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

Borja Martinez-Tellez\textsuperscript{1,2+*}, Mireia Adelantado-Renau\textsuperscript{3†}, Francisco M. Acosta\textsuperscript{1},
Guillermo Sanchez-Delgado\textsuperscript{1}, Antonio Martinez-Nicolas\textsuperscript{4,5}, Mariëtte R. Boon\textsuperscript{2}, Jose M.
Llamas-Elvira\textsuperscript{6,7}, Vicente Martinez-Vizcaíno\textsuperscript{8,9}, Jonatan R. Ruiz\textsuperscript{1}

\textsuperscript{1}PROFITH (PROmoting FITness and Health through Physical Activity) Research
Group, Department of Physical Education and Sports, Faculty of Sport Sciences,
University of Granada, Granada, Spain.

\textsuperscript{2}Department of Medicine, division of Endocrinology, and Einthoven Laboratory for
Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the
Netherlands.

\textsuperscript{3}LIFE Research Group, University Jaume I, Castellon, Spain.

\textsuperscript{4}Chronobiology Lab, Department of Physiology, College of Biology, University of
Murcia, Mare Nostrum Campus. IUIE, IMIB-Arrixaca, Spain.

\textsuperscript{5}Ciber Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain.

\textsuperscript{6}Servicio de Medicina Nuclear, Hospital Universitario Virgen de las Nieves, Granada,
Spain.

\textsuperscript{7}Biohealth Research Institute in Granada (ibs.GRANADA), Nuclear Medicine
Department. Granada, Spain.

\textsuperscript{8}Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca,
Spain.

\textsuperscript{9}Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile

*Corresponding author: borjammt@gmail.com

†These authors share first authorship
Address for correspondence: Borja Martinez-Tellez, MSc. Department of Physical Education and Sports, Faculty of Sport Sciences. University of Granada, Granada, Spain. Ctra de Alfacar s/n C.P. 18071. E-mail: borjammt@gmail.com; tel.: 0034 958242754; Fax: 0034 958244369

Acknowledgements
The authors would like to thank all the participants who took part in this investigation. This study is part of a Ph.D. Thesis conducted in the Biomedicine Doctoral Studies of the University of Granada, Spain. We are grateful to Alberto Quesada-Aranda for helping with the development of the Temperatus software (Free trial in http://proftd.ugr.es/temperatus). We are grateful to Ms. Carmen Sainz-Quinn for assistance with the English language. This study was supported by the Spanish Ministry of Economy and Competitiveness, Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI13/01393) and Retos de la Sociedad (DEP2016-79512-R), Fondos Estructurales de la Unión Europea (FEDER), by the Spanish Ministry of Education (FPU 13/04365), by the Fundación Iberoamericana de Nutrición (FINUT), by the Redes temáticas de investigación cooperativa RETIC (Red SAMID RD16/0022), by AstraZeneca HealthCare Foundation and by the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence; Scientific Unit of Excellence on Exercise and Health (UCEES). M.A.R is supported by a Predoctoral Research Grant from UJI (PREDOC/2015/13). A.M.N was supported by the Ministry of Economy and Competitiveness, the Instituto de Salud Carlos III through CIBERFES (CB16/10/00239) and grant 19899/GERM/15 (co-financed by FEDER).

Declaration of competing financial interests
The authors declare they have no actual or potential competing financial interests.
ABSTRACT

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

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

MATERIAL & METHODS: We quantified the levels of BAT by cold-induced $^{18}$F-FDG-PET/CT scan, and the Personal-ET and WT by iButtons, in 75 participants (74% women).

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

CONCLUSION: BAT volume and metabolic activity play a role in the relationship between Personal-ET and WT. Moreover, the data suggest that there are two distinct phenotypes: individuals who respond better to cold, both through non-shivering thermogenesis and peripheral vasoconstriction, and individuals who respond better to hot.
Keywords: Brown fat, thermoregulation, skin temperature, Temperatus®.
The regulation of core body temperature is one of the most critical functions of the human body (39). Core body temperature is regulated by behavioral and physiological mechanisms (3, 39). Behavioral strategies are voluntary and oriented responses that help to maintain core body temperature, such as modifying posture, wearing clothing in winter, or using cold-air-conditioning in summer (3). On the other hand, physiological mechanisms are involuntary responses that generate or dissipate heat. In mammals, four physiological mechanisms are particularly involved in thermoregulation (39): (i) water evaporation (sweating), (ii) control of the skin blood flow, (iii) non-shivering thermogenesis (NST), and (iv) shivering thermogenesis. These mechanisms constantly interact, and their main aim is to keep the core body temperature in a normal range.

