Effects of the Menstrual Cycle on Daily Blood Glucose Changes During Exercise and in Recovery in Aerobically Fit Athletes.

by

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Abstract

Background: Glucose monitoring is becoming increasingly popular in athletes who are hoping to optimize their nutrition, training and recovery. Glucose regulation is influenced by several factors, including the amount and type of exercise performed as well as circulating ovarian hormones in female athletes. As such, female athletes with fluctuating ovarian hormones across the menstrual cycle, may experience concurrent changes in blood glucose. Therefore, this study examines glucose during exercise and in recovery in female athletes in two distinct phases of the menstrual cycle.

Methods: 11 aerobically fit, naturally cycling female athletes were recruited to participate in this pilot study. Participants completed two standardized exercise sessions at two different phases of the menstrual cycle (i.e., early follicular phase (FP) and mid luteal phase (LP)). Continuous glucose monitors (CGM) were worn for the duration of the menstrual cycle and glucose values during exercise and in the 24 hours post exercise were analyzed.

Results: There were no significant differences in capillary glucose concentrations between the FP and LP immediately before (5.6 ± 0.7 vs 5.8 ± 0.5 mmol/L, p= 0.28) or after (5.7 ± 1.0 vs 5.9 ± 1.0 mmol/L, p= 0.28) exercise. Time spent with low interstitial glucose during exercise and in the 24-hour recovery window was minimal in both menstrual cycle phases, with the percent of time below 3.9 mmol/L being only 4 ± 6% and 3 ± 4% in the FP and the LP respectively (p = 0.82). Mean CGM glucose during exercise also did not change significantly between menstrual cycle phases, with mean values of 5.5 ± 1.2 and 5.8 ± 0.9 mmol/L in the FP and the LP respectively (p = 0.44). Despite the lack of change in glucose throughout the menstrual cycle, respiratory exchange ratio (RER) during exercise was higher during the LP compared to the FP (p = 0.04), suggesting

greater oxidation of carbohydrates at the same relative exercise intensity in the LP compared to the FP.

Conclusion: Overall, female athletes did not experience significant changes in glucose between the FP and LP during exercise and in recovery. Furthermore, they did not experience an increased amount of time with low glucose during 24 hours of recovery from exercise in the FP compared to the LP.

Preface

This thesis is an original work by Janai Marie Martens. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, project name “Daily Blood Glucose in Female Athletes,” No. Pro00129786, August 23, 2023. Data collection took place at the University of Alberta Physical Activity and Diabetes Laboratory. The primary investigator for the aforementioned pilot study was Dr. Normand Boulé and the study coordinator was Janai Martens. Co-investigators for the study include Dr. Jane Yardley, Dr. Zeinab Momeni, and Jordan Rees. The figures and tables included in the literature review from chapter 2 have been reproduced with appropriate permission from previous authors and publishers.

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Abbreviations

BG	Blood Glucose
CGM	Continuous Glucose Monitor
LP	Luteal Phase
FP	Follicular Phase
VO_2	Volume of Oxygen Consumption
RED-S	Relative Energy Deficiency in Sport
LEAF-Q	Low Energy Availability in Females Questionnaire
RER	Respiratory Exchange Ratio
MCG	Menstrual Cycle and Glucose

Chapter 1: Introduction

1.1 Background

Only 29% of sports medicine research is performed on female athletes (Paul et al., 2022). Although females make up approximately 50% of the population, they are largely underrepresented in sports science research. While female sport and exercise participation has increased exponentially over the past century, there is a lack of evidence-based performance, training, and nutritional guidelines that are customized for females (Elliott-Sale et al., 2021).

Nutrition, as a branch of sports science research, has garnered much attention over the years. Carbohydrates, in particular, are often studied in the context of sports nutrition because of their important contribution to exercise metabolism. Reduced carbohydrate availability can be a limiting factor for exercise performance because of potential substrate-limiting fatigue and/or decreased circulating glucose (Ivy et al., 1988).

