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## 1 Cold water effects on energy balance in healthy women during aqua-cycling

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**Running Title: Energy balance during aquatic exercise in women**

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**Abbreviations**

BMI	Body mass index
DXA	Dual-energy X-ray absorptiometry
EB	Energy balance
EE	Energy expenditure
EI	Energy intake
FFM	Fat-free mass
FM	Fat mass
HR	Heart rate
RER	Respiratory exchange ratio
REI	Relative energy intake
SD	Standard deviation
VO <sub>2</sub>	Oxygen consumption
VCO <sub>2</sub>	Carbon dioxide production
VE	Ventilation

## 2 **Abstract**

3 **Introduction:** While the popularity of aquatic physical activities continues to grow among  
4 women, the effects on energy expenditure and appetite control remain unknown. The objective  
5 of this study was to examine the effect of water temperature during aqua-cycling session on  
6 energy expenditure, rate of perceived exertion, energy intake, appetite sensations, and food  
7 reward in healthy premenopausal women. **Methods:** Participants completed three experimental  
8 session, in the postprandial condition, in a randomized order: a land control session (CON); an  
9 aqua-cycling session in 18°C (EXO18); an aqua-cycling session in 27°C (EXO27). Energy  
10 expenditure, food intake, appetite sensations, and food reward were investigated for each  
11 conditions. **Results:** EXO18 induced a significant increase in energy expenditure ( $p < 0.001$ )  
12 and oxygen consumption ( $p < 0.01$ ) compared to EXO27. CHO oxidation was higher in EXO18  
13 session compared to EXO27 and CON ( $p < 0.05$  and  $p < 0.001$ , respectively). While fat oxidation  
14 was higher in exercise sessions compared with CONT ( $p < 0.01$ ), no difference was observed  
15 between EXO18 and EXO27. Exercise sessions did not alter absolute energy intake session but  
16 induced a decrease in relative energy intake ( $p < 0.001$ ) and in hunger, desire to eat, and  
17 prospective food consumption compared with CON ( $p < 0.001$ ). We also show here that cold  
18 water exposure can increase EE while RPE is lower at the end of exercise session compared to  
19 same exercise at 27°C ( $p < 0.05$ ).

20 **Conclusion:** An exposure to a moderately cold water during aqua-cycling is an efficient  
21 strategy to promote increased energy expenditure and decreased hunger, which may be effective  
22 for energy balance management in healthy premenopausal women.

23 **Key words:** water, cold, food intake, exercise, women

24

25

## 26 INTRODUCTION

27 Regular exercise is well-known to have significant health benefits including the decrease in  
28 cardiometabolic risks (Janiszewski & Ross, 2009). It is thus essential to develop appropriate  
29 and achievable exercise programs to promote adherence, maximize engagement and favor  
30 health improvements. In the past few years, aquatic physical activities such as aqua-cycling,  
31 have gained popularity. This activity consists of pedaling against the water resistance using  
32 stationary immersible bicycle. Due to the physical properties and advantages of immersion (i.e.,  
33 non-weight-bearing activity and low joint impact), aqua- cycling seems appropriate for  
34 individuals wishing to begin or resume physical activity (Bergamin, Ermolao, Matten,  
35 Sieverdes, & Zaccaria, 2015; Schaun, Pinto, Praia, & Alberton, 2018; Yazigi et al., 2013). Most  
36 of the available investigations are feasibility studies (Morlock & Dressendorfer, 1974; Shapiro,  
37 Avellini, Toner, & Pandolf, 1981), or compare cardiorespiratory parameters in response to  
38 exercises performed in water versus dryland conditions (Ayme, Gavarry, Rossi, Desruelle, et  
39 al., 2014; Ayme, Gavarry, Rossi, Guieu, & Boussuges, 2014; Ayme, Rossi, Gavarry, Chaumet,  
40 & Boussuges, 2015; Bréchat et al., 1999; Garzon et al., 2017; Garzon et al., 2015; Sosner et al.,  
41 2016). While the effects of aqua-cycling on cardiorespiratory responses and energy expenditure  
42 have been investigated (Barbosa, Garrido, & Bragada, 2007; Pendergast, Moon, Krasney, Held,  
43 & Zamparo, 2015), little is known regarding its influence on energy balance (EB) despite its  
44 increasing popularity especially with women wishing to improve their body composition.

45 Although exercise induces increased energy expenditure (EE), it is now well-established that it  
46 can also affect energy intake (EI), appetite and food reward, depending on the exercise  
47 parameters (intensity, duration, induced EE, etc.) and on the individuals 'characteristics  
48 (Blundell, Gibbons, Caudwell, Finlayson, & Hopkins, 2015; Howe, Hand, & Manore, 2014;  
49 Miguet et al., 2018; Pomerleau, Imbeault, Parker, & Doucet, 2004; Rocha, Paxman, Dalton,  
50 Winter, & Broom, 2015). While few studies suggest that cold water exposure during exercise

