**Title:** SEX DIFFERENCES IN RECOVERY FROM SPRINT INTERVAL EXERCISE.

**Abstract**

The purpose of the study was to examine whether there were differences between males and females in energy metabolism following a bout of sprint interval training (SIT).Sixteen males (mean ± SD [95% CI] for age, stature, body mass and fat-free mass [FFM] of 25.4 ± 5.9 [22.3, 28.6] years, 181.3 ± 7.0 [177.6, 185.0] cm, 82.7 ± 13.3 [75.6, 89.8] kg, and 69.0 ±10.6 [63.4, 74.6] kgFFM, respectively) and 16 eumenorrheic females (26.1 ± 5.5 [23.1, 29.8] years, 164.1 ± 8.7 [159.5, 168.7] cm, 72.0 ± 15.4 [63.8, 80.2] kg, and 51.6 ± 8.5 [47.0, 56.1] kgFFM), tested in the mid-luteal phase of their menstrual cycle, completed a SIT protocol, consisting of 4 x 30-s Wingate sprints at 0.065% FFM. Respiratory variables were used to estimate energy metabolism following (post-SIT) and 24 h following the bout of SIT (24 h post-SIT). Compared to females, males had significantly higher post-SIT mean fat oxidation rates (0.10 g.min-1 and 0.17 g.min-1, respectively, *F*(1,30) = 34.82, *p* < 0.001, ƞp2 = 0.54), energy expenditure (1.28 ± 0.26 and 1.82 ± 0.40 kcal.min-1, respectively,*F*(1,30) = 20.759, *p* <0.001, ƞp2 = 0.41), excess post-exercise oxygen consumption values (1.91 ± 0.60 and 3.02 ± 1.58 L, *F*(1,30) = 6.882, *p* <0.014, ƞp2 = 0.19), and lower relative carbohydrate oxidation rates (0.0007±0.0013 and 0.0018±0.0007 g.min-1 per kgFFM, respectively, *F*(1,30) = 10.506, *p* < 0.003, ƞp2 = 0.26). The higher metabolic values post-SIT for the males compared to the females might be explained by the males having a greater FFM and having exercised at a higher exercise intensity. Practically, these findings could mean that, if prescribing SIT as a strength and conditioning professional, males and females could respond differently in terms of energy expenditure post-exercise.

**Key words:** Lipid metabolism; women; weight loss; high-intensity interval training.

**INTRODUCTION**

Low-volume, sprint interval training (SIT), whereby repeated short bouts of exercise are performed at very high intensities, is a time-efficient solution for not only improving aspects of health and fitness (21,22), but also for reducing and controlling fat mass (7,40,64). One of the reasons why SIT may assist with weight control is because the metabolic perturbations caused by the intensity of the intermittent exercise increases the magnitude and duration of excess post-exercise oxygen consumption (EPOC) (9,25,35) Fat oxidation also increases in the post-exercise period, in the presence of decreased muscle glycogen (34,60). Since total energy expenditure increases with each bout of intense exercise, SIT could potentially be used for weight management purposes.

Men and women differ in exercise-related energy metabolism. For instance, women, at a given exercise intensity, tend to have a reduced reliance on glycogen compared to men (48,56,61). In recovery from low- to moderate-intensity endurance exercise, substantially lower energy expenditure, lipolysis and fatty acid mobilization have been observed among women (28), suggested to be because women, in comparison to men, have better homeostatic control over return to resting metabolic values (15,26). In addition, EPOC has been found to be higher in males following one-hour of moderate-intensity exercise, when compared to age- and fitness-matched females, although when values were corrected for FFM, differences were found to be similar, suggesting that FFM had a significant relationship to the magnitude of the EPOC response (38). The lower fat oxidation and EPOC observed following exercise among women might explain why, in some SIT interventions, where women have been included in the sample, fat loss has not been as marked (2,32,40). There is a need to further investigate post-exercise metabolic differences between males and females (whilst also monitoring energy intake), as a result of a bout of SIT, in order to explore the usefulness of SIT for weight management.

