European Journal of Physical Education and Sport Science
ISSN: 2501 - 1235
ISSN-L: 2501 - 1235
Available on-line at: www.oapub.org/edu

DOI: 10.46827/ejpe.v11i7.5715 Volume 11 | Issue 7 | 2024

INVESTIGATING THE EFFECTS OF HORMONAL FLUCTUATIONS ASSOCIATED WITH MENSTRUAL CYCLE ON PEAK FAT OXIDATION DURING GRADED EXERCISE IN ENDURANCE-TRAINED WOMEN

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Abstract:
Background: The menstrual cycle (MC) and associated hormones have become a popular subject of discussion due to their potential impact on how women utilize energy sources during exercise. Previous research has reported MC does not affect performance and also MC does not affect peak fat oxidation (PFO). However, given the variability of hormonal concentrations over time their effects can be variable at different phases within the MC (1,2,7). **Purpose:** In this study, our aim was to assess if three different phases within the MC, mid follicular (MF), late follicular (LF) and mid-luteal (ML), had differing effects on peak fat oxidation and substrate utilisation due to varying hormones. **Methods:** A group of endurance-trained women (n=12) aged 37 (+/- 2.9 years) were randomly recruited and assigned to the mid-follicular (MF), late follicular (LF) or mid-luteal (ML) group. MC length was established, and within the MC the LF phase was firstly identified through the calendar method and subsequently through hormonal verification. Following calendar and hormonal verification of the LF phase, both MF and ML phases were identified using the same method. FATMAX tests combined with hormonal quantification were then conducted on each participant at each phase of interest following an overnight fast. **Results:** PFO occurred at 50 watts in the MF and LF phase with PFO occurring at 35 watts in the ML phase. PFO at the MF phase was 0.344g/min, it

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was 0.345g/min at LF and was 0.275g/min at the ML phase. Further analysis highlighted that there was no difference between PFO rates at MF and LF ($p>0.05$). There was a difference in PFO rates between MF and ML, but this difference was marginal but not significant ($p=0.11$). Differences in PFO rates between LF and ML were found to be significant ($p<0.05$). More in-depth analysis utilising a One-way ANOVA for fat oxidation rates at each stage across the complete FATMAX test found no significant difference at any stage in fat oxidation rates ($p>0.05$). When peak CHO oxidation was calculated, results outlined 1.78g/min during the MF phase, 1.79g/min during the LF phase and 2.02g/min during the ML phase and all at 125 watts. When CHO oxidation rates were statistically analysed at each intensity utilising a One Way Anova, there was no difference in CHO oxidation rate irrespective of the phase. When peak CHO was assessed via a T test in pairs, MF V LF ($p>0.05$), MF V LF ($p>0.05$) and LF V ML ($p=0.13$) there was no significant difference. When hormone concentrations were assessed in pairs (MF v LF, MF v ML & LF v ML) at the three points of interest within the MC, utilising the paired sample t-test it was noted that there was no significant difference between E3G concentrations at MF to LF ($p=0.12$), MF to ML ($p=0.29$) and LF to ML ($p=0.12$). Similarly, PdG concentrations were assessed, and it was highlighted that between MF to LF there was no difference ($p>0.05$), however, MF to ML and LF to ML concentrations of PdG were significantly different ($p<0.05$). LH concentrations were statistically analysed also employing the paired sample t-test, and it was observed to be significantly different from MF to LF ($p<0.05$) and LF to ML ($p<0.05$) but MF to ML was not significant ($P>0.05$). In conclusion, a significant difference in PFO was detected between LF and ML phases, but no difference in PFO was observed between MF and LF phases or MF and ML phases. Also, no difference in Fat oxidation or CHO oxidation was detected between any phase at each intensity.

Keywords: menstrual cycle, menstrual cycle phase, hormone fluctuations, peak fat oxidation, graded exercise & endurance

1. Introduction

The human body is a complex and intricate system, influenced by numerous factors that can impact its performance and function. One such factor that has garnered increasing attention in recent years is the MC in women. Beyond its role in reproductive health, the MC is associated with hormonal fluctuations that have been suggested to affect various aspects of a woman's physiology, including metabolism and exercise performance (1,3). Understanding the relationship between hormonal fluctuations during the MC and exercise metabolism is crucial for optimizing training strategies and enhancing athletic performance in women (1,4). This article aims to investigate the effects of these hormonal fluctuations on PFO and substrate utilisation during graded exercise in women, shedding light on how the MC may influence substrate utilization (Fat & Carbohydrate Oxidation) and ultimately impact overall exercise capacity. By investigating the intricate interplay

between hormones and substrate utilization, we can gain valuable insights into how MC can affect exercise. We can also get a better understanding of the variability of hormones associated with MC and how this variability can lead to differing effects from woman to woman (8,9,10).

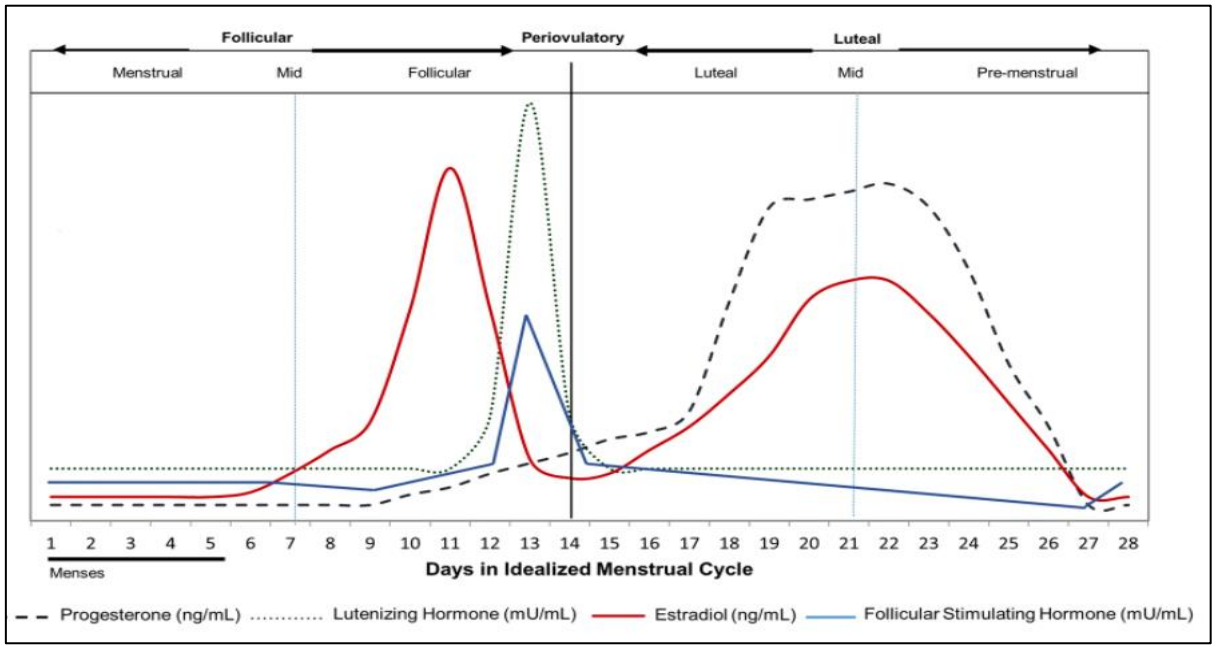

Figure 5.1: Menstrual cycle hormone fluctuations association with theoretical 28-day MC

1.1 Menstrual cycle

As mentioned, the MC is a complex process that plays a crucial role in the reproductive health of females but also influences many other processes. It is governed by a delicate interplay of hormones that orchestrate the various stages. The theoretical MC lasts approximately 28 days and is broken into three distinct phases: the Follicular phase, Ovulation and the Luteal phase (5,6). Each phase is characterised by alternating peaks and troughs of Oestrogen or Progesterone along with Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Before we attempt to understand how these hormones affect substrate utilisation we must first understand each phase and its characteristics (11,12).