51 can increase both EE (McArdle, Toner, Magel, Spina, & Pandolf, 1992; Sheldahl, Buskirk,  
52 Loomis, Hodgson, & Mendez, 1982) and EI (Crabtree & Blannin, 2015; White, Dressendorfer,  
53 Holland, McCoy, & Ferguson, 2005), the tested temperatures may not be generalizable to  
54 exercises common in classical exercise programs to promote health. The use of moderately cold  
55 water temperatures (18°C - 20°C) and their effect on overall EB needs to be investigated.  
56 Considering that the interplay between EE and EI is a fundamental feature of the long-term  
57 regulation of body weight, the physiological modulations in response to immersed cycling need  
58 to be explored to inform appropriate exercise programs. Thus, the objective of this study was  
59 to examine the effect of water temperature during immersed cycling on energy expenditure,  
60 energy intake, appetite sensations, and food reward in healthy, premenopausal women. We  
61 hypothesized that cycling in cold water would induce a negative energy balance compared with  
62 exercising with a warmer water temperature, by increasing energy expenditure and decreasing  
63 appetite sensation after exercise in cold condition.

64

## 65 **METHODS**

### 66 **Population**

67 This study was conducted on eleven women aged  $21.2 \pm 0.6$  y, recruited through  
68 advertisements. Their mean body mass was  $58.2 \pm 5.3$  kg, with a BMI of  $21.7 \pm 2.9$  kg.m<sup>-2</sup>,  
69 percentage of fat mass of  $22.2 \pm 4.5$  %, and FFM of  $42.5 \pm 2.8$  kg. All women had normal  
70 menstruation cycles (length of cycles:  $29 \pm 1$  days) for at least 1 year and had not taken any oral  
71 contraceptives for more than 1 year prior to the beginning of the study. Smokers, dieters,  
72 aquaphobic individuals, and individuals taking medication were excluded. Volunteers were not  
73 engaged in regular intense physical activities. This study was conducted in accordance with the  
74 Declaration of Helsinki and approved by the ethical authorities (Human Ethical Committee:  
75 CPP; authorization reference: 2019-A00353-54). This work has been registered as a Clinical

76 Trial (NCT03978975). Of note, the present analysis covers only data regarding the main  
77 objective of the overall project (Temperature effect), among normal weight participant only.  
78 All participants gave written informed consent.

79

## 80 **Experimental design**

81 All visit took place in a medical center. After a full medical examination to assess eligibility,  
82 the included participants were asked to complete a food preference questionnaire (which was  
83 used to compose the buffet meals presented during the experimental sessions). Each participant  
84 came to the laboratory on four separate occasions. The first visit aimed to assess anthropometry  
85 and body composition. Then, participants completed three experimental visits, in a postprandial  
86 state, in a randomized order during their luteal phase and separated by at least 7 days. The first  
87 was a control condition (CON); the second was a water-based cycling session at a temperature  
88 of 18°C (EXO18); the third was a water-based cycling session at a temperature of 27°C  
89 (EXO27). Subjects were informed if they were allocated to the control session or an exercise  
90 session but they didn't know which one of the two exercise sessions was planned. Thirty  
91 minutes after each session, an *ad libitum* lunch meal was provided and energy intake measured.  
92 Cardiorespiratory parameters, and rate of perceived exertion were assessed during the session.  
93 Before and after each session, appetite sensations, food preference and reward were assessed at  
94 different times.

95

### 96 **Visit 1: Anthropometry and body composition**

97 Height and body mass were determined using a standard wall-mounted stadiometer and digital  
98 scale (SECA, Les Mureaux, France), respectively. Body Mass Index (BMI) was calculated as  
99 body mass (kg) divided by height squared (m<sup>2</sup>). Fat mass (FM) and fat-free mass (FFM) were

100 assessed by dual-energy X-ray absorptiometry (DXA) (QDR4500A scanner, Hologic,  
101 Waltham, MA, USA).

102

### 103 **Visits 2, 3, and 4: Experimental sessions**

#### 104 *Sessions*

105 Subjects were submitted to three experimental session in a randomized order. Participants  
106 arrived at the laboratory at 11:00 am, three hours after a standardized breakfast which  
107 represented 9.5 to 10 kcal per kg of body mass (55% CHO, 30% lipids and 15% protein) (Isacco,  
108 Duché, & Boisseau, 2012). The participants were instructed to abstain from stimulants (coffee,  
109 tea) and from moderate-to-vigorous physical activity for 24 h prior to each session.

110 During the CON session, on land, women were asked to sit on a chair and to remain quiet and  
111 at rest during 40 min in a stable environmental temperature ( $22 \pm 0.5^{\circ}\text{C}$ ).

112 During the two exercise trials (EXO18; EXO27), participants performed exercise on a cycle  
113 ergometer, immersed in water at the waist level in an individual cabin, with trunk and head  
114 exposed to ambient air (aquabikecabine; Aquafit Technologie®, SIREM, Saint Maurice s/  
115 Beynost, France). They cycled for 40 min (~11:20am-12:00am) at 70% of their theoretical  
116 maximal heart rate ( $220 - \text{age}$ ) corresponding to similar intensity used in other studies (McArdle  
117 et al., 1992).

118 For each exercise session, the water temperature was continuously monitored by a thermometer,  
119 and a water renewal system maintained a constant water temperature. Similarly to the CON  
120 condition, ambient air temperature was stable ( $22 \pm 0.5^{\circ}\text{C}$ ).