Is it important, when including females in the sample population, to account for menstrual cycle, by highlighting whether females are eumenorrheic, and whether they are using some form of hormone-based contraception. Although it has been reported that males and females respond similarly following SIT with regard to EPOC (58), such studies have not controlled for menstrual cycle status (38,58). It is, therefore, important that, in a study of post-exercise energy metabolism, menstrual cycle is considered and controlled.

The purpose of the current study was to examine whether there were sex differences in energy metabolism following a single bout of SIT. It was hypothesized that EPOC and mean post-exercise fat/carbohydrate oxidation rates, as well as RER, and estimated energy expenditure among eumenorrheic women, completing a bout of SIT in the mid-luteal phase of their menstrual cycle, would be significantly different to those of men. Practically, these findings may influence prescription of strength and conditioning, in that SIT exercise, if being used for weight management purposes, might only be prescribed for males.

**METHODS**

**Experimental approach to the problem**

The study design was cross-sectional in nature, so that differences between males and females in mean post-exercise fat/carbohydrate oxidation rates, EPOC, RER, and energy expenditure following a bout of SIT could be examined. Subjects attended the laboratory on three separate occasions. The first occasion was to familiarise subjects with test procedures and to attain preliminary measures. On the second occasion, subjects completed the bout of SIT, with resting metabolic data, using indirect calorimetry, obtained before and after the exercise. Subjects returned for the third occasion, between 23 h and 24 h after the exercise session, so that resting metabolic data could again be obtained. The research design, whereby males and females are compared, with additional controls over menstrual cycle phase, follows the design of other, similar studies on determining sex-related differences in fuel metabolism during and following long-duration exercise (30,57). .The duration of the examination of the acute response also follows previous research designs (9,63).

**Subjects**

A total of 32 subjects, aged 18 to 38 years, who were physically active, but not involved in any form of SIT at the time of testing (or within the previous four months), volunteered for the study. Males (*n* = 16) and females (*n* = 16) were matched for physical activity (since this affects energy metabolism more so than maximal oxygen consumption or percent body fat (29)) using metabolic equivalents (METS) estimated from the International Physical Activity Questionnaire (IPAQ) short form (42). Subjects were excluded if they: Had a health or medical condition that prevented them from undertaking strenuous exercise; were smokers; or were using dietary supplements. Only eumenorrheic females were included; females using any form of hormonal contraception were excluded. Subject characteristics are given in Table 1. Subjects were supervised during their laboratory visits. The study had prior approval by the University Ethics’ Committee. The subjects were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study.

<INSERT TABLE 1 ABOUT HERE>

**Procedures**

*Preliminary measures*

Preliminary measures, taken during the familiarization visit included physical activity/health status, stature, body mass, and FFM; the latter was estimated from body density via the Siri equation using air displacement plethysmography (BodPod® 2007B, Life Measurement Inc., Concord, California, USA). In comparison to hydrodensitometry (14) and dual energy x-ray absorptiometry (41), the BodPod® has shown good validity for body composition assessment, with standard errors ranging from 2.2% to 3.7% body fat (17), although plethysmography is subject to errors due to using an equation to convert density into percent fat. Particular care was taken to minimise the effects of hair and clothing, since these could affect results (53).

*SIT trial and post-SIT measures*

Subjects attended the laboratory in the morning (between 07:00 h and 08:00 h) following an overnight fast of 12.4 ± 1.3 (95% CI 12.0, 12.9) h. On arrival, resting metabolic data were collected for 30 min via the use of a pre-calibrated gas analyzer (Cortex Metalyzer 3b, Biophysik GmbH, Leipzig, Germany), while subjects lay semi-supine. Resting metabolic data collection met all the best practice guidelines of Compher et al. (12), notably: abstinence from exercise, alcohol, caffeine; state of fasting; room temperature; pre-measurement rest period; rigorous adherence to guidelines to prevent air leaks; and recording of steady-state conditions. Following metabolic data collection, subjects completed the bout of SIT, which followed that described by Whyte et al. (64), and consisted of 4 x 30-s Wingate cycles at 0.065 kg FFM, with a 4.5-min recovery at 30 W between intervals. The mean ± SD load on the Wingate test was 4.5 ± 0.7 (95% CI 4.1, 4.9) kg for the males and 3.4 ± 0.6 (95% CI 3.1, 3.6) kg for the females. Capillary blood samples (5 µl) were taken from the fingertip at rest and immediately after the bout of SIT and analyzed using a pre-calibrated analyzer (Lactate Pro, Arkray, KDK Corporation, Kyoto, Japan) for the determination of lactate. Ratings of perceived exertion (RPE) (6) were noted immediately following each Wingate sprint. Post-exercise lactate concentration, RPE, and results from the Wingate sprints were used as indicators of exercise intensity. Obtained using software (Monark Anaerobic Test Software Version 3.3.0.0), peak powerwas taken asthe highest mechanical power generated during any 3 to 5 s period of the test, mean power asthe mean of the total power generated during the 30-s test period, and fatigue index as the rate of decline in power relative to the peak value.