1.2 The follicular phase (Day 1 to 14 approx.)

The Follicular phase of a 28-day MC lasts approximately 12 to 14 days from day 1 to day 14. Within this phase, there are further distinctive components. Day one of a 28-day MC starts with Menstruation, which typically lasts between 3 to 7 days. Menstruation is when a woman experiences her period. This is caused due to the lining of the uterus which had thickened in preparation for pregnancy, now sheds as blood and tissue. During this time, all four hormones, oestrogen, progesterone, LH and FSH, are at baseline concentrations (13,14).

1.3 Mid-follicular phase (MF)

During the MF phase of the MC, which typically occurs around day 7 of a 28-day MC the focus shifts to the development of ovarian follicles. This phase is initiated by the release of Follicle Stimulating Hormone (FSH) from the pituitary gland, which stimulates the growth and maturation of several follicles in the ovaries. As these follicles develop, they produce oestrogen, which helps thicken the uterine lining in preparation for a potential pregnancy. The dominant follicle continues to grow and produce more oestrogen, signalling to the body that it is ready for ovulation (11,14).

1.4 Late follicular phase

During the late follicular phase of the menstrual cycle, hormone levels continue to fluctuate as the body prepares for ovulation. Oestrogen levels rise steadily during this phase, reaching their peak just before ovulation occurs. This surge in oestrogen stimulates the thickening of the uterine lining in preparation for a potential pregnancy. Additionally, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play crucial roles in stimulating the maturation of ovarian follicles, with LH triggering the release of a mature egg from one of the follicles. As oestrogen levels peak towards the end of the late follicular phase, they signal to the body that it is time for ovulation to take place. This hormonal shift sets the stage for the next phase of the menstrual cycle, where an egg is released from the ovary and becomes available for fertilization (15).

1.5 Ovulation

In the latter stages of the follicular phase, as the follicles develop, they compete for dominance. The follicle that produces the most oestrogen becomes the dominant follicle, while the others regress. The rising levels of oestrogen from the dominant follicle trigger a surge in LH release from the pituitary gland. This LH surge is the key player in ovulation. The LH surge triggers a series of events within the dominant follicle, leading to the release of the mature egg from the ovary. This is ovulation, which typically occurs around day 14 of a regular 28-day menstrual cycle. The released egg is swept up by finger-like structures called fimbriae at the end of the fallopian tubes. These structures help guide the egg into the fallopian tube. The egg remains viable in the fallopian tube for 12-24 hours after ovulation, waiting for potential fertilization (16,17).

1.6 Luteal phase

The luteal phase of MC begins after ovulation and lasts for about 14 days, regardless of whether pregnancy occurs. During this phase, the remaining follicle forms a structure called the corpus luteum, which produces the hormone progesterone. Progesterone prepares the lining of the uterus for the implantation of a fertilized egg. If pregnancy does not occur, the corpus luteum degenerates, progesterone levels decrease, and the lining of the uterus sheds, leading to the next menstrual period. If a pregnancy occurs, the corpus luteum continues to produce progesterone, which is essential for maintaining a healthy

pregnancy. The menstrual cycle will then stop until after childbirth and breastfeeding have ended 12,18).

1.7 Mid-luteal phase

The mid-luteal phase typically occurs around days 21-23 after ovulation in a 28-day MC. Progesterone is the dominant hormone during this phase. Progesterone stimulates the growth and vascularization of the endometrium, creating a receptive environment for a fertilized egg. High progesterone levels prevent further ovulation during this cycle. Oestrogen levels remain moderately elevated compared to the follicular phase. They work synergistically with progesterone to support endometrial growth and preparation. LH Levels are low and stable in the mid-luteal phase, contrasting with the surge observed just before ovulation. FSH similar to LH, FSH levels are also low and stable during this phase (11,12).

1.8 Late luteal phase

During this phase, as mentioned previously, if pregnancy occurs, progesterone will continue to remain elevated in support of a healthy pregnancy, and if pregnancy does not occur, the corpus luteum degenerates, progesterone levels decrease, and the lining of the uterus sheds, leading to the next menstrual period, and the cycle repeats itself (11,12).

1.9 Substrate utilisation and hormones

When we review specific phases of the MC with consideration for substrate utilisation it becomes clearer as to why an understanding of the whole MC process is extremely important for the female endurance athlete. These considerations become even more relevant as the distance and duration of endurance events are becoming longer and longer, with an even greater need for a detailed understanding of each athlete's individual needs and how their endogenous fuel stores respond during exercise. If we consider recent research suggests that during the MF phase of the MC, when all sex hormones are at baseline levels, it is reported that females oxidise substrates similarly to males, given that hormones are low and, therefore, not having a significant effect. During the MF phase, females typically oxidise more fats for fuel at lower intensities, but as exercise intensity increases, substrate utilisation shifts from fats to carbohydrates. This is similar to how a male athlete would respond to exercise in terms of substrate utilisation (1,7).

As the MC moves forward and we move into the LF phase, oestrogen levels start to rise independently of progesterone and around day 12 of the 28-day MC, oestrogen peaks. Oestrogen is reported to shift metabolism towards fats for fuel (1). This is partially due to the increased concentrations of glycogen synthase, which increases with the increase in oestrogen. Glycogen Synthase has a glycogen sparring effect and promotes glycogen storage over glycogen utilisation. At this point (LF phase) in the MC, endurance performance may be enhanced due to increased utilisation of fats for fuel, partly due to the MC hormone profile creating this shift in metabolism.

