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1	The impact of 16-h heat exposure on appetite and food reward in adults
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- 25 Abstract 1
- 26

27 Heat exposure is thought to reduce energy intake (EI) but studies are sparse and results not always concordant. The aim of this study was to examine whether a 16-h exposure to 32°C leads 28 to reduced EI compared to a control session (22°C) and whether modifications in appetite 29 30 sensations or food reward are implied. Sixteen healthy, lean, and active participants (9 women and 7 men, 25±5 yo, body mass index: 22.0±2.4 kg.m⁻²) were passively exposed to two different 31 thermal temperatures from 4:00 pm to 8:00 am under controlled conditions. Hunger and thirst 32 scores were regularly assessed using visual analogue scales. A fixed dinner meal (3670±255 kJ) 33 was consumed at 7:30 pm and an ad libitum breakfast buffet (20 foods/drinks varying in 34 temperature, fat, and carbohydrate content) at 7:30 am. Components of reward (explicit liking 35 [EL] and implicit wanting [EI]) for fat and sweet properties of food were assessed before each 36 37 meal using the Leeds Food Preference Questionnaire (LFPQ). Ad libitum EI at breakfast did not differ between sessions (2319±1108 vs 2329±1141 kJ, in 22 and 32°C sessions, respectively; 38 39 p=0.955). While thirst scores were higher in the 32 than the 22°C session (p<0.001), hunger 40 scores did not differ (p=0.580). EL and IW for high fat foods relative to low fat foods were 41 decreased in 32 compared to 22° C before dinner and breakfast (*p*<0.001 for all). Although EI and hunger were not affected by a 16-h exposure to heat, modifications in food reward suggested a 42 reduction in the preference of high-fat foods. Future research should investigate whether 43 reduced EI in response to heat exposure is due to spontaneous selection of low-fat foods rather 44 45 than altered appetite sensations.

- 46
- 47 Keywords: LFPQ, hot conditions, thermal exposure, appetite, energy balance, hunger

CAS: composite appetite score EB : energy balance EE: energy expenditure EI: energy intake EL: explicit liking IW: implicit wanting LFPQ : Leeds Food Preference Questionnaire RH: relative humidity VAS: visual analogue scale

Abbreviations

48 **1. Introduction**

49 Athletes and military personnel are regularly exposed to energy deficits due to inadequate levels 50 of energy intake (EI) compared to their high levels of energy expenditure (EE) (Charlot, 2021; 51 Heydenreich, Kayser, Schutz, & Melzer, 2017; Logue et al., 2020; Tassone & Baker, 2017; Tharion et al., 2005). If sustained, this state may have direct deleterious consequences on their cognitive 52 53 (Giles et al., 2019; O'Leary, Wardle, & Greeves, 2020) and physical (Murphy, Carrigan, Philip 54 Karl, Pasiakos, & Margolis, 2018; O'Leary et al., 2020) performances. Seasonal scheduling and localization of competitions may impose athletes to train/compete in the heat during a 55 56 significant duration (de Korte, Bongers, Hopman, & Eijsvogels, 2021; Racinais, Casa, Brocherie, & Ihsan, 2019). For their part, militaries are often deployed in war zones characterized by a hot 57 58 climate (Ashworth, Cotter, & Kilding, 2020; Parsons, Stacey, & Woods, 2019). During acute heat exposures (< than 8 h), EI was found to be reduced in some studies compared to a 59 thermoneutral control session (Shorten, Wallman, & Guelfi, 2009; Wasse, King, Stensel, & 60 Sunderland, 2013; Zakrzewski-Fruer, Horsfall, Cottrill, & Hough, 2021). Given that EE is 61 62 unaltered or even slightly increased due to an rise in ventilation and sweat gland activity in this 63 thermal environment (Ocobock, 2016; Valencia, McNeill, Brockway, & Smith, 1992), energy 64 balance (EB; EI minus EE) is very likely to be negative during heat exposures. These decreases 65 were sometimes accompanied by reductions in appetite (assessment of hunger scores) (Kojima, Sasaki, Tsuchiya, & Goto, 2015; Wasse et al., 2013) and increases in the plasma levels of 66 anorexigenic hormones such as peptide YY (Shorten et al., 2009; Zakrzewski-Fruer et al., 2021). 67 These results imply that heat exposures may potentially worsen the already frequent and large 68 states of energy deficiency. 69

To date, these effects remain uncertain since the literature is sparse and some studies have 70 reported no modifications in appetitive responses (Ahmed et al., 2019; Faure et al., 2016). The 71 72 main reason for this lack of consistency is very likely the considerable variability of the protocols 73 (duration of exposure, choice of temperature, presence of physical exercise sessions, test meals, 74 clothing...) (Charlot, Faure, & Antoine-Jonville, 2017; Millet et al., 2021). Although a recent meta-75 analysis found a slight heat-induced anorexia (Millet et al., 2021), further studies are required to help draw more solid conclusions on the effects of heat exposure on appetite and EI. Particularly, 76 77 studies in which heat exposure is applied during a prolonged period (> 8 h) and during 78 sedentary conditions are required in order to eliminate the biases induced by physical exercise. 79 Food reward has been shown to strongly influence food choices and EI in both laboratory and

80 free-living settings (Dalton & Finlayson, 2014; French et al., 2014). However, food reward is

81 rarely assessed in extreme environments (Charlot et al., 2021; Karl et al., 2018) and never to our

82 knowledge during heat exposure. The construct of food reward and its sub-components of liking

and wanting may help elucidate the heat-induced anorexia that is often observed.

The aim of this study was therefore to assess the effects of 16-h passive heat exposure on EI assessed during an *ad libitum* breakfast and associated changes in appetite and food reward. It was hypothesized that heat exposure would exert an anorexigenic effect reflected by a decrease in hunger scores, and/or a decrease in liking and wanting for high fat foods, both resulting in lower EI.

