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Effect of heat exposure and exercise on food intake regulation: a randomized crossover study in young healthy men

Cécile Faure\textsuperscript{a}, Keyne Charlot\textsuperscript{a,h,c}, Stéphane Henri\textsuperscript{a,d}, Marie-Dominique Hardy-Dessources\textsuperscript{b,c}, Olivier Hue\textsuperscript{a}, Sophie Antoine-Jonville\textsuperscript{a}

\textsuperscript{a} Adaptation to Tropical Climate, Exercise and Health Laboratory, EA3596, University of the French West Indies, Pointe-à-Pitre, Guadeloupe, France
\textsuperscript{b} UMR Inserm 1134, University of the French West Indies, Guadeloupe
\textsuperscript{c} France-Laboratory of Excellence GR-Ex (The red cell: from genesis to death), PRES Sorbonne Paris Cité, Paris, France
\textsuperscript{d} Orthopedics and Trauma Center, Academic Hospital of Pointe-à-Pitre-Abymes, Guadeloupe, France

Corresponding author:
Dr. Sophie Antoine-Jonville
Laboratoire ACTES, Université des Antilles et de la Guyane
BP 250
97157 POINTE A PITRE Cédex
GUADELOUPE
s_anoine@ymail.com sophie.jonville@univ-ag.fr
Tel: (+ 590) 590 48 31 75 Fax: (+ 590) 590 48 31 79

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ABSTRACT

Objective
The effect of physical activity on food intake regulation may be moderated by environmental temperature. The aim of the study was to determine the single and combined effects of metabolic activity and temperature on energy intake and its hormonal regulation.

Methods
A randomized crossover study was conducted in the laboratory. Ten healthy and physically active young Afro-Caribbean men participated in four experimental sessions (rest at 22°C and 31°C and cycling at 60% of their maximal oxygen uptake at 22°C and 31°C, all for 40 minutes). Each test period was followed by a 30-minute recovery period and then an *ad libitum* meal. The main outcome measures were energy balance, subjective appetite, and plasma pancreatic polypeptide (PP), cholecystokinin (CCK) and ghrelin concentrations.

Results
Relative energy intake was significantly decreased whereas plasma PP was increased in the exercise conditions (*p*<0.004 and *p*<0.002, respectively). Postprandial levels of CCK were elevated only in the rest conditions. Exposure to heat induced a decrease in plasma ghrelin (*p*<0.031).

Conclusions
Exercise induced a short-term energy deficit. However, modifications in the hormonal regulation of food intake in response to short-term heat or heat and exercise exposure seem to be minor and did not induce changes in energy intake.

This trial was registered at clinicaltrials.gov as NCT02157233.

Abbreviations. **PP**: pancreatic polypeptide; **CCK**: cholecystokin; **rest-22°C**: rest at 22°C; **rest-31°C**: rest at 31°C; **ex-22°C**: exercise at 22°C; **ex-31°C**: exercise at 31°C; **V̇O₂max**: maximum oxygen uptake.
maximal oxygen uptake

Key words. Physical activity; Food intake regulation; Environment; Energy balance;
Metabolism
1. INTRODUCTION

Overweight and obesity have reached epidemic proportions throughout the world and are now the fifth leading risk for death worldwide [1]. The prevalence of their associated comorbidities has also risen, further increasing the healthcare costs [2]. A better understanding of the underlying mechanisms, starting with studies on healthy individuals, is crucial for the development of strategies to manage this epidemic. Along with genetic, physiological and behavioral factors, a fundamental cause of overweight and obesity is an imbalance between energy intake and expenditure. Mounting evidence suggests that the increased intake of energy-dense foods and the decrease in physical activity due to increasingly sedentary lifestyles are the main explanations for this imbalance [2].

The general mechanisms of energy balance regulation are well known, with most data coming from studies performed in temperate climates. The mechanisms rely mainly on the integration of signals that reflect the metabolic state of peripheral tissues. These signals are either orexigenic, like ghrelin, or anorexigenic, like pancreatic polypeptide (PP), cholecystokinin (CCK) or peptide YY [3]. The mechanisms of short-term post-exercise food intake regulation have been particularly informative. An increase in the secretion of anorexigenic factors and an inhibition of ghrelin secretion, which is orexigenic, have been described in subjects after they perform an acute exercise bout [4] or in response to overfeeding plus exercise [5]. These mechanisms contribute to a relative food intake inhibition (energy intake after accounting for the extra energy expended during the exercise), while an acute exercise bout has no meaningful effect on the subsequent absolute energy intake [6].