Subjects’ resting metabolic data were collected continuously for up to 60 min following the SIT bout. Twenty-four hours later, subjects reported to the laboratory, again in the morning following an overnight fast of 12.4 ± 1.0 (95% CI 12.1, 12.8) h, to determine whether there were any longer-term effects. Metabolic data were again collected, in the same manner as described above.

*Metabolic measures*

The initial 5 min of respiratory data were discarded, and steady state was deemed to have been achieved in each 5-min period thereafter, if the coefficient of variation (CV) for volume of oxygen consumed (O2) and volume of carbon dioxide produced (CO2) was ≤10% (12). Whole-body fat and carbohydrate oxidation rates were estimated from respiratory data using stoichiometric equations (19). Energy expenditure was estimated using the Weir equation (62). Excess post-exercise oxygen consumption, defined as the increased oxygen used above resting post-exercise levels (20), was taken as the total V̇O2 from the pre-exercise baseline measure subtracted from the total V̇O2 in the post-exercise period of the same duration. Measures of re-test reliability of the gas analysis were performed on a subsample of seven subjects, who were not involved in the main study, to ascertain whether resting measures of energy metabolism obtained from the gas analyzer could be repeated under similar pre-test conditions, without the effect of exercise.

*Menstrual cycle confirmation*

As a precaution to maintain high within-group reliability in the event of, albeit unlikely (48),cross-cycle perturbations in fat oxidation (8,23,39,66), female subjects were tested in the mid-luteal phase of their menstrual cycle. Menstrual cycle phase was initially estimated via diaries indicating menses and a urine test kit (One Step®, Home Health, Bushey, UK) to detect LH surge, and then confirmed from subsequent analysis of salivary progesterone and 17β-estradiol. Saliva samples were collected using the unstimulated passive drool technique. Samples were centrifuged at 1500 x g for 15 min, and the supernatant frozen at -80ºC. Salivary 17β-estradiol and progesterone were analyzed via ELISA technique using standardized enzyme immunoassay kits (Salimetrics, 1-3702 estradiol, and 1-1502 progesterone, respectively). The intra-assay CV ranges from 4-8.4% for progesterone and 7-8.1% for 17β-estradiol, and the sensitivities of the assays are 0.3671 pmol.L-1 for 17β-estradiol and 0.0159 nmol.L-1 for progesterone.

*Dietary measures*

Dietary intake, including fluid intake, was self-monitored for 24 h prior to the exercise bout, and during the study period (24 h post-exercise) using food diaries, which were analyzed using nutritional analysis software (Nutritics Academic Edition v4.267, Dublin, Ireland). Subjects were discouraged from using any dietary supplements and caffeine for the study duration. Consistent food recall, without variation in macronutrient contribution, was shown.

**Statistical analyses**

A total of 32 subjects were required to examine between-group differences, assuming an effect size of *F* = 0.138 (11), 80% power, and an alpha of 0.05 (11). A two-way mixed methods analysis of variance (ANOVA) was used to examine the effect of sex on resting metabolic data before (pre-SIT), after (post-SIT) and 24 h after (24 h post-SIT) the single bout of SIT, with post-hoc analysis using Bonferroni adjustment for multiple pairwise comparisons, if differences existed within-subjects, and Univariate analysis for between-subject effects and where interaction effects were significant. Dietary macronutrients 24 h prior to and during the study period were also analyzed using a two-way, mixed methods ANOVA, as were sex differences in Wingate results, EPOC, RPE and blood lactate concentration. To determine differences between the sexes in anthropometry, and estimated physical activity-related energy expenditure, an independent *t*-test was used. Re-test reliability of metabolic data and diet were calculated using 95% limits of agreement on the subsample of seven subjects (44). Data are described using mean ± SD, 95% confidence interval (95% CI), effect size (Cohen’s d for t-tests, and partial eta-squared [ƞp2] for ANOVA, and significance was accepted at the *p* ≤ 0.05 level. A posteriori linear regression analysis was undertaken to determine the extent to which FFM explained the variance in post-SIT fat oxidation.

