

1 **The mediating role of brown fat and skeletal muscle measured by 18F-**  
2 **Fluorodeoxyglucose in the thermoregulatory system in young adults**

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50 The authors declare they have no actual or potential competing financial interests.

51

52 **ABSTRACT**

53 **BACKGROUND:** Upon a cold exposure, brown adipose tissue (BAT) and skeletal  
54 muscles are activated as part of the thermoregulatory system, although the exact  
55 contribution of these tissues remains unknown. The personal level of environmental  
56 (Personal-ET) and wrist temperatures (WT) are measures of ambient and body  
57 temperature. Whether BAT or skeletal muscle activity is mediating the relationship  
58 between Personal-ET and WT has not been studied before.

59 **OBJECTIVES:** We examined whether BAT and skeletal muscles have a mediating  
60 role between Personal-ET and WT (as a proxy of peripheral  
61 vasoconstriction/vasodilation).

62 **MATERIAL & METHODS:** We quantified the levels of BAT by cold-induced <sup>18</sup>F-  
63 FDG-PET/CT scan, and the Personal-ET and WT by iButtons, in 75 participants (74%  
64 women).

65 **RESULTS:** We found that BAT volume and metabolic activity play a positive and  
66 significant role (up to 25.4%) in the association between Personal-ET and WT. In  
67 addition, we found that at the coldest temperatures, the participants with lower levels of  
68 WT (inducing higher peripheral vasoconstriction) had higher levels of BAT-outcomes,  
69 whereas in warmer temperatures we found that participants with higher levels of WT  
70 (inducing higher peripheral vasodilation) had lower levels of BAT-outcomes. We did  
71 not find any mediating role of skeletal muscle activity.

72 **CONCLUSION:** BAT volume and metabolic activity play a role in the relationship  
73 between Personal-ET and WT. Moreover, the data suggest that there are two distinct  
74 phenotypes: individuals who respond better to cold, both through non-shivering  
75 thermogenesis and peripheral vasoconstriction, and individuals who respond better to  
76 hot.

77 **Keywords:** Brown fat, thermoregulation, skin temperature, Temperatus®.

## 78 INTRODUCTION

79 The regulation of core body temperature is one of the most critical functions of the  
80 human body (39). Core body temperature is regulated by behavioral and physiological  
81 mechanisms (3, 39). Behavioral strategies are voluntary and oriented responses that help  
82 to maintain core body temperature, such as modifying posture, wearing clothing in  
83 winter, or using cold-air-conditioning in summer (3). On the other hand, physiological  
84 mechanisms are involuntary responses that generate or dissipate heat. In mammals, four  
85 physiological mechanisms are particularly involved in thermoregulation (39): (i) water  
86 evaporation (sweating), (ii) control of the skin blood flow, (iii) non-shivering  
87 thermogenesis (NST), and (iv) shivering thermogenesis. These mechanisms constantly  
88 interact, and their main aim is to keep the core body temperature in a normal range.

89 Skin temperature is a feedforward mechanism of the thermoregulatory system (39).  
90 When a change in the ambient temperature is detected by skin thermoreceptors, these  
91 trigger thermoregulatory responses that prevent any change in core body temperature  
92 (34). When humans are exposed to warm environments, peripheral blood vessels are  
93 dilated in order to promote heat loss (vasodilation), whereas in cold environments,  
94 peripheral blood vessels are constricted to prevent heat loss (vasoconstriction) (39). In  
95 animals, the engagement of specific thermoregulatory strategies is hierarchical (38). For  
96 instance, vasoconstriction occurs before NST, because vasoconstriction energy  
97 efficiency is higher than NST activation at least in mice models (26, 38). However,  
98 whether skin blood flow regulation mechanisms work hierarchically or concomitantly  
99 with NST activation or inhibition has not yet been studied in humans.

100 Both brown adipose tissue (BAT) and some skeletal muscles (40) play a role in NST.  
101 BAT is a specialized tissue for the rapid production of heat when the body is exposed to  
102 cold temperatures, which is mediated by the action of the uncoupling protein 1 (6). In

103 humans, BAT is mainly metabolically active upon cold exposure (9, 19, 41). However,  
104 BAT consume large quantities of energy expenditure in small mammals, although its  
105 contribution to NST in humans seems to be negligible, being the skeletal muscle the  
106 main effector of NST (5, 29, 40) and shivering (muscle contractions) during cold  
107 exposure (11). However, the contribution of BAT and skeletal muscle in the regulation  
108 of thermogenesis is largely unknown (1, 28, 40).

109 There are several ways to assess environmental temperature exposure (21, 31). Some  
110 studies quantified the personal level of environmental temperature (Personal-ET) (21),  
111 measured by an iButton during a period of 7 days. This iButton is always with the  
112 participant and should be in direct contact with the air (never with the skin) (25). This is  
113 thus a surrogate marker of temperature exposure of every individual. Other studies (4,  
114 20) quantified a proxy of skin blood flow mechanisms (16, 35) and chronobiology (37)  
115 outcomes attaching an iButton to the wrist, measuring the wrist temperature (WT),  
116 normally at the same time that the Personal-ET. Personal-ET is related to WT (21);  
117 however, since cold and warm exposures have a direct effect on activation or inhibition  
118 of BAT and skeletal muscle, it could be that these thermogenic tissues might have a  
119 mediating role between Personal-ET and WT.

120 Based on the aforementioned, we studied the mediating role of BAT and skeletal  
121 muscle activity [assessed by cold-induced  $^{18}\text{F}$ -Fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) uptake]  
122 between Personal-ET and WT in young healthy adults for 7 days (24 hours/day). In  
123 order to understand the physiological mechanisms, we examined whether the  
124 association of the number of hours exposed to a certain Personal-ET with BAT and  
125 skeletal muscle  $^{18}\text{F}$ -FDG uptake is mediated by WT as a surrogate marker of skin blood  
126 flow mechanisms.

127 **MATERIAL & METHODS**

128 A total of 90 (n=65 women) white Caucasian healthy adults aged  $21.9 \pm 2.3$  years old  
129 participated in the present study (Table 1). The participants were enrolled in the  
130 ACTIBATE study (36), an exercise-based randomized controlled trial (Clinical  
131 Trials.gov ID: NCT02365129). All participants were non-smokers, were not enrolled in  
132 a weight loss program, had a stable body weight (body weight changes  $<3$  kg) over the  
133 previous 3 months, were not physically active ( $<20$  minutes on  $<3$  days/week), did not  
134 take any medication, had no acute or chronic illness, and reported not to be regularly  
135 exposed to cold. The study was conducted in Granada (Southern Spain) between  
136 October and November in 2015 and 2016. The study protocol and informed consent  
137 were conducted in accordance with the Declaration of Helsinki (revision of 2013), and  
138 they were approved by the Human Research Ethics Committee of both the University of  
139 Granada (n° 924) and the Servicio Andaluz de Salud (Centro de Granada, CEI-  
140 Granada). A written informed consent was obtained from all the participants.