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

Both brown adipose tissue (BAT) and some skeletal muscles (40) play a role in NST. BAT is a specialized tissue for the rapid production of heat when the body is exposed to cold temperatures, which is mediated by the action of the uncoupling protein 1 (6). In
humans, BAT is mainly metabolically active upon cold exposure (9, 19, 41). However, BAT consume large quantities of energy expenditure in small mammals, although its contribution to NST in humans seems to be negligible, being the skeletal muscle the main effector of NST (5, 29, 40) and shivering (muscle contractions) during cold exposure (11). However, the contribution of BAT and skeletal muscle in the regulation of thermogenesis is largely unknown (1, 28, 40).

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

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

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

Wrist and Personal Environmental temperatures measurements

All participants wore 2 iButtons (DS-1922 L, Thermochron; resolution: 0.0625 °C; frequency: 10 min intervals; Maxim, Dallas, USA) for 7 days. One iButton was placed on the ventral side of the wrist of the non-dominant hand over the radial artery with a wrist band in order to determine WT. We instructed the participants to wear the iButton on the wrist for the whole day (even when asleep) and to take it off only when bathing or swimming. A second iButton was attached to a plastic fob and was used to quantify the Personal-ET. This iButton remained with the participant at all times but was never in direct contact with the body (21) or under clothing. During sleep-phases, the Personal-ET sensor was placed on the bedside table. The iButtons were programmed to start the recording at 06.00 and to finish 7 days later at 12.00 in the morning when the
18F-FDG positron emission tomography in combination with a computed tomography scan (18F-FDG positron emission tomography with computed tomography (PET/CT) scan was performed. The participants registered the non-wear periods in a diary during the 7 days. We excluded the non-wear periods as well as those participants with less than 5 valid days. For a day to be considered valid at least 75% of the day had to be registered (≥18 hours). All iButtons were programmed and analyzed with the Temperatus® software (http://profith.ugr.es/temperatus?lang=en). We calculated an average of the valid recordings for the 7 days for both WT and Personal-ET separately. Moreover, we calculated the number of hours per day that the participants were exposed to a certain temperature with a 1°C-range from 11 to 42°C for the Personal-ET (e.g. 11-11.99°C, 12-12.99°C, etc.) and from 29 to 37°C for WT (e.g. 29-29.99°C, 30-30.99°C, etc.).

**Personalized cooling protocol**

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

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

Body composition

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

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

To quantify the mediating role of BAT volume, activity (i.e. SUV\textsubscript{mean}, SUV\textsubscript{peak}), and metabolic activity, and skeletal muscle activity in the relationship between Personal-ET and WT, we conducted mediation analyses (15). In addition, we tested the mediating role of WT on the association of the number of hours per day exposed to a certain Personal-ET with BAT volume and activity, and skeletal muscle activity. We used the PROCESS macro version 3.0, model four, with 5,000 bias-corrected bootstrap samples and 95% confidence intervals. Bootstrapping is a nonparametric resampling procedure which does not require the assumption of normality of the sampling distribution (33).

The mediation was estimated using the indirect effect, which indicates the change on the effect of the independent variable on the outcome that can be endorsed to the proposed mediator. Indirect effects (a\*b paths) with confidence intervals not including zero are interpreted as statistically significant (14), which could occur regardless of the significance of the total effect (c path, effect of the independent variable on the dependent variable) and the direct effect (c’ path, effect on the dependent variable when both the independent and the mediator variables are included as independent variables) (15). To quantify how much of the total effect was due to the mediation, we calculated the percentage of mediation [(indirect effect / total effect) x 100], provided when the total effect was larger than the indirect effect with the same direction (15). All the analyses were performed using the IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp), and the level of significance was set at P<0.05.
RESULTS

Table 1 shows the characteristics of the participants. A total of 15 out of 90 participants were excluded because less than 5 valid days of temperature had been recorded. A total of 75 participants (74.6% women) were finally included in the analyses, with 6.3 ± 0.5 valid days. The average age was 21.9 ± 2.3 years old and with a BMI of 25.2 ± 4.8 kg/m².

