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

Joel Rocha[1*], Jenny Paxman[2], Caroline Dalton[3], Edward Winter[4] and David Broom[4]

[1]Department of Physiology, School of Medicine, Faculty of Health Sciences, Trinity College Dublin, D2

[2]Food and Nutrition Group, Sheffield Business School, Sheffield Hallam University, S1 1WB

[3]Biomedical Research Centre, Faculty of Health and Wellbeing, Sheffield Hallam University, S1 1WB

[4]Academy of Sport and Physical Activity, Faculty of Health and Wellbeing, Sheffield Hallam University, S10 2BP

*Corresponding author

Address for correspondence: Joel Rocha, Department of Physiology, School of Medicine, Faculty of Health Sciences, Trinity College Dublin, D2, Republic of Ireland

E-mail: Borgespj@tcd.ie

Telephone: +353 (0)1896 1937

Email addresses:

JP: J.R.Paxman@shu.ac.uk

CD: C.F.Dalton@shu.ac.uk

EW: E.M.Winter@shu.ac.uk

DB: D.R.Broom@shu.ac.uk

Abstract (314 words)

This study examined the effects of an acute bout of exercise of low-intensity on food intake and energy expenditure over four days in women taking oral contraceptives. Twenty healthy, active (n=10) and inactive (n=10) pre-menopausal women taking oral contraceptives completed two conditions (exercise and control), in a randomised, crossover fashion. The exercise experimental day involved cycling for one hour at an intensity equivalent to 50% of maximum oxygen uptake and two hours of rest. The control condition comprised three hours of rest. Participants arrived at the laboratory fasted overnight; breakfast was standardised and an *ad libitum* pasta lunch was consumed on each experimental day. Participants kept a food diary to measure food intake and wore an Actiheart to measure energy expenditure for the remainder of the experimental days and over the subsequent 3 days. There was a condition effect for absolute energy intake (exercise vs. control: 3363 ± 668 kJ vs. 3035 ± 752 kJ; p = 0.033, d = 0.49) and relative energy intake (exercise vs. control: 2019 ± 746 kJ vs. 2710 ± 712 kJ; $p < 0.001$, d = -1.00) at the *ad libitum* lunch. There were no significant differences in energy intake over the four days in active participants and 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 (mean difference = -1974 kJ; 95% CI -1048 to -2900 kJ, $p = 0.002$, $d = -0.89$). There was a group effect ($p = 0.001$, $d = 1.63$) for free-living energy expenditure, indicating that active participants expended more energy than inactive participants during this period. However, there were no compensatory changes in daily physical activity energy expenditure. These results support the use of low-intensity aerobic exercise as a method to induce a short-term negative energy balance in inactive women taking oral contraceptives.

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

Introduction

Regular exercise is prophylactic and promotes metabolic adaptations that improve physical and mental health (Bertheussen et al., 2011; Chaput et al., 2010; Tremblay & Therrien, 2006). In addition, the ability of exercise to disrupt energy balance through its effects on food intake and energy expenditure makes it important for the maintenance of adequate body mass and composition.

Exercise-induced behavioural and physiological compensatory responses in energy intake and/or non-exercise energy expenditure (King et al., 2007) might explain the high inter-variability responses of exercise interventions that are designed to reduce body mass. Additionally, these responses differ according to participants' habitual physical activity (Martins et al., 2008) and sex (Hagobian et al., 2010), therefore, it is important to control for these variables. Indeed, results from a recently published meta-analysis on the effect of acute exercise on subsequent (within 24 hours post-exercise) energy intake (Schubert et al., 2013) suggested that individuals who engage in less physical activity are more likely to experience an anorexic effect of exercise.

In addition, findings from our previous study (Rocha et al., 2013) suggest that active men compensate for an acute exercise-induced energy deficit quicker than inactive men. However, it is still not known if these findings occur in women.

Most studies investigating the effects of an acute bout of exercise on hunger and food intake in active (Finlayson et al., 2009; Hagobian et al., 2012; Lluch et al., 1998; Lluch et al., 2000; Larson-Meyer et al., 2012) and inactive women (George & Morganstein, 2003; Maraki et al., 2005; Reger et al., 1984; Tsofliou et al., 2003; Unick et al., 2010) have reported no changes in hunger and/or energy intake. Despite the majority of studies reporting a consistent lack of an acute effect of exercise on energy intake, most of these studies have assessed energy intake in only one subsequent meal one to two hours post exercise (Finlayson et al., 2009; George & Morganstein, 2003; Hagobian et al., 2012; Larson-Meyer et al., 2012; Tsofliou et al., 2003; Unick et al., 2010), so any compensation that may have occurred later on the day or during subsequent days was not measured.

According to the United Nations, oral contraceptives are the most common modern contraceptive method (including both reversible and non-reversible methods) in developed countries and the third most common in developing countries (United Nations Department of Economic and Social Affairs, 2009). Oral contraceptives (OCs) have now become a feature of everyday life, with globally, nearly 200 million women taking the "pill" packet on a daily basis (Chadwick et al., 2012). However, there is little evidence of the effects of exercise on appetite and energy intake in women taking OCs. For instance, only one study has provided information on the use of oral contraceptives by all participants (Hagobian et al., 2012), while several of these studies (George & Morganstein, 2003; Kissileff et al., 1990; Maraki et al., 2005; Reger et al., 1984) examined premenopausal women without controlling variables such as the regularity of the menstrual cycles, premenstrual or unusual menstrual symptoms, menstrual phase when testing and the use of hormonal contraceptive preparations. This is despite research suggesting higher energy intakes at the luteal phase than follicular phase and that women prone to premenstrual or unusual menstrual symptoms have greater fluctuations of energy intake and appetite (Dye & Blundell, 1997). Moreover, some studies examining the effects of OCs on energy intake reported an increase (Eck et al., 1997; Naessen et al., 2007) and others no difference (Bancroft & Rennie, 1993; McVay et al., 2011; Tucci et al., 2010).

