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An acute bout of cycling does not induce compensatory responses in pre-menopausal women not using hormonal contraceptives

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Abstract

There is a clear need to improve understanding of the effects of physical activity and exercise on appetite control. Therefore, the acute and short-term effects (three days) of a single bout of cycling on energy intake and energy expenditure were examined in women not using hormonal contraceptives. Sixteen active (n = 8) and inactive (n = 8) healthy pre-menopausal women completed a randomised crossover design study with two conditions (exercise and control). The exercise day involved cycling for one hour (50% of maximum oxygen uptake) and resting for two hours, whilst the control day comprised three hours of rest. On each experimental day participants arrived at the laboratory fasted, consumed a standardised breakfast and an *ad libitum* pasta lunch. Food diaries and combined heart rate-accelerometer monitors were used to assess free-living food intake and energy expenditure, respectively, over the subsequent three days. There were no main effects or condition (exercise vs control) by group (active vs inactive) interaction for absolute energy intake ($P > 0.05$) at the *ad libitum* laboratory lunch meal, but there was a condition effect for relative energy intake ($P = 0.004$, $\eta_p^2 = 0.46$) that was lower in the exercise condition (1417 ± 926 kJ vs. 2120 ± 923 kJ). Furthermore, post-breakfast satiety was higher in the active than in the inactive group ($P = 0.005$, $\eta_p^2 = 0.44$). There were no main effects or interactions ($P >$

0.05) for mean daily energy intake, but both active and inactive groups consumed less energy from protein (14 ± 3% vs. 16 ± 4%, $P = 0.016$, $\eta_p^2 = 0.37$) and more from carbohydrate (53 ± 5% vs. 49 ± 7%, $P = 0.031$, $\eta_p^2 = 0.31$) following the exercise condition. This study suggests that an acute bout of cycling does not induce compensatory responses in active and inactive women not using hormonal contraceptives, while the stronger satiety response to the standardised breakfast meal in active individuals adds to the growing literature that physical activity helps improve the sensitivity of short-term appetite control.

Keywords: Food intake; Energy expenditure; Appetite; Active; Inactive, Exercise.

Introduction

As a readily modifiable component of energy balance, exercise is a commonly promoted strategy for weight management. While some have questioned the role of exercise (without dietary restriction) as a means of eliciting weight loss (1), exercise appears to play an important role in the prevention of initial weight gain and the promotion of successful weight loss maintenance (2). However, it is becoming clear that marked heterogeneity exists in body mass responses to exercise (and other lifestyle, pharmacological and surgical) interventions designed to promote weight loss (3). High inter-individual variability could be explained by physiological and behavioural compensatory responses in energy intake and/or non-exercise energy expenditure (4).

Based on the work of Jean Mayer (5), research has started to examine how habitual physical activity moderates the sensitivity of short-term appetite control. A J-shaped relationship between physical activity and energy intake has been proposed

(6), with high levels of habitual physical activity associated with stronger homeostatic appetite control while low levels of physical activity are thought to be associated with dysregulated appetite (7). Despite this, few studies have directly compared the effects of acute exercise on appetite between active and inactive individuals (8-14), and studies typically only examine the impact of a bout of exercise on appetite and food intake at the subsequent meal or over the remainder of the day (8, 9, 12, 13, 15). This is of importance as a 'lag' in corrective responses elicited by acute energy deficit or surfeit has been noted. For example, Bray et al. (16) reporting that compensatory changes in EI are evident 2-5 days after dietary manipulation of energy intake, while Edholm (17) also reported a 2-day lag between increased daily energy expenditure and subsequent increases in daily energy intake. However, a corrective lag in energy intake or energy expenditure has not always been reported when one component of energy balance is perturbed (18).