The mediating role of BAT

Figure 1 shows the mediating effect of BAT volume, activity (i.e. SUV_{\text{mean}} and SUV_{\text{peak}}), BAT metabolic activity, and skeletal muscle activity (SUV_{\text{peak}}) in the relationship between Personal-ET and WT. Personal-ET was positively associated with WT (c path= 0.0763; P=0.0014) and negatively associated with BAT-related outcomes (volume, SUV_{\text{mean}}, SUV_{\text{peak}}, and metabolic activity, a path, all P<0.001, see Figure 1 panels A, B, C, D, respectively) and skeletal muscle activity (a path, P=0.0023, see Figure 1E). BAT-related outcomes and skeletal muscle activity were not significantly associated with WT (b path). After including the mediator variables in the model (see Figure 1 c’ path; all P<0.05), the direct effect of Personal-ET on WT remained statistically significant. The percentages of mediation of BAT volume and metabolic activity in the relationship between Personal-ET and WT were 25.4% and 23.9%, respectively. However, we did not observe any mediating effect of BAT activity (i.e., SUV_{\text{mean}} and SUV_{\text{peak}}) and skeletal muscle activity in the relationship between Personal-ET and WT (see Figure 1 panels: B, C, and E, respectively). These results persisted after controlling for sex, BMI, FMI, or LMI (data not shown). Furthermore, we repeated the analyses using BAT-related outcomes as well as skeletal muscle activity multiplied by lean body mass percentage (18) and the results remained unchanged (data not shown).
Figure 2A shows the mediating effect of WT in the relationship between the number of hours exposed to a certain Personal-ET and BAT-related outcomes (volume, SUV\textsubscript{peak}, and metabolic activity). The number of hours per day exposed to a warm Personal-ET was negatively associated with BAT volume (from 25°C to 28°C; \(c\) path; all P<0.05) and positively associated with WT (from 24°C to 27°C; \(a\) path; all P<0.05) (Table S1). WT was also negatively associated with BAT volume at this temperature range (\(b\) path; all P<0.05). The direct effect was only significant when examining the number of hours per day exposed to temperatures \(\geq \)26°C (\(c'\) path; all P<0.05) (Table S1). WT showed the highest percentage of mediation (57%) in the relationship between the number of hours exposed to 24°C and BAT volume in comparison with other ranges of warm temperatures (see Figure 2E). In addition, we observed that the number of hours per day exposed to a cold Personal-ET was positively related to BAT volume (from 14°C to 20°C; \(c\) path; all P<0.05) and negatively associated with WT (from 16°C to 20°C; \(a\) path; all P<0.05) (Table 2S). WT was negatively associated with BAT volume (\(b\) path; P<0.05) and the association between the number of hours exposed to a cold temperature (from 16°C to 19°C) and BAT volume persisted after including WT as a mediator (\(c'\) path; both; all P<0.05). The sign of the indirect effect changed during the ambient exposure, being positive during cold-ambient exposure and negative during warm-ambient exposures (see Figure 2B-G). Moreover, when the participants were exposed to a certain range of temperature in the thermoneutral zone, WT did not play a mediating role in BAT volume (from 21°C to 23°C, see Figures 2B and E). The mediation analyses were performed for the number of hours exposed to each degree of Personal-ET showing that the mediating effect disappeared at temperatures \(\geq\)28°C or \(\leq\)14°C, probably due to a lack of statistical power at these ranges (small number of participants
exposed to these extreme temperatures). The mediating role of WT was also observed in
the relationship between Personal-ET and BAT activity (i.e. SUV\textsubscript{peak} and metabolic
activity, see Figure 2 for the indirect effect: panels C and D, respectively, and for the
percentage of mediation: panels F and G, respectively; see Table S2 and S3 for further
details). Furthermore, we did not find a mediating effect of the WT on the association of
the number of hours exposed to a certain Personal-ET with SUV\textsubscript{mean} and skeletal muscle
activity (data not shown), as well as in upper (>29ºC) and lower (<13ºC) ranges of
temperature due to the lack of statistical power in these ranges (data not shown). The
results persisted after controlling by sex, BMI, LMI, FMI, or date when the evaluation
were performed (data not shown). Overall, the results persisted, when we repeated all
the analyses excluding data regarding the temperature ranges, for both WT and
Personal-ET, when the participants were asleep (data not shown). Moreover, we
repeated the analyses using other classifications of skeletal muscles (5) activity
(SUV\textsubscript{peak}) and the absence of a mediating role of this tissue persisted (data not shown).

**DISCUSSION**

The present study quantifies, for the first time, the mediating role of human BAT and
skeletal muscle cold-induced activity in the relationship between personal level of
environmental temperature and human wrist temperature as an indirect proxy of skin
blood flow. Intriguingly, the results show that BAT volume and metabolic activity
mediate up to 25.4% of the association between Personal-ET and WT. Moreover, the
results indicate that the association of the number of hours exposed to a certain
Personal-ET with BAT volume, SUV\textsubscript{peak}, and metabolic activity is mediated by WT at
temperatures from 14ºC to 20ºC and from 24ºC to 28ºC, but not in the thermoneutral
zone, as expected. We did not find a mediating role of human skeletal muscles or a
relationship between WT and skeletal muscles. We also found that the participants with
lower WT (inducing higher peripheral vasoconstriction) at the coldest temperatures had higher levels of BAT volume, SUV$_\text{peak}$ and metabolic activity, whereas the participants with higher WT (inducing higher peripheral vasodilation) at the warmest temperatures had lower levels of BAT volume, SUV$_\text{peak}$ and metabolic activity. These findings show how WT (as a proxy of blood flow) is related to BAT volume and activity (SUV$_\text{peak}$) in young adults. However, further studies are needed to elucidate the possible mechanisms behind these relationships.