89

90 2. Materials and methods

91 2.1. Design

Participants took part in four 16-h sessions separated by \sim 2 weeks in a climatic apartment (four 92 93 bedrooms, a living-room with a kitchen and a bathroom with a shower and a toilet) in which 94 ambient temperature was fixed at 22 or 32 °C (two sessions in each temperature). The measured ambient temperatures were 22.5 \pm 0.7 °C and 32.3 \pm 0.6 °C, respectively. Relative humidity was 95 96 fixed at ~40 \pm 5%. Four groups of 4 participants were composed and this composition remained similar during the study. The session order was randomly allocated and counterbalanced for the 97 98 4 groups. Just before 4:00 pm, participants arrived and were fitted with devices for continuous 99 measurements of heart rate, skin temperature, and ingestion of a thermal pill. Dressed in 100 standardized clothes (short sleeves tee-shirt made of cotton, shorts made of polyester, socks made of cotton, and synthetic sandals), the participants remained in the apartment until the next 101 102 morning (8:30 am). At 7:30pm, a dinner (pasta, yoghourt, and chocolate bar) served in 103 standardized quantity was consumed. At 9:30pm, they went to bed and were woken up at 104 7:00am. At 7:30 am, an *ad libitum* breakfast buffet was served with a large selection of foods and 105 drinks. Breakfast EI was calculated from the weighed quantity consumed. Food reward using the LFPQ was assessed just before the dinner and the breakfast. Subjective appetite and thermal 106 sensations were assessed throughout the sessions using visual analog scale (VAS). Fluid and 107 108 food intake and fluid loss (urine and sweat) were directly measured or calculated during the 109 sessions. The first session at each temperature was treated as a familiarization session. The 110 second session at each thermal condition was treated for analysis (~ 1 month apart). The 111 procedure is presented in Figure 1.

112

113 2.2. Participants

Sixteen healthy, young, lean, and active participants (9 women and 7 men) were recruited from the Institut de Recherche Biomédicale des Armées (age: 25 ± 5 yo; body mass: 64.6 ± 9.5 kg, height: 171 ± 7 cm, and body mass index: 22.0 ± 2.4 kg.m⁻²). Inclusion criteria were restraint score < 2 based on the Three-Factor Eating Questionaire-R18 (Karlsson, Persson, Sjöström, & Sullivan, 2000), regularly consuming at least 3 meals per day including breakfast, and have a sleep score < 5 based on the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, &</p>

Kupfer, 1989). They were also not eligible if they had been exposed to a hot climate (> 3 120 consecutive days, mean temperature > 30 °C) in the last 6 months before the study to ensure 121 122 that participants were not considered heat acclimated. Given that this study was realized in 123 spring 2021, they were not exposed to temperature above 20 °C in the 6 previous months. Female participants who used contraceptive medication and had irregular menstrual cycle 124 125 (absence of regular cycle for at least 3 consecutive months) were excluded. Female participants 126 had to realize the two sessions that were considered for analysis at the same phase of their menstrual cycle since core temperature regulation (Baker, Siboza, & Fuller, 2020), appetite and 127 EI (Asarian & Geary, 2013) fluctuated during the menstrual cycle higher. The second session was 128 therefore planned approximately 30 days (29 ± 4 d) after the first one. This study was conducted 129 130 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°2 (2021-A01238-33). 131 132 Written informed consent was obtained from all participants who received financial 133 remuneration for study completion.

134

135 *2.3. Measurements*

136 *2.3.1. Energy intake*

Participants were asked to eat the same breakfast at the same hour (depending on their habits) before each session. Since all the participants worked on site, they were instructed to eat at the mess at the same hour (between 11:30am and 12:30pm) and eat the same kind of meals (one salad, one main dish with meat or fish and pasta or rice on the side, one portion of cheese, one yoghurt, and one fruit). They were also instructed to drink at least 5 glasses of water (~ 1 L) on this day to avoid disparities in the hydration level and to not perform physical activities on the day and day before the study.

144 Dinner was composed of a main dish (carbonara, salmon, cheese, or meat balls pasta, Grande Box, Lustucru®, ~360 g),, a yoghurt (Vanilla, Chocolate, Lemon, or Coconut Yoghourt, Yaourt 145 146 Gourmand, Mamie Nova (125 g) and a bar of milk chocolate (Milka (17 g). Participants chose 147 the recipes/flavor of the first two items during a screening visit. During the first session, the full 148 portions were served but participants could choose to not finish them knowing that the same 149 quantities would be served during the remaining sessions with the obligation to finish them. Thus, food, quantities, and therefore EI was fixed during this meal. Two subjects chose to eat 2 150 yoghurts and two others ate only half of the chocolate bar. Mean EI of the meal was 3670 ± 255 151 kJ with a CHO/Fat/Protein distribution of $51 \pm 2\%$, $37 \pm 2\%$, and $13 \pm 1\%$. 152 153 A breakfast buffet was served ad libitum. It was composed of a selection of drinks, savory and

sweet and/or high-fat and low-fat foods, and extras/toppings. The details are presented in Table

155 1. The buffet was prepared outside the climatic apartment in a room at ~22°C. Some items were

served at room temperature (~ 22°C). Other were served cold (~ 4°C) and some were served hot 156 (~ 50°C). Pre-tests revealed that the gustatory properties of some items were impaired after 20-157 158 30 min in a room at 32 °C. To avoid this deterioration, the buffet was set on two large tables in a 159 temperate room (20-24 °C) outside the climatic apartment. These tables were then moved just before 7:30 am in order that participants may choose drinks and foods with similar gustatory 160 properties in both thermal conditions. Each item was served in large quantities (see Table 1). 161 162 Participants were informed that they were allowed to freely serve themselves and that they were not forced to finish their dish. They served themselves using specific cutlery and placed 163 each item in specific disposable dishes (small plates, bowl, cup, or goblet). An investigator then 164 weighed (Mettler Toledo, Columbus, OH, USA, accuracy: 0,1g) each dish with the served portion. 165 166 The same dish with remaining foods and drinks were weighed after breakfast. The difference between before and after the breakfast corresponded to the consumed quantities. They were 167 168 allowed to serve themselves three times in total. If available food portions decreased by approximately 50% of the starting portion, investigators refilled the concerned items to ensure 169 170 that served portions were similar between participants. They were previously informed that 171 food selection and EI were assessed since it was impossible to totally conceal the weighing 172 procedure. This latter phase was however done the most quick and discrete possible: after they served themselves, they had to leave their tray on a table in the main room and returned to their 173 respective room. The weighing lasted less than 30 seconds and their tray was then handed over 174 175 to the participants. Finally, all the procedure (food selection and eating) was done entirely 176 separately in order to avoid the well-known social facilitation of eating (Ruddock, Brunstrom, Vartanian, & Higgs, 2019). Before the beginning of the breakfast, participants remained in their 177 room with their door shut. They were invited one at the time to serve themselves at the buffet 178 and to eat alone in their room. The participants therefore never crossed during the whole 179 180 breakfast.

