

1 **The effect of ambient temperature during acute aerobic exercise on short term appetite,**
2 **energy intake and plasma acylated ghrelin in recreationally active males**

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48 **Abstract**

49 Ambient temperature during exercise may affect energy intake regulation. Compared with a
50 temperate (20°C) environment, 1 h of running followed by 6 h of rest tended to decrease energy
51 intake from two *ad libitum* meals in a hot (30°C) environment but increase it in a cool (10°C)
52 environment ($P=0.08$). Core temperature changes did not appear to mediate this trend; whether
53 acylated ghrelin is involved is unclear. Further research is warranted to clarify these findings.

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66 **Key words** ambient temperature, exercise, appetite, energy intake, acylated ghrelin, core
67 temperature

68 **Introduction**

69 The effects of exercise on appetite and energy intake are well documented from laboratory
70 studies (King et al. 2010, King et al. 2011, Martins et al. 2007, Ueda et al. 2009, Wasse et al.
71 2012). Most observe a transient suppression of appetite during exercise with no effect on
72 subsequent energy intake. However, these observations are not unanimous because a handful
73 of studies describe no effect of exercise on appetite (Ueda et al. 2009, Wasse et al. 2013) and
74 some authors report increases (Martins et al. 2007) or decreases (Ueda et al. 2009) in post-
75 exercise energy intake. Recent attention has focussed on how exercise affects concentrations
76 of circulating appetite-regulatory gut hormones. Exercising in more extreme environmental
77 conditions (altitude, temperature) may perturb the normal physiological responses to exercise
78 and could subsequently affect the acute regulation of appetite. It is already known that altitude
79 suppresses appetite (Tschöp et al. 1998), an effect that might be related to alterations in
80 concentrations of appetite-regulatory peptides (Tschöp et al. 1998, Wasse et al. 2012).
81 Although one laboratory study shows exercising in the heat may reduce relative energy intake
82 (Shorten et al. 2009) most reports suggesting appetite is suppressed in the heat are anecdotal
83 (Burke, 2001). Cold temperatures may exert the opposite effect with an increase in energy
84 intake reported after exercise in cold water (White et al. 2005). However, whether this is
85 directly related to the cold temperature *per se* is questionable because water immersion itself
86 increases energy intake (Halse et al. 2011). Physiological differences, such as in substrate
87 utilisation, are evident when individuals are exposed to cold air or cold water (Haman et al.
88 2006). Furthermore, Wiesner and colleagues (2010) report hormonal and metabolic responses
89 specific to exercise in water that do not occur with land-based exercise. For these reasons we
90 surmise that the appetite response to cold air could differ to that observed in cold water,
91 however because there is no evidence to substantiate this, research examining this notion is
92 warranted.

93 Core temperature changes have been postulated as a mechanism responsible for alterations in
94 energy intake in different environmental temperatures, however, this is not conclusive.
95 Changes in gut hormone responses may also be responsible for differences in energy intake
96 during exercise in the heat/cold. There are numerous hormones secreted from the
97 gastrointestinal tract that are involved in the control of energy homeostasis, particularly the
98 short-term regulation of energy intake. The majority of these hormones, which include
99 cholecystokinin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are secreted in the
100 post-prandial period and contribute to meal termination and satiety (Yu and Kim, 2012).
101 However, notable among the appetite-regulatory gut hormones is acylated ghrelin, unique in
102 being the only known gut hormone that stimulates appetite (Wren et al. 2001), and purported
103 to be a meal initiation factor (Cummings et al. 2001). Testament to the widespread distribution
104 of its receptor in both central and peripheral regions, ghrelin has numerous other biological
105 effects including being a potent stimulator of growth hormone secretion (Kojima et al. 1999)
106 as well as having important roles in immune function (Dixit and Taub, 2005) and glucose
107 metabolism (Verhulst and Depoortere, 2012). However, with a unique role as the only known
108 circulating appetite-stimulating gut hormone, it is unsurprising that examining the role of
109 ghrelin in energy homeostasis has become such a prolific area of research. Total ghrelin (des-
110 acyl and acyl ghrelin) is up-regulated after short term cold exposure and down-regulated after
111 short term heat exposure (Tomasik et al. 2005). How long these perturbations persist is
112 unknown due to the short duration of exposure in that study (30 minutes); whether these
113 alterations affect subsequent appetite and energy intake remains to be investigated. Inferences
114 from a study investigating total ghrelin may be limited because it is generally believed it is the
115 acylated fraction of ghrelin that is necessary for its appetite stimulatory effects (Broglia et al.
116 2004).