Other limitations include the use of *ad libitum* buffet-style meals (George & Morganstein, 2003; Reger et al., 1984, Tsofliou et al., 2003; Unick et al., 2010), the lack of definition of participants' inactivity (Reger et al., 1984; Tsofliou et al., 2003), the estimation of energy expenditure using heart rate equations (George & Morganstein, 2003; Maraki et al., 2005) and the lack of measurement of energy expenditure (Tsofliou et al., 2003). Therefore, the present study sought to overcome some of these limitations by controlling for participants' premenstrual or unusual menstrual symptoms, menstrual phase when testing and the use of hormonal contraceptive preparations. In addition, this study increased the observation period to four days and used well-controlled and validated methods to measure *ad libitum* energy intake in the laboratory and free-living energy expenditure.

No study has examined acute effects of an acute bout of exercise on food intake and physical activity energy expenditure while directly comparing active and inactive women taking oral contraceptives. Findings from this study will inform whether an exercise challenge will alter these groups' physical activity and energy intake over a number of days.

Methods

Participants

With institutional ethics approval, twenty-nine healthy women were recruited. Nine participants withdrew from the study stating personal reasons (n=4), not able to find suitable dates for the experimental days (n=3), not liking the breakfast provided (n=1) and feeling uncomfortable wearing the Actiheart (n=1). Therefore, 20 healthy, active (n=10; age 22.6 ± 3.6 years; body mass 61.4 ± 4.4 kg; body mass index 21.9 ± 1.3 $kg.m^{-2}$) and inactive (n=10; age 22.3 ± 3.2 years; body mass 60.1 ± 4.3 kg; body mass index 21.6 ± 2.0 $kg.m^{-2}$) women completed the study. Participants were non-smokers, had regular menstrual cycles (21-35 days), were not pregnant or lactating, had no known history of cardiovascular or metabolic diseases, were not dieting, had a stable body mass (± 2 kg) for 6 months before the study and were not taking any medication except oral contraceptives (16 participants were taking combined oral contraceptives and 4 progesterone-only pills). Severity of premenstrual symptoms was assessed through the shortened premenstrual assessment form (SPAF; Allen et al., 1991) that consists of 10 items rated on a scale from 1 (not present or no change from usual) to 6 (extreme change, perhaps noticeable even to casual acquaintances). The mean score for the SPAF for the active and inactive groups were 16.8 ± 6.8 and 17.6 ± 5.8, respectively with no participant scoring greater than 28 (scores greater than 30 are indicative of moderate premenstrual symptoms) (Allen et al., 1991). Participants mean score for cognitive restraint based on the revised version of the Three-Factor Eating Questionnaire (Karlsson et al., 2000) was 11.6 ± 3.1 for the active and 10.5 ± 3.3 for the inactive group with all participants having a cognitive restraint score lower than 18. Self-reported weekly physical activity assessed by a modified version of Godin Leisure-Time Exercise Questionnaire (GLTEQ) (Godin & Shepard, 1985) was used to allocate participants to the active (engaged in regular exercise and undertaken at least 150 minutes per week of moderate-intensity physical activity i.e., physical activity that noticeably increases breathing, sweating and heart rate and is between 12-14 in the 6-20 rating of perceived exertion scale), and inactive groups (did not engage in regular exercise and did not meet the minimum physical activity recommendation guidelines of 150 minutes of moderate-intensity physical activity per week) (Department of Health, 2004). Veracity of self-reported measures of physical activity was confirmed with a posteriori analysis of the Actiheart data. These data calculated individual Physical Activity Level (PAL) by dividing participants' total energy expenditure in a 24-hour period by their basal metabolic rate. The active group had a mean PAL of 1.79 ± 0.13 and the inactive 1.56 ± 0.15,which according to the classification of lifestyles in relation to PAL in adults (WHO, 2004) identified them as having an active to moderately active lifestyle (1.70-1.99) and a sedentary to light activity lifestyle (1.40-1.69), respectively.

Design and procedure

To minimise participant-expectancy effects, participants were blinded about the true purpose of the study (effects of an acute bout of exercise on immediate and subsequent three days energy intake and expenditure) and were informed that the investigation was assessing how food and physical activity affected mood.

Before the experimental days, participants attended the laboratory for one preliminary session consisting of two exercise tests (submaximal and maximal cycling tests), screening and habituation with all procedures. After the preliminary session, participants were allocated either to the active or inactive group and completed the study in a randomised, crossover fashion with approximately 4 weeks (time varied according to participants' menstrual cycle) between both conditions (exercise and control). Experimental days were booked during the first week they restarted taking the oral contraceptives or, if continuous, when a new pack was started. This control means that findings are limited to the mechanisms operating at the examined stages, however, this was undertaken to minimise possible effects of sex hormones in energy intake (Dye & Blundell, 1997) and expenditure (Bowen et al., 2011). The experimental days were completed on the same day of the week to control for dietary and physical activity habits. Additionally, participants were asked to refrain from consuming alcohol or caffeine and taking part in vigorous physical activity in the 24 hours prior to each experimental day and to record their food intake for two days before the first experimental day. This allowed participants to keep their activity patterns consistent between conditions and replicate their food intake during the two days before the second experimental day.