181

182	Table 1 – Compositi	on of foods and	drinks served i	in the <i>ad libitum</i>	breakfast buffet
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Item	Brand	Served portion	Energy content (kJ/100 g)	CHO (g/ 100g)	Fat (g/100 g)	Protein (g/100 g)
Drinks						
Milk*	Auchan	1000 mL	196	4.8	1.6	3.3
Orange juice*	Tropicana	1000 mL	162	8.9	0.0	0.8
Grape juice*	Auchan	1000 mL	295	16	0.5	0.5
Sweet foods						
Applesauce*	Andros	$1000 \mathrm{g}$	343	20.0	0.1	0.3
White cheese*	Auchan	1000 g	459	2.9	7.8	7.0
Corn flakes**	Kellog's	500 g	1568	87.0	0.6	4.5
Bread**	Heudebert	8 9-g slices	1679	73.0	7.3	11.0
Toast**	Harry's	8 25-g slices	1090	48.5	4.3	7.0
Chocolate cake**	Ker Čadélac	8 30-g cakes	1812	45.7	25.5	5.3
Extra/topping						
Strawberry jam*	Bonne Maman	100 g	997	59.0	0.1	0.4
Chocolate spread**	Rigoni di Asiago	100 g	2242	54.0	32.0	8.1
Honey**	Lune de Miel	100 g	1365	80.0	0.5	0.5
Cacao powder**	Nesquick	100 g	1540	78.9	3.6	5.1

Butter*	Auchan	8 portions of 12,5 g	3033	0.6	80.0	0.8
Savory foods						
Salami*	Herta	8 10-g slices	1818	1.0	39.0	20.0
Cheese*	Leerdamer	8 25-g slices	1471	0.1	27.5	26.0
Cooked ham*	Herta	8 42.5 slices	437	0.5	2.5	20.0
Mini-sausages***	Auchan	300 g	920	1.3	19.0	11.0
Hard-boiled eggs**	Matines	8 eggs	594	0.5	9.8	13.0
White beans***	Auchan	500 g	303	12	0.5	5.0

183 */**/*** The items were served cold/at room temperature/hot.

184

185 *2.3.2. Subjective ratings*

186 Appetite, thirst, and thermal assessments (thermal sensation, discomfort and preference) were assessed 7 times during each session. Appetite was separated into four different perceptions: 187 hunger, desire to eat, fullness, and prospective consumption. Appetite and liking were assessed 188 on 100 mm VAS presented on paper preceded by the following questions: "How hungry do you 189 190 feel?"; "How strong is your desire to eat something?"; "How full do you feel?"; and "What quantity of food would you be able to eat?". These scales were anchored with "not at all" and 191 192 "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 cm. The composite appetite 193 score (CAS), reflecting the responses to the four VAS questions, was included in the study as a 194 195 summary measure of appetite. CAS was calculated using the following formula: CAS = ((100 - 100))196 desire to eat) + (100 – fullness) + hunger + prospective consumption)/4 (Anderson, Catherine, 197 Woodend, & Wolever, 2002). These scales have been shown to have a good level of reliability 198 and reproducibility, and a certain level of predictive validity (Stubbs et al., 2000). Thirst was also 199 assessed at the same time points as appetite sensations. After dinner, liking of the food items 200 was assessed using a VAS preceded by the question: "Did you like this food item?". The self-201 selected portion size was also assessed for each item by the question "What amount of food would have been ideal?" These scales were anchored with "0% of the served portion" and 202 203 "200% of the served portion" at the left and right ends, respectively.

Thermal sensation ("How do you perceive your thermal environment?") was determined with the ASHRAE 7-pt scale (from -3, cold, to 3) and thermal comfort ("How do you perceive your thermal environment?") using a VAS with "very uncomfortable" and "very comfortable" at the left and right ends, respectively. Thermal preference ("What would you prefer at the moment?") was assessed using a 5-point NICOL scale (from 2, prefer much cooler to+2, prefer much warmer) (Humphreys, Nicol, & Raja, 2007).

Fatigue and sleep quality were assessed at 7:15am using a VAS preceded by the questions: "Do
you feel tired?" and "Were you satisfied of this night?", respectively, with "not at all" and
"extremely" at the left and right ends, respectively.

213

214 *2.3.3. Food reward*

215 Food reward for fat and sweet dimensions of foods was assessed using the Leeds Food 216 Preference Questionnaire (LFPQ) (Finlayson, King, & Blundell, 2008; Finlayson, King, & Blundell, 217 2007; Oustric et al., 2020). Briefly, liking and IW for 4 categories of food: high-fat-sweet, high-218 fat-savory, low-fat-sweet and low-fat-savory were assessed using a computerized task. Explicit liking (EL) was measured using 100-pt VAS for which participants had to answer to the question 219 220 "How pleasant would it be to taste this food now?" with "not at all" and "extremely" at the left 221 and right ends, respectively. Food images (n = 16) appeared individually on the screen in a randomized order. IW was assessed using a forced-choice procedure. Every image of each of the 222 223 4 food categories was compared to every other image of the 3 other categories (96 pairs in 224 total). Participants were instructed to respond as quickly as they could to indicate which food 225 they most wanted to eat at that moment. The IW score is therefore a combination of reaction time and frequency of selection (Oustric et al., 2020). EL and IW can be summarised as "appeal" 226 227 scores (called "appeal bias" in the LFPQ) for fat or sweet foods. Mean savoury scores may be subtracted from the mean sweet score to provide a "Taste appeal" score. The same procedure 228 229 may be applied to high fat relative to low fat foods for the "Fat appeal" score.

230

231 2.3.4. Temperatures and heart rate

232 Upon their arrival in the apartment, participants ingested a capsule containing a temperature 233 sensor (BodyCap, e-Celsius, Caen, France). The capsule is equipped with a memory chip which 234 allows storage of data in case of loss of connection and communicates with a monitor that remains with the participants during the whole session. The system was shown to accurately 235 represent fast changes in water temperature (Bongers, Daanen, Bogerd, Hopman, & Eijsvogels, 236 2018) and was judged reliable to assess core (Bogerd et al., 2018; Travers, Nichols, Farooq, 237 Racinais, & Periard, 2016) temperature. Given the timing of ingestion, we acknowledge that first 238 239 measurements (between 1 to 3 h) reflect gastric temperatures.

