in perceived appetite from a neutral temperature in the study conducted by Kojima *et al.* (2015).

Relative energy intake

In agreement with previous research, REI was lower during the exercise conditions compared with the resting control in the present study (Kojima *et al.*, 2016, Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Martins *et al.*, 2007). However, the environmental temperature in which the exercise was performed did not influence REI. These findings replicate those other studies, reporting no difference in REI when comparing exercise in the heat, cold and thermoneutral conditions (Faure *et al.*, 2016, Crabtree & Blannin, 2015, Shorten *et al.*, 2009). These studies suggest that exercise suppresses REI regardless of environmental temperature. This reduction in REI is due to the EE from exercise, as EI was unaffected.

Perceptions of appetite

This study detected no significant differences between conditions, this is in contrast to previous research showing exercise produces a transient decrease in perceptions of appetite. The findings of this study contrast with findings by Wasse *et al.* (2013), detecting a trend for lower overall feelings of hunger post-exercise (hours 1 and 1.5) in the heat, cold and thermoneutral conditions compared to control, although this was not associated with a decrease in EI. There was no difference in the response of perceived appetite to exercise in the cold and the heat in this study. This lack of difference in overall appetite is reinforced by the lack of change in plasma PYY and acylated ghrelin, suggesting no physiological stimulation for a change in appetite.

The change in environmental temperature in this study caused a little to no change in perceived appetite, and did not have the suppressive effect seen in other research (Kojima *et al.*, 2015, Wasse *et al.*, 2013). This may be due to the differences in experimental environmental temperature, as the 'hot' temperature in this study was lower than that utilised by Kojima *et al.* (2015) (36°C).

It is possible that in the present study, exercise intensity was not great enough to stimulate a decrease in plasma acylated ghrelin or an increase in plasma PYY to

reduce appetite. Exercise intensities of 70% of $\dot{V}O_{2max}$ have been shown to stimulate an increase in plasma PYY (Broom *et al.*, 2009) and a decrease in plasma acylated ghrelin (Larson-Meyer *et al.*, 2012). However, exercise at 70% of $\dot{V}O_{2max}$ may not be feasible for the target population of overweight and obese individuals.

Gut hormones

Peptide YY

This study found no significant changes in PYY over time or between conditions, these findings agree with those of some previous research reporting no increase in plasma PYY in response to an acute bout of aerobic exercise in neutral environmental temperatures compared to a resting control (Kojima *et al.*, 2016, Douglas *et al.*, 2015, Larson-Meyer *et al.*, 2012, Shorten *et al.*, 2009). However, some studies have produced findings in conflict, reporting an increase in plasma PYY after exercise in a neutral temperature compared to a resting control (Broom *et al.*, 2009, Ueda *et al.*, 2009a, Martins *et al.*, 2007). Of those studies, the study with an exercise protocol most closely resembling that of the present study (60-minute treadmill run at 61% of $\dot{V}O_{2max}$) was conducted by Broom *et al.* (2009) (60-minute run at 70% of $\dot{V}O_{2max}$). the difference in plasma PYY observed between these studies is likely due to the lower intensity exercise in the present study. This suggests that there may be an exercise intensity threshold that must be reached in order to affect appetite through a suppression of plasma PYY. Ueda *et al.* (2009a) also detected a significant increase in plasma PYY after 30 minutes cycling at 50 or 75% of $\dot{V}O_{2max}$ compared to rest. As that study used two different exercise intensities, one lesser and one greater than that used in this study, the differences observed are not likely due to exercise intensity. It may be that the test meal provided to participants by Ueda *et al.* (2009a) is responsible for the differences observed, those participants would have their gastric emptying slowed by exercise, this greater stomach fill increases the stimulation of PYY secretion. This may also be the cause of the increase in plasma PYY observed by Martins *et al.* (2007) following a 60-minute cycle at 65% maxHR compared to rest.

Kojima *et al.* (2016) observed no significant changes in plasma PYY following a 20 km (78 minutes) run compared to a resting control condition. The differences observed between this study and that conducted by Kojima *et al.* (2016) may have

been due to the population, Kojima *et al.* (2016) used a sample of male endurance runners, training for 2.5 hours six times a week. As the effects of prolonged aerobic exercise on plasma PYY have not been investigated, it is possible that the high training workload had the effect of blunting these individuals' plasma PYY response. Plasma PYY did not increase significantly compared to a resting control in response to a 40-minute treadmill run at 70% of $\dot{V}O_{2max}$ in a study by Shorten *et al.* (2009). The exercise duration is the main difference between the present study and Shorten *et al.* (2009), this is a possible reason for the discrepancies observed between the two studies as shorter duration exercise has previously been shown to have a lesser effect of plasma PYY (Ueda *et al.*, 2009a, Martins *et al.*, 2007).

The addition of manipulation of environmental temperature to exercise also did not have an effect on plasma PYY. Exercise in the heat and the cold did not result in a change in plasma PYY concentrations compared to rest or exercise in a thermoneutral environment. These findings are the same as those of Crabtree & Blannin (2015) and Kojima *et al.* (2015) finding no difference in plasma PYY after exercise in the cold compared to a neutral temperature. Furthermore, Kojima *et al.* (2015) and Shorten *et al.* (2009) also did not detect a difference in plasma PYY after exercise in the heat compared to exercise in a neutral temperature.

Acylated ghrelin

This study also produced findings in conflict with those of other studies investigating the effects of exercise in different environmental temperatures on acylated ghrelin, detecting significant change in acylated ghrelin over time (Crabtree & Blannin, 2015, Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). In a neutral temperature, plasma acylated ghrelin trended to follow previous literature and was suppressed as a result of aerobic exercise (Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Broom *et al.*, 2009, Broom *et al.*, 2007). However, there were also several studies producing contradictory findings to the present study (Douglas *et al.*, 2015, Kojima *et al.*, 2015, Broom *et al.*, 2009, Shorten *et al.*, 2009). Of those studies, Douglas *et al.* (2015) did not measure plasma acylated ghrelin at similar time points to the present study and is therefore not directly applicable for comparison. Following a 40-minute run at 70% of $\dot{V}O_{2max}$, Shorten *et al.* (2009) did not detect any difference from baseline or from a resting control. It is possible that the exercise duration in this study was not great enough to promote an increase in plasma acylated ghrelin, as only exercise durations in excess of 40 minutes has been shown to increase plasma

acylated ghrelin (Kojima *et al.*, 2016, Larson-Meyer *et al.*, 2012, King *et al.*, 2010, Broom *et al.*, 2007).

Surprisingly, exercise in the heat and the cold environments did not have the expected effect on plasma acylated ghrelin. An increase in environmental temperature blunted the exercise related decrease of plasma acylated ghrelin after meal 1, where there was a significant decrease in plasma acylated ghrelin in all other trials. This suggests a reduction in appetite sensitivity after exercise in the heat. In the cold, plasma acylated ghrelin was decreased immediately after exercise, producing findings similar to those of Kojima *et al.* (2015), detecting a transient decrease, in both studies plasma acylated ghrelin levels rose to near-baseline 30 minutes after exercise. Wasse *et al.* (2013) also detected a decrease in plasma acylated ghrelin from baseline but no difference between cold and thermoneutral conditions after a 60-minute treadmill run at 65% of $\dot{V}O_{2max}$, although this decrease was also transient and plasma acylated ghrelin rose again 60 minutes after exercise. A study by Crabtree & Blannin (2015) reported findings in conflict with those of the present study with regard to the response of plasma acylated ghrelin to an aerobic exercise bout in the cold. Crabtree & Blannin (2015) detected a significant increase in plasma acylated ghrelin following a 45-minute treadmill walk at 60% of $\dot{V}O_{2max}$. Population differences are a possible cause of the difference between the studies, Crabtree & Blannin (2015) studied an overweight population versus a normal weight population in the present study. This difference was investigated by Ueda *et al.* (2009b), finding that acylated ghrelin was higher in overweight than normal weight individuals at rest and after exercise. This may partly explain the differences observed between these studies.