On the experimental days, participants arrived at the laboratory between 8.00 and 9.30am after a 10-hour overnight fast with only water consumption permitted (Figure 1). On arrival participants consumed a standard breakfast within 15 minutes. On the exercise experimental day, participants rested for one hour, cycled for one hour at 50% of maximum oxygen uptake and then rested for another hour. On the control experimental day this was equivalent to three hours of rest (participants had to remain seated while working, reading or listening to music and were monitored to ensure that they abstained from any food related cues) from the end of breakfast until the beginning of lunch. After eating the *ad libitum* lunch participants were fitted with an Actiheart and given a food diary that was used to estimate three-day food intake and energy expenditure. At the end of the study participants were debriefed about the true purpose of the study.

Measures

Anthropometry

Procedures adhered to recommendations of the International Society for the Advancement of Kinanthropometry (ISAK). Stature, body mass, waist and hip circumference were measured as previously described (Rocha et al., 2013). Body Mass Index (BMI) was calculated as body mass in kilograms divided by the square of stature in meters. Waist circumference was divided by hip circumference to determine waist to hip ratio. Percentage of body fat was obtained via a bioelectrical impedance body composition analyser InBody720 (Derwent Healthcare Ltd, Newcastle upon Tyne, UK) according to the manufacturer’s instructions. Measurements were performed without shoes and socks with participants being instructed to slightly abduct their arms and remain still in the upright position. All bioelectrical impedance measurements were performed with the participants having fasted for at least two hours and without having engaged in any kind of exercise during that day.

Submaximal and maximal cycling tests

Before the tests, participants were allowed some time (no longer than 15 minutes) to warm-up and accustom themselves to cycle-ergometer exercise (model 874E, Monark, Sweden). The submaximal–incremental cycling test was completed to determine the relationship between exercise intensity and oxygen consumption. The test consisted of a maximum of 16 min of continuous cycling divided into four, 4-min stages. The pedalling rate was initially set at 60 rpm but participants were allowed to choose a different rpm if they felt uncomfortable or could not maintain this cadence. Initial exercise intensity was adjusted to individual activity status with inactive participants starting at 60 W and active at 60 W or 90 W. At the end of each 4-min stage, exercise intensity was increased by 30 W. Participants were required to undertake the entire test whilst seated. A calibrated MedGraphics CPX Ultima (Medical Graphics Ltd, Gloucester, UK) gas analysis system determined oxygen consumption and carbon dioxide production. A heart rate monitor (Polar F4, Polar Electro, Kempele, Finland) was used to assess heart rate continuously which was recorded every 15 s during the last minute of each stage. In addition, ratings of perceived exertion (Borg, 1973) were assessed during the same time periods.

After allowing for sufficient recovery from the sub-maximal test participants began the maximal oxygen uptake cycling test. The test involved cycling continuously through 3-min stages until volitional exhaustion. The initial pedalling rate was the same as the one chosen for the submaximal test and initial intensity of exercise was set equal to the last stage of the submaximal cycling test. At the end of each 3-min stage exercise intensity was increased by 30 W. Strong verbal encouragement was given to all participants throughout the test which was terminated when the participant failed to maintain cycling cadence for 20 consecutive seconds or signalled that they could not continue. To confirm that a true cycling-specific maximal oxygen consumption had been attained, two or more of the criteria were met: participant heart rate within 15 beats/min of age-predicted maximum heart rate (205.8–0.685(age)) (InBar et al., 1994), an increase in oxygen consumption ($\dot{V}O_2$) of less than 100 ml min_1 despite an increase in exercise intensity, and a RER greater than 1.15. The participants maximal oxygen consumption and oxygen cost of cycling was used to ascertain the exercise intensity necessary to elicit 50% of maximal oxygen consumption.

Breakfast and *ad libitum* lunch meal

Breakfast was standardised across conditions and quantities were determined based on individual body mass (23.6 kJ/kg of body mass).This meal consisted of a bowl of cereal (CornFlakes, Kellogg's, UK) with fresh semi-skimmed milk (Sainsbury, UK) and a glass of UHT orange juice (Drink Fresh, DCB Foodservice, UK). The *ad libitum* lunch meal consisted of durum wheat semolina conchiglie pasta (Granaria,Favellatos.r.l, Italy) served with tomato and mascarpone cheese sauce (FratelliSacla, S.p.A., Asti, Italy). This meal comprised 10.1% energy from protein, 67.2% carbohydrate and 22.7% fat, with an energy density of 7.4 kJ/g. Cooking and cooling times were standardised across conditions and the pasta and sauce meal was served on both experimental days at a temperature of 60-65°C.

Hunger ratings

Hunger ratings were assessed during the experimental trials with 100-mm paper version visual analogue scales (VAS) before and after breakfast, and at 30 min intervals thereafter until the end of lunch. The VAS was preceded by the question "how hungry do you feel?" anchored on the left by "not at all hungry" and on the right by "very hungry" (Flint et al., 2000). Participants placed a vertical mark through the line at the point which best matched their present feeling of hunger. The distance from the left anchor to the vertical mark was then measured with a ruler and used as the hunger score.

Laboratory energy expenditure

Expired air samples were collected in 150 L Douglas Bags (Harvard Apparatus, Edenbridge, Kent, UK) at 15 min intervals during the 60-minute exercise and rest period of the experimental days. Samples were analysed using an oxygen/carbon dioxide gas analyser (Dual Gas Analyser GIR250, Hitech Instruments, Luton, UK) which was calibrated before each analysis. A dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) determined expired air volumes that were corrected to STPD (standard temperature, pressure and dry gas). This method was used to ensure that participants cycled at 50% of their $\dot{V}O_{2max}$ and to estimate energy expenditure by indirect calorimetry (Frayn, 1983).