The effect of physical activity on energy intake is moderated by other factors, as well, including exercise intensity and duration, training status, sex, amount of body fat [6,7]. Among these factors, environmental temperature in which the exercise task is performed has...
not been extensively studied. However, further investigation on this factor is relevant for the
understanding of energy intake behaviors. Based on the perturbations of human [8,9] and
animal [10] feeding behaviors reported in hot environment, we hypothesized that heat and
exercise would interact to reduce relative energy intake. The aim of the study was thus to
determine the single and combined effects of metabolic activity and environmental
temperature on energy intake and its hormonal regulation.

2. SUBJECTS AND METHODS

2.1. Ethical approval

This trial was registered at clinicaltrials.gov as NCT02157233 and approved by the Regional
Ethics Committee (French Human Subject Review Committee of Limoges, France: CPP13-
018a/2013-A01037-38). The experiments were performed in accordance with the guidelines
set by the Declaration of Helsinki and written informed consents to participate in the study
were obtained from all subjects. The study sponsors were not involved in study design,
collection and analysis of data, writing the report or the decision to submit the manuscript for
publication.

2.2. Subjects

Participants were recruited from October 2013 to November 2014 by the research manager.
Advertisements posted on the University of the French West Indies Guadeloupian campus and
in surrounding sports centers were used.

Ten healthy and physically active men (age: 20.7 ± 1.7 yrs, height: 1.80 ± 0.06 m, body mass:
69.2 ± 7.1 kg, body mass index: 21.3 ± 1.7 kg.m⁻², fat mass: 7.2 ± 5.3 %) were included in the
study. They were all of Afro-Caribbean origin.
Subjects had stable body mass (±2 kg for more than 1 year, determined by self-report). They were nonsmokers, without major chronic disease (i.e., diabetes, coronary heart disease), and not taking medications known to influence food intake or body mass. They reported normal body mass at birth and were born at term. They had lived in the Caribbean area for more than 3 months and were thus well acclimated to a tropical climate. They reported no food allergies or restrictions and screening had excluded individuals with eating disorders [11]. They trained regularly with a mixture of endurance and resistance exercises. Their physical activity was within a range of 2000-6000 METs.min/week (2100-6700 kcal/week). The subjects were not aware of the specific purpose of the study.

2.3. Experimental design

The subjects underwent a familiarization and testing session, during which their maximal oxygen uptake (\(\text{VO}_{2\text{max}}\)) and anthropometric data were measured. They then participated in the experimental trials, which were administered in a randomized counterbalanced design over 4 days: a resting control in a neutral environmental temperature (rest-22°C), a resting control in a hot temperature (rest-31°C), exercise (cycling) in a neutral environmental temperature (ex-22°C) and exercise in a hot temperature (ex-31°C). They were instructed to have similar energy intake and to abstain from high-intensity exercise and alcohol the day before each session. This was carefully checked with food and activity journals. Sessions would be postponed in case of unacceptable quality or quantity dissimilarity. Subjects reported to the laboratory at 6:30 am after an overnight fast on four occasions at least one week apart.

The experiment was divided into three parts (Fig. 1): the "test" of 40 minutes in either the rest or the exercise condition, followed by a 30-minute rest period, and then an \textit{ad libitum} meal of 30 minutes. During the control trials, the subjects lay on an examination table for 40 minutes.
The exercise trials consisted of a 40 minutes cycling (Monark Weight Ergometer 814 E, Varberg, Sweden) at 60% of their \( \dot{V}O_{2\text{max}} \). The environmental temperatures were set at 22°C and 31°C for neutral and hot temperature, respectively, with 45% relative humidity, and stable temperature and humidity throughout each session.

Hydration was controlled during the entire study, as follows. During the 40-minute test period, the subjects were given 200 ml of water when they were resting and 400 ml of water when exercising, and they could drink as much as they wanted to. At T40, they were weighed and given water in an amount equal to that of the loss in body mass, to compensate the water loss. The water absorbed during the recovery period to compensate water loss was recorded. During the \textit{ad libitum} meal at the end of sessions, they had a quantity of water corresponding to 4 ml water/kg body mass.