Participants were also equipped with three cutaneous sensors. The mean of each measurement
was calculated to determine skin temperature. Then body temperature ([skin temperature +
core temperature]/2) was calculated.

243 Finally, a heart chest strap was fitted (Polar H10, Polar, Kempele, Finland) communicating with a

watch (Polar RC3, Polar, Kempele, Finland) to measure heart rate. The mean was calculated for
3 periods (before the night: 4:00 pm to 10:00 pm; night: 10:00 pm to 7:00 am; morning: 7:00 am
to 8:00 am).

247

248 2.3.5. Body mass modifications

Body mass was assessed three times (4:00 am, 9:30 pm, and 7:15 am). Food weight was controlled (dinner) or accurately assessed (breakfast) and was therefore easily determined. Fluid intake was measured using weighing of water bottles. Participants were given cool (~4°C)

252 500-mL water bottles that were replaced at the beginning of each of these three periods (before

- dinner: 4:00 pm to 7:30 pm; dinner and after: 7:30 pm to 10:00 pm: night until breakfast: 9:30
- 254 pm to 7:15 am). Their participant code was clearly written on their bottles and they were
- instructed to consume as much volume as they desired. If a bottle was finished before the end of
- the period, supplementary bottles were available in a fridge.
- To assess urine loss, a scale was placed very close to toilets. Participants had to weigh themselves before and after urination and to indicate their participant code and the hour on a record. The correct filling of the records were frequently checked with participants to ensure that no weighing were forgotten.
- Finally sweat loss was calculated using all the aforementioned measurements, considering that
 water loss through respiration was negligible (food and water intake body mass modification –
- 263 urine loss).
- 264

265 *2.3.6. Hydration level*

Urine was collected to assess hydration level. Urine specific gravity of the collected urine was
immediately measured with an automated dipstick analyzer (Clinitek Status+ Analyzer, Siemens,
Munich, Germany) using a Multistix10SG (Siemens, Munich, Germany). Values < 1.013 indicate
euhydration (Perrier, Bottin, Vecchio, & Lemetais, 2017), whereas values > 1.020 reflect
hypohydration (Logan-Sprenger & Spriet, 2013).

271

272 2.4. Statistical analyses

A priori power analysis for EI based on a recent meta-analysis (Millet et al., 2021) selecting 273 conventional α (0.05) and 1- β (0.80) levels observed that at least twelve participants were 274 required. All variables were checked for normality using a Shapiro-Wilk test followed by 275 skewness and kurtosis. In case of absence of normality, logarithmic transformation was 276 277 performed. If this transformation did not correct data normality, nonparametric tests were used. 278 In the case of repeated measurements, mixed-model repeated-measures ANOVA were used. 279 Thus, appetite and thirst-related VAS scores were compared using a 2x7 ANOVA (temperature 280 effect: 22 vs 32°C and time effect: 4:00 pm, 6:00 pm, 7:30 pm, 8:00 pm, 9:30 pm, 7:15 am, and 281 8:00 am). A 2x2 ANOVA was used to compare EL and IW Taste and Fat Appeal scores (temperature effect: 22 vs 32°C and meal effect: dinner vs breakfast). When the sphericity 282 assumption was violated (Mauchly's test), a Greenhouse-Geisser correction was used. Post hoc 283 284 analyses were performed using Bonferroni's tests. The effect of ambient temperature was assessed using Student t-tests or Wilcoxon rank-sum tests for the remainder of the 285 measurements. The differences were also examined using Cohen's effect size (ES): > 0.2 (small), 286

287 > 0.5 (moderate), and > 0.8 (large) (Cohen, 1988). Data are presented as the means ± SD.
288 Significance was determined as *p* < 0.05. Analyses were performed using Jamovi software (1.2.9
289 version, the Jamovi project; retrieved from https://www.jamovi.org).

290

291 **3. Results**

292 3.1. Breakfast energy intake

293 EI (Figure 2A and 2B) and macronutrient composition of the meal (in g or % of total EI; Figure 2A and 2C, respectively) were not different between the sessions (p > 0.411, d < 0.21). Analysis 294 295 of individual variability using graphs shows that results were very heterogeneous with 7 296 individuals increasing and 9 decreasing their EI during the 32 compared to the 22°C session. 297 Moreover participants' intake for specific items of the buffet was also not different (data not shown). Figure 2D presents the participants' food and drink selection during this meal. They 298 299 selected 5.8 \pm 2.3 and 5.1 \pm 2.1 items during the 22 and 32°C sessions, respectively (p = 0.102, d300 = 0.32).

301

302 *3.2. Appetite and dinner palatability*

No temperature effect was found for hunger score (p = 0.580) and CAS (p = 0.380). However, thirst was significantly higher in the 32 than the 22°C session (p < 0.001, d = 0.66).

At the dinner, palatability for pasta (p = 0.317, d = 0.26), yoghurt (p = 0.135, d = 0.14), and chocolate (p = 0.090, d = 0.45) were not significantly different between sessions (Table 2). Similarly, self-selected portion size was not different for pasta (p = 0.706, d = 0.21) and yoghourt (p = 0.061, d = 0.14), but was lower in the 32 than 22°C session for chocolate (p = 0.044, d = 0.60).

310 Table 2 -Subjective assessment of dinner with fixed quantities

	22 °C	32°C		
	Palatabi	lity (/10)		
Pasta	6.1 ± 1.9	5.6 ± 1.8		
Yoghurt	7.2 ± 2.0	7.0 ± 2.0		
Chocolate	7.4 ± 2.3	6.5 ± 2.7		
	Self-selected portion size (%)			
Pasta	128 ± 38	122 ± 33		
Yoghurt	119 ± 38	107 ± 22		
Chocolate	107 ± 44	90 ± 48*		

311 *different from 22°C (*p* < 0.05)

312

313 *3.3. Food reward*

A temperature effect was found in both EL (p < 0.001, d = 0.56, Figure 3A) and IW (p < 0.001, d = 0.77, Figure 3B) for high fat relative to low fat foods (fat appeal). Analysis of individual variability using graphs shows that among the 16 participants, a contradictory increase in the fat appeal score before dinner was observed in 6 and 4 participants for EL and IW, respectively.