121

#### 122 *Measurements*

123 *Metabolic and cardiorespiratory parameters*

124 After calibration, oxygen consumption ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), ventilation  
125 (VE) and heart rate (HR) were continuously recorded throughout the 40 min of each session  
126 using indirect calorimetry (K4b<sup>2</sup>, Cosmed, Rome, Italy) and HR monitor (Polar, V800,  
127 Kempele, Finland). Total energy expenditure (EE in kcal) over the 40 min and, specifically, at  
128 10, 20, 30 and 40 min of each session, was calculated as follows:  $\text{VO}_2$  ( $\text{L}\cdot\text{min}^{-1}$ ) x energy  
129 equivalent of oxygen x duration (min)(Zuntz N, 1901). Respiratory exchange ratio (RER;  
130  $\text{VCO}_2/\text{VO}_2$ ) and carbohydrate (CHO) and lipid oxidation rates were calculated at rest and over  
131 the entire period of each session and, specifically, at 10, 20, 30 and 40 min of each session:

$$132 \text{ CHO} = 4.585\text{VCO}_2 - 3.2255\text{VO}_2$$

$$133 \text{ Lipid} = 1.6946\text{VO}_2 - 1.7012\text{VCO}_2$$

134 where CHO and lipid are in  $\text{g}\cdot\text{min}^{-1}$ , and  $\text{VCO}_2$  and  $\text{VO}_2$  are in  $\text{l}\cdot\text{min}^{-1}$  (Péronnet & Massicotte,  
135 1991).  $\text{VO}_2$  and  $\text{VCO}_2$  were determined as the mean of the values during the last minutes of  
136 each stage.

#### 137 *Ad libitum meals and energy intake*

138 Participants were provided with an *ad libitum* buffet meal for lunch (12:00pm).

139 Food items were provided in excess of expected consumption and participants were instructed  
140 to eat until “comfortably satiated”. The food selection was covertly weighed by the  
141 investigators before and after the meal and participants were unaware of the quantity of calories  
142 served. Energy and macronutrient intakes were calculated using dietary analysis software  
143 (Bilnut 4.0 SCDA Nutrisoft software, France). Relative energy intake (REI) for the *ad libitum*  
144 lunch meal was calculated as the EI minus the net EE of each session.

#### 145 *Subjective appetite ratings*

146 Appetite ratings were assessed throughout the day using visual analogue scales (150 millimeter  
147 visual analogue scales, VAS) at baseline (fasted), immediately after breakfast, before and after

148 exercise, before and after lunch and 30 and 60 min after lunch (Flint, Raben, Blundell, &  
149 Astrup, 2000).

#### 150 *Food preference and reward*

151 The Leeds Food Preference Questionnaire (LFPQ; described in detail by Dalton and Finlayson)  
152 (Dalton & Finlayson, 2014) was administered before and after lunch to determine scores of  
153 implicit wanting and explicit liking for high-fat (>50% energy) or low-fat (<20% energy) foods  
154 matched for familiarity, sweetness, protein and acceptability (Finlayson, King, & Blundell,  
155 2008). Low fat scores were subtracted from high fat scores to obtain the fat appeal bias score;  
156 thus a positive score indicates greater liking or wanting towards high-fat compared to low-fat  
157 foods.

#### 158 *Rate of perceived exertion*

159 During each exercise session, at 20 (T20) and 40 (T40) min, the rate of perceived exertion  
160 (RPE) was assessed using the 6 to 20-point Borg scale, where 6 means “no exertion at all” and  
161 20 means maximal exertion (Borg, Hassmén, & Lagerström, 1987). During the screening visit,  
162 the range of sensations that corresponds to effort categories within the Borg scale was explained  
163 to the participants to familiarize them with it.

164

#### 165 **Statistics**

166 The sample size estimation was calculated according (i) to differences reported in the literature  
167 (White et al., 2005) and (ii) to effect-size bounds recommended by Cohen’s (Cohen, 1988) :  
168 small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8, “grossly perceptible and therefore  
169 large”). Power calculation based on previous work (White et al. 2005) suggested that a sample  
170 size of 11 participants would allow detection of at least 40% difference in energy intake  
171 between exercise conditions with a standard deviation of 40%, a probability of 0.05, and a beta  
172 level of 0.80. It have been added to the methods section. Statistical analyses were performed

173 using Stata software, Version 15 (StataCorp, College Station, TX, US). Continuous data were  
174 expressed as mean and standard-deviation and the assumption of normality was assessed using  
175 the Shapiro-Wilk test. The comparisons between sessions (CON, EXO27; EXO18) were  
176 performed using random-effects models for crossover designs, taking account of the following  
177 effects: session, sequence, *session x sequence* interaction and subject as random effect. The  
178 normality of residuals from these models was studied as aforementioned. In case of non-normal  
179 distribution, a logarithmic transformation was implemented. A Sidak's type I error correction  
180 was applied to perform multiple comparisons. Random-effects models were also used to  
181 measure time effect during each exercise session, (1) time, session and *time x session* interaction  
182 as fixed effects, and (2) subject as random-effect in order to model between and within  
183 participant variability. Analogous statistical analysis plan was performed to study assumptions  
184 of random-effects models and multiple comparisons. Appetite sensations were also compared  
185 with area under the curve (AUC) values using the trapezoid method.