**The mediating role of BAT**

We show that both BAT volume and metabolic activity have a mediating role in the relationship between Personal-ET and WT measured in daily living conditions independently of the sex, BMI, LMI and FMI. This indicates that participants with who were exposed to the same Personal-ET during the 7 day had different WT, which is explained, at least in part, by different levels of BAT volume or metabolic activity. Therefore, by every 1°C that the personal-ET is decreased, BAT volume would explain approximately an increase of 0.0194°C in WT. The relationship between Personal-ET and the WT daily pattern has been widely used in the field of chronobiology (21, 22). Several studies compared WT daily patterns in obese vs. normal-weight women (8), young vs. older men and women (4, 16), and men vs. women (20), and showed worse patterns (higher variability and higher daytime values) of WT in obese and older participants. These findings are also in accordance with those of human BAT studies, which showed that obese, older people, and men have lower BAT volume and activity (32). Therefore, we postulate that BAT volume and metabolic activity should be taken into account in further chronobiological studies using WT, especially in those studies, which only measured WT as a proxy of the circadian pattern without the inclusion of the Personal-ET. We established that based on the following facts: (i) the observed
mediating role of human BAT volume (and metabolic activity) in the relationship between Personal-ET and WT, (ii) the activation of BAT in cold ambient-temperatures (Personal-ET≤20ºC), (iii) that obese, older people, and men have lower BAT volume and activity as well as worse patterns of WT, and (iv) the circadian rhythms and, specifically core body temperature rhythms are all controlled by specific neural pathways in the anterior hypothalamus (39). For instance, Martinez-Nicolas et al. (21) studied the mediation role of WT in the relationship between Personal-ET and mean arterial blood pressure in summer and winter, and postulated that BAT could mediate this relationship. In this study, we show that this hypothesis might be true, although further studies are needed to fully understand the possible mechanisms behind these assumptions.

**The mediating role of WT**

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

In warm-ambient environments (from 24°C to 28°C), we observed that the higher the Personal-ET is, the higher WT is, which is associated with a lower BAT volume and activity. Therefore, by every hour exposed at 27°C (personal-ET), WT would increase and explain approximately a decrease of 3.2 ml of BAT volume. The skin has warm-sensitive neurons specially to perceive the warm exposures (39). However, there is some controversy as to which the main transient receptor potential (TRP) channel to be involved as a warm sensor is, the candidates being TRPV1, TPRV3, TPRV4, and TRPM2 (39). Therefore, there might be participants with higher or more number of TRP channels than others, and this fact could explain why there are different responses to the same stimulus, although further studies are needed. Regardless of the main TRP channel involved, our results suggest that when Personal-ET is high (hot), the body initiates some physiological response in order to preserve core temperature. Thus, the main physiological mechanism involved is to induce a peripheral vasodilation with an inhibition of human BAT (redistribution of blood flow to peripheral regions to dissipate heat). We also showed that the higher the WT is, the lower the levels of BAT volume and activity (inhibition of this tissue) are. For instance, two participants that spent the same time in warm ambient, the participant with higher WT also had lower levels of BAT volume, which might indicate more efficiency adapting to warm temperatures, which reciprocally would implicated less efficient response to cold.

Cold

In cold-ambient exposures (from 14°C to 19°C), we showed that the lower the Personal-ET is, the lower WT is, which is associated with higher BAT volume and activity. Therefore, by every hour exposed at 15°C (personal-ET), WT would decrease and explain approximately an increase of 2.5 ml of BAT volume. In the skin of the
peripheral parts of the body, there are also cold-sensitive neurons. These cold-sensitive neurons highly expressed levels of transient receptor potential cation channel subfamily M member 8 (TRMP8), which is the primary peripheral cold sensor in the thermoregulatory system (10). Animal models have shown that the inhibition of this sensor inhibits the behavioral and physiological responses to cooling (10). Taking this into consideration, we can postulate that there are individuals with a more efficient thermoregulatory system against cold stimuli, inducing a higher peripheral vasoconstriction and BAT activation in order to keep the core body temperature constant, which could be explained by a higher sympathetic tone. According to this, it might be possible for people with higher levels of human BAT to have a higher concentration of TRMP8, as well as different polymorphism of the gene TRMP8 might be associated with a better response to cold stimuli; however, these hypotheses have not been studied so far.

