24-h passive heat and cold exposures did not modify energy intake and appetite but strongly modify food reward

Maxime Coca[1,2α], Louis Besançon[3α], Mégane Erblang[2], Stéphanie Bourdon[1,2], Arnaud Gruel[1,2], Benoît Lepetit[1,2], Vincent Beauchamps[4,5], Blandine Tavard[6], Pauline Oustric[7], Graham Finlayson[8], David Thivel[9], Alexandra Malgoyre[1,2], Pierre-Emmanuel Tardo-Dino[1,2], Cyprien Bourrilhon[1,2], Keyne Charlot[1,2]*

[1]Unité de Physiologie des Exercices et Activités en Conditions Extrêmes, Département Environnements Opérationnels, Institut de Recherche Biomédicale des Armées, 1 place Général Valérie André, 91223 Brétigny Cedex, France.

[2]LBEPS, Univ Evry, IRBA, Université Paris Saclay, 91025 Evry, France.

[3]Hôpital d'instruction des armées Percy, 92140 Clamart, France.

[4]Unité Fatigue et Vigilance, Département Environnements Opérationnels, Institut de Recherche Biomédicale des Armées, 1 place Général Valérie André, 91223 Brétigny Cedex, France.

[5]EA 7330 VIFASOM, Université de Paris, 75004 Paris, France.

[6]Centre Interarmées du Soutien « Equipements Commissariats », Service du commissariat des armées, 78120 Rambouillet, France.

[7]Inserm, U1296 Unit, "Radiation: Defense, Health and Environment", Centre Léon Bérard, 28 rue Laënnec, 69008 Lyon, France

[8]Appetite Control Energy Balance Research Group, School of Psychology, Faculty of Medicine and Health, University of Leeds, Leeds LS2 9JT, United Kingdom.

[9]Laboratoire des adaptations Métaboliques à l'Exercice en conditions Physiologiques et Pathologiques (EA 3533), Université Clermont Auvergne, Clermont-Ferrand, France.

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114524000825
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

***Corresponding author: Phone:** +336.37.96.80.40, **E-mail:** keynecharlot@gmail.com, **Address:** IRBA 1 place général Valérie André 91223 Brétigny-sur-Orge France,

[α] Co First authors

Short title: Passive thermal exposures and energy intake

Abbreviations: CAS: composite appetite score, EE: energy expenditure; EI: energy intake, EL: explicit liking, ES: effect size, GLP-1: glucagon-like peptite-1, HR: heart rate, LFPQ: Leeds Food Preference Questionnaire, PYY: peptide YY, RQ: respiratory quotient, USG: urine specific gravity, VAS: visual analog scale, IW: implicit wanting

Acknowledgments

The authors would like to thank the participants for their conscientiousness and their kindness. They also want to thank the Fatigue & Vigilance laboratory and their chief Fabien Sauvet for letting us use their precious climatic apartment.

Financial support

None.

Conflict of interest

None.

Authorship

KC designed the study. MC, LB, ME, SB, AG, BL, VB, BT, PO, GF, DT, AM, PETD, CB, and KC carried out the study and collected the data. MC, LB, VB, and KC processed data and conducted the statistical analyses. SB and AG conducted the biological analysis. Laboratoire graphique (KC) designed the figures. MC, LB, and KC drafted the initial manuscript. All authors reviewed and revised the manuscript, approved the final manuscript as submitted, and agree to be accountable for all aspects of the work.

Abstract

Effects of acute thermal exposures on appetite appear hypothetical in reason of very heterogeneous methodologies. The aim of this study was therefore to clearly define the effects of passive 24-h cold (16°C) and heat (32°C) exposures on appetitive responses compared to a thermo neutral condition (24°C). Twenty-three healthy, young, and active male participants realised three sessions (from 1 pm) in a laboratory conceived like an apartment dressed with the same outfit (Clo=1). Three meals composed of three or four cold or warm dishes were served *ad libitum* to assess energy intake (EI). Leeds Food Preference Questionnaires were used before each meal to assess food reward. Subjective appetite was regularly assessed and levels of appetitive hormones (acylated ghrelin, GLP-1, leptin, and PYY) were assessed before and after the last meal (lunch). Contrary to the literature, total EI was not modified by cold or heat exposure ($p=0.120$). Accordingly, hunger scores ($p=0.554$) were not altered. Levels of acylated ghrelin and leptin were marginally higher during the 16 ($p=0.032$) and 32°C ($p<0.023$) sessions, respectively. Interestingly, implicit wanting for cold and low-fat foods at 32°C and for warm and high-fat foods at 16°C were increased during the whole exposure ($p < 0.024$). Moreover, cold entrées were more consumed at 32 °C ($p<0.062$) and warm main dishes more consumed at 16°C ($p<0.025$). Thus, passive cold and hot exposures had limited effects on appetite and it seems that offering some choice based on food temperature may help individuals to express their specific food preferences and maintain EI.

Keywords: Heat, Cold, Food intake, LFPQ, Food reward, rations

1. Introduction

Athletes are required to live, train, and compete under various climates, the two sides of the spectrum (cold and heat) presenting different challenges for event's organisers, coaches, and athletes[1,2,3,4,5] in order to mitigate the detrimental impact on health and performances. With climate change[6], athletes are at risk to more and more frequently face these adverse conditions[7]. Warfighters struggle with the same problems as athletes: numerous military operations in climatically-severe regions were conducted over the last two decades[8,9] raising concerns on their possibility to protect them from extreme thermal exposures[10,11] potentially jeopardising operational success.

Reaching adequate nutrition represents a major challenge for these populations frequently placed in cold and hot conditions. First, both athletes and warfighters frequently struggle to maintain their body mass independently from the climate[12,13,14,15]. In view of their high levels of energy expenditure (EE), increasing energy intake (EI) to avoid negative energy balance or energy deficiency may understandably be hard to reach and seems to be only feasible by increasing the frequency of eating occasions[16]. Second, heat and cold exposures increase EE at rest and during exercise[17,18] and worsen energy demands. Third, a recent meta-analysis[19] revealed a modest orexigenic effect (750 kJ increase in EI; Z = 2.35, p = 0.019, g = 0.44) of cold and a small anorexigenic effect of heat (635 kJ decrease in EI; Z = -2.29: p = 0.022, g = –0.39). Thus, if we consider all these aspects, we may expect that cold but more likely heat exposure will aggravate already frequent and severe energy deficient states that can lead to deleterious consequences on health, physiological functions[20], and cognitive[21] and physical performance[20,22].

However, the impact of heat and cold exposures on appetite and EI urgently requires scientific support. Indeed, a recent meta-analysis identified limited number of available evidences/studies, as well as a large methodological disparity between studies (participants characteristics, duration of exposure, choice of temperature, presence of physical exercise sessions, nature of the test meals, clothing...) that could blur the interpretation of the results. To date, it seems impossible to obtain a consensus to confirm the hypothetical and opposite effects of heat and cold exposures on EI. Moreover, the mechanisms implicated in these temperature-induced modifications in EI are poorly understood. There is therefore a need to

assess the isolated effects of thermal exposures on the different determinants implicated in food intake (subjective appetite, hormonal modifications, food reward,…).

Thus, the present study aimed to determine the effect of a 24-h exposure to cold (16°C) and hot (32°C) on EI compared to a thermoneutral (24°C) control exposure. We expected that 24-h EI will reduce and increase EI during the heat and cold exposure, respectively. A secondary objective was to link these modifications with modulations of appetite, plasma levels of hormones implicated in eating behaviour (ghrelin, leptin, glucagon-like peptide-1 [GLP-1], and peptide YY [PYY]), food reward, and olfactory and gustatory capacities.

2. Materials and Methods

2.1 Design

The protocol is presented in Figure 1. Participants took part in three 24-h sessions separated by at least 2 weeks in a laboratory organised as an apartment (four bedrooms, a living-room with a kitchen and a bathroom with a shower and a toilet) in which ambient temperatures were fixed either at 16°C, 24°C, and 32°C (one session for each temperature). Participants were split in six groups of four and this composition remained similar during the study to avoid biases related to social relationships. The session order was randomly allocated and counterbalanced for the six groups. Each 6 session orders had therefore been attributed once.

To date, the effects of thermal passive exposures have been mainly assessed during short durations (< 16 h) with one or two test meals[23,24,25,26,27]. The observation of these effects during a longer exposure (24 h) with three consecutive test meals would help identifying whether cold and/or heat exert an effect on appetite during more than one meal. The choice of 16 and 32°C were based on previous studies in which 30°C and 32°C was sufficient to modify EI[23]and food reward[27] compared to 20 and 22 °C, respectively and in which 16°C[28] compared to 18°C[25] was found more efficient to modify *ad libitum* EI. Moreover, 16°C seems to be the lowest acceptable (without shivering) temperature in light clothing[18]. Thus, these two extreme thermal exposures were judged sufficient to elicit modifications in appetite while being acceptable during 24 h in the same outfit. The control session was fixed at equal distance from 16 and 32°C (24°C), a condition that was perfectly comfortable in passive/slightly active conditions.

They arrived at 12:00pm to be equipped with devices used for continuous measurements (heart rate [HR], core and skin temperatures, duration of spontaneous light physical

activities). Participants then dressed in standardised clothes (tee-shirt made of cotton, jogging pants made of 50% cotton and 50% polyester, cotton socks, and synthetic sandals, 1 Clo). At 12:30pm participants ate a control meal with standardised quantities in a room at 22°C. At 1:00 pm, participants went inside the apartment until 1:30pm the next day. Three *ad libitum* meals were served at 7:00pm (dinner), 8:00am (breakfast), and 12:30pm the following day (lunch). Dinner and lunch were composed of a cold entrée, a hot main dish, bread, and a cold dessert. Breakfast was composed of a sweetened cottage cheese, a chocolate madeleine and orange juice. Participants slept between 10:30 pm and 7:00 am. They slept with the same clothing and were authorised to sleep with one or two blankets during the 16 and 24 °C sessions and with just one light sheet or one blanket during the 32 °C session. This was done based on some pre-tests that showed that sleep was strongly impacted with insufficient or too much covering during sleep. Food reward using the Leeds Food Preference Questionnaire (LFPQ) was assessed just before each meal. Subjective appetite and thermal sensations were assessed throughout the sessions using visual analogic scale (VAS). Body mass fluctuations, total entries (water and food intake) and total loss (urine and sweat loss) were directly measured or calculated. Blood samples were collected before (12:15pm) and after (1:10pm) the lunch to measure plasma hormones concentrations (ghrelin, leptin, PYY, GLP-1). To finish, olfactory and gustatory capacities were tested at 10:00am. Participants were authorised to engage in leisure activities (darts, table football, video games, board games, and reading) between tests. They were forbidden to bring work and were limited at 30 min of table football at distance from tests and measurements (30 min) to avoid too large EE.

2.2 Participants

A priori power analysis for EI based on a recent meta-analysis[19] (+750 kJ in the cold [Z = 2.35, p = 0.019, g = 0.44], -635 kJ in the heat [Z = -2.29: p = 0.022, g = -0.39]) selecting conventional α (0.05) and 1-β (0.80) levels and with an expected effect size of 0.61 (calculated from the previous results) observed that at least twenty participants were required (G*Power v3.1.9.4, Kiel, Germany). We therefore recruited twenty-four healthy, young, lean, male, and active participants. Women were supposed to be included in this study but logistical and temporal constraints have impeded us to include them (difficulty to control menstrual cycle while maintaining the composition of the groups and limited availability of the laboratory [4 months]). One participant dropped for a medical reason independent of the study. Twenty-three were therefore conserved for analysis (age: 30.0 ± 7.4 yo, 75.8 ± 8.9 kg, 178 ± 6 cm, and 13.2 ± 5.8 % of body fat mass). Inclusion criteria were restraint score < 50

based on the Three-Factor Eating Questionaire-R18[29] without dietary allergies and intolerances, regularly consuming at least 3 meals per day including breakfast, not following a specific diet, not on medication, and have a sleep score < 8 based on the Pittsburgh Sleep Quality Index[30]. They were also not eligible if they had been exposed to a hot or a cold climate (> 3 consecutive days, mean temperature > 30 °C or < 0 °C, respectively) in the last 3 months before the study to ensure that participants were not considered heat/cold acclimatised. Given that this study was realised in winter/spring 2022-2023, they were theoretically exposed to temperature between 5-20 °C during this period. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the French National Ethics Committee Sud Méditerranée n°IV (2022-A01862-41). It was also registered in Clinical Trials (MCT05584527). Written informed consent was obtained from all participants who received financial remuneration for study completion.