### 2.4. Anthropometric data

#### 2.4.1. Body mass, height and body mass index (BMI)

Body mass was measured without clothing, once before the test (physical activity or resting control) and once after, to determine mass loss due to sweating. Prior to bioelectric impedance analysis, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was then calculated.

#### 2.4.2. Bioelectric impedance analysis

The subjects underwent bioelectric impedance analysis, using an InBody S10 body composition analyzer with InBody 3.0 software (BioSpace) to determine their body fat mass. They lay supine and body mass and height were input into the analyzer. This instrument uses eight tactile electrodes, two of which are in contact with the thumb and middle finger of each hand, and two in contact with the bilateral aspects of the ankle joint of each foot.
2.5. Cardiopulmonary assessment

On their first visit to the laboratory, the subjects' $\dot{V}O_{2\text{max}}$ was measured with an incremental exercise test to exhaustion, performed on a cycle ergometer (Monark Weight Ergometer 814E, Varberg, Sweden). The increments were set at 15-20 W per minute according to the expected physical fitness so as to reach exhaustion within 12 minutes as recommended [12]. Complete metabolic data were collected using a breath-by-breath ergospirometry system (Metalyzer ® 3B, Cortex Biophysik GmbH, Germany). They had a $\dot{V}O_{2\text{max}}$ of 47.1 ± 7.4 ml.min$^{-1}$.kg$^{-1}$.

During the four experimental sessions, heart rate (PolarVantage, Polar Electro Oy, Kempele, Finland), oxygen consumption and respiratory exchange ratio (RER) (Metalyzer ® 3B, Cortex Biophysik GmbH, Germany) were assessed regularly (Fig. 1).

2.6. Core temperature and thermal comfort

The tympanic membrane is supplied with blood from the internal carotid artery just like the hypothalamus. This cranial location thus served as a substitute for the measurement of the inaccessible hypothalamic temperature [13].

Core temperature was recorded during the tests with a tympanic temperature probe (Smiths Medical, St Paul, MN, USA) placed in the aural canal near the tympanic membrane. The tympanic probe was held in position and isolated from the external environment with cotton, surgical tape (Transpore; 3M, London, Ontario, Canada), and an ear defender (model 1000; Mastercraft, Bobcaygeon, Ontario, Canada) as described by others [14].

Subjects were asked to assess their thermal comfort using a visual analog scale (VAS).
2.7. Energy balance

2.7.1. Energy expenditure

Energy expended during the tests was determined by indirect calorimetry (Metalyzer® 3B, Cortex Biophysik GmbH, Leipzig, Germany) and calculated using the Weir equation.

2.7.2. Assessment of energy intake

Prior to the study, individuals who were eligible for study inclusion were asked to describe what they usually ate for breakfast. Test meal items were evaluated for palatability before initiation of the study. We thus tried to offer the subjects familiar foods for breakfast in order to be as close as possible to the real-life meal situation. Indeed, Blundell et al. reported that this procedure results in a better assessment of energy intake [15].

Forty minutes after each experimental trial, the subjects were thus presented with an array of ham and cheese sandwiches of known energy composition (327 kcal/100g, according to the French food composition table [16]) for 30 minutes, during which time they were instructed to eat ad libitum until satiety was reached. The foods provided were in excess of the expected intakes.

Total energy intake from the breakfast meal was calculated by weighing all remaining food products after the participant had left the laboratory. This method has been shown to be reliable for assessing energy intake [17]. Total kilocalories were recorded. In addition, relative energy intake was calculated by correcting post-exercise energy intake for the energy cost of the exercise session as follows: \( \text{Rel EI} = \text{EI} - \text{EE} \), where Rel EI is the relative energy intake, EI is the absolute energy intake during the \textit{ad libitum} meal, and EE is the energy expenditure during the 40-minute test period.
2.8. Subjective appetite sensations

A VAS was used to measure subjective appetite sensations. Subjects were asked to rate their hunger (How hungry do you feel? “not hungry at all” to “as hungry as I’ve ever felt”), desire to eat (How strong is your desire to eat?: “very weak” to “very strong”), fullness (How full do you feel? “not full at all” to “very full”), and prospective food consumption (How much do you think you can eat? “nothing at all” to “a large amount”). The composite appetite score (CAS) reflects the responses to the four VAS questions and was included in the study as a summary measure of appetite. CAS was calculated using the following formula: \[ \text{CAS} = \frac{(100 - \text{satiety}) + (100 - \text{fullness}) + \text{desire to eat} + \text{prospective food intake}}{4} \] [18].