117 Given the importance for athletes to maintain appropriate energy balance during episodes of
118 training and competition which are frequently undertaken in a variety of environments,
119 clarification is required to establish whether land-based exercise in hot or cool environments
120 differentially affects appetite and energy intake and whether changes are related to alterations
121 in plasma acylated ghrelin concentrations or core temperature.

122

123 **Materials and Methods**

124 Two separate pilot studies were approved by both the Loughborough University and
125 Nottingham Trent University Ethics Committees. Eleven healthy, habitually active males
126 (mean \pm SD; age 21.1 ± 1.2 y, BMI 23.6 ± 2.2 kg/m², VO_{2peak} 56.7 ± 5.0 mL/kg/min) completed
127 a ‘heat study’ and ten healthy, habitually active males (mean \pm SEM; age 22.9 ± 2.5 y, BMI
128 23.1 ± 1.6 kg/m², VO_{2peak} 57.9 ± 7.3 mL/kg/min) completed a ‘cool study’. Participants were
129 free from metabolic and gastrointestinal abnormalities. Participants gave their written informed
130 consent to participate, completed a submaximal and maximal oxygen uptake test on a treadmill
131 (Woodway ELG 55; Weil am Rhein, Germany) and then completed two, 7 h trials in a
132 randomised order in an environmental chamber (Design Environmental, Gwent, UK). Trials
133 were separated by at least seven days. In the heat study one trial was completed in a temperate
134 environment (20°C; control) and the other in a hot environment (30°C; experimental), in the
135 cool study one trial was completed in a temperate environment (20°C; control) and the other in
136 a cool environment (10°C; experimental). Relative humidity was kept constant at 50%.
137 Participants fasted overnight prior to trials which commenced at ~9am. During the 24 h prior
138 to the first trial, participants weighed and recorded their food intake and then replicated this
139 before the second trial. Upon arrival, a cannula was inserted into an antecubital vein to enable
140 frequent sampling of venous blood and a rectal thermometer (Grant Instruments, UK) was self-

141 inserted ~10 cm past the anal sphincter for monitoring core body temperature. Clothing was
142 not standardised and participants were asked to wear clothing appropriate for the environmental
143 temperature throughout each visit. At the start of each trial, participants completed a 60 minute
144 treadmill run at a speed that elicited 65% of maximal oxygen uptake, followed by 6 h rest.
145 Blood samples for acylated ghrelin were collected at baseline (0), 0.5, 1, 2, 3, 4, 5.5, 6.5 and 7
146 h into pre-cooled 5 mL EDTA tubes that were pre-treated with 50 μ L of a solution containing
147 p-hydroxymercuribenzoic acid, phosphate buffered saline, and sodium hydroxide. After 10 min
148 centrifugation at 3500 rpm, 2 mL of plasma were dispensed into a plain tube and 200 μ L of 1
149 M hydrochloric acid was added before being centrifuged for 5 min at 3500 rpm. Appetite
150 sensations were measured every 30 minutes from baseline using validated 100 mm visual
151 analogue scales (Flint et al. 2000). Cold buffet-style meals were provided at 2 and 5.5 h to
152 assess *ad libitum* energy intake. Foods were presented in excess of expected consumption and
153 identical items were available to participants at both meals. These items were three varieties of
154 breakfast cereal, semi-skimmed milk, brown and white bread, cheese, ham, tuna, butter,
155 margarine, mayonnaise, chocolate chip cookies, salted crisps, muffins, Nutri-grain bars,
156 chocolate rolls, mini Mars bars, apples, bananas, oranges, fruit yoghurt, chocolate Nesquik and
157 orange juice. Acylated ghrelin concentrations were determined from plasma using a
158 commercially available ELISA (SPI BIO, Montigny le Bretonneux, France). All statistical
159 analyses were performed using the Statistical Package for the Social Sciences (SPSS) software,
160 version 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Differences in fasting and AUC
161 values for appetite perceptions, acylated ghrelin, core temperature and thermal sensations were
162 determined using Student's t-tests. Two-factor repeated measures ANOVA was used to
163 examine differences between trials for appetite perceptions, energy and macronutrient intake,
164 acylated ghrelin, core temperature and thermal sensations. Statistical significance was
165 accepted at the 5% level. Results are presented as mean \pm SD. Due to problems collecting

166 blood from one participant in the heat study, acylated ghrelin concentrations reported are for n
167 = 10. Changes in energy intake between control and experimental trials in each study were
168 calculated and these differences compared using an independent samples T-test. Effect sizes
169 were reported to facilitate comparison of the magnitude of the effect of hot and cool
170 temperatures on energy intake. Effect sizes were calculated in accordance with Cohen's
171 classification where 0.2, 0.5 and 0.8 are considered small, moderate and large effects,
172 respectively.