With regard to the effect of exercise in the heat on plasma acylated ghrelin, this study found differences from previous research (Kojima *et al.*, 2015, Wasse *et al.*, 2013, Shorten *et al.*, 2009). Plasma acylated ghrelin did not change significantly from baseline in this study following exercise in the heat. A study conducted by Kojima *et al.* (2015) detected a significant decrease from baseline in acylated ghrelin following exercise in the heat, Wasse *et al.* (2013) and Shorten *et al.* (2009) also detected a decrease after exercise in the heat, although not significant. It is possible that the greater temperature used in the heat condition in the Kojima *et al.* (2015) study (36°C) compared to the present study (29°C) is responsible for the discrepancies in the findings. Shorten *et al.* (2009) report findings dissimilar to the present study, detecting a suppression of plasma acylated ghrelin after exercise in the heat, though

this was not significant. This is likely due to the difference in environmental temperature, Shorten *et al.* (2009) used a temperature of 36°C to expose individuals to heat, compared to 29°C in the present study. Kojima *et al.* (2015) also detected a decrease in plasma acylated ghrelin post-exercise using a temperature of 36°C, suggesting the temperature used in the present study was not great enough to promote a suppression of plasma acylated ghrelin.

The findings of this study can be explained by the lack of change and difference in the gut hormones PYY and acylated ghrelin between conditions, this had the effect of not changing perceptions of appetite. As a result of this, there was no difference in the stimulus to consume food, causing there to be no difference in EI between conditions.

Limitations

There were several limitations to the present study. Firstly, the small sample size of eight young males may have been insufficient to detect significant differences in gut hormones. A post-hoc sample size calculation revealed that a sample size of 12 would have been needed to detect a significant difference in plasma acylated ghrelin iAUC and plasma PYY iAUC (G*Power, Heinrich Heine University, Düsseldorf, Germany).

Also, as this study used only male participants it is not possible to expand the findings to females, although the responses between males and females are suggested to be similar (Alajmi *et al.*, 2016). Furthermore, it is not known if children, adolescents or an older population would respond differently to the exercise and environmental interventions used in the present study.

Acylated ghrelin and PYY are not the only gut hormones thought to regulate appetite, it has been suggested that the gut hormones pancreatic polypeptide and GLP-1 suppress appetite (Schubert *et al.*, 2014). Had the present study measured those additional gut hormones, it may have been possible to gain a greater understanding of the effects of exercise and environmental temperature on appetite.

The relative discomfort of having a cannula and rectal thermistor inserted may have reduced participants desire to eat, reducing relative and total energy intake; this discomfort was reported anecdotally by several participants. Other participants

reported apprehension and anxiety regarding the insertion of the cannula. Although these effects would have occurred in each condition, it is possible that the effect would diminish in later visits as participants became accustomed to the discomfort and may override the possible intervention effects.

Implications

This study has reinforced the finding that exercise reduces REI. In addition, the finding of this study is that environmental temperature does not limit the REI reducing effect of exercise. This is an important finding for an overweight and obese population wishing to lose weight, as individuals can be reassured that REI will be suppressed regardless of environmental temperature, inducing an energy deficit, which will result in weight loss if chronically maintained.

Athletes and those involved in recreational physical activity should also be wary of the effects of exercise on appetite. Individuals should be aware that they will be at an energy deficit after exercise, and at risk of unwanted weight loss if a conscious effort is not made to account for the reduced EI.

Individuals entering extremely cold environments (mounting climbing, polar exploration) should also be aware of the reduction of REI due to not compensating for the increase in EE, resulting in a negative energy balance and weight loss. This is particularly important due to the risk to life in those extreme environments.

This study has provided the suggestion that exercise in the heat blunts the response of plasma acylated ghrelin to subsequent meals, reducing appetite sensitivity. This may be problematic to those seeking weight loss, as higher acylated ghrelin at the termination of a meal may increase EI in the next meal.

Directions for future research

The effect of repeated exercise sessions on appetite has not been widely investigated. This would be an important piece of research, both for athletes and the overweight and obese, as it would give an opportunity to produce recommendations on the long-term effects of recreational exercise or training with regard to changes in EI.

It may prove prudent to repeat the present study using an overweight or obese population, so as to assess the interventions' effectiveness in that target population. If an effect is shown in that population, a long-term study (> 3 months) may be conducted to assess its effectiveness as a weight loss protocol.

The heat condition in this study did not alter EI or promote a significant increase in PYY or decrease in acylated ghrelin using a temperature of 30°C. Other studies have detected changes in EI using a temperature of 36°C, suggesting a temperature threshold (Kojima *et al.*, 2015, Shorten *et al.*, 2009). A direct comparison of exercise in 30°C and 36°C would aid in identifying a temperature great enough to promote a decrease in appetite.

The exercise in the present study, 61% of $\dot{V}O_{2max}$, may also not have been of great enough intensity to produce changes in appetite, where other studies have detected reductions in appetite using 70% of $\dot{V}O_{2max}$ (Shorten *et al.*, 2009). This decrease from other research may be replicated if the present study were replicated using the intensity of 70% of $\dot{V}O_{2max}$.

There is currently very little literature on the differing effects of running and cycling on appetite. A single direct comparison suggests running and cycling have similar effects on appetite, however this requires further investigation (Wasse *et al.*, 2012). If the two modes prove equivalent in the reduction of appetite and energy intake, cycling may be a preferable option for the overweight and obese due to the reduced weight bearing nature and therefore reduced knee stress.

Overall, the results in this research area are conflicting, therefore the effects of environmental temperature manipulation and exercise on appetite requires further clarification in future research.

CONCLUSION

This study found that exercise reduced relative energy intake regardless of temperature, although this reduction did not correlate with either increases in plasma PYY or decreases in plasma acylated ghrelin. This decrease in relative energy intake was also not associated with any exercise related decrease in overall appetite. These findings are in conflict with those of other studies, which have found a decrease in energy intake after exercise in the heat compared with colder temperatures. Due to the limited evidence base, further research is required to understand the combined effects of exercise and environmental temperature on appetite and EI in a variety of populations.

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APPENDICES

A - Informed Consent Form

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

NAME: …… (Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.
- I will be required to attend 5 sessions in the laboratory (1 for preliminary measures and 4 for main trials) to complete the project.

As part of the study I will have to:

- Complete a maximal exercise test for the measurement of VO_{2max}
- Exercise in a hot (30 °C), cold (10°C), and thermoneutral (20°C) environments for 60 minutes
- Insert a rectal thermometer to measure core temperature
- Give blood samples to be analysed for a number of hormones
- Consume *ad libitum* meals

I am aware of any risks that may be involved with the project.

All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed …… (Participant) Date: ……

B - Physical Activity Readiness Questionnaire

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT _____ WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

C - Pre-Test Medical Questionnaire

PRE-TEST MEDICAL QUESTIONNAIRE

To be completed by all subjects before participating in practical sessions.

Name: …….
Age:…… Gender: M / F

1 Are you in good health? Yes / No
If no, please explain:

2 Are you pregnant or have you given birth in the last 6 months? Yes / No

3 How would you describe your present level of moderate activity?
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

4 Have you suffered from a serious illness or accident? Yes / No
If yes, please give particulars:

5 Are you recovering from an illness or operation? Yes / No
If yes, please give particulars:

6 Do you suffer, or have you ever suffered from:
Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No
Diabetes? Yes / No
Epilepsy? Yes / No
High blood pressure? Yes / No
Heart conditions or circulation problems:
(angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack, other)?
Do you have chest pains at any time? Yes / No
Do you suffer from fainting/blackouts/dizziness? Yes / No
Is there any history of heart disease in your family? Yes / No

7 Are you currently taking medication ? Yes / No
If yes, please give particulars:

8 Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? If yes, please give particulars: Yes / No

9 Have you had to consult your doctor, or had hospital treatment within the last six months? Yes / No

10 Have you, or are you presently taking part in any other laboratory experiment? Yes / No

11. Are you currently fitted with a pacemaker? Yes / No

12. Do you have any food allergies or intolerances? Yes / No

If yes, please state what this allergy or intolerance is……

PLEASE READ THE FOLLOWING CAREFULLY:

Persons will be considered ***unfit to do the experimental exercise task*** if they:

have a fever, suffer from fainting spells or dizziness;
have suspended training due to a joint or muscle injury;
have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
have anaphylactic shock symptoms to needles, probes or other medical-type equipment.
have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)
have a history of infectious diseases (e.g. HIV, Hepatitis B); and, if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

I have read the statement above and confirm that I am **not** considered ***unfit to do the experimental exercise task***:

Name of subject (please print) _____

Signature of Subject _____ Date: _____

Name of Experimenter (please print) _____

Signature of Experimenter _____ Date: _____

DECLARATION

I hereby volunteer to be a subject in experiments/investigations during the period of 20___**.**

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Name of subject (please print) _____

Signature of Subject _____ Date: _____

Name of Experimenter (please print) _____

Signature of Experimenter _____ Date: _____

D - Blood Screening Form

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?
b. Have you had jaundice within the previous year?
c. Have you ever had any form of hepatitis?
d. Have you any reason to think you are HIV positive?
e. Have you ever been involved in intravenous drug use?
f. Are you a haemophiliac?
g. Is there any other reason you are aware of why taking blood might be hazardous to your health?
h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response.