There is also a paucity of studies focusing specifically on the appetite responses to exercise in women, but existing studies typically reported no changes in hunger and/or energy intake (19). However, whether sex differences exist in the appetitive and body mass responses to exercise has been debated (20), and inconsistency in these sex-based responses may in part relate to the lack of control of appetite-modulating variables such as menstrual cycle, menstrual symptoms or use of hormonal contraceptives. As hormonal contraceptive use is rarely identified, this limits understanding of how such medication moderates the impact of exercise on appetite control. Our previous study examining women taking oral contraceptives (11) demonstrated there were no significant differences in energy intake over the four days in active participants. However, there was a suppression of energy intake on the first day after the exercise experimental day compared with the same day of the control

condition in inactive participants. As a follow on, this study aimed to examine the immediate and short-term effects (i.e. subsequent three days) of a single bout of cycling on appetite, energy intake and energy expenditure in physically active and inactive pre-menopausal women not taking hormonal contraceptives.

Material and methods

Participants

Twenty-three healthy pre-menopausal women not taking oral contraceptives volunteered, but seven participants withdrew because of time constraints. Therefore, 16 active (n = 8; age 21.9 ± 4.0 years; Body Mass Index (BMI) 22.2 ± 2.0 $kg.m^{-2}$) and inactive (n = 8; age 24.5 ± 3.5 years; BMI 23.0 ± 3.1 $kg.m^{-2}$) women completed the study. Participants had regular menstrual cycles (21-35 days), stable body mass (±2 kg during the previous six months), no history of cardiovascular or metabolic diseases, were non-smokers and not taking medication, pregnant or lactating. Participants were blinded to the true purpose of the study (i.e. advertised as effects of food and exercise on mood) to minimise participant-expectancy effects. The study was approved by the Faculty of Health and Wellbeing Research Ethics Committee, Sheffield Hallam University and all participants provided written informed consent.

Participants were categorised as active and inactive according to their self-reported weekly physical activity (Godin Leisure-Time Exercise Questionnaire (21)). Active participants engaged in regular exercise and met the minimum PA guidelines (22) whilst the inactive did not. A posteriori analysis of the combined heart rate and accelerometer (Actiheart) data was used to confirm the veracity of the self-reported

measure. Calculated Physical Activity Level (PAL) (total daily energy expenditure divided by basal metabolic rate) was 2.04 ± 0.23 (range 1.72-2.30) for the active and 1.49 ± 0.16 (range 1.24-1.74) for the inactive group.

Design and procedures

After completing preliminary assessment, participants undertook two, four-day experimental conditions (one laboratory based and 3 free-living days) in a randomised, crossover fashion with approximately four weeks between each condition (participants' menstrual cycle defined exact time). Experimental laboratory days were scheduled on the same day of the week during the early to mid-follicular phase (days 5-9) of the menstrual cycle. Participants recorded their food intake for two days before the first experimental condition and replicated this intake before the second experimental condition, and were asked to abstain from caffeine, alcohol and vigorous physical activity 24 hours before each experimental condition.

Experimental laboratory days started between 8.00 and 9.30am with participants having fasted for 10-hour overnight (Figure 1). The day commenced with a standard breakfast, followed by either 3 hours of rest (control condition- CON) or two hours of rest separated by one hour of cycling at 50% of maximal oxygen consumption (exercise condition- EX). Following this 3 hour period, participants consumed an *ad libitum* lunch and were then provided with a combined heart rate and accelerometer monitor (Actiheart, Cambridge Neurotechnology, Cambridge, UK) and a food diary that were used to estimate energy intake and expenditure over the following 3 days.

Preliminary Assessment

Anthropometry

Body mass (model 424; Weylux; Hallamshire Scales Ltd, Sheffield, UK) and stature (Harpenden, Holtain Ltd, Crymmych, Wales) were measured to the nearest 0.05 kg and 0.01 m, respectively, and BMI was calculated from the above measures. Percentage body fat was determined via bioelectrical impedance (InBody720, Derwent Healthcare, Newcastle, UK) according to the manufacturer's instructions. These measurements were performed with participants fasted for at least two hours and having refrained from undertaking exercise and voiding beforehand.