Free-living energy expenditure

Free-living energy expenditure was estimated using an Actiheart (Cambridge Neurotechnology, Cambridge, UK) that was attached to each participant's chest (lower position described in Brage et al., 2006) using electrocardiogram (ECG) electrodes (E4 T815 Telectrode, Surrey, UK). The Actihearts were set up to collect data in "HR variability" and record activity every 15 seconds. Participants were told to wear the monitor at all times, when awake or asleep including when washing or swimming. At the end of the three-day period, participants returned the Actihearts and the data were downloaded using a docking station and analysed using its commercial software. Heart rate and accelerometer data were converted to energy expenditure using the revised branched group calibration equation (Brage et al., 2007).

Laboratory energy intake

On each experimental day, participants ate their breakfast and *ad libitum* lunch alone in individual air-conditioned testing cubicles equipped with Sussex Ingestion Pattern

Monitors (SIPM). During lunch, participants were not given a specific time to finish eating but were instructed to "eat as much or as little as they wanted". Food intake (in grams) was covertly monitored using the SIPM, which consists of a concealed digital balance (KMB-TM, Kern, Germany) connected to a PC computer. To ensure participants did not use the empty plate as an external cue to end their meal, the SIPM was programmed to prompt the participant to call the experimenter, using a call button, once at least 300 g were consumed to receive a refill. This process was repeated until the participants indicated that they had finished eating. A separate side plate was provided for participants to place cutlery when not eating with them (e.g. still chewing food) to ensure the weight of cutlery did not interfere with the food weighing process.

Free-living energy intake

Participants were instructed to weigh and record all items of food and drink consumed both at home and outside the home in food diaries for the remainder of the experimental days and subsequent three days. All participants received guidance on how to complete the dietary record and measure food portions. When weighing was not possible, participants were asked to estimate portion sizes using standard household measures. Immediately upon receipt, food diaries were reviewed in the presence of the participant to ensure completeness and legibility, with any missing or unclear items being corrected. Food diaries were analysed to estimate energy and macronutrient intake using the dietary analysis software NetWisp (version 3.0; Tinuviel Software, Warrington, UK).

Percentage of energy compensation

Percentage of energy compensation was calculated for the *ad libitum* lunch meal, and for each one of the daily energy intakes (i.e. experimental day and subsequent 3 days).

To calculate the percentages compensation for the *ad libitum* lunch meals and for each day the following formulas were applied:

[(lunch energy intake in exercise condition - lunch energy intake in control condition) / (net exercise-induced energy expenditure)] x 100

[(energy intake of day A in exercise condition - energy intake of day A in control condition) / (net exercise-induced energy expenditure)] x 100

In the latter, A denotes the day for which the percentage compensation is being calculated.

When positive, the percentage compensation values indicated that over the analysed period of time, energy intake was greater in the exercise than in the control condition while negative values indicated a greater intake in the control than in the exercise condition. A value of 100% indicated complete compensation of the net exercise-induced energy expenditure (i.e. the excess energy intake at the exercise compared with the control condition matched for the net exercise-induced energy expenditure). A value of 0% indicates no compensation (i.e. energy intake was the same in both conditions).

Statistical analyses

Statistical Package for the Social Sciences program for windows (SPSS 19.0, Chicago, IL) was used for all analyses. Data were checked for normal distribution using histograms and Shapiro-Wilk tests. Homogeneity of variance and sphericity were checked using Levene's and Mauchley's test, respectively. Area under the curve (AUC) values for hunger were calculated using the trapezoidal rule. Net exercise-induced energy expenditure was calculated as (energy expenditure during the 60 min cycling period - energy expended during equivalent control period). Relative energy intake was calculated as lunch energy intake minus the net exercise-induced energy expenditure or the resting energy expenditure for the exercise and control condition, respectively.

Differences between groups for baseline characteristics, work rate, relative intensity of exercise (% of $\dot{V}O_{2max}$), ratings of perceived exertion (RPE) during exercise and net exercise-induced energy expenditure were assessed by independent Student's t-tests. Percentages of energy compensation were compared between groups using a one-way ANOVA with the Welch test (when homogeneity of variance was violated). Two-way mixed-model ANOVAs (Group × Condition) compared the experimental day's lunch energy intake, energy expenditure, heart rate and respiratory exchange ratio (RER). Three-way mixed-model ANOVAs (Group × Condition × Time) compared subjective hunger ratings, body mass on the experimental days, daily energy intake and expenditure and macronutrient intakes. In these 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 could not be 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 when statistical significance or large effect sizes were present. Cohen's d (standardised mean difference) effect sizes were calculated by dividing the difference between means by the pooled standard deviation thus reflecting differences expressed in standard deviation units. According to Cohen's (1988) guidelines, effect sizes were conservatively interpreted as small (0.2), medium (0.5), and large (0.8) effects. In addition, 95% confidence intervals were determined for energy intake, macronutrient intake, energy expenditure and percentage of energy compensation. Means and standard deviations (mean ± SD) are presented for all outcomes unless otherwise stated. Statistical significance was accepted at the 5% level.

Results

Baseline characteristics

Participant baseline characteristics are presented in Table 1. Active participants had greater $\dot{V}O_{2max}$ and lower percentage of body fat than inactive participants ($p < 0.05$). There were no differences in age, stature, body mass, BMI and waist-to-hip ratio.