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

E - Breakfast Habits Questionnaire

PID: _____ ***Technician Initials:*** _____ ***Date:*** _____

Breakfast Habits Questionnaire

Please read every question carefully. Choose the box that fits your answer best and fill it in. This is not a test so there are no wrong answers. Also, nobody who knows you will look at your questionnaire once you have finished it.

1. **How often do you usually have breakfast?** Mark one box for weekdays and one box for weekend.

Weekdays (Mon-Fri)

- ☐ I never have breakfast on weekdays
- ☐ One day
- ☐ Two days
- ☐ Three days
- ☐ Four days
- ☐ Five days

Weekend (Sat-Sun)

- ☐ I never have breakfast on the weekend
- ☐ I usually have breakfast on only one day of the weekend (Saturday OR Sunday)
- ☐ I usually have breakfast on both weekend days (Saturday AND Sunday)

2. **What time do you normally have breakfast?**

Weekdays

☐☐:☐☐ AM / PM

Weekend

☐☐:☐☐ AM / PM

3. **Where do you normally have breakfast?** Mark one box for weekdays and one box for weekend.

Weekdays

- ☐ At home
- ☐ On way to work/school/University
- ☐ Other: _____

Weekend

- ☐ At home
- ☐ On way to work/ school/University
- ☐ Other: _____

4. **What do you normally eat and drink for breakfast?** Please provide details next to the relevant category.

Weekdays

☐ Ready-to-eat cereal

- Whole grain _____
- Refined grain_____

☐ Cooked cereal (e.g. porridge) _____

☐ Bread/Toast

- Type of bread: _____ Spreads _____
- Other details _____

☐ Meat/fish/eggs _____

☐ Other _____

☐ Drinks

- Hot drinks (e.g. tea, coffee)_____
- Cold drinks (e.g. pure fruit juice, milk) _____

Weekends

☐ Ready-to-eat cereal

- Whole grain _____
- Refined grain_____

☐ Cooked cereal (e.g. porridge) _____

☐ Bread/Toast

- Type of bread: _____ Spreads _____
- Other details _____

☐ Meat/fish/eggs _____

☐ Other _____

☐ Drinks

- Hot drinks (e.g. tea, coffee)_____
- Cold drinks (e.g. pure fruit juice, milk) _____

5. **If you ever skip breakfast, why do you skip breakfast?** Mark one box for weekdays and one box for weekend.

Weekdays	**Weekend**
☐ Not hungry (lack of appetite)	☐ Not hungry (lack of appetite)
☐ Feel nauseated/ weak/ tired	☐ Feel nauseated/ weak/ tired
☐ Do not like the food	☐ Do not like the food
☐ No motivation to prepare breakfast	☐ No motivation to prepare breakfast
☐ Lack of time	☐ Lack of time
☐ Would rather sleep	☐ Would rather sleep
☐ To help lose weight	☐ To help lose weight
☐ Other: _____	☐ Other: _____

6. **Do you smoke?**

☐ Yes ☐ No

7. **Have you smoked previously?** If 'yes', please insert approximate dates.

☐ Yes, from _____ to _____ ☐ No

Thank you

F - The Three-Factor Eating Questionnaire

The Three-Factor Eating Questionnaire

Please read each statement and select from the multiple choice options the answer that indicates the frequency with which you find yourself feeling or experiencing what is being described in the statements below.

1. When I smell a delicious food, I find it very difficult to keep from eating, even if I have just finished a meal.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

2. I deliberately take small helpings as a means of controlling my weight.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

3. When I feel anxious, I find myself eating.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

4. Sometimes when I start eating, I just can't seem to stop.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

5. Being with someone who is eating often makes me hungry enough to eat also.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

6. When I feel blue, I often overeat.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

7. When I see a real delicacy, I often get so hungry that I have to eat right away.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

8. I get so hungry that my stomach often seems like a bottomless pit.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

9. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

10. When I feel lonely, I console myself by eating.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

11. I consciously hold back at meals in order not to weight gain.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

12. I do not eat some foods because they make me fat.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

13. I am always hungry enough to eat at any time.
Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

14. How often do you feel hungry?
Only at meal times (1)/ sometimes between meals (2)/ often between meals (3)/almost always (4)

15. How frequently do you avoid "stocking up" on tempting foods?
Almost never (1)/ seldom (2)/ moderately likely (3)/ almost always (4)

16. How likely are you to consciously eat less than you want?
Unlikely (1)/ slightly likely (2)/ moderately likely (3)/ very likely (4)

17. Do you go on eating binges though you are not hungry?
Never (1)/ rarely (2)/ sometimes (3)/ at least once a week (4)

18. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself? 

G - Experimental Food Rating Scale

Please rate the following items honestly
Breakfast:

Kelloggs Bran Flakes
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Weetabix
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

White Bread
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Brown Bread
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Strawberry Jam
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Butter
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Banana
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Apple
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Semi-Skimmed Milk
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Orange Juice
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

Pasta Meal:

Pasta with Vegetable Tomato Sauce
(Dislike Extremely) 1 2 3 4 5 6 7 8 9 (Like Extremely)

H - Food Diary

FOOD DIARY INSTRUCTIONS

- **Everything** that you eat and drink in the 24 h before your first main trial should be **recorded** in this **diary** and **weighed using the digital scales provided**.

- You will be required to **replicate this diet in the 24 h before your remaining three main trials**, so it important that you only consume foods and drinks that you can replicate (for example, do not eat a home-cooked meal that someone else has cooked, if you will not be able to eat this again before your remaining trials).

- Please make sure you fill in all the columns for each food/drink item:

    1. **Date and time of day** – the date and time you had the food/drink (you only need to write the date at the beginning of each day).
    2. **Brand name** – for example, Kelloggs or Heinz.
    3. **Description** – as much detail as possible. Please tell us the manufacturer's name (e.g. Kelloggs, Heinz) and cooking method (e.g. grilled, roast, boiled).
    4. **Amount served** – This must be the exact weight served in grams; most snack foods will have the weight of the food on the packet so you can write this in your diary.
    5. **Leftovers** – This is the amount that you did not eat or drink in grams (e.g. apple cores, crusts of bread).

- This information is important for understanding our results from the study, so it is very important that you **avoid missing things out or making it up!** Thank you!

Date and time of day	Brand name (e.g. Heinz, Tesco, Kel-logs)	Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)	Amount served (grams)	Leftovers (grams)

I – Participant Information Sheet

INFORMATION SHEET
The effect of environmental temperature during exercise on appetite and appetite-regulatory hormones.

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer, we thank you for your participation. If you decide not to take part, there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?
The purpose of the study is to investigate whether exercise performed in different environmental temperatures affects energy intake and appetite-regulatory hormones.

What type of participant is needed?
Males aged between 18-35 years who are not heat acclimated are eligible to participate in the study. A health screen questionnaire must be completed by each individual who volunteers to participate in the study. Those with the following conditions may be excluded from the study for their own safety: musculoskeletal injury that has affected normal movement within the last month, disturbance of vision, congenital heart disease, uncontrolled exercise-induced asthma, diabetes, epilepsy and chronic obstructive pulmonary disease (COPD). As it is not feasible to list every medical condition, it is possible that those with other medical conditions, not given above, may be excluded from the study once identified.

What will participants be asked to do?
Participants will be required to attend the laboratory on five separate occasions. During the first visit preliminary measurements will be taken, including height, weight and Bodpod to measure body composition. This visit will also include a submaxial incremental exercise test and a maximal oxygen uptake (VO_{2max}) test where you will be asked to exercise to volitional exhaustion. You will also be familiarised with the environmental chamber and range of temperatures in which you will be asked to perform exercise during sessions 2-5 (i.e., 10°C, 20°C and 30°C). For sessions 2-5, you will be asked to complete the following in the fasted state:

- 60 minutes of treadmill exercise at 70% VO_{2max} in a thermoneutral (20°C) environment
- 60 minutes of treadmill exercise at 70% VO_{2max} in a cold (10°C) environment
- 60 minutes of treadmill exercise at 70% VO_{2max} in a hot (30°C) environment
- Rest for 60 min in a thermoneutral environment

After completing one of the 60 min treatments above, you will be asked to rest in a thermoneutral (20°C) environment for 4 hours. Participants will be provided with a two *ad libitum* meals – one at 30 min and the other at 4 h post-treatment. Throughout the trials, measures of thermal sensation, blood samples and subjective feelings of hunger and temperature will be taken. A rectal thermometer will be used for the measurement of core temperature. Please also note that you will be required to record your food and drink intake the day before visit 2 and replicate this the day before visits 3, 4 and 5. You will be asked to consume a high-carbohydrate meal (e.g., rice- or pasta-based) at approximately 20:00 h the evening before main trials, as you will be required to exercise for 60 minutes in the fasted state the next morning.

