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Acute exposure to a hot ambient temperature reduces energy intake but does not affect gut hormones in men during rest

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Abstract

This study examined the effect of ambient temperature on energy intake, perceived appetite and gut hormone responses during rest in men. Thirteen men (age 21.5 (SD 1.4) years; BMI 24.7 (SD 2.2) $kg \cdot m^{-2}$) completed three, 5.5-h conditions in different ambient temperatures: i) cold (10˚C), ii) thermoneutral (20˚C), and iii) hot (30˚C). A standardised breakfast was consumed after fasting measures, and an *ad libitum* lunch provided at 4 to 4.5 h. Blood samples (analysed for plasma acylated ghrelin, total peptide tyrosine-tyrosine (PYY) and total glucagon-like peptide (GLP-1) concentrations), perceived appetite and thermoregulatory responses were collected throughout. Linear mixed models were used for statistical analyses. *Ad libitum* energy intake was 1243 (SD 1342) kJ higher in 10˚C and 1189 (SD 1219) kJ higher in 20˚C versus 30°C ($P = 0.002$). Plasma acylated ghrelin, total PYY and GLP-1 concentrations did not differ significantly between the conditions ($P \geq 0.303$). Sensitivity analyses for the 4-h pre-lunch period showed that perceived overall appetite was lower in both 30˚C and 10°C when compared with 20˚C ($P \leq 0.019$). In conclusion, acutely resting in a hot compared with a thermoneutral and cold ambient temperature reduced lunchtime *ad libitum* energy intake in healthy men. Suppressed perceived appetite may have contributed to the reduced energy intake in the hot compared with thermoneutral ambient temperature, whereas gut hormones did not appear to play an important role.

Introduction

The health and economic burden of managing preventable diseases caused by excess adiposity is not sustainable over the long-term[1]. Of concern is that approaches to prevent gains in body fat through moderating energy intake have often not been effective for a multitude of complex inter-linked reasons that may include motivation, food cravings and will-power[2]. Appetite contributes to the control of energy intake and is regulated by numerous physiological, psychological, social and environmental factors[3]. One environmental factor that may be relatively practical to manipulate (e.g., through heated rooms, clothing, sauna, hot baths, or outdoor exposure to hot climates) is temperature. Yet, few studies have determined the appetite responses to different ambient temperatures, particularly under resting conditions. During 48 to 60-h exposures to different ambient temperatures within a relatively narrow range (16 versus 22˚C, and 27 versus 22˚C) at rest in metabolic chambers, findings of two similar studies suggest that *ad libitum* energy intake increases at lower temperatures[4,5]. Further, the reported differences in energy intake were related to changes in core body temperature[4,5]. There is also pilot data demonstrating that a 2 hour exposure to a warm (26–27°C) versus thermoneutral (19–20°C) ambient temperature resulted in a trend for reduced *ad libitum* energy intake; yet, the difference was not significant, possibly because the sample size was not sufficient[6]. Energy intake, however, was not affected in response to a 2.5 hour exposure to a mild cold (18ºC) versus thermoneutral (24ºC) ambient temperature, despite a trend towards higher perceived hunger[7]. Similarly, the amount of food consumed at breakfast, lunch and dinner was unaffected when comparing exposure to 28°C, 32°C, 36°C, and 38°C, even though subjective appetite at lunch was lower in the higher temperatures, perhaps due to the narrow range of temperatures and because the meals were not served *ad libitum*[8]. To complement these findings, the majority of studies that have manipulated ambient temperature during exercise show that exercise performed in the heat decreases and exercise performed in the cold increases subsequent energy intake and perceived appetite when compared with thermoneutral temperatures[9-15], whilst others have shown no effect[15,16]. Thus, findings from the existing literature are not consistent.

It has been proposed that appetite responses to different ambient temperatures may be regulated in part by gut hormones, including the orexigenic hormone acylated ghrelin and the anorexigenic hormones peptide tyrosine-tyrosine (PYY) and glucagon-like peptide (GLP-1)[9,10,13-17]. Yet, studies investigating gut hormone responses to different ambient temperatures during rest are sparse. The limited evidence to date has shown that 30 minutes resting at 2°C and 30°C increased and decreased plasma total ghrelin concentrations, respectively, when compared with 20 °C, although whether this contributed to differences in energy intake was not assessed[17]. In support, a study examining the

independent and combined effects of 40 minutes heat exposure and exercise reported that a hot (31°C) versus thermoneutral (22°C) ambient temperature decreased plasma total ghrelin with a trend for a reduction in subjective appetite; however, ambient temperature did not affect energy intake[16]. Importantly, these studies conducted at rest did not measure the acylated fraction of ghrelin, which is responsible for ghrelin's effects on appetite[18]. Studies that have manipulated ambient temperature during exercise rather than rest have reported mixed findings for total ghrelin[14-16, 19], acylated ghrelin [9,10,13,15,19] and PYY[13-15]. Further, only one study appears to have measured GLP-1, which did not change in response to exercise performed in hot or cold ambient temperatures[15]. These variable responses may be due to inter-study differences in participant characteristics, the temperatures used to elicit 'hot' (30 to 36°C), 'thermoneutral' (20 to 25°C) and 'cold' (2 to 12°C), whether ambient temperature was manipulated for the entire trial duration or at a certain time point (e.g., during exercise only, or before *ad libitum* meal consumption), exercise characteristics, and test meal characteristics. Moreover, exercise exerts an independent effect on appetite that may interact with ambient temperature in studies that have included an exercise component[20].

Due to the inconsistent findings and lack of existing data conducted under resting conditions, the independent effect of ambient temperature on energy intake, appetite and gut hormones remains unknown. Among the resting studies to date, none have compared a continuum of temperatures from cold to hot to establish the possible dose-response relationship with appetite-related variables. The primary aim of this study was to compare the acute effects of cold, thermoneutral and hot ambient temperatures during rest on lunchtime *ad libitum* energy intake in men. The secondary aims were to examine the effects of ambient temperature on gut hormone (acylated ghrelin, PYY and GLP-1), perceived appetite and thermoregulatory responses.

Experimental methods

Participants

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Institute of Sport and Physical Activity Research Ethics Committee at the University of Bedfordshire (approval number: 2016ISPAR003). Data collection took place between January 2016 and January 2017. Healthy men aged 18 to 30 years were recruited. Written informed consent was obtained from all participants. A questionnaire was completed to screen participants for potential health conditions that may affect their eligibility to participate or the study outcomes, including dietary allergies and intolerances, blood borne diseases, congenital heart disease, diabetes, high blood pressure, epilepsy, respiratory

conditions, musculoskeletal injury that affected normal movement within the last month, and disturbance of vision. All of the participants had a healthy body fat %, with a range of 8.0 to 18.2%[21,22]. Participants confirmed verbally that they had not been exposed to either hot or cold environments that would be atypical of the local area and may have resulted in a degree of acclimatisation within three months prior to their inclusion in the study; for example, a holiday. Those who were exposed did not partake in the study. Participants also confirmed that they consumed breakfast habitually (i.e., at least four days per week). Prior to the experimental conditions, height was measured to the nearest 0.01 m using a stadiometer (Harpenden, Holtain Ltd., Crymych, UK), body mass was measured to the nearest 0.1 kg using a digital balance scale (Tanita BC 418 MA analyser, Tanita Corporation, Japan) and body fat was measured to the nearest 0.1 % via air displacement plethymography (Bod Pod, Cosmed, Middlesex, UK). The participants were also familiarised with study procedures and equipment.

