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

3  
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28 **ABSTRACT**

29 **Objective**

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

33 **Methods**

34 A randomized crossover study was conducted in the laboratory. Ten healthy and physically  
35 active young Afro-Caribbean men participated in four experimental sessions (rest at 22°C and  
36 31°C and cycling at 60% of their maximal oxygen uptake at 22°C and 31°C, all for 40  
37 minutes). Each test period was followed by a 30-minute recovery period and then an *ad*  
38 *libitum* meal. The main outcome measures were energy balance, subjective appetite, and  
39 plasma pancreatic polypeptide (PP), cholecystokinin (CCK) and ghrelin concentrations.

40 **Results**

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

45 **Conclusions**

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

49 This trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT02157233.

50

51 **Abbreviations.** PP: pancreatic polypeptide; CCK: cholecystokinin; rest-22°C: rest at 22°C;  
52 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

## 57 1. INTRODUCTION

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

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

78 The effect of physical activity on energy intake is moderated by other factors, as well,  
79 including exercise intensity and duration, training status, sex, amount of body fat [6,7].  
80 Among these factors, environmental temperature in which the exercise task is performed has

81 not been extensively studied. However, further investigation on this factor is relevant for the  
82 understanding of energy intake behaviors. Based on the perturbations of human [8,9] and  
83 animal [10] feeding behaviors reported in hot environment, we hypothesized that heat and  
84 exercise would interact to reduce relative energy intake. The aim of the study was thus to  
85 determine the single and combined effects of metabolic activity and environmental  
86 temperature on energy intake and its hormonal regulation.

## 87 **2. SUBJECTS AND METHODS**

### 88 **2.1. Ethical approval**

89 This trial was registered at clinicaltrials.gov as NCT02157233 and approved by the Regional  
90 Ethics Committee (French Human Subject Review Committee of Limoges, France: CPP13-  
91 018a/2013-A01037-38). The experiments were performed in accordance with the guidelines  
92 set by the Declaration of Helsinki and written informed consents to participate in the study  
93 were obtained from all subjects. The study sponsors were not involved in study design,  
94 collection and analysis of data, writing the report or the decision to submit the manuscript for  
95 publication.

### 96 **2.2. Subjects**

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

100 Ten healthy and physically active men (age:  $20.7 \pm 1.7$  yrs, height:  $1.80 \pm 0.06$  m, body mass:  
101  $69.2 \pm 7.1$  kg, body mass index:  $21.3 \pm 1.7$  kg.m<sup>-2</sup>, fat mass:  $7.2 \pm 5.3$  %) were included in the  
102 study. They were all of Afro-Caribbean origin.

103 Subjects had stable body mass ( $\pm 2$  kg for more than 1 year, determined by self-report). They  
104 were nonsmokers, without major chronic disease (i.e., diabetes, coronary heart disease), and  
105 not taking medications known to influence food intake or body mass. They reported normal  
106 body mass at birth and were born at term. They had lived in the Caribbean area for more than  
107 3 months and were thus well acclimated to a tropical climate. They reported no food allergies  
108 or restrictions and screening had excluded individuals with eating disorders [11]. They trained  
109 regularly with a mixture of endurance and resistance exercises. Their physical activity was  
110 within a range of 2000-6000 METs.min/week (2100-6700 kcal/week). The subjects were not  
111 aware of the specific purpose of the study.

### 112 **2.3. Experimental design**

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

124 The experiment was divided into three parts (Fig. 1): the "test" of 40 minutes in either the rest  
125 or the exercise condition, followed by a 30-minute rest period, and then an *ad libitum* meal of  
126 30 minutes. During the control trials, the subjects lay on an examination table for 40 minutes.

127 The exercise trials consisted of a 40 minutes cycling (Monark Weight Ergometer 814 E,  
128 Varberg, Sweden) at 60% of their  $\dot{V}O_{2max}$ . The environmental temperatures were set at 22°C  
129 and 31°C for neutral and hot temperature, respectively, with 45% relative humidity, and stable  
130 temperature and humidity throughout each session.

