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Metz, L, Isacco, L, Beaulieu, K orcid.org/0000-0001-8926-6953 et al. (4 more authors)
(2021) Cold-Water Effects on Energy Balance in Healthy Women During Aqua-Cycling.
International Journal of Sport Nutrition and Exercise Metabolism. ISSN 1526-484X

<https://doi.org/10.1123/ijsnem.2020-0177>

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<https://doi.org/10.1123/ijsnem.2020-0177>. © 2021 Human Kinetics, Inc.

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

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Clinical trial number: NCT03978975

ID: 2019-A00353-54

URL:

<https://clinicaltrials.gov/ct2/results?cond=&term=03978975&cntry=FR&state=&city=&dist=>

Words: 3100

Tables: 2

Figures: 3

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 ₂	Oxygen consumption
VCO ₂	Carbon dioxide production
VE	Ventilation

Abstract

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

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

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

INTRODUCTION

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

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

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

METHODS

Population

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

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

Experimental design

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

Visit 1: Anthropometry and body composition

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

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

Visits 2, 3, and 4: Experimental sessions

Sessions

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

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

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

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

Measurements

Metabolic and cardiorespiratory parameters

After calibration, oxygen consumption (VO_2), carbon dioxide production (VCO_2), ventilation (VE) and heart rate (HR) were continuously recorded throughout the 40 min of each session using indirect calorimetry (K4b², Cosmed, Rome, Italy) and HR monitor (Polar, V800, Kempele, Finland). Total energy expenditure (EE in kcal) over the 40 min and, specifically, at 10, 20, 30 and 40 min of each session, was calculated as follows: VO_2 ($\text{L}\cdot\text{min}^{-1}$) x energy equivalent of oxygen x duration (min) (Zuntz N, 1901). Respiratory exchange ratio (RER; VCO_2/VO_2) and carbohydrate (CHO) and lipid oxidation rates were calculated at rest and over the entire period of each session and, specifically, at 10, 20, 30 and 40 min of each session:

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

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

where CHO and lipid are in $\text{g}\cdot\text{min}^{-1}$, and VCO_2 and VO_2 are in $\text{l}\cdot\text{min}^{-1}$ (Péronnet & Massicotte, 1991). VO_2 and VCO_2 were determined as the mean of the values during the last minutes of each stage.

Ad libitum meals and energy intake

Participants were provided with an *ad libitum* buffet meal for lunch (12:00pm). Food items were provided in excess of expected consumption and participants were instructed to eat until “comfortably satiated”. The food selection was covertly weighed by the investigators before and after the meal and participants were unaware of the quantity of calories served. Energy and macronutrient intakes were calculated using dietary analysis software (Bilnut 4.0 SCDA Nutrisoft software, France). Relative energy intake (REI) for the *ad libitum* lunch meal was calculated as the EI minus the net EE of each session.

Subjective appetite ratings

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

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

Food preference and reward

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

Rate of perceived exertion

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

Statistics

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

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

RESULTS

Metabolic and cardiorespiratory parameters

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

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

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

Absolute and relative energy intake

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

Subjective appetite ratings

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

Food preference and reward

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

Rate of perceived exertion

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

DISCUSSION

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

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

condition is associated with higher EE at a similar relative exercise intensity (White et al., 2005). This was concomitant with a higher VO_2 and a decrease in RPE at the end of exercise. In addition, in the present study, subjects were asked to pedal at a constant pace to maintain the fixed HR. An explanation for our increased EE could be that they exercised at a higher speed during the cold compared to warm temperature session. Indeed, cold exposure during exercise can induce a significant decrease in HR (Gagnon et al., 2013) which could partly explain the increased oxygen consumption and respiratory demand in the cold compared with the aqua-cycling session at 27°C matched for HR. In fact, cold exposure have been shown to induce increased central blood volume and activation of the baroreceptor reflex, both mechanisms associated with decreased HR (Gagnon et al., 2013). We also found here that the increase of EE during the 18°C condition was concomitant with an increase in carbohydrate oxidation and no change in lipid oxidation, which is different (Gagnon et al., 2020; Gagnon et al., 2013; Timmons et al., 1985) or in accordance (Haman et al., 2005; White et al., 2005) with others studies. Some methodological differences may explain the variability of results found in literature like the condition of cold exposure (i.e., climatic chamber vs. water immersion), the large range of temperature (from -10°C to 22°C) and the nutritional status of individuals (e.g., fasting vs. post-prandial) which is essential when investigating substrate utilization. Finally, we show here that cold water exposure can increase EE while RPE is lower at the end of exercise compared to session at 27°C. It is worth noting that while RPE values were similar at 20 min of exercise, the RPE discrepancy emerging at 40 min occurred in parallel with a drop in VO_2 values from 30 to 40 min of exercise at 18°C (no more significant difference in VO_2 between the two exercise sessions). It would have been relevant to report RPE values at each 10 min of exercise to decipher if similar RPE pattern was observed with exercise duration and better understand these adaptations.

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

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

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

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

ACKNOWLEDGEMENTS, AUTHORSHIPS, DECLARATIONS.

The authors would like to thank the participants who gave their time to complete this pilot study. We are also grateful to the Aquafit Technology-SIREM Company who lent our laboratory the specific aquacabine. Finally, we'd like to express a special thanks to UGECAM nutrition in Clermont Ferrand who has been a partner of this work. LM, DM, PB and TD designed the study; LM, TD, LI, NF and KB were in charge of the experimental sessions and data collection; LM, TD, IL, PB, KB and NF analyzed the data; all authors significantly contributed to the writing and revision of the manuscript. The authors have neither financial conflict nor other conflicts of interest to disclose. The authors declare that the results of the study are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.

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

	CON	EXO18	EXO27
Cardiorespiratory and metabolic parameters			
VO ₂ (ml.min ⁻¹)	297.7 ± 69.6	1790.6 ± 348.1 ^{***,μμ}	1478.6 ± 175.9 ^{***}
EE (kcal)	57.5 ± 13.2	351.2 ± 65.5 ^{***,μμμ}	289.7 ± 31 ^{***}
HR (bpm)	72 ± 10	136 ± 4 ^{***}	133 ± 9 ^{***}
VE (L.min ⁻¹)	8 ± 1.8	38.6 ± 7.5 ^{***,μμ}	34.2 ± 4.6 ^{***}
RER	0.88 ± 0.08	0.93 ± 0.07	0.93 ± 0.08
CHO oxidation rates (g.min ⁻¹)	0.2 ± 0.06	1.5 ± 0.5 ^{***,μ}	1.3 ± 0.3 ^{***}
Lipid oxidation rates (g.min ⁻¹)	0.07 ± 0.04	0.29 ± 0.25 ^{***}	0.26 ± 0.14 ^{***}
Energy intake			
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 ^{***}	293.7 ± 184.5 ^{***}

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

CON: control; EXO18: exercise session performed on an underwater cycle ergometer in 18°C; EXO27: exercise session performed on an underwater cycle ergometer in 27°C; VO₂: oxygen consumption; EE: energy expenditure; HR: heart rate; VE: ventilation; RER: respiratory exchange ratio; CHO: carbohydrates; EI: energy intake; REI: relative energy intake.

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
Wanting	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
Liking	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.

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Figures legends

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

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

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

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

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

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

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

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

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

477 Figure 1

481 Figure 2

A **B** **C** **D**

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485 Figure 3

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