- This decreased to 2 and 1 before breakfast indicating that results were more homogeneousbefore breakfast than before dinner.
- A meal effect was found for sweet relative to savoury foods (taste appeal) in both EL (p < 0.001,
- 321 d = 2.58, Figure 3C) and IW (p < 0.001, d = 3.72, Figure 3D), responses being higher before
- 322 breakfast than before dinner. Finally, a meal*temperature interaction in taste appeal score in IW
- only (p = 0.029). Post hoc tests revealed no further meaningful differences.
- 324

325 3.4. Heat-related perceptive and physiological modifications

- Core, skin, and body temperatures and heart rate were all higher in the 32 than the 22°C session
- 327 (p < 0.001 for all, d = 1.03, 2.40, 2.03, and 1.14, respectively; Table 3).
- 328 Water intake (p < 0.001, d = 3.06), total intake (p < 0.001, d = 3.33), sweat loss (p < 0.001, d = 3.33)
- 329 2.86), and total loss (p < 0.001, d = 2.89) were higher in the 32 than the 22°C session while urine
- loss (p = 0.041, d = 0.56) and body mass modifications (p = 0.012, d = 0.72) were lower (Table 3)
- 331 Thermal sensation (p < 0.001, d = 3.44) and discomfort (p < 0.001, d = 1.67) were higher and
- thermal preference lower (p < 0.001, d = 3.30) in the 32 than the 22°C session (Table 3).
- 333 Sleep duration reduction in the 32 than the 22°C session was not significant (p = 0.084) and
- 334 sleep analysis revealed only slight modifications in the 32 than the 22°C session. However, this
- analysis was not the primary objective of the article and will not be presented. Fatigue at the
- morning was not different between sessions and sleep quality was assessed lower in the 32 than
- 337 the 22°C session (p = 0.004, d = 1.46).

338 Table 3 – Thermal-related perceptive and physiological markers

	22 °C	32°C
Core Temperature (°C)	36.7 ± 0.3	37.0 ± 0.3***
Skin Temperature (°C)	34.7 ± 0.6	35.9 ± 0.4 ***
Body Temperature (°C)	35.7 ± 0.4	$36.4 \pm 0.3^{***}$
Heart Rate (bpm)	65.9 ± 6.1	73.0 ± 6.3***
Δ Body mass (kg)	0.38 ± 0.40	$-0.05 \pm 0.50^{*}$
Food intake (kg)	1.00 ± 0.26	1.05 ± 0.27
Water intake (kg)	0.60 ± 0.31	1.50 ± 0.50***
Total intake (kg)	1.60 ± 0.38	2.55 ± 0.53***
Urine loss (kg)	0.91 ± 0.33	0.79 ± 0.32*
Sweat loss (kg)	0.30 ± 0.18	$1.81 \pm 0.64^{***}$
Total loss (kg)	1.21 ± 0.44	2.60 ± 0.66***
Thermal sensation (-3 ;+3)	-0.49 ± 0.55	$1.44 \pm 0.49^{***}$
Thermal discomfort (/10)	3.7 ± 1.9	6.3 ± 1.1**
Thermal preference (-2;+2)	0.34 ± 0.37	$-0.93 \pm 0.40^{***}$
Sleep duration (min)	478 ± 26	456 ± 62
Fatigue score (/10)	3.77 ± 2.00	4.84 ± 2.37
Sleep quality (/10)	6.99 ± 2.11	3.82 ± 2.23**

339 Different from 22°C (**p* < 0.05 ; ***p* < 0.01 ; ****p* < 0.001)

340 USG was found slightly lower at 4:00 pm in the 32 than the 22°C session $(1.020 \pm 0.006 \text{ vs} 1.024 \pm 0.005$, respectively; p = 0.036, d = 0.72). USG were found similar between 32 and 22°C sessions 342 at 9:30 pm $(1.019 \pm 0.006 \text{ vs} 1.019 \pm 0.004$, respectively; p = 1.000) and 7:15 am $(1.026 \pm 0.006 \text{ vs} 1.027 \pm 0.007$, respectively; p = 0.270).

344

345 4. Discussion

346

The aim of this study was to assess the impact of heat exposure on hunger sensations and EI and its impact on food reward before two different meals (dinner and breakfast) during a long sedentary period (16 h). Contrary to our hypothesis, heat exposure did not modify appetite or *ad libitum* breakfast EI and only very slightly impacted subjective assessments (palatability and self-selected portion size) of the fixed dinner meal. However, liking and wanting for high-fat foods relative to low fat foods were decreased before both meals.

353

Several studies have reported modifications of appetite and EI during or after heat exposure. 354 355 Subjective hunger was found diminished just after (Kojima et al., 2015) and up to 2 h (Wasse et al., 2013) after an exercise session in the heat compared to a thermoneutral condition. In a 356 357 recent study (Zakrzewski-Fruer et al., 2021) with a design similar to this study (sedentary 4-h 358 exposure at 30 °C compared to 20 °C), appetite scores were decreased during the whole exposure. Ad libitum EI at lunch was found to decrease by 1189 kJ ± 1219 kJ in the same study. 359 360 Concordant results were found after an exercise session using two consecutive cold buffet-style 361 meals (-1400 ± 2345 kJ at 30 compared to 20°C, *p* = 0.08 (Wasse et al., 2013)) and a buffet-type breakfast (EI increased after an exercise session at 25°C compared to a rest session at 25 °C but 362 not after the same exercise at 36 °C (Shorten et al., 2009)). Although effects were less 363

pronounced in other studies (Ahmed et al., 2019; Faure et al., 2016; Laursen et al., 2017), a 364 365 recent meta-analysis (Millet et al., 2021) highlighted a very likely anorexigenic effect of heat 366 independently of performing an exercise session. Real-life condition studies are very rare but 367 results from a recent one (Zheng, Li, & Wang, 2019) suggested blunted levels of hunger on hot days (34-36 °C) compared to milder ones (28-30 °C). Although this study was designed to focus 368 369 on the effects of heat exposure in removing or limiting numerous biases (no physical exercise, 370 standard and similar clothing, and *ad libitum* water intake), hunger scores and EI during an *ad libitum* buffet breakfast were not influenced by the thermal environment therefore contradicting 371 results from the aforementioned studies. This lack of effect remains to understand and explain 372 373 especially when a very similar study (Zakrzewski-Fruer et al., 2021) found a strong and 374 homogenous response during exposure to heat.