2.9. Hormones involved in food intake regulation

Upon arrival, a catheter was inserted into an antecubital vein for blood sampling. Venous blood was drawn into Vacutainer tubes containing EDTA before, during, and after rest/exercise and in the postprandial state. The blood was transferred from the Vacutainer tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases. After centrifugation at 1,600xg for 15 minutes at 4°C, the plasma was collected and kept at -70°C until analysis. Plasma PP, CCK and ghrelin concentrations were measured from these samples, using Enzyme Immunoassay kits and according to the manufacturer’s protocols (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA).

2.10. Statistics

The required number of participants was \textit{a priori} calculated using G*power 3.1 for Mac. Alpha error probability threshold was 0.05. The calculations were performed based on expected relative energy intake, with different effect size and different power between 0.80
and 0.95, and 0.85 for correspondence among repeated measures. Resulting required sample size varied. Ten appeared as feasible and sufficient to evidence an effect size of 0.4, with 0.80 power. All results were analyzed with the SPSS v.20 software package (SPSS Inc., Chicago, IL, USA). A probability value of $p<0.05$ was considered statistically significant. Data are presented as means ± SDs except in figures where SEMs are used.

Three-factor repeated-measures analyses of variance were performed to analyze the effects of metabolic activity (2 levels: rest and exercise), ambient temperature (2 levels: neutral and hot), time (4, 6 or 8 levels), and their interaction on plasma hormone concentrations, tympanic temperature, thermal comfort, composite appetite sensations and cardiopulmonary data.

Two-factor repeated-measures analyses of variance were performed to analyze the effects of metabolic activity, ambient temperature and their interaction on energy expenditure, absolute energy intake, relative energy intake and water intake.

Data were tested for sphericity using Mauchly's test and if the assumption of sphericity was violated, the Greenhouse–Geisser correction was undertaken to adjust the degrees of freedom. Tukey's post-hoc tests were performed to identify mean differences among conditions when the effect of time x metabolic activity, time x temperature, or time x temperature x metabolic activity was significant. Otherwise, only significant simple effects or interaction effects are reported.

3. RESULTS

3.1. Cardiopulmonary assessment (Fig. 2)

Oxygen uptake and RER (Fig. 2a and 2c) were affected by the metabolic activity, time and their interaction ($p<0.001$). Heart rate (Fig. 2b) was significantly increased in the exercise conditions ($p<0.001$). It was also higher in the hot conditions than in the neutral ones.
There was an effect of time (p<0.001) on heart rate, and the interaction of metabolic activity x temperature x time was significant (p<0.043).

### 3.2. Temperature and thermal comfort

The variation from T0 tympanic temperature was significantly affected by the interaction of metabolic activity x temperature x time was significant (p=0.001), exclusively due to higher values at all times in the Ex-31°C session as compared to the others (all p<0.001). Metabolic activity, time, temperature and their interactions significantly affected thermal comfort (Fig. 3), except for the metabolic activity x temperature x time interaction, which was not significant.

### 3.3. Water intake

Water intakes during the 40-min test, water intakes to compensate water loss and during the meal and total water intakes are reported in table 1. Exercise increased water intake during the 40-minute test period (p<0.001) and during the recovery to compensate water loss (p=0.002), as well as the total water intake (p<0.001). Water intake during the 40-minute test period and total water intake were also affected by temperature (p<0.001 and p=0.019, respectively). The combined effects of metabolic activity and temperature on total water intake approached significance (p=0.072).

### 3.4. Energy balance and appetite

The energy expended during the 40-minute test periods was 45 ± 6, 46 ± 6, 389 ± 22 and 385 ± 26 kcal, in the rest-22°C, rest-31°C, ex-22°C and ex-31°C conditions, respectively. It was only affected by metabolic activity (p<0.001). Absolute energy intake did not differ between the four experimental conditions (1042 ± 330, 1039 ± 217, 1156 ± 236 and 1090 ± 296 kcal in
the rest-22°C, rest-31°C, ex-22°C and ex-31°C conditions, respectively), neither was energy intake reported to body mass (all p>0.258 for metabolic activity, temperature and interaction).