141 *Wrist and Personal Environmental temperatures measurements*

142 All participants wore 2 iButtons (DS-1922 L, Thermochron; resolution:  $0.0625$  °C;  
143 frequency: 10 min intervals; Maxim, Dallas, USA) for 7 days. One iButton was placed  
144 on the ventral side of the wrist of the non-dominant hand over the radial artery with a  
145 wrist band in order to determine WT. We instructed the participants to wear the iButton  
146 on the wrist for the whole day (even when asleep) and to take it off only when bathing  
147 or swimming. A second iButton was attached to a plastic fob and was used to quantify  
148 the Personal-ET. This iButton remained with the participant at all times but was never  
149 in direct contact with the body (21) or under clothing. During sleep-phases, the  
150 Personal-ET sensor was placed on the bedside table. The iButtons were programmed to  
151 start the recording at 06.00 and to finish 7 days later at 12.00 in the morning when the

152 <sup>18</sup>F-FDG positron emission tomography in combination with a computed tomography  
153 scan (<sup>18</sup>F-FDG) positron emission tomography with computed tomography (PET/CT)  
154 scan was performed. The participants registered the non-wear periods in a diary during  
155 the 7 days. We excluded the non-wear periods as well as those participants with less  
156 than 5 valid days. For a day to be considered valid at least 75% of the day had to be  
157 registered ( $\geq 18$  hours). All iButtons were programmed and analyzed with the  
158 Temperatus® software (<http://profith.ugr.es/temperatus?lang=en>). We calculated an  
159 average of the valid recordings for the 7 days for both WT and Personal-ET separately.  
160 Moreover, we calculated the number of hours per day that the participants were exposed  
161 to a certain temperature with a 1°C-range from 11 to 42°C for the Personal-ET (e.g. 11-  
162 11.99°C, 12-12.99°C, etc.) and from 29 to 37°C for WT (e.g. 29-29.99°C, 30-30.99°C,  
163 etc.).

#### 164 *Personalized cooling protocol*

165 The personalized cooling protocol has been explained in detail elsewhere (24). Briefly,  
166 the participants entered a mild-cold room (around 19.5°C), and they were asked to wear  
167 a water perfused cooling vest (Polar Products Inc., Ohio, USA). We determined the  
168 participant's shivering threshold, reducing the water temperature gradually until  
169 shivering occurred. Shivering was determined both visually by researchers as well as  
170 self-reported by the participants. After 48-72 hours, we exposed the participants to 2  
171 hours at their personalized temperature to induce maximum non shivering  
172 thermogenesis (above  $\sim 4^\circ\text{C}$ ) (17). After 1 hour of cold exposure, we injected a bolus of  
173 <sup>18</sup>F-FDG ( $\sim 185\text{MBq}$ ), and we increased the water temperature 1°C in order to prevent  
174 shivering. After 2 hours of cold exposure we performed the PET/CT scan from the atlas  
175 vertebrae to the thoracic vertebra 6. The evaluations were performed in 4 different  
176 weeks among 2 months (from October to November 2016) in Granada, Spain.



177 *Quantification of <sup>18</sup>F-FDG uptake by BAT and skeletal muscle*

178 We quantified BAT volume and activity following the recently published  
179 recommendations (7). PET/CT images were analyzed using the Beth Israel plugin for  
180 FIJI (24) software by BMT with the supervision of a nuclear medicine physician. We  
181 applied an individualized standardized uptake value (SUV) threshold [ $1.2/(\text{lean body}$   
182  $\text{mass/body mass})$ ] (7) with a fixed range of Hounsfield units (HU, -190 to -10). We  
183 quantified BAT volume and activity (i.e.  $\text{SUV}_{\text{mean}}$ ,  $\text{SUV}_{\text{peak}}$ ). We computed BAT  
184 metabolic activity as  $\text{BAT volume} \times \text{SUV}_{\text{mean}}$  (24) as well as the <sup>18</sup>F-FDG uptake by a  
185 reference tissue (descending aorta). We quantified the <sup>18</sup>F-FDG uptake ( $\text{SUV}_{\text{peak}}$ ) of  
186 several skeletal muscles between the atlas vertebrae and the thoracic vertebra 4. We  
187 drew a single region of interest (ROI) from 1 slice in paracervical, sternocleidomastoid,  
188 scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid,  
189 pectoralis major, and triceps brachii muscles from both left and right sides of the body  
190 (5, 13). An average of both sides including all skeletal muscles was calculated in order  
191 to obtain a single representative value of the skeletal muscle glucose uptake of the upper  
192 part of the body. Our protocol has shown a high inter-observer reliability, regardless of  
193 the threshold applied to quantify BAT (23).

194 *Body composition*

195 Body composition was assessed on a separate day by Dual Energy X-ray  
196 Absorptiometry (HOLOGIC, Discovery Wi) (36). The participants' weight and height  
197 were measured without shoes and wearing a T-shirt and shorts using a SECA scale and  
198 stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and we  
199 calculated body mass index (BMI) ( $\text{kg/m}^2$ ).

200 *Statistical analysis*

201 The descriptive characteristics of the study sample are presented as mean and standard  
202 deviation (SD) unless otherwise stated. There was no sex interaction (all  $P > 0.10$ ) in any  
203 of the study variables, thus we conducted the analyses in men and women together.

204 To quantify the mediating role of BAT volume, activity (i.e.  $SUV_{mean}$ ,  $SUV_{peak}$ ), and  
205 metabolic activity, and skeletal muscle activity in the relationship between Personal-ET  
206 and WT, we conducted mediation analyses (15). In addition, we tested the mediating  
207 role of WT on the association of the number of hours per day exposed to a certain  
208 Personal-ET with BAT volume and activity, and skeletal muscle activity. We used the  
209 PROCESS macro version 3.0, model four, with 5.000 bias-corrected bootstrap samples  
210 and 95% confidence intervals. Bootstrapping is a nonparametric resampling procedure  
211 which does not require the assumption of normality of the sampling distribution (33).  
212 The mediation was estimated using the indirect effect, which indicates the change on the  
213 effect of the independent variable on the outcome that can be endorsed to the proposed  
214 mediator. Indirect effects ( $a*b$  paths) with confidence intervals not including zero are  
215 interpreted as statistically significant (14), which could occur regardless of the  
216 significance of the total effect (c path, effect of the independent variable on the  
217 dependent variable) and the direct effect (c' path, effect on the dependent variable when  
218 both the independent and the mediator variables are included as independent variables)  
219 (15). To quantify how much of the total effect was due to the mediation, we calculated  
220 the percentage of mediation  $[(\text{indirect effect} / \text{total effect}) \times 100]$ , provided when the  
221 total effect was larger than the indirect effect with the same direction (15). All the  
222 analyses were performed using the IBM SPSS Statistics for Windows version 22.0  
223 (Armonk, NY: IBM Corp), and the level of significance was set at  $P < 0.05$ .