375

The main explanation we can offer for the discrepancy in findings is related to the nature of the 376 test meal. Zakrzewski-Fruer et al (Zakrzewski-Fruer et al., 2021) selected an ad libitum pasta 377 dish served at lunch after 4 h of heat exposure (30 °C vs 20 °C). In the present study, a buffet 378 379 breakfast containing foods and drinks varying in temperature (served cold, at ambient 380 temperature, or hot), fat content (from low to high-fat), and taste (savory or sweet) was retained. This choice was made for two main reasons: 1) this procedure has been shown reliable 381 382 and reproducible (Chaput et al., 2016; McNeil, Riou, Razmjou, Cadieux, & Doucet, 2012; Thivel, 383 Genin, Mathieu, Pereira, & Metz, 2016), 2) the variety of items partially reproduced the variety of the food list used in the LFPQ allowing therefore observation of similar responses between 384 actual intake and LFPQ responses (Dalton, Blundell, & Finlayson, 2013; Kapoor et al., 2021). 385 Moreover, since we only considered the measurements realized during the second session of 386 both thermal conditions, we theoretically removed the likely over-consumption and selection of 387 388 different items at each session for the purpose of variety (Stubbs, Johnstone, Mazlan, Mbaiwa, & 389 Ferris, 2001). This choice did not lead to modifications of EI when exposed to heat and only 390 induced slight differences in food selection between thermal conditions indicating that 391 participants mostly ate the same kind of items. These observations are only partially surprising. Indeed, contrary to other meals, breakfast composition characterized by strong habits. In a 392 393 survey realized in 771 Irish adults, only 17% modified the composition of their breakfast each day (Gibney & Uzhova, 2019). Moreover, specifically in France, a large majority of children and 394 395 adults (~90%) consumed a breakfast with a high content in carbohydrates (Gibney et al., 2018; Lepicard, Maillot, Vieux, Viltard, & Bonnet, 2017). Thus, even with a buffet theoretically allowing 396 397 the choice of items with different fat and carbohydrates content, it cannot be excluded that this 398 usual high level of carbohydrates intake may conceal the expression of modifications of preferences for the different items. In these conditions, EI would have required very strong 399

400 modulators to be affected, and an increase of 10 °C was probably not sufficient to change these401 strong habits.

402

Two other hypotheses surrounding the choice of the buffet breakfast were: 1) low hunger 403 404 scores, and 2) the duration between the end of the meal and the end of the study. Concerning the 405 former point, we did not expect such low hunger scores (~ 4 cm on a 10-cm scale) since we 406 respected participants' habits in terms of sleep duration, sleep usual awakening time, and delay 407 between awakening and breakfast initiation and since we recruited habitual breakfast eaters. 408 Amongst the 16 participants, only two ate their breakfast with hunger scores higher than 5 cm 409 and most of them probably ate in the absence of hunger. Studies in which hunger is assessed 410 before breakfast generally report hunger scores twice the levels in the present study (6-8 cm) (Robert A. Corney, Horina, Sunderland, & James, 2015; McNeil et al., 2017; Radcliffe et al., 2021; 411 412 Thomas, Higgins, Bessesen, McNair, & Cornier, 2015; Zakrzewski-Fruer et al., 2021). However, 413 analysis of these protocols revealed a 60 to 90 min delay between the awakening and the VAS 414 filling allowing hunger to grow. In a study in which hunger scores were assessed at 7:30 am, 30 415 min after awakening, 3 to 4-cm response were reported indicating that the hunger scores in the 416 present study were not unusual. Nevertheless, this could participate to the lack of expression of 417 environmental effect on subjective scales, hidden by a certain cognitive inertia classically 418 described for all subjective assessments during the 15 to 60 minutes following awakening 419 (Hilditch & McHill, 2019). In these conditions of low hunger VAS, the observation of a reduction 420 in EI is less likely. Concerning the second hypothesis, one recent study highlighted an effect of 421 the defined postprandial delay on breakfast EI. It was found that EI was lower when participants 422 knew that they could leave the laboratory as soon as the 30-min period allocated to eat elapsed 423 compared to an imposed 1 or 3-h stay after (Palmer, Irwin, McCartney, Cox, & Desbrow, 2021). 424 Participants were obliged to remain in their rooms until 8:15 am (45 min after the beginning of 425 the meal). They were then allowed to remove equipment and to take a shower. Considering the 426 results of this study, which was not published when the present study was designed, it is 427 possible that participants have rushed to leave the unit, altering their food intake. It is difficult to 428 weigh the importance of this possible explanation, but it is quite reasonable to think that the 429 procedure used in the present study at least partly blunted food/drink choice and intake.

430

An effect of heat exposure on food reward was examined for the first time in the present study. It
has already been used in extreme environments such as cold (Charlot et al., 2021) or hypoxia
(Aeberli et al., 2013; Karl et al., 2018) but never in a laboratory context with a control condition.
In the present study, a clear heat-induced decrease of liking and wanting for high-fat foods
(decrease in the fat appeal score for EL and IW) was found before each meal (dinner and

breakfast) suggesting that being acutely exposed to heat may potentially reduce the selection of 436 437 high-fat foods in favor of lower fat ones. Moreover, this response was found in the large majority 438 of participants (~90% before breakfast) reflecting its robustness. However, these specific 439 modifications of food reward were not accompanied by coherent modifications of food choice or 440 EI contrary to previous studies linking LFPQ responses and actual choices (Dalton et al., 2013) 441 or self-reported preference for fat taste and high-fat food intake (Chmurzynska et al., 2021). As 442 explained earlier, the modalities of the *ad libitum* test meal selected for this study were possibly not the best option to objectify these modifications in food reward. Moreover, one might argue 443 444 that, despite being able to choose foods with different fat content, participants did not correctly identify the fatness of proposed foods blurring therefore the associations between the responses 445 446 during the LFPQ and the choices made during the buffet. Interestingly, subjective assessment of 447 the three imposed items during the dinner showed that chocolate bar, the item with the highest 448 relative fat content, was the one that was the most depreciated in the 32 vs 22 °C session. It remains hypothetical since these foods were not specifically chosen to observe such 449 450 relationships but it supports the link between heat exposure and preference for high-fat foods. 451 Studies assessing the direct influence of acute the direct influence of acute or long heat exposure 452 on food choice is almost non-existent. Studies on seasonal variations indicate that EI is higher in 453 winter than summer (Stelmach-Mardas et al., 2016) probably through modifications in food 454 choice (fruits and vegetables, cereals dairy). However, these changes were likely influenced by 455 their availability (pre or post-harvest) and effects of heat are therefore hypothetical.