**RESULTS**

On the subsample of seven subjects, 95% limits of agreement, standard error of difference, and 95% CI of bias for fat oxidation rates were ± 0.017 (0.003 and -0.009 to + 0.008) g.min-1, for carbohydrate oxidation rates were ± 0.07 (0.01 and -0.04 to +0.03)g.min-1 and for energy expenditure were , ± 0.42, (0.08 and -0.20 to +0.20) kcal.min-1.

In the sample of females, hormonal-based contraception had not been used for at least 7 months before starting the study. Seven of the female subjects had never used hormone-based contraceptives. None of the females had been pregnant in the previous six months and none were breastfeeding. There were no reports of past diagnosed menstrual irregularities (hirsutism, polycystic ovary syndrome, pituitary dysfunction, thyroid dysfunction, premature ovarian failure or other hormone-related condition). No female subjects reported having any menstrual irregularities at the time of testing. Testing took place 17.6±4.4 d after menses. Mean oestradiol and progesterone values were 1.76±0.44 (95% CI 1.51 to 2.02) pmol.L-1 and 243.5±176.3 (95% CI 141.7 to 345.3) nmol.L-), respectively. Estradiol and progesterone were within the normal range for this test kit and for our laboratory. Two subjects were excluded from participation at the baseline measure stage, as they did not meet the inclusion criteria for menstrual cycle status.

Mean values for metabolic variables pre-SIT, post-SIT and 24 h post-SIT for the males and females are given in Table 2. Since mean FFM for males (69.0 ± 10.6, 95% CI 63.4, 74.6) was higher (*t*(30) = -5.131, *p* < 0.001, d = 1.81) than FFM for females (51.6 ± 8.5, 95% CI 47,0, 56.1), relevant values were expressed relative to FFM (Table 2).

<INSERT TABLE 2 ABOUT HERE>

There were significant interaction effects for sex and time for absolute fat oxidation rates (F2,60 = 28.699, p < 0.001), fat oxidation rates relative to FFM (F2,60 = 17.157, p < 0.001), carbohydrate oxidation rates (F2,60 = 9.126, p < 0.001), carbohydrate oxidation rates relative to FFM (F2,60 = 3.348, p = 0.042), energy expenditure (F2,60 = 5.805, p = 0.005), EPOC (F1,30 = 9.736, p = 0.004), RER (F2,60 = 7.55, p = 0.001), and METS (F2,60 = 4.248, p = 0.019), but not for EPOC, energy expenditure or RER relative to FFM. The significant within-subject differences (for all subjects) over time for absolute and scaled fat oxidation rates, carbohydrate oxidation rates, energy expenditure, and values for METS (Table 2), occurred because of the post-SIT values, which were significantly (*p* < 0.001) higher than the values pre-SIT and 24 h after the SIT bout. Post-SIT RER within-subject values were significantly (*p* > 0.001) lower than on the other two test occasions (Table 2).

From post-hoc analyses, mean values for energy expenditure was significantly (*p* ≤ 0.01) higher for males than females at all time points, but not when expressed relative to FFM (Table 2). Only the post-SIT fat oxidation rates were significantly (*F*(1,30) = 34.82, *p* < 0.001, ƞp2 = 0.54) higher for males than females; mean values pre-SIT and 24-h post SIT were not significantly different between the sexes. Likewise, only the post-SIT scaled (per kg FFM) carbohydrate oxidation rates were significantly (*F*(1,30) = 10.506, *p* = 0.003, ƞp2 = 0.26) higher for females compared to values for males, and the EPOC post-SIT values were significantly (*F*(1,30) = 6.882, *p* < 0.014, ƞp2 = 0.19) lower for females compared to those for males (Table 2). Scaled RER values were significantly lower for males compared to females at all times points. There were no significant differences for all other variables between the sexes.