Submaximal cycling test

A submaximal cycling test was undertaken to determine the relationship between oxygen consumption and exercise intensity in order to determine the workload needed to elicit 50% of maximum oxygen uptake during the exercise condition. After 15 minutes of warm-up, participants completed four, 4-min exercise stages at 60 rpm using a Monark cycle ergometer (model 874E, Monark, Sweden). Initial intensity was set according activity status (inactive participants: 60W; active: 60 or 90W) with 30W increases at the end of each stage. Oxygen consumption and carbon dioxide production were determined using a breath-by-breath gas analysis system (CPX Ultima, Medical Graphics, Gloucester, UK), which was calibrated before each test using a 3-liter syringe and gases of known concentration. Heart rate was assessed continuously during exercise (Polar F4, Kempele, Finland).

Maximal cycling test

A maximal cycling test was also undertaken to determine the participants' maximal oxygen consumption in which participants cycled continuously through 3-min stages until volitional exhaustion. Initial exercise intensity was equal to that of the last stage of the submaximal cycling test and workload increased by 30W at the end of each stage. Participants were given strong verbal encouragement throughout and the test which ended when participants could not continue or failed to maintain the pedalling rate for 20 consecutive seconds. Cycling-specific maximal oxygen consumption was confirmed as attained, when two or more of the following criteria were met: heart rate within 15 beats.min^{-1} of predicted maximum heart rate (205.8–0.685(age)) (23), an increase in oxygen consumption ($\dot{V}O_2$) of less than 100 ml.min^{-1} despite an increase in exercise intensity, and a respiratory exchange ratio (RER) greater than 1.15.

Experimental Days

Breakfast meal

Upon arrival, participants consumed a breakfast meal comprising a bowl of cereal (CornFlakes, Kellogg's, UK) with fresh semi-skimmed milk (Sainsbury, UK) and a glass of orange juice (Drink Fresh, DCB Foodservice, UK) with a mean energy content of 12.8% from protein, 76.5% from carbohydrate and 9.6% from fat. Breakfast was standardised between conditions, and quantities determined based on individual body mass (23.6 kJ/kg of body mass) (10, 11). Participants ate individually in air-conditioned testing cubicles equipped with Sussex Ingestion Pattern Monitors (SIPM).

Exercise and control periods

Following breakfast consumption, participants rested for 60 minutes in a seated position. Participants were allowed to read and undertake work in a laboratory devoid of any food-related cues. During CON, participants remained at rest for a further 120 minutes (180 minutes in total). However, during EX, participants cycled at 50% of maximal oxygen consumption for 60 minutes, and then rested for 60 minutes (seated devoid of any food-related cues). During the exercise bout and equivalent period of rest during CON, indirect calorimetry was used to estimate energy expenditure (and ensure participants exercised at the target intensity during EX) (24). Expired air was collected (Harvard Apparatus, Kent, UK) and analysed (GIR250 combined O_2/CO_2 gas analyser, Hitech Instruments, Luton, UK) at 15 min intervals using Douglas Bags during the 60 minute period of exercise or rest.

Ad libitum lunch meal

An *ad libitum* lunch meal was provided to participants after the 180 minute period of rest (CON) or rest/exercise (EX). This was comprised of durum wheat semolina conchiglie pasta (Granaria,Favellatos.r.l, Italy) with tomato and mascarpone cheese sauce (FratelliSacla, S.p.A., Asti, Italy). Energy content was 10.1% from protein, 67.2% carbohydrate and 22.7% fat, with an energy density of 7.4 kJ/g. Participants ate in isolation and care was taken to standardise the test meals. Food was served to participants on each occasion using the same dinnerware and cutlery, and the same verbal script was used by researcher when interacting with participants. Cooking and cooling times were standardised across conditions and the pasta and sauce meal was served to participants in individual air-conditioned testing cubicles on both experimental days at a temperature of 60-65°C. Participants were instructed to ‘‘eat as

much or as little as they wanted". The SIPM were used to covertly measure food intake in grams and prompt the participant to call the researcher, by pressing a call button, once at least 300 g of the lunch meal had been consumed. Following this, the researcher would provide a refill to ensure the empty plate was not used as an external cue to end their meal. This step was repeated until participants indicated that they had finished eating.