2.3 Period of normalisation

Participants were asked to eat the same supplied foods (same foods [cold entrée, hot main dish, and dessert] quantities, and timing) for the dinner the day before and breakfast and lunch the day of session. The composition of these meals was similar to the *ad libitum* meals to accustom them to these kinds of foods but with different recipes to avoid monotony. Mean EI was 9.27 ± 1.36 MJ (45 ± 3, 34 ± 2, and 21± 2% from carbohydrate, fat and protein, respectively) during this period. They were also instructed to drink at least 1.5 L of water from the previous evening to avoid disparities in the hydration level and to not perform physical activities on the day before the study (level of activity controlled by an accelerometer). Finally, they were instructed to respect their sleeping habits during the 2 previous nights. The objective of this period of normalisation was to obtain a similar physiological basal state before each session.

2.4 Control measurements

2.4.1 Room temperature and hygrometry

The experimental sessions took place in the Institut de Recherche Biomédicale des Armées (IRBA)'s climatic apartment in which the temperature but not hygrometry can be regulated. Twelve thermal sensors (ibuttons, Maxim Integrated, Sunnyvale, CA, USA) were placed in several locations in the apartment (4 in the living room and 2 in each room) to measure temperature and hygrometry continuously to ensure that they were in line with the desired conditions. The mean temperature and hygrometry for the 3 sessions were 17.3 ± 0.4 °C, 46.9

± 9.7 % ; 24.3 ± 0.2 °C, 29.5 ± 8.4 % ; 32.1 ± 0.6 °C, 22.2 ± 3.5 %, respectively. Thus, at the exception of 16 °C for which temperature was slightly higher than expected, we managed to perfectly reach the target values.

2.4.2 Core and skin temperature

Upon their arrival in the climatic apartment, participants ingested a thermometric pill (Body Cap, e-Celsius, Caen, France) that was equipped with a memory chip allowing storage of data in case of loss of connection and communicated with a monitor that remains with the participants during the whole session. Participants were also equipped with two cutaneous sensors (ibutton, Maxim Integrated, Sunnyvale, CA, USA), one on the left superior part of the chest, the other on the 1/3 superior front of the right thigh) to measure skin temperature (mean of the two measurements). Skin temperature measured on the chest and thigh showed acceptable agreement with mean skin temperature measured on 8 sites[31].

2.4.3 Body mass modifications

Body mass was regularly assessed (1:00pm, 4:00pm, 7:00pm, 7:30pm, 10:00pm., 7:30am, 8:00 am, 10:00am, 12:00am, and 12:30am) with a balance (Mettler Toledo ICS 425d, Greifensee, Switzerland, accurate to 20 g) to follow fluctuations. Food and fluid intake were measured using weighings (electronic kitchen scale, Lacor, Bergara, Spain, accurate to 5 g) after each meal and weighings of water bottles realised at the same time as body mass, respectively. Large water bottles (1.5 L) were left at their disposal either outside (at room temperature) or kept in the fridge (at 4°C) at their convenience. They were instructed to consume as much water as they desired. Urinary and faecal discharges were auto-assessed using a scale placed very close to toilets. Participants had to weigh themselves before and after urination/defecation. The correct filling of the records was frequently checked to ensure that no weighing was forgotten. Sweat loss was calculated using all the preceding measurements, considering that water loss though respiration was negligible.

2.4.4 Hydration level

Urine was collected (12:00pm, 9:40pm,11:15am) to assess hydration level, using an automated dipstick analyser (Clinitek Status + Analyser, Siemens, Munich, Germany) and Multistix10SG (Siemens Munich, Germany). The urine specific gravity (USG) values < 1.013 indicate hyperhydration[32], whereas USG values > 1.020 reflect hypohydration[33]. Subjective colour analysis using the Armstrong scale[34] was also used to monitor the hydration status of subjects.

2.4.5 Subjective thermal ratings

Thermal sensation and thermal comfort (« How do you perceive your thermal environment? ») were regularly determined with the ASHRAE 7-pt scale (from -3 cold to 3 heat) and with VAS with very uncomfortable and very comfortable at the left and right ends[35].

2.4.6 Sleep duration and quality

The sleep characteristics and duration were recorded by a measurement of cerebral activity and sleep phase analysis. Participants wore a wireless DREEM2 headband (SAS, Paris, France) that automatically recorded physiological sleep data in real time (EEG accelerometer and pulse oximeter). This alternative to polysomnography has been validated[36]. Fatigue using a 100-mm VAS “Are you tired?” was assessed at 9:45pm, 7:45am, and 11:45am with “not at all” and “extremely” at the left and right ends.

2.4.7 Energy expenditure

Resting metabolism was assessed at 1:00 pm to ensure that they were in similar metabolic state then three times in the preprandial period at distance from the previous meal to limit the effect of the thermal effect of foods (5:30pm, 7:00am, 11:30am) by indirect calorimeter using a metabolic monitor (Q-NRG, Cosmed, Rome, Italy). The participants were placed in a comfortable lying position during the 15-minute measurement. Mean oxygen uptake and carbon dioxide production were then determined from the more stable sample lasting at least 6 min. Respiratory quotient (RQ) was also collected from the same sample. The investigators performed a visual check to ensure that the participants were awake during measurements.

An accelerometer (MotionWatch 8, CamNTech, Papworth Everard, UK) was fitted at the left wrist during the whole session. Durations (in min) of sedentary activities (< 1.5 MET), light activities (between 1.5 and 3.0 METs), moderate activities (between 3.0 and 4.5 METs), and

vigorous activities (> 4.5 METs) during the 24 h were determined using the device software (MotionWare 1.0.27, CamNTech, Papworth Everard, UK). The duration of spontaneous light physical activities (> 1.5 MET) was then determined.

2.4.8 Heart rate

HR was continuously measured using a heart chest belt (Polar H10, Polar, Kempele, Finland) communicating with a watch (Polar RC3, Kempele, Finland).

2.5 Main measurements

2.5.1 Energy intake quantity

Each test meal was served *ad libitum* and was composed of 3 items (plus bread for dinner and lunch) served in a large bowl, plate, or jug (list and composition of served food in Supplementary file 1). In case of special diets (pesco-vegetarian), a choice was offered for some items to replace meat by fish or vegetarian options (n = 2). Lunch and dinner foods came from usual French military rations. EI between consumption of field rations and home diets were found similar[37] even during three weeks[38] confirming that these foods could be served without expecting low consumption. Participants ate alone with no distraction (not allowed to use their phone or read a book) in their room to avoid eating while being influenced by social facilitation[39] and occupation[40] and served themselves in their plate or bowl using specific cutlery. Participants were instructed to signal the end of their meal by knocking at their door allowing us to calculate the meal duration. They were also instructed to taste each item even if they did not want it to be able to assess food palatability. Thirty minutes after the beginning of the meal, plates were cleared and were weighed. The differences between before and after the meal corresponded to the consumed quantities. Consumed quantities were then used to calculate EI and macronutrients intake.

2.5.2 Subjective ratings

Appetite and thirst were assessed 10 times per session including just before and after the meals (30 min after the start of the meals). Appetite was separated into four different perceptions: hunger, desire to eat, fullness, and prospective consumption. Appetite was measured using 100-mm VAS presented on paper preceded by the following questions; « Are you hungry? »; « How strong is your desire to eat? »; « How full do you feel? » and « What quantity of food would you able to eat? ». These scales were anchored with « not at all » and « extremely » at the left and right ends respectively. The distance from the extreme left to the

participant's vertical dash represented the rating score expressed in mm (0 to 100). The composite appetite score (CAS)[41], reflecting the responses to the four VAS questions, was included in the study as a summary measure of appetite. CAS was calculated using the following formula: CAS= [hunger + desire to eat + (100-fullness) + prospective consumption]/4. Thirst was also assessed at the same time points as appetite sensations. We also considered food palatability that was assessed using VAS: « Did you like this food? ».

2.5.3 Food reward

Food reward was assessed using the LFPQ[42,43,44]. Two versions were used: the original one comparing appeal for high-fat and low-fat foods (fat appeal) and sweet and savory food (taste appeal) using foods usually consumed in France[45] and a new one created for this study comparing appeal for cold and hot foods/drinks (temperature appeal) and fluid and solid items (texture appeal). The latter was created following the recommendations listed proposed by Oustric *et al.*[44]. Temperature of the served foods appears to be differentially appreciated according to the thermal environment. Indeed, it is well demonstrated that oral temperature sensing in the mouth may influence ingestive acceptance[46] and that cold drinks/foods may be perceived as more pleasant in the heat[47] through higher ability to satiate thirst[48]. Other dimensions such as the texture, the color, and/or the taste are interconnected with food/drink temperature since these dimensions may be influenced by the coldness/warmth of ingested items or influence the perception of the served temperature[49,50,51,52]. The methodology of food selection is described in the Supplemental file 2. This questionnaire was not planned before breakfast to not overburden participants.

LFPQ is a 2-phase computerised task. One task consists in answering the question « How pleasant would it be to taste this food now? » using 100-pt VAS. Food images (n = 16) appeared individually on the screen in a randomised order. Each food image was shown to the participants beforehand to ensure the adequacy between the image and its interpretation. The mean score for each group of foods (low-fat, high-fat, savory, and sweet for the first questionnaire and hot, cold, fluid, and solid for the 2nd one) corresponded to explicit liking (EL). The other score reflecting the implicit wanting (IW) was assessed using a covertly timed forced choice procedure. Every image of each of the 4 food categories was compared to every other image of the 3 other categories (96 pairs in total). Participants were instructed to respond as quickly as they could to indicate which food they most wanted to eat at that moment. The IW score is therefore a combination of reaction time and frequency of

selection[44]. Each questionnaire required 5 to 10 min to be achieved. EL and IW scores are presented as appeal bias in order to show the relative preference for one dimension (e.g. low-fat) in comparison to the opposite one (e.g. high-fat). A positive score in this example would indicate a relative preference for high-fat and a negative one a preference for low-fat, the higher or lower the value, the higher the preference for the respective dimension.

2.5.4 Olfactory and gustatory capacities

Two series of tests were carried out to analyse the olfactory and gustatory capacities using the ODOFIN taste strip and ODOFIN sniffing sticks (Burghart Messtechnik GmbH, Holm, Germany). While the impact of thermal temperature on these capacities was never assessed, it is strongly suggested that smell and taste functions modify feeding behaviour[53] and that the hunger state may affect these functions[54]. It was interesting to know in this context whether heat and cold exposures that are expected to modify hunger and food intake may also affect olfactory and gustatory capacities. These tests proposed a semi-objective evaluation of the olfactory and gustatory capacities of the subjects using 4 tests. The olfactory threshold test attempts to define the subject's limit olfactory capacity using a gradient of odorant power (strength: 16 = low to 1 = high). For the olfactory discrimination test, the subject has to recognise 1 different odour from 2 other proposals. The olfactory identification test consists of recognising the proposed odour among 4 possibilities. Finally, the taste discrimination test aims to evaluate the participant's ability to detect the four tastes (sweet, salty, bitter and acid). The first series of tests (olfactory discrimination and gustatory discrimination at 5:00pm) allowed the subjects to become accustomed to the complexity of the tests. The second series of tests (all test at 10:00am) was preferentially chosen to study the impact of the temperature on the subjects' olfactory and gustatory abilities. Each test (threshold, olfactory discrimination and identification, and taste discrimination) was performed according to the manufacturer's instructions. The same investigator was previously assigned to each test in order to limit operator-dependent bias.

2.5.5 Hormones

A plasma assay of hormones known to modulate appetite (active ghrelin (acylated), leptin, active GLP-1 and total PYY) was performed using the Luminex™ Technology technique using customised panels (Milliplex Human Metabolic Hormone Magnetic Bead Panel, Merck Millipore, Burlington, MA, USA). The LUMINEX technique combines the principle of Elisa assay and flow cytometry. These hormones were judged the most relevant to explain the modulation of EI in response to thermal environments. Two samples were taken respectively at 12:15pm (15 min before lunch) and at 1:10pm (10 min after lunch) in order to analyse the prandial effect. The samples were taken on a 4-ml EDTA tube. Immediately after collection, a mixture of Pefabloc SC (Merck Millipore, Burlington, MA, USA) and DCPP IV inhibitor (Merck Millipore, Burlington, MA, USA), both enzyme blockers, was added in whole blood, to inhibit the deleterious effects of proteases. Within 30 minutes of collection, they were centrifuged (10 minutes at 2000 G in a refrigerated centrifuge) to isolate plasma that was then deposited in 5 aliquots (1 for each hormone and 1 as a backup) and frozen at -80 °C. This allows a multiplex analysis of several protein targets with high precision. All the assays were performed by the same investigators. Given that total PYY results contained too many outliers (> 35%), Elisa tests (Human total PYY ELISA, Merck Millipore, Burlington, MA, USA) were done again to correct this problem. All assays were run in duplicate. When intra-assay coefficients of variation exceeded 25%, measurements were rerun. Mean intra-assay coefficients of variation were 10.0, 7.9, 6.3, and 4.9 for acylated ghrelin, active GLP-1, leptin, and total PYY, respectively.