224

225

226 **RESULTS**

227 Table 1 shows the characteristics of the participants. A total of 15 out of 90 participants  
228 were excluded because less than 5 valid days of temperature had been recorded. A total  
229 of 75 participants (74.6% women) were finally included in the analyses, with  $6.3 \pm 0.5$   
230 valid days. The average age was  $21.9 \pm 2.3$  years old and with a BMI of  $25.2 \pm 4.8$   
231  $\text{kg/m}^2$ .

232 **The mediating role of BAT**

233 Figure 1 shows the mediating effect of BAT volume, activity (i.e.  $\text{SUV}_{\text{mean}}$  and  
234  $\text{SUV}_{\text{peak}}$ ), BAT metabolic activity, and skeletal muscle activity ( $\text{SUV}_{\text{peak}}$ ) in the  
235 relationship between Personal-ET and WT. Personal-ET was positively associated with  
236 WT ( $c$  path= 0.0763;  $P=0.0014$ ) and negatively associated with BAT-related outcomes  
237 (volume,  $\text{SUV}_{\text{mean}}$ ,  $\text{SUV}_{\text{peak}}$ , and metabolic activity,  $a$  path, all  $P<0.001$ , see Figure 1  
238 panels A, B, C, D, respectively) and skeletal muscle activity ( $a$  path,  $P=0.0023$ , see  
239 Figure 1E). BAT-related outcomes and skeletal muscle activity were not significantly  
240 associated with WT ( $b$  path). After including the mediator variables in the model (see  
241 Figure 1  $c'$  path; all  $P<0.05$ ), the direct effect of Personal-ET on WT remained  
242 statistically significant. The percentages of mediation of BAT volume and metabolic  
243 activity in the relationship between Personal-ET and WT were 25.4% and 23.9%,  
244 respectively. However, we did not observe any mediating effect of BAT activity (i.e.,  
245  $\text{SUV}_{\text{mean}}$  and  $\text{SUV}_{\text{peak}}$ ) and skeletal muscle activity in the relationship between Personal-  
246 ET and WT (see Figure 1 panels: B, C, and E, respectively). These results persisted  
247 after controlling for sex, BMI, FMI, or LMI (data not shown). Furthermore, we repeated  
248 the analyses using BAT-related outcomes as well as skeletal muscle activity multiplied  
249 by lean body mass percentage (18) and the results remained unchanged (data not  
250 shown).

## 251 **The mediating role of WT**

252 Figure 2A shows the mediating effect of WT in the relationship between the number of  
253 hours exposed to a certain Personal-ET and BAT-related outcomes (volume,  $SUV_{peak}$ ,  
254 and metabolic activity). The number of hours per day exposed to a warm Personal-ET  
255 was negatively associated with BAT volume (from 25°C to 28°C; *c* path; all  $P < 0.05$ )  
256 and positively associated with WT (from 24°C to 27°C; *a* path; all  $P < 0.05$ ) (Table S1).  
257 WT was also negatively associated with BAT volume at this temperature range (*b* path;  
258 all  $P < 0.05$ ). The direct effect was only significant when examining the number of hours  
259 per day exposed to temperatures  $\geq 26^\circ\text{C}$  (*c'* path; all  $P < 0.05$ ) (Table S1). WT showed the  
260 highest percentage of mediation (57%) in the relationship between the number of hours  
261 exposed to 24°C and BAT volume in comparison with other ranges of warm  
262 temperatures (see Figure 2E). In addition, we observed that the number of hours per day  
263 exposed to a cold Personal-ET was positively related to BAT volume (from 14°C to  
264 20°C; *c* path; all  $P < 0.05$ ) and negatively associated with WT (from 16°C to 20°C; *a* path;  
265 all  $P < 0.05$ ) (Table 2S). WT was negatively associated with BAT volume (*b* path;  
266  $P < 0.05$ ) and the association between the number of hours exposed to a cold temperature  
267 (from 16°C to 19°C) and BAT volume persisted after including WT as a mediator (*c'*  
268 path; both; all  $P < 0.05$ ). The sign of the indirect effect changed during the ambient  
269 exposure, being positive during cold-ambient exposure and negative during warm-  
270 ambient exposures (see Figure 2B-G). Moreover, when the participants were exposed to  
271 a certain range of temperature in the thermoneutral zone, WT did not play a mediating  
272 role in BAT volume (from 21°C to 23°C, see Figures 2B and E). The mediation analyses  
273 were performed for the number of hours exposed to each degree of Personal-ET  
274 showing that the mediating effect disappeared at temperatures  $\geq 28^\circ\text{C}$  or  $\leq 14^\circ\text{C}$ ,  
275 probably due to a lack of statistical power at these ranges (small number of participants

276 exposed to these extreme temperatures). The mediating role of WT was also observed in  
277 the relationship between Personal-ET and BAT activity (i.e.  $SUV_{peak}$  and metabolic  
278 activity, see Figure 2 for the indirect effect: panels C and D, respectively, and for the  
279 percentage of mediation: panels F and G, respectively; see Table S2 and S3 for further  
280 details). Furthermore, we did not find a mediating effect of the WT on the association of  
281 the number of hours exposed to a certain Personal-ET with  $SUV_{mean}$  and skeletal muscle  
282 activity (data not shown), as well as in upper ( $>29^{\circ}C$ ) and lower ( $<13^{\circ}C$ ) ranges of  
283 temperature due to the lack of statistical power in these ranges (data not shown). The  
284 results persisted after controlling by sex, BMI, LMI, FMI, or date when the evaluation  
285 were performed (data not shown). Overall, the results persisted, when we repeated all  
286 the analyses excluding data regarding the temperature ranges, for both WT and  
287 Personal-ET, when the participants were asleep (data not shown). Moreover, we  
288 repeated the analyses using other classifications of skeletal muscles (5) activity  
289 ( $SUV_{peak}$ ) and the absence of a mediating role of this tissue persisted (data not shown).