Experimental design

Each participant completed three, 5.5 h experimental conditions at a different ambient temperature in an environmental chamber (custom built from TIS Services, Medstead, Hampshire, UK): 10˚C (cold), 20˚C (thermoneutral), and 30˚C (hot). The environmental chamber was 4.8 (length) x 4.2 (width) x 2.7 (height) m with a temperature range of +1 to 50˚C and an accuracy of ± 1˚C for temperature and ± 2% for relative humidity. The order of the three conditions was pre-determined using a computer-based random number generator according to an incomplete Latin square design. There was a 7 to 14 day washout period between the experimental conditions, which took place at the same time of day to control for circadian variation and to reflect typical breakfast (~09:00) and lunch (~13:00) times.

Participants were instructed to refrain from alcohol and caffeine consumption and to not take part in any strenuous physical activity for the 24 h preceding each experimental condition. Participants were also asked to complete a 24 h weighed food diary prior to their first experimental condition and not to consume any energy-providing nutrients from 21:00 onwards; dietary intakes (quantity and timings) were replicated in the 24 h before the subsequent experimental conditions. Each participant consumed 500 mL of water (this equated to ~ 5-7 mL·kg body mass^{-1}) 2 h before arriving to the laboratory to promote a euhydrated state and thus limit the need for additional water consumption during the experimental conditions[23]. To reflect real-life situations and for health and safety reasons, the participants were given prior knowledge of the ambient temperature of each condition they would be completing so that clothes could then be chosen accordingly, with the

exception that clothes tailored for extreme environments were not permitted (e.g., thermal jackets, coats and gloves). This approach is in line with previous research[10].

Experimental protocol

Participants arrived at the laboratory at 08:30 after a 12 h overnight fast. On arrival, participants were fitted with skin temperature thermistors (Grant, EUS-UVS5-0, Wessex Power, Dorset, UK) located on the upper arm, chest, thigh and calf using adhesive tape, a rectal thermometer (YSI, 401, Yellow springs, Ohio, US) inserted 10 cm past the anal sphincter to monitor core temperature, and a heart rate monitor (Polar FS1, Polar, Kempele, Finland). A urine sample was collected and osmolality was measured (Atago Vitech Scientific, Pocket PAL-OSMO, West Sussex, UK) to confirm participants were euhydrated, i.e., urine osmolality < 700 mOsm/kg H_2O^{-1}[23]. Subsequently, an intravenous cannula was inserted into an antecubital vein and two fasting baseline blood samples were collected 5 min later within a thermoneutral ambient temperature. Participants then entered the environmental chamber, which was set at 10˚C, 20˚C or 30˚C. Participants remained seated throughout each condition and were permitted to complete work on a laptop that did not contain any appetite-related cues. After 5 min of entering the chamber, each participant consumed a standardised breakfast meal. Blood samples were then collected at 0.5, 1, 1.5, 2, 3, 4, 5 and 5.5 h during the postprandial period (i.e., 0.5 h intervals after eating and 1 h intervals for all remaining time points). An *ad libitum* pasta meal was provided at 4 to 4.5 h. Perceptions of hunger, satisfaction, fullness and prospective food consumption were assessed using 100 mm visual analogue scales (VAS) at baseline (fasted) and then every 30 min after consuming breakfast. Overall appetite score was calculated as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness[24]. In line with previous research, water was available *ad libitum* and the amount consumed was recorded[4-6, 9-11,15]. The only exception was that water was not permitted in the hour prior to or during the *ad libitum* lunch due to the possible influence on energy intake[25]. Body mass was recorded at baseline and on cessation of each experimental condition once all equipment, such as skin thermistors and rectal probes, had been removed. Relative humidity was controlled at 50% for all conditions[10]. Ambient temperature, relative humidity and heart rate were recorded every 30 min; core temperature and skin temperature were recorded every 10 min.

Blood sampling and chemistry

Blood samples were collected into pre-chilled EDTA vacuettes (Vacuette, Greiner Bio-One, Austria). From each sample, 50µL blood samples were collected into two heparinised microhaematocrit tubes for determination of haematocrit and a 20µL sample into a microcuvette for

determination of haemoglobin concentrations to assess changes in plasma volume[26]. One vacuette was immediately centrifued at 1500 × *g* for 10 min at 4°C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant was placed into separate cryovials and stored at −80°C until later analysis of total PYY and total GLP-1. To prevent the degradation of acylated ghrelin, a solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) (this was 10μL per mL of blood) was added to one EDTA vacuette. This vacuette was then spun in a refrigerated centrifuge at 1500 × *g* for 10 min at 4°C. The plasma supernatant was then placed into a storage tube and 100μL of hydrochloric acid (HCl) per 1 mL of plasma was added to preserve acylated ghrelin[27]. Thereafter, the sample was spun at 1500 × *g* for 5 min at 4°C prior to storage at −80°C pending acylated ghrelin analysis. Commercially available enzyme immunoassays were used according to manufacturer's instructions to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total GLP-1 (Millipore, Watford, UK) and total PYY (Millipore,Watford, UK). To eliminate interassay variation, samples from each participant were analysed in the same run. The intra-assay coefficient of variation was 3.0% for acylated ghrelin and total PYY and 8.2% for total GLP-1.

Meals

The standardised breakfast consisted of bread, cheese, jam, orange juice and milk. The meal provided 25 $kJ \cdot kg^{-1}$ of body mass (6 $kcal \cdot kg^{-1}$ of body mass) and the macronutrient content was 17% protein, 33% fat, and 46% carbohydrate. Participants were instructed to consume the meal within 10 min. The consumption time of the breakfast was recorded and participants were instructed to replicate this in subsequent conditions. *Ad libitum meals* have been shown to be sensitive to differences in energy intake in response to resting[4-7] and performing exercise[9-13] in different temperatures. The *ad libitum* pasta meal consisted of penne pasta (Everyday Value, Tesco, Dundee, UK) and chunky vegetable tomato sauce (Everyday Value, Tesco, Dundee, UK) cooked and prepared per manufacturer's instructions. The total energy content of the meal was 8326 kJ (1990 kcal) with 81.4% of energy from carbohydrate, 5.2% from fat and 13.4% from protein. None of the participants consumed the entire amount provided. The pasta meal was served warm, 10 minutes after preparation. Participants were instructed to serve their food into a separate bowl and were told: 'we ask that you continue eating until you have satisfied your hunger'. The participants had 30 min to consume the *ad libitum* pasta meal in an isolated area within the environmental chamber to remove any social influences. To determine the quantity eaten, the *ad libitum* meal was weighed pre- and post- consumption.