The potential effects or lack of effect become more difficult to predict when we move into the ML luteal phase. The variability of effects on substrate utilisation at this point could be due to both oestrogen and progesterone being elevated simultaneously. How substrate utilisation is affected here is potentially due to a number of factors. The first factor to consider is the change or magnitude of change in oestrogen concentrations from MF to ML. The second factor to consider here is the ratio of oestrogen to progesterone at the ML phase (1). A systematic review conducted by Oosthuyse & Bosch (2010) mentioned that when the magnitude of change of oestrogen from MF to ML was 4:1 (nmol:nmol) or greater and the ratio of oestrogen to progesterone at the ML phase was in excess of 18:1 (pmol:nmol) there was potential for improved endurance performance in events such as a time trial (1). However, when the ratio of change was measured at lesser concentrations, there was no improvement, and, in some cases, it was performance degrading. It is worth mentioning that various studies have outlined many different effects which elevated progesterone can impose on females, which have the potential to affect performance, separate to effecting substrate utilisation (19, 20, 21, 22). Some of these implications reported due to increased progesterone would be increased basal body temperature, which is widely reported to rise following ovulation when progesterone rises. Elevated progesterone is also proposed to increase sweat rate which increases salt losses, reduced plasma volume, and heat tolerance is reduced which can all lead to earlier fatigue (23).

With consideration for substrate utilisation and the three specific phases within the MC, the MF phase is when all sex hormones are at baseline, the LF phase is when oestrogen is elevated in isolation, and the ML phase is when both oestrogen and progesterone are elevated. We decided to conduct a project investigating the potential effects imposed on substrate utilisation and PFO during graded exercise at these distinct phases.

2. Material & Methods

2.1 Subjects

Fifteen females (37+/- 2.9 years) actively engaged in endurance-type exercise completed pre-screening and informed consent before being invited to participate in this study (See Appendix 5A & 5B). Twelve participants completed the three FATMAX tests and one VO2 Max test, with five of the twelve completing the extra FATMAX test for reliability purposes. Two participants completed two tests and then had to withdraw due to overseas commitments, with one participant withdrawing due to a prolonged respiratory infection. The study was examined and approved by the South East Technological University (SETU) ethics committee. All participants were informed via letters to clubs, information fliers, informed consent forms and Q&A sessions in relation to the format of the study and testing protocols (See Appendix 5C, 5D & 5E). Their sports included triathlon, marathon, and other ultra-distance events. Only data for those who completed the study is included in the results.

2.2 Equipment

- Mira hand-held fertility analyser,
- Mira max wands for Oestrogen (E3G), Progesterone (PdG) and Luteinizing Hormone (LH),
- Keto-mojo hand-held glucose analyser,
- Keto-mojo glucose strips,
- Moxus Metabolic Cart,
- Lode Corival resistance simulating stationary bicycle.

2.3 Identifying MC phases and verification (See Appendix 5G & 5H)

The MC phases of interest were the mid-follicular (MF), phase or low hormone phase, the late-follicular (LF) and the mid-luteal phase (ML). MC length was set at 100%, with time of ovulation at 50%. MF was then set at 25%, with LF from 40 – 45 % and ML at 75%, as previously applied by Frandsen et al. (3). Applying the below formula, the MF, LF and ML phases were identified;

- MF = MC length x 0.25,
- LF = MC length x 0.40,
- LF = MC length x 0.45,
- ML = MC length x 0.75.

MC length is the number of days from the start of menses, through one complete cycle and back to the start of menses (theoretical model suggests 28 days +/- 5 days). Following phase identification using the above formula, the Mira fertility analyser was used to verify the pre-ovulation oestrogen spike over 3 days, which characterises the LF phase. Oestrogen concentrations can be determined (using the Mira fertility analyser and Max wand) from urinary analysis of the oestrogen metabolite esterone-3-glucuronide (E3G)[19,20]. Identification of the oestrogen spike would confirm the LF phase. Following confirmation of the LF oestrogen spike and verification of calendar dates, three FATMAX tests were scheduled, one at MF, one at LF and one at ML. The progesterone peak which characterises the LF phase, was also measured through urinary analysis of the metabolite pregnanediol glucuronide (PdG). PdG was also measured using the Mira fertility analyser (24,25). Once phases were identified and verified, MC was mapped and test dates highlighted (See Appendix I)

2.4 Alternating test sequence

To minimise as much as possible the learning effect of testing, the first test for each participant was alternated to a different phase from the proceeding participant. This ensured, that from the 12 participants, four commenced testing during their MF phase, 4 commenced testing during their LF phase, and four commenced testing during their ML phase of their MC.

2.5 Participant pre-test protocol (Day before testing)

The day preceding each test, participants were asked to;

- Refrain from exercise,
- Record their diet (test days 2 & 3 participants would follow as closely as possible the same meal plan),
- Consume their last meal before 9pm.

2.6 Participant pre-test protocol (Morning of testing, completed at home)

- No pre-test meal (over-night fasted).
- Participant collects a sample of the first urine of the day.
- Dip the Mira max wand in the urine sample for 15 to 20 seconds.
- Remove the wand and place it in the mire fertility analyser.
- Check Mira fertility analyser has paired with mire app.
- Mira fertility analyser starts a countdown (16 minutes) and uploads results to Mira app.
- The Mira Max wand and analyser deliver E3G, PdG and LH concentration.

2.7 Participant pre-FATMAX Protocol (Stationary Bike – Lode Corival)

- Participant arrives at SETU Arena, Human Performance Lab (HPL) in a fasted state.
- Pre-test hormones are recorded on a test sheet from Mira app.
- A finger prick blood sample is collected and analysed for blood glucose. This is to verify that fasting blood glucose concentrations are in keeping with published norms.
- Participant is set up on Lode Corival stationary bike with settings recorded (saddle height, handle bar position.
- Head piece, mouth piece and nose clip are fixed in place on the participant and checked for comfort and operation.
- Moxus metabolic cart breath collection pipework is connected to the participant and Moxus starts to analyse inhaled and expired air. (not recording at this point).

2.8 FATMAX test protocol (Stationary Bike)

- Following set-up; participant details are inputted into the Moxus desktop PC.
- Test and data collection commences with the participant seated on the bike, no pedalling for 3 minutes.
- Within the final minute of each 3-minute stage a blood glucose and a blood beta-hydroxybutyrate (BHB) samples are collected from the fingertip, analysed on a handheld analyser and recorded on the participant test sheet.
- At 3 minutes, stage 1 of cycling commences with a resistance of 35 watts.
- Resistance increases by 15 watts every 3 minutes until a resistance of 125 watts is maintained for 3 minutes or a respiratory exchange ratio of 1.0 is achieved for 1 minute.

- Following one of the aforementioned being achieved, the test is concluded and data collection is halted. All data is anonymised and stored on the Moxus desktop in the SETU Arena, HPL.
- Participants cool down, and are supplied with water and an energy gel.

2.9 VO2 Max Protocol (Stationary Bike)

- The VO2 Max test for each participant commenced following the completion of their third FATMAX test (fourth if they were partaking in reliability testing).
- Participants were allowed three minutes to ready themselves between FATMAX completion and VO2 Max start.
- VO2 Max test began at 50 watts for 30 seconds.
- Resistance increased by 25 watts every 30 seconds.
- The VO2 Max test concluded when either the participant reached fatigue and could not continue or when they were unable to maintain the required resistance, and the test was stopped by the researcher.