The relative energy intake (Fig. 4) was significantly decreased in the exercise conditions compared with the rest conditions (p=0.004).

The composite appetite sensation (Fig. 5) was only affected by time (p<0.001). The effect of temperature did not reach significance (p=0.073), with a trend towards reduced appetite in the hot conditions.

3.5. Markers of food intake regulation

Plasma PP (Fig. 6) was increased in the exercise conditions (p=0.002) compared with rest conditions. It was also affected by time (p<0.001), with postprandial PP being higher than at all other time points.

CCK (Fig. 7) was affected by time (p=0.005), with a significant increase at T100 compared with T40 and T55 (p=0.006 and 0.002, respectively). The metabolic activity x time interaction was also significant (p=0.005).

The meal induced an increase in CCK only in the rest conditions (p=0.006 and p<0.001 for rest-22°C and rest-31°C, respectively). In the rest-31°C condition, postprandial CCK was higher than at all other time points (p<0.001).

Plasma ghrelin (Fig. 8) was affected by the environmental temperature, with higher levels in the neutral conditions (p=0.031).
4. DISCUSSION

The main findings of this first randomized crossover study including rest and exercise in neutral and hot ambient temperatures and focused on energy balance regulation were: 1) exercise induces a decrease in the relative energy intake; 2) heat does not further modify this effect in these acclimated subjects; 3) CCK and PP were differently affected by the metabolic activity, while ghrelin was decreased in the heat.

A suppressive effect of exercise on appetite, referred to as short-term exercise-induced anorexia, has been described [19]. The anorexic effect of exercise is more likely to be observed in obese/overweight or sedentary individuals. Highly fit and physically active people do not exhibit the same response to exercise [6,20,21]. Our findings confirm the general observation that absolute energy intake is not affected by metabolic activity, although this observation is not unanimous.

Nevertheless, the increase in energy expenditure caused by increased metabolic activity was not compensated for by increased energy intake. A few studies have reported that increased energy requirements do not always immediately trigger increased energy intake and appetite and, consequently, exercise is likely to induce negative energy balance [6]. The cause of this exercise-induced energy deficit is not precisely known but several mechanisms have been proposed. They include the improvement of satiety by increasing the postprandial sensitivity to ingested nutrients consumed in post-exercise meals [22].

Such an energy deficit, if repeated over a longer period, could have significant implications in terms of weight management. The question can also be analyzed from the point of view of the resting sessions, which are likely to induce positive energy balance on longer term. This study fuels the idea of an inability to compensate for inactivity by reducing energy intake [22].
Contrary to our hypothesis, the environmental temperature did not seem to modify the energy intake subsequent to exercise in these acclimated subjects. Similarly to what we observed, a previous study reported no difference in absolute and relative energy intake after exercise performed in the heat and a neutral environment [8].

Given its randomized crossover design, this study could investigate the mechanisms of energy balance after exercise in the heat. The focus on CCK and PP was motivated by the putative theoretical impact of heat exposure on gastro-intestinal tract releases due to blood flow redistribution. We observed an elevation of these anorexigenic factors (i.e., CCK and PP) in response to food intake, as expected [23,24]. Total ghrelin levels known as an orexigenic factor, however, were not affected by food intake. Similar observations were reported, with no modification in ghrelin levels 30 minutes after the ingestion of a meal, following rest or exercise [25]. Although there is a close relationship between total and the biologically active appetite stimulating acylated ghrelin it can not be excluded that after exercise and/or exposure to heat this relationship is somewhat different.

We found small effects of exercise on the appetite hormone levels, as reported in other studies [4]. CCK and PP, the two anorexigenic factors measured in our study, seem to be differently affected by metabolic activity. Whereas PP was higher in the exercise conditions, CCK increased only after food intake in the rest conditions. The increased secretion of PP in the exercise conditions is in accordance with a recent meta-analysis [6]. This is very compatible with previous observations reporting that not only does acute exercise increase fasting plasma PP [26], but it also raises postprandial PP levels [27].

