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The effect of ambient temperature during acute aerobic exercise on short term appetite, energy intake and plasma acylated ghrelin in recreationally active males

Lucy K Wasse¹, James A King¹,², David J Stensel¹,² and Caroline Sunderland³.

¹School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, LE11 3TU, UK

²NIHR Leicester-Loughborough Diet, Lifestyle & Physical Activity Biomedical Research Unit

³Sport, Health and Performance Enhancement (SHAPE) Research Group, School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK

Corresponding author:
Lucy K Wasse
Gastrointestinal Centre
University of Manchester
Clinical Sciences Building
Stott Lane
Salford
M6 8HD
Email: lucy.wasse@manchester.ac.uk
Tel: +44 (0)161 206 1447

Email address of authors:
James A King: J.A.King@lboro.ac.uk
David J Stensel: D.J.Stensel@lboro.ac.uk
Caroline Sunderland: caroline.sunderland@ntu.ac.uk

Current affiliations:
Lucy K Wasse
Gastrointestinal Centre
Institute of Inflammation and Repair
University of Manchester
Stott Lane
M6 8HD
lucy.wasse@manchester.ac.uk
Abstract
Ambient temperature during exercise may affect energy intake regulation. Compared with a temperate (20°C) environment, 1 h of running followed by 6 h of rest tended to decrease energy intake from two ad libitum meals in a hot (30°C) environment but increase it in a cool (10°C) environment (P=0.08). Core temperature changes did not appear to mediate this trend; whether acylated ghrelin is involved is unclear. Further research is warranted to clarify these findings.

Key words ambient temperature, exercise, appetite, energy intake, acylated ghrelin, core temperature
Introduction

The effects of exercise on appetite and energy intake are well documented from laboratory studies (King et al. 2010, King et al. 2011, Martins et al. 2007, Ueda et al. 2009, Wasse et al. 2012). Most observe a transient suppression of appetite during exercise with no effect on subsequent energy intake. However, these observations are not unanimous because a handful of studies describe no effect of exercise on appetite (Ueda et al. 2009, Wasse et al. 2013) and some authors report increases (Martins et al. 2007) or decreases (Ueda et al. 2009) in post-exercise energy intake. Recent attention has focussed on how exercise affects concentrations of circulating appetite-regulatory gut hormones. Exercising in more extreme environmental conditions (altitude, temperature) may perturb the normal physiological responses to exercise and could subsequently affect the acute regulation of appetite. It is already known that altitude suppresses appetite (Tschöp et al. 1998), an effect that might be related to alterations in concentrations of appetite-regulatory peptides (Tschöp et al. 1998, Wasse et al. 2012). Although one laboratory study shows exercising in the heat may reduce relative energy intake (Shorten et al. 2009) most reports suggesting appetite is suppressed in the heat are anecdotal (Burke, 2001). Cold temperatures may exert the opposite effect with an increase in energy intake reported after exercise in cold water (White et al. 2005). However, whether this is directly related to the cold temperature per se is questionable because water immersion itself increases energy intake (Halse et al. 2011). Physiological differences, such as in substrate utilisation, are evident when individuals are exposed to cold air or cold water (Haman et al. 2006). Furthermore, Wiesner and colleagues (2010) report hormonal and metabolic responses specific to exercise in water that do not occur with land-based exercise. For these reasons we surmise that the appetite response to cold air could differ to that observed in cold water, however because there is no evidence to substantiate this, research examining this notion is warranted.
Core temperature changes have been postulated as a mechanism responsible for alterations in energy intake in different environmental temperatures, however, this is not conclusive. Changes in gut hormone responses may also be responsible for differences in energy intake during exercise in the heat/cold. There are numerous hormones secreted from the gastrointestinal tract that are involved in the control of energy homeostasis, particularly the short-term regulation of energy intake. The majority of these hormones, which include cholecystokinin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are secreted in the post-prandial period and contribute to meal termination and satiety (Yu and Kim, 2012). However, notable among the appetite-regulatory gut hormones is acylated ghrelin, unique in being the only known gut hormone that stimulates appetite (Wren et al. 2001), and purported to be a meal initiation factor (Cummings et al. 2001). Testament to the widespread distribution of it’s receptor in both central and peripheral regions, ghrelin has numerous other biological effects including being a potent stimulator of growth hormone secretion (Kojima et al. 1999) as well as having important roles in immune function (Dixit and Taub, 2005) and glucose metabolism (Verhulst and Depoortere, 2012). However, with a unique role as the only known circulating appetite-stimulating gut hormone, it is unsurprising that examining the role of ghrelin in energy homeostasis has become such a prolific area of research. Total ghrelin (des-acyl and acyl ghrelin) is up-regulated after short term cold exposure and down-regulated after short term heat exposure (Tomasik et al. 2005). How long these perturbations persist is unknown due to the short duration of exposure in that study (30 minutes); whether these alterations affect subsequent appetite and energy intake remains to be investigated. Inferences from a study investigating total ghrelin may be limited because it is generally believed it is the acylated fraction of ghrelin that is necessary for its appetite stimulatory effects (Broglio et al. 2004).
Given the importance for athletes to maintain appropriate energy balance during episodes of training and competition which are frequently undertaken in a variety of environments, clarification is required to establish whether land-based exercise in hot or cool environments differentially affects appetite and energy intake and whether changes are related to alterations in plasma acylated ghrelin concentrations or core temperature.