3. Results

In Figure 5.2 below, E3G concentrations are displayed from data gathered when verifying the pre-ovulation or LF oestrogen spike (E3G urine concentration) of the MC. When concentrations were statistically analysed using a paired sample t-test across the 3 days, it was highlighted that there was a significant difference in E3G from day 1 to day 2 ($p<0.05$), day 1 and day 3 were not significantly different ($p>0.05$) and day 2 and day 3 were not significant, but this was borderline ($p=0.07$).

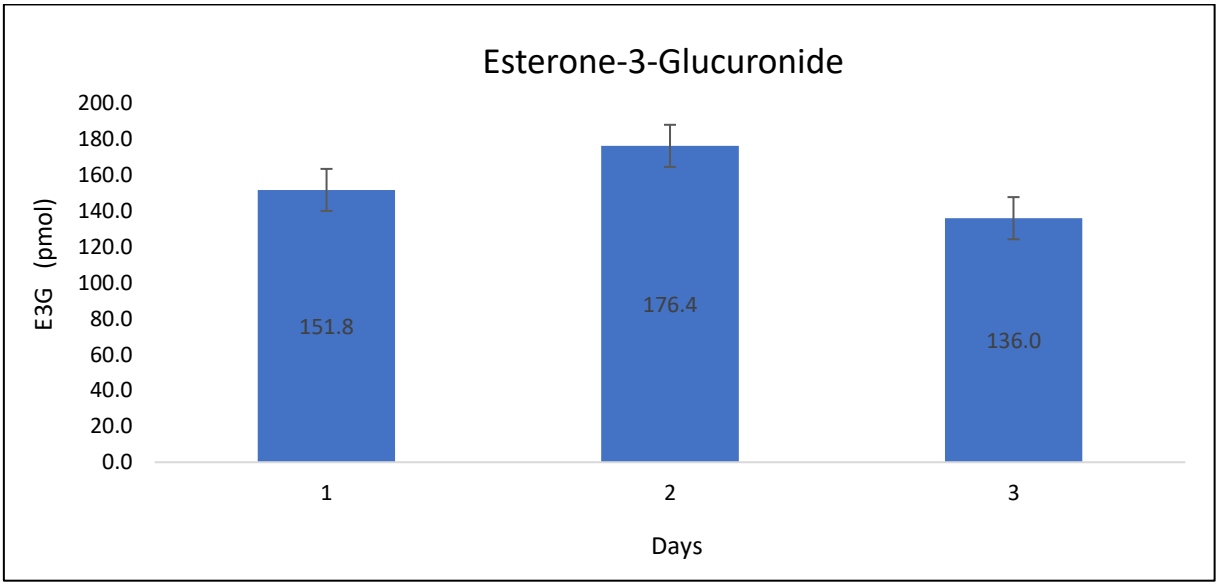

Figure 5.2: Mean E3G concentrations with standard error (SE) across 3 days of MC phase identification

Table 5.1 shows the mean E3G, PdG and LH concentrations with standard error which were gathered during phase verification. Similarly to the statistical analysis conducted on E3G concentrations, PdG and LH concentrations were assessed utilising a paired sample t-test across the three days of phase verification. PdG concentrations were found to be similar across all 3 days with no significant differences ($p>0.05$). LH concentrations were found to be similar on days 1 and 2 ($p>0.05$), days 1 and 3 were significantly different ($p<0.05$), and days 2 and 3 were not significantly different, but this again was borderline ($p=0.09$).

Table 5.1: MC hormone concentrations & standard error across 3 days of phase verification (SE=Standard Error)

MC Hormones & Standard Error	Day 1	Day 2	Day 3
Mean E3G (ng/ml)	151.8	176.4	136.0
SE	27.9	33.3	19.3
Mean PdG (ug/ml)	2.6	2.3	2.3
SE	0.30	0.28	0.39
Mean LH (IU/L)	3.2	3.4	4.1
SE	0.52	0.40	0.50

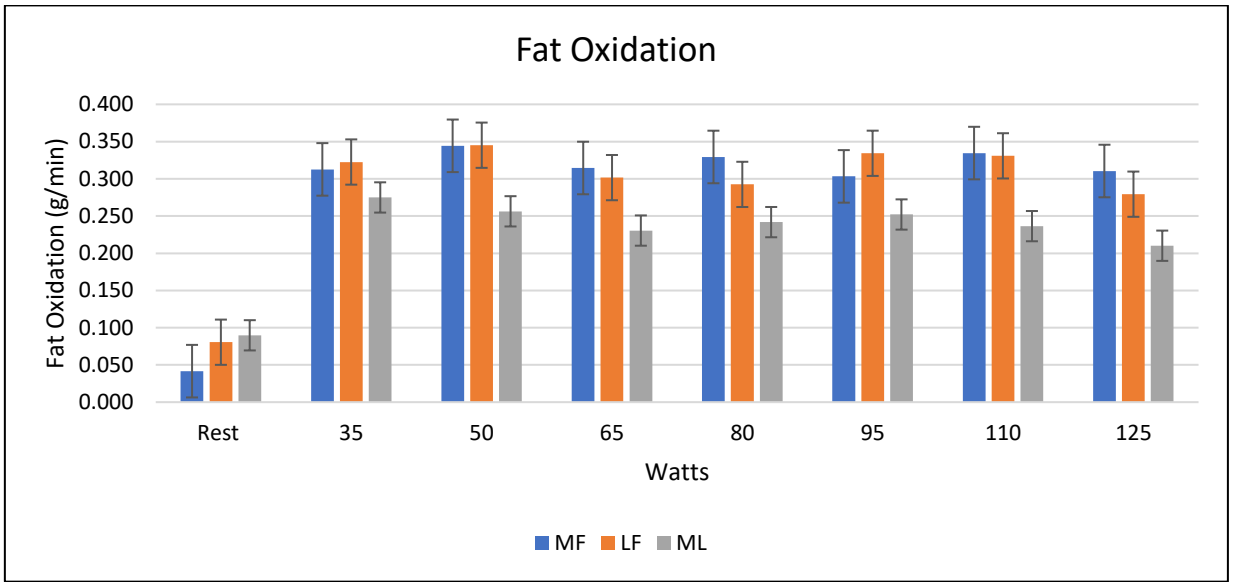

Figure 5.3: Mean fat oxidation across FATMAX tests @ MF, LF & ML phases with SE

Above, in Figure 5.2, is shown the different fat oxidation rates at each stage during the FATMAX test. PFO occurred at 50 watts in the MF and LF phase with PFO occurring at 35 watts in the ML phase. PFO at the MF phase was 0.344g/min, it was 0.345g/min at LF and was 0.275g/min at the ML phase. Further analysis highlighted that there was no difference between PFO rates at MF and LF ($p>0.05$). There was a difference in PFO rates between MF and ML, but this difference was marginal but not significant ($p=0.11$). Differences in PFO rates between LF and ML were found to be significant ($p<0.05$). More in-depth analysis utilising a One Way ANOVA for fat oxidation rates at each stage across the complete FATMAX test found no significant difference at any stage in fat oxidation

rates (p>0.05). When hormone concentrations (Table 5.2) were assessed at the three points of interest within the MC, utilising the paired sample T-test, it was noted that there was no significant difference between E3G concentrations at MF to LF (p=0.12), MF to ML (p=0.29) and LF to ML (p=0.12). Similarly, PdG concentrations were assessed, and it was highlighted that between MF to LF, there was no difference (p>0.05). However, MF to ML and LF to ML concentrations of PdG were significantly different (p<0.05). LH concentrations were statistically analysed also employing the paired sample t-test and it was observed to be significantly different from MF to LF (p<0.05) and LF to ML (p<0.05) but MF to ML was not significant (P>0.05).