Calculations

Mean skin temperature was calculated using the following equation[28]: $T_{sk} = 0.3*(T_{arm}+T_{chest}) + 0.2*(T_{calf}+T_{thigh})$, where T_{sk} = mean skin temperature, T_{arm} = arm skin temperature, T_{chest} = chest skin temperature, T_{calf} = calf skin temperature and T_{thigh} = thigh skin temperature. Mean body temperature was calculated as follows[29]: $0.8(T_{rec}) + 0.2(T_{sk})$, where T_{rec} = core temperature and T_{sk} = mean skin temperature.

In addition to absolute concentrations, plasma hormone concentrations are presented relative to baseline concentrations (i.e., delta) to minimise the potential influence of day-to-day biological variation[10]. Total area under the curve (AUC) values for gut hormone, perceived appetite and thermoregulatory data were calculated using the trapezoid rule. Correcting for plasma volume change did not produce different results for significant gut hormone analyses; thus, the uncorrected data are provided.

Statistical analyses

Statistical analyses were completed using SAS (University Edition, SAS Institute, Inc., Cary, NC). Normality of the data were checked using Quantile-Quantile plots. Linear mixed models were used to examine differences in all outcome variables with either condition (for fasting data, AUC data and *ad libitum* energy intake) or condition and time (for perceived appetite, gut hormone, and thermoregulatory responses) included as fixed factors. All linear mixed models included a random effect for each participant and were adjusted for period (order) effects[30]. Baseline concentrations were included as a covariate for gut hormone and perceived appetite analyses, as recommended to minimise artifactual effects due to random differences at baseline[31]. Where significant condition and/or condition by time interactions were found, post hoc analysis was performed using the Holm–Bonferroni correction for multiple comparisons; data from each individual time point were compared between the conditions for significant condition by time interactions[32]. Sensitivity analyses were completed for the pre-lunch period (i.e., 0 to 4 h) for gut hormone and perceived appetite data to remove the possible confounding effect of the *ad libitum* lunch. Statistical significance was accepted as $P \leq 0.05$. Absolute standardised effect sizes (ES) are provided to supplement important findings (i.e., significant effects between the individual conditions), with 0.2 considered the minimum important difference, 0.5 moderate and 0.8 large[33]. Results are presented as mean ± standard deviation (SD) in the text and tables or mean ± standard error (SEM) in the figures for clarity.

Justification of sample size

Sample size estimations were based on our primary outcome variable, energy intake. For primary obesity prevention, an energy deficit of 418 kJ·day^{-1} (100 kcal·day^{-1}) is recommended to prevent excess weight gain in 90% of the U.S population; thus, we deemed this a clinically meaningful difference in *ad libitum* energy intake between the conditions[34]. The expected SD for energy intake at an *ad libitum* pasta meal in healthy men is ~460 kJ (~110 kcal) based on our previous work using an identical meal[35] and research using similar meals[36,37]. Based on these values, it was estimated that 13 participants would be needed to detect a meaningful between-condition difference in *ad libitum* energy intake (Cohen's d = 0.90) at 80% power and an alpha level of 0.017 to account for multiple comparisons using the Bonferroni correction. To account for a potential 20% drop-out rate, sixteen participants were recruited.

Results

Participant characteristics

The final sample included 13 participants. Three participants withdrew for the following reasons: time constraints (*n* 2) and feeling nauseous during cannulation insertion and blood draws (*n* 1). The physical characteristics of the final sample are shown in Table 1.

Thermoregulatory responses

Thermoregulatory responses for each experimental condition with the main effects of condition and time, the condition by time interaction and individual time point differences are shown in Fig. 1. Baseline thermoregulatory data did not differ between the conditions ($P \geq 0.132$). The significantly lower core temperature, mean skin temperature and mean body temperature in 10˚C versus 20˚C and 30˚C and in 20˚C versus 30˚C ($P < 0.0001$ for all) were complemented by large effect sizes (d = 1.12 to 6.19). Between-condition analyses of the AUC data for thermoregulatory variables produced similar results.

Indicators of hydration

Change in body mass from baseline to the cessation of each condition did not differ significantly between the conditions ($P = 0.915$). Plasma volume change from baseline differed significantly between the conditions ($P = 0.049$) and there was a significant condition by time interaction ($P = 0.006$), but no significant differences between the individual conditions or at any time points were found after adjusting for multiple comparisons ($P \geq 0.055$). The total volume of water consumed throughout each condition differed significantly between the conditions ($P = 0.003$). More water was consumed in 30˚C versus 10˚C and 20˚C (estimated marginal means (SEM): 1070 (164) mL for 30˚C, 485 (167) mL for 20˚C and 543 (171) mL for 10˚C; $P \leq 0.014$; d = 0.86 to 0.96).

Ad libitum energy intake

The mean and individual *ad libitum* energy intake responses for each experimental condition are shown in Fig. 2. *Ad libitum* energy intake was 1243 kJ (297 kcal) higher in 10˚C (P = 0.002; d = 0.82) and 1188 kJ (284 kcal) higher in 20˚C (P = 0.002; d = 0.79) when compared to 30°C with no significant difference between 10˚C and 20˚C (P = 1.000). All individual responses followed this pattern, with the exception that two participants had a higher energy intake in 30˚C versus 10˚C (see Fig. 2).

Perceived appetite

Baseline perceived appetite variables did not differ between the conditions ($P \geq 0.459$). All perceived appetite variables changed significantly over time ($P < 0.0001$), but there were no main effects of condition and no condition by time interactions ($P \geq 0.090$ for all). Perceived appetite AUC values did not differ between the conditions ($P \geq 0.415$ for all). Following sensitivity analyses for the pre-lunch period, the main effect of condition became significant for hunger, fullness, satisfaction, prospective food consumption and overall appetite ($P \leq 0.012$ for all). Pre-lunch hunger was lower in 10˚C versus 20˚C (P = 0.011; d=0.35), pre-lunch fullness was lower in 20˚C versus 30˚C (P = 0.010; d = 0.34), pre-lunch satisfaction was lower in 20˚C versus 30˚C (P = 0.007; d = 0.45), pre-lunch prospective food consumption was lower in 10˚C versus 20˚C (P = 0.002; d = 0.34) and in 30˚C versus 20˚C (P = 0.012; d = 0.35) and pre-lunch overall appetite was lower in 10˚C versus 20˚C (P = 0.019; d = 0.30) and in 30˚C versus 20˚C (P = 0.003; d = 0.37). The perceived overall appetite responses to each experimental condition are shown in Fig. 3.