Materials and Methods

Two separate pilot studies were approved by both the Loughborough University and Nottingham Trent University Ethics Committees. Eleven healthy, habitually active males (mean ± SD; age 21.1 ± 1.2 y, BMI 23.6 ± 2.2 kg/m², VO₂peak 56.7 ± 5.0 mL/kg/min) completed a ‘heat study’ and ten healthy, habitually active males (mean ± SEM; age 22.9 ± 2.5 y, BMI 23.1 ± 1.6 kg/m², VO₂peak 57.9 ± 7.3 mL/kg/min) completed a ‘cool study’. Participants were free from metabolic and gastrointestinal abnormalities. Participants gave their written informed consent to participate, completed a submaximal and maximal oxygen uptake test on a treadmill (Woodway ELG 55; Weil am Rhein, Germany) and then completed two, 7 h trials in a randomised order in an environmental chamber (Design Environmental, Gwent, UK). Trials were separated by at least seven days. In the heat study one trial was completed in a temperate environment (20°C; control) and the other in a hot environment (30°C; experimental), in the cool study one trial was completed in a temperate environment (20°C; control) and the other in a cool environment (10°C; experimental). Relative humidity was kept constant at 50%. Participants fasted overnight prior to trials which commenced at ~9am. During the 24 h prior to the first trial, participants weighed and recorded their food intake and then replicated this before the second trial. Upon arrival, a cannula was inserted into an antecubital vein to enable frequent sampling of venous blood and a rectal
thermometer (Grant Instruments, UK) was self-inserted ~10 cm past the anal sphincter for monitoring core body temperature. Clothing was not standardised and participants were asked to wear clothing appropriate for the environmental temperature throughout each visit. At the start of each trial, participants completed a 60 minute treadmill run at a speed that elicited 65% of maximal oxygen uptake, followed by 6 h rest. Blood samples for acylated ghrelin were collected at baseline (0), 0.5, 1, 2, 3, 4, 5.5, 6.5 and 7 h into pre-cooled 5 mL EDTA tubes that were pre-treated with 50µL of a solution containing p-hydroxymercuribenzoic acid, phosphate buffered saline, and sodium hydroxide. After 10 min centrifugation at 3500 rpm, 2 mL of plasma were dispensed into a plain tube and 200 µL of 1 M hydrochloric acid was added before being centrifuged for 5 min at 3500 rpm. Appetite sensations were measured every 30 minutes from baseline using validated 100 mm visual analogue scales (Flint et al. 2000). Cold buffet-style meals were provided at 2 and 5.5 h to assess *ad libitum* energy intake. Foods were presented in excess of expected consumption and identical items were available to participants at both meals. These items were three varieties of breakfast cereal, semi-skimmed milk, brown and white bread, cheese, ham, tuna, butter, margarine, mayonnaise, chocolate chip cookies, salted crisps, muffins, Nutri-grain bars, chocolate rolls, mini Mars bars, apples, bananas, oranges, fruit yoghurt, chocolate Nesquik and orange juice. Acylated ghrelin concentrations were determined from plasma using a commercially available ELISA (SPI BIO, Montigny le Bretonneux, France). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Differences in fasting and AUC values for appetite perceptions, acylated ghrelin, core temperature and thermal sensations were determined using Student’s t-tests. Two-factor repeated measures ANOVA was used to examine differences between trials for appetite perceptions, energy and macronutrient intake, acylated ghrelin, core temperature and thermal sensations. Statistical...
significance was accepted at the 5% level. Results are presented as mean ± SD. Due to problems collecting blood from one participant in the heat study, acylated ghrelin concentrations reported are for n = 10. Changes in energy intake between control and experimental trials in each study were calculated and these differences compared using an independent samples T-test. Effect sizes were reported to facilitate comparison of the magnitude of the effect of hot and cool temperatures on energy intake. Effect sizes were calculated in accordance with Cohen’s classification where 0.2, 0.5 and 0.8 are considered small, moderate and large effects, respectively.