2.6 Statistical analyses

All variables were checked for normal distribution using a Shapiro-Wilk test. If Gaussian distribution was not respected, nonparametric tests (Friedman's test) were used. In the case of repeated measurements, mixed-model repeated-measures ANOVA were used. Thus, VAS scores (appetite, thirst, and thermal scales) were compared using a 3x10 ANOVA (temperature effect: 16°C *vs* 24°C *vs* 32°C and time effect). A 3x3 ANOVA was used to compare EL and IW Taste and Fat Appeal bias scores (temperature effect: 16°C *vs* 24°C *vs* 32°C and meal effect: dinner *vs* breakfast *vs* lunch). A 3x2 ANOVA was used to compare EL and IW Texture and Temperature Appeal bias scores (temperature effect: 16°C *vs* 24°C *vs* 32°C and meal effect: dinner *vs* lunch). All these variables were normally distributed. For the remaining variables, 3x1 repeated-measures ANOVAs (or Friedman's test) were used

(temperature effect: 16°C *vs* 24°C *vs* 32°C). When the sphericity assumption was violated (Mauchly's test), a Greenhouse-Geisser correction was used. Post hoc analyses were performed using Bonferroni's tests (Conover's test in case of use of Friedman's test). The differences were also examined using Cohen's effect size (ES): > 0.2 (small), > 0.5 (moderate), and > 0.8 (large)[55]. Data are presented as the means ± SD. Significance was determined as $p < 0.050$. However, since it is frequently recommended to eradicate the categorisation based on this threshold[56], we mentioned all results with $p < 0.100$ and post hoc tests were conducted with a former *p value* lower than 0.100. Analyses were performed using JASP software (0.16.4.0 version, Amsterdam, Netherlands).

3. Results

All detailed results are available in the Supplementary file 3.

3.1 Control measurements

3.1.1. Core and skin temperature and heart rate

Statistical analyses revealed temperature effects for diurnal HR, core and skin temperature, and for spontaneous physical activity. Results of post hoc tests are presented in the Figure 2. Core temperature (Figure 2A) and skin temperature (Figure 2B), and HR (Figure 2C), were largely higher during the 32°C compared to the 16 °C session and slightly-to-largely lower during the 16 °C compared to the 24 °C session. Core and skin temperature were moderately-to-largely higher during the 32 °C compared to the 24 °C session. Results from the nocturnal period are available in the supplementary file 2. Spontaneous physical activity was slightly-to-moderately higher during the 16 °C than the 24 °C and the 32 °C sessions (Figure 2E).

3.1.2. Energy expenditure

A temperature effect was found for EE (Figure 2D). It was slightly higher during the 16 and 32 °C sessions compared to 24 °C session (+5.42 ± 6.57 % and +5.07 ± 9.55 % during the 16 and 32 °C sessions, respectively). RQ was not modified by temperature exposures (supplemental file 3).

3.1.3. Body mass modifications and hydration level

Temperature effects were found for body mass modification, water intake, food intake, total intake, urine loss, sweat loss, total loss (Figure 3A for details), and USG ($p = 0.028$). Water,

food, and total intake were slightly-to-largely higher during the 32 than the 24 °C session. Body mass modifications were moderately-to-largely higher during the 32 and the 24 °C sessions compared to the 16 °C session. Water and total intakes were largely higher during the 32 than the 16 °C session. Water intake was moderately lower during the 16 than the 24 °C session. Sweat and total losses were largely higher during the 32 than the 24 and 16 °C sessions. Sweat loss was moderately lower during the 16 and the 24 °C session. Urine and feces loss was moderately lower during the 32 than the 16 °C session.

Finally post hoc test revealed that USG was slightly higher during the 16 compared to the 24 and 32 °C sessions (p = 0.055, d = 0.475 for both). USG and urine colour results are presented in the Supplementary file 3.

3.1.4. Subjective thermal ratings

Temperature and time effects were found for thermal sensation (Figure 3B) and discomfort (Figure 3C). Moreover, a time x temperature interaction was found for thermal discomfort. Post hoc tests results are presented in Figure 2B and 2C. Thermal sensation was largely higher in the 32 than the 24°C and the 16 °C sessions and largely lower in the 16 than the 24°C session. Thermal discomfort was largely higher in the 16 and the 32 °C than the 24 °C sessions. Thermal sensation was slightly lower at 8:00 pm compared to the basal value in all sessions. Thermal discomfort slightly increased after the sleep period in the 16 and 32 °C sessions.

3.1.5. Sleep and fatigue

Thermal environment had no impact on sleep quality and duration and on subjective feelings of fatigue before and after the night (Supplementary file 3).

3.2 Main measurements

3.2.1. Food intake, meal duration, and palatability

Thermal environment did not modify 24-h EI (p = 0.120 ; 14.49 ± 2.64, 14.06 ± 2.85, and 14.96 ± 2.99 MJ, for 16, 24 and 32°C sessions, respectively). However, a temperature effect was found for EI at the dinner, and breakfast, EI being slightly higher during the 32 than the 24 °C sessions (Figures 4A and 4B). Meal durations (Figures 4D to 4F) and macronutrients intake (Supplementary file 3) were not impacted by thermal exposures.

A temperature effect was found for the entrées, main dishes, and orange juice intake (Table 1). Entrée at dinner was slightly more consumed during 32 than during 16 °C session and entrée at lunch was slightly less consumed during 16 than during 24 °C session. Main dish was slightly more consumed during the 16 than the 24 °C session. Orange juice was slightly more consumed during the 32 than the 24 °C session.

During dinner, the hot main dish was fully consumed 3, 1, and 2 times and the bread 5, 3, and 3 times during the 16, 24, and 32°C sessions, respectively. During breakfast, sweet cottage cheese was fully consumed 0, 2, and 5 times, madeleines 0, 0, and 1 time, and orange juice 2, 1, and 5 times during the 16, 24, and 32°C sessions, respectively. During lunch, the dessert was fully consumed 1, 1, and 2 times and the bread 4, 3, and 4 times during the 16, 24, and 32°C sessions, respectively.

Palatability was found to be impacted by temperature only for cottage cheese and madeleine at the breakfast (Table 1). Cottage cheese was moderately more appreciated during the 32 than the 16 °C session. Madeleine was slightly less appreciated during the 32 than the 16 °C session.

3.2.2. Subjective ratings for the level of hunger and thirst

ANOVA revealed no temperature effect for CAS (Figure 5). Thirst was however impacted by temperature. Post hoc tests indicated that thirst levels were slightly higher during the 32 than the 16 and 24 °C sessions.

3.2.3 Olfactory and gustatory capacities

Temperature had no effect on the different scores assessing olfactory and gustatory capacities (Supplementary file 3).

3.2.4. LFPQ

A temperature effect was found for EL and IW Fat appeal biases, EL-Taste appeal bias, and EL and IW Temperature appeal biases but not for texture appeal bias. Post hoc tests are summed up in the Figures 6 and 7. Briefly, EL and IW for high-fat foods were slightly-to-moderately reduced during the 32 compared than the 16 and 24 °C sessions. IW for high fat-fat foods was slightly increased during the 16 than the 24 °C session. EL for sweet foods was slightly increased during the 32 compared to the 16 °C session. EL and IW for cold foods

were largely higher during the 32 than the 16 and the 24 °C sessions and EL and IW for hot foods were largely higher during the 16 than the 24 °C session.

Meal effects were found for EL and IW Fat appeal biases, EL and IW Taste appeal biases, IW Texture appeal bias, and IW Temperature appeal bias. Fat appeal biases were moderately higher during breakfast than during lunch indicating a larger preference towards the high-fat foods during breakfast. Taste appeal biases were largely higher during breakfast than dinner and lunch indicating a larger preference towards sweet foods. IW-Taste appeal was slightly lower during dinner than lunch indicating a larger preference towards solid foods. Finally, IW-Temperature appeal bias was slightly lower during dinner than lunch indicating a larger preference towards cold foods.

A meal effect was found for EL-FAT bias, IW-FAT bias, EL-Taste bias, IW-Taste bias, and IW-Texture bias. EL and IW for high-fat foods was moderately lower at lunch compared to breakfast. EL and IW for sweet foods was largely higher at breakfast compared to dinner and lunch. IW for solid foods slightly decreased at lunch compared to dinner.

3.2.5. Plasma levels of hormones

A temperature effect was found for acylated ghrelin, active GLP-1 before lunch, and leptin after lunch (Figure 8). Post hoc revealed that acylated ghrelin was slightly higher during the 16 °C session compared to the two other ones. GLP-1 was slightly higher during the 16°C compared to the 32 °C session. Leptin was slightly higher in the 32 °C session compared to two other ones. A meal effect was found for acylated ghrelin, active GLP-1, and total PYY with levels being lower for ghrelin and higher for active GLP-1 and total PYY after compared to before.

4. Discussion

Contrary to our hypothesis, neither a 24-h cold nor heat exposures modified total EI in young, active, and healthy men. In accordance, feelings of hunger and plasma levels of hormones involved in appetite modulation were also not altered. EI was even slightly increased during the first two test meals in the hot session (32 °C) compared to the thermoneutral one (24 °C), an observation that is in opposition to previous ones. However, food reward was strongly impacted by both thermal environments: towards low-fat, sweet and cold foods in hot conditions and high-fat, savory, and hot foods in cold conditions. Since test meals allowed

choices between cold and warm foods, participants were able to eat more of the foods they preferred in the specific thermal environment, maintaining therefore EI.

4.1. Physiological and subjective impact of thermal exposures

To control the subjective and objective impact of thermal exposures on participants is important for two reasons: 1) to check that the selection of temperature/hygrometry was successful and 2) to ensure that the eventual modifications in food intake-related variables could be serenely linked to the thermal impact. As previously pointed by a recent meta-analysis[19], this monitoring is rather weak in most studies and overall very inconsistent. Given the wide range of temperatures (from -140 °C to 18°C and from 30 to 36 °C in cold and hot conditions, respectively) and durations of exposure (3 min to 24 h) and the realisation or not of physical exercises that were sometimes done in immersion, this control appeared essential. Moreover, the lack of standardised clothing across conditions in half of the studies and the absence of details about the ability to maintain the experimental environment, at the exception of some studies[23,57,58], reinforces this need.

In the present study, we tried our best to control these thermal aspects and to limit the differences between sessions. In these conditions, we confirmed that 16 and 32 °C conditions had a large impact on physiological variables (core and skin temperature, HR, resting EE, urine and sweat loss for the 32°C session only), and some behavioural ones (spontaneous PA in the 16 °C session and water intake in the 32°C session). Moreover, participants felt the environment as slightly cool/cool and slightly warm/warm in cold and hot sessions, respectively and similarly uncomfortable in both sessions compared to the control 24 °C session. One interesting result was the similarity of temperatures and HR during sleep between sessions. This was due to our will not to impose the number of blankets. This choice, however, allowed the participants to sleep well. Thus, the possible effects of sleep deprivation on appetite[59,60] in the morning measurements were at best marginal. Comparisons with similar studies with passive thermal exposures at similar temperatures[23,25,28] showed that the impact of cold (10-18 °C *vs* 20-24°C in the cold *vs* control sessions, respectively) on resting EE (+6.8%), HR (-2.4%), and core (-1.2 to -1.1%) and skin temperature (-13.2 to -6.5%) was concordant in the present paper (+5.4%, -4.8%, -0.4, and -7.3%, respectively). The smaller decrease in core temperature was very likely due to the increase in spontaneous PA in the 16 °C session (Figure 2E), a compensatory behaviour, objectified in mice[61] but not in humans[62], that may have slightly increased internal heat

production. In the heat, core and skin temperature increases were lower in the present work (+0.25 and +4.5%, respectively) than in the study of Zakrzewski-Fruer et al[23] (+1.1 and +10.3%, respectively). The difference between the hot and the neutral conditions was larger in the latter study than in the present one (+10 *vs* + 8 °C) but it remains insufficient to explain the lesser impact of heat exposure in ours. The possibility to engage in some light activities alone or in group may be a potent hypothesis.