Body mass during the experimental days

There were no main or interaction effects ($p > 0.05$) for body mass on the exercise (active start vs. end: 61.1 ± 5.6 kg vs. 61.0 ± 5.6 kg; inactive start vs. end: 61.1 ± 4.3 kg vs. 61.0 ± 4.4 kg) and control experimental days (active start vs. end: 61.1 ± 5.5 kg vs. 61.0 ± 5.5 kg; inactive start vs. end: 60.6 ± 4.2 kg vs. 60.6 ± 4.2 kg).

Hunger ratings

There was a main effect of time ($p < 0.001$) for hunger ratings but there were no interactions or other main effects ($p > 0.05$) (Fig. 2). Differences in hunger ratings were also evaluated using AUC values for the time before and after breakfast (08:45-09:00), the following hours until lunch (09:00-12:00), and the time before and after lunch (12:00-12:20). There was a main effect of time ($p < 0.001$) for hunger AUC values but no interactions or other main effects ($p > 0.05$).

Exercise responses and energy expenditure on the experimental days

The active participants exercised at a higher work rate than the inactive (70.3 ± 11.4 W vs. 57.4 ± 14.2 W; $p = 0.039$), however, the relative intensity of exercise and ratings of perceived exertion were not different between the active and inactive groups (51.2 ± 2.2% vs. 54.0 ± 7.5% of $\dot{V}O_{2max}$; $p = 0.27$; RPE: 11.9 ± 1.6 vs. 11.7 ± 1.2; $p = 0.79$). There were no main or interaction effects for RER ($p > 0.05$) and only a condition ($F(1,18) = 709.5$; $p < 0.001$) effect for heart rate that, as anticipated, was different between the control and the exercise experimental day (72 ± 11 bpm vs. 131 ± 14 bpm, $p < 0.001$). Similarly, there was only a condition ($p < 0.001$) effect for the energy expenditure during the 60 minutes of exercise (1345 ± 195 kJ) and equivalent resting period (325 ± 41 kJ) and no differences between the net exercise-induced energy expenditure of active and inactive participants (1078 ± 132 kJ vs. 964 ± 239 kJ; $p = 0.227$, $d = 0.60$). There was a group effect for total energy expenditure (active vs. inactive: 6389 ± 1036 kJ vs. 4949 ± 841 kJ, $p = 0.001$, $d = 1.61$) and physical activity energy expenditure (active vs. inactive: 2780 ± 857 kJ vs. 1571 ± 727 kJ, $p < 0.001$, $d = 1.60$) during the remainder of the experimental days. There were no other main or interaction effects ($p > 0.05$).

Ad libitum lunch energy intake on experimental days

The energy intake at the *ad libitum* lunch meal for active and inactive participants on both experimental days is presented in Table 2. There was only a condition (p = 0.033, d = 0.49) effect for absolute energy intake at the *ad libitum* lunch with a higher absolute energy intake in the exercise than the control condition (exercise vs. control: 3363 ± 668 kJ vs. 3035 ± 752 kJ). After adjustment of absolute energy intake for the energy expended during the 60 min of exercise/rest (relative energy intake, REI), there was a condition effect ($F(1,18) = 19.723$; $p < 0.001$, $d = -1.00$) with a lower REI in the exercise than the control condition (2019 ± 746 kJ vs. 2710 ± 712 kJ).

Daily energy expenditure

Total free-living energy expenditure indicated that active participants expended more energy than inactive participants over the course of the three days ($F(1,18) = 15.817$; $p = 0.001$, $d = 1.63$, mean difference = 1573 kJ; 95% CI 597 to 2548 kJ). This difference can be explained by the differences in physical activity energy expenditure during this period, which was higher in the active than the inactive group (3639 ± 787 kJ vs. 2363 ± 767 kJ, $p < 0.001$). There were no other main effects or interactions ($p>$ 0.05) for daily energy expenditure.

Daily energy intake

Daily energy intake for both groups is shown in Figure 3. One participant in the inactive group did not complete the full four-day food diary, therefore analyses were made with 10 active and 9 inactive participants. There was a time (p = 0.003) and group (p = 0.036) effect and a trend with a large effect size for a condition x group x time interaction (p = 0.056; d = 0.80) for daily energy intake. Pairwise comparisons showed that energy intake was greater on the experimental days (10180 ± 1670 kJ) than the subsequent first (8535 ± 2511 kJ, p = 0.027, d = 0.81), second (8531 ± 2330 kJ, p = 0.022, d = 0.84) and third (8364 ± 2459 kJ, p = 0.024, d = 0.91) days and that inactive participants had a higher mean energy intake over the four days than the active group (9431 ± 1168 kJ vs. 8385 ± 1364 kJ, p = 0.036, d = -0.86). Post hoc analysis did not show any differences in the active group and inactive participants had only a decrease in energy intake on the first day after the exercise experimental day compared with the same day of the control condition (mean difference = -1974 kJ; 95% CI -1048 to -2900 kJ, p = 0.002, d = -0.89).

Daily macronutrient intake

There were no main or interaction effects for the percentage of energy consumed from protein, fat and carbohydrate (p> 0.05) but there was a trend with a large effect size for condition x group interaction for energy consumed from fat (p = 0.055, d = -1.35). Post hoc analysis demonstrated that only the active group consumed less fat in the exercise than the control condition (mean difference = -6%; 95% CI -11 to -2%, p = 0.012, d = -1.10). No other differences were observed in the inactive and active group.