Data from the four sprints are given in Table 3. There were no significant interaction effects for these variables. Significant differences between males and females were apparent for peak and mean power output, even when adjusted for FFM. Apart from absolute values for peak power output, and fatigue index, values significantly decreased over time. Blood lactate concentration at rest was 1.48 ± 0.90 (95% CI 0.99, 1.96) mmol.L-1 for males and 1.38 ± 1.05 (95% CI 0.82, 1.94) mmol.L-1 for females. At the end of the four sprints, blood lactate concentration was 12.84 ± 2.26 (95% CI 11.63, 14.04) mmol.L-1 for males, and 11.30 ± 1.86 (95% CI 10.31, 12.29) mmol.L-1 for females. Blood lactate concentration differed significantly (*F*(1,30) = 827.514, *p* < 0.001, ƞp2 = 0.97) over time, but not for sex (*F*(1,30) = 3.504, *p* = 0.071, ƞp2 = 0.11).

<INSERT TABLE 3 ABOUT HERE>

Dietary intakes for males and females are given in Table 4. There were no significant differences between pre-SIT and 24 h post-SIT values for calorie intake, carbohydrate, fat, and protein intakes. Males had a significantly (*F*[1,30] = 38.399, *p* < 0.001, ƞp2 = 0.56) higher calorie intake than did females. When expressed per kg FFM, there were no significant differences in carbohydrate, fat and protein intakes between males and females. There were no significant interaction effects for these variables.

<INSERT TABLE 4 ABOUT HERE>

**DISCUSSION**

In the current study, absolute post-SIT fat oxidation rates and estimated energy expenditure were higher among males compared to eumenorrheic females (tested in the mid-luteal phase of their menstrual cycle (as confirmed through hormonal analysis) and relative values (per FFM) for carbohydrate oxidation rates were lower (having large effects sizes) for females. The hypotheses for differences in these variables, were therefore accepted. The findings potentially indicate that males, as a result of a single bout of SIT, might be in a greater negative energy balance after SIT. Males have been observed to lose greater fat mass than females in response to some SIT interventions (7,40,64), which potentially could be explained by males having a greater fat oxidation and energy expenditure following SIT compared to females, as was observed in the current study.

In terms of estimated absolute fat oxidation, the males, in this study, had higher rates immediately after the initial recovery from the SIT bout than those observed for females (Table 2). The reason for this sex difference in post-SIT fat oxidation rates could have been because of the males having a higher proportion of FFM than the females, since, when fat oxidation rates were expressed relative to FFM in the current study, differences between the sexes post-SIT, were no longer significant (Table 2). Indeed, the posteriori linear regression analysis suggested that FFM explained 58% of the variance in absolute, post-SIT fat oxidation. Higher fat oxidation rates post-exercise have previously been explained by individuals having a greater relative muscle mass (2,10,61). That said, fat oxidation rates, relative to FFM, were still, in the current study, 24% greater for males than they were for females and the effect size was still high; carbohydrate rates, even when scaled to FFM, were also lower among females compared to males. Furthermore, there may have been measurement error for FFM determination (4); for instance, if FFM was underestimated in females, the scaled differences in fat oxidation would not be as marked. It can be concluded that fat oxidation rates may be higher in females in the immediate recovery period following a bout of SIT, but that FFM, possibly due to differences in muscle properties (15,61), may have, to some extent, accounted for these observed sex-related differences .