## 290 **DISCUSSION**

291 The present study quantifies, for the first time, the mediating role of human BAT and  
292 skeletal muscle cold-induced activity in the relationship between personal level of  
293 environmental temperature and human wrist temperature as an indirect proxy of skin  
294 blood flow. Intriguingly, the results show that BAT volume and metabolic activity  
295 mediate up to 25.4% of the association between Personal-ET and WT. Moreover, the  
296 results indicate that the association of the number of hours exposed to a certain  
297 Personal-ET with BAT volume,  $SUV_{peak}$ , and metabolic activity is mediated by WT at  
298 temperatures from  $14^{\circ}C$  to  $20^{\circ}C$  and from  $24^{\circ}C$  to  $28^{\circ}C$ , but not in the thermoneutral  
299 zone, as expected. We did not find a mediating role of human skeletal muscles or a  
300 relationship between WT and skeletal muscles. We also found that the participants with

301 lower WT (inducing higher peripheral vasoconstriction) at the coldest temperatures had  
302 higher levels of BAT volume,  $SUV_{peak}$  and metabolic activity, whereas the participants  
303 with higher WT (inducing higher peripheral vasodilation) at the warmest temperatures  
304 had lower levels of BAT volume,  $SUV_{peak}$  and metabolic activity. These findings show  
305 how WT (as a proxy of blood flow) is related to BAT volume and activity ( $SUV_{peak}$ ) in  
306 young adults. However, further studies are needed to elucidate the possible mechanisms  
307 behind these relationships.

### 308 **The mediating role of BAT**

309 We show that both BAT volume and metabolic activity have a mediating role in the  
310 relationship between Personal-ET and WT measured in daily living conditions  
311 independently of the sex, BMI, LMI and FMI. This indicates that participants with who  
312 were exposed to the same Personal-ET during the 7 day had different WT, which is  
313 explained, at least in part, by different levels of BAT volume or metabolic activity.  
314 Therefore, by every 1°C that the personal-ET is decreased, BAT volume would explain  
315 approximately an increase of 0.0194°C in WT. The relationship between Personal-ET  
316 and the WT daily pattern has been widely used in the field of chronobiology (21, 22).  
317 Several studies compared WT daily patterns in obese vs. normal-weight women (8),  
318 young vs. older men and women (4, 16), and men vs. women (20), and showed worse  
319 patterns (higher variability and higher daytime values) of WT in obese and older  
320 participants. These findings are also in accordance with those of human BAT studies,  
321 which showed that obese, older people, and men have lower BAT volume and activity  
322 (32). Therefore, we postulate that BAT volume and metabolic activity should be taken  
323 into account in further chronobiological studies using WT, especially in those studies,  
324 which only measured WT as a proxy of the circadian pattern without the inclusion of  
325 the Personal-ET. We established that based on the following facts: (i) the observed

326 mediating role of human BAT volume (and metabolic activity) in the relationship  
327 between Personal-ET and WT, (ii) the activation of BAT in cold ambient-temperatures  
328 (Personal-ET $\leq$ 20°C), (iii) that obese, older people, and men have lower BAT volume  
329 and activity as well as worse patterns of WT, and (iv) the circadian rhythms and,  
330 specifically core body temperature rhythms are all controlled by specific neural  
331 pathways in the anterior hypothalamus (39). For instance, Martinez-Nicolas et al. (21)  
332 studied the mediation role of WT in the relationship between Personal-ET and mean  
333 arterial blood pressure in summer and winter, and postulated that BAT could mediate  
334 this relationship. In this study, we show that this hypothesis might be true, although  
335 further studies are needed to fully understand the possible mechanisms behind these  
336 assumptions.

### 337 **The mediating role of WT**

338 All the physiological mechanisms of the thermoregulatory system seem to be  
339 orchestrated in the preoptic area (POA) of the hypothalamus (39). In addition to the  
340 peripheral tissues, the temperature of the brain is an input into the thermoregulatory  
341 system (12). One of the hypotheses explaining why human BAT is placed in the  
342 cervical and supraclavicular zone is because, as a thermogenic tissue, its main function  
343 is to regulate the temperature of the blood going to the brain (2, 42). Several studies  
344 have shown that human BAT activation is related to an increase in the blood flow in  
345 BAT (28, 30). Based on these results, the present study postulate that the increase in  
346 BAT activation (blood flow) could result in a redistribution of the blood in the  
347 peripheral part of the body during a cold stimulus which is moved into BAT in order to  
348 generate heat, since BAT is highly irrigated (27). In contrast, during a warm ambient,  
349 the blood flow in the peripheral part of the body increases at the same time as BAT  
350 blood flow and activation decrease.

351 *Warm*

352 In warm-ambient environments (from 24°C to 28°C), we observed that the higher the  
353 Personal-ET is, the higher WT is, which is associated with a lower BAT volume and  
354 activity. Therefore, by every hour exposed at 27°C (personal-ET), WT would increase  
355 and explain approximately a decrease of 3.2 ml of BAT volume. The skin has warm-  
356 sensitive neurons specially to perceive the warm exposures (39). However, there is  
357 some controversy as to which the main transient receptor potential (TRP) channel to be  
358 involved as a warm sensor is, the candidates being TRPV1, TRPV3, TRPV4, and  
359 TRPM2 (39). Therefore, there might be participants with higher or more number of  
360 TRP channels than others, and this fact could explain why there are different responses  
361 to the same stimulus, although further studies are needed. Regardless of the main TRP  
362 channel involved, our results suggest that when Personal-ET is high (hot), the body  
363 initiates some physiological response in order to preserve core temperature. Thus, the  
364 main physiological mechanism involved is to induce a peripheral vasodilation with an  
365 inhibition of human BAT (redistribution of blood flow to peripheral regions to dissipate  
366 heat). We also showed that the higher the WT is, the lower the levels of BAT volume  
367 and activity (inhibition of this tissue) are. For instance, two participants that spent the  
368 same time in warm ambient, the participant with higher WT also had lower levels of  
369 BAT volume, which might indicate more efficiency adapting to warm temperatures,  
370 which reciprocally would implicate less efficient response to cold.

371 *Cold*

372 In cold-ambient exposures (from 14°C to 19°C), we showed that the lower the Personal-  
373 ET is, the lower WT is, which is associated with higher BAT volume and activity.  
374 Therefore, by every hour exposed at 15°C (personal-ET), WT would decrease and  
375 explain approximately an increase of 2.5 ml of BAT volume. In the skin of the



376 peripheral parts of the body, there are also cold-sensitive neurons. These cold-sensitive  
377 neurons highly expressed levels of transient receptor potential cation channel subfamily  
378 M member 8 (TRMP8), which is the primary peripheral cold sensor in the  
379 thermoregulatory system (10). Animal models have shown that the inhibition of this  
380 sensor inhibits the behavioral and physiological responses to cooling (10). Taking this  
381 into consideration, we can postulate that there are individuals with a more efficient  
382 thermoregulatory system against cold stimuli, inducing a higher peripheral  
383 vasoconstriction and BAT activation in order to keep the core body temperature  
384 constant, which could be explained by a higher sympathetic tone. According to this, it  
385 might be possible for people with higher levels of human BAT to have a higher  
386 concentration of TRMP8, as well as different polymorphism of the gene TRMP8 might  
387 be associated with a better response to cold stimuli; however, these hypotheses have not  
388 been studied so far.