Unlike for PP, very few data are available on the effects of acute exercise on CCK. Observations partly contrary to our finding have been reported, with one study suggesting that acute exercise increased plasma CCK levels [28]. The main reason for this discrepancy stands
probably in the differences of intensity and duration. The study by Bailey et al. involves
maximal incremental exercise performed in less than 20 minutes, which generates metabolic
stimuli not comparable to the ones raised by our exercise conditions. Our results are similar to
the findings for chronic exercise, with stable levels of CCK [28] while those of PP are
increased [29]. Moreover, one study reported that CCK levels were reduced in response to
food intake in women athletes, as compared with sedentary controls, when energy intake was
increased [30]. This could be paralleled to the reduced levels of postprandial CCK after
exercising, and suggests that energy expenditure may modify the CCK response to food
intake. We are not aware of investigations on CCK in response to exercise in the heat. To our
knowledge, we are the first to provide evidence that hot environmental temperature does not
further modify the effect of exercise on CCK levels. This would mean that despite probable
different blood flow in the gastro-intestinal tract, CCK release would be preserved. CCK then
appears as a robust anorexigenic factor maintaining its function whatever the ambient
temperature variation (within the range we tested here). Also, this discards the
thermoregulatory mechanisms as the main triggers of CCK inhibition in response to exercise.

Metabolic activity did not affect plasma ghrelin, however, as evidenced earlier by other
studies in healthy subjects [31-33]. Indeed, total plasma ghrelin levels were not modified after
high-intensity exercise [25], but a few data support the idea that an acute bout of exercise
influences appetite by suppressing levels of acylated ghrelin [4]. Desacyl ghrelin (not
measured in our study), in contrast to acylated ghrelin, induces a negative energy balance by
decreasing food intake [34]. This may have contributed to the post-exercise energy deficit
observed in our study.

Ghrelin levels were affected by the environmental temperature, whereas temperature had no
effect on the plasma levels of CCK or PP. This agrees with recent findings that the
hypothalamic mRNA levels of CCK were not modified after heat exposure in chickens [35].

As the outside temperature in Guadeloupe is about 28°C with 80% relative humidity (day and night average), the subjects underwent cold exposure when they entered the 22°C room, as was reflected by their reports of lower thermal comfort in the 22°C conditions, even at baseline. Since acute exposure to cold increases plasma ghrelin levels [36], acclimation of our participants to tropical climate may explain the higher ghrelin level at baseline and all through the session, even though not significantly so at single time points, in the 22°C condition.

Apart from this specific initial response, our data basically confirm some of those recently published by Kojima et al. [37] whereas disagreements can be explained by differences in fitness of the participants. They also provide suspicions that acclimation is a potential modulator factor, and that ghrelin is a fast and sensitive responder to relative cold exposure.

One of the strengths of this study resides in its design. To the best of our knowledge, this is the first study with subjects who participated in both rest and exercise sessions, in neutral and hot ambient temperatures. Moreover, water intake was carefully controlled, unlike most of the studies investigating food intake and its regulation. The role of water intake in decreased hunger and food intake has not been extensively studied. A few studies have reported that water consumed before and during a meal does not modify energy intake in young subjects [38,39], and a recent one showed that hydration status had no impact on post-exercise appetite or energy intake [40]. In older and obese older adults, however, water consumption reduces energy intake [38]. As the results seem to differ depending on the situation and the population under study, and given that both exercise and heat induce water loss, we chose an experimental design that controlled for hydration status to avoid any potential bias.

The limitations of this study are its very acute nature, the lack of inclusion of women, the small number of subjects and the low values for body fat mass, the latter two of which impede
generalization to overweight or obese populations. Moreover, as water intake was controlled, the subjects of our study seem to have drunk less water than in a previous study [8], and this may not actually reflect what would have occurred in real life. Last, conversely to most studies, the subjects involved in the experiment were acclimated to heat, which makes comparisons difficult.