## 186 **RESULTS**

### 187 *Metabolic and cardiorespiratory parameters*

188 Total EE induced by both exercise session was higher compared to the CON session ( $p < 0.001$ ).  
189 The EXO18 session displayed a significantly higher total EE than the EXO27 session ( $p < 0.001$ )  
190 (Table 1). Similarly,  $VO_2$  was significantly higher during both exercise session than CON  
191 session ( $p < 0.001$ ) and EXO18 exhibited significantly greater  $VO_2$  than EXO27 ( $p < 0.007$ )  
192 (Table 1). Specifically, EXO18 induced higher  $VO_2$  at 10 ( $p < 0.001$ ), 20 ( $p < 0.004$ ) and 30  
193 ( $p < 0.002$ ) min of exercise compared with EXO27. No significant difference in  $VO_2$  was  
194 observed between EXO18 and EXO27 at 40 min (Figure 1A). RER was not significantly  
195 different between the three sessions (Table 1).

196 Mean HR and VE were higher during exercise compared with CON session ( $p < 0.001$ ), while  
197 only VE was significantly higher during EXO18 compared to EXO27 ( $p < 0.008$ ) (Table 1 and  
198 figure 1B). HR in the two exercise session was slightly lower than expected.

199 Concerning substrate oxidation, CHO and lipid oxidation rates were higher throughout the  
200 EXO18 and EXO27 sessions compared with CON session ( $p < 0.001$ ). No significant difference  
201 was observed in lipid oxidation between EXO18 and EXO27 for the sessions overall or at 10,  
202 20, 30 and 40 min of exercise. CHO oxidation was higher during EXO18 compared to EXO27  
203 ( $p < 0.03$ ) over the entire period (Table 1) and at 10 ( $p < 0.001$ ), 20 ( $p < 0.02$ ), 30 ( $p < 0.03$ ) and 40  
204 min ( $p < 0.04$ ) (Figure 1C & D).

#### 205 *Absolute and relative energy intake*

206 There was no difference in total EI and macronutrient intake between sessions. REI was  
207 significantly lower in EXO18 and EXO27 compared to the CON session ( $p < 0.001$ ) with no  
208 difference between EXO18 and EXO 27 sessions (Table 1).

#### 209 *Subjective appetite ratings*

210 Before the experimental session (exercise or rest control), hunger, desire to eat, and prospective  
211 consumption values were significantly higher during CON session compared with EXO18 and  
212 EXO27 sessions ( $p < 0.001$ ). Total AUC values for hunger, desire to eat, and prospective food  
213 consumption were significantly higher in CON session compared to EXO18 and EXO27  
214 sessions ( $p < 0.001$ ) (Figure 1A, B, C and D).

#### 215 *Food preference and reward*

216 As detailed in Table 2, no condition (exercise vs. control), time (pre- vs. post-meal) or  
217 interaction (time x condition) effect was found for Wanting or Liking.

#### 218 *Rate of perceived exertion*

219 No significant difference in RPE at 20 min was observed between EXO18 and EXO27 while  
220 RPE at 40 min was significantly lower in EXO18 compared to EXO27 ( $p < 0.03$ , Figure 3).

## 221 **DISCUSSION**

222 This is, to our knowledge, the first study investigating the impact of different water temperature  
223 of aqua-cycling on EE, EI, appetite sensations, and food reward in healthy premenopausal  
224 women. Our results, suggest that cold water exposure (18°C) during aqua-cycling leads to a  
225 higher EE compared with a 27°C temperature. Importantly, both aqua-cycling sessions induced  
226 a decrease in REI and appetite sensations, compared with a control session, suggesting that  
227 practice of aqua-cycling could be a promising weight management strategy.

228 While exercising, heat is produced during skeletal muscle contraction thus, a water temperature  
229 of 27°C is classically used in aquatic center for swimming use rather than thermoneutral  
230 temperature (i.e., 33-35°C) (Barbosa, Marinho, Reis, Silva, & Bragada, 2009). Many studies  
231 have investigated the effect of cold exposure on physiological outcomes using climatic chamber  
232 or water immersion (Crabtree & Blannin, 2015; Gagnon et al., 2020; Gagnon et al., 2013;  
233 McArdle, Magel, Lesmes, & Pechar, 1976; McArdle et al., 1992; Shorten, Wallman, & Guelfi,  
234 2009). Nevertheless, the range of studied temperatures remains wide (from -10°C to 36°C) with  
235 significant methodological heterogeneity, which makes any comparison difficult. The available  
236 evidence seems to indicate an increase in EE and lipid oxidation during cold exposure (Gagnon  
237 et al., 2020; Gagnon et al., 2013; McArdle et al., 1976; Timmons, Araujo, & Thomas, 1985),  
238 with few studies examining the acute responses to exercise in cold water on EB (McArdle et  
239 al., 1976; McArdle et al., 1992; White et al., 2005). McArdle et al. (McArdle et al., 1992)  
240 showed in men and women, that oxygen consumption was higher in a cold water condition  
241 (20°C) at rest and during low intensity exercise ( $\leq 35\%$   $VO_{2max}$ ) compared with warmer water  
242 temperature (28°C). However, the difference in oxygen consumption was no longer significant  
243 between 20°C and 28°C during moderate intensity exercise (i.e., 40% to 66%  $VO_{2max}$ ). In young  
244 healthy men, White et al. (2005) found that 45 min of aqua-cycling at 60% of  $VO_{2max}$  at 20°C  
245 did not influence EE compared to 33°C, which is not in line with the present results where cold