Plasma acylated ghrelin, total PYY and total GLP-1 concentrations

Fig. 4 shows the delta gut hormone responses to the three experimental conditions with the main effects of condition and time, and the condition by time interaction. Plasma total GLP-1 data was available for nine out of the 13 participants due to funding reasons. Baseline gut hormone concentrations did not differ between the conditions ($P \geq 0.081$). There were no between-condition differences in AUC data for the gut hormones ($P \geq 0.099$). Sensitivity analyses for the pre-lunch period produced similar results.

Discussion

This was the first study to directly compare the acute appetite responses to cold, thermoneutral, and hot ambient temperatures under resting conditions. The main findings were that energy intake was

reduced during an *ad libitum* lunch in response to acute exposure to a hot compared with a cold and thermoneutral ambient temperature in healthy men. The reduced lunchtime energy intake in the heat compared with thermoneutral ambient temperature coincided with lower perceived appetite between breakfast and lunch, indicating that this may be an important mediating factor. Conversely, acylated ghrelin, total PYY and total GLP-1 did not appear to contribute to the reduced energy intake in the heat.

The lower energy intake in the hot compared to thermoneutral and cold ambient temperature in our study was found even after an acute exposure, i.e., the four hours preceding and during lunch. This extends previous research showing 48 to 60-hour exposures to higher ambient temperatures within a relatively narrow range (16 versus 22˚C, and 27 versus 22˚C) at rest in metabolic chambers reduce *ad libitum* energy intake[4,5], indicating that much shorter exposures can exert similar effects. Further, a 2-hour exposure to a warm (26–27°C) versus thermoneutral (19–20°C) environment resulted in a trend for a 100 kcal reduced energy intake[6]. It is possible that this difference was not significant because a sample size estimation was not completed (sample size was based on feasibility) and a parallel-group design (rather than a crossover design) was used, while the longer exposure duration and 'hot' rather than 'warm' temperature in our study could explain why we found a reduction in energy intake of almost three times the magnitude. Our findings also complement research showing reduced energy intake in the hours after exercise performed in hot compared to thermoneutral ambient temperatures[10], suggesting that such effects can be seen without the exercise component. Interestingly, studies showing no effect of exercise in hot ambient temperatures on energy intake have included Afro-Caribbean men who were acclimated to the heat and thus potentially not sensitive to it's appetite-suppressing effects[16], which may have also been the case in the Canadian Armed Forces members[15]. The lack of difference in energy intake between the cold and thermoneutral temperatures in our study is in accordance with research comparing a 2.5-hour 'mild cold' (18°C) with a thermoneutral (24°C) resting exposure[7]. Thus, there may not be a 'dose-response' relationship between ambient temperature during rest and energy intake, with effects seen in hot temperatures only. This lack of effect of resting in the cold also indicates that the increased energy intake in response to exercise performed in the cold[9-12] may have been due to an interaction with exercise, although some have found no effect when manipulating temperature during exercise[15]. The reason for the disparities between studies is likely related to inter-study differences in participant characteristics, study designs and methods. Given the limited data conducted during rest, future research is required to confirm our findings.

In addition to being statistically significant, the 297 and 284 kcal reductions in energy intake were associated with large and moderate effect sizes in the hot compared with cold and thermoneutral ambient temperatures, respectively, and exceed the 100 kcal·day^{-1} energy deficit that has been recommended to prevent excess weight gain in 90% of the US population[34]. Thus, if repeated on a daily basis, our findings may have clinical relevance for primary obesity prevention. Further, it was possible to almost triple this recommended daily energy deficit in just one meal during the day, suggesting that manipulating ambient temperature in the hours before and during lunch may only be required for one meal every three days. In terms of real-life application, temperature appears to be relatively practical to manipulate when compared with other environmental factors that influence appetite (e.g., altitude); for example, through heated rooms, clothing, sauna, hot baths, or outdoor exposure to hot climates. Further, the temperatures selected in our study simulated real life temperatures and are thus ecologically valid. That said, research with shorter exposure times or potentially serving the meal in a thermoneutral ambient temperature may improve the practical application of our findings. Given that most of the evidence has manipulated ambient temperature during exercise rather than rest, our findings may be particularly important for individuals who would benefit from reductions energy intake, but have barriers to performing exercise at the moderate to vigorous intensities required to elicit suppressions in appetite[20]. As there are reported acute cardiometabolic health benefits from acute energy restriction[38,39], our findings may also have clinical relevance for cardiometabolic disease prevention. That said, longer term trials are required to directly determine the impact on obesity and cardiometabolic disease risk.

Among the complex mechanisms that regulate energy intake, perceptions in appetite may have contributed to the reduction in energy intake in the hot ambient temperature in our study. When examining the 4-hour period from baseline to lunch, perceived appetite differed between the conditions, but not in a dose-response manner. As may be expected, perceived appetite was suppressed in the hot compared with thermoneutral ambient temperature, which may have contributed to the reported difference in *ad libitum* energy intake between these ambient temperatures. This finding aligns with research conducted under resting conditions, where perceived appetite was reduced at higher temperatures when comparing 28°C, 32°C, 36°C, and 38°C[8]. Somewhat in contrast to these findings, perceived appetite was also lower in the cold when compared with the thermoneutral ambient temperature; although, there was no difference in *ad libitum* energy intake between these ambient temperatures. Previous work has not compared perceived appetite responses to cold and thermoneutral temperatures under resting conditions, but has shown an increase in hunger in response to the 'mild cold', i.e., 18ºC versus 24ºC[7] or no difference when comparing 16ºC with 22ºC[4]. Taken together, our findings suggest that the

relationship between ambient temperature and appetite may not occur in a dose-response manner and it is possible that both hot and cold temperatures can suppress perceptions in appetite when compared with thermoneutral temperatures. In contrast, there is evidence that exercise performed in the cold stimulates perceived appetite[10,14,15] and exercise performed in the heat suppresses perceived appetite[10,15]. Thus, exercise and rest in the cold may exert different effects on appetite perceptions, a question that remains to be examined. It is also possible that the temperature of the *ad libitum* meal may partly explain the apparent discourse of the effects of the cold on perceived appetite and energy intake. Indeed, the *ad libitum* meal was served warm; as such, the increased energy intake in the cold may have occurred in an attempt to control core temperature regardless of appetite perceptions during the previous four hours. Likewise, the warm meal may have discouraged food intake during the hot condition. Thus, it would be interesting to determine whether similar findings would be seen with meals served cold or at room temperature. It should also be noted that appetite perceptions did not differ between the conditions when the post-lunch period was included. Thus, it appears that differences in lunchtime energy intake attenuated the effect of ambient temperature on perceived appetite, which would be worth investigating with longer duration post- *ad libitum* lunch periods.