Thus, 16 °C and 32 °C temperatures were sufficient to elicit moderate to large physiological, behavioural, and subjective modifications compared to the 24 °C control session. Participants started therefore their three test meals in very different conditions.

4.2. EI, hunger, and hormones

Literature indicates a possible orexigenic effect of cold exposure (increases in hunger, EI, and ghrelin levels)[24,58,63,64,65,66] and an anorexigenic effect of heat exposure (decreases in hunger, EI, and increases in leptin and PYY levels)[23,24,67,68,69]. It was therefore logically expected to confirm these opposite and suggested effects during a 24-h period with three meals in which participants were not exercising and other biases were annulled or limited. Surprisingly, 24-h EI was not different between sessions. More surprisingly, EI was slightly higher during the first two meals (dinner and breakfast) in the hot condition compared to the neutral one, this result being in total opposition with previous ones. Accordingly, participants did not initiate their meals with different levels of subjective hunger and similar gustatory and olfactory capacities. The latter assessments were considered exploratory since only indirect evidence[53,54] supported a hypothetical effect of thermal exposures. Even if these results contradict most of the existing literature, they remain in total accordance with those of our previous study[27] in which no modification of hunger score and EI was found after 16 h in the heat (32°C) compared to a neutral condition (22 °C). Our main hypothesis at this time was the tendency to not modify habits during breakfast[70] used as a test meal reducing therefore the possibility to modify selection of foods and consumed amounts. The fact that subjective hunger and EI were not modified (and even slightly increased in the 32°C session for the latter) during 3 consecutive meals weaken this proposition but demonstrates instead the robustness of the absence of anorexigenic effect of heat. Surprisingly some hormonal modifications (at the lunch initiated 23h30 after the beginning of exposure) in favor with an anorexigenic effect of heat (increase in postlunch leptin at 32°C) and orexigenic effect of cold (increase in prelunch acylated ghrelin at 16°C) were observed. However the physiological

impact of these modifications was judged small and partially in disagreement with the literature[63,71]. One might argue that these modifications were not attributed to the thermal environment, but rather caused by the higher food intake during dinner and particularly during breakfast at 32°C. The absence of previous blood samplings (for example in a fasted state before breakfast) precluded reliable interpretations.

Given the myriad of protocols used in this specific field[19], to identify the hypothesis explaining the discrepancy of results seems a hard and risky task. The impact of the realisation of physical activities during thermal exposures is very likely major but make all comparisons with passive exposures hazardous since the production of internal heat during exercise counteracts the effects of ambient temperature to an extent. Moreover, at the exception of some studies[24,26], participants were placed in a temperate environment after exercise until the test meals. Thus the decay of thermal impact during exercise between exercise and assessment of EI is another bias to consider.

Direct comparisons with studies using passive exposure are therefore more reasonable. Cold exposures (10-18 °C *vs* 20-24 °C) induced marginal effects on EI and appetite[23,25,28]. The results of the present study are totally in agreement with these previous studies despite large cold-induced perceptive and physiological alterations. An increased EI during cold exposure (10 *vs* 20°C) was found only in the study of Wasse *et al.*[24]. However, this 6-h exposure started with a 1-h exercise session. In these conditions, it is difficult to know how this activity may have altered the effects of cold exposure on appetite. For example, contrary to passive studies[23,28], core temperature was similar in both exposures. This lack of effect may be logically explained by the exercise-induced heat production that may persist several hours. On the other hand, the fact that participants were "able to wear whatever clothing they wished"[24] may also have mitigated cold-induced physiological effects. Another explanation could be this excess may have generated higher heat production related to the thermal effect of food. Indeed, Westerterp-Plantenga *et al.*[28] reported a correlation between overeating at 16°C (compared to 22°C) and the attenuation of rectal core body temperature. These results suggest that humans may unconsciously modulate food intake to increase heat production and therefore limit cold-induced heat dissipation. This adaptive behaviour well-documented in mammals[72] and also observed in warmer conditions (reduction of food intake to avoid increase in core temperature)[73], required further investigation in humans if we considered the scarcity of publications. These examples perfectly illustrate how even small discrepancies

between protocols (presence of physical activities and the choice of clothing) complicate interpretations and comparisons between studies.

Passive heat exposure was found to significantly reduce hunger score and EI by 1189 ± 1219 kJ[23]. Hypotheses may be proposed based on the differences in protocols. A recent study[74] demonstrated that the shorter the holding times in a laboratory following a test meal, the lower the EI. We can suppose that this effect is more important in individuals placed in uncomfortable conditions and that they rushed their meal to leave the experimentation the soonest. While participants stayed several hours in the laboratory after the two first meals in the present study, those in the Zakrzewski-Fruer *et al.*[23] study remained only one hour. It is however impossible to know whether this effect operated here. Results from the study of Wasse *et al.*[24], in which EI was found slightly lower during two successive test buffet meals at 30°C compared to the 20°C control session, suggest that this effect is rather unlikely to operate. Another difference was the activities that were authorised during the study. Socialisation was authorised and encouraged through the supply of several leisure activities in the present study while no socialisation (one participant per session) and one sedentary task ("work on a laptop") was authorised in the previous study[23]. In addition to probably amplify physiological impact of ambient temperature, remaining completely sedentary in a laboratory setting without many stimulating activities may also improve self-awareness and therefore the quality of VAS fillings but also reduce the number of psychological cues compared to a context closer to daily-life[75]. Nevertheless the impact on the hunger scales filling remained to be demonstrated especially since no difference in perceived appetite was observed between free-living and laboratory controlled conditions[76].

4.3. Food reward and food choices

The impact of thermal exposures on food reward and preference is scarcely studied. We performed a pilot study on the impact of a 16-h passive exposure to heat[27] and field studies during a 15-day expedition in the cold[77,78] using an adapted paper version of the LFPQ[79]. While no meaningful modifications of food preferences were observed during the latters, robust decreases in IW and EL for high-fat foods were observed during heat passive exposures[27]. Moreover, Motoki et al.[50] identified a negative relationship between food preference for savory foods and their perceived warmth in warm conditions (27-30 °C), whereas this relationship was positive in cooler ones (20-23°C). We globally confirmed in

the present study these results (large increase in food reward for low-fat and cold foods/drinks and a slight increase in EL for sweet foods). Cold exposure induced opposite modifications in food reward (increase in IW for high-fat foods, increases in EL and IW for warm foods/drinks, and a slight increase in EL for savory foods). Correlations between LFPQ scores and both intake and food choice are regularly evidenced[80,81]. However, these associations may sometimes be hard to highlight[27,82] in adverse situations (altitude and heat exposure, respectively) when it was not possible to propose a buffet composed of the same foods used in the LFPQ. Since the aim of this study was to mimic real-life conditions using meals composed of traditional French dishes, we did not gather the optimal conditions to observe these associations. However, the analysis of food intake (Table 1) revealed interesting tendencies. Indeed, cold entrée intake was slightly higher at 32 °C than 16 °C at dinner and slightly lower at 16 °C than 24 °C at lunch. Moreover, hot main dish intake was higher at 16 than 24 °C at both meals. Finally, orange juice, which was served cold, was more consumed at 32 than 24 °C. These observations were in line with the modifications of food reward for warm/cold foods. Concerning the latter, we might argue that the slightly higher levels of thirst at 32°C compared to 24°C just before breakfast (p = 0.023, d = 0.429) partially explained this result and the higher EI observed during the 32°C session. If participants' intake was driven by thirst and if we consider the low satiating effect of fluids, it is possible that the greater EI during breakfast was not only due to an effect of heat on appetite. If orange juice was removed from the EI calculation, the effect of heat exposure compared to control session was reduced but not totally removed (4078 ± 1170 *vs* 4573 ± 1310 kJ [p = 0.062; d = 0.37] with orange juice and 3340 ± 1118 vs 3698 ± 1209 kJ [p = 0.143 ; d = 0.32] without orange juice in the 24 and 32°C sessions, respectively). In these conditions, it is therefore possible that heat-induced higher levels of thirst may have enhanced EI during breakfast but it remains to be demonstrated.

It is therefore possible that this small but ecological choice may have allowed participants to adjust their intake according to the modifications in food reward maintaining EI. Some comparisons with similar studies support this idea. In the study of Zakrzewski-Fruer *et al.*[23], a hot pasta dish was served in large quantities after an exposure to 20 or 30 °C. EI was significantly lower in the 30 °C condition. Based on the present results, it is very likely that participants were less interested in this only dish and ate less of it. In our previous study[27], a breakfast buffet composed of cold, temperate, and hot foods/drinks was served after 16 h at 22 or 32 °C. With this test meal, EI was not different between the conditions. Finally, cold

sandwiches were served after a 75 min exposure at 31 or 22 °C[26]. No modification was observed apparently contradicting this hypothesis. Nevertheless participants were French West Indies natives and subjective thermal scales revealed that the 32°C session was considered as comfortable/temperate and the 21°C session as slightly uncomfortable/cool. Thus, the effect of passive heat exposure was very likely not addressed in this study. Further studies are required to confirm the possibility that EI and appetite are barely impacted by heat exposure as long as choices are possible (using foods with different temperature). Indeed, EI were slightly decreased after an exercise session realised at 30/36 °C compared to 20/25 °C[24,67] while buffet composed of foods served at different temperatures were used as test meals. The isolated impact of physical exercise in combination with exposure to extreme temperature remained to be elucidated.

4.4. Limitations

Numerous efforts were done to limit and avoid bias that often interferes with the interpretation of the results. This is why temperatures were selected based on literature and previous studies from our laboratory to potentially induce modifications in appetite during a passive exposure. Moreover, this thermal impact was controlled using devices that were almost imperceptible for participants and the same outfit adapted to 24 °C was imposed to avoid thermal compensation by adding layers that often occur during cold exposures[23,24,83].

Management of water intake was subjected to several possibilities and *ad libitum* intake was privileged since it was shown that participants were able to replace the higher sweat loss during heat exposures almost perfectly maintaining levels of hydration similar between sessions[23,27]. The present results confirmed the efficiency of this choice. One might argue that drinking high volumes of water, as it was the case in the 32°C session, may reduce subsequent food intake[84], but since EI was slightly higher in this hot condition, this effect was very unlikely.

The study occurred between January and April in a period with cold to temperate temperatures (5 to 20 °C). The aim was to avoid participants to be heat acclimatised and less sensitive to the heat exposure. However, it is possible that participants were partly acclimatised to the mild cold climate reducing therefore the impact of cold exposure and potentially explaining the absence of modifications of EI in the cold condition.

Food intake may be assessed through a myriad of protocols[85,86,87], each choice being accompanied by strengths and weaknesses. We chose to privilege an ecological solution

using a three-course meals (plus bread for dinner and lunch) that corresponded to the French standards and that was already used in different contexts[88,89]. It was moreover concordant with the fact that participants were left free to live in this 'apartment'. The choice to use military ration may be criticised given that military foods are preconceived to be inferior to commercial foods[90] especially when eaten in comfortable conditions. However, military rations consumed *ad libitum* during several weeks induced similar EI than fresh foods or usual diets[37,38,91] strongly suggesting that these foods may be found appropriate to daily life. However, it is possible that despite the consumption of similar foods during the normalisation period in order to improve familiarisation, some items (entrées mostly) were rated less than moderately good. It is difficult to known what would have been the results if all foods were rated above average.

Finally, the interpretation of the results is limited to this specific population (young, active, healthy young men). The effects on women, older individuals, overweight/obese and with a metabolic or psychological pathology remain to be assessed.

5. Conclusions

Passive exposures to heat (32 °C) and cold (16 °c) did not alter EI assessed at 3 successive meals during a 24-h period compared to a neutral control condition (24 °C) contrary to our hypothesis. Hunger scores, plasma hormonal levels, and gustatory and olfactory capacities were accordingly not modified. However, food reward for fatness, taste, and temperature of foods were deeply altered with heat and cold exposure. These modifications were likely to be involved in the higher consumption of cold foods during heat exposure and warm foods during cold exposure. It was therefore hypothesised that offering some choice based on food temperature may help individuals to express their specific food preferences and maintain EI.

Thus, these results suggested that offering cold or warm foods in hot or cold conditions may enhance food intake. This may appear challenging for athletes and military personnel that may eat in unusual and/or uncomfortable conditions. However, these logistical constraints may be interesting to solve if it may limit loss of body mass on a longer basis.