### 389 **The mediating role of skeletal muscles**

390 Skeletal muscles are involved in the thermoregulatory responses during cold exposure  
391 (5, 40). Interestingly, we did not observe an effect of the skeletal muscle activity (as  
392 measured by the  $^{18}\text{F}$ -FDG uptake) in the relationship between Personal-ET and WT.  
393 This lack of mediating effect does not necessarily mean that skeletal muscle is not  
394 involved in cold-induced thermogenesis. This lack of effect might be due to the fact that  
395 the cold-ambient temperatures were not cold enough to induce skeletal muscle  
396 activation, or because the  $^{18}\text{F}$ -FDG tracer is not a good marker of skeletal muscle  
397 metabolism (40).

398 We postulate, however, that there are participants who respond better (i.e. responders)  
399 than others to cold exposures, and others that respond better to warm exposures, yet  
400 further studies are needed. This assumption is also based on the fact that some people

401 could have an overexpression of POA neuron levels or TRMP8 or TRP channels (39),  
402 making the thermoregulatory system more efficient, or maybe in the brain the areas  
403 involved in the thermoregulatory system are different. This cross-sectional and  
404 observational study should be replicated in older participants and using other nuclear  
405 tracers such as  $^{15}\text{O-O}_2$ , [ $^{11}\text{C}$ ]-acetate (40), or adenosine perfusion, a vasodilator that  
406 seems to activate human BAT (23). Moreover, we know that during sleep phases humans  
407 can lose at least 25% of their total thermoregulatory capacity. Since our aim was to  
408 study the mediating role of human BAT during 7 days (even in sleep phases) we keep  
409 these analyses as main results, although excluding the sleep phase did not alter the  
410 results (data not shown). Moreover, in this study the level of clothing during the  
411 measurements were not evaluated. Future experimental studies are warranted to  
412 elucidate the possible mechanisms behind this efficiency in the thermoregulatory  
413 system and new therapies that could be developed to improve this physiological system.

#### 414 **CONCLUSIONS**

415 We show that BAT volume and metabolic activity mediate the relationship between  
416 Personal-ET and WT. Moreover, our data support that the individuals who were  
417 exposed to lower environmental temperatures and at the same time had lower wrist skin  
418 temperature, concomitantly had higher BAT volume. We also observed the opposite  
419 effect when the participants were exposed to warmer temperatures, which indicates a  
420 redistribution of the blood flow between the peripheral part of the body and BAT  
421 activation/inhibition in order to keep the core body temperature constant. Future  
422 interventional studies should try to find strategies to improve the thermoregulatory  
423 system and its relationship with metabolic diseases.

424

425 **AUTHOR CONTRIBUTIONS**

426 Conception and design of research: B.M.T. and J.R.R.; B.M.T., F.A.M, G.S.D., and  
427 J.M.L.E. performed the experiments; M.A.R., B.M.T., V.M.V., and J.R.R. analyzed the  
428 data; All authors interpreted the results; M.A.R. and B.M.T. prepared the figures and  
429 drafted manuscript; All authors critically revised the manuscript and approved the final  
430 version.

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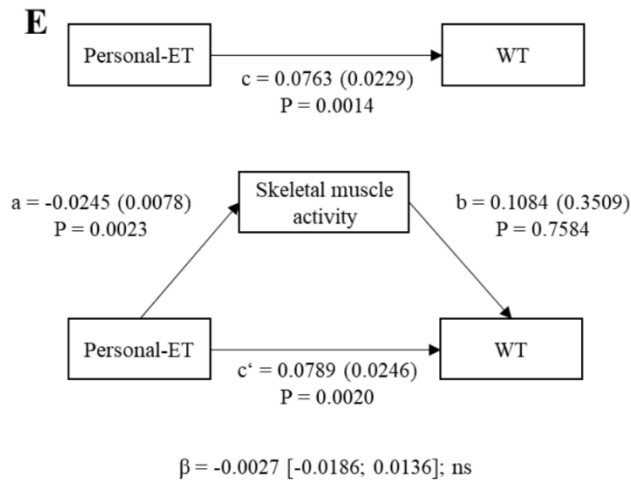
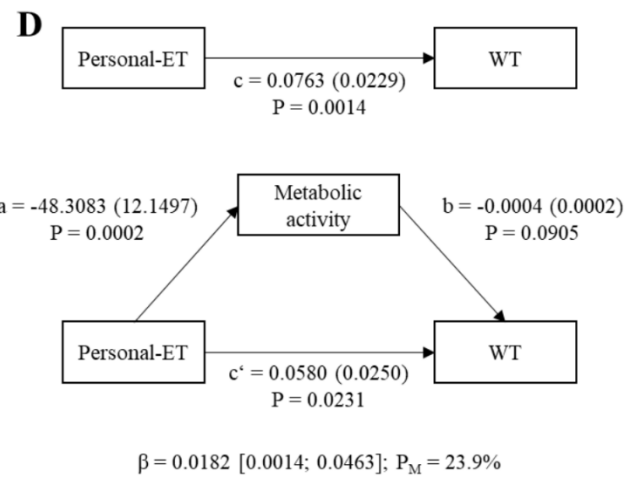
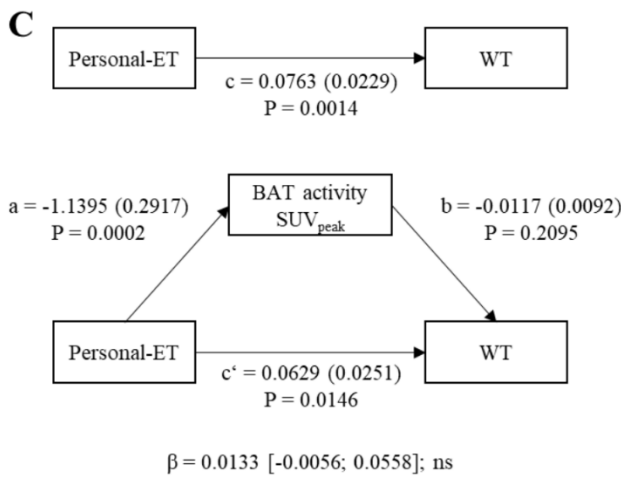
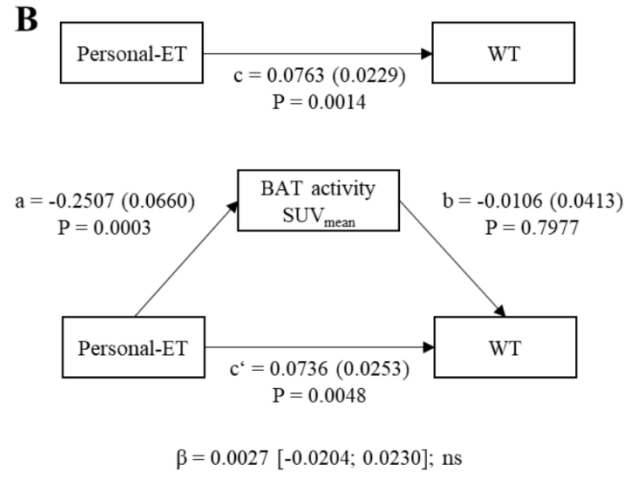
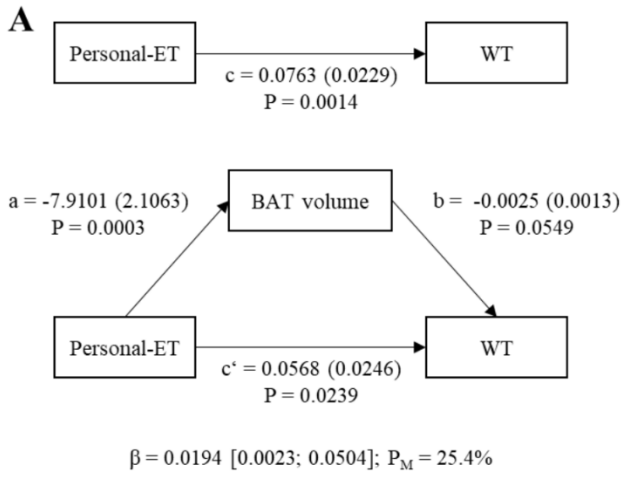
578