173

174 **Results**

175 *Appetite*

176 Compared with the temperate environment, the hot and cool temperatures modulated the
177 appetite response to exercise with AUC values for hunger and prospective food consumption
178 being lower by 15% and 12% respectively prior to the first meal (0 – 2 h) in the heat trial
179 compared with the temperate trial ($P < 0.05$). Over the same time period, AUC values for
180 satisfaction and fullness were 27% lower ($P < 0.05$) and 23% lower ($P = 0.07$) in the cool trial
181 compared with the temperate trial. There were no other significant differences apparent over
182 this time period, or across the entire 7 h trial (Table 1).

183 *Energy intake*

184 The total energy intake and the change in energy intake in response to different ambient
185 temperatures varied widely between individuals (Figure 1). In the heat study, there was a trend
186 for a reduction in cumulative energy intake in the hot trial compared with the temperate trial
187 by 1400 ± 2401 kJ ($P = 0.08$; Figure 1). The opposite trend was apparent in the cool study
188 where participants increased their energy intake by 1450 ± 2345 kJ ($P = 0.08$; Figure 1) in the

189 cool trial compared with the temperate trial. The effect sizes for the difference in energy intake
190 between the temperate and their respective hot and cool trials were moderate ($d = -0.5$ for the
191 heat trial and 0.5 for the cool trial). There was a main effect of time in the heat study ($P < 0.05$)
192 with participants consuming more at the morning meals than the afternoon meals, however
193 energy intake was reduced by a similar extent ($\sim 12\%$) at both the morning and afternoon meals
194 in the hot compared with the temperate trial. There was no difference in energy intake
195 consumed at the morning and afternoon meals in the cool study, and energy intake tended to
196 be increased by a similar extent at each meal in the cool trial. When the delta values in energy
197 intake between the temperate and experimental trial in each study were compared (using an
198 independent samples T test), a significant difference was evident ($P = 0.013$).

199 *Acylated ghrelin*

200 No main effects of temperature on acylated ghrelin concentrations were observed in either
201 study although a trial x time interaction was evident in both ($P < 0.05$). However, post hoc
202 analysis did not reveal differences between trials at any time points. A main effect of time
203 showed that acylated ghrelin concentrations were suppressed at the end of exercise from
204 baseline values (Figures 2a and 2b). Delta values in acylated ghrelin concentrations from
205 baseline until the end of the exercise bout between the temperate and experimental trial in each
206 study were compared (using an independent samples T test) and a significant difference was
207 evident ($P < 0.05$). Despite differences upon cessation of exercise, acylated ghrelin values were
208 similar between trials within each study immediately prior to consumption of the first *ad*
209 *libitum* meal at 2 h.

210

211 *Core temperature*

212 Core temperature was significantly elevated on completion of exercise in the hot compared
213 with the temperate trial ($38.9 \pm 0.4^{\circ}\text{C}$ vs. $38.5 \pm 0.5^{\circ}\text{C}$ respectively; $P < 0.001$) but was similar
214 thereafter. Core temperature was similar at all times between the temperate and cool trials.

215

216 **Discussion**

217 Results from these pilot studies indicate that the environmental temperature during and after
218 acute exercise may transiently modulate appetite and short term energy intake but it is unlikely
219 that changes in core temperature mediated these changes and it is uncertain whether changes
220 in acylated ghrelin concentrations are involved. Total energy intake from two *ad libitum* meals
221 during a 7 h trial tended to be decreased in 30°C and increased in 10°C compared with a neutral
222 20°C environment. No individual meal was responsible for this trend, with the change in energy
223 intake being consistent across both *ad libitum* meals indicating a persistence of effect of
224 ambient temperature on energy intake. Although most individuals within each study respond
225 similarly (ie: increased energy intake in the cool, decreased energy intake in the heat) there is
226 a wide variation in individual responses (Figure 1). However, these findings give some support
227 to the anecdotal and limited empirical evidence that ambient temperature may modulate
228 appetite and energy intake. Furthermore, this research indicates that the effect persists when
229 acute exercise is undertaken, and expands upon current literature by extending beyond the
230 immediate post-exercise meal.