This protocol was designed to confirm tendencies surfacing from a restricted amount of publications, the obtained results finally raised more interrogations. Indeed, it remains to challenge the proposed hypothesis in comparing EI during hot/cold exposures and neutral ones using different menus adapted or not to the expected food reward modifications observed in the heat. Moreover, including physical exercises during these exposures appeared essential since the most concerned populations (athletes and soldiers) are not supposed to remain sedentary.

References

1. Castellani JW, Young AJ (2012) Health and performance challenges during sports training and competition in cold weather. *Br J Sports Med* **46**, 788-791.

2. Bubnis MA, Hulsopple C (2022) Human Performance and Injury Prevention in Cold Weather Environments. *Curr Sports Med Rep* **21**, 112-116.

3. Gatterer H, Dunnwald T, Turner R *et al.* (2021) Practicing Sport in Cold Environments: Practical Recommendations to Improve Sport Performance and Reduce Negative Health Outcomes. *International journal of environmental research and public health* **18**.

4. Gibson OR, James CA, Mee JA *et al.* (2020) Heat alleviation strategies for athletic performance: A review and practitioner guidelines. *Temperature (Austin)* **7**, 3-36.

5. Racinais S, Hosokawa Y, Akama T *et al.* (2023) IOC consensus statement on recommendations and regulations for sport events in the heat. *Br J Sports Med* **57**, 8-25.

6. Bernard P, Chevance G, Kingsbury C *et al.* (2021) Climate Change, Physical Activity and Sport: A Systematic Review. *Sports Med* **51**, 1041-1059.

7. Yamasaki L, Nomura S (2022) Global warming and the Summer Olympic and Paralympic games: a perspective from the Tokyo 2020 Games. *Environmental health and preventive medicine* **27**, 7.

8. Sullivan-Kwantes W, Haman F, Kingma BRM *et al.* (2021) Human performance research for military operations in extreme cold environments. *J Sci Med Sport* **24**, 954-962.

9. Parsons IT, Stacey MJ, Woods DR (2019) Heat Adaptation in Military Personnel: Mitigating Risk, Maximizing Performance. *Front Physiol* **10**, 1485.

10. Periard JD, DeGroot D, Jay O (2022) Exertional heat stroke in sport and the military: epidemiology and mitigation. *Exp Physiol* **107**, 1111-1121.

11. Sullivan-Kwantes W, Cramer M, Bouak F *et al.* (2021) Environmental Stress in Military Settings. In *Handbook of Military Sciences* [AS (ed.), editor]: Crown.

12. Heydenreich J, Kayser B, Schutz Y *et al.* (2017) Total Energy Expenditure, Energy Intake, and Body Composition in Endurance Athletes Across the Training Season: A Systematic Review. *Sports medicine - open* **3**, 8.

13. Logue DM, Madigan SM, Melin A *et al.* (2020) Low Energy Availability in Athletes 2020: An Updated Narrative Review of Prevalence, Risk, Within-Day Energy Balance, Knowledge, and Impact on Sports Performance. *Nutrients* **12**.

14. Charlot K (2021) Negative energy balance during military training: The role of contextual limitations. *Appetite* **164**, 105263.

15. Tassone EC, Baker BA (2017) Body weight and body composition changes during military training and deployment involving the use of combat rations: a systematic literature review. *Br J Nutr* **117**, 897-910.

16. Burke LM, Slater G, Broad EM *et al.* (2003) Eating patterns and meal frequency of elite Australian athletes. *Int J Sport Nutr Exerc Metab* **13**, 521-538.

17. Valencia ME, McNeill G, Brockway JM *et al.* (1992) The effect of environmental temperature and humidity on 24 h energy expenditure in men. *Br J Nutr* **68**, 319-327.

18. McInnis K, Haman F, Doucet E (2020) Humans in the cold: Regulating energy balance. *Obes Rev* **21**, e12978.

19. Millet J, Siracusa J, Tardo-Dino PE *et al.* (2021) Effects of Acute Heat and Cold Exposures at Rest or during Exercise on Subsequent Energy Intake: A Systematic Review and Meta-Analysis. *Nutrients* **13**.

20. O'Leary TJ, Wardle SL, Greeves JP (2020) Energy Deficiency in Soldiers: The Risk of the Athlete Triad and Relative Energy Deficiency in Sport Syndromes in the Military. *Frontiers in nutrition* **7**, 142.

21. Beckner ME, Lieberman HR, Hatch-McChesney A *et al.* (2023) Effects of energy balance on cognitive performance, risk-taking, ambulatory vigilance and mood during simulated military sustained operations (SUSOPS). *Physiol Behav* **258**, 114010.

22. Murphy NE, Carrigan CT, Philip Karl J *et al.* (2018) Threshold of Energy Deficit and Lower-Body Performance Declines in Military Personnel: A Meta-Regression. *Sports Med* **48**, 2169-2178.

23. Zakrzewski-Fruer JK, Horsfall RN, Cottrill D *et al.* (2021) Acute exposure to a hot ambient temperature reduces energy intake but does not affect gut hormones in men during rest. *Br J Nutr* **125**, 951-959.

24. Wasse LK, King JA, Stensel DJ *et al.* (2013) Effect of ambient temperature during acute aerobic exercise on short-term appetite, energy intake, and plasma acylated ghrelin in recreationally active males. *Applied Physiology, Nutrition, and Metabolism* **38**, 905-909.

25. Langeveld M, Tan CY, Soeters MR *et al.* (2016) Mild cold effects on hunger, food intake, satiety and skin temperature in humans. *Endocrine connections* **5**, 65-73.

26. Faure C, Charlot K, Henri S *et al.* (2016) Effect of heat exposure and exercise on food intake regulation: A randomized crossover study in young healthy men. *Metabolism* **65**, 1541-1549.

27. Charlot K, Millet J, Pasquier F *et al.* (2022) The impact of 16-h heat exposure on appetite and food reward in adults. *Appetite* **177**, 106144.

28. Westerterp-Plantenga MS, van Marken Lichtenbelt WD, Strobbe H *et al.* (2002) Energy metabolism in humans at a lowered ambient temperature. *Eur J Clin Nutr* **56**, 288-296.

29. Karlsson J, Persson LO, Sjöström L *et al.* (2000) Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. *Int J Obes Relat Metab Disor* **24**, 1715-1725.

30. Buysse DJ, Reynolds CF, 3rd, Monk TH *et al.* (1989) The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* **28**, 193-213.

31. MacLean BL, MacLean K, Stewart IB *et al.* (2021) Monitoring heat strain: the effect of sensor type and location on single-site and mean skin temperature during work in the heat. *International archives of occupational and environmental health* **94**, 539-546.

32. Perrier ET, Bottin JH, Vecchio M *et al.* (2017) Criterion values for urine-specific gravity and urine color representing adequate water intake in healthy adults. *Eur J Clin Nutr* **71**, 561-563.

33. Logan-Sprenger HM, Spriet LL (2013) The acute effects of fluid intake on urine specific gravity and fluid retention in a mildly dehydrated state. *J Strength Cond Res* **27**, 1002-1008.

34. Armstrong LE, Maresh CM, Castellani JW *et al.* (1994) Urinary indices of hydration status. *International journal of sport nutrition* **4**, 265-279.

35. Humphreys MA, Nicol JF, Raja IA (2007) Field Studies of Indoor Thermal Comfort and the Progress of the Adaptive Approach. *Advances in Building Energy Research* **1**, 55 - 88.

36. Arnal PJ, Thorey V, Debellemaniere E *et al.* (2020) The Dreem Headband compared to polysomnography for electroencephalographic signal acquisition and sleep staging. *Sleep* **43**.

37. Ahmed M, Mandic I, Lou W *et al.* (2023) Dietary Intakes From Ad Libitum Consumption of Canadian Armed Forces Field Rations Compared With Usual Home Dietary Intakes and Military Dietary Reference Intakes. *Mil Med* **188**, e205-e213.

38. McClung HL, Armstrong NJ, Hennigar SR *et al.* (2020) Randomized Trial Comparing Consumption of Military Rations to Usual Intake for 21 Consecutive Days: Nutrient Adequacy and Indicators of Health Status. *Journal of the Academy of Nutrition and Dietetics* **120**, 1791-1804.

39. Ruddock HK, Brunstrom JM, Vartanian LR *et al.* (2019) A systematic review and meta-analysis of the social facilitation of eating. *Am J Clin Nutr* **110**, 842-861.

40. Ogden J, Coop N, Cousins C *et al.* (2013) Distraction, the desire to eat and food intake. Towards an expanded model of mindless eating. *Appetite* **62**, 119-126.

41. Anderson GH, Catherine NL, Woodend DM *et al.* (2002) Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *Am J Clin Nutr* **76**, 1023-1030.

42. Finlayson G, King N, Blundell J (2008) The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control. *Appetite* **50**, 120-127.

43. Finlayson G, King N, Blundell JE (2007) Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. *Physiol Behav* **90**, 36-42.

44. Oustric P, Thivel D, Dalton M *et al.* (2020) Measuring food preference and reward: Application and cross-cultural adaptation of the Leeds Food Preference Questionnaire in human experimental research. *Food Quality & Preference* **80**.

45. Thivel D, Oustric P, Beaulieu K *et al.* (2023) Development, sensitivity and reliability of a French version of the Leeds food preference questionnaire (LFPQ-fr) for the evaluation of food preferences and reward. *Physiol Behav* **267**, 114187.

46. Lemon CH (2021) Tasting temperature: neural and behavioral responses to thermal stimulation of oral mucosa. *Current opinion in physiology* **20**, 16-22.

47. Burdon CA, Johnson NA, Chapman PG *et al.* (2012) Influence of beverage temperature on palatability and fluid ingestion during endurance exercise: a systematic review. *International journal of sport nutrition and exercise metabolism* **22**, 199-211.

48. Eccles R, Du-Plessis L, Dommels Y *et al.* (2013) Cold pleasure. Why we like ice drinks, ice-lollies and ice cream. *Appetite* **71**, 357-360.

49. Labbe D, Almiron-Roig E, Hudry J *et al.* (2009) Sensory basis of refreshing perception: role of psychophysiological factors and food experience. *Physiol Behav* **98**, 1-9.

50. Motoki K, Saito T, Nouchi R *et al.* (2020) Cross-Modal Correspondences Between Temperature and Taste Attributes. *Front Psychol* **11**, 571852.

51. Suzuki M, Kimura R, Kido Y *et al.* (2017) Color of hot soup modulates postprandial satiety, thermal sensation, and body temperature in young women. *Appetite* **114**, 209-216.

52. Ma Z, Paudel U, Foskett JK (2023) Effects of temperature on action potentials and ion conductances in type II taste-bud cells. *American journal of physiology Cell physiology* **325**, C155-C171.

53. Cameron JD, Goldfield GS, Doucet E (2012) Fasting for 24 h improves nasal chemosensory performance and food palatability in a related manner. *Appetite* **58**, 978-981.

54. Hanci D, Altun H (2016) Hunger state affects both olfactory abilities and gustatory sensitivity. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies* **273**, 1637-1641.

55. Cohen J (1988) Statistical Power Analysis for the Behavioral Sciences. *2nd ed Hillsdale (NJ): Lawrence Erlbaum Associates*, 567 p-567 p.

56. Amrhein V, Greenland S, McShane B (2019) Scientists rise up against statistical significance. *Nature* **567**, 305-307.

57. Metz L, Isacco L, Beaulieu K *et al.* (2021) Cold-Water Effects on Energy Balance in Healthy Women During Aqua-Cycling. *Int J Sport Nutr Exerc Metab*, 1-8.

58. White LJ, Dressendorfer RH, Holland E *et al.* (2005) Increased caloric intake soon after exercise in cold water. *International journal of sport nutrition and exercise metabolism* **15**, 38-47.

59. Brondel L, Romer MA, Nougues PM *et al.* (2010) Acute partial sleep deprivation increases food intake in healthy men. *Am J Clin Nutr* **91**, 1550-1559.

60. Chamorro R, Garrido M, Algarin C *et al.* (2023) A single night of moderate at-home sleep restriction increases hunger and food intake in overweight young adults. *Nutrition* **108**, 111962.

61. Presby DM, Jackman MR, Rudolph MC *et al.* (2019) Compensation for cold-induced thermogenesis during weight loss maintenance and regain. *Am J Physiol Endocrinol Metab* **316**, E977-E986.

62. Celi FS, Brychta RJ, Linderman JD *et al.* (2010) Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. *European journal of endocrinology* **163**, 863-872.

