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RESEARCH ARTICLE

An Investigation into the Effects of a 12-week Sleep Low Train Low Intervention on Fat Oxidation and Exercise Performance in Recreationally Endurance-Trained Women

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ABSTRACT

Background: **Combining training stimuli with nutritional interventions to maximise the training effect has become the topic of numerous research studies and reviews The Sleep Low Train Low (SLTL) protocol has become popular amongst endurance athletes as a method of increasing fat oxidation and thereby reduce glycogen dependence in events in excess of 90 minutes. The SLTL protocol was adopted for this study.**

Purpose: **It was hypothesised that implementing a 12-week SLTL protocol would increase fat oxidation and beta-hydroxybutyrate (BHB) production during exercise without negatively effecting performance.**

Methods: **25 women who are endurance trained were recruited. 21 women (mean age 41.9 years +/−7 years) completed the 12-week training intervention. 12 incorporated a sleep low train low element, while 9 were pre-fuelled for all sessions (pre and post testing consisted of a FATMAX/VO2 max test, 20-minute functional threshold power test and 90 minute endurance power test on a bike ergometer). Menstrual cycle (MC) phase and function where appropriate were established, tracked and accounted for using questionnaires/test data sheets and morning oral temperature.**

Results: **Analysis highlighted a significant increase in mean peak fat oxidation (PFO in g/min) ($p < 0.05$) and BHB production @90watts ($p < 0.05$) from pre to post within the FAST group. Also, the FAST group experienced a significant change in PFO when compared to the FED group from pre to post ($p < 0.05$). The FED group experienced a reduction in PFO, but this was not significant ($p = 0.21$). BHB production in the FED group reduced significantly at rest, at 60 watts and 90 watts ($p < 0.05$) from pre to post. Blood lactate adaptations were similar in both groups with the FAST group exhibiting a significant decrease in blood lactate at 120 watts and 150 watts ($p < 0.05$) with the FED group displaying a significant decrease at 90 watts and 150 watts ($p < 0.05$). VO2 max and 90-minute endurance power significantly improved from pre to post (FASTED $p < 0.05$, FED <0.05) in both groups. Functional Threshold Power (FTP) increased significantly in the FAST group ($p < 0.05$) but the increase in the FED group ($p = 0.11$) was not significant. The results of this study suggest that adopting a 12-week Sleep Low Train Low protocol in conjunction with regular high-intensity training significantly increases fat oxidation and BHB production in women who are recreationally endurance trained without negatively impacting performance.**

Keywords: Fat oxidation, performance, sleep low train low, women.

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1. Introduction

Increasing the intensity of fat oxidation during exercise in endurance athletes has long been the goal of various training protocols (Marquet *et al*., 2016; Volek *et al*., 2015). There are several protocols suggested to achieve an increase in fat oxidation during exercise, one of which is the SLTL protocol (Bartlett *et al*., 2015; Hulston *et al*., 2010; Lane *et al*., 2015; Marquet *et al*., 2016; Waterworth *et al*., 2020; Yeo *et al*., 2008). Sleeping low refers to sleeping in a low carbohydrate state, which is achieved by partaking in high-intensity (glycogen-depleting) exercise for approximately 60 to 90 minutes. This is followed by either no post-training meal or a meal with a very low carbohydrate (CHO) content. The combined effect of high-intensity glycogen-depleting exercise is followed by a low CHO meal or no meal, and sleeping low results in low CHO availability and increased fat oxidation during subsequent fasted exercise (Gonzalez *et al*., 2016). Incorporating an SLTL element into an endurance training program is suggested to increase fat oxidation and spare glycogen during exercise (Impey *et al*., 2016).

When implementing an SLTL regime, some thought must be given to the potential effects of repeatedly training in a glycogen-depleted state. Position stands published by the Institute of Performance Nutrition (IOPN) and the International Society of Sports Nutrition (ISSN) suggest that overuse of fasted-type training like SLTL could result in diminished immune function, chronic calorie deficit, and altered MC (Aragon *et al*., 2017; Sims *et al*., 2023). During this study, to assess sustained physiological stress, which could manifest as any or all of the aforementioned, we analysed pre and post-c-reactive protein (CRP) levels in both groups. C-reactive protein (CRP) is a protein produced by the liver that increases in response to inflammation in the body. CRP is widely used as a marker of chronic systemic inflammation and has been extensively studied in the context of cardiovascular disease risk assessment. Inflammation plays a crucial role in the body's response to injury or infection, and CRP is produced as part of the inflammatory response (Clyne & Olshaker, 1999).

The SLTL concept more commonly referred to as fasted training, has been the subject of several systematic reviews (Aird *et al*., 2018; Gillen *et al*., 2013; Vieira *et al*., 2016). There appears to be a shared view that SLTL increases fat oxidation during exercise, but the consensus in relation to performance is conflicting. Many authors report negative effects on performance when compared to training in the fed state (De Jonge, 2003; Oosthuyse & Bosch, 2010; Tarnopolsky *et al*., 1990). Research also supports the consensus that no effect or positive effects on performance are reported compared to training in the fed state (Anis *et al*., 2009; Levy & Chu, 2019; Maughan *et al*., 2010). However, most studies investigating the SLTL concept are conducted on males or male/female mixed groups with no consideration for MC (Aird *et al*., 2018; Van Proeyen *et al*., 2011; Vieira *et al*., 2016).

The gender bias, which is slowly shifting, appears to be a result of the difficulty of standardising protocols for females around MC. The difficulty arises due to fluctuating sex hormones across the MC and the variability in the concentration of these hormones from woman to woman (Hackney, 2016). The implied effect fluctuating sex hormone concentrations (estrogen and progesterone) can have on performance and fuel utilisation has meant a lack of research projects applying SLTL-type protocols to females (De Jonge, 2003; Devries, 2016; Elliott-Sale *et al*., 2020; McNulty *et al*., 2020; Oosthuyse & Bosch, 2010; Pivarnik *et al*., 1992). During the early to mid-follicular (MF) phase (day 0 to day 7 of a theoretical 28-day MC) when sex hormones are low (low hormone phase), it is proposed that females and males are similar in terms of fuel utilisation during exercise (Riddell *et al*., 2003). During this phase of the MC, the effects of oestrogen and progesterone are reduced due to their lower concentrations. It is reported that performance is not affected by hormones during the MF phase due to low oestrogen and progesterone concentrations and increased glycogen availability compared to the late follicular (LF) phase (Tarnopolsky *et al*., 1990). During the LF phase (day 10 to day 13 of a theoretical 28-day MC), there is an increase in estrogen, which has been correlated to an increase in endurance performance (McLay *et al*., 2007; Oosthuyse *et al*., 2005). When oestrogen is elevated, it is alleged to increase lipolysis and inhibit glycogen utilisation at rest and during exercise (Bunt, 1990; Hackney, 1999). When estrogen concentrations are elevated, it is suggested that glycogen synthase activity be increased, which promotes glycogen storage and has a glycogen sparring effect (Beckett & Toth, 2002). Also, when oestrogen increases in isolation, it is reported to increase fatty acid mobilisation and the capacity to utilise fats for fuel. The combination of glycogen sparring and increased fat mobilisation caused by elevated oestrogen would appear to be beneficial for endurance performance (Hackney, 1999; Jurkowski *et al*., 1981; Oosthuyse *et al*., 2005).