In hot ambient temperatures, the reduced splanchnic blood flow and blood flow redistribution to the skin for heat dissipation has been proposed to alter the stimulation and secretion of gut-derived appetite hormones, which could, in turn, affect energy intake[10]. Yet, concentrations of acylated ghrelin, total PYY and total GLP-1 did not differ significantly between the different ambient temperatures in our study. For comparison, studies investigating gut hormones during rest in different ambient temperatures are sparse. Nevertheless, resting for 30 minutes at 2 °C and 30 °C increased and decreased plasma total ghrelin concentrations, respectively, compared with 20 °C in healthy men. Thus, perhaps our 'cold' condition was not extreme enough, or perhaps the disparity with our findings was because we measured acylated rather than total ghrelin[17]. Indeed, total ghrelin also decreased in response to 40 min of heat exposure during rest or exercise when compared to a thermoneutral ambient temperature in Afro-Caribbean men[16]. Somewhat in agreement with our findings, previous reports show that acute exposures to different ambient temperatures during exercise do not affect acylated ghrelin[10,13,19], although others show that exercise performed in the cold increases acylated ghrelin when compared with thermoneutral ambient temperatures[9,15]. Regarding PYY, our findings complement research showing no effect of ambient temperature during exercise on total PYY concentrations[14,15]; yet, others report that exercise performed in hot ambient temperatures increases total PYY concentrations[13]. These

inconclusive findings and the limited current research conducted at rest warrants future research on gut hormone responses to different ambient temperatures.

It is possible that variable nature of gut hormone responses limited our ability to detect significant differences between the different ambient temperatures in our study, particularly because our study was conducted at rest rather than exercise, where differences may be more pronounced due to the additional impact of the exercise bout[20]. As recommended, we controlled for baseline (fasting) differences within our analyses[31]. Nevertheless, it is important to note that there was a trend for lower baseline concentration of acylated ghrelin in the hot experimental condition (P = 0.081). Further, total plasma PYY did not follow what may be considered the 'usual' pattern over time in the thermoneutral ambient temperature due to the reduction to below fasting concentrations within four hours of consuming breakfast. As there were no outliers, it is difficult to explain this response. That said, a possible contributing factor may have been the slightly higher fasting concentration in the thermoneutral condition (the difference between the thermoneutral and hot and cold conditions was 3.8-4.8 times that of the difference between the cold and hot conditions). This could indicate that our attempts to minimise day-to-day variation in gut hormones (e.g., by replicating diet and minimising physical activity in the days prior to the experimental trials) may have not been sufficient. Indeed, inter- and intra-individual variability in appetite-related variables is a current topic of considerable interest and should be a serious consideration in future research[3,7,40].

It is unlikely that differences in hydration or water intake between the conditions were major contributing factors to the reported differences in energy intake in our study. Indeed, hydration status does not appear to affect subjective appetite or energy intake, regardless of subjective thirst and fluid intake[41,42]. Further, any differences in sweat loss and hydration status in our study were expected to be minimal as the participants remained sedentary throughout; this was supported by our finding that body mass and plasma volume change were not reduced in the hot ambient temperature when compared with the cold and thermoneutral temperature. Although some previous research on ambient temperature and appetite has controlled water consumption[14,16], the majority has permitted *ad libitum* water consumption, which attempts to replace potential water loss from sweating in the heat and improves ecological validity[4-6,9-11,13,15]. Thus, water was available *ad libitum* in our study, but, importantly, participants were not permitted water in the hour prior to or during the *ad libitum* lunch. Indeed, immediate pre-meal water consumption reduces *ad libitum* energy intake in young men[25], whereas consuming water 30 minutes prior to meal consumption does not[43]. As such, the reduced energy intake in the heat was most likely a direct effect of the

ambient temperature with the higher water consumption playing, if any, a very minimal contribution.

Limitations of our study include the acute exposure; thus, chronic interventions are required to examine whether the reduced energy intake in a hot ambient temperature is sustained over longer periods. Indeed, individuals may begin to compensate in terms of, not only energy intake, but other components of energy balance, including energy expenditure. On this note, the current study did not measure energy expenditure, which increases acutely in lower ambient temperatures through both resting and physical activity energy expenditure[4,5]. Additional factors not measured in this study may mediate the relationship between ambient temperature and energy intake include other appetite-regulating hormones (e.g., leptin), *ad libitum* meal temperature, thermal sensation and tolerance, gastric emptying[44], perceptions of hydration and thirst, pro-opiomelanocortin neurons in the hypothalamus via animal studies[45]; such factors require examination to provide a more comprehensive understanding of appetite-related responses to ambient temperature. Further, our study and previous research[13-15] has measured total PYY rather than PYY_{3-36}, which is more potent in stimulating satiety[46]. The lack of standardised clothing in our study increased the ecological validity of our finding and is line with previous research[10]. However, this approach could have limited the effect of ambient temperature between the conditions even though each exposure exerted the expected thermoregulatory responses. To allow comparisons with much of the related literature[4,10-14,16-17,19], our findings are based on young healthy men. Individual characteristics, such as sex and weight status, may affect appetite responses[3,20] and there is limited data in overweight/obese populations[9] and women[5]; thus, future research with such populations is needed.

In conclusion, findings from the present study show meaningful reductions in lunchtime *ad libitum* energy intake in response to acutely resting in a hot compared with thermoneutral and cold ambient temperature. Possible mediating factors for the reduction in energy in the hot compared with thermoneutral ambient temperature were reduced perceptions of appetite, whereas we found no evidence for a role of acylated ghrelin, total PYY or total GLP-1. Due to the distinct lack of data conducted under resting conditions, further research with different populations and exposure durations would be valuable.

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Conflict of Interest

None.

Authorship

J. K. Z.-F. and J. H. designed the study (project conception, development of overall research plan, and study oversight). R. N. H. and D. C. completed data collection (hands-on conduct of the experiments). J. K. Z-F. and R. N. H. completed data analyses. J. K. Z.-F. performed the statistical analyses and drafted the manuscript. J. H. and R. N. H. commented on the manuscript and J. K. Z.-F. had primary responsibility for final content. All authors have read and approved the final manuscript.