Results

Appetite

Compared with the temperate environment, the hot and cool temperatures modulated the appetite response to exercise with AUC values for hunger and prospective food consumption being lower by 15% and 12% respectively prior to the first meal (0 – 2 h) in the heat trial compared with the temperate trial (P < 0.05). Over the same time period, AUC values for satisfaction and fullness were 27% lower (P < 0.05) and 23% lower (P = 0.07) in the cool trial compared with the temperate trial. There were no other significant differences apparent over this time period, or across the entire 7 h trial (Table 1).

Energy intake

The total energy intake and the change in energy intake in response to different ambient temperatures varied widely between individuals (Figure 1). In the heat study, there was a trend for a reduction in cumulative energy intake in the hot trial compared with the temperate trial by 1400 ± 2401 kJ (P = 0.08; Figure 1). The opposite trend was apparent in the cool
study where participants increased their energy intake by $1450 \pm 2345$ kJ ($P = 0.08$; Figure 1) in the cool trial compared with the temperate trial. The effect sizes for the difference in energy intake between the temperate and their respective hot and cool trials were moderate ($d = -0.5$ for the heat trial and 0.5 for the cool trial). There was a main effect of time in the heat study ($P < 0.05$) with participants consuming more at the morning meals than the afternoon meals, however energy intake was reduced by a similar extent (~12%) at both the morning and afternoon meals in the hot compared with the temperate trial. There was no difference in energy intake consumed at the morning and afternoon meals in the cool study, and energy intake tended to be increased by a similar extent at each meal in the cool trial. When the delta values in energy intake between the temperate and experimental trial in each study were compared (using an independent samples T test), a significant difference was evident ($P = 0.013$).

**Acylated ghrelin**

No main effects of temperature on acylated ghrelin concentrations were observed in either study although a trial x time interaction was evident in both ($P < 0.05$). However, post hoc analysis did not reveal differences between trials at any time points. A main effect of time showed that acylated ghrelin concentrations were suppressed at the end of exercise from baseline values (Figures 2a and 2b). Delta values in acylated ghrelin concentrations from baseline until the end of the exercise bout between the temperate and experimental trial in each study were compared (using an independent samples T test) and a significant difference was evident ($P < 0.05$). Despite differences upon cessation of exercise, acylated ghrelin values were similar between trials within each study immediately prior to consumption of the first *ad libitum* meal at 2 h.
Core temperature

Core temperature was significantly elevated on completion of exercise in the hot compared with the temperate trial (38.9 ± 0.4°C vs. 38.5 ± 0.5°C respectively; $P < 0.001$) but was similar thereafter. Core temperature was similar at all times between the temperate and cool trials.

Discussion

Results from these pilot studies indicate that the environmental temperature during and after acute exercise may transiently modulate appetite and short term energy intake but it is unlikely that changes in core temperature mediated these changes and it is uncertain whether changes in acylated ghrelin concentrations are involved. Total energy intake from two ad libitum meals during a 7 h trial tended to be decreased in 30°C and increased in 10°C compared with a neutral 20°C environment. No individual meal was responsible for this trend, with the change in energy intake being consistent across both ad libitum meals indicating a persistence of effect of ambient temperature on energy intake. Although most individuals within each study respond similarly (ie: increased energy intake in the cool, decreased energy intake in the heat) there is a wide variation in individual responses (Figure 1). However, these findings give some support to the anecdotal and limited empirical evidence that ambient temperature may modulate appetite and energy intake. Furthermore, this research indicates that the effect persists when acute exercise is undertaken, and expands upon current literature by extending beyond the immediate post-exercise meal.