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

## 138 **2.4. Anthropometric data**

### 139 ***2.4.1. Body mass, height and body mass index (BMI)***

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

### 144 ***2.4.2. Bioelectric impedance analysis***

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



## 150 **2.5. Cardiopulmonary assessment**

151 On their first visit to the laboratory, the subjects'  $\dot{V}O_{2\max}$  was measured with an incremental  
152 exercise test to exhaustion, performed on a cycle ergometer (Monark Weight Ergometer  
153 814E, Varberg, Sweden). The increments were set at 15-20 W per minute according to the  
154 expected physical fitness so as to reach exhaustion within 12 minutes as recommended [12].  
155 Complete metabolic data were collected using a breath-by-breath ergospirometry system  
156 (Metalyzer<sup>®</sup> 3B, Cortex Biophysik GmbH, Germany). They had a  $\dot{V}O_{2\max}$  of  $47.1 \pm 7.4$   
157  $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ .

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

## 161 **2.6. Core temperature and thermal comfort**

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

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

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

171

172 **2.7. Energy balance**

173 **2.7.1. Energy expenditure**

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

176 **2.7.2. Assessment of energy intake**

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

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

187 Total energy intake from the breakfast meal was calculated by weighing all remaining food  
188 products after the participant had left the laboratory. This method has been shown to be  
189 reliable for assessing energy intake [17]. Total kilocalories were recorded. In addition, relative  
190 energy intake was calculated by correcting post-exercise energy intake for the energy cost of  
191 the exercise session as follows:  $Rel\ EI = EI - EE$ , where Rel EI is the relative energy intake,  
192 EI is the absolute energy intake during the *ad libitum* meal, and EE is the energy expenditure  
193 during the 40-minute test period.

## 194 **2.8. Subjective appetite sensations**

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

## 203 **2.9. Hormones involved in food intake regulation**

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

## 213 **2.10. Statistics**

214 The required number of participants was *a priori* calculated using G\*power 3.1 for Mac.  
215 Alpha error probability threshold was 0.05. The calculations were performed based on  
216 expected relative energy intake, with different effect size and different power between 0.80

217 and 0.95, and 0.85 for correspondence among repeated measures. Resulting required sample  
218 size varied. Ten appeared as feasible and sufficient to evidence an effect size of 0.4, with 0.80  
219 power. All results were analyzed with the SPSS v.20 software package (SPSS Inc., Chicago,  
220 IL, USA). A probability value of  $p < 0.05$  was considered statistically significant. Data are  
221 presented as means  $\pm$  SDs except in figures where SEMs are used.

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

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

229 Data were tested for sphericity using Mauchly's test and if the assumption of sphericity was  
230 violated, the Greenhouse–Geisser correction was undertaken to adjust the degrees of freedom.

231 Tukey's post-hoc tests were performed to identify mean differences among conditions when  
232 the effect of time  $\times$  metabolic activity, time  $\times$  temperature, or time  $\times$  temperature  $\times$  metabolic  
233 activity was significant. Otherwise, only significant simple effects or interaction effects are  
234 reported.

### 235 **3. RESULTS**

#### 236 **3.1. Cardiopulmonary assessment (Fig. 2)**

237 Oxygen uptake and RER (Fig. 2a and 2c) were affected by the metabolic activity, time and  
238 their interaction ( $p < 0.001$ ). Heart rate (Fig. 2b) was significantly increased in the exercise  
239 conditions ( $p < 0.001$ ). It was also higher in the hot conditions than in the neutral ones

240 (p=0.003). There was an effect of time (p<0.001) on heart rate, and the interaction of  
241 metabolic activity x temperature x time was significant (p<0.043).

### 242 **3.2. Temperature and thermal comfort**

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

### 249 **3.3. Water intake**

250 Water intakes during the 40-min test, water intakes to compensate water loss and during the  
251 meal and total water intakes are reported in table 1. Exercise increased water intake during the  
252 40-minute test period (p<0.001) and during the recovery to compensate water loss (p=0.002),  
253 as well as the total water intake (p<0.001). Water intake during the 40-minute test period and  
254 total water intake were also affected by temperature (p<0.001 and p=0.019, respectively). The  
255 combined effects of metabolic activity and temperature on total water intake approached  
256 significance (p=0.072).