As outlined by the American Diabetes Association, blood glucose (BG) levels in healthy individuals should remain between 3.9 mmol/L and 10.0 mmol/L (American Diabetes Association, 2022). Large excursions from this normoglycemic range can pose a myriad of health problems, both chronic and acute (Daryabor et al., 2020). Limited existing studies show that hypoglycemia, defined as BG levels <3.9 mmol/L, can sometimes occur in healthy individuals (Flockhart et al., 2021; Skroce et al., 2024).

A century ago, researchers began looking at the potential performance implications of low BG. In 1924, Levine et al. recorded BG levels in participants running the Boston Marathon. It was discovered that most of the participants had low BG levels and high fatigue after the marathon was completed (Levine et al., 1924). Thus, the researchers speculated that low BG could be a source of fatigue during and after the marathon. A year later, the research group studied participants running in the same Boston Marathon, but this time while consuming carbohydrates during the race. The participants who consumed carbohydrates during the marathon had both higher BG levels and better running performance (faster finishing time) compared to the year previous (Gordon et al., 1925). Thus it was concluded that low BG corresponds to fatigue and diminished endurance performance, while normal BG levels prevent this fatigue from occurring (Levine et al., 1924).

Since then, several other studies have emerged, further linking low BG with decreased neuromuscular performance (Tornberg et al., 2017), decreased cognitive performance (Graveling et al., 2013; Verhulst et al., 2022), decreased thermoregulation (Brun et al., 2001), and decreased immune health (Scharhag et al., 2006). Furthermore, it is now acknowledged that exercising individuals may be more susceptible to exercise-induced hypoglycemia postprandially (Kondo et al., 2019; Kuipers et al., 1999; Porter et al., 2020). Other studies suggest that elite endurance athletes experience greater disruptions in glycemia compared to non-athletes, making endurance athletes more susceptible to hypoglycemic and hyperglycemic events (Flockhart et al., 2021).

As such, adequate glucose levels are assumed to be important in maintaining optimal health and performance. Many nutritional guidelines are aimed at maximizing glucose availability during sport performance. As outlined in a position statement by the American College of Sports Medicine, one of the primary goals for nutrient consumption during exercise is to provide sufficient carbohydrate intake to maintain adequate BG levels for exercise performance (D. T. Thomas et al., 2016).

Glucose monitoring devices, such as continuous glucose monitors (CGM) are now being marketed towards athletes. Companies, such as Supersapiens and Nutrisense are promoting CGM use in athletes with claims such as “master your metabolism,” “optimize nutrition, performance, and recovery,” and “never bonk again” (Supersapiens, n.d.). Although CGM is currently being marketed towards exercisers, the practical applications and utility of these devices from a sport performance standpoint is still unknown (Bowler et al., 2022). As outlined by a recent review, there are very few studies performed involving CGM use in athletes, with even fewer involving standardized exercise, looking at glucose profiles long term, nor looking specifically at glucose profiles in menstruating female athletes (Bowler et al., 2022).

Recently, the International Society for Sports Nutrition developed a position stand that considers female-specific nutritional concerns (Sims et al., 2023). This position stand outlines the need for female-specific considerations to nutritional recommendations and studies, given that there are sex-differences that exist in exercise and recovery metabolism. Females exhibit an increased reliance on fat as a substrate for oxidation over carbohydrates, compared to males exercising at the same relative, submaximal intensity (Devries et al., 2006). Furthermore, one study found that glucose levels decreased more in sedentary females during moderate exercise compared to sedentary males (Porter et al., 2020). Additionally, active females tend to have lower 24-hour

mean glucose profiles compared to active males (Bowler et al., 2024; Skroce et al., 2024). Because of these notable sex differences, examining glucose levels must be done with due consideration to sex-specific physiology.