Hunger ratings and satiety

Throughout the laboratory period of EX and CON, ratings of perceived hunger were assessed using visual analogue scales (VAS) (Figure 1). The VAS were 100-mm in length preceded by the question "how hungry do you feel?" and anchored at each end by "not at all hungry" and "very hungry". Participants were unable to refer to their previous ratings when completing each VAS. The use of VAS for the measurement of subjective appetite has previously been shown to be valid and reproducible (25).

The suppression of hunger per calorie of intake for the breakfast meal was calculated using the satiety quotient (SQ) (26). As the SQ reflects the capacity of a meal to modulate the strength of postprandial satiety, the SQ was calculated for CON only (as the exercise bout of EX will have independently influenced hunger and SQ ratings). The SQ was calculated using the following formula based on the hunger ratings before, immediately after and 30, 60, 90, 120, 150 and 180 minutes post-consumption, with a higher SQ indicative of a greater satiating efficiency:

$$\text{SQ (mm/kcal)} = \frac{\text{(rating before eating episode - rating after eating episode)}}{\text{energy of the food consumed}} \text{ x } 100$$

Free-living energy expenditure and energy intake

Following completion of the *ad libitum* lunch meal, participants were provided with a dietary record and a combined accelerometer and heart rate monitor (Actiheart, Cambridge Neurotechnology, Cambridge, UK) to measure free-living food intake and energy expenditure, respectively, for the remainder of the experimental day and over the subsequent three days. Participants received guidance on how to complete the diet diary, and were instructed to weigh and record all items consumed. In cases where weighing was not possible (e.g. eating at a restaurant), participants were asked to use standard household measures to estimate portion sizes. Dietary data was analysed using NetWisp software (3.0; Tinuviel, Warrington, UK) to estimate energy and macronutrient intake. During the same period, participants wore a combined accelerometer and heart rate monitor on their chest using electrocardiogram (ECG) electrodes (E4 T815 Telectrode, Surrey, UK). These monitors recorded activity every 15s and participants were instructed to wear the device at all times. A revised branched group calibration equation (27) was used to convert heart rate and accelerometer data to energy expenditure.

Statistical analyses

All analyses were undertaken with SPSS for windows (22.0, Chicago, IL). Histograms and Shapiro-Wilk tests were used to check for normal distribution whilst Levene's and Mauchley's tests were used to check for homogeneity of variance and sphericity, respectively. Relative energy intake (REI) was calculated as the difference between lunch energy intake and the net exercise-induced energy expenditure (exercise condition) or the resting energy expenditure (control condition).

Independent Student's t-tests and a Welch's t-test were used to assess between group differences for participants' characteristics and relative exercise intensity, respectively. Two-way mixed-design factorial ANOVAs (Group × Time of day) and (Group × Condition) were used to examine the SQ and experimental day's lunch energy intake, respectively. Three-way mixed-design factorial ANOVAs (Group × Condition × Time) were used to analyse subjective hunger ratings, daily energy intake and energy expenditure and macronutrient intakes. In the latter analyses energy intake on the experimental day was calculated by summing participants' energy intake throughout the day (breakfast + *ad libitum* lunch + remainder of experimental day). However, the same formula was not applied to macronutrient intake because the macronutrient values for breakfast and lunch of the experimental day were fixed. Therefore, macronutrient intake for the experimental day is limited to the free-living period of that day (i.e. remainder of the experimental day).

Post hoc tests were performed using Bonferroni adjustments. Standardised mean difference effect sizes (Cohen's *d*) were calculated by dividing the mean difference by the pooled standard deviation whereas partial eta squared (η_p^2) were calculated by dividing the sum of squares of the effect by the sum of squares of the effect plus the sum of squares of the error associated with the effect (28). All outcomes are presented as means and standard deviations (mean ± SD) unless otherwise stated. Statistical significance was accepted as $P < 0.05$.