Percentages of energy compensation

Percentages of energy compensation are presented in Figure 4. There were no significant between group differences for the percentages of energy compensation for the *ad libitum* lunch (active: 43 ± 67% vs. inactive: 16 ± 67%; p = 0.63, d = 0.42), experimental day (active: 109 ± 208% vs. inactive: -49 ± 216%; p = 0.08, d = 0.78), day one (active: 53 ± 346% vs. inactive: -176 ± 150%; p = 0.053, d = 0.90), day two (active: -91 ± 293% vs. inactive: 161 ± 371%; p = 0.21, d = -0.80) or day three (active: 34 ± 267% vs. inactive: 1 ± 253%; p = 0.40, d = 0.13) after the experimental day. Nevertheless, the moderate to large effect sizes for the experimental day, day one and day two after the experimental day suggest possible between group compensatory differences on these days. The cumulative percentage of energy compensation over the four days was not significantly different between groups (active: 104 ± 718% vs. inactive: -62 ± 631%; $p = 0.32$, $d = 0.26$).

Discussion

The present study is the first to examine the effects of an acute bout of low-intensity aerobic exercise on immediate and subsequent three-day energy intake and expenditure in active and inactive women taking oral contraceptives. The main findings arising from this study are that an acute bout of low-intensity aerobic exercise elicited an increase in *ad libitum* energy intake, did not induce significant changes in energy intake over the free-living period in active participants and induced a suppression of energy intake on the first day after the experimental day in inactive participants. Additionally, groups did not differ in physical activity energy expenditure between conditions suggesting that there were no acute compensatory changes to physical activity.

In contrast to our previous study (Rocha et al., 2013), there were no differences between the net exercise-induced energy expenditure in active and inactive women participants in the present study. This occurred despite both groups exercising at the same relative intensity and is possibly explained by the differences between groups' aerobic capacity being less in the present study. There were no changes in body mass between conditions suggesting that participants remained in energy balance during the period of time between the first and the second experimental day. Moreover, participants remained in fluid balance during the laboratory period of the experimental days as no changes were observed in body mass from the start to the end of the exercise/rest periods.

There were no differences in subjective hunger ratings either between groups or conditions in this study. This finding is in agreement with recent studies in active (Finlayson et al., 2009) and inactive women (Unick et al., 2010) and men (Rocha et al., 2013). However, the relationship between exercise intensity and hunger has not been consistently reported in women making it difficult to ascertain if this finding is attributable to the low exercise intensity (≈50% of $\dot{V}O_{2max}$) used in this study. For instance, previous studies have reported no effects on hunger using cycling (King et al., 1996), decreases after running (Reger et al., 1984) and an increase after a combination of aerobic and resistance exercise (Maraki et al., 2005), suggesting that, in women, the acute effect of exercise on hunger is also determined by the type of exercise undertaken.

There was an overall condition effect on absolute energy intake at the *ad libitum* lunch meal that was greater during the exercise than the control experimental day. This finding is not supported by previous studies in active (Hagobian et al., 2012; Lluch et al., 2000; Larson-Meyer et al., 2012) and inactive women (George & Morganstein, 2003; Maraki et al., 2005; Unick et al., 2010) which have reported a lack of an exercise-induced effect on absolute energy intake at the meal immediately after exercise. However, as previously discussed, different research designs and methodological limitations make comparisons difficult. Nevertheless, findings from the present study could be explained by a psychological drive to use food as a reward for exercising (King *et al*., 2007) or by exercise-induced changes in the hedonic response to food (Finlayson et al., 2009). In contrast, adjustment of energy intake for the energy expended during the exercise/rest period showed that both groups had a lower REI after exercise than control, suggesting that, similar to previous research in active (Hagobian et al., 2012, Pomerleau et al., 2004) and inactive women (Unick et al., 2010), participants maintained a short-term negative energy balance.

In this study, there were no significant differences in energy intake during the remainder of the experimental day or subsequent three days in the active group, a finding consistent with the only study examining the effects of exercise on daily energy intake in women (Pomerleau et al., 2004). Conversely, the inactive group had a lower energy intake on the first day after the exercise experimental day compared with control and no other differences in the remaining days. This is a novel finding and suggests that, as with our previous study in men (Rocha et al., 2013), an acute bout of exercise elicits a delayed response in inactive individuals. Despite the lack of significant differences in free-living energy intake in the active group, the mean percentages of energy compensation in the current study elicited a similar pattern to those previously observed in men (Rocha et al., 2013) suggesting that active participants may compensate quicker than inactive participants. In the present study, the energy compensation of active women was close to 100% within the experimental day (109%) whilst the same was not observed in inactive women (-49%). In addition, inactive women reduced their energy intake (-176%) on day one after experimental day before increasing it (161%) on day two after the experimental day providing further support to a more sensitive short-term appetite control in active than inactive individuals. When examining the cumulative percentage of energy compensation over the four days there were no statistical significant differences between groups.

Nevertheless, these values still provides important information regarding each group's overall energy compensation over the 4 days with the active group compensating for approximately all their net exercise-induced energy expenditure (104%) whereas the inactive group increased their exercise-induced energy deficit (-62%). For weight management, the latter values would, if sustained over greater durations, translate to weight loss. However, it is important to acknowledge that it is still not known what threshold, if one exists, separates active from inactive individuals. Hence, these results might not be applicable in the long-term as inactive participants will eventually become active and be able to immediately compensate for the exercise-induced energy deficits.

There were no differences between daily macronutrient intake in the exercise and control condition in the inactive group. However, the active group consumed less energy from fat over the four days of the exercise than the control condition, which is possibly explained by being more motivated to eat foods associated with restoring the expended energy (Blundell et al., 2003). Total energy expenditure and physical activity energy expenditure during the free-living period of the study were not different between conditions suggesting that both groups maintained their physical activity. These results agree with our previous findings in men (Rocha et al., 2013) suggesting that an acute bout of low-intensity aerobic exercise does not elicit compensatory changes in daily physical activity energy expenditure in premenopausal women taking oral contraceptives.