246 condition is associated with higher EE at a similar relative exercise intensity (White et al.,  
247 2005). This was concomitant with a higher  $\text{VO}_2$  and a decrease in RPE at the end of exercise.  
248 In addition, in the present study, subjects were asked to pedal at a constant pace to maintain the  
249 fixed HR. An explanation for our increased EE could be that they exercised at a higher speed  
250 during the cold compared to warm temperature session. Indeed, cold exposure during exercise  
251 can induce a significant decrease in HR (Gagnon et al., 2013) which could partly explain the  
252 increased oxygen consumption and respiratory demand in the cold compared with the aqua-  
253 cycling session at 27°C matched for HR. In fact, cold exposure have been shown to induce  
254 increased central blood volume and activation of the baroreceptor reflex, both mechanisms  
255 associated with decreased HR (Gagnon et al., 2013). We also found here that the increase of  
256 EE during the 18°C condition was concomitant with an increase in carbohydrate oxidation and  
257 no change in lipid oxidation, which is different (Gagnon et al., 2020; Gagnon et al., 2013;  
258 Timmons et al., 1985) or in accordance (Haman et al., 2005; White et al., 2005) with others  
259 studies. Some methodological differences may explain the variability of results found in  
260 literature like the condition of cold exposure (i.e., climatic chamber vs. water immersion), the  
261 large range of temperature (from -10°C to 22°C) and the nutritional status of individuals (e.g.,  
262 fasting vs. post-prandial) which is essential when investigating substrate utilization. ~~Finally, we~~  
263 ~~show here that cold water exposure can increase EE while RPE is lower at the end of exercise~~  
264 ~~compared to session at 27°C. It is worth noting that while RPE values were similar at 20 min~~  
265 ~~of exercise, the RPE discrepancy emerging at 40 min occurred in parallel with a drop in  $\text{VO}_2$~~   
266 ~~values from 30 to 40 min of exercise at 18°C (no more significant difference in  $\text{VO}_2$  between~~  
267 ~~the two exercise sessions). It would have been relevant to report RPE values at each 10 min of~~  
268 ~~exercise to decipher if similar RPE pattern was observed with exercise duration and better~~  
269 ~~understand these adaptations.~~

270 While cold water exposure may be an attractive strategy to increase EE, it has also been shown  
271 to increase EI (Crabtree, Chambers, Hardwick, & Blannin, 2014; Shorten et al., 2009; White et  
272 al., 2005) leading to no significant modification in EB. The present results indicate that  
273 compared to the dryland resting, both bouts of aqua-cycling induced a significant decrease in  
274 REI and AUC for appetite feelings (i.e., hunger, prospective food consumption, and desire to  
275 eat). There was no difference due to the water temperature. Interestingly, this result indicates  
276 that cold immersed exercise does not necessarily increase subsequent food intake, as it has been  
277 previously suggested (White et al., 2005). Appetite feelings were significantly higher leading  
278 up to the sedentary period during the dryland control session compared with ratings leading up  
279 to the immersed exercise sessions. It could reflect an anticipatory effect, indicating that subjects  
280 who knew that they were going to exercise rated appetite sensations at a lower level rather than  
281 the influence of the immersed exercise *per se* (Barutcu, Witcomb, & James, 2019). In addition,  
282 no difference in hedonic preference for high-fat foods was observed between conditions which  
283 may back-up the non-compensation in EI subsequent to exercise. Future studies should thus  
284 investigate EI, appetite feelings and hedonic responses for the rest of the day and subsequent  
285 days to see if any compensation appears.

286 We have to note several limitations in our study. First we focused on a theoretical percentage  
287 of HR that could be influenced by immersion (Alberton, Antunes, et al., 2013; Alberton, Kanitz,  
288 et al., 2013). We could not rule out that the intensity was slightly lower than 70% as aquatic  
289 immersion can induce a decrease in HR at rest and during exercise. Secondly we used a  
290 standardized breakfast and gave recommendation for the day before each session. However we  
291 did not control and calibrate the food intake on the day before the experimentation, we cannot  
292 exclude that it could have influenced our results on EB. Finally those who were randomly  
293 assigned to the two exercise session consecutively knew beforehand that their next visit would

294 be the control session. This last point could have indirectly influenced some subjective appetite  
295 rating.

296 To conclude, this study is the first to our knowledge to show that cold, aqua-cycling exercise  
297 can be a strategy to increase EE without increasing absolute food intake in healthy young  
298 women. The use of the aquatic environment for exercise, and more specifically aqua-cycling,  
299 could be considered in future health management programs. Future studies should thus focus  
300 on chronic effects of different aqua-cycling modalities and EI responses to determine  
301 appropriate programs to induce long term control and/or improvement in body composition and  
302 health in women.