### 257 **3.4. Energy balance and appetite**

258 The energy expended during the 40-minute test periods was  $45 \pm 6$ ,  $46 \pm 6$ ,  $389 \pm 22$  and  $385$   
259  $\pm 26$  kcal, in the rest-22°C, rest-31°C, ex-22°C and ex-31°C conditions, respectively. It was  
260 only affected by metabolic activity (p<0.001). Absolute energy intake did not differ between  
261 the four experimental conditions ( $1042 \pm 330$ ,  $1039 \pm 217$ ,  $1156 \pm 236$  and  $1090 \pm 296$  kcal in

262 the rest-22°C, rest-31°C, ex-22°C and ex-31°C conditions, respectively), neither was energy  
263 intake reported to body mass (all  $p > 0.258$  for metabolic activity, temperature and interaction).

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

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

### 269 **3.5. Markers of food intake regulation**

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

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

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

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

281

282

283 **4. DISCUSSION**

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

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

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

302 Such an energy deficit, if repeated over a longer period, could have significant implications in  
303 terms of weight management. The question can also be analyzed from the point of view of the  
304 resting sessions, which are likely to induce positive energy balance on longer term. This study  
305 fuels the idea of an inability to compensate for inactivity by reducing energy intake [22].

306 Contrary to our hypothesis, the environmental temperature did not seem to modify the energy  
307 intake subsequent to exercise in these acclimated subjects. Similarly to what we observed, a  
308 previous study reported no difference in absolute and relative energy intake after exercise  
309 performed in the heat and a neutral environment [8].

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

320 We found small effects of exercise on the appetite hormone levels, as reported in other studies  
321 [4]. CCK and PP, the two anorexigenic factors measured in our study, seem to be differently  
322 affected by metabolic activity. Whereas PP was higher in the exercise conditions, CCK  
323 increased only after food intake in the rest conditions. The increased secretion of PP in the  
324 exercise conditions is in accordance with a recent meta-analysis [6]. This is very compatible  
325 with previous observations reporting that not only does acute exercise increase fasting plasma  
326 PP [26], but it also raises postprandial PP levels [27].

327 Unlike for PP, very few data are available on the effects of acute exercise on CCK.  
328 Observations partly contrary to our finding have been reported, with one study suggesting that  
329 acute exercise increased plasma CCK levels [28]. The main reason for this discrepancy stands



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

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

352 Ghrelin levels were affected by the environmental temperature, whereas temperature had no  
353 effect on the plasma levels of CCK or PP. This agrees with recent findings that the

354 hypothalamic mRNA levels of CCK were not modified after heat exposure in chickens [35].  
355 As the outside temperature in Guadeloupe is about 28°C with 80% relative humidity (day and  
356 night average), the subjects underwent cold exposure when they entered the 22°C room, as  
357 was reflected by their reports of lower thermal comfort in the 22°C conditions, even at  
358 baseline. Since acute exposure to cold increases plasma ghrelin levels [36], acclimation of our  
359 participants to tropical climate may explain the higher ghrelin level at baseline and all through  
360 the session, even though not significantly so at single time points, in the 22°C condition.  
361 Apart from this specific initial response, our data basically confirm some of those recently  
362 published by Kojima et al. [37] whereas disagreements can be explained by differences in  
363 fitness of the participants. They also provide suspicions that acclimation is a potential  
364 modulator factor, and that ghrelin is a fast and sensitive responder to relative cold exposure.

365 One of the strengths of this study resides in its design. To the best of our knowledge, this is  
366 the first study with subjects who participated in both rest and exercise sessions, in neutral and  
367 hot ambient temperatures. Moreover, water intake was carefully controlled, unlike most of the  
368 studies investigating food intake and its regulation. The role of water intake in decreased  
369 hunger and food intake has not been extensively studied. A few studies have reported that  
370 water consumed before and during a meal does not modify energy intake in young subjects  
371 [38,39], and a recent one showed that hydration status had no impact on post-exercise appetite  
372 or energy intake [40]. In older and obese older adults, however, water consumption reduces  
373 energy intake [38]. As the results seem to differ depending on the situation and the population  
374 under study, and given that both exercise and heat induce water loss, we chose an  
375 experimental design that controlled for hydration status to avoid any potential bias.