Limitations in this study should be acknowledged. Participants were young healthy women taking oral contraceptives, therefore the findings might not apply to women not taking oral contraceptives, and older or obese adults. Controlling for participants menstrual cycle means that findings are limited to the mechanisms operating at the examined stages, however, this was undertaken to minimise possible effects of sex hormones in energy intake (Dye & Blundell, 1997) and expenditure (Bowen et al., 2011). Energy intake is affected by other factors that could not be controlled in the free-living so it may be that observed differences in energy intake did not arise from physiological regulatory mechanisms but from behavioural/psychological (e.g. emotional states) and/or environmental factors (e.g. presence of other people at meal times). Despite not being statistically significant the percentage of energy compensation group differences on the experimental day and subsequent day one and day two elicited moderate to large effect sizes and therefore it is possible that the low sample size in our study could have limited the statistical power to detect differences in free-living energy intake. Finally, caution should be taken when interpreting energy intake and expenditure data collected in the free-living because this is highly dependent on participants' compliance with methods and instructions making it more susceptible to errors in data collection.

Conclusions

This study demonstrated that an acute bout of low-intensity aerobic exercise did not elicit changes in hunger but increased energy intake at the meal immediately after exercise. Moreover, it induced a decrease in relative energy intake after exercise in both active and inactive pre-menopausal women taking oral contraceptives. There were no significant differences in active participants' daily energy intake over the four days whereas the inactive group decreased their daily energy intake on the first day after the exercise experimental day compared to control suggesting a delayed exercise-induced suppression of energy intake. The percentages of energy compensation have also provided further support to a more sensitive short-term appetite control in active than inactive individuals. Moreover, there were no concomitant compensatory changes in daily physical activity energy expenditure. These findings support the use of low-intensity aerobic exercise to induce a short-term negative energy balance in inactive women, which if sustained, would translate to weight loss.

Conflict of interest

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

Authors' contributions

J.R. conceived the study, recruited the participants, collected the data, performed the data analysis and wrote the manuscript. D.B., J.P, E.W. and C.D. obtained the funding, contributed to the design of the study and critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Engineering for Life (EFL) and the Engineering and Physical Sciences Research Council (EPSRC) for their help financing this research and all the volunteers for their participation in this study.

References

Allen, S. S., McBride, C. M., &Pirie, P. L. (1991). The shortened premenstrual assessment form. *The Journal of Reproductive Medicine*, 36, 769-772.

Bancroft, J., & Rennie, D. (1993).The impact of oral contraceptives on the experience of perimenstrual mood, clumsiness, food craving and other symptoms. *Journal of Psychosomatic Research*, 37, 195-202.

Bertheussen, G. F., Romundstad, P. R., Landmark, T., Kaasa, S., Dale, O., & Helbostad, J. L. (2011). Associations between physical activity and physical and mental health–a HUNT 3 study.*Medicine* & *Science* in *Sports* & *Exercise*, 43, 1220-1228.

Blundell, J. E., Stubbs, R. J., Hughes, D. A., Whybrow, S.,& King, N. A. (2003).Cross talk between physical activity and appetite control: Does physical activity stimulate appetite? *Proceedings of the Nutrition Society*, 62, 651-661.

Borg, G. A. V. (1973). Perceived exertion: A note on "history" and methods. *Medicine and Science in Sports*, 5, 90-93.

Bowen, R. S., Turner, M. J., & Lightfoot, J. T. (2011). Sex hormone effects on physical activity levels. *Sports Medicine*, 41, 73-86.

Brage, S., Brage, N., Ekelund, U., Luan, J., Franks, P. W., Froberg, K., et al. (2006). Effect of combined movement and heart rate monitor placement on physical activity estimates during treadmill locomotion and free-living. *European Journal of Applied Physiology*, 96, 517-524.

Brage, S., Ekelund, U., Brage, N., Henninngs, M. A., Froberg, K., Franks, P. W., & Wareham, N. J. (2007). Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity. *Journal of Applied Physiology*, 103, 682–92.

Chadwick, K. D., Burkman, R. T., Tornesi, B. M., & Mahadevan, B. (2012). Fifty years of “the pill”: risk reduction and discovery of benefits beyond contraception, reflections, and forecast. *Toxicological Sciences*, 125, 2-9.

Chaput, J. P., Klingenberg, L., Rosenkilde, M., Gilbert, J. A., Tremblay, A., & Sjödin, A. (2010). Physical activity plays an important role in body weight regulation. *Journal of Obesity, 2011*.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Department of Health. (2004). At least five a week: evidence on the impact of physical activity and its relationship to health, *Chief Medical Officer Report*. London.

Dye, L., & Blundell, J. (1997). Menstrual cycle and appetite control: Implications for weight regulation. *Human Reproduction*, 12, 1142.

Eck, L., Bennett, A., Egan, B., Ray, J., Mitchell, C., Smith, M., et al. (1997). Differences in macronutrient selections in users and nonusers of an oral contraceptive. *American Journal of Clinical Nutrition*, 65, 419.

Finlayson, G., Bryant, E., Blundell, J. E., & King, N. A. (2009). Acute compensatory eating following exercise is associated with implicit hedonic wanting for food. *Physiology & Behavior*, 97, 62-67.

Flint, A., Raben, A., Blundell, J. E.,& Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders*, 24, 38-48.

Frayn, K. N. (1983).Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*, 55, 628-634.

George, V. A., &Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite,* 40, 43-46.

Godin, G., &Shepard, S. (1985).The Godin Leisure-Time Exercise Questionnaire. A simple method to assess exercise behavior in the community. *Canadian Journal of Applied Sport Sciences*, 10, 141-146.