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307 nutrition in Clermont Ferrand who has been a partner of this work. LM, DM, PB and TD  
308 designed the study; LM, TD, LI, NF and KB were in charge of the experimental sessions and  
309 data collection; LM, TD, IL, PB, KB and NF analyzed the data; all authors significantly  
310 contributed to the writing and revision of the manuscript. The authors have neither financial  
311 conflict nor other conflicts of interest to disclose. The authors declare that the results of the  
312 study are presented clearly, honestly and without fabrication, falsification or inappropriate  
313 data manipulation. The results of the present study do not constitute endorsement by ACSM.

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435 Hirchwald,.
- 436
- 437

438 Table 1. Cardiorespiratory and metabolic parameters and energy intake during CON, EXO18 and EXO27  
 439 sessions.

	CON	EXO18	EXO27
<b>Cardiorespiratory and metabolic parameters</b>			
VO <sub>2</sub> (ml.min <sup>-1</sup> )	297.7 ± 69.6	1790.6 ± 348.1 <sup>***,μμ</sup>	1478.6 ± 175.9 <sup>***</sup>
EE (kcal)	57.5 ± 13.2	351.2 ± 65.5 <sup>***,μμμ</sup>	289.7 ± 31 <sup>***</sup>
HR (bpm)	72 ± 10	136 ± 4 <sup>***</sup>	133 ± 9 <sup>***</sup>
VE (L.min <sup>-1</sup> )	8 ± 1.8	38.6 ± 7.5 <sup>***,μμ</sup>	34.2 ± 4.6 <sup>***</sup>
RER	0.88 ± 0.08	0.93 ± 0.07	0.93 ± 0.08
CHO oxidation rates (g.min <sup>-1</sup> )	0.2 ± 0.06	1.5 ± 0.5 <sup>***,μ</sup>	1.3 ± 0.3 <sup>***</sup>
Lipid oxidation rates (g.min <sup>-1</sup> )	0.07 ± 0.04	0.29 ± 0.25 <sup>***</sup>	0.26 ± 0.14 <sup>***</sup>
<b>Energy intake</b>			
Total EI (kcal)	640.8 ± 230.9	640.5 ± 213.5	583.5 ± 176.9
CHO (%)	58.6 ± 16.4	61.2 ± 6.1	57.9 ± 10.5
Lipids (%)	18.1 ± 11	16.2 ± 6.5	20.2 ± 8.2
Proteins (%)	20.7 ± 6.7	17.9 ± 3.9	17.3 ± 5.1
REI (kcal)	583.3 ± 221.4	287.7 ± 207.1 <sup>***</sup>	293.7 ± 184.5 <sup>***</sup>

440 \*\*\*: significantly different from CON at p<0.001; μ; μμ; μμμ: significantly different from EXO27 at  
 441 p<0.05, p<0.01 and p<0.001, respectively.

442 CON: control; EXO18: exercise session performed on an underwater cycle ergometer in 18°C; EXO27:  
 443 exercise session performed on an underwater cycle ergometer in 27°C; VO<sub>2</sub>: oxygen consumption; EE:  
 444 energy expenditure; HR: heart rate; VE: ventilation; RER: respiratory exchange ratio; CHO:  
 445 carbohydrates; EI: energy intake; REI: relative energy intake.

446

447

448 Table 2. Liking and wanting fat bias scores pre- and post- meal in the control (CON), and exercise  
 449 (EXO18, EXO27) conditions.

		CON	EXO18	EXO27
<b>Wanting</b>	Pre-meal	6.4 ± 22.6	13.3 ± 25.3	9.4 ± 28.2
	Post-meal	16.6 ± 21.5	20.2 ± 17.2	17.4 ± 26.8
<b>Liking</b>	Pre-meal	6.8 ± 21.6	3.1 ± 13.4	7.2 ± 19.2
	Post-meal	7.2 ± 11.7	6.3 ± 8.2	6.2 ± 8.9

450 CON: control; EXO18: exercise session performed on an underwater cycle ergometer in 18°C; EXO27:  
 451 exercise session performed on an underwater cycle ergometer in 27°C.

452

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455 Figures legends

456 Figure 1. Oxygen consumption (A), heart rate (B), CHO (C) and fat (D) oxidation kinetic during  
457 exercise.

458 \*, \*\*, \*\*\*: significantly different between EXO18 and EXO27 at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ,  
459 respectively.

460 EXO18: exercise session performed on an underwater cycle ergometer in 18°C; EXO27:  
461 exercise session performed on an underwater cycle ergometer in 27°C; HR: heart rate;  $VO_2$ :  
462 oxygen consumption; CHO: carbohydrates.

463

464 Figure 2. Subjective hunger (A), satiety (B), Prospective Food Consumption (C) and Desire to  
465 eat (D) kinetics (left side) and absolute area under the curve (Abs AUC, right side).

466 \*\*\*: significantly different from CON at  $p < 0.001$ .

467 CON: control; EXO18: exercise session performed on an underwater cycle ergometer in 18°C;  
468 EXO27: exercise session performed on an underwater cycle ergometer in 27°C; AUC: area  
469 under the curve; Abs: absolute.