Results

Baseline characteristics and relative exercise intensity during EX

Participant characteristics are presented in Table 1. While there were no differences in age (t(14) = -1.38, P = 0.188, d = -0.74), stature (t(14) = 0.77, P = 0.454, d = 0.41), body mass (t(14) = -1.44, P = 0.888, d = -0.08) and BMI (t(14) = -0.64, P = 0.534, d = -0.34) between groups, active participants had greater $\dot{V}O_{2max}$ (mean difference = 12.7 $ml.kg^{-1}min^{-1}$; t(14) = 7.53, P < 0.001, d = 4.03) and lower percentage of body fat (mean difference = -9.3%; t(14) = -3.69, P = 0.002, d = -1.97) than inactive participants. By design, relative exercise intensity during EX did not differ between active and inactive groups (50.1 ± 2.1% vs. 55.2 ± 9.5% of $\dot{V}O_{2max,}$ respectively; t(7.69) = -1.50, P = 0.17, d = -0.80). However, exercise-induced energy expenditure during EX was higher in the active group than the inactive group (mean difference = 335 kJ; 95% CI 95 to 576 kJ, t(14) = 2.99, P = 0.01, d = 1.60).

Hunger, satiety quotient and laboratory *ad libitum* energy intake

Hunger changed over time (F(3.1, 43.5) = 44.623, P < 0.001, η_p^2 = 0.76) but there were no differences between conditions (F(1, 14) = 0.002, P = 0.962, η_p^2 < 0.01) or groups (F(1, 14) = 0.112, P = 0.743, η_p^2 = 0.01) (Fig. 2).

Satiety quotient decreased over time (F(2, 29) = 13.609, P < 0.0001, η_p^2 = 0.49), and was higher in the active than inactive group (14.7 ± 4.3 $mm.kcal^{-1}$ vs. 7.7 ± 4.1 $mm.kcal^{-1}$, F(1, 14) = 11.031, P = 0.005, η_p^2 = 0.44) (Figure 3) but there was no time*group interaction (F(2, 29) = 0.716, P = 0.501, η_p^2 = 0.05).

There were no differences between conditions (F(1, 14) = 1.962, P = 0.183, η_p^2 = 0.12), groups (F(1, 14) = 2.311, P = 0.151, η_p^2 = 0.14), or a group*condition interaction (F(1, 14) = 0.599, P = 0.452, η_p^2 = 0.04) for absolute energy intake (Table 2), however, there was a condition effect for relative energy intake (F(1,14) = 11.735, P = 0.004, η_p^2 = 0.46) which was lower in EX than CON (1417 ± 926 kJ vs. 2120 ± 923 kJ, respectively).

Free-living daily energy and macronutrient intakes

Due to an incomplete food diary, one participant in the inactive group was excluded from the analyses, therefore analyses were made with 8 active and 7 inactive participants per group. There were no differences between days (F(3, 39) = 0.943, P = 0.429, η_p^2 = 0.07), conditions (F(1, 13) = 0.399, P = 0.538, η_p^2 = 0.03), groups (F(1, 13) = 1.506, P = 0.241, η_p^2 = 0.10) or interactions (all P > 0.622) for daily energy intake on the free-living days (Figure 4). There was a condition effect for the percentage of energy consumed from protein (F(1, 13) = 7.644, P = 0.016, η_p^2 = 0.37) and carbohydrates (F(1, 13) = 5.887, P = 0.031, η_p^2 = 0.31), such that participants consumed more carbohydrates and less protein during EX than CON (CHO: 53 ± 5% vs. 49 ± 7%; Protein: 14 ± 3% vs. 16 ± 4%, respectively). There were no differences for fat intake (all P > 0.106).