Hagobian, T. A., & Braun, B. (2010). Physical activity and hormonal regulation of appetite: Sex differences and weight control. *Exercise and Sport Sciences Reviews,* 38, 25.

Hagobian, T. A., Yamashiro, M., Hinkel-Lipsker, J., Streder, K., Evero, N., & Hackney, T. (2012). Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Applied Physiology, Nutrition, and Metabolism,* 38, 66-72.

Inbar, O., Oten, A., Scheinowitz, M., Rotstein, A., Dlin, R., & Casaburi, R. (1994). Normal cardiopulmonary responses during incremental exercise in 20-70-yr-old men. *Medicine & Science in Sports & Exercise*, 26, 538-546.

Karlsson, J., Persson, L-O., Sjöström, L., & Sullivan, M. (2000). Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. *International Journal of Obesity*, 24, 1715-25.

King, N. A., Caudwell, P., Hopkins, M., Byrne, N. M., Colley, R., Hills, A. P., Stubbs, J. R., & Blundell, J. E. (2007).Metabolic and behavioral compensatory responses to exercise interventions: Barriers to weight loss. *Obesity,* 15, 1373-1383.

King, N. A., Snell, L., Smith, R. D., & Blundell, J. E. (1996).Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition,* 50, 663-667.

Kissileff, H., Pi-Sunyer, F., Segal, K., Meltzer, S., & Foelsch, P. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition,* 52, 240.

Larson-Meyer, D. E., Palm, S., Bansal, A., Austin, K. J., Hart, A. M., & Alexander, B. M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *Journal of Obesity,*2012.

Lluch, A., King, N. A., & Blundell, J. E. (2000). No energy compensation at the meal following exercise in dietary restrained and unrestrained women. *British Journal of Nutrition*, 84, 219-225.

Lluch, A., King, N., & Blundell, J. (1998).Exercise in dietary restrained women: No effect on energy intake but change in hedonic ratings. *European Journal of Clinical Nutrition,* 52, 300-307.

Maraki, M., Tsofliou, F., Pitsiladis, Y., Malkova, D., Mutrie, N., & Higgins, S. (2005). Acute effects of a single exercise class on appetite, energy intake and mood. is there a time of day effect? *Appetite,* 45, 272-278.

Martins, C., Morgan, L., & Truby, H. (2008). A review of the effects of exercise on appetite regulation: An obesity perspective. *International Journal of Obesity,* 32, 1337-1347.

McVay, M., Copeland, A., & Geiselman, P. (2011). Eating disorder pathology and menstrual cycle fluctuations in eating variables in oral contraceptive users and non-users. *Eating Behaviors,* 12, 49-55.

Naessen, S., Carlström, K., Byström, B., Pierre, Y., & Lindén Hirschberg, A. (2007). Effects of an antiandrogenic oral contraceptive on appetite and eating behavior in bulimic women. *Psychoneuroendocrinology*, 32, 548-554.

Pomerleau, M., Imbeault, P., Parker, T., Doucet, E. (2004). Effects of exercise intensity and appetite in women. *American Journal of Clinical Nutrition*, 80, 1230–1236

Reger, W. E., Allison, T. G., &Kurucz, R. L. (1984). Exercise, post-exercise metabolic rate and appetite. In F. L. Katch (Ed.), *The 1984 olympic scientific congress proceedings, vol. 2: Sport, health, and nutrition.* (pp. 115-23). Champaign, IL: Human Kinetics Publishers, Inc.

Rocha, J., Paxman, J., Dalton, C., Winter, E., & Broom, D. (2013). Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive men. *Appetite*, 71, 369-378.

Schubert, M. M., Desbrow, B., Sabapathy, S., & Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite*, 63, 92-104.

Tremblay, A. T. A., & Therrien, F. T. F. (2006). Physical activity and body functionality: Implications for obesity prevention and treatment. *Canadian Journal of Physiology and Pharmacology*, 84, 149-156.

Tsofliou, F., Pitsiladis, Y., Malkova, D., Wallace, A., & Lean, M. (2003). Moderate physical activity permits acute coupling between serum leptin and appetite–satiety measures in obese women. *International Journal of Obesity*, 27, 1332-1339.

Tucci, S., Murphy, L., Boyland, E., Dye, L., & Halford, J. (2010). Oral contraceptive effects on food choice during the follicular and luteal phase of the menstrual cycle: A laboratory based study. *Appetite*, 55, 388-392.

Unick, J. L., Otto, A. D., Goodpaster, B. H., Helsel, D. L., Pellegrini, C. A., & Jakicic, J. M. (2010). Acute effect of walking on energy intake in overweight/obese women. *Appetite,* 55, 413-419.

United Nations Department of Economic and Social Affairs (2009) World contraceptive use at http://www.un.org/esa/population/publications/WCU 2009/Main.html

WHO. (2004). Human energy requirements: Report of a joint FAO/WHO/UNU expert consultation. Food and Nutrition technical report series No. 1, Rome.

Figures captions

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

Figure 2. Subjective feelings of hunger (n=10 per group; means ± SEM). Hatched rectangles are consumption of meals; dark rectangle is equivalent to the 60 minutes cycling period.

Figure 3. Daily energy intake (n=10 for active and n=9 for inactive; means ± SEM). *Means significantly different between conditions (p = 0.002, d = -0.89).

Figure 4. Percentages of energy compensation (N=10 for active and N=9 for inactive; means ± SEM); Exp. = Experimental. Dashed line indicates complete compensation (100%) of the exercise-induced energy expenditure.