231 These findings are important for recreational and competitive athletes. Exercise, in the absence
232 of compensatory increases in food intake, can produce a short term negative energy balance
233 which may be efficacious for weight loss. The present findings provide some support for the
234 suggestion previously proposed by Shorten et al (2009) that exercising outdoors in the heat
235 may be preferable to exercising in an air conditioned gym if a more negative energy balance is

236 desired. From an athlete's perspective where optimal nutritional strategies can aid performance,
237 exercising in the heat could be detrimental if an athlete voluntarily consumes less food at a
238 subsequent meal which could lead to inadequate refuelling before ensuing events and could
239 impair performance or recovery. Conversely, high energy intakes, particularly if above energy
240 requirements could be detrimental to an athlete's post-exercise nutritional strategy. Given that
241 ambient temperatures of approximately 11°C, (similar to that used within the cool study), can
242 be advantageous to performance during prolonged moderate intensity exercise (Nimmo 2004)
243 the findings from the present study that exercise and rest in cool temperatures of 10°C tend to
244 increase post-exercise energy intake should be considered.

245 Previously, changes in core temperature or gut hormone concentrations (namely PYY) have
246 been suggested to mediate the change in energy intake after exercise in different environmental
247 temperatures (Shorten et al. 2009, White et al. 2005). In the study by White and colleagues,
248 despite inverse relationships between core temperature and energy intake being described,
249 actual changes in core temperature were small (0.3°C) which may be insufficient to affect
250 appetite. Furthermore the studies by White et al (2005) and Shorten et al (2009) used tympanic
251 temperature to assess core temperature. That method of core temperature measurement is
252 reportedly not valid when exercising in the heat in a laboratory (Ganio et al. 2009). In our
253 studies, we used rectal temperature (a valid and reliable method of measuring core temperature
254 during rest and exercise) to regularly monitor core temperature. We observed similar core
255 temperatures across trials within each study and core temperature differed only at the end of
256 the exercise bout in the hot trial compared with the temperate trial. Thus, our findings would
257 suggest that core temperature does not drive the changes in energy intake after acute exercise
258 followed by rest in different environmental temperatures.

259 Tomasik and colleagues (2005) examined the effect of ambient temperature on total ghrelin
260 concentrations and found concentrations were increased after 30 mins at 2°C and decreased

261 after 30 mins at 30°C. However, neither appetite nor energy intake were assessed so it is
262 unknown whether changes in total ghrelin affected subsequent appetite and energy intake. In
263 the present studies, there was not a consistent effect of ambient temperature on acylated ghrelin
264 concentrations. Given the complex mechanisms by which appetite and food intake are
265 regulated, it is likely that a combination of factors coordinate the food intake responses to
266 exercise and rest in different ambient temperatures that we observed here. It has been shown
267 that thermal perceptions are important inputs in the self-selection of exercise intensity, and
268 thermal sensation and thermal discomfort can control thermoregulatory behaviour (Schlader et
269 al. 2011). Participants felt “comfortable” in both temperate trials, and despite being able to
270 wear whatever clothing they wished, reported feeling “cool” in the trial at 10°C and “hot” in
271 the trial at 30°C. Hence thermal status may also be involved in feeding responses although this
272 is speculative.

273

274 The gastrointestinal system does not simply exist as a reservoir for food and drink but plays a
275 key role in the regulation of appetite and maintenance of energy balance. As well as directly
276 influencing appetite, hormones including ghrelin and PYY that are secreted from within the
277 gastrointestinal tract also affect gastric motility, gastric emptying and gastrointestinal blood
278 flow. Relationships between these gastric parameters and appetite perceptions such as hunger
279 and fullness have been observed and reviewed (Delzenne et al. 2010). The presence of an
280 intragastric balloon can decrease hunger and increase fullness, without decreasing subsequent
281 energy intake or affecting concentrations of appetite-regulatory peptides (Oesch et al. 2006,
282 Rigaud et al. 1995). In the present studies participants were free to consume water *ad libitum*
283 during trials, thus differences in hunger in the first 2 hours of the hot trial could just be a
284 consequence of stomach distension after water ingestion which was greater than in the
285 temperate trial (data not shown). However, it is unlikely that the trend for a reduction in energy

286 intake observed in the heat study was due to differences in stomach distension as this alone is
287 reportedly not sufficient to affect gut hormone concentrations or energy intake. Furthermore
288 the decrement in energy intake persisted at the afternoon meal when appetite ratings were
289 similar between trials.