A stable bicarbonate pool is required to reliably estimate V̇CO2 and fat oxidation rates (52). The fat oxidation rates following SIT might have been overestimated in the current study, likely an artefact of metabolic CO2 retention, as a result of bicarbonate pool replenishment (16,37). This increased CO2 in expired air could also lead to an artificially reduced RER (below the metabolic respiratory quotient), found in recovery from SIT (25,43,45). Thus, RER and estimated fat and carbohydrate oxidation rates using V̇CO2 are not deemed to be precise measures of fuel utilization, especially after SIT (5), since the duration of the CO2 retention has been reported to last beyond 60 min (28). Resting metabolic data had sufficient re-test reliability, since limits of agreement data on a subsample of subjects were narrow and well within typical variability margins (12). The low measurement error was much less than the differences observed between the sexes (e.g., 75% less for fat oxidation); The study would have, however, benefitted from a further examination of reliability for repeat measures of metabolic variables following SIT. Nevertheless, differences between males and females for absolute fat oxidation rates and RER were still observed in the current study when analyzed post-SIT. Future research is needed to examine whether males and females differ in their post-SIT bicarbonate pool replenishment, in order to more objectively account for the current findings.

In a review of 18 studies, it was concluded, that differences between men and women in fat oxidation following endurance exercise only occurred in a post-absorptive state, where women's fat oxidation was attenuated (27). The overnight fast in the current study might explain the greater absolute fat oxidation rates in males compared to females following the bout of SIT; the overnight fast was, however, necessary to control for the thermic effects of food. In a real-life, applied setting, when SIT is undertaken in energy-rich conditions, differences in fat oxidation rates between males and females may not be detectable. More research could be undertaken to determine fat oxidation rates in replete/normal conditions after a bout of SIT between males and females.

There was no significant difference due to sex for EPOC and effect size was small; hence the hypothesis for this variable was rejected. The measurement of EPOC in the current study was limited to a time frame (up to 60 min post-exercise, and a bout recorded at 24 h after exercise), rather than analyzing the accumulated EPOC over time. The EPOC was also not measured between intervals of SIT. The total EPOC was, therefore, underestimated. Furthermore, to measure EPOC accurately, ideally a control baseline measure on a separate day is needed, due to circadian effects on resting metabolic rate (24). Because testing in the current study was limited to a menstrual cycle phase, it was not possible for a control day to be implemented; a separate test day might also have been subject to other day-to-day variability (50). Because of the relationship between exercise intensity and EPOC magnitude and duration (36,45,65), the difference in EPOC between males and females (which occurred when only post-SIT data were analyzed) could be explained by differences in exercise intensity. However, although absolute and relative power output on each Wingate bout were higher for males than those for females, with high effects sizes, suggesting that exercise intensity was also higher, there were no differences in RPE and lactate, which can also be used as indicators of exercise intensity, although again, these markers are subject to sex- and hormone-related differences (18,31,49). Further examination of the potential differences in fat oxidation rates, energy expenditure, and EPOC between the sexes after SIT and their mechanisms is warranted.

In the current study, metabolic variables, in both the males and the females, had returned to baseline 24 h after the SIT bout. Other researchers have also found that fat oxidation and energy expenditure following a bout of SIT were transient (33,65), although Whyte et al. (63) found that post-SIT fat oxidation was still elevated between 18 h and 22 h. The speed of recovery to baseline could be dependent on the training status of subjects (54,63). In the current study, based on estimated energy expenditure scores from IPAQ, the cohort described themselves as being moderate to highly active, which might explain the quicker recovery to baseline.

One of the advantages of the current study was that menstrual cycle phase was controlled and similar for all female subjects. In studies, in which some fat loss among women in response to SIT has been reported, there were no controls over menstrual cycle phase (1,59). Being amenorrhoeic or using hormone-based contraception has, in the past, been found to interfere with fat metabolism, glucose use, and energy expenditure (3,8,13,39,46,55,66), although a more recent review of studies suggests no effect (47). Differences in response to SIT over the menstrual cycle could be investigated in future studies.

**PRACTICAL APPLICATIONS**

The findings of the current study, in that energy expenditure and fat oxidation rates were higher for males than they were for females immediately after a bout of SIT, potentially driven by differences in FFM between males and females, may explain why SIT might be more successful as a fat-loss intervention in men compared to women. Since a high capacity to oxidize fat and to expend energy following exercise is advantageous for the maintenance of body mass and metabolic health (51), SIT may favor males over females, a finding of importance for strength and conditioning professionals, end users, and clinicians. As a practical, take-home message, females, possibly in part due to a lower FFM compared to males, differ in how they metabolize fat and carbohydrate following SIT, which may have implications for weight loss over time. Males may benefit more than do females from undertaking SIT for fat loss.

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