The hormone profile of the MC and associated effects become more complex when in the mid-luteal (ML) phase (around day 21 of a theoretical 28-day MC). Oestrogen and progesterone are elevated simultaneously. The effects of oestrogen have been mentioned previously, but progesterone acts as an antagonist to oestrogen (Oosthuyse & Bosch, 2010). Progesterone increases core temperature, protein catabolism and total sodium losses, which are all counter-productive to endurance performance (Bonekat *et al*., 1987). Research-based evidence reported that the combined effect of sex hormones

TABLE I: Overview of Nutritional Intervention for the FAST and FED Groups

	3:30 PM High carb meal (>3g/kg/bw)	6:30–7:30 PM High-intensity track session	Post track session		6–7 AM	7–8 AM low-intensity bike/turbo session	Post training
FAST group			Low carb meal (approx 10g CHO)	Sleep low	No breakfast		Resume own SSD
FED group			Resume own SSD	Sleep refuelled	CHO drink 1-hour pre-training (1g/kg/bw)		Resume own SSD

Note. *SSD: Self-selected diet. *Outside of the times highlighted above, FAST and FED group participants followed their preferred self-selected diet, meeting a prescribed caloric content to support the training load.

on performance is variable, and the experiences of each individual are changeable (D'Eon *et al*., 2002; Frankovich & Lebrun, 2000; Oosthuyse & Bosch, 2010). The variability is potentially due to the ratio of oestrogen to progesterone (pmol: mmol). The potential for enhanced performance increases when there is a large increase in oestrogen from MF to ML and a high oestrogen-to-progesterone ratio during the ML phase. When there is a small increase in estrogen from MF to ML, combined with a low estrogen-to-progesterone ratio during the ML phase, the potential for performance enhancement is reduced (D'Eon *et al*., 2002; Devries, 2016; Frankovich & Lebrun, 2000; Oosthuyse & Bosch, 2010).

Considering the above, a 12-week Sleep Low Train Low intervention was conducted to investigate its effects on substrate utilisation, performance and the inflammatory marker CRP in women who are recreational endurance athletes. The impact of the menstrual cycle phase on substrate utilisation was accounted for by conducting the FATMAX test for each eumenorrheic participant during the same menstrual cycle phase (+/−1 day) and conducting the post-intervention performance based on a similar point within the MC as pre-intervention performance tests (+/−3 days).

2. Method

2.1. Subjects

25 females actively engaged in endurance-type exercise for a minimum of two years and completed pre-screening and informed consent forms before being invited to participate in this study. 21 participants completed the study. The study was examined and approved by the Waterford Institute of Technology ethics committee. Each participant was briefed on the study plan, the format of pre- and post-testing, and the nutritional and training intervention involved. All participants were aged between 18–60 years old (mean age 41.9 years +/−7 years). Their sports included triathlon, marathon, Open Water Swimming and other ultra-distance events. Only participant data for those who completed the study is included in the results.

2.2. 12-Week Nutrition and Training Intervention

Table I shows an overview of the nutrition and training intervention from Monday to Tuesday for both groups. There was no training on a Wednesday, and this format was repeated on Thursday and Friday. One other 60–90-minute session was completed by each participant on Saturday or Sunday. The FAST group conducted this session fasted, and the FED group conducted it fed.

2.3. Session Rate of Perceived Exertion (RPE)

Session RPE using the modified 10-point Borg scale was recorded within 10 minutes of session completion (Stannard *et al*., 2010). Session RPE was submitted at the end of each training week. RPEs were recorded, and the training load was tracked using a formatted Excel file.

TABLE II: Group Calorie and Macronutrient Intake Collected via Pre-intervention Two-day Food Diary

Group	Day 1				Day 2			
FAST	Calorie (kcal)	CHO (g)	FAT (g)	PRO (g)	Calorie (kcal)	CHO (g)	FAT (g)	PRO (g)
Average	1768	238	55	83	1739	227	58	77
Standard deviation	132	40	13	19	210	42	14	16
FED	Day 1				Day 2			
Average	1831	226	74	95	1828	186	78	98
Standard deviation	167	55	13	25	246	50	25	20

TABLE III: Calorie and Macronutrient Recommendations for Weight Maintenance During Intervention

Group				
FAST	Calorie (kcal)	CHO (g)	FAT (g)	PRO (g)
Average	1890	259	57	92
Standard deviation	171	24	6	21
FED	Calorie (kcal)	CHO (g)	FAT (g)	PRO (g)
Average	1931	265	57	86
Standard deviation	122	17	4	6

2.4. Pre/Post-Testing and Randomisation

Pre-intervention testing consisted of one laboratory-based test (FATMAX/VO2 max), two field-based performance tests (functional threshold power and 90-minute endurance power) and the collection of fasted (following a 10–12 hour overnight fast) intravenous blood samples. There was a minimum of three days between each test. When participants reported for testing, they completed a questionnaire and test data sheet. On each questionnaire and test data sheet, participants stated the number of days since their first day of menstruation. This document was also used to record preferred bike settings and the results from the test they were partaking in. Four such documents were completed during pre-testing (one before each test and one before intravenous blood samples). Participants were randomly assigned to either a Fasted (FAST) Group or a Fed (FED) Group based on the results of a pre-VO2 max test.

2.5. Nutrition Diary

A two-day pre-intervention food diary was recorded and analysed for calorie content and macronutrient intake (see Table II). Participants were advised individually, based on food diary analysis and future training load prescription, if they needed to increase or decrease their calorie intake and/or alter their diet composition. Throughout the 12-week intervention, five individual two-day food diaries were collected for each participant during weeks two, four, six, eight and ten. These diaries were used to monitor participant calorie intake and avoid a calorie deficit (see Table III).