The mediating role of skeletal muscles

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

We postulate, however, that there are participants who respond better (i.e. responders) than others to cold exposures, and others that respond better to warm exposures, yet further studies are needed. This assumption is also based on the fact that some people
could have an overexpression of POA neuron levels or TRMP8 or TRP channels (39),
making the thermoregulatory system more efficient, or maybe in the brain the areas
involved in the thermoregulatory system are different. This cross-sectional and
observational study should be replicated in older participants and using other nuclear
tracers such as $^{15}$O-O$_2$, $[^{11}]$C-acetate (40), or adenosine perfusion, a vasodilator that
seems to active human BAT (23). Moreover, we know that during sleep phases humans
can lose at least 25% of their total thermoregulatory capacity. Since our aim was to
study the mediating role of human BAT during 7 days (even in sleep phases) we keep
these analyses as main results, although excluding the sleep phase did not alter the
results (data not shown). Moreover, in this study the level of clothing during the
measurements were not evaluated. Future experimental studies are warranted to
elucidate the possible mechanisms behind this efficiency in the thermoregulatory
system and new therapies that could be developed to improve this physiological system.

CONCLUSIONS

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

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

REFERENCES


6. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological


27. **Muzik O, Mangner TJ, Leonard WR, Kumar A, Granneman JG.**


29. **Muzik O, Mangner TJ, Leonard WR, Kumar A, Janisse J, Granneman JG.**


40. U Din M, Raiko J, Saari T, Kudomi N, Tolvanen T, Oikonen V, Teuho J,


Table 1. Characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% women)</td>
<td>74.6%</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.9 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>41.3 ± 9.6</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>26.9 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>37.6 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>BAT volume (ml)</td>
<td>69.0 ± 61.3</td>
<td></td>
</tr>
<tr>
<td>BAT activity (SUV_{mean} g/ml)</td>
<td>3.7 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>BAT activity (SUV_{peak} g/ml)</td>
<td>11.0 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>Wrist Temperature (ºC)</td>
<td>34.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Personal-ET (ºC)</td>
<td>22.8 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean and standard deviation, unless otherwise stated. BAT: brown adipose tissue, Personal-ET: Personal level of environmental temperature, SUV: standardized uptake value.
Figure 1. Mediation models of the relationship between personal levels of environmental temperature and wrist skin temperature with (A) BAT volume (ml), (B) SUV_{mean} (g/ml), (C) SUV_{peak} (g/ml), (D) metabolic activity (calculated as BAT volume x BAT SUV_{mean}), and (E) skeletal muscle activity (g/ml) included as mediator variables, respectively. Paths a, b, c, and c’ are presented as unstandardized coefficients (Standard Error, SE). $\beta = \text{indirect effect (a*b paths)}$; [lower limit confident interval: upper limit confident interval], lower and upper levels for 95% confidence interval of the indirect effect based on 5000 bootstraps. The results are shown as unstandardized coefficients (Standard Error, SE) and bias corrected 95% CI based on 5000 bootstraps.

Personal ET: personal levels of environmental temperature; WT: wrist skin temperature; BAT: Brown adipose tissue; $P_M$: percentage of mediation; SUV: Standardized uptake value; WT: wrist temperature.

ns: non-significant.
A

\[ c = 0.0763 (0.0229) \]
\[ P = 0.0014 \]

\[ a = -7.9101 (2.1063) \]
\[ P = 0.0003 \]

\[ b = -0.0025 (0.0013) \]
\[ P = 0.0549 \]

\[ c' = 0.0568 (0.0246) \]
\[ P = 0.0239 \]

\[ \beta = 0.0194 [0.0023; 0.0504]; P_{\text{maj}} = 25.4\% \]

B

\[ c = 0.0763 (0.0229) \]
\[ P = 0.0014 \]

\[ a = -0.2507 (0.0660) \]
\[ P = 0.0003 \]

\[ b = -0.0106 (0.0413) \]
\[ P = 0.7977 \]

\[ c' = 0.0736 (0.0253) \]
\[ P = 0.0048 \]

\[ \beta = 0.0027 [-0.0204; 0.0230]; \text{ns} \]

C

\[ c = 0.0763 (0.0229) \]
\[ P = 0.0014 \]

\[ a = -1.1395 (0.2917) \]
\[ P = 0.0002 \]

\[ b = -0.0117 (0.0092) \]
\[ P = 0.2095 \]

\[ c' = 0.0629 (0.0251) \]
\[ P = 0.0146 \]

\[ \beta = 0.0133 [-0.0056; 0.0558]; \text{ns} \]

D

\[ c = 0.0763 (0.0229) \]
\[ P = 0.0014 \]

\[ a = -48.3083 (12.1497) \]
\[ P = 0.0002 \]

\[ b = -0.0004 (0.0002) \]
\[ P = 0.0905 \]

\[ c' = 0.0580 (0.0250) \]
\[ P = 0.0231 \]

\[ \beta = 0.0182 [0.0014; 0.0463]; P_{\text{maj}} = 23.9\% \]