470

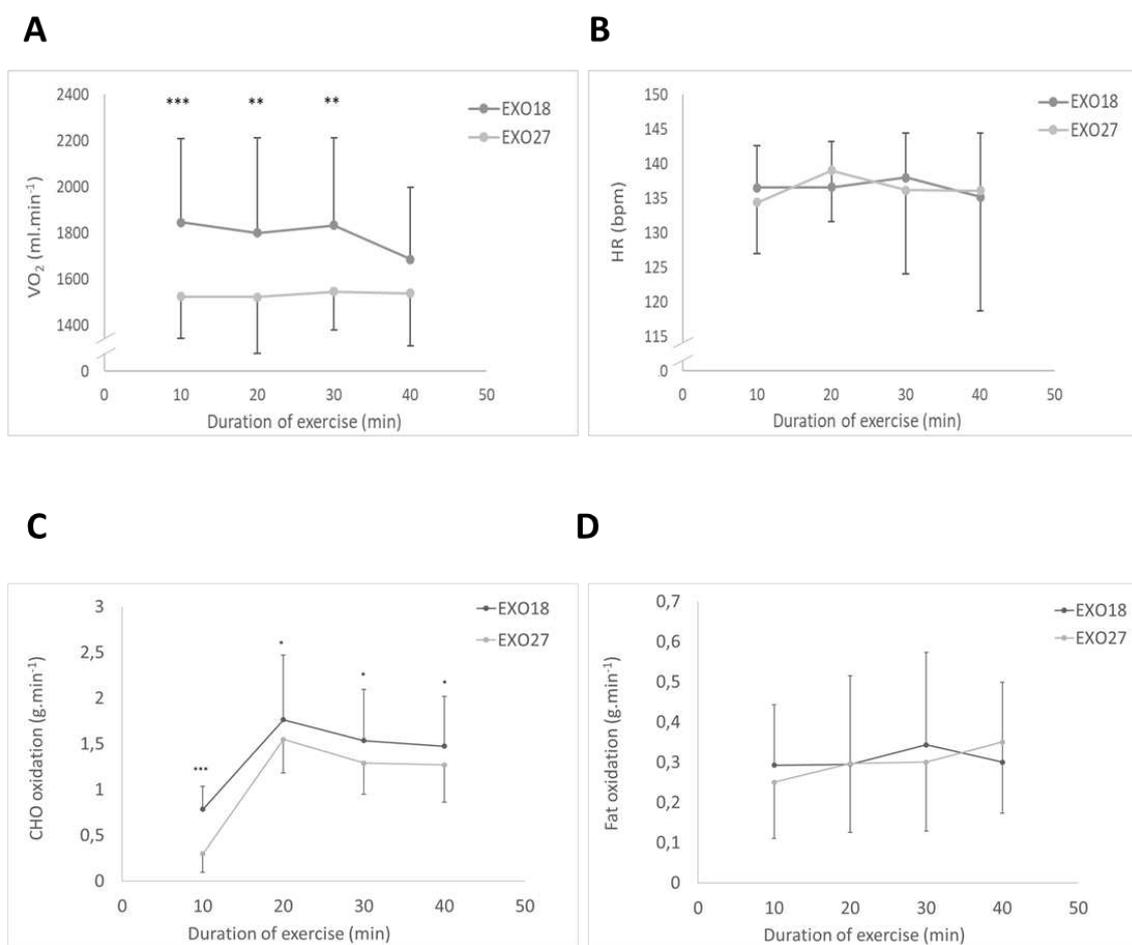
471 Figure 3. Rate of perceived exertion at 20 and 40 min of exercise.

472 \*: significantly different between EXO18 and EXO27 at  $p < 0.05$ .

473 EXO18: exercise session performed on an underwater cycle ergometer in 18°C; EXO27:  
474 exercise session performed on an underwater cycle ergometer in 27°C; RPE: rate of perceived  
475 exertion.

476

477 Figure 1



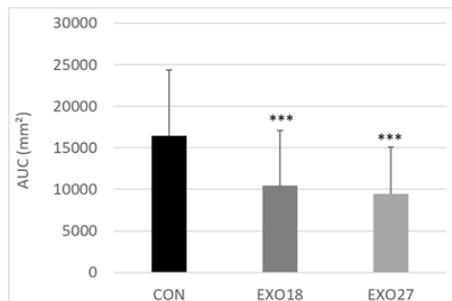
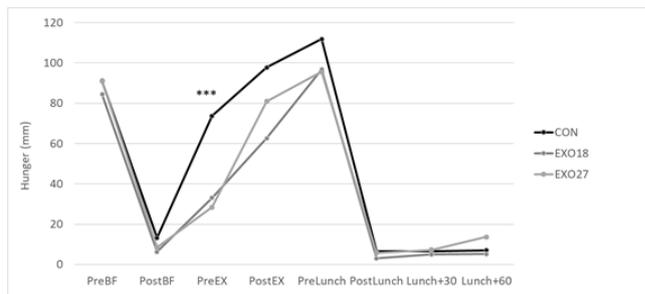
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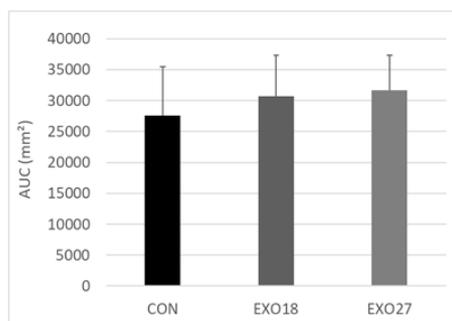
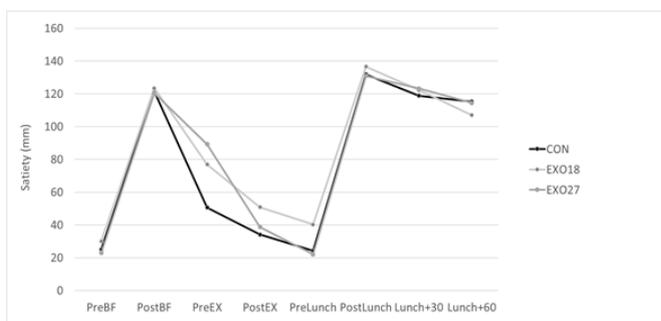
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481 Figure 2