2.6. Menstrual Cycle Phase or Status

The menstrual cycle phase or status was established during pre-testing where appropriate. This was achieved through a number of questionnaires which established the length (in days) of the last menstrual cycle and the number of days since the first day of menstruation (first day of bleeding). Participants who were post menopause or taking oral contraception were noted and re-tested on or near the same dates (see Table IV). Before post-testing, the menstrual cycle phase was re-established in eumenorrheic participants. This was achieved through a combination of questionnaires (as above) and collated oral temperature readings (see Fig. 1). This allowed for post-testing to be conducted at a similar time point within the menstrual cycle phase as pre-testing. Pre- to post-menstrual cycle phase alignment for post-intervention FATMAX testing was prioritised due to the potential effect of hormone fluctuations on substrate utilisation during this test.

2.7. FATMAX/VO2 Max Test

Participants reported to the Human Laboratory in Waterford Institute of Technology WIT Arena following an overnight fast for the FATMAX Test. The FATMAX test is a method for measuring the maximal rate of fat oxidation during incremental exercise and can also be utilised to measure VO2 max (30). Both VO2 and VCO2 were measured through breath-by-breath analysis using the Moxus metabolic cart. The FATMAX test commenced at 60 watts on a bike ergometer and increased by 30 watts every three minutes. The intensity continued to increase by 30 watts every three minutes until

TABLE IV: Number of Participants Taking Oral Contraception, Post Menopausal or Time of Menstrual Cycle Phase During Pre- and Post-Testing

No. of participants	Menstrual cycle status
3	Post menopausal
4	Early follicular
3	Late follicular
9	Late luteal
2	Oral contraception

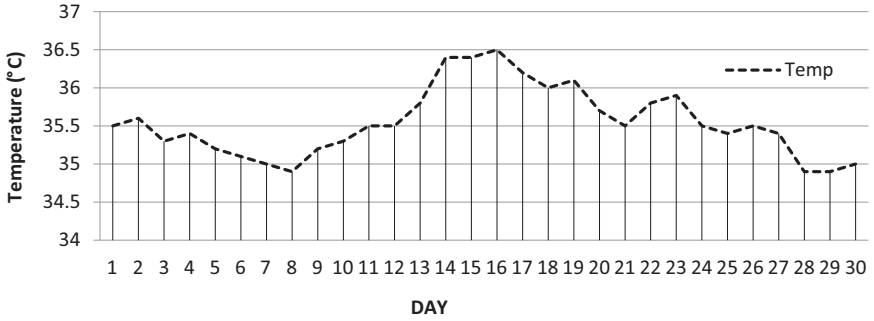

Fig. 1. Mean oral temperature for 16 eumenorrheic participants in the 4 weeks before post-testing.

a respiratory exchange ratio (RER) of 1.0 or greater was established for one minute. At this point, the VO2 max test commenced with the intensity increasing by 30 watts every 30 seconds until the participant could no longer maintain the required intensity. Capillary blood samples were collected at rest, in the final minute of every three-minute stage, and upon completion of the VO2 Max test from the fingertip. These samples were immediately analysed for blood lactate and blood BHB.

2.8. Capillary Blood Samples

Capillary blood samples were collected from the fingertip at rest, in the final minute of each three-minute stage and post VO2 max test. These samples were analysed immediately for blood lactate concentration (blood lactate, mmol/l) and blood beta-hydroxybutyrate concentration (BHB, mmol/l). Finger prick blood lactate samples were measured using the Nova Biomedical lactate plus analyser, and the BHB measurements were collected and measured using the Keto Mojo B-Ketone analyser.

2.9. Intravenous Blood Samples

Intravenous blood samples were collected from the median cubital vein pre and post-intervention by a qualified phlebotomist. Samples were labelled and spun, and serum was separated and stored at −80 degrees Celsius.

2.10. C-reactive Protein Analysis

Intravenous blood samples were removed from storage and allowed to thaw at room temperature. Samples were then analysed for c-reactive protein pre and post-intervention using the Assay Genie high-sensitivity double antibody sandwich Elisa kit (range 31.25–2000 pg/ml). A dilution factor of 4000 was applied when following the manufacturer's protocol for analysis.

2.11. Heart Rate and Blood Oxygen Saturation

During the FATMAX test, heart rate (HR) and blood oxygen saturation (SpO2) were recorded using an Innovo pulse oximeter.

2.12. Functional Threshold Power (FTP) Test

Participants reported to the Watershed Leisure Facility (Kilkenny) for a 30-minute pre-test. Participants were advised not to partake in strenuous exercise 48 hours before testing. Participants were in a fed state, having consumed their preferred pre-race meal three hours before the trial. Testing was conducted in a group format (Group 1, n = 12; Group 2, n = 13, randomly assigned). Group 1 completed their FTP test first, then their 90-minute endurance power test 3 days later. Group 2 completed their 90-minute endurance power test first, followed by their FTP test 3 days later. Each participant was assigned a Keiser M3 bike ergometer, which records power (watts), average power (watts), cadence (rpm), HR (bpm), distance (km), speed (km/hr), average speed (km/hr). Each bike ergometer was paired with a participant for pre and post-testing to reduce error and increase repeatability. To control the environment, music was played to mask other sounds. The same music was

TABLE V: Pre-FTP Repeatability Test Results on Keiser M3 Ergometers

	No. of tests	Mean power (watts)	Mean cadence 80 (rpm)
Test 1 (pre)	21	105	80.2
Test 2 (pre)	21	104.1	80.1
Test 1 (post)	21	104.7	80.4
Test 2 (post)	21	103.1	80.3

Note. Single factor ANOVA was used to assess mean power and mean cadence for statistically significant differences in terms of repeatability of tests. (mean power $p = 0.91$, mean cadence $p = 0.86$).

TABLE VI: Pre-90-minute Endurance Power Repeatability Test Results on Keiser M3 Ergometers

	No. of tests	Mean power (watts)	Mean cadence 80 (rpm)
Test 1 (pre)	21	104.1	80.0
Test 2 (pre)	21	105.0	80.4
Test 1 (post)	21	104.8	80.4
Test 2 (post)	21	105.5	80.5

Note. Single factor ANOVA was used to assess mean power and mean cadence for statistically significant differences in terms of repeatability of tests. (mean power $p = 0.93$, mean cadence $p = 0.84$).

repeated for the duration of pre- and post-testing. Before the FTP test commenced, each participant cycled for 1 minute with a resistance of 12 at a cadence of 80 rpm. After one minute, they recorded average power and average speed. They then reset the bike and repeated this test to ensure the bike was recording accurately and participants were familiar with the bike operation. This pre-test protocol was repeated for pre- and post-testing to ensure bike ergometers displayed accurate and repeatable measures (see Table V).