**Table 1.** Characteristics of the study participants

	n=75
579	
580	Sex (% women) 74.6%
581	Age (years) 21.9 ± 2.3
582	Body mass index (kg/m <sup>2</sup> ) 25.2 ± 4.8
583	Lean mass (kg) 41.3 ± 9.6
584	Fat mass (kg) 26.9 ± 9.5
585	Fat mass (%) 37.6 ± 7.0
586	BAT volume (ml) 69.0 ± 61.3
587	BAT activity (SUV <sub>mean</sub> g/ml) 3.7 ± 1.9
588	BAT activity (SUV <sub>peak</sub> g/ml) 11.0 ± 8.5
589	Wrist Temperature (°C) 34.0 ± 0.7
590	Personal-ET (°C) 22.8 ± 3.1

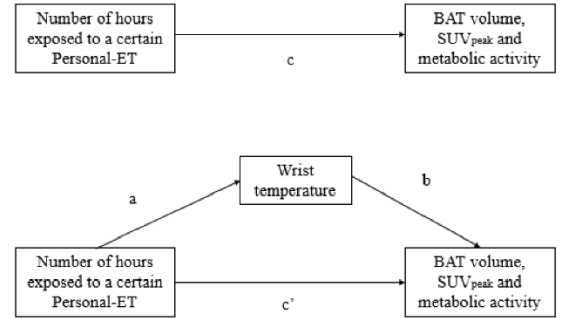
590 Data are presented as mean and standard deviation,  
 591 unless otherwise stated. BAT: brown adipose tissue,  
 592 Personal-ET: Personal level of environmental  
 temperature, SUV: standardized uptake value.

593 **Figure 1.** Mediation models of the relationship between personal levels of environmental temperature and  
594 wrist skin temperature with (A) BAT volume (ml), (B)  $SUV_{mean}$  (g/ml), (C)  $SUV_{peak}$  (g/ml), (D) metabolic  
595 activity (calculated as BAT volume x  $SUV_{mean}$ ), and (E) skeletal muscle activity (g/ml) included as  
596 mediator variables, respectively.  
597 Paths a, b, c, and c' are presented as unstandardized coefficients (Standard Error, SE).  $\beta$  = indirect effect  
598 ( $a*b$  paths); [lower limit confident interval: upper limit confident interval], lower and upper levels for  
599 95% confidence interval of the indirect effect based on 5000 bootstraps.  
600 The results are shown as unstandardized coefficients (Standard Error, SE) and bias corrected 95% CI  
601 based on 5000 bootstraps.  
602 Personal ET: personal levels of environmental temperature; WT: wrist skin temperature; BAT: Brown  
603 adipose tissue;  $P_M$ : percentage of mediation; SUV: Standardized uptake value; WT: wrist temperature.  
604 ns: non-significant.

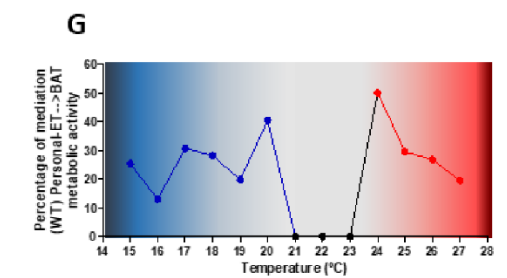
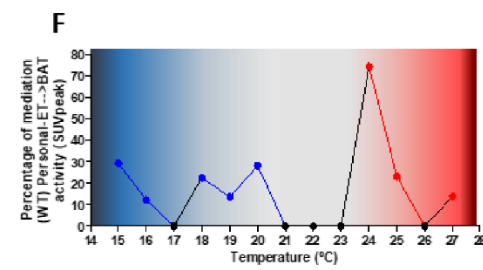
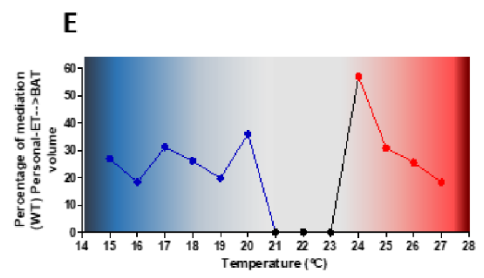
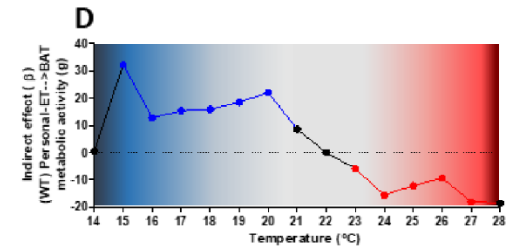
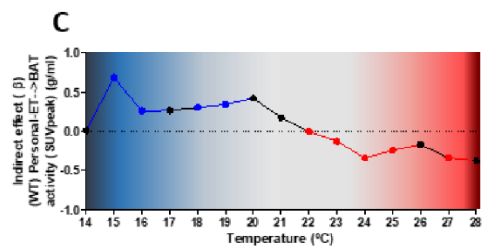
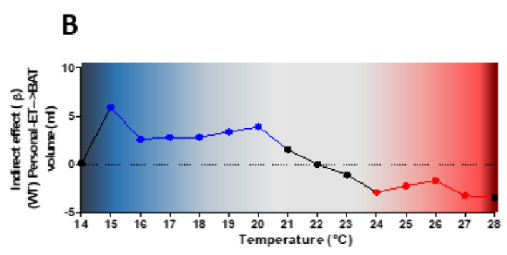