63. Crabtree DR, Blannin AK (2015) Effects of exercise in the cold on Ghrelin, PYY, and food intake in overweight adults. *Medicine and science in sports and exercise* **47**, 49-57.

64. Dressendorfer RH (1993) Effect of internal body temperature on energy intake soon after aerobic exercise. *Medicine & Science in Sports & Exercise* **S42**, 228-228.

65. Halse RE, Wallman KE, Guelfi KJ (2011) Postexercise Water Immersion Increases Short-Term Food Intake in Trained Men. *Medicine & Science in Sports & Exercise* **43**, 632-638.

66. Kojima C, Kasai N, Kondo C *et al.* (2018) Post-Exercise Whole Body Cryotherapy (-140 degrees C) Increases Energy Intake in Athletes. *Nutrients* **10**.

67. Shorten AL, Wallman KE, Guelfi KJ (2009) Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *The American journal of clinical nutrition* **90**, 1215-1221.

68. Kojima C, Sasaki H, Tsuchiya Y *et al.* (2015) The influence of environmental temperature on appetite-related hormonal responses. *Journal of physiological anthropology* **34**, 22-22.

69. Zheng G, Li K, Wang Y (2019) The Effects of High-Temperature Weather on Human Sleep Quality and Appetite. *International journal of environmental research and public health* **16**.

70. Gibney MJ, Uzhova I (2019) Breakfast: Shaping Guidelines for Food and Nutrient Patterns. *Nestle Nutr Inst Workshop Ser* **91**, 133-142.

71. Charlot K, Faure C, Antoine-Jonville S (2017) Influence of Hot and Cold Environments on the Regulation of Energy Balance Following a Single Exercise Session: A Mini-Review. *Nutrients* **9**.

72. Terrien J, Perret M, Aujard F (2011) Behavioral thermoregulation in mammals: a review. *Frontiers in bioscience (Landmark edition)* **16**, 1428-1444.

73. Bernhard MC, Li P, Allison DB *et al.* (2015) Warm Ambient Temperature Decreases Food Intake in a Simulated Office Setting: A Pilot Randomized Controlled Trial. *Frontiers in nutrition* **2**, 20.

74. Palmer B, Irwin C, McCartney D *et al.* (2021) The impact of post-prandial delay periods on ad libitum consumption of a laboratory breakfast meal. *Appl Physiol Nutr Metab* **46**, 1290-1297.

75. Blundell J, de Graaf C, Hulshof T *et al.* (2010) Appetite control: methodological aspects of the evaluation of foods. *Obes Rev* **11**, 251-270.

76. Aberg S, Palmnas-Bedard M, Karlsson T *et al.* (2023) Evaluation of Subjective Appetite Assessment under Free-Living vs. Controlled Conditions: A Randomized Crossover Trial Comparing Whole-Grain Rye and Refined Wheat Diets (VASA-Home). *Nutrients* **15**.

77. Charlot K, Chapelot D, Colin P *et al.* (2020) Daily energy balance and eating behaviour during a 14-day cold weather expedition in Greenland. *Appl Physiol Nutr Metab*.

78. Charlot K, Chapelot D, Siracusa J *et al.* (2021) An augmented food strategy leads to complete energy compensation during a 15-day military training expedition in the cold. *Physiol Rep* **9**, e14591.

79. Charlot K, Malgoyre A, Bourrilhon C (2018) Proposition for a shortened version of the Leeds Food Preference Questionnaire (LFPQ). *Physiol Behav* **199**, 244-251.

80. Griffioen-Roose S, Mars M, Finlayson G *et al.* (2011) The effect of within-meal protein content and taste on subsequent food choice and satiety. *Br J Nutr* **106**, 779-788.

81. Dalton M, Blundell J, Finlayson GS (2013) Examination of food reward and energy intake under laboratory and free-living conditions in a trait binge eating subtype of obesity. *Front Psychol* **4**, 757.

82. Aeberli I, Erb A, Spliethoff K *et al.* (2013) Disturbed eating at high altitude: influence of food preferences, acute mountain sickness and satiation hormones. *European journal of nutrition* **52**, 625-635.

83. Ahmed M, Mandic I, Lou W *et al.* (2019) Comparison of dietary intakes of Canadian Armed Forces personnel consuming field rations in acute hot, cold, and temperate conditions with standardized infantry activities. *Military Medical Research* **6**, 26.

84. McKay NJ, Belous IV, Temple JL (2018) Increasing water intake influences hunger and food preference, but does not reliably suppress energy intake in adults. *Physiol Behav* **194**, 15-22.

85. Gibbons C, Hopkins M, Beaulieu K *et al.* (2019) Issues in Measuring and Interpreting Human Appetite (Satiety/Satiation) and Its Contribution to Obesity. *Current obesity reports* **8**, 77-87.

86. Gregersen NT, Flint A, Bitz C *et al.* (2008) Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *Am J Clin Nutr* **87**, 1277-1281.

87. Thivel D, Genin PM, Mathieu ME *et al.* (2016) Reproducibility of an in-laboratory test meal to assess ad libitum energy intake in adolescents with obesity. *Appetite* **105**, 129-133.

88. Charlot K, Chapelot D (2013) Energy compensation after an aerobic exercise session in high-fat/low-fit and low-fat/high-fit young male subjects. *Br J Nutr* **110**, 1133-1142.

89. Charlot K, Chapelot D (2019) Comparison of energy-matched high-intensity interval and moderate-intensity continuous exercise sessions on latency to eat, energy intake, and appetite. *Appl Physiol Nutr Metab* **44**, 665-673.

90. de Graaf C, Cardello AV, Matthew Kramer F *et al.* (2005) A comparison between liking ratings obtained under laboratory and field conditions: the role of choice. *Appetite* **44**, 15-22.

91. Hirsch E, Kramer FM (1993) Situational influences on food intake. In *Nutritional needs in hot environments*, pp. 215-242 [ComnrIo medicine, editor]: Washington, D.C: National Academy Press.

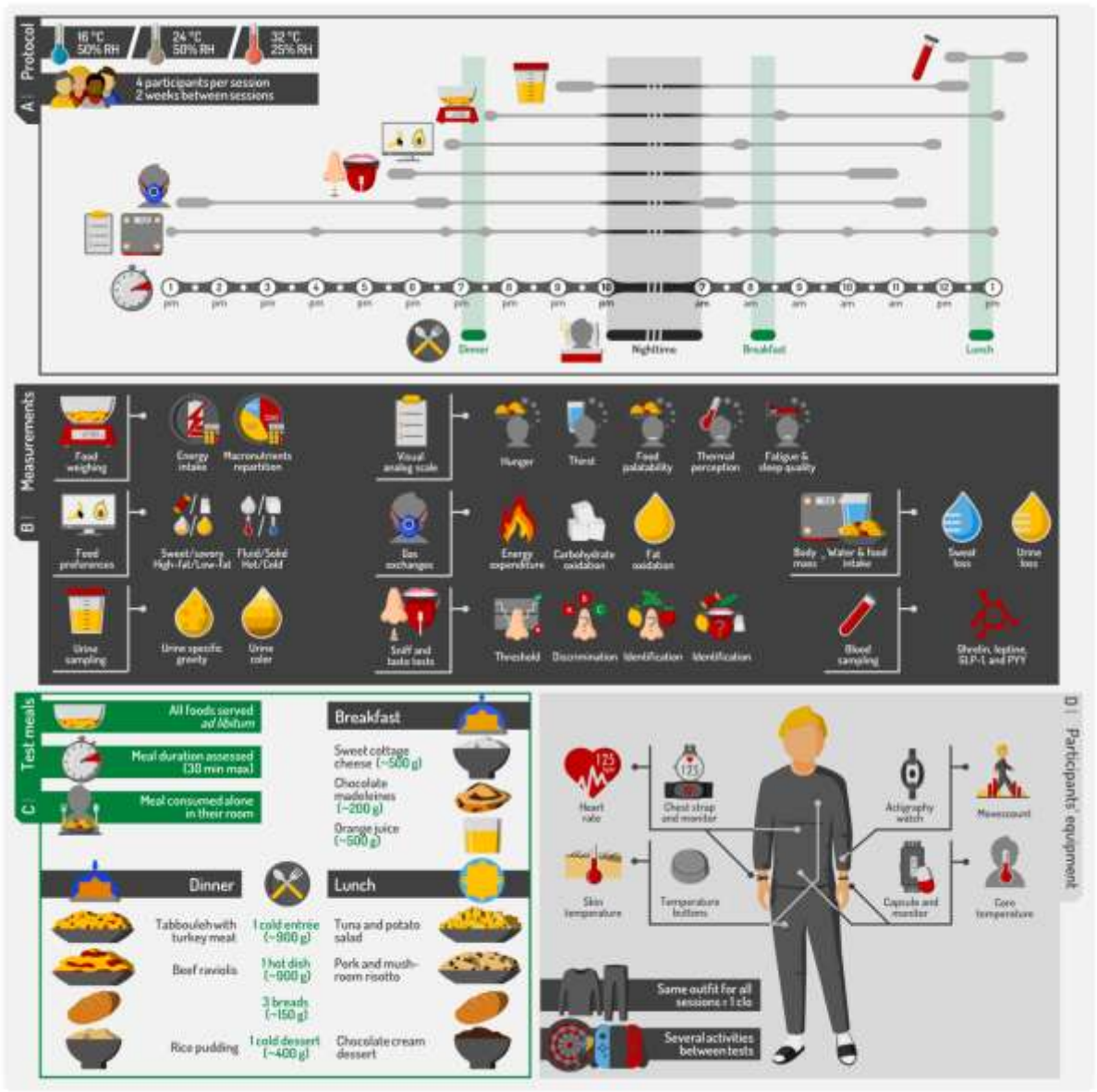

Figure 1. Study protocol.

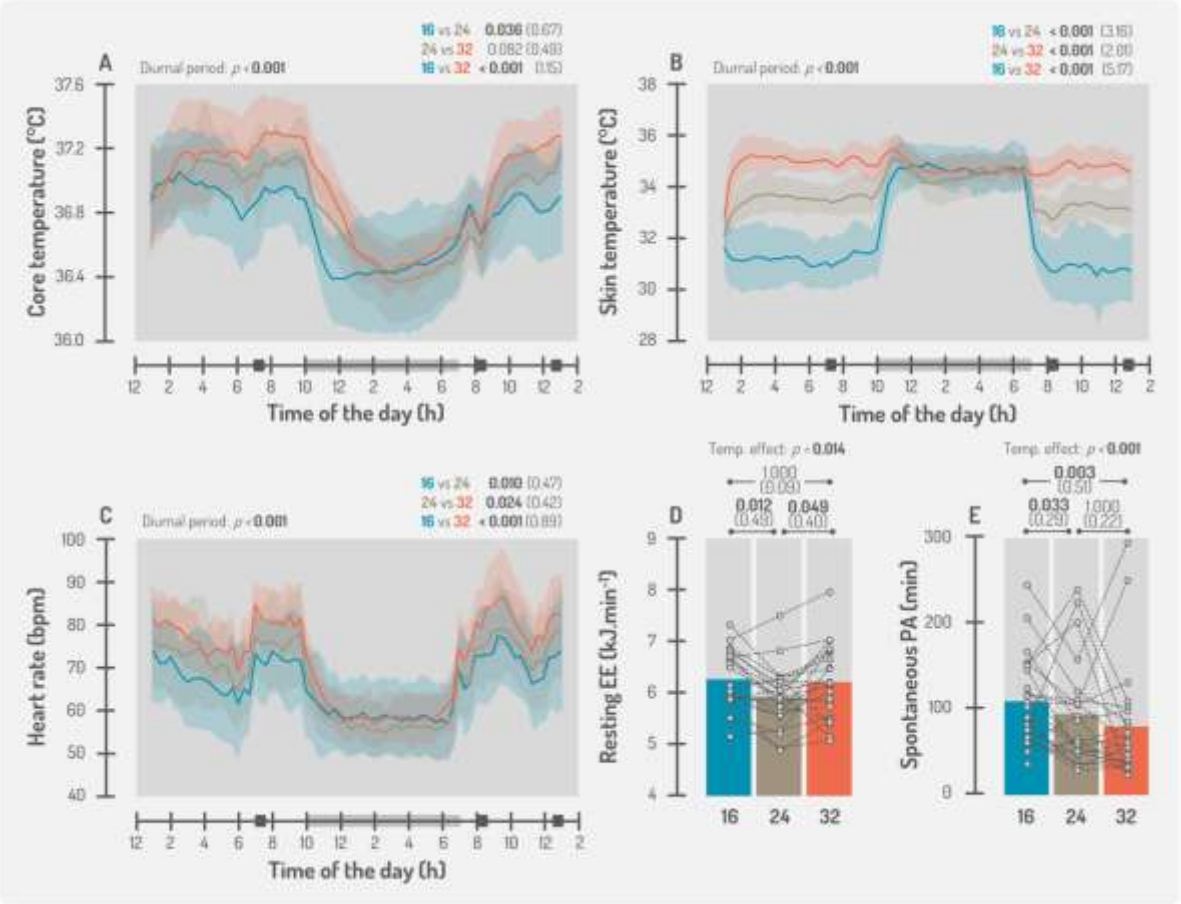

Figure 2. Thermophysiological modifications and spontaneous physical activity. Solid Lines represent the mean values in each session and light surfaces represent SD (Figures A, B, and C). The light grey rectangle represents the sleep period and the three dark grey rectangles represent the meals. In Figures D and E, dotted lines represent individual values and rectangles the mean values for each session. EE: energy expenditure, PA: physical activity. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