What are the possible risks of taking part in the study?
Participants will not be placed under any unnecessary physical or mental stress throughout the duration of the study. Potential risks of participating are as follows:
- Consent – Participants will be informed of the study and what they will be required do. A consent form will be completed before test measurements commence.
- Anonymity – The data collected would not in any way be linked to specific participants. Data collected will be either locked in a filing cabinet by a University member of staff or either in a password protected folder on a computer.
- BodPod – During visit 1. claustrophobia may occur when inside the Bodpod. However the participant will be made aware of the emergency release button.
- Blood sampling – Blood samples will be taken by a trained researcher. A certified first aider will be on-site whilst blood sampling occurs and all procedures will be given special care. Samples will be collected in a clean and sterile environment to avoid the chance of infection and all wounds will be treated until bleeding has stopped and then covered to reduce the risk of infection.
- Exposure to extreme heat or cold – Risks associated with extreme heat are hyperthermia – this may include muscle cramps, fast pulse and loss of consciousness. Risks associated with the cold are hypothermia – this may include shivering, pale skin and loss of consciousness. To prevent these symptoms occurring, core temperature will be monitored throughout all trials using a rectal thermometer. Please note that there are risks associated with of inserting anything into the body can cause shock (e.g., the rectal probe); specific safety precautions will be carried out in case of any medical emergencies to ensure the participant is safe (e.g., the door will remain unlocked in case medical attention is required if the participant suffers anaphylactic shock).
- Physical stress during exercise - Participants will be informed of the exercise protocol and all safety procedures will be explained before testing commences. A safety mat will always be present behind the treadmill and clear of any equipment, in order to minimise the risk of injury. For the main trials, you will be asked to exercise in the fasted state. It is possible that you may experience low blood glucose levels and feelings of dizziness or sickness, particularly if you are not used to completing fasted exercise. To minimise the risk of low blood glucose, we ask you to consume a high-carbohydrate meal the evening before the main trials. We can also provide you with food at the laboratories if needed. A first aider will be present at all times within the laboratories. A researcher will be present at all times during exercise to ensure participants are not in any discomfort. Exercise will be terminated if participants feel ill or are in discomfort or pain.

What if you decide you want to withdraw from the project?
If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?
Everyone that participates in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?
Questions are always welcome and you should feel free to ask myself, Matthew Horner (matthew.horner@study.beds.ac.uk), or the staff supervising the project (Dr Julia Fruer:

Julia.Fruer@beds.ac.uk; Dr. John Hough: John.Hough@beds.ac.uk) any questions at anytime.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.
This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

J – Ethics Documentation

UNIVERSITY OF BEDFORDSHIRE

Research Ethics Scrutiny

When completing this form please ensure that you read and comply with the following:

Researchers must demonstrate clear understanding of an engagement with the following:

1. *Integrity* - The research has been carried out in a rigorous and professional manner and due credit has been attributed to all parties involved.
2. *Plagiarism* - Proper acknowledgement has been given to the authorship of data and ideas.
3. *Conflicts of Interest* - All financial and professional conflicts of interest have been properly identified and declared.
4. *Data Handling* - The research draws upon effective record keeping, proper storage of date in line with confidentiality, statute and University policy.
5. *Ethical Procedures* - Proper consideration has been given to all ethical issues and appropriate approval sought and received from all relevant stakeholders. In addition the research should conform to professional codes of conduct where appropriate.
6. *Supervision* - Effective management and supervision of staff and student for whom the researcher(s) is/are responsible
7. *Health and Safety*- Proper training on health and safety issues has been received and completed by all involved parties. Health and safety issues have been identified and appropriate assessment and action have been undertaken.

The **Research Institutes** are responsible for ensuring that all researchers abide by the above. It is anticipated that ethical approval will be granted by each Research Institute. Each Research Institute will give guidance and approval on ethical procedures and ensure they conform to the requirements of relevant professional bodies. As such Research Institutes are required to provide the University Research Ethics Committee with details of their procedures for ensuring adherence to relevant ethical requirements. This applies to any research whether it be, or not, likely to raise ethical issues. Research proposals involving vulnerable groups; sensitive topics; groups requiring gatekeeper permission; deception or without full informed consent; use of personal/confidential information; subjects in stress, anxiety, humiliation or intrusive interventions must be referred to the University Research Ethics Committee.

Research projects involving participants in the NHS will be submitted through the NHS National Research Ethics Service (NRES). The University Research Ethics Committee will normally accept the judgement of NRES (it will never approve a proposal that has been rejected by NRES), however NRES approval will need to be verified before research can commence and the nature of the research will need to be verified.

Where work is conducted in collaboration with other institutions ethical approval by the University and the collaborating partner(s) will be required.

The **University Research Ethics Committee** is a sub-committee of the Academic Board and is chaired by a member of the Vice Chancellor's Executive Group, appointed by the Vice-Chancellor and includes members external to the University

Research Misconduct: Allegations of Research Misconduct against staff or post graduate (non-taught) research students should be made to the Head of the Research Graduate School

UNIVERSITY OF BEDFORDSHIRE

Research Bid: Ethical Issues (STAFF RESEARCH)

Section A must be completed by the proposer and the form should then be submitted with a copy of the research proposal to the Director of the Research Institute. To be completed prior to the onset of any research activity.

Proposer: Dr. Julia Fruer & Dr. John Hough
Research Institute: Institute for Sport and Physical Activity Research (ISPAR)

Proposal short title: The effect of environmental temperature on appetite regulation and energy intake during exercise in men

SECTION A To be completed by the candidate

The candidate is required to summarise in the box below the ethical issues involved in the research proposal and how they will be addressed. In any proposal involving human participants the following should be provided:

- clear explanation of how informed consent will be obtained,
- how will confidentiality and anonymity be observed,
- how will the nature of the research, its purpose and the means of dissemination of the outcomes be communicated to participants,
- how personal data will be stored and secured
- if participants are being placed under any form of stress (physical or mental) identify what steps are being taken to minimise risk

If protocols are being used that have already received UREC ethical approval then please specify. Roles of any collaborating institutions should be clearly identified. Reference should be made to the appropriate professional body code of practice.

Answer the following question by ringing/deleting **yes** or **no** as appropriate:

1. Does the study involve vulnerable participants or those unable to give informed consent (e.g. children, people with learning disabilities, your own students)?
   No

   If **YES**: Have/will researchers be DBS checked?
   Yes No

2. Will the study require permission of a gatekeeper for access to participants (e.g. schools, self-help groups, residential homes)?
   No

3. Will it be necessary for participants to be involved without consent (e.g. covert observation in non-public places)?
   No

4. Will the study involve sensitive topics (e.g. sexual activity, substance abuse)?
   No

5. Will blood or tissue samples be taken from participants?
   Yes

6. Will the research involve intrusive interventions (e.g. drugs, hypnosis, physical exercise)?
   Yes

7. Will financial or other inducements be offered to participants (except reasonable expenses)?
   No

8. Will the research investigate any aspect of illegal activity?
   No

9. Will participants be stressed beyond what is normal for them?
   Yes

10. Will the study involve participants from the NHS (e.g. patients) or participants who fall under the requirements of the Mental Capacity Act 2005?

    No

If you have answered yes to any of the above questions or if you consider that there are other significant ethical issues then details should be included in your summary above. If you have answered yes to Question 1 then a clear justification for the importance of the research must be provided.

*Please note if the answer to Question 10 is yes then the proposal should be submitted through **NHS research ethics approval procedures** to the appropriate **NRES**. The University Research Ethics Committee should be informed of the outcome

Background

Obesity prevention is highlighted as a national priority in Public Health England's 2015-16 Annual Plan. As the success of traditional weight management approaches has been variable, improved cost-effective approaches are required. This study focuses on the impact of altering environmental temperature during exercise.