290

291 Gastric emptying may influence ingestive behaviour and although the volume of a meal
292 influences gastric emptying, nutrients within that meal may play a greater role in affecting
293 gastric emptying due to feedback from the intestine in response to nutrients in the gut lumen
294 which affect secretion of peptides including CCK and PYY. Exercise in the heat does not
295 generally affect gastric emptying rate when participants are hydrated, however emptying rates
296 may vary dependent on the hydration status of participants. Rehrer and colleagues (1990)
297 observed that dehydration delays gastric emptying of carbohydrate beverages. We did not
298 quantify gastric emptying rates in these studies, so it is not possible to associate any changes
299 in gastric emptying with alterations in energy intake. There is limited literature regarding the
300 effect of cold ambient temperatures on gastric emptying, but unlike in the heat, dehydration
301 will less likely be a factor impacting upon gastric emptying rate. In rats, cold ambient
302 temperature normalises a delayed gastric emptying response induced by abdominal surgery
303 (Stengel et al. 2010). However, it is unclear what effect the cool temperature in the present
304 study would have on normal gastric emptying responses to food and fluid ingestion. Since
305 ghrelin stimulates gastric motility and accelerates gastric emptying, Stengel and colleagues
306 (2010) proposed that the normalised gastric emptying response was due to increased acylated
307 ghrelin concentrations after cold exposure. Given the relationship between gut hormones,
308 appetite and gastrointestinal function, perturbations in concentrations of gut hormones may act
309 in concert to affect gastric function and appetite as well as directly influencing appetite
310 regulatory areas within the brain.

311

312 Although our study benefits from the longer period of follow-up than in other research, there
313 are some limitations which should be addressed in future. Due to the wide variability in energy
314 intake responses between individuals further research is necessary to confirm these findings
315 with greater participant numbers. This research focussed on concentrations of the appetite-
316 stimulatory gut hormone, acylated ghrelin. However, satiety hormones secreted from the gut
317 and adipose tissue which include, but are not limited to, PYY, GLP-1 and leptin also play
318 integral roles in the regulation of appetite and energy intake. In future, it would be prudent to
319 quantify concentrations of these and other hormones involved in appetite regulation in
320 conjunction with acylated ghrelin to improve understanding of how these hormones may be
321 perturbed in response to exercising in different ambient temperatures. This is particularly
322 important because of the discordance between our findings and those of Shorten et al (2009)
323 who did not observe any alterations in acylated ghrelin after exercise in the heat, but attribute
324 a reduction in energy intake to elevated concentrations of PYY. Finally, since there may be sex
325 differences in the way exercise affects appetite regulatory hormones and appetite, it would be
326 of value to also study female participants.

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351 **References**

352 Broglio, F., Gottero, C., Prodam, F., Gauna, C., Mucciolo, G., Papotti, M., et al. 2004. Non-
353 acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated
354 ghrelin in humans. *J. Clin. Endocrinol. Metab.* **89**(6): 3062 – 3065. PMID: 15181099.

355 Burke, L.M. 2001. Nutritional needs for exercise in the heat. *Comp. Biochem. Physiol. A. Mol.*
356 *Integr. Physiol.* **128**(4): 735 – 748. PMID: 11282317.

357 Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., and Weigle, D.S.
358 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans.
359 *Diabetes.* **50**(8): 1714 – 1719. PMID: 11473029.

360 Delzenne, N., Blundell, J., Brouns, F., Cunningham, K., De Graaf, K., Lluch, A., et al. 2010.
361 Gastrointestinal targets of appetite regulation in humans. *Obes. Rev.* **11**(3): 234 – 250. PMID:
362 20433660.

363 Dixit, V.D. and Taub, D.D. 2005. Ghrelin and immunity: a young player in a old field. *Exp.*
364 *Gerontol.* **40**(11): 900 – 910. PMID: 16233968.

365 Flint, A., Raben, A., Blundell, J.E., and Astrup, A. 2000. Reproducibility, power and validity
366 of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int. J.*
367 *Obes. Relat. Metab. Disord.* **24**(1): 38 – 48. PMID: 10702749.

368 Ganio, M.S., Brown, C.M., Casa, D.J., Becker, S.M., Yeargin, S.W., McDermott, B.P., et al.
369 2009. Validity and reliability of devices that assess body temperature during indoor exercise in
370 the heat. *J. Athl. Train.* **44**(2): 124 – 135. PMID: 19295956.

371 Halse, R.E., Wallman, K.E., and Guelfi, K.J. 2011. Postexercise water immersion increase
372 short-term food intake in trained men. *Med. Sci. Sports. Exerc.* **43**(4): 632 – 638. PMID:
373 20798665.

374 Haman, F. 2006. Shivering in the cold: from mechanisms of fuel selection to survival. *J. Appl.*
375 *Physiol.* **100**(5): 1702 – 1708. PMID: 16614367.

376 King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. 2010. Influence of prolonged
377 treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin.
378 *Appetite.* **54**(3): 492 – 498. PMID: 20152871

379 King J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., et al. 2011.
380 Differential acylated ghrelin, peptide YY3-36, appetite and food intake responses to equivalent
381 energy deficits created by exercise and food restriction. *J. Clin. Endocrinol. Metab.* **96**(4):
382 1114 – 1121. PMID: 21270331.

