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

Cécile Faure, Keyne Charlot, Stéphane Henri, Marie-Dominique Hardy-Dessources, Olivier Hue, Sophie Antoine-Jonville

► To cite this version:

Cécile Faure, Keyne Charlot, Stéphane Henri, Marie-Dominique Hardy-Dessources, Olivier Hue, et al.. Effect of heat exposure and exercise on food intake regulation: a randomized crossover study in young healthy men. *Metabolism*, 2016, 65 (10), pp.1541-1549. 10.1016/j.metabol.2016.07.004 . hal-01496661

HAL Id: hal-01496661

<https://hal.science/hal-01496661>

Submitted on 27 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Cécile Faure^a, Keyne Charlot^{a,b,c}, Stéphane Henri^{a,d},

Marie-Dominique Hardy-Dessources^{b,c}, Olivier Hue^a, Sophie Antoine-Jonville^a

^a Adaptation to Tropical Climate, Exercise and Health Laboratory, EA3596, University of the French West Indies, Pointe-à-Pitre, Guadeloupe, France

^b UMR Inserm 1134, University of the French West Indies, Guadeloupe

^c France-Laboratory of Excellence GR-Ex (The red cell: from genesis to death), PRES Sorbonne Paris Cité, Paris, France

^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_antoine@ymail.com

sophie.jonville@univ-ag.fr

Tel: (+ 590) 590 48 31 75

Fax: (+ 590) 590 48 31 79

Disclosures: The authors declare no conflict of interest.

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: cholecystokinin; 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; $\dot{V}O_{2max}$:

53 maximal oxygen uptake

54

55 **Key words.** Physical activity; Food intake regulation; Environment; Energy balance;

56 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 ($\dot{V}O_{2\max}$) 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 *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 $\dot{V}O_{2\max}$. 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 *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' $\dot{V}O_{2\max}$ 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 $\dot{V}O_{2\max}$ 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: $Rel\ EI = EI - EE$, where Rel EI is the relative energy intake, EI is the absolute energy intake during the *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: $CAS = ((100 - \text{satiety}) + (100 - \text{fullness}) + \text{desire to eat} + \text{prospective food intake})/4$ [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 *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 $\pm$ 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 $\times$ metabolic activity, time $\times$ temperature, or time $\times$ temperature $\times$ 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

($p=0.003$). 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.

ACKNOWLEDGEMENTS. We warmly thank the subjects and the nurse and physician (Véronique Conord and Thibault Philippe) for their great involvement. The authors also thank Cathy Carmeni for her excellent revision and suggestions. SAJ expresses her warm thanks to Pr. Anderson's team at the Department of Nutritional Sciences and Physiology for inspiring this research project.

FUNDING. This project was supported by grants from the European Social Fund and Region Guadeloupe (No CR/12-116), the French Ministry of Overseas Territories (No 0123-C001-D971/2013), and the European Regional Development Fund (PO No 1/1.4/-31793). The funders had no role in the study design, data collection and analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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.

REFERENCES

1. WHO. Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. 2009. Available at: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf (Accessed 22 Jan 2016).
2. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nat Rev Endocrinol* 2013;9:13–27. doi:10.1038/nrendo.2012.199.
3. Sanchez-Lasheras C, Konner AC, Bruning JC. *Front Neuroendocrinol* 2010;31:4–15. doi:10.1016/j.yfrne.2009.08.002.
4. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Med* 2014;44:387–403. doi:10.1007/s40279-013-0120-3.
5. Hagobian TA, Sharoff CG, Braun B. Effects of short-term exercise and energy surplus on hormones related to regulation of energy balance. *Metabolism* 2008;57:393–8. doi:10.1016/j.metabol.2007.10.016.
6. Schubert MM, Desbrow B, Sabapathy S, Leveritt M. Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 2013;63:92–104. doi:10.1016/j.appet.2012.12.010.
7. Fuqua JS, Rogol AD. Neuroendocrine alterations in the exercising human: implications for energy homeostasis. *Metabolism* 2013;62:911–21. doi:10.1016/j.metabol.2013.01.016.
8. Shorten AL, Wallman KE, Guelfi KJ. Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *Am J Clin Nut* 2009;90:1215–21. doi:10.3945/ajcn.2009.28162.Am.
9. Wasse LK, King JA, Stensel DJ, Sunderland C. Effect of ambient temperature during acute aerobic exercise on short-term appetite, energy intake, and plasma acylated ghrelin in recreationally active males. *Appl Physiol Nutr Metab = Physiol Appl Nutr Metab* 2013;38:905–9. doi:10.1139/apnm-2013-0008.