606 **Figure 2.** A) Mediation models of the relationship between the number of hours exposed to a certain  
607 Personal-ET and BAT-related outcomes in young adults. Path  $c$  shows the association between  
608 independent and dependent variables. Arrows  $a \times b$  show the natural indirect effect ( $\beta$ ) pathway, and  $c'$   
609 shows the natural direct effect pathway. B) Indirect effects ( $\beta$ ) of the simple mediation analyses of wrist  
610 temperature on the association between the number of hours exposed to each degree of Personal-ET (from  
611 14°C to 28°C) and BAT volume, whereas panels C and D refer to BAT  $SUV_{peak}$  and metabolic activity,  
612 respectively. E)  $P_M$  of the simple mediation analyses of wrist temperature on the association between the  
613 number of hours exposed to each degree of personal-ET (from 14°C to 28°C) and BAT volume, whereas  
614 panels F and G refer to BAT  $SUV_{peak}$  and metabolic activity, respectively. Black dots represent that 0 was  
615 in the 95% confidence interval of the indirect effect, and, therefore, the mediation was considered non-  
616 statistically significant ( $P > 0.05$ ). Red and blue dots mean that the mediation analysis was statistically  
617 significant but with a different direction. BAT: brown adipose tissue; Personal-ET: personal level of  
618 environmental temperature; WT: wrist skin temperature;  $P_M$ : Percentage of mediation.  
619

**A**



$\beta$  = indirect effect (Fig. 2B, C and D);  $P_M$ (%; Fig. 2E, F and G)



**Table S1.** Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and brown adipose tissue volume.

Independent variable	Total effect (c)	Direct effect (c')	Path a	Path b	Indirect effect (a*b)	BC 95% CI Lower Upper	P <sub>M</sub> (%)
<i>Number of hours per day exposed to</i>							
28°C	-24.1511 (8.5478)**	-20.7341 (8.2251)*	0.1172 (0.0952)	-29.1556 (10.0126)**	-3.4170	-13.6843; 2.2662	
27°C	-17.5167 (4.8821)***	-14.2999 (4.9035)**	0.1296 (0.0545)*	-24.8155 (10.1490)*	<b>-3.2168</b>	-8.9958; -0.5952	18.36
26°C	-6.5085 (1.9827)**	-4.8443 (2.0725)*	0.0702 (0.0211)**	-23.7003 (10.6935)*	<b>-1.6642</b>	-3.4272; -0.3454	25.57
25°C	-7.2114 (2.8879)*	-4.9839 (2.9130)	0.0814 (0.0307)**	-27.3753 (10.6111)*	<b>-2.2275</b>	-5.4156; -0.4822	30.89
24°C	-5.0423 (3.6912)	-2.1706 (3.6547)	0.0925 (0.0384)*	-31.0297 (10.7150)**	<b>-2.8717</b>	-5.8755; -0.9852	56.95
23°C	-0.1435 (2.5060)	0.9153 (2.3847)	0.0318 (0.0265)	-33.3129 (10.4289)**	-1.0588	-2.6913; 0.0416	
22°C	-1.7610 (2.8817)	-1.7698 (2.7173)	-0.0003 (0.0309)	-32.7633 (10.3079)**	0.0087	-1.6455; 2.1567	
21°C	1.5069 (3.5844)	-0.0470 (3.4213)	-0.0474 (0.0379)	-32.7773 (10.4483)**	1.5539	-0.6785; 4.8597	
20°C	10.8615 (5.0206)*	6.9503 (5.0396)	-0.1373 (0.0529)*	-28.4826 (10.6645)**	<b>3.9112</b>	1.1979; 9.3971	36.01
19°C	17.0622 (5.2043)**	13.6895 ( 5.1955)*	-0.1305 (0.0575)*	-25.8529 (10.2149)*	<b>3.3727</b>	0.5235; 9.4524	19.77
18°C	10.7468 (3.2756)**	7.9320 (3.4450)*	-0.1199 (0.0347)***	-23.4791 (10.7603)*	<b>2.8148</b>	0.5623; 8.0286	26.19
17°C	9.0101 (2.7922)**	6.1971 (3.0982)*	-0.1271 (0.0282)***	-22.1406 (11.3763)	<b>2.8130</b>	0.3676; 6.3115	31.22
16°C	14.1431 (4.0529)***	11.5538 (4.0487)**	-0.1021 (0.0452)*	-25.3649 (10.1354)*	<b>2.5893</b>	0.4701; 6.8149	18.31
15°C	21.6970 (9.6794)*	16.0500 (9.5090)	-0.2054 (0.1042)	-28.8089 (10.4058)**	<b>5.9170</b>	1.0768; 16.2034	26.94

14°C                      23.2957 (8.7497)\*\*      23.1941 (8.2016)\*\*      -0.0031 (0.0979)      -32.6534 (9.8079)\*\*      0.1015      -8.4056; 8.6450

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps.

BC: Bias corrected; CI: confidence interval; P<sub>M</sub>: percentage of mediation.

Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.

p-values indicating associations between study variables = \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Table S2.** Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and standardized uptake value peak.