Furthermore, females also contend with fluctuations in BG throughout the menstrual cycle, due to changing ovarian hormone levels. Ovarian hormones impact several biological systems, including substrate metabolism and BG. Research is limited on changes in BG throughout the menstrual cycle in healthy individuals, however some studies suggest that females have lower overall resting BG during the follicular phase (FP) of the menstrual cycle compared to the luteal phase (LP) (Dey et al., 2019; Lin et al., 2023). Furthermore, one particular study also identified lower BG during the FP of the menstrual cycle compared to the LP, when performing endurance exercise (Zderic et al., 2001). However, studies pertaining to BG levels in exercising female athletes across the menstrual cycle have been limited to within-laboratory blood tests and fail to account for the 24 hours pre and post exercise intervention. To our knowledge, there are no existing studies that examine the BG profiles in female athletes during both standardized exercise and recovery, and throughout the duration of the menstrual cycle. Since BG has the potential to influence performance and recovery, and because the menstrual cycle can impact BG, examining BG levels in female athletes, at certain points of the menstrual cycle, is an important contribution to existing literature.

1.2 Problem Statement

This study seeks to examine the changes in BG throughout the menstrual cycle (early FP vs mid-LP), including the prevalence of hypoglycemia during exercise and in recovery in aerobically-trained female athletes.

1.3 Hypothesis

There will be greater time spent with low BG (<3.9 mmol/L) in the early-FP versus the mid-LP of the menstrual cycle, during exercise and in the 24-hour recovery, in female athletes who are naturally cycling.

1.4 Outcomes

Based on the glycemic targets from the American Diabetes Association, primary and secondary outcome measurements are as follows.

Primary outcome: Time <3.9 mmol/L during the intervention and in the 24 hours after exercise.

Secondary outcome: Time in normal BG range (3.9-8.0 mmol/L), standard deviation (a measure of glucose variability), mean glucose, time >8.0 mmol/L, mean glucose during exercise, 0-6am mean glucose, respiratory exchange ratio (RER) during exercise, heart rate during exercise, and food intake the day before, of, and after the exercise intervention (American Diabetes Association, 2022).

1.5 Definitions

As per the Canadian Institute for Health Research descriptions of sex and gender terminology, this study will be using female as referring to the biological attributes of sex rather than the terminology of women to describe the social construct of gender (Canadian Institute of Health Research, n.d.).

This study will be using the terminology of naturally cycling to describe participants who have a menstrual cycle length between 21-35 days and have a luteinizing hormone surge around ovulation. The terminology of naturally cycling will be used, as recommended by Smith et al. (2022), as opposed to eumenorrheic female participants because the current study will not be performing serum hormone collection and analysis and therefore cannot determine actual ovarian hormone concentrations. Testing females who are naturally cycling is considered silver standard if blood tests are unable to be collected (Smith et al., 2022).

The terminology of BG is used to describe glucose levels from either a CGM or a capillary blood glucose draw. Although CGMs do not take direct blood glucose measurements, they use an algorithm to convert interstitial glucose to predicted blood glucose (FDA, 2018).

1.6 Limitations

As mentioned above, this study will not be performing serum hormone analysis and thus has some limitations associated with assuming menstrual cycle phases. We are assuming that because of a luteinizing hormone peak and basal body temperature increase around ovulation, females will meet threshold criteria for a eumenorrheic cycle. However, since we are not confirming hormone concentrations using a blood sample, there is a chance that some participants

could be experiencing menstrual irregularities (eg., luteal phase deficiency) and/or low ovarian hormone levels for which we fail to account for in the present study. According to recommendations by Janse et al., if serum hormone collection and analysis is not feasible for a project, researchers should at least use urinary luteinizing hormone tests to predict ovulation (Janse et al., 2019).

Another limitation of the study described in chapter 3, is the decision to analyze BG during and post exercise only in the early FP (phase 1) and mid-LP (phase 4) of the menstrual cycle, as defined by Elliott-Sale et al. (2021). The decision to examine phase 1 vs phase 4 accounts for the greatest difference in hormonal milieu while considering both estrogen and progesterone effects. This biphasic comparison however, ignores the late FP (phase 2) and ovulatory (phase 3) differences, where estrogen is high and progesterone is low.