Following bike checks and setups, each individual had their preferred bike settings recorded (saddle height, handlebar height, preferred resistance) and commenced a pre-test warm-up (10 minutes). Following the warm-up, each participant had the opportunity to fine-tune their setup. At this point, the final setup settings were recorded. These settings were replicated for post-testing. Participants were briefed again on the nature of the test, the duration of the test and the goal of the test. The FTP test commenced at 8 PM. The participants could check their bike screen for the first 60 seconds to ensure the bike ergometer was recording. At this point, all screens were turned to face the floor and were not visible until test completion. Participants cycled for 20 minutes at their best effort. After the 20 minutes, average power and distance were recorded. Athletes then commenced a 110-minute cooldown.

2.13. 90-minute Endurance Power Test

The pre- and post-test protocol and location for the 90-minute endurance test were the same as those for the FTP test (see FTP section). Participants were in a fed state, having consumed their preferred pre-race meal three hours before the trial. Athletes were advised that this is a 90-minute test and should not be placed in the same manner as the FTP test. Athletes were allowed drinks during the test. Their drink of choice was noted, and they were advised and reminded post-testing that they should use the same drink type and adopt the same hydration strategy as they did during pre-testing (Table VI).

2.14. Resting Metabolic Rate (RMR) Calculation

Pre-intervention, participants' basic metrics (height, weight, age) were collected, and using the Mifflin St. Jeor resting metabolic rate (RMR) formula (see below), their RMR was calculated. Based on this RMR calculation combined with an average step count and considering their pre-scheduled training load, each participant was given an individually tailored calorie count that would support training without weight loss or gain. It should be noted that any such formula assumes all individuals of the same age, gender, height, and weight will have the same RMR. This is not the case and so there are potential errors (Frankenfield, 2013; Frankenfield *et al*., 2005).

2.15. Mifflin St. Jeor RMR Calculation

RMR was calculated using the Mifflin St. Jeor equation for females (Mifflin *et al.*, 1990);
*Women: (10 × **weight** in kg) + (6.25 × **height** in cm) – (5 × age in years) – 161.*

2.16. Fat Oxidation Calculation

Fat oxidation was calculated using the Frayn (1983) equation:

$$Fax\ Oxidation\ g/\min = 1.67\ (Vo2) - 1.67\ (Vo2) - 1.92\,(n)$$

N or urinary nitrogen was estimated to be zero, with no energy production arising from the catabolism of protein in the liver.

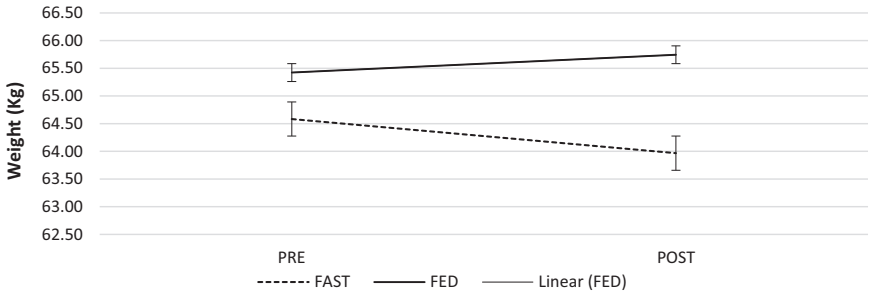

Fig. 2. Mean weight pre- and post-intervention for the FAST and FED groups with standard error.

TABLE VII: CRP Values for Each Group at Pre- and Post-Intervention with Standard Deviation and p Value

	CRP pre-intervention (mg/l)	Standard deviation pre	CRP post-intervention (mg/l)	Standard deviation post	P value
FAST group	0.95	0.81	1.1	0.83	$p > 0.05$
FED group	1.5	0.92	1.5	0.68	$p > 0.05$

2.17. Statistical Analysis

Statistical analysis was conducted using the IBM SPSS statistics v25 software package in conjunction with Microsoft Excel 2010. Data was assessed via Excel 2010 software and was found to be normally distributed. Inter-group analysis was assessed from pre- to post-test through a t-test, with intra-group analysis conducted via ANOVA. Other statistical analysis tests were conducted where appropriate. Statistical analysis was conducted on all pre- and post-data, such as fat oxidation rate, BHB, VO2 max, blood lactate, FTP, 90-minute endurance power, and session RPE. All mean, standard deviation, standard error and p values are presented in the following results.

3. Results

All variables were found to be normally distributed. No significant change was recorded in body weight for FAST or FED groups from pre to post-intervention ($p > 0.05$, FAST: Pre = 64.58 Kg +/−10.7 Kg, Post = 63.97 Kg +/−10.1 Kg; FED: Pre = 65.42 Kg +/−5.0 Kg, Post = 65.74 Kg +/−5.0 Kg; see Fig. 2).

3.1. C-Reactive Protein

CRP was assessed pre and post-intervention. No significant change in CRP was observed either within groups or between groups (Table VII).

3.2. FATMAX

Rates of fat oxidation were assessed from pre to post within the FAST and FED groups. Also, the magnitude of change in fat oxidation (g/min) from pre to post within the FAST group vs. the magnitude of change within the FED group was assessed. The FAST group observed an increase in PFO during the FATMAX test, which was significant (pre @ 0.30 g/min, post @ 0.40 g/min, $p < 0.01$). The FED group experienced a reduction in PFO during the FATMAX test, but this reduction was not significant (pre @ 0.38 g/min, post @ 0.35 g/min, $p = 0.21$). The overall change within the FAST group vs. the FED group was significant ($p < 0.05$). PFO occurred at 60 watts for both groups pre and post-intervention.

3.3. Beta-Hydroxybutyrate (BHB)

BHB production was also analysed at rest and during the FATMAX test at intensities from 60 watts to 150 watts. Within the FAST group, an increase in BHB concentration was observed from pre to post. This increase was significant at 90 watts ($p < 0.05$). Within the FED group, a reduction in BHB occurred during the FATMAX test from pre to post. At rest, 60 and 90 watts, this reduction was significant ($p < 0.05$). From 120 watts to 150 watts, BHB concentration was similar at pre and post-testing values ($p > 0.05$) for both groups. When the overall change in BHB concentration was analysed between the groups, it was noted that at 90 watts, the change was significant ($p < 0.05$).

