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

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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 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 ± 1.7 yrs, height: 1.80 ± 0.06 m, body mass:
101 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
102 study. They were all of Afro-Caribbean origin.

103 Subjects had stable body mass (± 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 $\dot{V}O_{2\max}$. 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[®] 3B, Cortex Biophysik GmbH, Germany). They had a $\dot{V}O_{2\max}$ of 47.1 ± 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[®] 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[®] 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 ± 6 , 46 ± 6 , 389 ± 22 and 385
259 ± 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 ± 330 , 1039 ± 217 , 1156 ± 236 and 1090 ± 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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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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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).

	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 $p < 0.001$.