Table 5.2: Mean hormone concentrations @ MF, LF & ML during FATMAX testing with SE

	MF	***LF***	***ML***
Mean E3G (ng/ml)	104.7	180.8	124.2
SE	13.37	44.01	12.40
Mean PdG (ug/ml)	4.4	3.3	11.9
SE	0.73	0.84	2.78
Mean LH (IU/L)	4.9	10.2	4.8
SE	0.88	1.14	0.56

In Figure 5.3, the BHB concentrations are presented. It can be observed that peak BHB occurred at 65 watts during the MF phase (0.35mmol/L) but was noted at 50 watts during the LF (0.35mmol/L) and ML (0.38mmol/L) phases. However, when peak BHB was statistically assessed by means of a one-way ANOVA, it was highlighted that there was no difference in BHB concentrations.

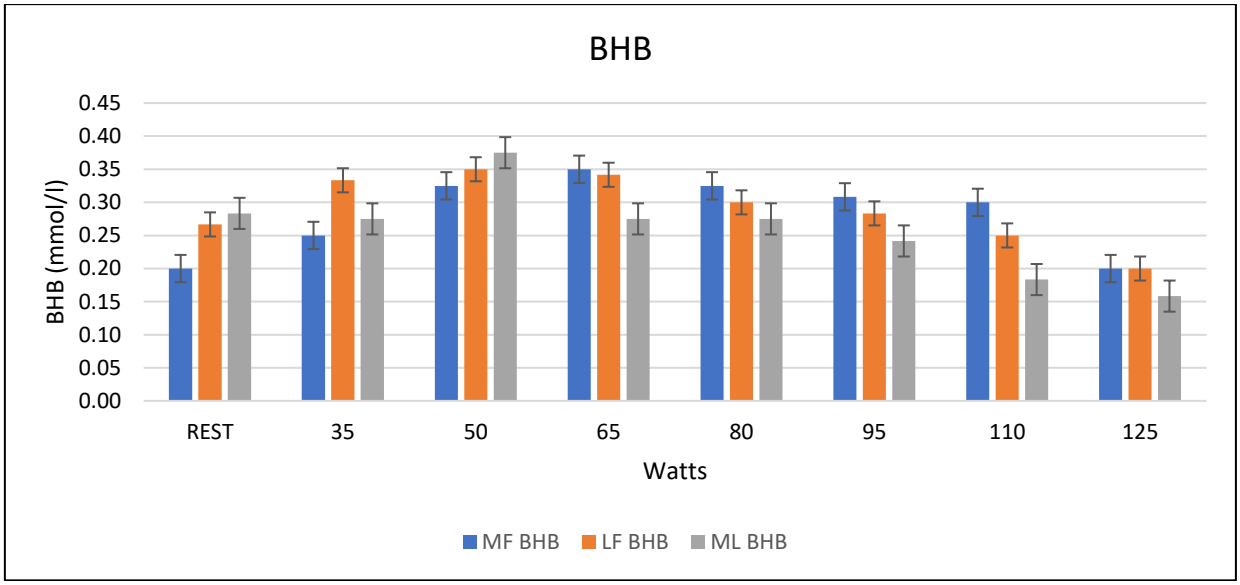

Figure 5.4: Mean BHB concentrations at each stage during FATMAX test @ MF, LF & ML phases with SE

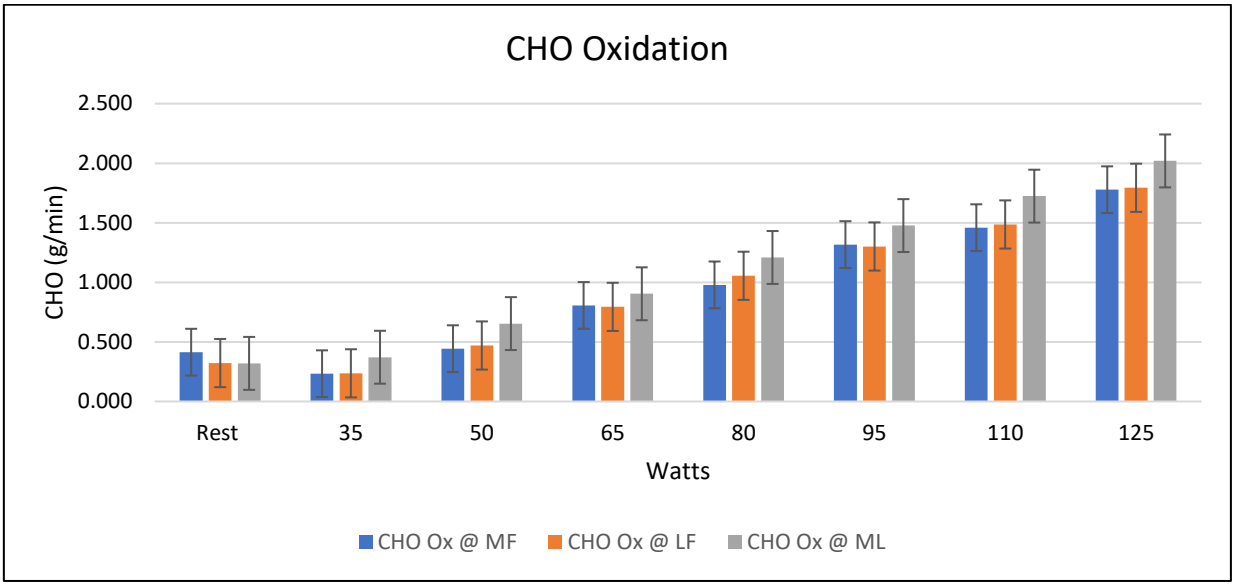

Figure 5.5: Mean CHO oxidation at each stage during FATMAX test @ MF, LF & ML phases with SE

Figure 5.4 displays mean CHO Oxidation rates across the FATMAX test during each phase of the MC. Peak CHO oxidation occurred at 125 watts in all phases. Peak CHO oxidation was 1.78g/min during the MF phase, 1.79g/min during the LF phase and 2.02g/min during the ML phase. When the CHO oxidation rate was statistically analysed at each intensity utilising a one-way ANOVA, there was no difference in CHO oxidation rate irrespective of the phase. When peak CHO was assessed via a T-test in pairs, MF V LF ($p>0.05$), MF V LF ($p>0.05$) and LF V ML ($p=0.13$), there was no significant difference. However, it is worth noting that the LF and ML peak CHO oxidation rate was not significantly different; it was marginal.