Free-living daily energy expenditure

Due to incomplete heart-rate and accelerometer monitor data in two participants (removed due to skin irritation), analyses are for 7 active and 7 inactive participants. During the three free-living days after the experimental laboratory days, TEE was different between groups (F(1, 12) = 14.141, P = 0.003, η_p^2 = 0.54), with the active

group expending more energy (mean difference = 3527 kJ; 95% CI 2148 to 4906 kJ). This difference is primarily due to a higher PAEE of the active group (active vs. inactive: 5244 ± 1791 kJ vs. 2189 ± 879 kJ; F(1, 12) = 19.336, $P = 0.001$, $\eta_p^2 = 0.62$). However, there were no differences in TEE (exercise vs control: 10984 ± 2861 kJ vs. 10284 ± 2097 kJ, F(1, 12) = 2.825, $P = 0.119$, $\eta_p^2 = 0.19$) and PAEE (exercise vs control: 4034 ± 2338 kJ vs. 3399 ± 1726 kJ, F(1, 12) = 2.861, $P = 0.117$, $\eta_p^2 = 0.19$) between conditions during the three days after the experimental days.

Discussion

This study examined the effects of an acute bout of cycling on the immediate and subsequent free-living energy intake and PAEE in active and inactive pre-menopausal women not using hormonal contraceptives. There were no differences between EX and CON for *ad libitum* lunch intake on the laboratory test days, or daily energy intake and PAEE during the subsequent free-living period. These data therefore suggest that a bout of aerobic exercise does not elicit acute or delayed compensatory in total daily energy intake or PAEE. Interestingly though, active individuals displayed a stronger satiety response to the standardised breakfast meal used during the laboratory test days compared to their inactive counterparts, adding to the growing literature indicating that an individual's habitual physical activity status moderates the sensitivity of short-term appetite control (7).

Consistent with previous research (19), the present study failed to observe any acute differences between CON and EX for subjective hunger or absolute energy intake during the *ad libitum* lunch meal. As such, after adjusting for energy expended during the exercise/rest period, lunch REI was lower in the exercise condition. These findings are consistent with a recent meta-analysis indicating that acute bouts of

aerobic exercise are effective in inducing acute energy deficits (at the mean or group level, at least) (19). When high intensity exercise is used (≥70% of $\dot{V}O_{2max}$), there is evidence of 'exercise-induced anorexia', such that hunger is transiently suppressed post-exercise (29). However, this effect is not always seen following low intensity exercise (such as that used in the present study).

While a 2-5 day 'lag' in energy intake compensation has been noted following dietary perturbations to energy balance (16, 30, 31), whether such corrective responses in energy intake exist after exercise-induced perturbations has received less attention. In the present study, there was no evidence of delayed compensation in energy intake (or expenditure) during the three free-living days subsequent to the bout of cycling used in the present study. However, whether delayed compensation is seen following exercise-induced energy deficits of a greater magnitude, or when repeated exercise-induced energy deficits are induced over consecutive days, is unclear. This is of particular importance given that exercise interventions often report that losses in body mass are lower than would be expected based on objective measures of exercise-induced energy expenditure (32).

In agreement with previous studies (7), no difference in absolute EI at the laboratory *ad libitum* lunch meal was seen between the active and inactive individuals following the 60 min bout of cycling (despite a greater exercise-induced energy expenditure in active individuals). However, greater SQ was observed in the active than inactive group following the standardised laboratory breakfast meal, indicating that the meal produced more subjective postprandial satiety in active individuals than inactive individuals. Indeed, this was despite a tendency for high fasting hunger levels in the active group. Using a preload test meal paradigm, active males and females have previously been shown to be better able to adjust energy intake to the energy

content of a prior preload than inactive individual (7, 13, 15). Furthermore, medium-term exercise training in previously inactive males and females has been shown to increase hunger in the fasted state and the SQ response to fixed energy meals (33, 34).