3.4. Blood Lactate

As highlighted in Fig. 6 and Table VIII, blood lactate concentrations at rest were similar in both groups at pre and post-testing. However, the FAST and FED groups displayed a significant decrease in blood lactate across a range of intensities during post-testing when compared to pre-test values. From

TABLE VIII: FAST and FED Group Pre- and Post-Intervention Mean Maximal Rate of Fat Oxidation (PFO @ 60watts) with Standard Deviation

	FAST (Fat Ox g/min)	SD	FED (Fat Ox g/min)	SD
Mean fat Ox pre	0.30	0.10	0.40	0.09
Mean fat Ox post	0.38*	0.10	0.35	0.10

Note. *denotes a significant increase in FATMAX from pre to post.

pre to post-blood, lactate concentrations were significantly lower in the FAST group at intensities 120 and 150 watts ($p < 0.05$). The FED group also experienced reductions in blood lactate from pre to post at 60, 90 and 150 watts ($p < 0.05$). The change in blood lactate concentration was observed in the FAST group at post-testing. When compared to the change observed in the FED group, it was not significant ($p > 0.05$), and both groups exhibited a similar reduction.

3.5. VO2 Max

Both groups experienced an increase in VO2 max from pre to post ($p < 0.01$). The increase in their VO2 max scores was significant. The overall change within the FAST group from pre to post was analysed vs. the overall change within the FED group from pre to post and this was found to be similar ($p = 0.24$).

3.6. Function Threshold Power (FTP)

When functional threshold power was assessed pre vs. post within the FAST group, the change was significant ($p < 0.05$), with mean ftp increasing from 119 (+/−18) watts to 127 (+/27) watts. An increase in FTP was also observed in the FED group from a pre value of 120 (+/−9) watts to a post value of 127 (+/−17) watts, but this increase was not significant ($p = 0.11$). This was potentially due to the individual change (9.9 watts per participant) in the FED group being less than the mean individual change (13.3 watts) within the FAST group. The overall change in the FAST group was similar to the overall change in the FED group, with no difference observed ($p = 0.81$).

3.7. 90-Minute Endurance

Results from the 90-minute endurance power test highlighted similar increases within both groups from pre to post. The FAST group experienced a significant increase from pre to post, pre = 81 (+/−23) watts to post = 96 (+/−30) watts (FAST $p < 0.01$). The Fed group also experienced a significant increase, changing from pre = 80 (+/−10) watts to post = 92 (+/−15) watts (FED $p < 0.05$). The analysis of overall change in FAST vs. FED was not significant ($p = 0.5$) as both groups experienced similar adaptations.

3.8. Rate of Perceived Exertion (RPE)

Session RPE was recorded for 18 low-intensity sessions and 18 high-intensity sessions. The FAST group RPE rating was higher for both low and high-intensity sessions when compared to the FED Group (FAST RPE V FED RPE low-intensity session $p < 0.05$), (FAST RPE v FED RPE high-intensity sessions $p < 0.05$). The mean RPE for the FAST group during the low-intensity sessions was 5.0 (+/−0.54), with the FED group recording an RPE of 4.7 (+/−0.68 for the same sessions. The FAST group mean score for the high-intensity sessions was 4.9 (+/−0.34), while the FED group mean score for the same session was 4.3 (+/−0.52).

4. Discussion

When analysing the results of this study, it is important to consider the potential benefits and drawbacks of implementing a sleep train-type regime with a female cohort of endurance athletes. The objective of adding a sleep train component to an endurance training regime is to increase the contribution of energy from fat and, in doing so, reduce the carbohydrate cost of exercise. Some of the potential benefits such as a increased contribution to enrgy from fats as experienced by the FAST group are outlined in Figs. 3, 4, and 5 and Table VIII. One of the main reasons outlined in previous research and position stands that sleep low train low protocols are not implemented with women is the risk of chronic calorie deficit and the associated complications (Aragon *et al*., 2017; Kerksick *et al*., 2017; Sims *et al*., 2023). We attempted to overcome this risk through individual calorie prescription with consideration for future training load (Frankenfield *et al*., 2005; Frankenfield, 2013). Resting Metabolic Rate (RMR) calculations using the Mifflin St. Jeor RMR formula for each participant were conducted combined with future training load calorie cost and average step count to inform the individual's daily calorie requirements. This was accompanied by observational food diaries

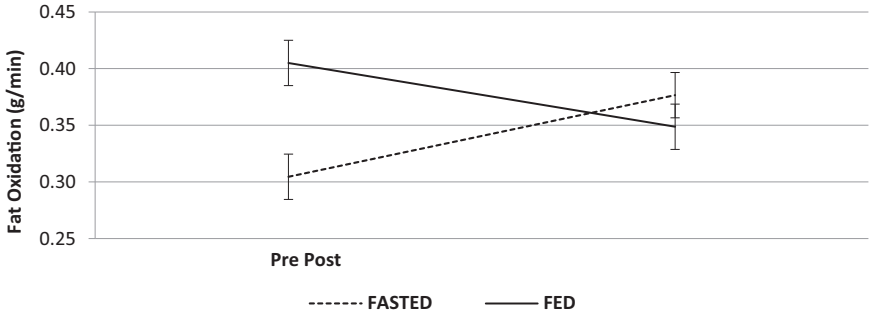

Fig. 3. Pre- and post-intervention group means for peak fat oxidation (g/min) ($p < 0.05$) with standard deviation.

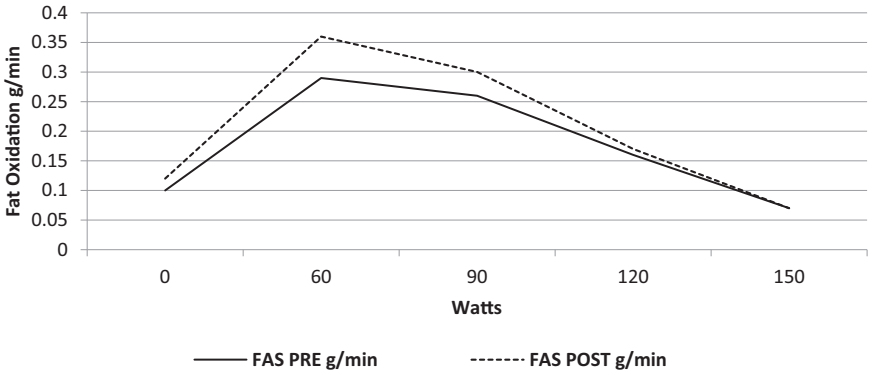

Fig. 4. Mean Fat Oxidation (g/min) for the FAST group pre and post-intervention across the FATMAX test.