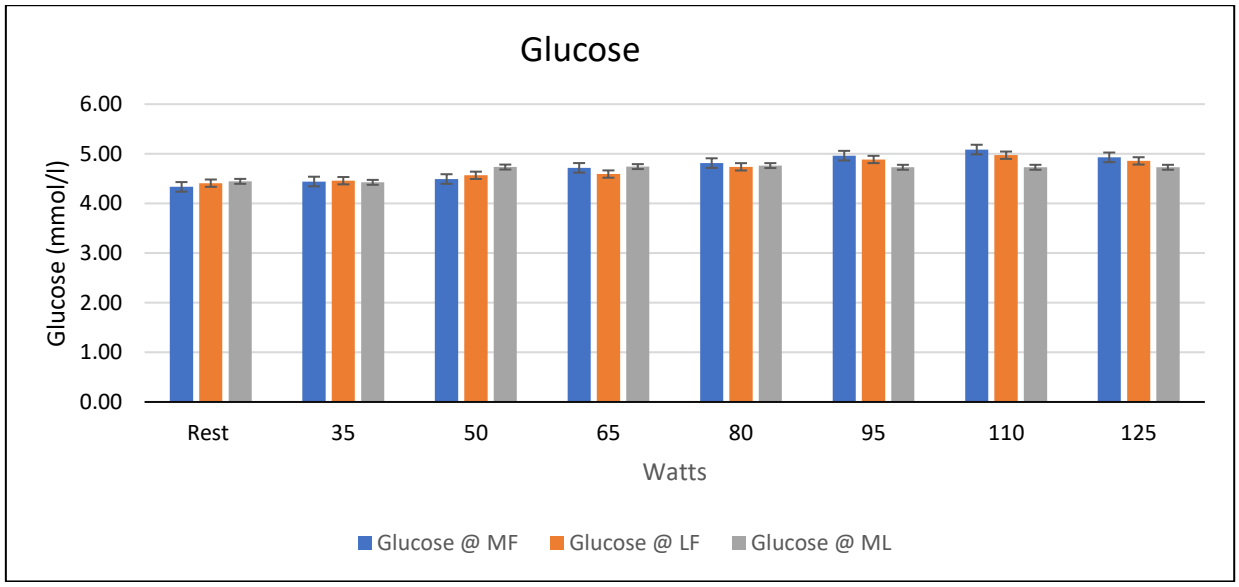

Figure 5.6: Mean Glucose concentration at each stage during FATMAX test @ MF, LF & ML phases with SE

Figure 5.5 presents glucose concentrations across the FATMAX test at each intensity. Peak glucose concentrations occurred at 110 watts at both MF and LF phases. Peak glucose concentration occurred at 80 watts in the ML phase. At the MF and LF phases, peak glucose concentration was 5.09mg/dL and 4.97mg/dL, respectively. At the ML phase, it was 4.76mg/dL. There was no difference in glucose concentrations at any intensity or MC phase.

4. Discussion

When reviewing the results obtained in this study, similarities can be observed between other studies, such as that conducted by Frandsen et al. (7). Results from the Frandsen study reported no difference in PFO across the three phases MF, LF and ML. Likewise, the results outlined above detected no difference between MF and LF or MF and ML. However, a significant difference in PFO was detected between LF (0.345g/min) and ML (0.275g/min). When further analysing this difference and reviewing the hormone concentrations, we notice that although there was no difference in E3G concentrations between MF (104.7ng/ml) to LF (180.8ng/ml), MF to ML (124.2ng/ml) or LF to ML, these fluctuations in E3G were in keeping with the typical profile of the theoretical MC (5). There was a significant difference in PdG concentrations between MF (4.4ug/ml) to ML (11.9ug/ml) and LF (3.3ug/ml) to ML. This difference, again, is typical of the hormone profile of the MC and potentially offers support to the theory that increased progesterone concentration associated with the ML phase of the MC could impose an impact on PFO (1,5). It's also worth noting here that in conjunction with PFO being significantly different when comparing LF and ML phases, the intensity at which PFO occurred was lower during the ML (35 watts) phase when compared to both MF (50 watts) and LF (50 watts). This again offers support to the theory that increased progesterone can affect PFO (1).

In contrast to these findings, both mean Fat oxidation and CHO oxidation rates were assessed throughout the FATMAX test. Fat and CHO oxidation was calculated and analysed at each intensity during the FATMAX test and at each of the three phases of interest. No difference in either mean FAT or CHO oxidation was detected at any intensity in any phase (MF v LF, MF v ML and LF v ML).

When BHB results were analysed it was highlighted that there was no difference in peak BHB concentrations. It is worth noting that peak BHB occurred at 65 watts during the MF phase and at 50 watts during the LF and ML phases. BHB is synthesised in the liver from fatty acids before being delivered to the muscles during exercise (26). The time delay that occurs due to BHB being synthesised in the liver may explain why PFO occurred at 50 watts in the MF phase and why peak BHB occurred at 65 watts. Similarly, PFO occurred at 35 watts in the ML phase, and peak BHB occurred at 50 watts. When mean BHB concentrations were assessed at each intensity during the FATMAX test and across the three phases, there was only a significant difference detected at 110 watts ($p<0.05$). When this difference was further scrutinised, it was discovered that there was no difference in mean BHB between MF and LF; however, the difference occurred

between MF and ML as well as LF and ML, which highlights the ML as the area where the difference occurred. Again, this difference at the ML could be a result of the increased progesterone concentrations associated with this phase. However, considering all pre and post-intensity analysis highlighted no difference in BHB concentration, it is possible that the difference experienced at 110 watts is just an anomaly in the results.

Glucose concentrations were stable throughout the FATMAX test and there was no difference in glucose at any phase or intensity during the FATMAX test. Glucose concentrations collected at rest before the commencement of the FATMAX test would suggest that all participants adhered to the overnight fast requested and all participants presented in a fasted state. Previous research has suggested that the maintenance of blood glucose levels whilst exercising in a fasted state is a result of the body's desire to maintain glycaemia through the production of hepatic glucose (27).

When considering the above results, it would certainly suggest that mean fat and CHO oxidation are not affected by hormones associated with MC at any phase during graded exercise. These results also suggest there is no difference in PFO in the MF or LF phases however, the progesterone spike associated with the ML phase appears to have the potential to suppress PFO and create a difference when analysed versus the LF phase. Also, mean BHB and mean Glucose concentrations analysed throughout the FATMAX test at all the intensities and MC phases would support the hypothesis that hormones associated with MC do not affect or have a limited effect on substrate utilisation.

Nonetheless, all the data displayed and discussed above is based on the statistical analysis of group means, which can often conceal outliers. Given one of the main topics of conversation when discussing the MC and associated hormones is the variability of MC from female to female (28). Should we, therefore, display the results from outliers in isolation, as these outliers often highlight how, in this case, MC hormones can affect PFO and substrate utilisation across the phases? These outliers can also highlight how not every female falls under the theoretical model for MC.