A small number of studies suggest that acute exercise in the heat suppresses appetite and energy intake to a greater extent than exercise in the cold, but these findings are variable and the mechanisms involved are poorly understood (Wasse et al. 2013; Kojima et al. 2015). For example, exercise in cold has been shown to attenuate the exercise-induced reduction in hunger when compared with exercise in a hot and thermoneutral environment in healthy men (Kojima et al. 2015). Furthermore, energy intake (EI) was lower after exercise in a hot enviornment compared with thermoneutral environment (Wasse et al. 2013), and higher in cold compared with thermoneutral environment (Crabtree et al.; Wasse et al. 2013). However, others have not found a difference in EI after exercise in the heat when compared with a thermoneutral environment; rather, EI was greater after exercise at a neutral temperature, but not in the heat, when compared with a resting control (Shorten et al. 2009).

Research on the mechanisms involved in explaining changes in appetite after exposure to different environmental temperatures has produced conflicting findings. Although cold exposure while at rest has been shown to increase total ghrelin concentrations (Tomasik et al. 2005), exercise performed in hot or cold environments did not affect acylated ghrelin (Shorten et al. 2009; Wasse et al. 2013) or total ghrelin (Kojima et al. 2015). Similalrly, some report that exercise in the heat increased PYY (Shorten et al. 2009), while others have shown no effect of environmental temperature during exercise on PYY (Crabtree et al. 2015; Kojima et al. 2015). In adiditon to the unequivocal findings associated with this line of research, the study designs that have been employed have limations, including failing to include a direct measure of EI (Kojimia et al. 2015), to a resting control trial (Kojima et al. 2015; Wasse et al. 2013) and to measure the active form of ghrelin (Kojima et al. 2015).

The unique contribution of this proposal to determine the impact of exercise performed in a cold, thermoneutral and hot environment on appetite-regulating hormones (acylated ghrelin, PYY and Glucagon like peptide-1) and EI.

The specific aims are as follows:

- To examine the effect of exercise performed in different environmental temperatures on appetite and appetite-regulatory hormones.
- To examine the effect of exercise performed in different environmental temperatures on *ad libitum* energy intake.

Methods

Participants

Following ethical approval, 15 males aged 18-35 years will be recruited. Prior to data collection, all participants will be given an information sheet (see Appendix A) which will outline the requirements of the study. Any further information required from the participants will be provided before they sign an informed consent sheet (see Appendix B). A Physical Activity Readiness questionnaire (PAR–Q) and Pre-test Medical Questionnaire (see Appendix C), and a blood screening form (see Appendix D) will also be completed to ensure that no participants will complete this study with underlying health concerns or issues that would put the participant or the experimenter at risk (e.g. blood borne diseases). The participant will be made aware that they are able to withdraw from the study at any point and it will not impact them in a negative way.

Preliminary measurements

Prior to the experimental trials, anthropometric measures of stature (Stadiometer, Harpenden, HAR- 92.602, Holtain), body mass (Scales, Tanita, BWB0800, Allied Weighing, Conwy, UK) and percentage body fat (via BodPod measurement) will be measured. The use of the BodPod will adhere to a standard protocol, following guidance from Easton and Rilley (2002). To gain an understanding of habitual breakfast consumption, all participants will be asked to complete a brief questionnaire (Appendix E).

Participants will then be asked to complete a submaximal and maximal exercise test on a treadmill in temperate conditions. The submaximal test will consist of 4 x 4 min stages at intensities ranging between approximately 30-80% of maximal oxygen uptake ($\dot{V}O_{2max}$) at a gradient of 1% starting at 5 km/h increasing by 1.5 km/h every minute. A maximal exercise test will then be completed after a rest of 5-10 min. This protocol will start at a 1% incline and the gradient will be increased by 1% every minute until volitional exhaustion. The initial speed will be set at the speed corresponding to a heart rate (HR) of approximately 160 beats min^{-1} (measured during the submaximal exercise test). Expired gas will be analysed during the entire period of testing using a breath-by-breath cardiopulmonary exercise testing system (MetaLyzer 3B, Cortex, Leipzig, Germany) and values of oxygen (VO_2) consumption and carbon dioxide (VCO_2) production will be recorded. Heart rate will also be recorded continuously using short-range radio telemetry. Ratings of perceived exertion (RPE) will be assessed using the 6-20 Borg scale during the final minute of each stage. $\dot{V}O_{2max}$ will be calculated as the maximum $\dot{V}O_2$ value averaged over 60s during the test using a rolling average. Subsequently, treadmill speed eliciting 70% $\dot{V}O_{2max}$ will be estimated using the submaximal and maximal exercise test data.

Experimental Design
Using a repeated measures cross-over design, participants will complete four conditions in a counterbalanced order: 1) exercise in a thermoneutral (20˚C; EX-NEU) environment 2) exercise in the heat (30˚C; EX-HEAT), 3) exercise in the cold (10˚C; EX-COLD), and 4) resting control in a thermoneutral environment (CON). Each condition will be completed for 60 min in an environmental chamber and in the fasted state. The exercise will be a 60 min treadmill run at the speed elicitng 70% maximum oxygen uptake and at a gradient of 1%. Relative humidity will be 50% for all conditions. Upon leaving the chamber, participants will rest in a thermoneutral environment for 4 hours and measures of EI, perceived appetite, appetite-regulaitng hromones and substrate oxidation will be taken. The study design is outlined in Figure 1.

For all main trials, participants will attend the laboratories at ~08:30 after an overnight fast (no food or drink for at least 12 hours) and will be instructed not to consume any alcohol or caffeine and to have not taken part in any strenuous physical activity for the previous 24 hours. Participants will also be directed to complete a 24 h weighed food diary prior to their first main trial and replicate their dietary intake in the 24 h period before subsequent main trials (Appendix F).

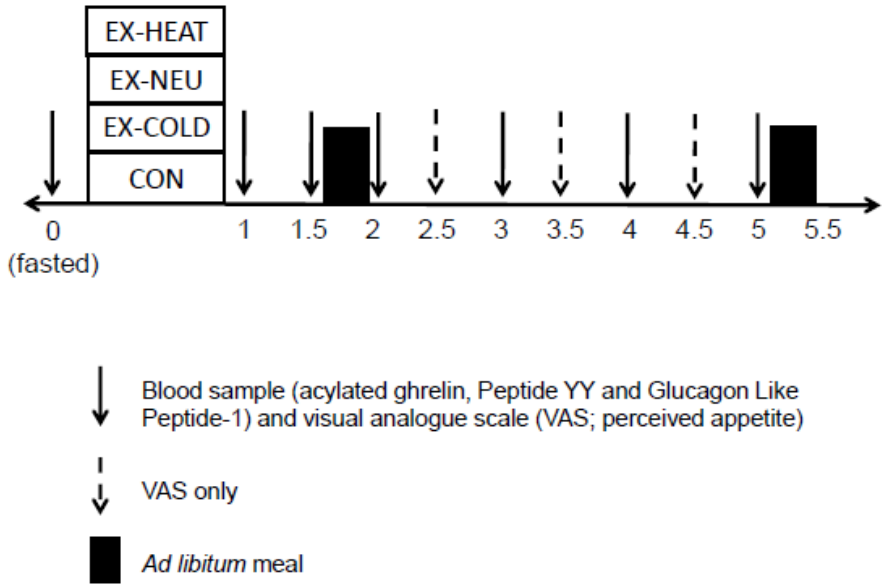

Figure 1. Study design with four conditions. EX-HEAT = exercise performed in the heat; EX-COLD = exercise performed in the cold; EX-NEU = exercise performed in a thermoneutral environment; CON = rest in a thermoneutral environment.

Energy expenditure and substrate oxidation
Energy expenditure and substrate oxidation will be estimated via online gas analysis (MetaLyzer 3B, Cortex, Leipzig, Germany) and indirect calormetry (Frayn, 1983) while in the environemntal chamber during the exercise and control trials at 0-10 min (with the first 5 min disgauarded from analysis), 30-35 min and 55-60 min and for the first 20 minutes after leaving the environmental chamber. For resting measurements, participants will lay supine on a bed and the first 5 min of the sample will be disguarded from analysis.

Blood sampling
On arrival to the laboratory, an intravenous cannula will be inserted into an antecubital vein by trained researcher (with an up to date hepatitis B vaccination). Blood samples will be collected at 0 (fasted), 1, 1.5, 2, 3, 4 and 5 h. All blood collections will be completed by a

trained research student or staff member. Blood samples will be collected into pre-chilled EDTA Vacutainers (BD, Oxford, UK). From each sample, 20-µL blood samples will be collected into two heparinised microhaematocrit tubes for determination of haematocrit and a 10 µL sample into a microcuvette for determination of haemoglobin concentration to assess changes in plasma volume (Dill & Costill, 1974). One Vacutainer will be immediately centrifued at 1500 × *g* for 10 min at 4°C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant will then be placed into separate cryovials and stored at −80°C until later analysis of total PYY. To prevent the degradation of acylated ghrelin a solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) (this will be 10µL per mL of blood) will be added to one EDTA vacuette. This vacuette will then be spun in a refrigerated centrifuge at 1500 × *g* for 10 min at 4°C. The plasma supernatant will then be placed into a storage tube and 100 µL of hydrochloric acid (HCL) per 1 mL of plasma will be added to preserve acylated ghrelin (Hosoda *et al.*, 2004). Thereafter, the sample will be spun at 1500 × *g* for 5 min at 4°C prior to storage at −80°C until later analysis of acylated ghrelin.