456

457 No effect of the temperature in the taste appeal score (liking or wanting for savory over sweet items) was observed in this study. This may be contradictory with some articles suggesting a 458 link between between hydration level, exercise-induced salt loss, and/or thirst sensation and the 459 460 preference for salt (Leshem, Abutbul, & Eilon, 1999; Takamata, Mack, Gillen, & Nadel, 1994). It 461 was indeed hypothesized that sodium loss in sweat may increase desire for salt and savory foods 462 and drinks in order to prevent more loss and to maintain osmoregulation. However, this 463 mechanism is contradicted by several studies in which sodium loss did not induce excess salt 464 appetite (Carroll et al., 2019; Leshem, 2009). The present results rather support the last hypothesis. The effect of heat exposure on other dimensions of food would be interesting to 465 assess starting with the temperature of the served foods. Indeed, it is now well demonstrated 466 467 that oral temperature sensing in the mouth may influence ingestive acceptance (Lemon, 2021) and that cold drinks but also cold foods may be perceived as more pleasant in the heat (Burdon, 468 469 Johnson, Chapman, & O'Connor, 2012) through higher ability to satiate thirst (Eccles, Du-Plessis, 470 Dommels, & Wilkinson, 2013). Other dimensions such as the texture, the color, and/or the taste 471 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

473 (Labbe et al., 2009; Motoki, Saito, Nouchi, & Sugiura, 2020; Suzuki et al., 2017).

474

475 Assessing appetite and food intake is a complex task (Gibbons, Hopkins, Beaulieu, Oustric, & Blundell, 2019) requiring making choices during the design of the protocol (Gregersen et al., 476 477 2008; Horner, Byrne, & King, 2014; Laan, Leidy, Lim, & Campbell, 2010; Thivel et al., 2016) that 478 may lead to limited conclusions. Adding the thermal matter into this field necessarily adds supplemental limitations. As explained in this recent meta-analysis (Millet et al., 2021), no 479 consensus exists in the adequate modalities of thermal exposure (temperature, humidity, 480 duration of exposure, clothing, water intake management). The choices made may be therefore 481 482 somewhat arbitrary and may influence the obtained results. In this article, a lot of effort was made to ensure that ambient temperature was the only difference between sessions. Clothing 483 484 activities and hydration status were similar. We found that heat-related physiological (core, skin, and body temperatures, HR, sweat loss) and subjective markers (thermal discomfort, sensation, 485 486 and preference) were strongly impacted by the exposure to 32 °C compare to 22 °C. These 487 differences, although not surprising since similar studies (Faure et al., 2016; Zakrzewski-Fruer 488 et al., 2021) indicate that the exposure to 32°C induced very large and concordant differences in 489 physiological and subjective thermal markers. Moreover, although providing them free access to 490 cool water during and between the meals to avoid imposing them large amounts of water that 491 may be felt as uncomfortable as others (Wasse et al., 2013), water loss was replaced by ad libitum water intake leading to neutral water balance. Participants were therefore at worse 492 slightly less hydrated in the 32 than the 22 °C. Since, severe dehydration (between -1 and -3% of 493 body mass) does not appear to modify EI (Carroll et al., 2019; Robert Anthony Corney, 494 Sunderland, & James, 2015) even during a semi-solid breakfast (Robert A. Corney et al., 2015), 495 496 heat-induced perturbations in water balance were not certainly considered a bias in the present 497 study. Another potential limitation of this study would the influence of heat on sleep duration 498 and quality that may be altered during heat exposure (Libert et al., 1991; Okamoto-Mizuno & 499 Mizuno, 2012; Zheng et al., 2019). Only marginal alterations of sleep architecture (data not 500 shown), duration, and fatigue score were reported in this study. This result is crucial in the 501 context of the present study since sleep restriction may modify EI (Radcliffe et al., 2021; 502 Soltanieh, Solgi, Ansari, Santos, & Abbasi, 2021) and food reward (McNeil et al., 2017), However, 503 these perturbations seem observable with less than 6-h sleep (Soltanieh et al., 2021) and/or if 504 sustained over several days (Calvin et al., 2013). Milder sleep deprivation/perturbation as in the 505 present study was generally not associated with EI modifications (Morrison et al., 2021). It was 506 concluded that all measurements related to food intake were unlikely to be influenced by heat-507 induced sleep-alterations. Finally, a slight effect of differences in menstrual cycle may exist.

Indeed it has been clearly showed that EI is higher during the luteal than the midfollicular and the periovulatory phases (Asarian & Geary, 2013). Study constraints (4 participants per group, and 2-d inertia of temperature modifications in the apartment) impeded us to plan the sessions in the same phase for each female participant. However, at the exception of one participant that realized both sessions in different phases (luteal then periovulatory phases), the eight other were in the same phase (5 in luteal, 2 in the early-follicular, and 1 in the midfollicular phase). It is therefore possible that results for women may have been slightly altered.

515

516 **5. Conclusions**

517

In this study, 16-h passive exposure to heat (32 °C, 40 % RH) modified food reward with a 518 decrease in liking and wanting for high fat relative to low fat foods in comparison to a 519 thermoneutral condition (22 °C, 40% RH). Surprisingly, hunger feelings were not modified 520 during the whole exposure and the absence of modifications in actual food choice or EI during an 521 522 ad libitum breakfast buffet did not reflect the changes in food reward. Although these apparent 523 contradictory results remain to be understood, the lower preference for high-fat foods may 524 explain the heat-induced decrease in EI observed in previous studies indicating that acute EI 525 modifications are not necessarily driven by hunger modifications but could be altered by reward 526 value of energy dense foods. The effects of a longer exposure with several successive meals and 527 the analysis of food reward for other dimensions (such as temperature and texture) that may be impacted by ambient temperature should be assessed to better identify the effects of heat 528 529 exposure on appetite. Moreover, the effect of gender on heat-induced modifications of EI and food reward deserved to be specifically assessed since men and women are known to differently 530 experience similar thermal environments (Karjalainen, 2007). This was not the primary aim of 531 532 this study and it lacked of statistical power to realize adequate analyses.