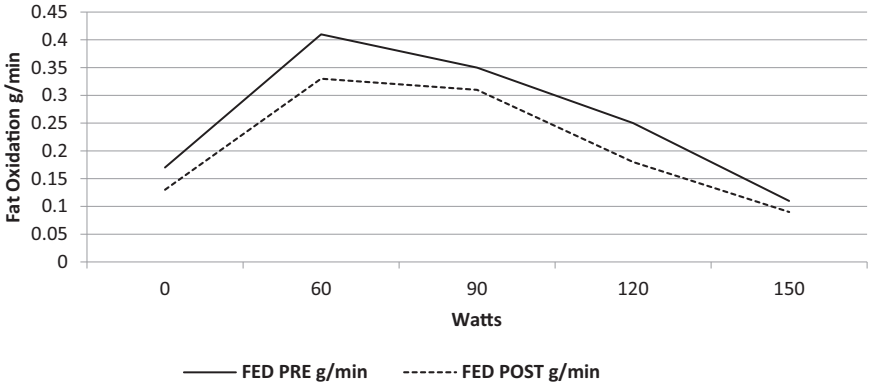

Fig. 5. Mean Fat Oxidation (g/min) for the FED group pre- and post-intervention across the FATMAX test.

throughout the study to ensure calorie prescriptions were being adhered to. Pre and post-mean weight for both groups would suggest this was successful (FAST pre-to-post mean weight 64.58 to 63.97 kg $p > 0.05$ FED pre-to-post mean weight 65.42 to 65.74 Kg $p > 0.05$). Calorie intake to support training load and ensure weight homeostasis was a critical component in determining the success or failure of this study.

Another potential drawback previously outlined to implementing a train-low regime is the increased risk of compromised immune function again due to chronic calorie deficit combined with increased training load (Kerksick *et al.*, 2017). Compromised immune function can manifest in many forms, but any form of inflammation, infection, or trauma will be accompanied by a rapid increase in CRP (Du Clos, 2000). Similarly, the resolution of any such inflammation, infection or trauma will be accompanied by a reduction in CRP (Du Clos, 2000). A 12-week exercise program comprising of either two (control group $n = 12$) or four (intervention group $n = 12$) aerobic sessions per week (each session 45–60 minutes) conducted with obese adolescents found no change in CRP from baseline to completion of the intervention (see Table VII). Similarly, we found baseline CRP levels in the FASTED and FED groups did not change significantly from pre to post-following 12 weeks of training, which included a sleep low train low element (FAST CRP pre 0.95 mg/l, post 1.1 mg/l, $p < 0.05$, FED CRP pre 1.5 mg/l, post 1.5 mg/l $p < 0.05$). The stated CRP levels are in keeping with the average ranges outlined by the American Heart Foundation as normal (average range 1–3 mg/l) (Wong *et al.*, 2008). This was potentially due to the less stressful method of reaching a fasted state for training through an overnight

TABLE IX: FAST AND FED GROUP PRE- AND POST-INTERVENTION MEAN MAXIMAL RATE OF BHB PRODUCTION AND STANDARD DEVIATION

	FAST (BHB mmol/l)	FED (BHB mmol/l)
Mean BHB prod pre	0.31 (0.17) @ 60w	0.67 (0.43) @ 90w
Mean BHB prod post	0.42 (0.36) @ 90w **	0.30 (0.35) @ rest -*

Note. **denotes a significant increase in BHB concentration and the intensity at which maximal concentration of BHB occurred. -*denotes a significant reduction in BHB concentration and the intensity at which it occurred.

TABLE X: BLOOD LACTATE CONCENTRATION FOR THE FAST AND FED GROUPS DURING FATMAX TEST PRE- AND POST-INTERVENTION

	@ REST	@ 60Watts	@ 90Watts	@ 120Watts	@ 150Watts
FASTED PRE blood lactate (mmol/l)	1.22	1.33	1.86	3.39	5.68
FED PRE blood lactate (mmol/l)	1.41	1.58	1.82	2.78	5.34
FASTED POST blood lactate (mmol/l)	1.13	1.28	1.58	2.59*	3.58*
FED POST blood lactate (mmol/l)	1.32	1.24*	1.34	2.23*	3.53*

Note. *denotes a significant reduction from pre to post in blood lactate at the specific wattage.

TABLE XI: MEAN VO2 MAX RESULTS PRE- AND POST-INTERVENTION WITH STANDARD DEVIATION

	FAST (ml/kg/min)	FED (ml/kg/min)
Mean Vo2 max pre	34.6 (6.9)	35.5 (2.4)
Mean Vo2 max post	40.5 (7.9)*	39.9 (3.9)*

Note. *denotes a significant change from pre to post in VO2 Max.

TABLE XII: MEAN AND STANDARD DEVIATION RPE VALUES FOR FASTED AND FED SESSIONS

RPE for session	Low-intensity	Standard deviation	High-intensity	Standard deviation
FAST group	5.0*	0.54	4.9*	0.34
FED group	4.7	0.68	4.3	0.52

Note. *denotes a significant difference between the FAST and FED groups when Low ($p < 0.05$) or High ($p < 0.05$) intensity session RPE was analysed.

sleep (naturally fasted). Other methods of reaching a fasted state, like that implemented in the twice-a-day training model, would appear to be more stressful when the fasted state is being achieved during waking hours (Nakamura *et al*., 2016; Stannard *et al*., 2010). Importantly, CRP levels would suggest that combining a chronic sleep low train regime with an overnight fast did not compromise immune function and result in other complications.