Plasma samples will be analysed for acylated ghrelin PYY and GLP-1 concentrations via commercially available enzyme linked immunosorbent assays (ELISA). To eliminate interassay variation, samples from each participant will be analysed in the same run. Haematocrit and haemoglobin concentrations will also be analysed.

Temperature and humidity
On arrival at the laboratory, participants will be fitted with a heart rate monitor (Polar, FS1, Kempele, Finland), and a rectal thermometer (Henleys, 400H,, Hertfordshire, UK) inserted 10 cm past the anal sphincter. Humidty and the environmental temperature will be recorded every 30 min. Core temperature and skin temperature will be measured every 5 min via a rectal thermometer and skin thermistors, respectively. Clothing will be standardised with participants asked to wear the same clothing for all of the experimental trials.

Hydration
All participants will be asked to consume 500 ml (this is ~ 5-7 mL/kg body mass) of water 2 h before arrival at the laboratory to promote a euhydrated state (Sawka *et al.*, 2007) Hydration status will be measured on arrival before the start of the trial via urine osmolarity to confirm a euhydrated state (< 600 mOsmol/kg). During the trial participants will be allowed to consume water *ad libitum* for each condition. Fluid consumed throughout each condition will be recorded. Body mass will be measured in the fasted state and post exercise (in order to assess the fluid lost during exercise) and at the end of the trial.

Perceived appetite
Perceptions of hunger, satisfaction, fullness, prospective food consumption and breakfast palatability will be assessed using 100-mm visual analogue scales (VAS) every 30 min from baseline.

Ad libitum energy intake
Ad libitum meals will be provided at 1.5 h and at 5 h for the quantification of EI. The participants will have 30 minutes to consume the *ad libitum* meals. The foods provided for both meals will be in excess of expected consumption and of precisely known quantity and nutrient composition. At 1.5 h, participants will be provided with an *ad libitum* buffet-type breakfast meal where participants will be presented with an array of breakfast foods for 30 min during which time they will be instructed to eat until satiety is reached. The breakfast will consist of foods and drinks considered regular choices for breakfast are of varied macronutrient composition (i.e., breakfast cereals, milk, bread, croissants, spreads, yogurt, fruit, juice, cold meats, and cheese). An *ad libitum* pasta meal will be provided at 5 h. This will consist of pasta (uncooked 500 g) and tomato sauce (500 g) (total energy content: 1990 kcal) cooked and prepared according to standardised manufacturer's preparation guidelines.

The meals will be presented in the same way for each trial and each participant. For example, the pasta will be presented in a standardised large bowl and with a standardised spoon. A standardised plate and cuttlerly (fork, spoon and knife) whereby the participants can scoop the amount desired onto the plate will also be provided.

The food and drink items in the *ad libitum* meals will be weighed before and after in order to measure EI using the same set of kitchen scales (Salter,HoMedics Group Ltd, UK). Total kilojoules, along with the quantity of consumed carbohydrate, protein, and fat and the percentage of energy intake from solid foods and drinks will be recorded. In addition, relative energy intake (REI) will be calculated by correcting postexercise energy intake for the energy cost of the exercise session above the resting energy expenditure.

References

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Risks and how they will be minimised/avoided

1*. Informed consent* - All participants will be provided with an information sheet (Appendix A) detailing the nature and purpose of the study. A consent form (Appendix B) will be read and signed by participants before any testing commences.

2. Confidentiality - All data and information collected regarding the participants will be securely stored on a password protected computer and hard copies will be stored in a locket filing cabinet at the University of Bedfordshire. If publication of the study is achieved the participants will be informed and all data will be anonymous and not linked to individual participants.

3. *Anonymity* - All results and information collected will be securely stored at the University of Bedfordshire. Only relevant staff members will have access. As previously mentioned if this research is published all data will be anonymous. All participants will be identified using unique codes, rather than using names. The data collected will be linked to specific participants, however only senior members of the reserach team will have access.

4*. Rectal thermometer* – This study requires core temperature to be measured via a disposable rectal thermometer that will be placed 10cm past the anal sphincter. The thermometer will be sterile and the participants will insert the thermometer themselves in a private area. Instructions will be provided verbally on how to do this. The participant will always be accompanied by an experimenter during this procedure. The experimenter will

remain outside the door of the area being used by the participant. The participant will be wearing a HR monitor that will be conitinually monitored by the experimenter. This will allow the experimenter to monitor for an incident of a raised heart rate caused by increase stress in the participant or anaphalytic shock (an extremely low probability to occur). The door will remain unlocked in case medical attention is required if the participant suffers anaphylactic shock.

5. Exposure to extreme heat or cold – Core temperature will be monitored throughout all trials using a rectal thermometer to prevent this reaching dangerous levels (2˚C elevation above baseline/and elevation above 39.7˚C. There are risks associated with of inserting anything into the body can cause shock (e.g., the rectal probe); specific safety precautions are carried out in case of any medical emergencies to ensure the participant is safe. These include wearing a HR monitor in order for the researcher to identify if anaphlactoid shock occurs if HR drops dramatically. Furthermore, the insertion will occur in a private room in which the door will not be locked in case the researcher needs to enter should complications occur. If anaphylactoid shock does occur the participant will be placed in the supine position with their feet elevated to regain lucidity. Core temperature will be recorded every 5 minutes but will be monitored more regularly throughout the protocol in case a dramatic rise occurs.

6*. Physical stress during exercise* - Participants will be informed of the exercise protocol and all safety procedures will be explained before testing commences. A safety mat will always be present behind the treadmill and clear of any equipment, in order to minimise the risk of injury. A first aider will be present at all times within the laboratories so that if an incident occurs, first aid will be immediately provided. A researcher will be present at all times during exercise to ensure participants are not in any discomfort. Exercise will be terminated if participants feel ill or are in discomfort or pain.

7. *VO_{2max}* - The study requires a measurement of VO_{2max} where participants are required to run to volitional exhaustion. Risks involved may be vomiting and feeling faint. The participant will be monitored carefully throughout the maximal test and can stop at any point. Those with the following conditions will be excluded from the study for their own safety: musculoskeletal injury that has affected normal movement within the last month, disturbance of vision, congenital heart disease, uncontrolled exercise-induced asthma, diabetes, epilepsy and chronic obstructive pulmonary disease (COPD). As it is not feasible to list every medical condition, it is possible that those with other medical conditions may be excluded from the study once identified. Participants will be informed of the exercise protocol and all safety procedures will be explained before testing commences. A first aider will be present at all times within the laboratories so that if an incident occurs, first aid will be immediately provided. At least two researchers will be present at all times during exercise to ensure participants are not in any discomfort. Exercise will be terminated if participants feel ill or are in discomfort or pain and will be monitored.

8. *Use of treadmill* – Participants will be made aware that appropriate footwear must be worn to avoid trips and slips. The treadmill will change between different speeds during the VO_{2max} therefore the participants will be counted down (3, 2, and 1) to avoid unexpected changes. When demounting from the treadmill it will be made certain that the belt has fully stopped. In case of a fall a blue padded mat will be positioned behind the treadmill to minimise injury. The participants will be made aware that they can withdraw at any point.

9. *Blood sampling* - Venous blood sampling from the antecubital vein will be carried out in accordance with the BASES blood sampling safety procedures. This will ensure the safety of both participants and the research team. Laboratory coats, goggles and disposable gloves will be worn every time blood samples are being handled. The person collecting the blood will be trained and have an up-to-date Hepatitus B vaccination. Particular attention will be paid to cover any cuts on the hands using waterproof dressings. Likewise, cannulas used

during the cannulation method will not be re-used, but immediately put into a sharps bin. If containers or vials become externally contaminated, they will be cleaned immediate

All participants will be pre-screened with the use of a blood screening form (see appendix D) prior to blood sampling to clear any risk of blood borne viruses, which could put the researchers at risk. If the participant answers 'yes' to any of the questions, the participant will not be eligible for the blood sample procedure and will be excluded from the study.