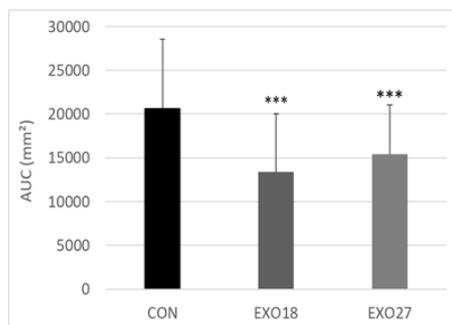
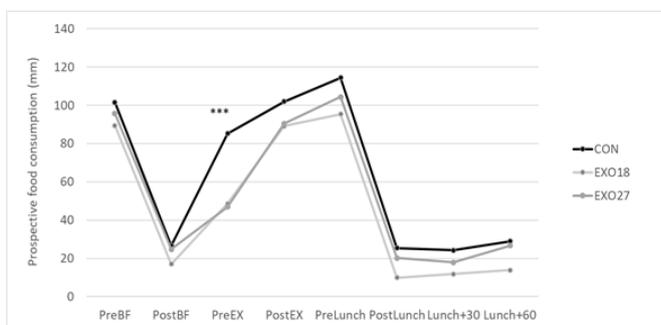
**A**



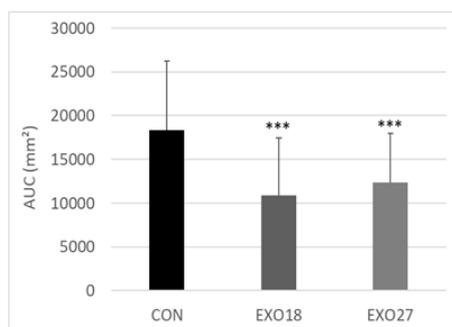
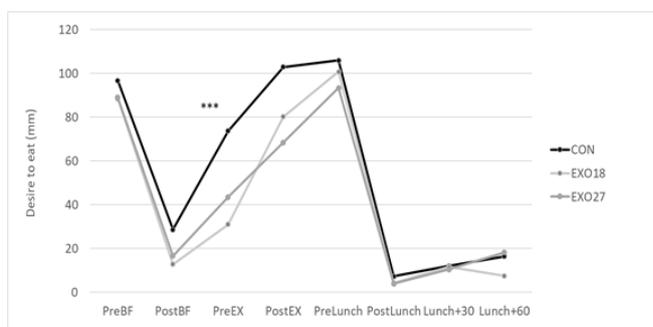
**B**



**C**



**D**

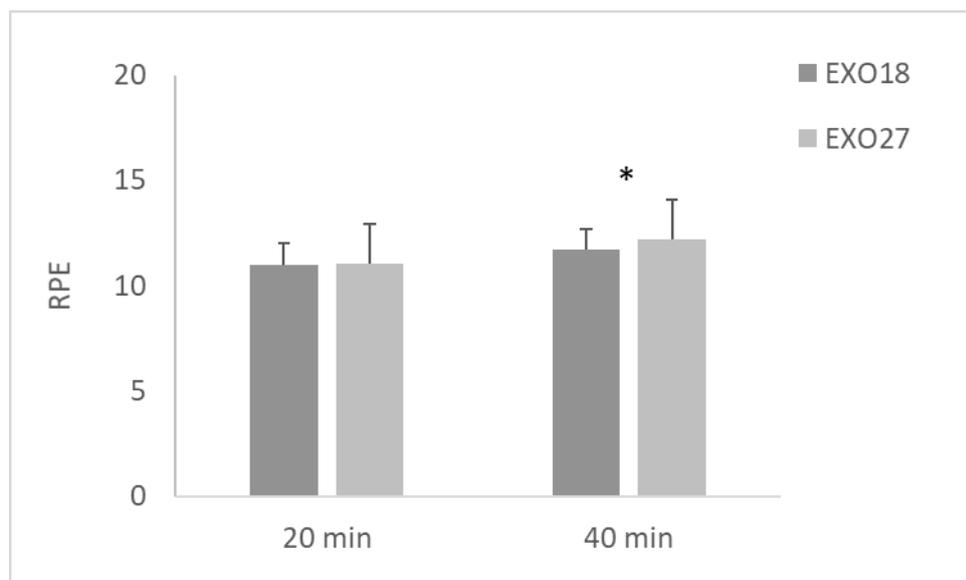


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485 Figure 3



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