533 534

535 Ethical statement

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°2 (2021-A01238-33). Written informed consent was obtained from all participants who received financial remuneration for study completion.

540

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545 **Conflict of interest**

- 546 No potential conflicts of interest relevant to this article were reported.
- 547

548 Authors contribution

- 549 KC and JM designed the study. KC, JM, FP, PVB, JM, FS, PETD, and investigators group realized the
- 550 experiment and collected the data. KC and JM processed data and conducted the statistical
- analyses. Laboratoire graphique (KC) designed the figures. 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.
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557 Data availability statement

- 558 The datasets generated and analyzed during the current study are available from the
- 559 corresponding author upon reasonable request.

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825 Figure legends

826

827 Figure 1 - Study protocol. RH = relative humidity, LFPQ = Leeds Food Preference
828 Questionnaire.

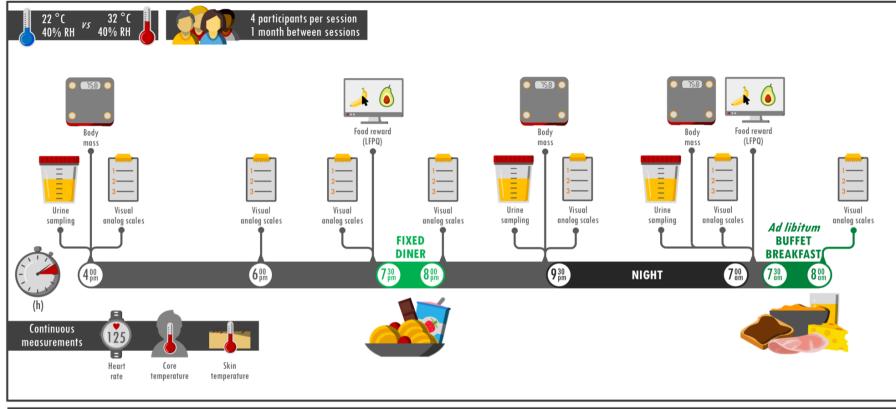
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830 Figure 2 – Absolute (A) and relative (C) macronutrient macronutrient intake, energy intake (B) and the amount of consumed items (D) at the ad libitum breakfast-buffet. For 831 Figures A and C, each bar indicates individual data ranged by total energy intake and relative 832 carbohydrate intake, respectively. Horizontal lines indicate the mean for each macronutrient. 833 For Figure B, bars indicate the means and the grey lines the individual values. For Figure D, grey 834 835 bars indicated the number of items that were selected in both conditions by the same participants. Blue and red bars exceeding the grey bar indicated the number of items that were 836 837 only selected in 22 and 32°C sessions, respectively. PRO = protein, CHO = carbohydrates. 838

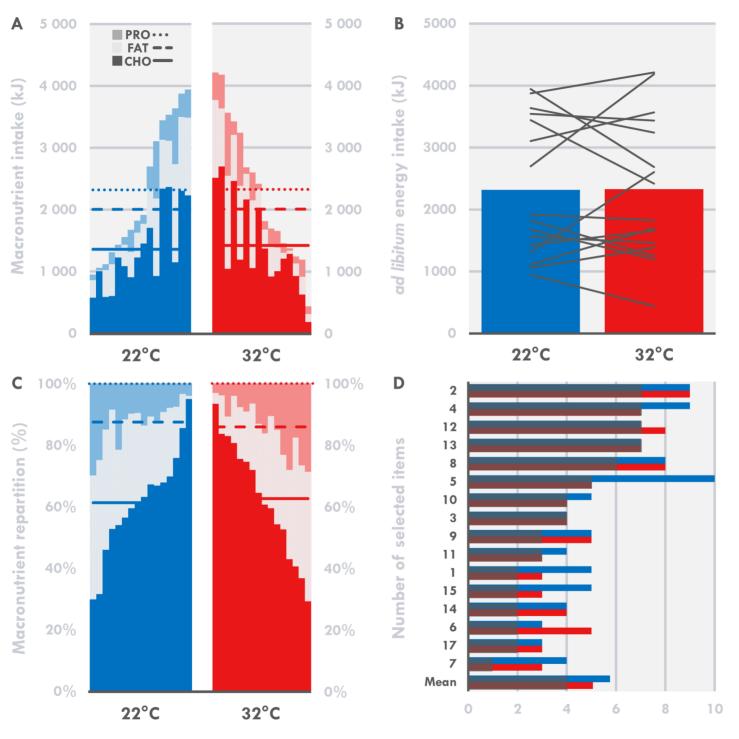
Figure 3 - Hunger (A) and thirst (B) scores during the whole exposure. Means ± SD. 0 h =
16:00 pm. Grey bars symbolizes meals and black bar the sleep period. *** different from 22°C
session (p < 0.001).

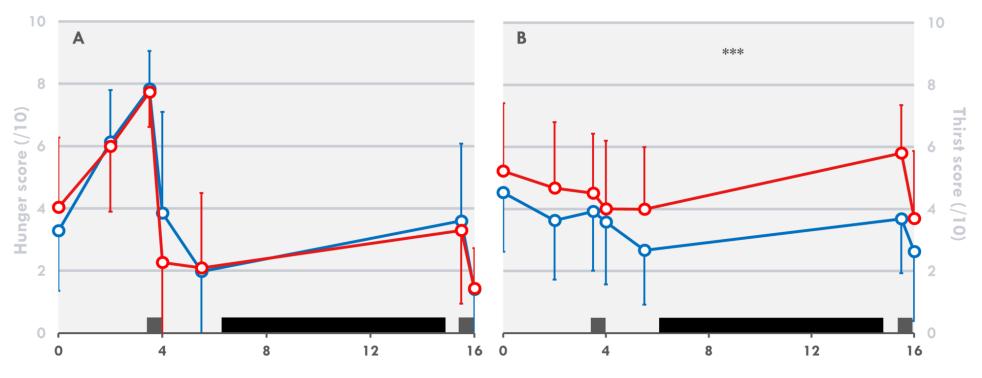
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Figure 4 – Fat and Taste appeal scores before dinner and breakfast. Values for explicit liking (A and C, respectively) and implicit wanting (B and D, respectively). The bars indicates the means and the grey lines the individual values. ***different from 22°C session (p < 0.001); $\alpha \alpha \alpha$ different from the dinner (p < 0.001).









Time from the beginning of the exposure (h)