10. Blood analysis - The bloods will need to be analysed, risks involved cross - contamination. There may be chemical spills when using the ELISA. Therefore the experimenters will be trained and any spills will be cleaned immediately following the appropriate standard operating procedures (SOPs) and risk assessments (RAs).

11. *Disinfecting equipment* - Researchers will use disposable gloves when disinfecting equipment. The cortex mask and turbine will be cleaned by immersing the Turbine in Milton solution. Leaving it for 10 minutes. Then rinsing the Turbine in tap water in a beaker. DO NOT HOLD UNDER A RUNNING TAP as this will damage the delicate turbine. Change the water in the beaker, and repeat. Finally, rinse the turbine in a beaker of distilled water. Dry as much as possible by waving in air (do not use mechanical driers or towels or tissues). Dip the Housing in Milton solution, then rinse under a running tap and dry. Do not clean the Electronic assembly. This must not get wet. Re-assemble as shown in diagram. Rinse the mask in warm water, then immerse in Milton for 10 minutes. Rinse thoroughly (Under the tap). Dry with paper towels.

12. *Allergies to test meals* - To reduce the risk of participants having any kind of reaction to the meals provided, a health screen questionnaire (Appendix E) will be completed by the participants before beginning of the study. This will identify any allergies the participants may have to the meals, e.g. lactose or gluten intolerance. If any participant's allergies to any of the foods provided in the test meals, they will not be able to participate in the study.

13. *Bodpod* - Claustrophobia could occur when inside the Bodpod. However the participant will be made aware of the emergency release button and told that they can leave the Bodpod at any time if they feel uncomfortable. Participants will also be provided with a robe to minimsie possible feelings of embarrassment that are associated with wearing minimal clothing.

Proposer declaration

I understand that I cannot collect any data until the application referred to in this form has been approved by all relevant parties. I agree to carry out the research in the manner specified. If I make any changes to the approved method I will seek further ethical approval for any changes.

Signature of Proposer: **Dr. Julia Fruer** Date: 18/10/2016

This form together with a copy of the research proposal should be submitted to the Research Institute Director for consideration by the Research Institute Ethics Committee/Panel

Note you cannot commence collection of research data until this form has been approved

SECTION B To be completed by the Research Institute Ethics Committee:

Comments:

Reviewer:

Recommendation of Reviewer:

Signature of Reviewer: Date:

Signature Chair of Research Institute Ethics Committee:

Dr Laura Charalambous Date:

Dr Laura Charalambous Date:

K – Statistical Output

Relative Energy Intake

Descriptive Statistics

	Mean	Std. Deviation	N
REI_CON	2359.125	784.3845	8
REI_COLD	1385.250	547.3716	8
REI_NEU	1586.375	750.3936	8
REI_HOT	1589.500	753.3091	8

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
condition	.326	6.409	5	.274	.692	.990	.333

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept
Within Subjects Design: condition

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
condition	Sphericity Assumed	4440153.62	3	1480051.21	14.462	.000
	Greenhouse-Geisser	4440153.62	2.077	2137357.87	14.462	.000
	Huynh-Feldt	4440153.62	2.970	1495092.15	14.462	.000
	Lower-bound	4440153.62	1.000	4440153.62	14.462	.007
Error(condition)	Sphericity Assumed	2149089.37	21	102337.589		
	Greenhouse-Geisser	2149089.37	14.542	147786.813		
	Huynh-Feldt	2149089.37	20.789	103377.590		
	Lower-bound	2149089.37	7.000	307012.768		

Pairwise Comparisons

Measure: MEASURE_1

(I) condition	(J) condition	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	973.875*	174.293	.005	340.178	1607.572
	3	772.750*	189.809	.028	82.640	1462.860
	4	769.625*	187.720	.027	87.111	1452.139
2	1	-973.875*	174.293	.005	-1607.572	-340.178
	3	-201.125	155.186	1.000	-765.352	363.102
	4	-204.250	152.346	1.000	-758.151	349.651
3	1	-772.750*	189.809	.028	-1462.860	-82.640
	2	201.125	155.186	1.000	-363.102	765.352
	4	-3.125	67.600	1.000	-248.907	242.657
4	1	-769.625*	187.720	.027	-1452.139	-87.111
	2	204.250	152.346	1.000	-349.651	758.151
	3	3.125	67.600	1.000	-242.657	248.907

Based on estimated marginal means

*. The mean difference is significant at the

b. Adjustment for multiple comparisons: Bonferroni.

PYY

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
TIME	.001	21.651	14	.168	.447	.825	.200
CONDITION	.345	3.961	5	.567	.622	.979	.333
TIME * CONDITION	.000	.	119	.	.165	.340	.067

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept
Within Subjects Design: TIME + CONDITION + TIME * CONDITION

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	Sphericity Assumed	28158.116	5	5631.623	3.903	.009
	Greenhouse-Geisser	28158.116	2.234	12604.140	3.903	.048
	Huynh-Feldt	28158.116	4.123	6829.427	3.903	.016
	Lower-bound	28158.116	1.000	28158.116	3.903	.105
Error(TIME)	Sphericity Assumed	36071.199	25	1442.848		
	Greenhouse-Geisser	36071.199	11.170	3229.239		
	Huynh-Feldt	36071.199	20.615	1749.731		
	Lower-bound	36071.199	5.000	7214.240		
CONDITION	Sphericity Assumed	3143.272	3	1047.757	1.064	.394
	Greenhouse-Geisser	3143.272	1.867	1683.303	1.064	.379
	Huynh-Feldt	3143.272	2.938	1069.852	1.064	.393
	Lower-bound	3143.272	1.000	3143.272	1.064	.350
Error (CONDITION)	Sphericity Assumed	14773.954	15	984.930		
	Greenhouse-Geisser	14773.954	9.337	1582.367		
	Huynh-Feldt	14773.954	14.690	1005.700		
	Lower-bound	14773.954	5.000	2954.791		
TIME * CONDITION	Sphericity Assumed	7102.651	15	473.510	1.205	.287
	Greenhouse-Geisser	7102.651	2.478	2866.732	1.205	.342
	Huynh-Feldt	7102.651	5.101	1392.514	1.205	.335
	Lower-bound	7102.651	1.000	7102.651	1.205	.322
Error (TIME*CONDITION)	Sphericity Assumed	29475.564	75	393.008		
	Greenhouse-Geisser	29475.564	12.388	2379.352		
	Huynh-Feldt	29475.564	25.503	1155.769		
	Lower-bound	29475.564	5.000	5895.113		

Acylated Ghrelin

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.002	18.894	14	.285	.531	1.000	.200
condition	.223	5.584	5	.363	.538	.757	.333
time * condition	.000	.	119	.	.205	.567	.067

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept
Within Subjects Design: time + condition + time * condition

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	22912.448	5	4582.490	9.869	.000
	Greenhouse-Geisser	22912.448	2.656	8625.546	9.869	.001
	Huynh-Feldt	22912.448	5.000	4582.490	9.869	.000
	Lower-bound	22912.448	1.000	22912.448	9.869	.026
Error(time)	Sphericity Assumed	11607.841	25	464.314		
	Greenhouse-Geisser	11607.841	13.282	873.970		
	Huynh-Feldt	11607.841	25.000	464.314		
	Lower-bound	11607.841	5.000	2321.568		
condition	Sphericity Assumed	5819.060	3	1939.687	1.179	.351
	Greenhouse-Geisser	5819.060	1.615	3604.189	1.179	.343
	Huynh-Feldt	5819.060	2.271	2562.750	1.179	.349
	Lower-bound	5819.060	1.000	5819.060	1.179	.327
Error(condition)	Sphericity Assumed	24683.468	15	1645.565		
	Greenhouse-Geisser	24683.468	8.073	3057.672		
	Huynh-Feldt	24683.468	11.353	2174.150		
	Lower-bound	24683.468	5.000	4936.694		
time * condition	Sphericity Assumed	5802.121	15	386.808	2.104	.019
	Greenhouse-Geisser	5802.121	3.070	1890.080	2.104	.141
	Huynh-Feldt	5802.121	8.506	682.114	2.104	.053
	Lower-bound	5802.121	1.000	5802.121	2.104	.207
Error (time*condition)	Sphericity Assumed	13789.733	75	183.863		
	Greenhouse-Geisser	13789.733	15.349	898.419		
	Huynh-Feldt	13789.733	42.530	324.232		
	Lower-bound	13789.733	5.000	2757.947		