References

1. Scarborough P, Bhatnagar P, Wickramasinghe KK *et al.* (2011) The economic burden of ill health due to diet, physical inactivity, smoking, alcohol and obesity in the UK: an update to 2006-07 NHS costs. *J Public Health (Oxf)* **33**, 527-535.
2. Lean MEJ, Astrup A, Roberts SB (2018) Making progress on the global crisis of obesity and weight management. *BMJ* **361**, k2538.
3. Hopkins M & Blundell JE (2006) Energy balance, body composition, sedentariness and appetite regulation: pathways to obesity. *Clin Sci (Lond)* **130**, 1615-1628.
4. Westerterp-Plantenga MS, van Marken Lichtenbelt WD, Strobbe H *et al.* (2002) Energy metabolism in humans at a lowered ambient temperature. *Eur J Clin Nutr* **56**, 288-296.
5. Westerterp-Plantenga MS, van Marken Lichtenbelt WD, Cilissen C *et al.* (2002) Energy metabolism in women during short exposure to the thermoneutral zone. *Physiol Behav* **75**, 227-235.
6. Bernhard MC, Li P, Allison DB *et al.* (2015) Warm ambient temperature decreases food intake in a simulated office setting: a pilot randomized controlled trial. *Front Nutr* **24**, 2:20.
7. Langeveld M, Tan CY, Soeters MR *et al.* (2016) Mild cold effects on hunger, food intake, satiety and skin temperature in humans. *Endocr Connect* **5**, 65-73.
8. Zheng G, Li K, Wang Y (2019) The effects of high-temperature weather on human sleep quality and appetite. *Int J Environ Res Public Health* **18**, 16(2). pii: E270.
9. Crabtree DR & Blannin AK (2015) Effects of exercise in the cold on ghrelin, PYY, and food intake in overweight adults. *Med Sci Sports Exerc* **47**, 49-57.
10. Wasse LK, King JA, Stensel DJ *et al.* (2013) Effect of ambient temperature during acute aerobic exercise on short-term appetite, EI, and plasma acylated ghrelin in recreationally active males. *Appl Physiol Nutr Metab* **38**, 905-909.
11. White LJ, Dressendorfer RH, Holland E *et al.* (2005) Increased caloric intake soon after exercise in cold water. *Int J Sport Nutr Exerc Metab* **15**, 38-47.
12. Dressendorfer RH (1993) Effect of internal body temperature on energy intake soon after aerobic exercise. *Med Sci Sports Exerc* **25**, Suppl. 5, S42.
13. Shorten AL, Wallman KE, Guelfi KJ (2009) Acute effect of environmental temperature during exercise on subsequent EI in active men. *Am J Clin Nutr* **90,** 1215-1221.
14. Kojima C, Sasaki H, Tsuchiya Y *et al.* (2015) The influence of environmental temperature on appetite-related hormonal responses. *J Physiol Anthropol* **34**, 22.
15. Mandic I, Ahmed M, Rhind S *et al.* (2019) The effects of exercise and ambient temperature on dietary intake, appetite sensation, and appetite regulating hormone concentrations. *Nutr Metab (Lond)* **16**, 29.

16. Faure C, Charlot K, Henri S *et al.* (2016) Effect of heat exposure and exercise on food intake regulation: A randomized crossover study in young healthy men. *Metabolism* **65**, 1541-1549.
17. Tomasik P, Sztefko K, Pizon M (2005) The effect of short-term cold and hot exposure on total plasma ghrelin concentrations in humans. *Horm Metab Res* **37**, 189-190.
18. Broglio F, Gottero C, Prodam F *et al.* (2004) Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. *J Clin Endocrinol Metab* **89**, 3062-3065.
19. Laursen TL, Zak RB, Shute RJ *et al.* (2017) Leptin, adiponectin, and ghrelin responses to endurance exercise in different ambient conditions. *Temperature (Austin)* **4**, 166-175.
20. Hazell TJ, Islam H, Townsend LK *et al.* (2016) Effects of exercise intensity on plasma concentrations of appetite-regulating hormones: Potential mechanisms. *Appetite* **98**, 80-88.
21. Gallagher D, Heymsfield SB, Heo M *et al.* (2000) Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *Am J Clin Nutr* **72**, 694–701.
22. Bray GA. Contemporary Diagnosis and Management of Obesity and the Metabolic Syndrome. Newtown, PA: Handbooks in Health Care (2003).
23. Sawka MN, Burke LM, Eichner ER *et al.* (2007) American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* **39**, 377-390.
24. Stubbs RJ, Hughes DA, Johnstone AM *et al.* (2000) The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* **84**, 405-415.
25. Corney RA, Sunderland C, James LJ (2016) Immediate pre-meal water ingestion decreases voluntary food intake in lean young males. *Eur J Nutr* **55,** 815-819.
26. Dill D & Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Phys* 37, 247-248.
27. Hosoda H, Doi K, Nagaya N *et al.* (2004) Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clin Chem* **50**, 1077-1080.
28. Ramanathan NL (1963) A new weighting system for mean surface temperaature of the humn body. *J Appl Phys* **19**, 531-533.
29. Colin J, Timbal J, Houdas Y *et al.* (1971) Computation of mean body temperature from rectal and skin temperatures. *J Appl Physiol* **31**, 484–489.
30. Senn S (1993) *Cross-over trials in clinical research*. Chichester, UK: Wiley.

31. Blundell J, de Graaf C, Hulshof T *et al*. (2010) Appetite control: methodological aspects of the evaluation of foods. *Obes Rev* **11**, 251-270.
32. Atkinson G (2002) Analysis of repeated measurements in physical therapy research: multiple comparisons amongst level means and multifactorial designs. *Phys Ther Sport* **3**, 191–203.
33. Cohen J (1988) *Statistical Power Analysis for the Behavioural Sciences*, 2nd ed. Hillsdale, NJ, USA: Lawrence Erlbaum Associates.
34. Hill JO, Peters JC, Wyatt HR (2009) Using the energy gap to address obesity: a commentary. *J Am Diet Assoc* **109**, 1848-1853
35. Hough J, Esh C, Mackie P *et al.* Daily-running exercise may induce incomplete energy intake compensation: a 7-day crossover trial. *Appl Physiol Nutr Metab*. Published online: 13 Deccember 2019. doi: 10.1139/apnm-2019-0603.
36. Chowdhury EA, Richardson JD, Tsintzas K *et al.* (2015) Carbohydrate-rich breakfast attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in lean adults. *Br J Nutr* **114**, 98-107.
37. Gregersen NT, Flint A, Bitz C *et al.* (2008) Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *Am J Clin Nutr* **87**, 1277-1281.
38. Antoni R, Johnston KL, Collins AL *et al.* (2016) Investigation into the acute effects of total and partial energy restriction on postprandial metabolism among overweight/obese participants. *Br J Nutr* **115**, 951-959.
39. Maraki M, Magkos F, Christodoulou N *et al.* (2010) One day of moderate energy deficit reduces fasting and postprandial triacylglycerolemia in women: the role of calorie restriction and exercise. *Clin Nutr* **29**, 459-463.
40. Goltz FR, Thackray AE, Atkinson G *et al*. (2019) True Interindividual Variability Exists in Postprandial Appetite Responses in Healthy Men But Is Not Moderated by the FTO Genotype. *J Nutr* **149**, 1159-1169.
41. Corney RA, Horina A, Sunderland C *et al*. (2015) Effect of hydration status and fluid availability on ad-libitum energy intake of a semi-solid breakfast. *Appetite* **91**, 399-404.
42. Corney RA, Sunderland C, James LJ (2015) The effect of hydration status on appetite and energy intake. *J Sports Sci* **33**, 761-768.
43. Van Walleghen EL, Orr JS, Gentile CL *et al*. (2007) Pre-meal water consumption reduces meal energy intake in older but not younger subjects. *Obesity* **15**, 93-99.
44. Stengel A, Goebel M, Luckey A *et al.* (2010) Cold ambient temperature reverses abdominal surgery-induced delayed gastric emptying and decreased plasma ghrelin levels in rats. *Peptides* **31**, 2229-2235.