10. Rhoads RP, Baumgard LH, Suagee J K, Sanders SR. Nutritional Interventions to Alleviate the Negative Consequences of Heat Stress 1 , 2 2013;267–76. doi:10.3945/an.112.003376.during.
11. de Lauzon B, Romon M, Deschamps V, Lafay L, Borys J-M, Karlsson J, et al. The Three-Factor Eating Questionnaire-R18 is able to distinguish among different eating patterns in a general population. *J Nutr* 2004;134:2372–80.
12. Arena R, Myers J, Williams MA., Gulati M, Kligfield P, Balady GJ, et al. Assessment of functional capacity in clinical and research settings: A scientific statement from the American Heart Association committee on exercise, rehabilitation, and prevention of the council on clinical cardiology and the council on cardiovascular n. *Circulation* 2007;116:329–43. doi:10.1161/CIRCULATIONAHA.106.184461.
13. Zubieta-Calleja G, Paulev P. Thermo-Regulation, Temperature and Radiation. In: *New Human Physiology*. 2nd ed. Available from: <http://www.zuniv.net/physiology/book/chapter21.html> (Accessed 22 Jan 2016).
14. Jay O, Bain AR, Deren TM, Sacheli M, Cramer MN. Large differences in peak oxygen uptake do not independently alter changes in core temperature and sweating during exercise. *Am J Physiol Regul Integr Comp Physiol* 2011;301:R832–41. doi:10.1152/ajpregu.00257.2011.
15. Blundell JE, De Graaf K, Finlayson G et al. Measuring food intake, hunger, satiety and satiation in the laboratory. In: Allison DB, Baskin ML, ed. *Handbook of Assessment Methods for Eating Behaviours and Weight-Related Problems: Measures, Theory and Research*. 2nd ed. Newbury Park, California: Sage Publications. 2012. 283–325.
16. ANSES. Ciquel tables. 2013. Available at: <https://pro.anses.fr/TableCIQUAL/ind>. (Accessed 22 Jan 2016).
17. Arvaniti K, Richard D, Tremblay A. Reproducibility of energy and macronutrient intake and related substrate oxidation rates in a buffet-type meal. *Br J Nutr* 2000;83:489–95.

- 466 18. Anderson GH, Catherine NLA, Woodend DM, Wolever TMS. Inverse association between
467 the effect of carbohydrates on blood glucose and subsequent short-term food intake in young
468 men. *Am J Clin Nutr* 2002;76:1023–30.
- 469 19. King NA, Burley VJ, Blundell JE. Exercise-induced suppression of appetite: effects on food
470 intake and implications for energy balance. *Eur J Clin Nutr* 1994;48:715–24.
- 471 20. Jokisch E, Coletta A, Raynor HA. Acute energy compensation and macronutrient intake
472 following exercise in active and inactive males who are normal weight. *Appetite* 2012;58:722–
473 9. doi:10.1016/j.appet.2011.11.024.
- 474 21. Martins C, Truby H, Morgan LM. Short-term appetite control in response to a 6-week exercise
475 programme in sedentary volunteers. *Br J Nutr* 2007;98:834–42.
476 doi:10.1017/S000711450774922X.
- 477 22. Blundell JE, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy
478 balance: impact of exercise. *Obes Rev* 2015;16 Suppl 1:67-76. doi: 10.1111/obr.12257.
- 479 23. Moran TH. Gut peptides in the control of food intake. *Int J Obes (Lond)* 2009;33 Suppl 1:S7–
480 10. doi:10.1038/ijo.2009.9.
- 481 24. Murphy KG, Bloom SR. Gut hormones in the control of appetite. *Exp Physiol* 2004;89:507–
482 16. doi:10.1113/expphysiol.2004.027789.
- 483 25. Erdmann J, Tahbaz R, Lippl F, Wagenpfeil S, Schusdziarra V. Plasma ghrelin levels during
484 exercise - effects of intensity and duration. *Regul Pept* 2007;143:127–35.
485 doi:10.1016/j.regpep.2007.05.002.
- 486 26. Hilsted J, Galbo H, Sonne B, Schwartz T, Fahrenkrug J, de Muckadell OB, et al.
487 Gastroenteropancreatic hormonal changes during exercise. *Am J Physiol* 1980;239:G136–40.
- 488 27. Greenberg GR, Marliss EB, Zinman B. Effect of exercise on the pancreatic polypeptide
489 response to food in man. *Horm Metab Res = Horm Und Stoffwechselforsch = Horm Metab*
490 1986;18:194–6. doi:10.1055/s-2007-1012268.