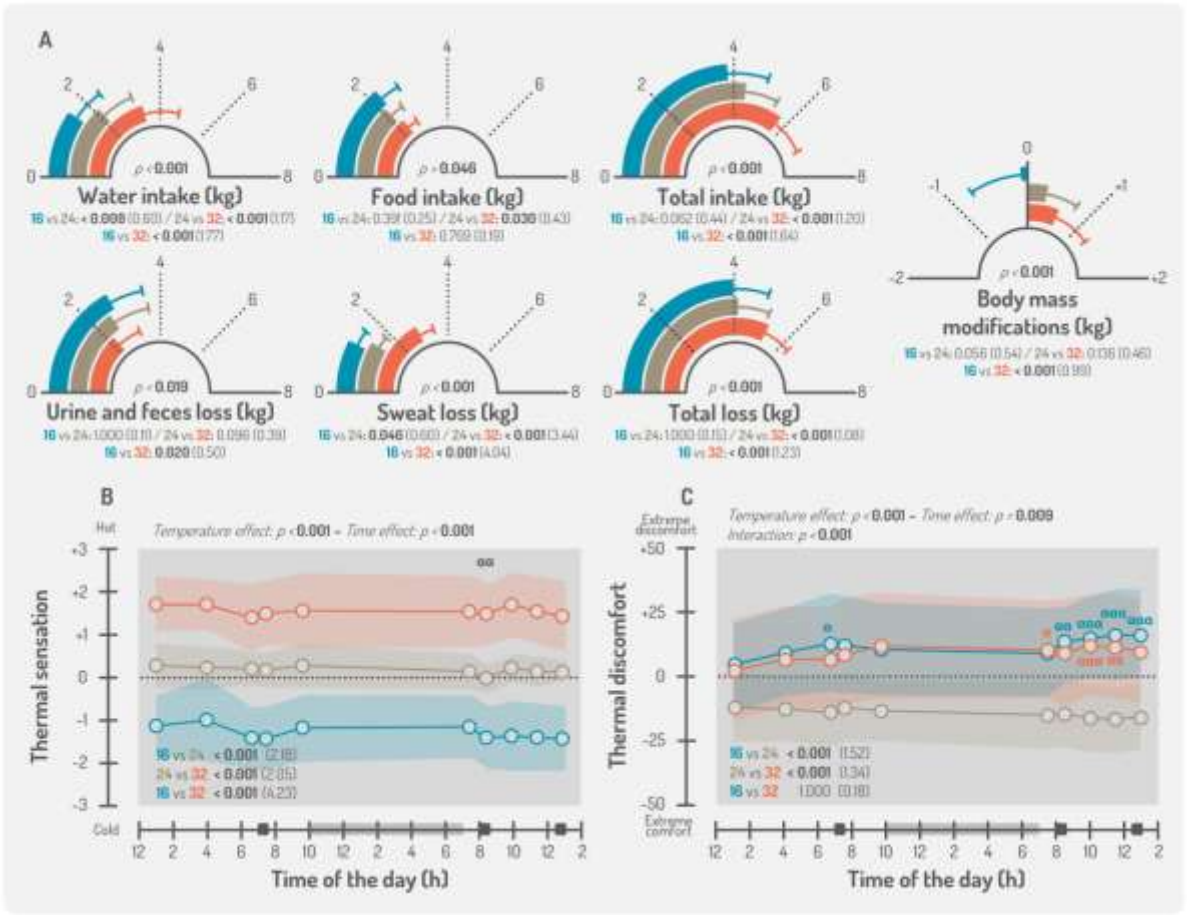

Figure 3. Body mass modifications analysis (A) and thermal sensation (B) and discomfort (C). Figures A: Data are presented in means ± SD. Figures B and C: solid Lines represent the mean values in each session and light surfaces represent SD. The light grey rectangle represents the sleep period and the three dark grey rectangles represent the meals. $^{\alpha}$different from basal measurements (1:00pm) ($^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$; $^{\alpha\alpha\alpha}p < 0.001$, in grey: time effect for all sessions, in colour: time effect only for the respective session). P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

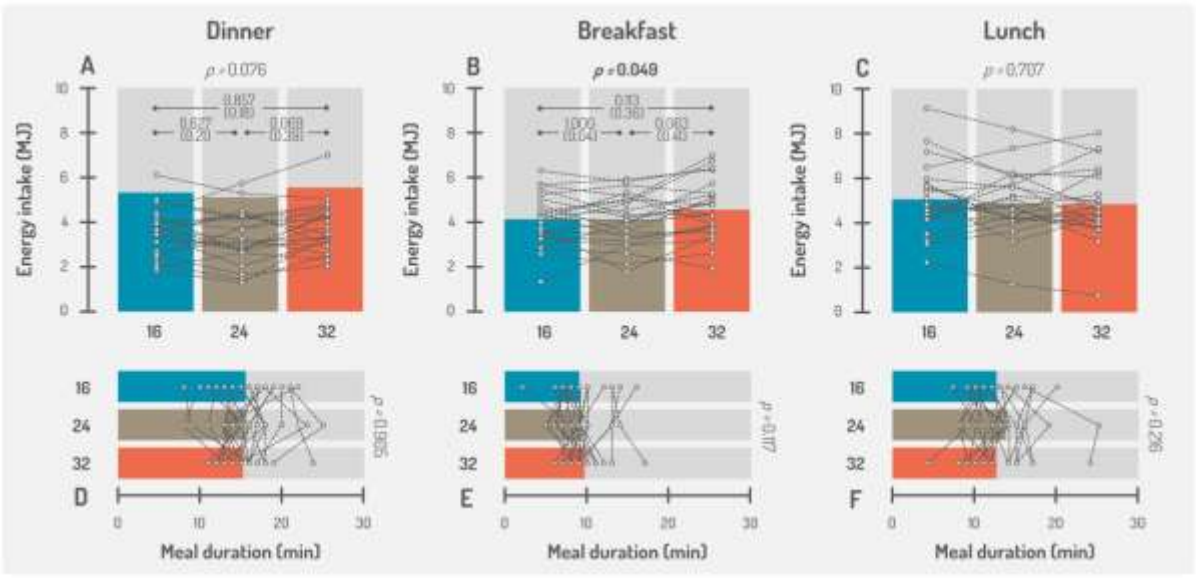

Figure 4. Energy intake and meal duration for dinner (A), breakfast (B), and lunch (C). Dotted lines represent individual values and rectangles the mean values for each session. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

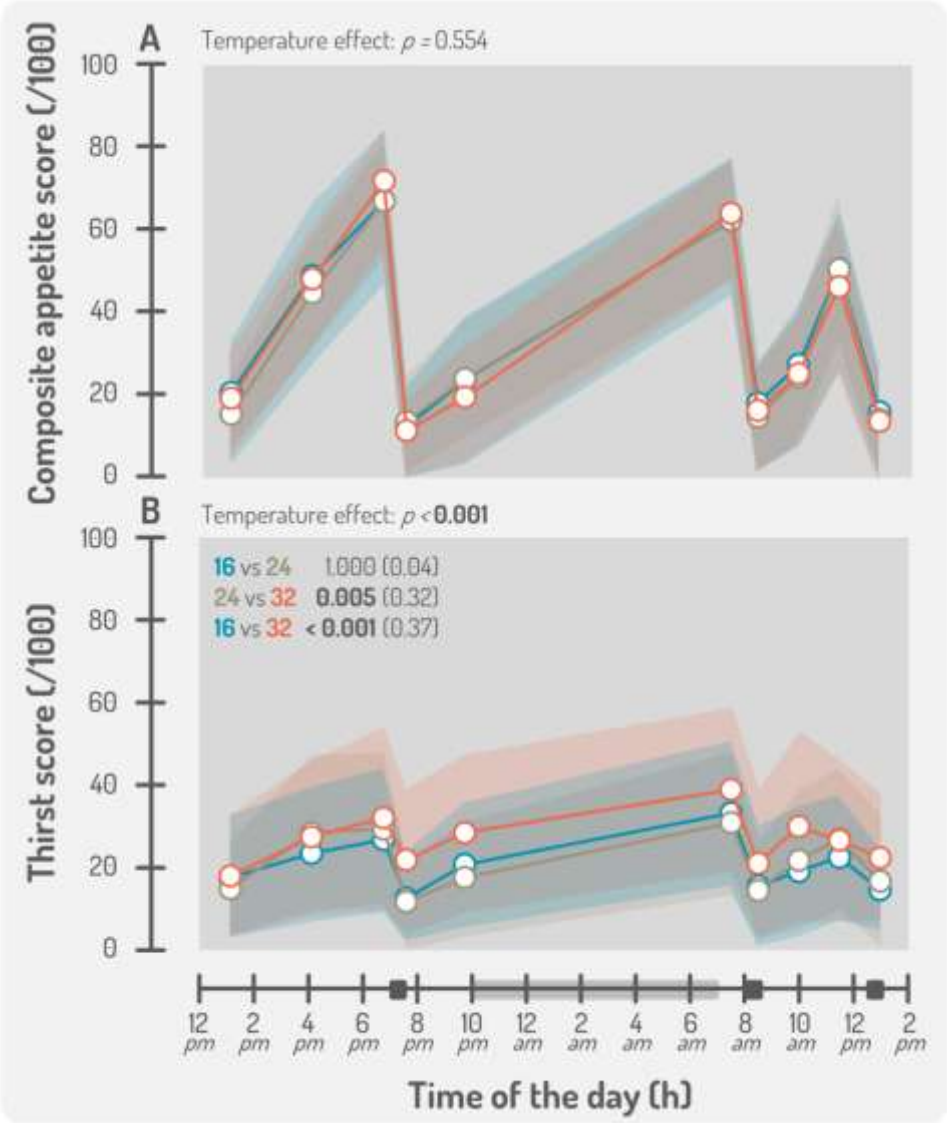

Figure 5. Composite appetite score (A) and thirst (B) sensations during the whole sessions. Solid Lines represent the mean values in each session and light surfaces represent SD. The light grey rectangle represents the sleep period and the three dark grey rectangles represent the meals. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