While the underlying mechanisms remain to be determined, the present data support the notion that active individuals have better short-term appetite control than their inactive counterparts, which over the longer-term, may help with body mass regulation. Indeed, while it could be argued that any differences between the active and inactive group may reflect differences in body composition rather than physical activity levels *per se*, these differences in body composition actually serve to further emphasise the importance of physical activity in body mass management. These differences in body composition may be important in the regulation of appetite as fat-free mass, as the main determinant of resting metabolic rate, has recently been shown to play an important role in day-to-day food intake (35). Furthermore, while high levels of habitual activity are thought to improve the sensitivity of short-term appetite control, potentially due to enhanced gut mediated satiety signalling (7), inactivity may amplify hedonic states and behavioural traits favouring overconsumption indirectly through increased adiposity (7). However, further research specifically examining the mechanisms through which habitual inactivity moderates appetite regulation is needed.

During the three day free-living period, there were no differences in energy expenditure between EX and CON, suggesting that a single bout of exercise did not alter PAEE over subsequent days. These results are in agreement with our previous studies in men (10) and women taking oral contraceptives (11), suggesting that a single bout of low-intensity cycling does not elicit a transient suppression in hunger,

or compensatory changes in daily physical activity energy expenditure, irrespective of habitual physical activity, sex or use of oral contraceptives.

While there were no differences in daily energy intake between EX and CON, both active and inactive groups consumed less energy from proteins and more from carbohydrates over the free-living days of EX than during CON. While it is acknowledged that the magnitude of these changes was small, the effect of exercise on dietary macronutrient selection/preference has received little attention. Indeed, as the effect of exercise on food intake has primarily been limited to the subsequent 24-hour period, the impact of long-term exercise training on macronutrient intake remains unclear. The change in macronutrient intake observed here could be explained by participants being motivated to seek specific foods to restore energy stores or preferences for tastes associated with the carbohydrates needed to replenish the glycogen stores (36). The ability of an acute bout of exercise to improve psychological wellbeing (37, 38) could also be related to changes in protein intake. For instance, lower energy intake of protein during the first 10 days of the menstrual cycle (includes period over which the experimental studies were completed) has been associated with higher ratings of wellbeing in healthy women not taking oral contraceptives (39).

It should be noted that these findings are in contrast to our previous study in which inactive women taking oral contraceptives demonstrated a suppression of energy intake on the day following exercise (11). Given the study design and the participant characteristics did not differ other than the use of oral contraceptives, it is plausible to suggest that this discrepancy may partially be accounted for by the effect of such medication on appetite. Indeed, in a combined analysis of data from our present and that collected in our previous study (*see supplementary online material*),

examination of the total mean energy intake over the 4 days revealed an interaction between activity status and oral contraceptives ($P = 0.038$). Energy intake was higher in inactive women taking oral contraceptives (OC) compared to inactive women not taking oral contraceptives (Non-OC) (9419 ± 939 vs 7543 ± 2312 kJ, respectively; $P = 0.043$), but no difference was seen between OC and Non-OC active women (OC vs Non-OC: 8385 ± 1037 vs 8905 ± 1987 kJ, $P = 0.483$). The mechanisms responsible for this effect remain unclear but highlights future studies should consider OC use as a potential confounding factor. Inactive women energy intake in the present study was lower than that previously seen in our previous study (11), and thus, there may have been a 'floor effect' where further reductions in energy intake were not seen. Further research is now required to confirm these findings and determine the precise influence of hormonal contraceptives on exercise-induced compensatory responses.

Limitations include participants being young healthy women; therefore findings might not apply to other populations. Ovarian hormones (e.g. estradiol) were not measured in the present study (or our previous study), so their impact on appetite regulation could not be directly assessed. Sample size may have limited the power to detect differences in energy intake during the free-living period of the study and examine for differences between physical activity groups, however, this was due to the highly controlled experimental environment. Moreover, sample size is in the range of similar studies (40, 41, 42). The *ad libitum* test meal was offered at a fixed time to ensure that differences in time did not affect energy intake. Nevertheless, allowing the participants to choose the time of their next meal may have revealed further effects. It is important to be cautious when interpreting free-living energy intake and expenditure data because the available methods are heavily dependent on participants' compliance with instructions. Finally, combined heart-rate and accelerometer data

was converted to energy expenditure using a revised branched group calibration equation and not calibrated to each participant individually.