45. Vicent MA, Mook CL, Carter ME (2018) POMC neurons in heat: A link between warm temperatures and appetite suppression. *PLoS Biol* **16**, e2006188.
46. Chelikani PK, Haver AC, Reidelberger RD (2004) Comparison of the inhibitory effects of PYY(3-36) and PYY(1-36) on gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* **287**. R1064-1070.

Figure legends

Fig. 1 Thermoregulatory responses in the 10˚C, 20˚C and 30˚C experimental conditions using a Latin square design (*n* 13). A standardised breakfast meal was consumed at 0 h and an *ad libitum* lunch meal was consumed at 4 to 4.5 h. Values are means with error bars to represent the standard error of mean (SEM). *Significant main effect of experimental condition and significant condition by time interaction using linear mixed models ($P < 0.0001$). $^{\#}$Significant main effect of time using linear mixed models ($P \leq 0.0002$). Significant difference using linear mixed models with the Holm–Bonferroni correction for multiple comparisons between: †10˚C and 30˚C; $^{\delta}$20˚C and 30˚C; $^{\phi}$10˚C and 20˚C ($P \leq 0.050$ for all).

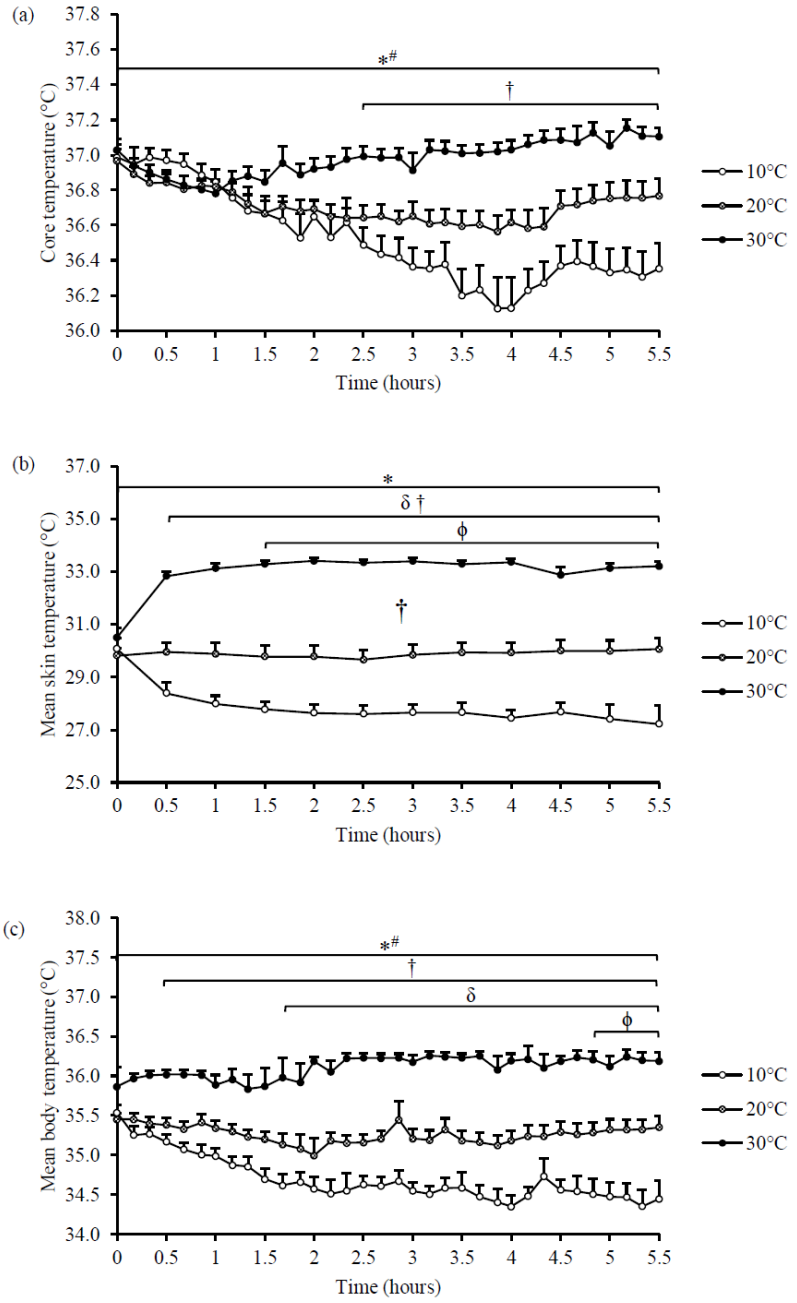

Fig. 2 *Ad libitum* energy intake in the 10˚C, 20˚C and 30˚C experimental conditions using a Latin square design (*n* 13). Bars represent means with standard error of means (SEMs) represented by error bars. Lines represent individual responses. *Significant main effect of condition (P = 0.001) with higher *ad libitum* energy intake in 10˚C and 20˚C compared with 30˚C using linear mixed models with the Holm–Bonferroni correction for multiple comparisons (P = 0.002).