These findings are important for recreational and competitive athletes. Exercise, in the absence of compensatory increases in food intake, can produce a short term negative energy
balance which may be efficacious for weight loss. The present findings provide some support for the suggestion previously proposed by Shorten et al (2009) that exercising outdoors in the heat may be preferable to exercising in an air conditioned gym if a more negative energy balance is desired. From an athlete’s perspective where optimal nutritional strategies can aid performance, exercising in the heat could be detrimental if an athlete voluntarily consumes less food at a subsequent meal which could lead to inadequate refuelling before ensuing events and could impair performance or recovery. Conversely, high energy intakes, particularly if above energy requirements could be detrimental to an athlete’s post-exercise nutritional strategy. Given that ambient temperatures of approximately 11ºC, (similar to that used within the cool study), can be advantageous to performance during prolonged moderate intensity exercise (Nimmo 2004) the findings from the present study that exercise and rest in cool temperatures of 10ºC tend to increase post-exercise energy intake should be considered. Previously, changes in core temperature or gut hormone concentrations (namely PYY) have been suggested to mediate the change in energy intake after exercise in different environmental temperatures (Shorten et al. 2009, White et al. 2005). In the study by White and colleagues, despite inverse relationships between core temperature and energy intake being described, actual changes in core temperature were small (0.3ºC) which may be insufficient to affect appetite. Furthermore the studies by White et al (2005) and Shorten et al (2009) used tympanic temperature to assess core temperature. That method of core temperature measurement is reportedly not valid when exercising in the heat in a laboratory (Ganio et al. 2009). In our studies, we used rectal temperature (a valid and reliable method of measuring core temperature during rest and exercise) to regularly monitor core temperature. We observed similar core temperatures across trials within each study and core temperature differed only at the end of the exercise bout in the hot trial compared with the temperate trial.
Thus, our findings would suggest that core temperature does not drive the changes in energy intake after acute exercise followed by rest in different environmental temperatures.

Tomasik and colleagues (2005) examined the effect of ambient temperature on total ghrelin concentrations and found concentrations were increased after 30 mins at 2°C and decreased after 30 mins at 30°C. However, neither appetite nor energy intake were assessed so it is unknown whether changes in total ghrelin affected subsequent appetite and energy intake. In the present studies, there was not a consistent effect of ambient temperature on acylated ghrelin concentrations. Given the complex mechanisms by which appetite and food intake are regulated, it is likely that a combination of factors coordinate the food intake responses to exercise and rest in different ambient temperatures that we observed here. It has been shown that thermal perceptions are important inputs in the self-selection of exercise intensity, and thermal sensation and thermal discomfort can control thermoregulatory behaviour (Schlader et al. 2011). Participants felt “comfortable” in both temperate trials, and despite being able to wear whatever clothing they wished, reported feeling “cool” in the trial at 10°C and “hot” in the trial at 30°C. Hence thermal status may also be involved in feeding responses although this is speculative.

The gastrointestinal system does not simply exist as a reservoir for food and drink but plays a key role in the regulation of appetite and maintenance of energy balance. As well as directly influencing appetite, hormones including ghrelin and PYY that are secreted from within the gastrointestinal tract also affect gastric motility, gastric emptying and gastrointestinal blood flow. Relationships between these gastric parameters and appetite perceptions such as hunger and fullness have been observed and reviewed (Delzenne et al. 2010). The presence of an intragastric balloon can decrease hunger and increase fullness, without decreasing subsequent energy intake or affecting concentrations of appetite-regulatory peptides (Oesch et al. 2006,
Rigaud et al. 1995). In the present studies participants were free to consume water *ad libitum* during trials, thus differences in hunger in the first 2 hours of the hot trial could just be a consequence of stomach distension after water ingestion which was greater than in the temperate trial (data not shown). However, it is unlikely that the trend for a reduction in energy intake observed in the heat study was due to differences in stomach distension as this alone is reportedly not sufficient to affect gut hormone concentrations or energy intake. Furthermore the decrement in energy intake persisted at the afternoon meal when appetite ratings were similar between trials.

Gastric emptying may influence ingestive behaviour and although the volume of a meal influences gastric emptying, nutrients within that meal may play a greater role in affecting gastric emptying due to feedback from the intestine in response to nutrients in the gut lumen which affect secretion of peptides including CCK and PYY. Exercise in the heat does not generally affect gastric emptying rate when participants are hydrated, however emptying rates may vary dependent on the hydration status of participants. Rehrer and colleagues (1990) observed that dehydration delays gastric emptying of carbohydrate beverages. We did not quantify gastric emptying rates in these studies, so it is not possible to associate any changes in gastric emptying with alterations in energy intake. There is limited literature regarding the effect of cold ambient temperatures on gastric emptying, but unlike in the heat, dehydration will less likely be a factor impacting upon gastric emptying rate. In rats, cold ambient temperature normalises a delayed gastric emptying response induced by abdominal surgery (Stengel et al. 2010). However, it is unclear what effect the cool temperature in the present study would have on normal gastric emptying responses to food and fluid ingestion. Since ghrelin stimulates gastric motility and accelerates gastric emptying, Stengel and colleagues (2010) proposed that the normalised gastric emptying response was due to increased acylated
ghrelin concentrations after cold exposure. Given the relationship between gut hormones, appetite and gastrointestinal function, perturbations in concentrations of gut hormones may act in concert to affect gastric function and appetite as well as directly influencing appetite regulatory areas within the brain.