Overall Appetite

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.000	.	44	.	.249	.371	.111
condition	.458	4.466	5	.490	.751	1.000	.333
time * condition	.000	.	377	.	.148	.367	.037

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept
Within Subjects Design: time + condition + time * condition

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	171346.841	9	19038.538	46.326	.000
	Greenhouse-Geisser	171346.841	2.238	76562.295	46.326	.000
	Huynh-Feldt	171346.841	3.340	51304.730	46.326	.000
	Lower-bound	171346.841	1.000	171346.841	46.326	.000
Error(time)	Sphericity Assumed	25891.264	63	410.972		
	Greenhouse-Geisser	25891.264	15.666	1652.700		
	Huynh-Feldt	25891.264	23.379	1107.482		
	Lower-bound	25891.264	7.000	3698.752		
condition	Sphericity Assumed	1970.465	3	656.822	1.297	.302
	Greenhouse-Geisser	1970.465	2.252	875.002	1.297	.304
	Huynh-Feldt	1970.465	3.000	656.822	1.297	.302
	Lower-bound	1970.465	1.000	1970.465	1.297	.292
Error(condition)	Sphericity Assumed	10636.581	21	506.504		
	Greenhouse-Geisser	10636.581	15.764	674.752		
	Huynh-Feldt	10636.581	21.000	506.504		
	Lower-bound	10636.581	7.000	1519.512		
time * condition	Sphericity Assumed	2328.065	27	86.225	.634	.919
	Greenhouse-Geisser	2328.065	3.984	584.391	.634	.642
	Huynh-Feldt	2328.065	9.903	235.087	.634	.778
	Lower-bound	2328.065	1.000	2328.065	.634	.452
Error (time*condition)	Sphericity Assumed	25688.437	189	135.918		
	Greenhouse-Geisser	25688.437	27.886	921.188		
	Huynh-Feldt	25688.437	69.321	370.573		
	Lower-bound	25688.437	7.000	3669.777		

L – Mean + SD For Graphs Presented

Mean body temperature

PID	MBT_CON_0	MBT_CON_30	MBT_CON_1	MBT_CON_1.5	MBT_CON_2	MBT_CON_2.5	MBT_CON_3	MBT_CON_3.5	MBT_CON_4	MBT_CON_4.5	MBT_CON_5
Mean	34.9692143	34.7026429	34.4773571	34.351125	34.3706821	34.2578571	34.3628036	34.5745536	34.5010714	34.5352857	34.5394464
SD	0.25274013	0.37831294	0.46672288	0.49875381	0.51483042	0.58454358	0.65584821	0.86726841	0.561882	0.55661889	0.54983997
PID	MBT_COLD_0	MBT_COLD_0.5	MBT_COLD_1	MBT_COLD_1.5	MBT_COLD_2	MBT_COLD_2.5	MBT_COLD_3	MBT_COLD_3.5	MBT_COLD_4	MBT_COLD_4.5	MBT_COLD_5
Mean	35.5015	35.286375	35.6494375	35.2331875	34.9053281	34.6877969	34.6720781	34.6543125	34.6390625	34.5244063	34.4937188
SD	0.44498443	0.95374737	1.00459841	0.31320549	0.28768757	0.19409366	0.34892687	0.40285442	0.46285098	0.59229468	0.73367824
PID	MBT_NEU_0	MBT_NEU_0.5	MBT_NEU_1	MBT_NEU_1.5	MBT_NEU_2	MBT_NEU_2.5	MBT_NEU_3	MBT_NEU_3.5	MBT_NEU_4	MBT_NEU_4.5	MBT_NEU_5
Mean	35.8295	36.7668125	37.3495	35.8319375	35.1766875	34.9265156	34.8693594	34.7035938	34.7328906	34.6870625	34.5931719
SD	0.28271894	1.59056679	1.61117703	0.3754883	0.35727457	0.23094452	0.17570686	0.42906914	0.29122988	0.35324406	0.29242068
PID	MBT_HOT_0	MBT_HOT_0.5	MBT_HOT_1	MBT_HOT_1.5	MBT_HOT_2	MBT_HOT_2.5	MBT_HOT_3	MBT_HOT_3.5	MBT_HOT_4	MBT_HOT_4.5	MBT_HOT_5
Mean	35.87775	36.638375	37.6049375	36.3965469	35.3972188	34.9410469	34.7789531	34.5770969	34.8459531	34.6587656	34.6227031
SD	0.19774569	0.19207081	0.20255272	0.30477551	0.17513447	0.3021785	0.4533425	0.83513356	0.45534958	0.66561623	0.71425857

PYY

	CONTROL						
Time	0	1	1.5	2	3	5	
Average	0	-6.3645	-17.575	38.84883	28.62933	18.45783	
SD	0	19.2699	27.07996	64.77196	29.28007	29.27907	
	COLD						
Time	0	1	1.5	2	3	4	5
Average	0	0.8815	-19.113	25.25733	19.06567	10.49683	6.109
SD	0	34.87431	21.92728	37.05521	32.33712	40.93828	43.76261
	NEUTRAL						
Time	0	1	1.5	2	3	4	5
Average	0	14.543	10.081	36.73867	18.91533	13.825	1.1855
SD	0	26.20719	34.72284	20.55676	25.40799	28.28546	28.90986
	HOT						
Time	0	1	1.5	2	3	4	5
Average	0	5.046333	-19.2908	28.0465	6.089833	3.393167	-11.8302
SD	0	24.1237	16.20606	40.18566	26.63713	47.66723	42.94442

Acylated Ghrelin

	CONTROL						
Time	0	1	1.5	2	3	5	
Average	0	9.811333	2.009	-15.5145	-40.2563	-13.1288	
SD	0	20.08815	38.56375	23.55532	26.59144	37.28348	
	COLD						
Time	0	1	1.5	2	3	4	5
Average	0	-7.406	4.579667	-25.8545	-40.3472	-11.7765	-2.567
SD	0	13.06359	20.5145	6.979979	10.24531	58.38009	28.74664
	NEUTRAL						
Time	0	1	1.5	2	3	4	5
Average	0	-26.1927	-24.4413	-33.7038	-39.2935	-46.4348	-17.5522
SD	0	29.23986	29.99248	11.17763	19.30002	10.70991	19.13409
	HOT						
Time	0	1	1.5	2	3	4	5
Average	0	2.859667	4.366667	-12.7027	-22.4123	-17.7975	11.84433
SD	0	32.32574	20.94331	20.99489	24.26556	26.37252	29.71372

Overall Appetite

	OVERALL_CON_0	OVERALL_CON_1	OVERALL_CON_1.5	OVERALL_CON_2	OVERALL_CON_2.5	OVERALL_CON_3	OVERALL_CON_3.5	OVERALL_CON_4	OVERALL_CON_4.5	OVERALL_CON_5
Mean	73.34375	80.9375	87.15625	10.34375	15.1875	31.09375	34.40625	45.75	50.84375	65.96875
SD	17.02621	13.50446	7.537925	5.493401	12.75858	20.40787	25.45126	24.47958	30.35944	18.58159
	OVERALL_10_0	OVERALL_10_1	OVERALL_10_1.5	OVERALL_10_2	OVERALL_10_2.5	OVERALL_10_3	OVERALL_10_3.5	OVERALL_10_4	OVERALL_10_4.5	OVERALL_10_5
Mean	67.34375	77.0625	79.3125	13.0625	14.8125	24.125	31.03125	38.375	51.15625	56.875
SD	27.59786	12.95097	15.6626	10.64756	8.439649	16.82101	20.47186	23.58685	23.14818	22.52419
	OVERALL_20_0	OVERALL_20_1	OVERALL_20_1.5	OVERALL_20_2	OVERALL_20_2.5	OVERALL_20_3	OVERALL_20_3.5	OVERALL_20_4	OVERALL_20_4.5	OVERALL_20_5
Mean	69.625	69.40625	82.8125	11.40625	19.15625	25.9375	38.5625	45.84375	53.59375	57.5
SD	15.96816	20.42317	15.04027	10.60318	17.61439	25.64028	28.43217	31.08052	25.09337	23.06087
	OVERALL_30_0	OVERALL_30_1	OVERALL_30_1.5	OVERALL_30_2	OVERALL_30_2.5	OVERALL_30_3	OVERALL_30_3.5	OVERALL_30_4	OVERALL_30_4.5	OVERALL_30_5
Mean	70.4375	74.6875	83.3125	20.78125	21.65625	29.6875	40.28125	51.46875	60.09375	67.59375
SD	18.5293	15.85974	9.629521	22.78723	15.70654	20.91469	23.38438	23.64259	18.20515	20.50999