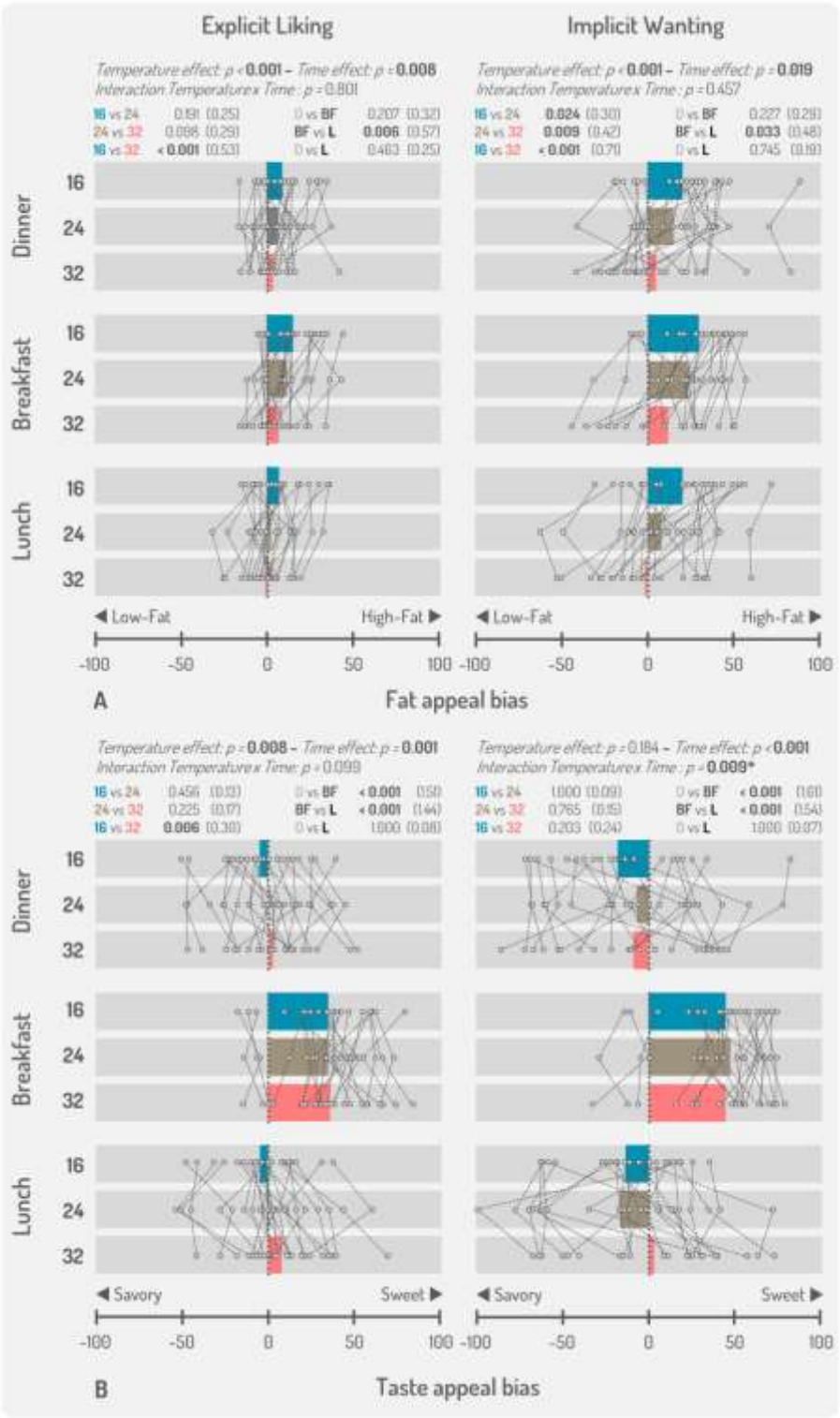

Figure 6. Fat (A) and Taste (B) appeal biases using the Leeds food preference questionnaire (LFPQ). Temp = temperature. Dotted lines represent individual values and rectangles the mean values for each session. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

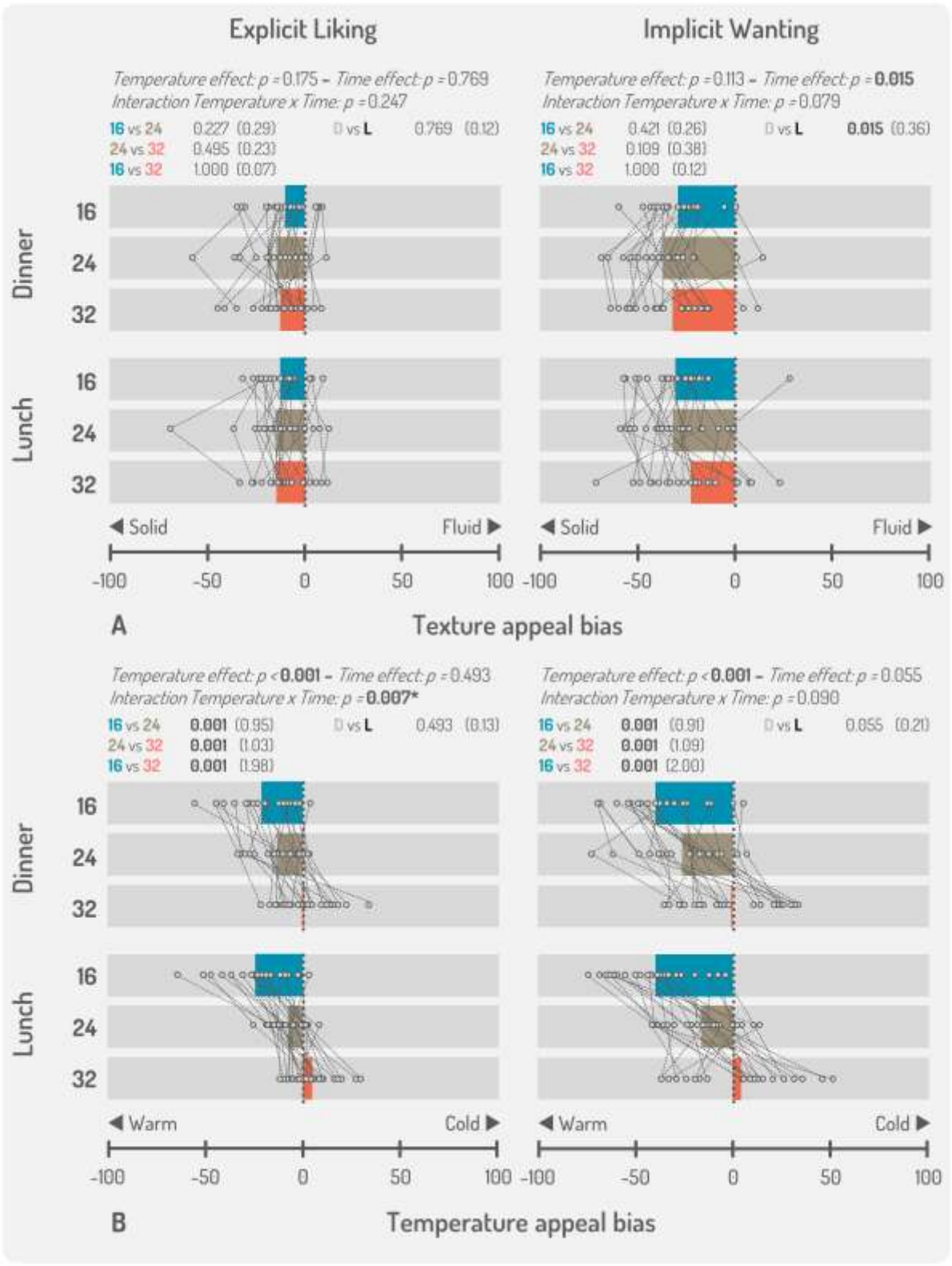

Figure 7. Texture (A) and Temperature (B) appeal biases using the Leeds food preference questionnaire (LFPQ). Temp = temperature. Dotted lines represent individual values and rectangles the mean values for each session. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

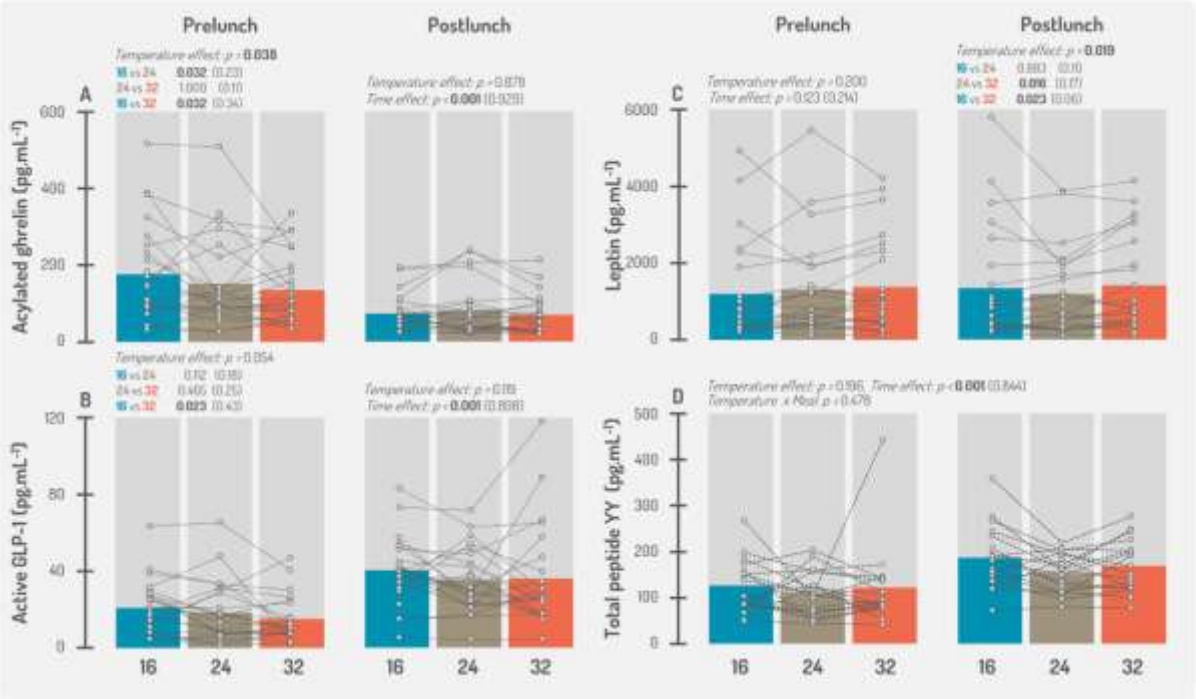

Figure 8. Plasma levels of acylated ghrelin (A), glucagon-like peptide-1 (GLP-1; B), leptin (C), and Peptide YY (PYY; D). Dotted lines represent individual values and rectangles the mean values for each session. P values lower than 0.05 are highlighted in bold and effect sizes are indicated into brackets.

Table 1. Food intake and palatability

	16 °C	**24 °C**	**32°C**	**ANOVA p value**	**16 vs 24 p value (d)**	**24 vs 32 p value (d)**	**16 vs 32 p value (d)**
Food intake at dinner (g)							
Entrée (cold)	150 ± 144	173 ± 109	210 ± 146	0.078	0.913 (0.17)	0.622 (0.27)	**0.011** (0.44)
Main dish (warm)	533 ± 188	453 ± 161	485 ± 197	**0.023**	**0.023** (0.44)	0.929 (0.18)	0.228 (0.26)
Dessert (cold)	115 ± 81	137 ± 88	143 ± 82	0.107			
Bread (temperate)	64 ± 38	66 ± 38	65 ± 43	0.918			
Food intake at breakfast (g)							
Cottage cheese (cold)	261 ± 114	269 ± 120	298 ± 122	0.155			
Madeleine (temperate)	124 ± 46	119 ± 49	132 ± 60	0.326			
Juice (cold)	299 ± 162	298 ± 137	358 ± 184	0.059	1.000 (0.01)	0.062 (0.37)	0.256 (0.37)
Food intake at lunch (g)							
Entrée (cold)	110 ± 137	139 ± 130	159 ± 153	0.074	0.084 (0.21)	0.660 (0.14)	0.191 (0.35)
Main dish (warm)	404 ± 165	339 ± 152	333 ± 148	**0.011**	**0.025** (0.42)	1.000 (0.03)	0.103 (0.45)
Dessert (cold)	132 ± 89	146 ± 86	139 ± 101	0.589			
Bread (temperate)	59 ± 41	58 ± 36	46 ± 44	0.206			
Palatability at dinner (/100)							
Entrée (cold)	39 ± 31	47 ± 31	46 ± 30	0.161			
Main dish (warm)	70 ± 16	67 ± 16	67 ± 17	0.575			
Dessert (cold)	44 ± 33	43 ± 32	47 ± 34	0.423			
Bread (temperate)	58 ± 26	56 ± 29	62 ± 27	0.457			
Palatability at breakfast (/100)							
Cottage cheese (cold)	62 ± 21	71 ± 17	73 ± 19	0.058	0.160 (0.47)	1.000 (0.10)	0.065 (0.57)
Madeleine (temperate)	78 ± 21	76 ± 22	72 ± 21	0.066	0.498 (0.10)	0.118 (0.22)	**0.028** (0.32)
Juice (cold)	68 ± 23	67 ± 23	71 ± 24	0.580			
Palatability at lunch (/100)							
Entrée (cold)	35 ± 31	36 ± 30	37 ± 32	0.260			
Main dish (warm)	60 ± 27	56 ± 31	60 ± 22	0.989			
Dessert (cold)	55 ± 31	50 ± 30	52 ± 31	0.525			
Bread (temperate)	51 ± 35	55 ± 32	54 ± 30	0.570			

Mean ± SD. P values < 0.05 are highlighted in bold.