E

\[ c = 0.0763 (0.0229) \]
\[ P = 0.0014 \]

\[ a = -0.0245 (0.0078) \]
\[ P = 0.0023 \]

\[ b = 0.1084 (0.3509) \]
\[ P = 0.7584 \]

\[ c' = 0.0780 (0.0246) \]
\[ P = 0.0020 \]

\[ \beta = -0.0027 [-0.0186; 0.0136]; \text{ns} \]
Figure 2. A) Mediation models of the relationship between the number of hours exposed to a certain Personal-ET and BAT-related outcomes in young adults. Path c shows the association between independent and dependent variables. Arrows a x b show the natural indirect effect (β) pathway, and c’ shows the natural direct effect pathway. B) Indirect effects (β) of the simple mediation analyses of wrist temperature on the association between the number of hours exposed to each degree of Personal-ET (from 14°C to 28°C) and BAT volume, whereas panels C and D refer to BAT SUV$_{peak}$ and metabolic activity, respectively. E) $P_M$ of the simple mediation analyses of wrist temperature on the association between the number of hours exposed to each degree of personal-ET (from 14°C to 28°C) and BAT volume, whereas panels F and G refer to BAT SUV$_{peak}$ and metabolic activity, respectively. Black dots represent that 0 was in the 95% confidence interval of the indirect effect, and, therefore, the mediation was considered non-statistically significant ($P>0.05$). Red and blue dots mean that the mediation analysis was statistically significant but with a different direction. BAT: brown adipose tissue; Personal-ET: personal level of environmental temperature; WT: wrist skin temperature; $P_M$: Percentage of mediation.
\[ \beta = \text{indirect effect (Fig. 2B, C and D); } Ps(\%), \text{Fig. 2E, F and G)} \]
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.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Number of hours per day exposed to</th>
<th>Total effect (c)</th>
<th>Direct effect (c')</th>
<th>Path a</th>
<th>Path b</th>
<th>Indirect effect (a*b)</th>
<th>BC 95% CI Lower</th>
<th>BC 95% CI Upper</th>
<th>Pm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28°C</td>
<td>-24.1511 (8.5478)**</td>
<td>-20.7341 (8.2251)*</td>
<td>0.1172 (0.0952)</td>
<td>-29.1556 (10.0126)**</td>
<td>-3.4170</td>
<td>-13.6843; 2.2662</td>
<td>3.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27°C</td>
<td>-17.5167 (4.8821)**</td>
<td>-14.2999 (4.9035)**</td>
<td>0.1296 (0.0545)*</td>
<td>-24.8155 (10.1490)*</td>
<td>-3.2168</td>
<td>-8.9958; -0.5952</td>
<td>18.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26°C</td>
<td>-6.5085 (1.9827)**</td>
<td>-4.8443 (2.0725)*</td>
<td>0.0702 (0.0211)**</td>
<td>-23.7003 (10.6935)*</td>
<td>-1.6642</td>
<td>-3.4272; -0.3454</td>
<td>25.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>-7.2114 (2.8879)*</td>
<td>-4.9839 (2.9130)</td>
<td>0.0814 (0.0307)**</td>
<td>-27.3753 (10.6111)*</td>
<td>-2.2275</td>
<td>-5.4156; -0.4822</td>
<td>30.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24°C</td>
<td>-5.0423 (3.6912)</td>
<td>-2.1706 (3.6547)</td>
<td>0.0925 (0.0384)</td>
<td>-31.0297 (10.7150)**</td>
<td>-2.8717</td>
<td>-5.8755; -0.9852</td>
<td>56.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23°C</td>
<td>-0.1435 (2.5060)</td>
<td>0.9153 (2.3847)</td>
<td>0.0318 (0.0265)</td>
<td>-33.3129 (10.4289)*</td>
<td>-1.0588</td>
<td>-2.6913; 0.0416</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22°C</td>
<td>-1.7610 (2.8817)</td>
<td>-1.7698 (2.7173)</td>
<td>-0.0003 (0.0309)</td>
<td>-32.7633 (10.3079)**</td>
<td>0.0087</td>
<td>-1.6455; 2.1567</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21°C</td>
<td>1.5069 (3.5844)</td>
<td>-0.0470 (3.4213)</td>
<td>-0.0474 (0.0379)</td>
<td>-32.7773 (10.4483)**</td>
<td>1.5539</td>
<td>-0.6785; 4.8597</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20°C</td>
<td>10.8615 (5.0206)*</td>
<td>6.9503 (5.0396)</td>
<td>-0.1373 (0.0529)*</td>
<td>-28.4826 (10.6645)**</td>
<td>3.9112</td>
<td>1.1979; 9.3971</td>