Any application of a low-training regime when performance is also a consideration must be beneficial in terms of outcome and how that outcome is supported through substrate utilisation. Ideally, control (FED) and intervention (FAST) groups should display the same or similar outcomes, with differences only noticeable when substrates to support the performance are analysed. As outlined in the results, both the FAST and FED groups experienced significant increases ($p < 0.05$) from pre to post in VO2 Max (see Fig. 10 and Table XI), functional threshold power (see Fig. 11) and 90-minute endurance power (see Fig. 12). These increases would all benefit endurance performance outcomes (Bassett & Howley, 2000; Sørensen *et al*., 2019). Both groups also displayed similar deceases in blood lactate from pre to post which again would be beneficial towards improved performance (see Fig. 9 and Table X) However, when FATMAX test results were analysed in both groups, peak fat oxidation had increased significantly in the FAST group ($p < 0.05$) from pre to post but decreased in the FED group from pre to post. This decrease was not significant ($p = 0.21$). It is worth noting at this point that the FAST group noted a higher RPE score for both fasted and fed sessions when compared to the FED group (see Table XII). Also, BHB production increased significantly in the FAST group @ 90 watts ($p < 0.05$) and decreased in the FED @ rest, 60 and 90 watts ($p < 0.05$). If we consider previous research outlines that optimally fuelled, an athlete can store approximately 90 minutes of carbohydrates and essentially an unlimited source of fats to fuel exercise (Burke, 2010; Jeukendrup, 2008). Any shift towards utilising more fats during performance would be beneficial, especially when events extend past 90 minutes. This becomes even more important, considering an average marathon takes approximately 240 minutes, while an average IRONMAN takes 12 to 15 hours (Knechtle *et al*., 2019; Lehto, 2016). This research would suggest that a train-low regime could prolong endogenous carbohydrate stores while utilising the greater fat stores during exercise.

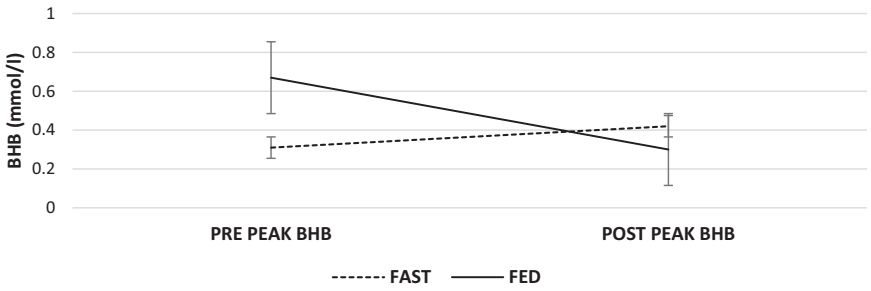

Fig. 6. Pre- and post-intervention peak BHB concentrations for the FAST and FED groups with standard error.

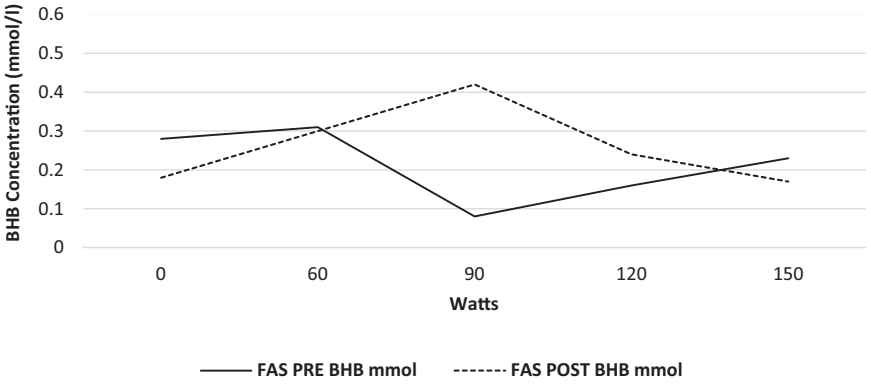

Fig. 7. Blood BHB FASTED Group during FATMAX test, pre- and post-intervention.

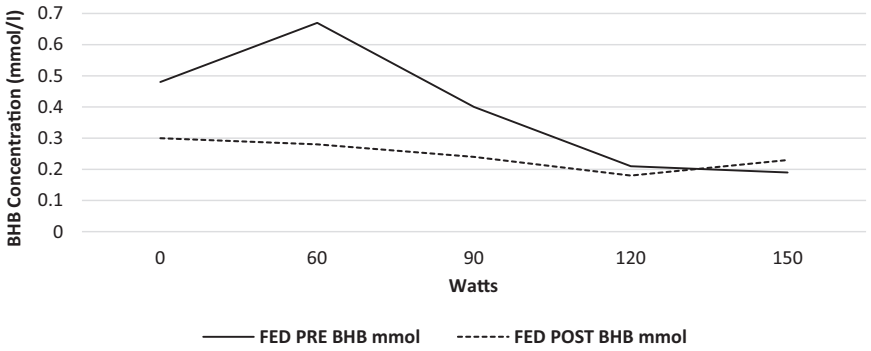

Fig. 8. Blood BHB FED Group during FATMAX test, pre- and post-intervention.

4.1. Acute Adaptation

The increased availability of BHB and Fats with an elevated contribution to energy from fats in the FAST group following the 12-week intervention is potentially due to a number of factors, both acute and chronic. When exercise is conducted in a fasted state, it has been reported that circulating concentrations of adrenaline and nor-adrenaline (catecholamines) can be elevated (Aird *et al*., 2018; Impey *et al*., 2016). When adrenaline and nor-adrenaline are elevated, it increases the mobilisation and availability of free fatty acids for energy production. With excess fat mobilisation and subsequent saturation of fatty acid transport across cell membranes, the liver converts the excess fats into acetoacetate and BHB (ketones) (Vanitallie & Nufert, 2003). These ketones can be re-distributed by the liver and converted back into Acetyl Coa in the muscle for energy production through the Krebs cycle. The liver, however, cannot utilise ketones for fuel as it lacks the required enzyme for this process (beta ketoacyl-CoA transferase). Interestingly, the FED group experienced a reverse in fat oxidation and BHB concentration from pre to post-intervention. This reverse occurred both at rest and during FATMAX/VO2 Max testing. Considering both groups completed the same training protocol, with only a slight alteration in feeding times and diet, the effect was increased fat oxidation in the FAST group. It decreased fat oxidation within the FED group during exercise. It is worth noting again that FATMAX/VO2 Max tests were conducted after an overnight fast (10–12 hours) for both groups. See Figs. 6, 7, and 8 and Table IX for an over view of the responses experienced by both groups during this 12 week intervention.