383 Kojima, M., Hosoda, H., Date, Y. Nakazato, M., Matsuo, H., and Kangawa, K. 1999. Ghrelin
384 is a growth-hormone-releasing acylated peptide from stomach. *Nature.* **402**(6762): 656 – 660.
385 PMID: 10604470.

386 Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. 2007. Effects of exercise on gut
387 peptides, energy intake and appetite. *J. Endocrinol.* **193**(2): 251 – 258. PMID: 17470516

388 Nimmo, M. 2004. Exercise in the cold. *J. Sports. Sci.* **22**(1): 898 – 915. PMID: 15768724.

389 Oesch, S., Rüegg, C., Fischer, B., Degen, L., and Beglinger, C. 2006. Effect of gastric
390 distension prior to eating on food intake and feelings of satiety in humans. *Physiol. Behav.*
391 **87**(5): 903 – 910. PMID: 16549077.

392 Rehrer, N.J., Beckers, E.J., Brouns, F., ten Hoor, F., and Saris, W.H. 1990. Effects of
393 dehydration on gastric emptying and gastrointestinal distress while running. *Med. Sci. Sports.*
394 *Exerc.* **22**(6): 790 – 795. PMID: 2287256.

395 Rigaud, D., Trostler, N., Rozen, R., Vallot, T., and Apfelbaum, M. 1995. Gastric distension,
396 hunger and energy intake after balloon implantation in severe obesity. *Int. J. Obes. Relat. Metab.*
397 *Disord.* **19**(7): 489 – 495. PMID: 8520639.

398 Schlader, Z.J., Simmons, S.E., Stannard, S.R., and Mündel, T. 2011. The independent roles of
399 temperature and thermal perception in the control of human thermoregulatory behaviour.
400 *Physiol. Behav.* **103**(2): 217 – 224. PMID: 21315099.

401 Shorten, A.L., Wallman, K.E., and Guelfi, K.J. 2009. Acute effect of environmental
402 temperature during exercise on subsequent energy intake in active men. *Am. J. Clin. Nutr.*
403 **90**(5): 1215 – 1221. PMID: 19793848.

404 Stengel, A., Goebel, M., Luckey, A., Yuan, P.Q., Wang, L., and Taché, Y. 2010. Cold ambient
405 temperature reverses abdominal surgery-induced delayed gastric emptying and decreased
406 plasma ghrelin levels in rats. *Peptides.* **31**(12): 2229 – 2235. PMID: 20817059.

407 Tomasik, P.J., Sztefko, K., and Pizon, M. 2005. The effect of short-term cold and hot exposure
408 on total plasma ghrelin concentrations in humans. *Horm. Metab. Res.* **37**(3): 189 – 190. PMID:
409 15824975.

410 Tschöp, M., Strasburger, C.J., Hartmann, G., Biollaz, J., and Bärtsch, P. 1998. Raised leptin
411 concentrations at high altitude associated with loss of appetite. *Lancet.* **352**(9134): 1119 – 1120.
412 PMID: 9798594.

413 Ueda, S.Y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. 2009. Changes
414 in gut hormone levels and negative energy balance during aerobic exercise in obese young
415 males. *J. Endocrinol.* **201**(1): 151 – 159. PMID: 19158129

416 Verhulst, P.J. and Depoortere, I. 2012. Ghrelin's second life: from appetite stimulator to
417 glucose regulator. *World. J. Gastroenterol.* **18**(25): 183 – 195. PMID: 22783041.

418 Wasse, L.K., Sunderland, C., King, J.A., Batterham, R.L., and Stensel, D.J. 2012. Influence of
419 rest and exercise at a simulated altitude of 4,000m on appetite, energy intake, and plasma
420 concentrations of acylated ghrelin and peptide YY. *J. Appl. Physiol.* **112**(4): 552 – 559. PMID:
421 22114179.

422 Wasse, L.K., Sunderland, C., King, J.A., Miyashita, M., and Stensel, D.J. 2013. The influence
423 of vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin
424 concentrations in lean young men. *Appl. Physiol. Nutr. Metab.* **38**(1): 1 – 6. PMID: 23368821.

425 White, L.J., Dressendorfer, R.H., Holland, E., McCoy, S.C., and Ferguson, M.A. 2005.
426 Increased caloric intake soon after exercise in cold water. *Int. J. Sport. Nutr. Exerc. Metab.*
427 **15**(1): 38 – 47. PMID: 15902988.