Although our study benefits from the longer period of follow-up than in other research, there are some limitations which should be addressed in future. Due to the wide variability in energy intake responses between individuals further research is necessary to confirm these findings with greater participant numbers. This research focussed on concentrations of the appetite-stimulatory gut hormone, acylated ghrelin. However, satiety hormones secreted from the gut and adipose tissue which include, but are not limited to, PYY, GLP-1 and leptin also play integral roles in the regulation of appetite and energy intake. In future, it would be prudent to quantify concentrations of these and other hormones involved in appetite regulation in conjunction with acylated ghrelin to improve understanding of how these hormones may be perturbed in response to exercising in different ambient temperatures. This is particularly important because of the discordance between our findings and those of Shorten el al (2009) who did not observe any alterations in acylated ghrelin after exercise in the heat, but attribute a reduction in energy intake to elevated concentrations of PYY. Finally, since there may be sex differences in the way exercise affects appetite regulatory hormones and appetite, it would be of value to also study female participants.
Acknowledgements

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References


Table 1. Effect of environmental temperature on appetite responses assessed using visual analogue scales.

<table>
<thead>
<tr>
<th></th>
<th>Preprandial (0 – 2 h) mm · 2h</th>
<th>Total trial (0 – 7 h) mm · 7h</th>
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<tbody>
<tr>
<td><strong>Hunger</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate</td>
<td>127 ± 10</td>
<td>258 ± 59</td>
</tr>
<tr>
<td>Heat</td>
<td>108 ± 15*</td>
<td>239 ± 82</td>
</tr>
<tr>
<td>Temperate</td>
<td>99 ± 40</td>
<td>247 ± 76</td>
</tr>
<tr>
<td>Cool</td>
<td>113 ± 32</td>
<td>258 ± 80</td>
</tr>
<tr>
<td><strong>Satisfaction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate</td>
<td>42 ± 7</td>
<td>365 ± 23</td>
</tr>
<tr>
<td>Heat</td>
<td>50 ± 8</td>
<td>349 ± 52</td>
</tr>
<tr>
<td>Temperate</td>
<td>70 ± 4</td>
<td>379 ± 48</td>
</tr>
<tr>
<td>Cool</td>
<td>51 ± 23*</td>
<td>356 ± 67</td>
</tr>
<tr>
<td><strong>Fullness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate</td>
<td>41 ± 7</td>
<td>361 ± 32</td>
</tr>
<tr>
<td>Heat</td>
<td>43 ± 8</td>
<td>358 ± 64</td>
</tr>
<tr>
<td>Temperate</td>
<td>58 ± 45</td>
<td>371 ± 54</td>
</tr>
<tr>
<td>Cool</td>
<td>45 ± 27</td>
<td>352 ± 72</td>
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<tr>
<td><strong>PFC</strong></td>
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<td></td>
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<tr>
<td>Temperate</td>
<td>154 ± 10</td>
<td>329 ± 54</td>
</tr>
<tr>
<td>Heat</td>
<td>135 ± 9*</td>
<td>317 ± 55</td>
</tr>
<tr>
<td>Temperate</td>
<td>118 ± 37</td>
<td>318 ± 72</td>
</tr>
<tr>
<td>Cool</td>
<td>129 ± 30</td>
<td>325 ± 69</td>
</tr>
</tbody>
</table>

Note. Values are mean ± SD, *n* = 11 (heat study), *n* = 10 (cool study). PFC, prospective food consumption.

*Significantly lower than respective temperate trial (*p* < 0.05)
**Figure Legends**

**Fig. 1.** Changes in energy intake (kJ) between temperate and cool trials (black columns, \( n = 10 \)) and temperate and hot trials (grey columns, \( n = 11 \)). Each column represents one participant. Solid black line indicates the mean increase in energy intake in the cool trial compared with the temperate trial, dashed black line indicates the mean decrease in energy intake in the hot trial compared with the temperate trial; difference between studies \( P = 0.013 \) (independent samples T-test).

**Fig. 2.** Plasma acylated ghrelin concentrations during the temperate (○) and heat (■) trials (a), and during the temperate (○) and cool (▲) trials (b). Values are mean ± SEM, \( n = 10 \). The black rectangle indicates the treadmill run and solid black arrows indicate the *ad libitum* buffet meals.