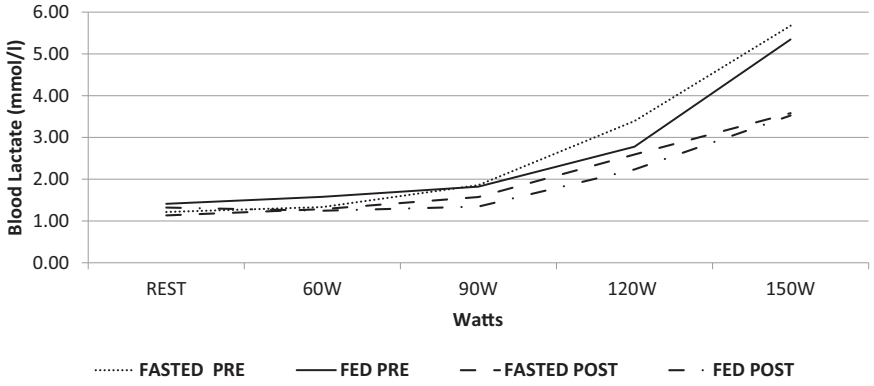

Fig. 9. Blood lactate production at rest pre-test and during the FATMAX test pre- and post-intervention.

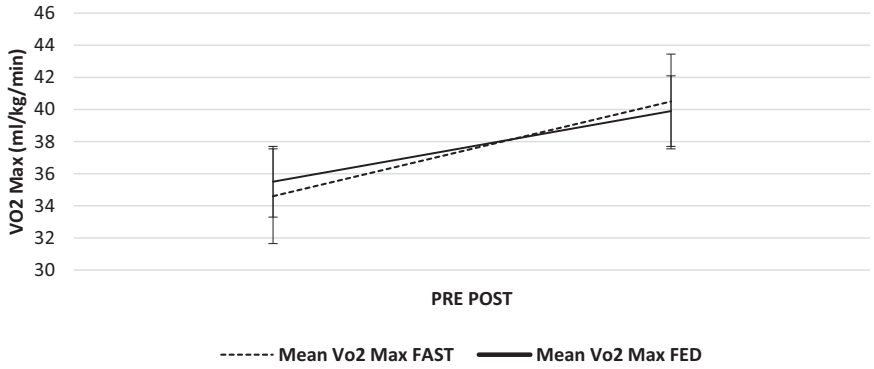

Fig. 10. VO2 max results pre- and post-intervention for FAST and FED Groups with standard error.

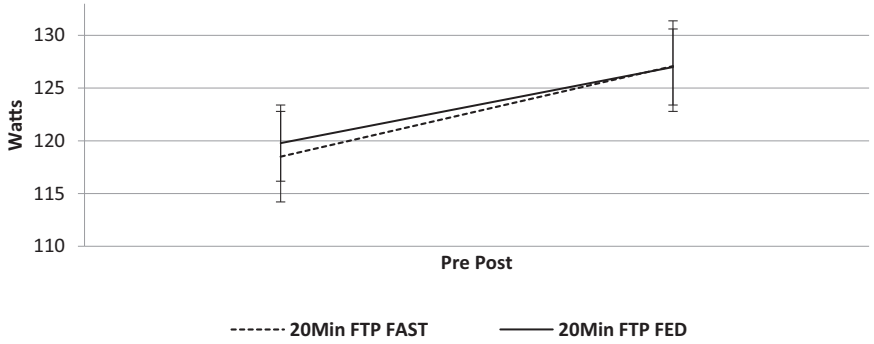

Fig. 11. FTP pre- and post-intervention for the FAST and FED Groups with standard error.

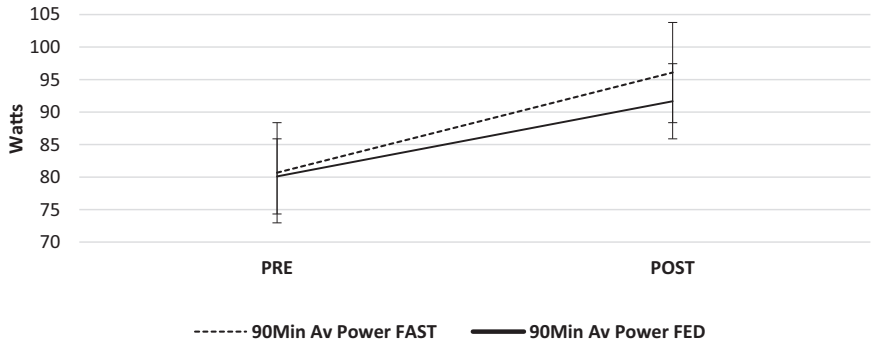

Fig. 12. 90 Minute Endurance Power pre- and post-intervention for the FAST and FED Groups with standard error.

4.2. Chronic Adaptation

Increased fat oxidation during exercise following a chronic train low protocol has been reported to result from mitochondrial biogenesis. Mitochondrial biogenesis refers to the process of creating new mitochondria in the body. The creation of new mitochondria is stated to be caused by a cascade which commences when AMP-activated protein kinase (AMPK), a cell regulatory system, is activated

due to a low ATP:ADP (adenosine tri-phosphate:adenosine di-phosphate) ratio (glycogen depletion). AMPK, in turn, activates peroxisome proliferator-activated receptor-gamma coactivator one alpha (pgc1-alpha), a transcription coactivator. PGC1-alpha, in turn, starts the chain of transcription and translation where DNA is transcribed to RNA, and in turn, RNA is translated to produce proteins to create new mitochondria (Hood, 2009; Pengam *et al*., 2021). The FAST group repeatedly creates a cellular environment where the ATP:ADP ratio is being manipulated towards this scenario. There is the potential that increased fat oxidation during exercise is due to chronic mitochondrial biogenesis, which leads to increased mitochondrial density and, in turn, the ability to utilise more fats for energy production.

5. Limitations

The main limitation observed following the analysis of this research project was that the FED group recorded a higher pre-intervention peak fat oxidation rate than the FAST group. At the outset of the project, consideration was given to group allocation for participants. Given the project's performance-based approach, it was decided that groups would be formed based on VO2 Max scores. There was no significant difference in VO2 Max between groups at the outset. The difference in peak fat oxidation rates of each group at pre-intervention was only highlighted when the project had commenced. We found that the FED group had more run-based athletes (weight-bearing) when group participants were analysed individually. In contrast, the FAST group consisted of more Swim/Bike athletes (non-weight bearing). Previous research suggests runners experience higher rates of fat oxidation than cyclists (Achten *et al*., 2003; Capostagno & Bosch, 2010). Considering this, there may have been a level of fat adaptation already in place within the FED group due to the higher frequency of Runners within this group.

6. Future Research

During this project, the menstrual cycle phase was established and accounted for through questionnaires and oral temperature measurements. In future research projects, menstrual cycle hormone tracking in conjunction with exercise across an intervention would allow for further analysis of the effect sex hormones could impose on substrate utilisation during exercise.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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