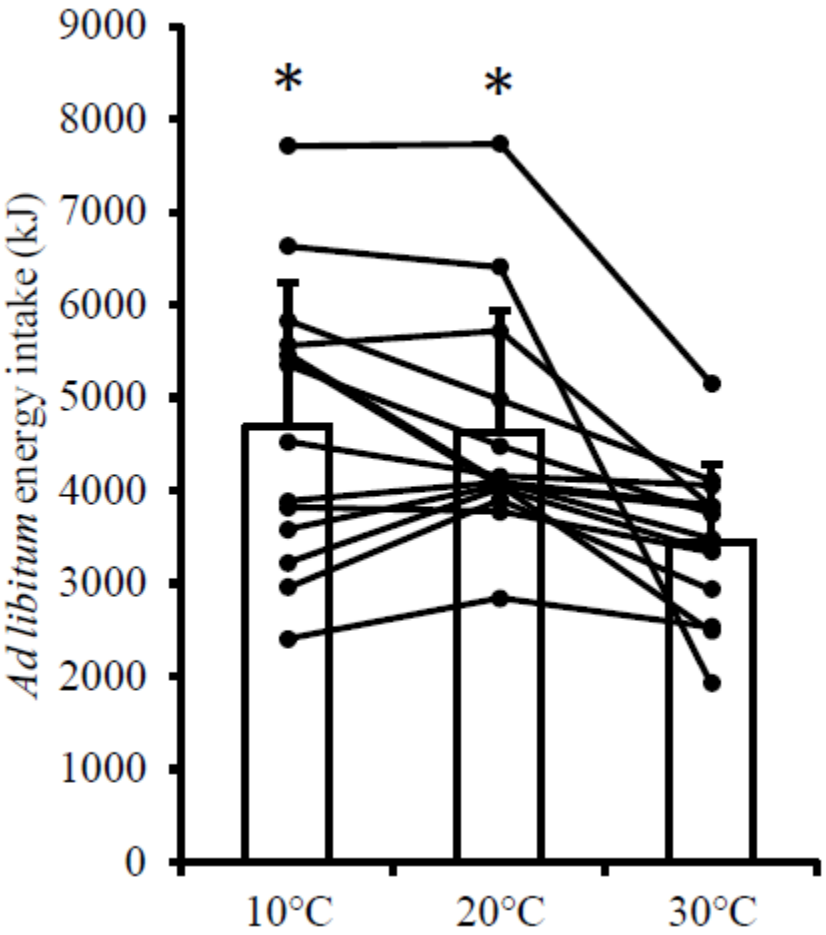

Fig. 3 Perceived overall appetite in the 10˚C, 20˚C and 30˚C experimental conditions using a Latin square design (*n* 13). A standardised breakfast meal was consumed at 0 h and an *ad libitum* lunch meal was consumed at 4 to 4.5 h. Values are means with error bars to represent the standard error of mean (SEM). Sensitivity analyses using linear mixed models for the pre-lunch period showed a significant difference between: *20˚C and 30˚C; †10˚C and 20˚C ($P \leq 0.019$).

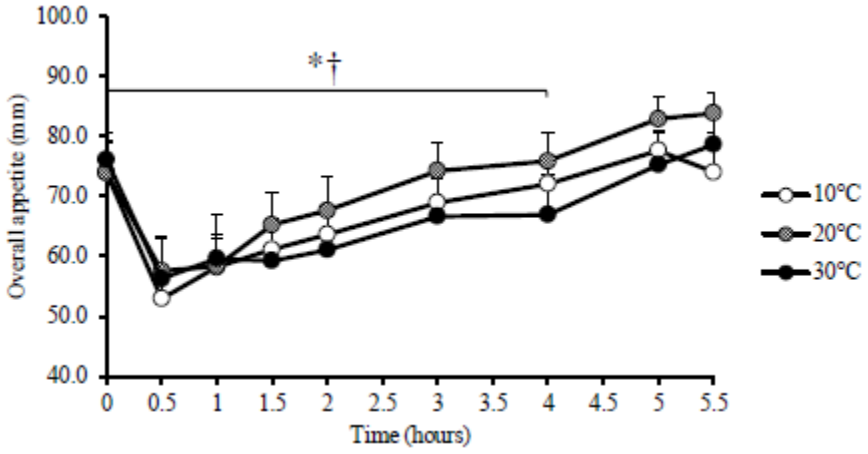

Fig. 4 Plasma concentrations of delta (i.e., change from baseline) acylated ghrelin (a), total PYY (b) and total GLP-1 (c) for the 10˚C, 20˚C and 30˚C experimental conditions using a Latin square design. A standardised breakfast meal was consumed at 0 h and an *ad libitum* lunch meal was consumed at 4 to 4.5 h. Values are means with error bars to represent the standard error of mean (SEM). *Significant main effect of time ($P \leq 0.032$), non-significant main effect for condition and non-significant condition by time by interaction ($P \geq 0.303$) regardless of whether expressed as delta or absolute concentrations.

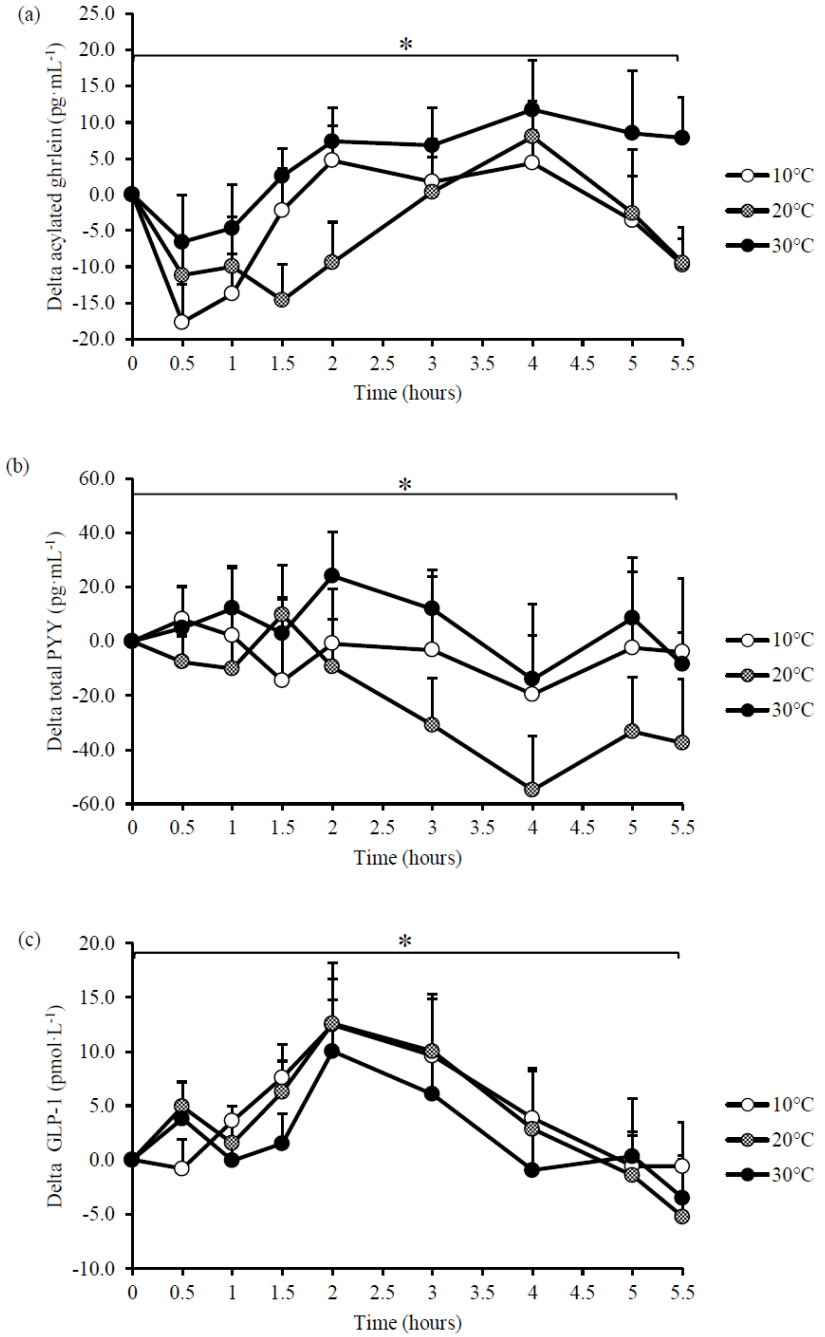

Table 1. Participant characteristics[1]

	Mean	SD
Age (y)	21.5	1.4
Stature (m)	1.77	0.05
Body mass (kg)	77.3	9.8
Body fat %	15.5	3.1
BMI ($kg \cdot m^{-2}$)	24.7	2.2

[1] *n* 13.