376 The limitations of this study are its very acute nature, the lack of inclusion of women, the  
377 small number of subjects and the low values for body fat mass, the latter two of which impede

378 generalization to overweight or obese populations. Moreover, as water intake was controlled,  
379 the subjects of our study seem to have drunk less water than in a previous study [8], and this  
380 may not actually reflect what would have occurred in real life. Last, conversely to most  
381 studies, the subjects involved in the experiment were acclimated to heat, which makes  
382 comparisons difficult.

383 In conclusion, exercise induced a short-term energy deficit. However, modifications in the  
384 hormonal regulation of food intake in response to short-term exposures to heat or to heat and  
385 exercise seem to be minor and did not induce any changes in energy intake. Long-term studies  
386 are required to comprehend the role of environmental temperature among the modulators for  
387 energy balance. This would allow a better understanding of the association between exercise  
388 training and performance, and body mass regulation in tropical and hot environments.  
389 Overweight individuals would also be an interesting target for further studies. Since this  
390 population seems to present specific patterns of response to environmental temperature [41],  
391 further studies should also be conducted on less fit and lean subjects to apprehend whether  
392 similar conclusions would apply to physical activity interventions for people involved in  
393 weight management programs.

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399

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406

407

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409

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

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528 overweight adults. *Med Sci Sports Exerc* 2015;47:49–57.

529 **FIGURE CAPTIONS**

530 **Figure 1. Experimental design.** The subjects participated in 4 experimental sessions in  
531 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  
532 or at 31°C, all for 40 minutes). This test period was followed by a 30-minute recovery period  
533 and then an *ad libitum* meal. Energy balance and plasma pancreatic polypeptide (PP),  
534 cholecystokinin (CCK) and ghrelin levels were determined.

535  
536 **Figure 2. Cardiopulmonary characteristics of the subjects over time.** Mean ( $\pm$  SEM) oxygen  
537 uptake ( $\dot{V}O_2$ ), heart rate and respiratory exchange ratio (RER) in each of the 4 study  
538 conditions. Some error bars are too small to appear.

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

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

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

544  
545 **Figure 3.** Mean ( $\pm$  SEM) visual analogic scale (VAS) score for subjective thermal comfort in  
546 each of the 4 study conditions. Single effects of metabolic activity, time and temperature (all  
547  $p < 0.001$ ); combined effects of metabolic activity x temperature ( $p = 0.007$ ), metabolic activity  
548 x time, and temperature x time ( $p < 0.001$ ). Metabolic activity x temperature x time interaction  
549 was not significant ( $p = 0.060$ ).

550  
551 **Figure 4.** Mean ( $\pm$  SEM) relative energy intake in each of the 4 study conditions. \*Single effect  
552 of metabolic activity:  $p = 0.004$ .

553

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

556  
557 **Figure 6.** Mean ( $\pm$  SEM) plasma pancreatic polypeptide (PP) in each of the 4 study  
558 conditions. Single effects of metabolic activity:  $p = 0.002$  (ex>rest) and time:  $p < 0.001$   
559 (T100>T0, T40, T55).

560  
561 **Figure 7.** Mean ( $\pm$  SEM) plasma cholecystokinin (CCK) in each of the 4 study conditions.  
562 Single effect of time:  $p = 0.005$  (T100 > T40, T55) and combined effects of metabolic activity  
563 x time:  $p = 0.005$ . Post-hoc analysis: in the rest-22°C condition: \* T100 significantly different  
564 from T55 ( $p = 0.006$ ); in the rest-31°C condition: # T100 significantly different from T0, T40  
565 and T55 ( $p < 0.001$ ).

566  
567 **Figure 8.** Mean ( $\pm$  SEM) plasma ghrelin in each of the 4 study conditions. Single effect of  
568 temperature:  $p = 0.031$  (22°C>31°C).

	<b>sed-22°C</b>	<b>sed-31°C</b>	<b>ex-22°C</b>	<b>ex-31°C</b>	
<b>Water intake during the 40 min-test (g)</b>	(51 ± 63)	116 ± 74)**	(164 ± 76)	287 ± 87)	#
<b>Water intake to compensate hydric loss (g)</b>	(30 ± 95)	10 ± 32) *	(180 ± 193)	354 ± 259)	
<b>Water intake during the meal (g)</b>	277 ± 29	278 ± 29	256 ± 88	259 ± 64	
<b>Total water intake (g)</b>	(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  $p < 0.001$ .