In conclusion, exercise induced a short-term energy deficit. However, modifications in the hormonal regulation of food intake in response to short-term exposures to heat or to heat and exercise seem to be minor and did not induce any changes in energy intake. Long-term studies are required to comprehend the role of environmental temperature among the modulators for energy balance. This would allow a better understanding of the association between exercise training and performance, and body mass regulation in tropical and hot environments. Overweight individuals would also be an interesting target for further studies. Since this population seems to present specific patterns of response to environmental temperature [41], further studies should also be conducted on less fit and lean subjects to apprehend whether similar conclusions would apply to physical activity interventions for people involved in weight management programs.
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DISCLOSURE STATEMENT. The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS. CF and SAJ designed the study. CF, KC, SH and SAJ supervised the experimental sessions. CF and KC were responsible for execution of plasma analysis. CF, KC and SAJ were responsible for data analysis and interpretation. CF, KC, SH, MDHD, OH and SAJ were responsible for writing and editing the manuscript. All of the above-mentioned authors take full responsibility for the paper as a whole, i.e., conception and design, ethics, data, analysis, and interpretation. They all approved the final version.
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FIGURE CAPTIONS

**Figure 1. Experimental design.** The subjects participated in 4 experimental sessions in random order (rest at either 22°C or 31°C and exercise at 60% of their \( \dot{V}O_2 \text{max} \) at either 22°C or at 31°C, all for 40 minutes). This test period was followed by a 30-minute recovery period and then an *ad libitum* meal. Energy balance and plasma pancreatic polypeptide (PP), cholecystokinin (CCK) and ghrelin levels were determined.

**Figure 2. Cardiopulmonary characteristics of the subjects over time.** Mean (± SEM) oxygen uptake (\( \dot{V}O_2 \)), heart rate and respiratory exchange ratio (RER) in each of the 4 study conditions. Some error bars are too small to appear.

a. Oxygen uptake: single effects of metabolic activity and time, and their interaction (p<0.001). Open triangles are overlapped by closed triangles.

b. Heart rate: single effects of metabolic activity (p<0.001), temperature (p=0.003) and time (p<0.001), and their interaction (p=0.043).

c. RER: single effects of metabolic activity and time, and their interaction (p<0.001).

**Figure 3.** Mean (± SEM) visual analogic scale (VAS) score for subjective thermal comfort in each of the 4 study conditions. Single effects of metabolic activity, time and temperature (all p<0.001); combined effects of metabolic activity x temperature (p=0.007), metabolic activity x time, and temperature x time (p<0.001). Metabolic activity x temperature x time interaction was not significant (p=0.060).

**Figure 4.** Mean (± SEM) relative energy intake in each of the 4 study conditions. *Single effect of metabolic activity: p=0.004.
Figure 5. Mean (± SEM) composite appetite score in each of the 4 study conditions. Single effect of time: p<0.001.

Figure 6. Mean (± SEM) plasma pancreatic polypeptide (PP) in each of the 4 study conditions. Single effects of metabolic activity: p=0.002 (ex>rest) and time: p<0.001 (T100>T0, T40, T55).

Figure 7. Mean (± SEM) plasma cholecystokinin (CCK) in each of the 4 study conditions. Single effect of time: p=0.005 (T100 > T40, T55) and combined effects of metabolic activity x time: p=0.005. Post-hoc analysis: in the rest-22°C condition: * T100 significantly different from T55 (p=0.006); in the rest-31°C condition: # T100 significantly different from T0, T40 and T55 (p<0.001).

Figure 8. Mean (± SEM) plasma ghrelin in each of the 4 study conditions. Single effect of temperature: p=0.031 (22°C>31°C).
### Table 1

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<th></th>
<th>sed-22°C</th>
<th>sed-31°C</th>
<th>ex-22°C</th>
<th>ex-31°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake during the 40 min-test (g)</td>
<td>(51 ± 63)</td>
<td>116 ± 74**</td>
<td>(164 ± 76)</td>
<td>287 ± 87#</td>
</tr>
<tr>
<td>Water intake to compensate hydric loss (g)</td>
<td>(30 ± 95)</td>
<td>10 ± 32*</td>
<td>(180 ± 193)</td>
<td>354 ± 259</td>
</tr>
<tr>
<td>Water intake during the meal (g)</td>
<td>277 ± 29</td>
<td>278 ± 29</td>
<td>256 ± 88</td>
<td>259 ± 64</td>
</tr>
<tr>
<td>Total water intake (g)</td>
<td>(359 ± 104)</td>
<td>403 ± 80**</td>
<td>(600 ± 218)</td>
<td>900 ± 238#</td>
</tr>
</tbody>
</table>

*: single effect of metabolic activity with $p<0.01$; **: single effect of metabolic activity with $p<0.001$; #: single effect of temperature with $p<0.05$; ##: single effect of temperature with $p<0.001$. 