<td>36.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19°C</td>
<td>17.0622 (5.2043)**</td>
<td>13.6895 (5.1955)*</td>
<td>-0.1305 (0.0575)*</td>
<td>-25.8529 (10.2149)*</td>
<td>3.3727</td>
<td>0.5235; 9.4524</td>
<td>19.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td>10.7468 (3.2756)**</td>
<td>7.9320 (3.4450)</td>
<td>-0.1199 (0.0347)**</td>
<td>-23.4791 (10.7603)*</td>
<td>2.8148</td>
<td>0.5623; 8.0286</td>
<td>26.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17°C</td>
<td>9.0101 (2.7922)**</td>
<td>6.1971 (3.0982)</td>
<td>-0.1271 (0.0282)**</td>
<td>-22.1406 (11.3763)</td>
<td>2.8130</td>
<td>0.3676; 6.3115</td>
<td>31.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16°C</td>
<td>14.1431 (4.0529)**</td>
<td>11.5538 (4.0487)**</td>
<td>-0.1021 (0.0452)*</td>
<td>-25.3649 (10.1354)*</td>
<td>2.5893</td>
<td>0.4701; 6.8149</td>
<td>18.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15°C</td>
<td>21.6970 (9.6794)*</td>
<td>16.0500 (9.5090)</td>
<td>-0.2054 (0.1042)</td>
<td>-28.8089 (10.4058)**</td>
<td>5.9170</td>
<td>1.0768; 16.2034</td>
<td>26.94</td>
<td></td>
</tr>
</tbody>
</table>
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; \( P_M \): 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.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Total effect (c)</th>
<th>Direct effect (c')</th>
<th>Path a</th>
<th>Path b</th>
<th>Indirect effect (a*b)</th>
<th>BC 95% CI Lower Upper</th>
<th>( P_M ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hours per day exposed to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28ºC</td>
<td>(-3.6344 (1.1794)**)</td>
<td>(-3.2599 (1.1593)**)</td>
<td>0.1172 (0.0952)</td>
<td>-3.1947 (1.4113)*</td>
<td>-0.3744</td>
<td>-1.7238; 0.2237</td>
<td></td>
</tr>
<tr>
<td>27ºC</td>
<td>(-2.4402 (0.6799)**)</td>
<td>(-2.1041 (0.6951)**)</td>
<td>0.1296 (0.0545)*</td>
<td>-2.5924 (1.4387)</td>
<td><strong>-0.3360</strong></td>
<td>-1.0857; -0.0057</td>
<td>13.77</td>
</tr>
<tr>
<td>26ºC</td>
<td>(-0.8976 (0.2765)**)</td>
<td>(-0.7923 (0.2937)*)</td>
<td>0.0702 (0.0211)**</td>
<td>-2.3975 (1.5152)</td>
<td>-0.1683</td>
<td>-0.4264; 0.0264</td>
<td></td>
</tr>
<tr>
<td>25ºC</td>
<td>(-1.0210 (0.4016)*)</td>
<td>(-0.7839 (0.4128)*)</td>
<td>0.0814 (0.0307)**</td>
<td>-2.9145 (1.5036)</td>
<td><strong>-0.2372</strong></td>
<td>-0.6959; -0.0130</td>
<td>23.23</td>
</tr>
<tr>
<td>24ºC</td>
<td>(-0.4558 (0.5178)*)</td>
<td>(-0.1163 (0.5213)*)</td>
<td>0.0925 (0.0384)*</td>
<td>-3.6683 (1.5283)*</td>
<td><strong>-0.3395</strong></td>
<td>-0.7859; -0.0673</td>
<td>74.49</td>
</tr>
<tr>
<td>23ºC</td>
<td>(0.1326 (0.3487)*)</td>
<td>(0.2571 (0.3384)*)</td>
<td>0.0318 (0.0265)</td>
<td>-3.9171 (1.4800)**</td>
<td><strong>-0.1245</strong></td>
<td>-0.3651; -0.0015</td>
<td>N/A</td>
</tr>
<tr>
<td>22ºC</td>
<td>(-0.1011 (0.4022)*)</td>
<td>(-0.1021 (0.3877)*)</td>
<td>(-0.0003 (0.0309)*)</td>
<td>-3.7612 (1.4708)*</td>
<td>0.001</td>
<td>-0.2129; 0.2276</td>
<td></td>
</tr>
<tr>
<td>21ºC</td>
<td>(0.1960 (0.4993)*)</td>
<td>(0.0180 (0.4870)*)</td>
<td>(-0.0474 (0.0379)*)</td>
<td>-3.7529 (1.4872)*</td>
<td>0.1779</td>
<td>-0.0555; 0.6272</td>
<td></td>
</tr>
<tr>
<td>20ºC</td>
<td>(1.5091 (0.6993)**)</td>
<td>(1.0842 (0.7154)**)</td>
<td>(-0.1373 (0.0529)*)</td>
<td>-3.0942 (1.5139)*</td>
<td><strong>0.4249</strong></td>
<td>0.0396; 1.1213</td>
<td>28.16</td>
</tr>
<tr>
<td>19ºC</td>
<td>(2.5401 (0.7171)**)</td>
<td>(2.1937 (0.7299)**)</td>
<td>(-0.1305 (0.0575)*)</td>
<td>-2.6546 (1.4351)</td>
<td><strong>0.3463</strong></td>
<td>0.0104; 1.1236</td>
<td>13.63</td>
</tr>
</tbody>
</table>
### 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.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Total effect (c)</th>
<th>Direct effect (c&lt;sup&gt;′&lt;/sup&gt;)</th>
<th>Path a</th>
<th>Path b</th>
<th>Indirect effect (a*b)</th>
<th>BC 95% CI Lower Upper</th>