Table 5.3: Mean E3G, PdG, PFO and Peak CHO for Participant No.10

Participant No.	MC Phase	Mean E3G (ng/ml)	Mean PdG (ug/ml)	PFO (g/min)	Peak CHO Ox (g/min)
10	@ MF	88	10	0.292 @ 80 watts	2.24 @ 125 watts
10	@ LF	134	4	0.453 @ 50 watts	2.25 @ 125 watts
10	@ ML	110	6	0.376 @ 95 watts	1.98 @ 125 watts
7	@ MF	144	5.3	0.384 @ 50 watts	2.23 @ 125 watts
7	@ LF	171	2.3	0.505 @ 50 watts	1.90 @ 125 watts
7	@ ML	127	30	0.321 @ 35 watts	2.23 @ 125 watts

We can observe from some of the individual results outlined in Table 5.3 that participant No.10 exhibited E3G concentrations that followed the profile of the theoretical MC (5). However, participant No.10's PdG profile did not, as we detected a progesterone spike in the MF phase. A progesterone or PdG spike outside of the ML phase is not typical but there are several reasons why this can occur, which are outside the scope of this article. This progesterone spike coupled with low oestrogen concentrations, then appears to affect both fat and CHO oxidation. We can clearly observe that at the MF phase, a PFO of 0.292gmin/min and a peak CHO oxidation of 2.24 g/min for participant No. 10. Alternatively, for participant No.10, when PdG concentrations are lower PFO is higher as observed in the LF and ML phases. When we statistically analysed both CHO oxidation and Fat oxidation for all the intensities during the FATMAX test at the three phases of interest by way of a single factor ANOVA, we found there was no difference in CHO Oxidation. There was, however, a significant difference in fat oxidation ($p<0.05$). We then further scrutinised the results using a T-test to assess the three phases against each other in pairs, MF v LF, MF v ML and LF v ML, to detect where the difference was. We discovered that there was a significant difference between MF v LF $p<0.05$) and between MF v ML ($p<0.05$). However, there was no difference between LF v ML. It appears that this difference in fat oxidation, which occurred at the MF point, was caused by a high progesterone spike, which occurred at a point within the MC that was not in keeping with the theoretical MC profile.

When participant no. 10's results are analysed in terms of an endurance event such as an Olympic distance triathlon, which would take approximately 3 to 4 hours to complete, the impact of such variations in substrate utilisation becomes very relevant. If we consider participant no.10 to be oxidising fat at a rate of approximately 18g/hour @ an intensity of 80 watts, this equates to 162 calories per hour from fat which in isolation will not support exercise for this duration. If we then consider that @ 125 watts this athlete will be oxidising CHO's at a rate of 134g/hour. Allowing for nutritional norms which suggest that athletes who practice nutrition can absorb approximately 90g of CHO per hour, oxidising CHO's at 134g/hour means this athlete is oxidising her fuel faster than she can absorb it (29). This, again, will become problematic for the athlete in events that last longer. It can also be noticed that during the LF phase for participant no. 10 that CHO is being oxidised at a similar rate to MF. However, potentially due to the much greater concentration of E3G or oestrogen and the much lesser concentration of PdG or progesterone, PFO is much higher. We can observe a PFO rate of 27g/hour, which equates to 243 calories per hour from fat. This, again in isolation, will not support exercise for this duration alone, but potentially lessens the demand on CHO's at lower intensities.

When assessing the data from participant no. 7 we can again observe some variations which differ from the theoretical norms. For example, hormone fluctuations associated with the theoretical MC would suggest that oestrogen or E3G concentrations at the MF phase would be lower than those observed at the ML. However, for participant no.7 a greater concentration of E3G was detected at MF when compared to ML. This variation could be due to an earlier surge in oestrogen, which has been reported in

previous research. For example, D'Souza et al. (4) have reported that when analysing hormone fluctuation associated with MC, they found that in some cases, hormone spikes were recorded earlier or later than the theoretical model would predict, again highlighting the potential differences from female to female. Also, it can be noticed from participant no.7's results that when E3G is high during the LF phase, and PdG is lowest, PFO is increased (0.505g/min @ 50 watts), and CHO oxidation is decreased (1.90g/min @ 125 watts). Alternatively, when participant no.7 experiences the ML PdG spike, her PFO rate is decreased (0.321g/min @ 35 watts), but her CHO oxidation rate (2.23g/min @ 125 watts) is increased in comparison to the LF phase.

Similar to participant no. 7, other participants (no. 2 & 3) exhibited hormone profiles that differ slightly from the theoretical norm. Participant no. 2 recorded higher concentrations of E3G (day 1-90ng/ml, day 2-84.4ng/ml, day 3-157ng/ml) on day 3 of hormone verification, which could indicate a later ovulation. Participant no. 3 noted an earlier spike in E3G (day 1-154ng/ml, day 2-143ng/ml, day 3-89ng/ml), which may indicate an earlier ovulation. This data again highlights the slight variations from the norm and the potential for varying effects from female to female.

5. Conclusion

When we consider the results outlined above and that from the Frandsen et al. (7) study along with the suggestions outlined in the Oosthuyse and Bosch review (1) there are similarities and differences. The similarities in results discovered with the Frandsen study were many but there was one main variation being that PFO appeared to be affected by MC hormone fluctuations when the LF and ML phases were assessed against each other. Frandsen et al. found no difference ($p>0.05$) in PFO at any stage. Likewise, the data above, in certain cases appeared to support the views outlined in the Oosthuyse and Bosch review and in other cases, went in contrast to it. It is the view from this study, considering the results above and previous research, that the effects on PFO and substrate utilisation from hormones associated with MC are variable due to numerous factors. Factors such as variations in hormone concentrations, variations in the timing of hormone fluctuations that differ from the norm, and variations in the length of MC can all singularly or in parallel have an impact or not on PFO and substrate utilisation. Due to this variability, attempting to find differences when analysing substrate utilisation in a group format will continuously yield different results. Therefore, it is our consensus that the effect of MC hormones on substrate utilisation should be assessed individually, case by case, with the results being relevant to the individual at a specific time point. As hormones change over time, so does their effect.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest, financial or otherwise, that could compromise the integrity of this proposal.

About the Authors

Eoin Molloy PhD(c) begain his studies at Waterford Institute of Technology, Waterford, Ireland from which he holds a BSc (Hon 1:1) in Sports Coaching and Performance. Upon graduating from WIT, Eoin continued his studies and completed a Post graduate Diploma in Performance Nutrition (with Distinction) through the Institute of Performance Nutrition. Whilst completing his PgDip, Eoin decided to also set out on the PhD track investigating performance and health markers of fasted training on female recerational endurance athletes. The aim of Eoin's research is to gain a better understanding of the effects of fasted training and menstrual cycle phase on endurance performance and substrate utilisation in female athletes. The objective being to clearly demonstrate the importance of female specific training programs derived from female speicifc research.