428 Wiesner, S., Birkenfeld, A.L., Engeli, S., Haufe, S., Brechtel, L., Wein, J., et al. 2010.
429 Neurohumoral and metabolic response to exercise in water. *Horm. Metab. Res.* **42**(5): 334 –
430 339. PMID: 20178064.

431 Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., et al. 2001.
432 Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.*
433 **86**(12): 5992. PMID: 11739476.

434 Yu, J.H. and Kim, M.S. 2012. Molecular mechanisms of appetite regulation. *Diabetes. Metab.*
435 *J.* **36**(6): 391 – 398. PMID: 23275931.

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459 **Table 1.** Effect of environmental temperature on appetite responses assessed using visual

460 analogue scales.

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462 Note. Values are mean ± SD, *n* = 11 (heat study), *n* = 10 (cool study). PFC, prospective food
463 consumption.

	Preprandial (0 – 2 h) mm · 2h	Total trial (0 – 7 h) mm · 7h
Hunger		
Temperate	127 ± 10	258 ± 59
Heat	108 ± 15*	239 ± 82
Temperate	99 ± 40	247 ± 76
Cool	113 ± 32	258 ± 80
Satisfaction		
Temperate	42 ± 7	365 ± 23
Heat	50 ± 8	349 ± 52
Temperate	70 ± 4	379 ± 48
Cool	51 ± 23*	356 ± 67
Fullness		
Temperate	41 ± 7	361 ± 32
Heat	43 ± 8	358 ± 64
Temperate	58 ± 45	371 ± 54
Cool	45 ± 27	352 ± 72
PFC		
Temperate	154 ± 10	329 ± 54
Heat	135 ± 9*	317 ± 55
Temperate	118 ± 37	318 ± 72
Cool	129 ± 30	325 ± 69

464 *Significantly lower than respective temperate trial (*p* < 0.05)