<th>P&lt;sub&gt;M&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hours per day exposed to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>28ºC</strong></td>
<td>-142.2746 (49.6038)**</td>
<td>-123.6264 (48.0546)*</td>
<td>0.1172 (0.0952)</td>
<td>-159.1181 (58.4978)**</td>
<td>-18.6482</td>
<td>-76.7198; 13.6812</td>
<td>12.72</td>
</tr>
<tr>
<td><strong>27ºC</strong></td>
<td>-92.7638 (28.7954)**</td>
<td>-74.7342 (29.0376)*</td>
<td>0.1296 (0.0545)*</td>
<td>-139.0875 (60.1009)*</td>
<td>-18.0296</td>
<td>-50.4684; -2.6810</td>
<td>19.44</td>
</tr>
<tr>
<td><strong>26ºC</strong></td>
<td>-34.8199 (11.6524)**</td>
<td>-25.4847 (12.2166)*</td>
<td>0.0702 (0.0211)**</td>
<td>-132.9461 (63.0354)*</td>
<td>-9.3352</td>
<td>-19.6154; -1.8476</td>
<td>26.31</td>
</tr>
<tr>
<td><strong>25ºC</strong></td>
<td>-41.1781 (16.8086)*</td>
<td>-29.0344 (17.0508)</td>
<td>0.0814 (0.0307)**</td>
<td>-149.2399 (62.1105)*</td>
<td>-12.1436</td>
<td>-31.4411; -2.6588</td>
<td>29.49</td>
</tr>
<tr>
<td><strong>23ºC</strong></td>
<td>-2.0202 (14.5630)</td>
<td>3.7930 (13.9632)</td>
<td>0.0318 (0.0265)</td>
<td>-182.8941 (61.0639)**</td>
<td>-5.8132</td>
<td>-15.5371; -0.0279</td>
<td>N/A</td>
</tr>
<tr>
<td>Temperature</td>
<td>Unstandardized Coefficient (SE)</td>
<td>BC 95% Confidence Interval of the Indirect Effect</td>
<td>p-values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------</td>
<td>-----------------------------------------------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22°C</td>
<td>-8.0621 (16.7643)</td>
<td>-8.1103 (15.9204)</td>
<td>0.0482</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.0003 (0.0309)</td>
<td>-1.806196 (60.3934)**</td>
<td>-9.8282; 11.9435</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21°C</td>
<td>6.3637 (20.8440)</td>
<td>-2.2449 (20.0209)</td>
<td>8.6086</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.0474 (0.0379)</td>
<td>-1.815810 (61.1410)**</td>
<td>-3.0637; 29.3434</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C</td>
<td>54.4724 (29.4166)</td>
<td>32.4111 (29.6347)</td>
<td>22.0613</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.1373 (0.0529)*</td>
<td>-1.60658 (62.7118)*</td>
<td>6.3974; 55.8110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19°C</td>
<td>93.9165 (30.4749)**</td>
<td>75.3119 (30.5755)*</td>
<td>18.6046</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.1305 (0.0575)*</td>
<td>-1.426087 (60.1146)*</td>
<td>2.8291; 52.5241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18°C</td>
<td>56.6625 (19.2841)**</td>
<td>40.7224 (20.3314)*</td>
<td>15.9400</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.1199 (0.0347)**</td>
<td>-1.329587 (63.5043)*</td>
<td>3.4256; 45.2335</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17°C</td>
<td>50.0828 (16.3261)**</td>
<td>34.6886 (18.1741)</td>
<td>15.3941</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.1271 (0.0282)**</td>
<td>-1.1211656 (66.7343)</td>
<td>1.9813; 38.6296</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16°C</td>
<td>98.7912 (22.6644)**</td>
<td>85.9709 (22.8526)**</td>
<td>12.8203</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.1021 (0.0452)*</td>
<td>-1.1255885 (57.2095)*</td>
<td>2.8726; 34.6266</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>127.3577 (56.2649)*</td>
<td>95.0696 (55.6223)</td>
<td>32.288</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.2054 (0.1042)</td>
<td>-1.1572056 (60.8681)*</td>
<td>5.3280; 88.6667</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14°C</td>
<td>162.8793 (49.7349)**</td>
<td>162.3202 (46.8374)**</td>
<td>0.5591</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.0031 (0.0979)</td>
<td>-1.1798669 (56.0109)**</td>
<td>-40.2083; 48.2821</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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; PM: 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.