- 491 28. Bailey DM, Davies B, Castell LM, Newsholme EA, Calam J. Physical exercise and
492 normobaric hypoxia: independent modulators of peripheral cholecystokinin metabolism in
493 man. *J Appl Physiol* 2001;90:105–13.
- 494 29. Hurley RS, Bossetti BM, O'Dorisio TM, Tenison EB, Welch MA, Rice RR. The effect of
495 exercise training on body weight and peptide hormone patterns in normal weight college-age
496 men. *J Sports Med Phys Fitness* 1991;31:52–6.
- 497 30. Hirschberg AL, Lindholm C, Carlstrom K, Von Schoultz B. Reduced serum cholecystokinin
498 response to food intake in female athletes. *Metabolism* 1994;43:217–22.
- 499 31. Shiiya T, Ueno H, Toshinai K, Kawagoe T, Naito S, Tobina T, et al. Significant lowering of
500 plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocr J*
501 2011;58:335–42.
- 502 32. Dall R, Kanaley J, Hansen TK, Moller N, Christiansen JS, Hosoda H, et al. Plasma ghrelin
503 levels during exercise in healthy subjects and in growth hormone-deficient patients. *Eur J*
504 *Endocrinol* 2002;147:65–70.
- 505 33. Kraemer RR, Castracane VD. Exercise and humoral mediators of peripheral energy balance:
506 ghrelin and adiponectin. *Exp Biol Med (Maywood)* 2007;232:184–94.
- 507 34. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, et al. Stomach regulates
508 energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 2005;54:18–24.
509 doi:10.1136/gut.2004.038737.
- 510 35. Lei L, Hepeng L, Xianlei L, Hongchao J, Hai L, Sheikahmadi A, et al. Effects of acute heat
511 stress on gene expression of brain-gut neuropeptides in broiler chickens. *J Anim Sci*
512 2013;91:5194–201. doi:10.2527/jas.2013-6538.
- 513 36. Tomasik PJ, Sztefko K, Pizon M. The effect of short-term cold and hot exposure on total
514 plasma ghrelin concentrations in humans. *Horm Metab Res = Horm Und Stoffwechselforsch =*
515 *Horm Metab* 2005;37:189–90. doi:10.1055/s-2005-861296.

- 516 37. Kojima C, Sasaki H, Tsuchiya Y, Goto K. The influence of environmental temperature on
517 appetite-related hormonal responses. *J Physiol Anthropol* 2015;34:22. doi:10.1186/s40101-
518 015-0059-1.
- 519 38. Van Walleghen EL, Orr JS, Gentile CL, Davy BM. Pre-meal water consumption reduces meal
520 energy intake in older but not younger subjects. *Obesity (Silver Spring)* 2007;15:93–9.
521 doi:10.1038/oby.2007.506.
- 522 39. Rolls BJ, Bell EA, Thorwart ML. Water incorporated into a food but not served with a food
523 decreases energy intake in lean women. *Am J Clin Nutr* 1999;70:448–55.
- 524 40. Kelly PJ, Guelfi KJ, Wallman KE, Fairchild TJ. Mild dehydration does not reduce
525 postexercise appetite or energy intake. *Med Sci Sports Exerc* 2012;44:516–24.
526 doi:10.1249/MSS.0b013e318231c176.
- 527 41. Crabtree DR, Blannin AK. Effects of exercise in the cold on ghrelin, PYY and food intake in
528 overweight adults. *Med Sci Sports Exerc* 2015;47:49–57.

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 $\dot{V}O_2$ 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 ($\pm$ 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 ($\pm$ 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 ($\pm$ SEM) relative energy intake in each of the 4 study conditions. *Single effect of metabolic activity: $p = 0.004$.

Figure 5. Mean ($\pm$ SEM) composite appetite score in each of the 4 study conditions. Single effect of time: $p < 0.001$.

Figure 6. Mean ($\pm$ 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 ($\pm$ 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 ($\pm$ SEM) plasma ghrelin in each of the 4 study conditions. Single effect of temperature: $p = 0.031$ (22°C > 31°C).

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

*: 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 $p < 0.001$.