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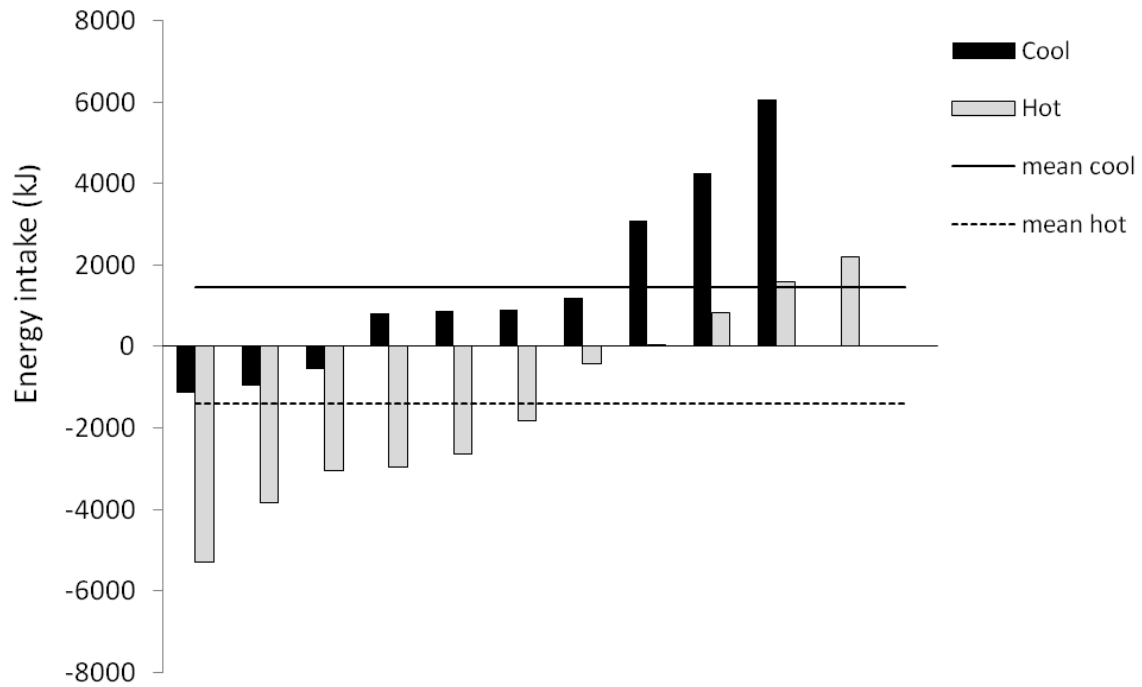
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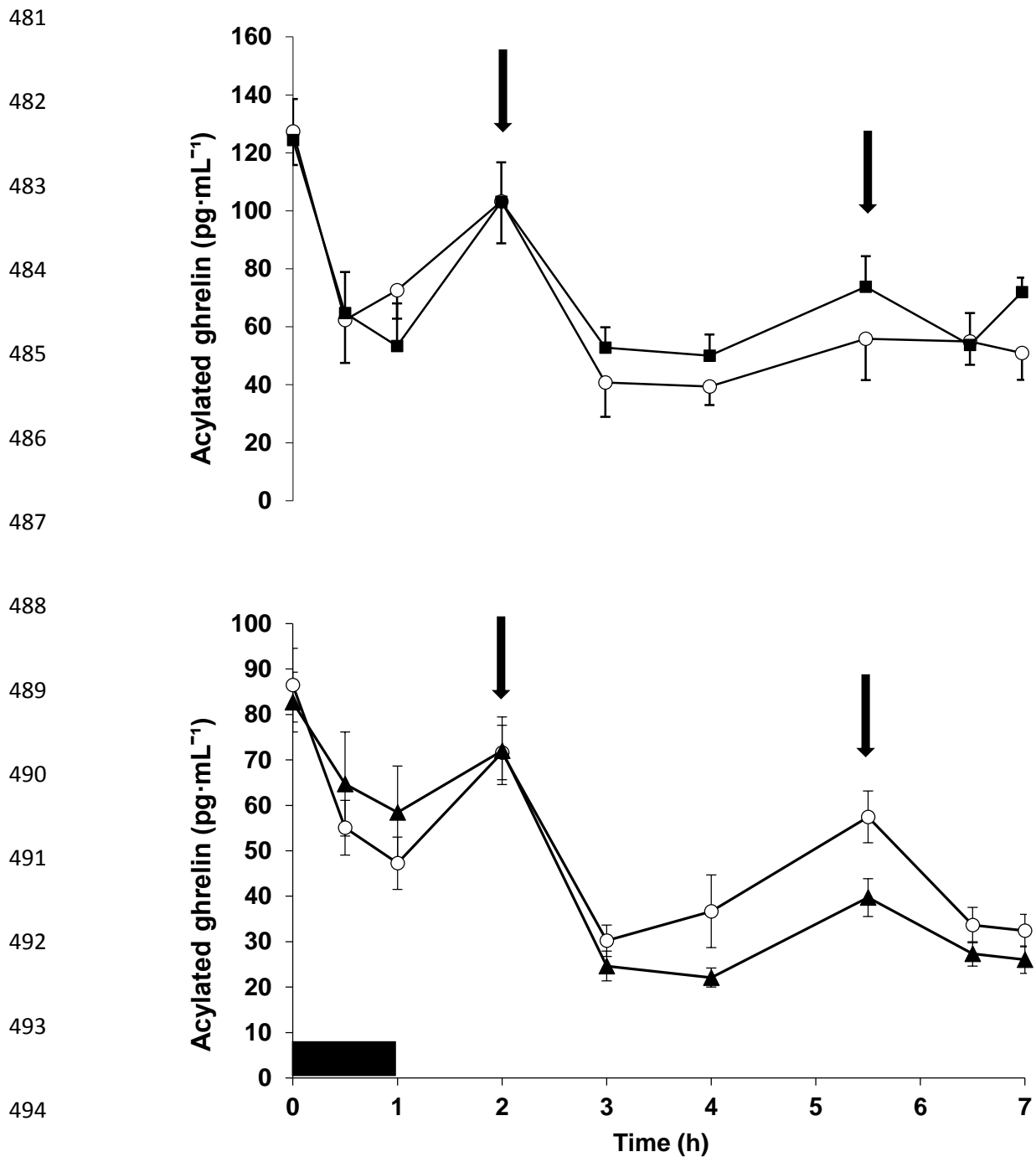
472 **Figures**



473

474 **Fig. 1.** Changes in energy intake (kJ) between temperate and cool trials (black columns, $n =$
 475 10) and temperate and hot trials (grey columns, $n = 11$). Each column represents one participant.
 476 Solid black line indicates the mean increase in energy intake in the cool trial compared with
 477 the temperate trial, dashed black line indicates the mean decrease in energy intake in the hot
 478 trial compared with the temperate trial; difference between studies $P = 0.013$ (independent
 479 samples T-test).

480



496 **Fig. 2.** Plasma acylated ghrelin concentrations during the temperate (○) and heat (■) trials (a),
 497 and during the temperate (○) and cool (▲) trials (b). Values are mean ± SEM, (n = 10). The
 498 black rectangle indicates the treadmill run and solid black arrows indicate the *ad libitum* buffet
 499 meals.