This study demonstrated that an acute bout of low-intensity cycling did not elicit changes in hunger and lunch energy intake in active and inactive women not using hormonal contraceptives. However, exercise induced a decrease in relative energy intake meaning that an acute energy deficit persisted after lunch. The stronger subjective satiety response to the standardised breakfast meal in active women also supports a growing body of evidence demonstrating more sensitivity in short-term appetite control in habitually active individuals. There were no differences in energy intake and expenditure during the remainder of the experimental day or any of the subsequent three days between conditions. These findings support the use of low-intensity aerobic exercise to induce a short-term negative energy balance in women not taking hormonal contraceptives and a stronger satiety response in active individuals. Together with findings from our previous study, the present study also suggests that future studies should consider OC use as a potential confounding factor.

Conflict of interest

None of the authors had any conflict of interest regarding any aspect of this study.

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595 # Tables

596 **Table 1.** Participants' baseline characteristics

	Active	**Inactive**
Age (years)	21.9 ± 4.0	24.5 ± 3.5
Stature (m)	1.68 ± 0.07	1.65 ± 0.07
Body mass (kg)	62.1 ± 5.8	62.7 ± 9.9
BMI ($kg.m^{-2}$)	22.2 ± 2.0	23.0 ± 3.1

Body fat (%) *	23.6 ± 5.7	32.8 ± 4.2
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1}) **	38.8 ± 4.2	26.1 ± 2.3
Cognitive restraint scale (TFEQ)	11.6 ± 3.0	11.0 ± 3.4
Severity of premenstrual symptoms (SPAF)	18.1 ± 5.8	17.6 ± 5.9

N=8 per group; values presented as mean ± SD.

BMI = body mass index; $\dot{V}O_{2max}$ = maximal oxygen consumption; TFEQ = three-factor eating questionnaire; SPAF = shortened premenstrual assessment form.

* Means significantly different ($P < 0.01$).

** Means significantly different ($P < 0.001$).

Table 2. *Ad libitum* lunch meal energy intake

	Active	**Inactive**
Absolute EI during EX (kJ)	2965 ± 583	2458 ± 1296
Absolute EI during CON (kJ)	2843 ± 1099	2033 ± 619
Relative EI during EX (kJ)*	1503 ± 452	1331 ± 1319
Relative EI during CON (kJ)	2518 ± 1108	1723 ± 601

N=8 per group; values presented as mean ± SD; EI = energy intake. EX = exercise condition; CON = control condition. Relative energy intake (REI) is the difference between lunch energy intake and the net exercise-induced energy expenditure (exercise condition) or the resting energy expenditure (control condition).

* Condition effect ($F(1,14) = 11.735$; $P = 0.004$, $\eta_p^2 = 0.46$).

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Figures captions

Figure 1. Schematic representation of the laboratory period of the experimental days.

Figure 2. Subjective feelings of hunger (n = 8 per group; means ± SEM). Hatched rectangles are consumption of meals; dark rectangle is equivalent to the 60 minutes cycling period.

Figure 3. Satiety quotient (n = 8 per group; means ± SEM) Hatched rectangles represent consumption of breakfast and *ad libitum* lunch.

Figure 4. Daily energy intake (n = 8 for active and n = 7 for inactive; means ± SEM).

Supplementary file. Combined 3-way mixed model ANOVA of total 4-day EI data from the present study (n = 8 for active non-OC, n = 7 for inactive non-OC; means ± SEM) and from Rocha, J., Paxman, J., Dalton, C., Winter, E., & Broom, D. Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive pre-menopausal women taking oral contraceptives. *Appetite, 89*, 183-191, Elsevier, 2015 study (n = 10 for active OC, n = 9 for inactive OC; means ± SEM). * denotes $P < 0.05$ Inactive OC vs Non-OC.