Dr. Maria Murphy-Griffin is a full-time lecturer in the department undergraduate programmes since 2002. She is interested in both exercise for health and in sport and performacne and have a particular interest in exercise/sport physiology. Dr. Maria Murphy-Griffin has been awarded a BA in Physical Education and mathematics (UL), an MSc in sport Science (Loughborough University, Leics) and a PhD in Exercise and the Heart (UL).

Professor Michael Harrison has worked at WIT since 1999. He is Head of the Department of Sport and Exercise Science and a former Dean of the School of Science and Computing. Michael's research, undertaken in conjunction with University Hospital Waterford and UPMC Whitfield, considers the impact of exercise for clinical populations, including age-related frailty, vascular disease and cancer. He has a specific interest in immune cell subsets that exert positive and negative influences on the processes that underlie ageing and vascular disease. Michael is Chair of the Register of Exercise Professionals in Ireland and sits on the Professional Standards Committee of the European Register of Exercise Professionals. He has been part of different national working groups and local initiatives to establish community-based exercise services for individuals with chronic health conditions and sits on the Advisory Board of the Exercise is Medicine Ireland National Centre. Michael holds a Fellowship from the American College of Sports Medicine.

References

1. Oosthuyse T, Bosch AN. The effect of the menstrual cycle on exercise metabolism: implications for exercise performance in eumenorrheic women. Sports medicine. 2010 Mar;40:207-27.
2. de Jonge XA. Effects of the menstrual cycle on exercise performance. Sports medicine. 2003 Sep;33:833-51.

3. Fernandez-del-Valle M. Exercise Metabolism and Menstrual Cycle. In The Active Female: Health Issues throughout the Lifespan 2023 Feb 26 (pp. 119-127). Cham: Springer International Publishing.
4. D'Souza AC, Wageh M, Williams JS, Colenso-Semple LM, McCarthy DG, McKay AK, Elliott-Sale KJ, Burke LM, Parise G, MacDonald MJ, Tarnopolsky MA. Sex Differences in the Response to Exercise Training: Menstrual cycle hormones and oral contraceptives: a multimethod systems physiology-based review of their impact on key aspects of female physiology. Journal of Applied Physiology. 2023 Dec 12;135(6):1284.
5. Reed BG, Carr BR. The normal menstrual cycle and the control of ovulation.
6. Itriyeva K. The normal menstrual cycle. Current problems in pediatric and adolescent health care. 2022 May 1;52(5):101183.
7. Frandsen J, Pistoljevic N, Quesada JP, Amaro-Gahete FJ, Ritz C, Larsen S, Dela F, Helge JW. Menstrual cycle phase does not affect whole body peak fat oxidation rate during a graded exercise test. Journal of Applied Physiology. 2020 Mar 13.
8. Hackney AC, McCracken-Compton MA, Ainsworth B. Substrate responses to submaximal exercise in the midfollicular and midluteal phases of the menstrual cycle. International Journal of Sport Nutrition and Exercise Metabolism. 1994 Sep 1;4(3):299-308.
9. Fernandez-del-Valle M. Exercise Metabolism and Menstrual Cycle. InThe Active Female: Health Issues throughout the Lifespan 2023 Feb 26 (pp. 119-127). Cham: Springer International Publishing. Kravitz L, Cazares A, Mermier C. Women, Hormones, Metabolism and Energy Expenditure.
10. Kravitz L, Cazares A, Mermier C. Women, Hormones, Metabolism and Energy Expenditure.
11. Guyton AC. Text book of medical physiology. China; 2006.
12. Reed BG, Carr BR. The normal menstrual cycle and the control of ovulation.
13. Mihm M, Gangooly S, Muttukrishna S. The normal menstrual cycle in women. Animal reproduction science. 2011 Apr 1;124(3-4):229-36.
14. Messinis IE, Messini CI, Dafopoulos K. The role of gonadotropins in the follicular phase. Annals of the New York Academy of Sciences. 2010 Sep;1205(1):5-11.
15. Miro F, Parker SW, Aspinall LJ, Coley J, Perry PW, Ellis JE. Relationship between follicle-stimulating hormone levels at the beginning of the human menstrual cycle, length of the follicular phase and excreted estrogens: the FREEDOM study. The Journal of Clinical Endocrinology & Metabolism. 2004 Jul 1;89(7):3270-5.
16. Jones BC, Hahn AC, DeBruine LM. Ovulation, sex hormones, and women's mating psychology. Trends in Cognitive Sciences. 2019 Jan 1;23(1):51-62.
17. Su HW, Yi YC, Wei TY, Chang TC, Cheng CM. Detection of ovulation, a review of currently available methods. Bioengineering & translational medicine. 2017 Sep;2(3):238-46.

18. Kraemer RR, Heleniak RJ, Tryniecki JL, Kraemer GR, Okazaki NJ, Castracane VD. Follicular and luteal phase hormonal responses to low-volume resistive exercise. Medicine & Science in Sports & Exercise. 1995 Jun 1;27(6):809-17.
19. Stachenfeld NS, Silva C, Keefe DL. Estrogen modifies the temperature effects of progesterone. Journal of applied physiology. 2000 May 1.
20. Baker FC, Siboza F, Fuller A. Temperature regulation in women: effects of the menstrual cycle. Temperature. 2020 Jul 2;7(3):226-62.
21. DALSKY GP. Effect of progesterone level on substrate utilization during endurance running. Brigham Young University; 1982.
22. Oosthuyse T, Bosch AN, Jackson S. Cycling time trial performance during different phases of the menstrual cycle. European journal of applied physiology. 2005 Jun;94:268-76.
23. Rodriguez-Giustiniani P, Rodriguez-Sanchez N, Galloway SD. Fluid and electrolyte balance considerations for female athletes. European journal of sport science. 2022 May 4;22(5):697-708.
24. Bouchard T, Yong P, Doyle-Baker P. Establishing a Gold Standard for Quantitative Menstrual Cycle Monitoring. Medicina. 2023 Aug 23;59(9):1513.
25. Bouchard TP, Fehring RJ, Mu Q. Quantitative versus qualitative estrogen and luteinizing hormone testing for personal fertility monitoring. Expert review of molecular diagnostics. 2021 Dec 2;21(12):1349-60.
26. Newman JC, Verdin E. β-Hydroxybutyrate: a signaling metabolite. Annual review of nutrition. 2017 Aug 21;37:51-76.
27. Ozcan L, Wong CC, Li G, Xu T, Pajvani U, Park SK, Wronska A, Chen BX, Marks AR, Fukamizu A, Backs J. Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. Cell metabolism. 2012 May 2;15(5):739-51.
28. Fehring RJ, Schneider M, Raviele K. Variability in the phases of the menstrual cycle. Journal of Obstetric, Gynecologic & Neonatal Nursing. 2006 May 1;35(3):376-84.
29. Jeukendrup AE, McLaughlin J. Carbohydrate ingestion during exercise: effects on performance, training adaptations and trainability of the gut. Sports nutrition: More than just calories-triggers for adaptation. 2011;69:1-8.