Independent variable	Total effect (c)	Direct effect (c')	Path a	Path b	Indirect effect (a*b)	BC 95% CI Lower Upper	P <sub>M</sub> (%)
<i>Number of hours per day exposed to</i>							
28°C	-3.6344 (1.1794)**	-3.2599 (1.1593)**	0.1172 (0.0952)	-3.1947 (1.4113)*	-0.3744	-1.7238; 0.2237	
27°C	-2.4402 (0.6799)***	-2.1041 (0.6951)**	0.1296 (0.0545)*	-2.5924 (1.4387)	<b>-0.3360</b>	-1.0857; -0.0057	13.77
26°C	-0.8976 (0.2765)**	-0.7293 (0.2937)*	0.0702 (0.0211)**	-2.3975 (1.5152)	-0.1683	-0.4264; 0.0264	
25°C	-1.0210 (0.4016)*	-0.7839 (0.4128)	0.0814 (0.0307)**	-2.9145 (1.5036)	<b>-0.2372</b>	-0.6959; -0.0130	23.23
24°C	-0.4558 (0.5178)	-0.1163 (0.5213)	0.0925 (0.0384)*	-3.6683 (1.5283)*	<b>-0.3395</b>	-0.7859; -0.0673	74.49
23°C	0.1326 (0.3487)	0.2571 (0.3384)	0.0318 (0.0265)	-3.9171 (1.4800)**	<b>-0.1245</b>	-0.3651; -0.0015	N/A
22°C	-0.1011 (0.4022)	-0.1021 (0.3877)	-0.0003 (0.0309)	-3.7612 (1.4708)*	0.001	-0.2129; 0.2276	
21°C	0.1960 (0.4993)	0.0180 (0.4870)	-0.0474 (0.0379)	-3.7529 (1.4872)*	0.1779	-0.0555; 0.6272	
20°C	1.5091 (0.6993)*	1.0842 (0.7154)	-0.1373 (0.0529)*	-3.0942 (1.5139)*	<b>0.4249</b>	0.0396; 1.1213	28.16
19°C	2.5401 (0.7171)***	2.1937 (0.7299)**	-0.1305 (0.0575)*	-2.6546 (1.4351)	<b>0.3463</b>	0.0104; 1.1236	13.63



<b>18°C</b>	1.3436 (0.4626)**	1.0383 (0.4931)*	-0.1199 (0.0347)***	-2.5464 (1.5403)	<b>0.3053</b>	0.0136; 0.9144	22.72
<b>17°C</b>	1.2252 (0.3901)**	0.9553 (0.4389)*	-0.1271 (0.0282)***	-2.1244 (1.6115)	0.2699	-0.0720; 0.7686	
<b>16°C</b>	2.1308 (0.5563)***	1.8689 (0.5667)**	-0.1021 (0.0452)*	-2.5652 (1.4187)	<b>0.2619</b>	0.0080; 0.7930	12.29
<b>15°C</b>	2.3449 (1.3674)	1.6561 (1.3661)	-0.2054 (0.1042)	-3.3535 (1.4950)*	<b>0.6888</b>	0.0485; 2.0564	29.37
<b>14°C</b>	2.5672 (1.2404)*	2.5555 (1.1931)*	0.1172 (0.0979)	-3.7495 (1.4268)*	0.0117	-0.9257; 0.9111	

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps. BC: Bias corrected; CI: confidence interval; P<sub>M</sub>: percentage of mediation; N/A: non-applicable according to statistical assumptions specified previously. Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold. p-values indicating associations between study variables = \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Table S3.** Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and metabolic activity.

<b>Independent variable</b>	<b>Total effect (c)</b>	<b>Direct effect (c')</b>	<b>Path a</b>	<b>Path b</b>	<b>Indirect effect (a*b)</b>	<b>BC 95% CI Lower Upper</b>	<b>P<sub>M</sub>(%)</b>
<i>Number of hours per day exposed to</i>							
<b>28°C</b>	-142.2746 (49.6038)**	-123.6264 (48.0546)*	0.1172 (0.0952)	-159.1181 (58.4978)**	-18.6482	-76.7198; 13.6812	
<b>27°C</b>	-92.7638 (28.7954)**	-74.7342 (29.0376)*	0.1296 (0.0545)*	-139.0875 (60.1009)*	<b>-18.0296</b>	-50.4684; -2.6810	19.44
<b>26°C</b>	-34.8199 (11.6524)**	-25.4847 (12.2166)*	0.0702 (0.0211)**	-132.9461 (63.0354)*	<b>-9.3352</b>	-19.6154; -1.8476	26.81
<b>25°C</b>	-41.1781 (16.8086)*	-29.0344 (17.0508)	0.0814 (0.0307)**	-149.2399 (62.1105)*	<b>-12.1436</b>	-31.4411; -2.6588	29.49
<b>24°C</b>	-31.1133 (21.4178)	-15.5447 (21.3620)	0.0925 (0.0384)*	-168.2222 (62.6304)**	<b>-15.5686</b>	-32.7741; -4.8195	50.04
<b>23°C</b>	-2.0202 (14.5630)	3.7930 (13.9632)	0.0318 (0.0265)	-182.8941 (61.0639)**	<b>-5.8132</b>	-15.5371; -0.0279	N/A

<b>22°C</b>	-8.0621 (16.7643)	-8.1103 (15.9204)	-0.0003 (0.0309)	-180.6196 (60.3934)**	0.0482	-9.8282; 11.9435	
<b>21°C</b>	6.3637 (20.8440)	-2.2449 (20.0209)	-0.0474 (0.0379)	-181.5810 (61.1410)**	8.6086	-3.0637; 29.3434	
<b>20°C</b>	54.4724 (29.4166)	32.4111 (29.6347)	-0.1373 (0.0529)*	-160.658 (62.7118)*	<b>22.0613</b>	6.3974; 55.8110	40.5
<b>19°C</b>	93.9165 (30.4749)**	75.3119 (30.5755)*	-0.1305 (0.0575)*	-142.6087 (60.1146)*	<b>18.6046</b>	2.8291; 52.5241	19.81
<b>18°C</b>	56.6625 (19.2841)**	40.7224 (20.3314)*	-0.1199 (0.0347)***	-132.9587 (63.5043)*	<b>15.9400</b>	3.4256; 45.2335	28.13
<b>17°C</b>	50.0828 (16.3261)**	34.6886 (18.1741)	-0.1271 (0.0282)***	-121.1656 (66.7343)	<b>15.3941</b>	1.9813; 38.6296	30.74
<b>16°C</b>	98.7912 (22.6644)***	85.9709 (22.8526)***	-0.1021 (0.0452)*	-125.5885 (57.2095)*	<b>12.8203</b>	2.8726; 34.6266	12.98
<b>15°C</b>	127.3577 (56.2649)*	95.0696 (55.6223)	-0.2054 (0.1042)	-157.2056 (60.8681)*	<b>32.288</b>	5.3280; 88.6667	25.35
<b>14°C</b>	162.8793 (49.7349)**	162.3202 (46.8374)***	-0.0031 (0.0979)	-179.8669 (56.0109)**	0.5591	-40.2083; 48.2821	

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps. BC: Bias corrected; CI: confidence interval; P<sub>M</sub>: percentage of mediation; N/A: non-applicable according to statistical assumptions specified previously. Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold. p-values indicating associations between study variables = \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.