1.7 Significance

As glucose monitoring technology continues to be marketed towards athletes, there is an increased need for research evaluating the practicality and feasibility of CGM use. Although life-threatening glycemic events are unlikely to occur in this female athlete population, episodes of hypoglycemia can still pose major threats to exercise performance and recovery (Amiel, 1998; Bowler et al., 2022; Gleeson et al., 1998; Nybo, 2003; Rustad et al., 2016; Smith et al., 2016; Tornberg et al., 2017). Glycemic regulation, measured using CGMs is disturbed in elite endurance athletes, particularly with excessive training loads (Flockhart et al., 2021). Furthermore, as a result of sex-based differences in glucose metabolism and ovarian hormone influence on several biological systems, it is important to consider how the menstrual cycle may impact BG regulation during and post exercise. Continuous glucose monitoring in female athletes throughout the menstrual cycle has yet to be studied.

Chapter 2: Literature Review

2.1 Physiology of the Menstrual Cycle

Throughout the menstrual cycle, women experience varying levels of luteinizing hormone, follicle-stimulating hormone, estrogen, and progesterone (Farage et al., 2009). Each of these hormones contribute to the cyclical nature of the menstrual cycle while also affecting other physiological processes (Brown et al., 2021; Campbell & Febbraio, 2001; Driver et al., 2005; Shechter et al., 2010; White et al., 2011; Zderic et al., 2001). In order to fully understand the menstrual cycle, its various phases, and the symptoms that accompany each phase, it is important to have an understanding of each ovarian hormone mentioned.

Luteinizing Hormone

Luteinizing hormone is released from the anterior pituitary gland in response to gonadotropin releasing hormone (Nedresky & Singh, 2022). Luteinizing hormone regulates ovulation and length of the menstrual cycle by causing an ovum to be released from the ovaries (Choi & Smitz, 2014). Luteinizing hormone also drives the production of progesterone by the corpus luteum during the LP (Choi & Smitz, 2014). Furthermore, luteinizing hormone may also have a role in aiding with cognitive processes and preventing neurodegeneration and cognitive pathologies (Blair et al., 2015).

Follicle Stimulating Hormone

Follicle stimulating hormone is released by the anterior pituitary gland, in response to gonadotropin releasing hormone (Orlowski & Sarao, 2022). This hormone plays a role in sexual development by causing follicular development and by driving estrogen production (Orlowski & Sarao, 2022).

Estrogen

Estrogen refers to a group of steroid hormones including estradiol, estriol, and estrone (Delgado & Lopez-Ojeda, 2022). These ovarian hormones play a role in developing sex characteristics (Delgado & Lopez-Ojeda, 2022), bone health (Emmanuelle et al., 2021), immune system health (Hamilton et al., 2017), regenerative processes (Tiidus, 2003), vascular health

(Mendelsohn, 1999), excretory system functioning, post-exercise muscle repair (Bar et al., 1988; Enns & Tiidus, 2010), glucose metabolism (Mauvais-Jarvis et al., 2013), neurodevelopmental processes (Gillies & McArthur, 2010), and diet modulation in healthy females (Krishnan et al., 2016).

Progesterone

Progesterone is another ovarian hormone that fluctuates throughout the menstrual cycle. This hormone does not become significantly present until post ovulation (Elliott-Sale et al., 2021). The ovaries and the adrenal cortex release progesterone in response to a surge in luteinizing hormone around ovulation (Cable & Grider, 2022). Apart from playing a large role in reproduction and menstrual cycle function, progesterone also ensures proper immune function and leukocyte development, continued development of myelin, and the development of bone mineral density along with estrogen (Cable & Grider, 2022)

The mean length of the menstrual cycle is between 27-29 days, however the length of what is considered a "normal" cycle can be anywhere between 21-35 days (Elliott-Sale et al., 2021). Menstrual cycle phases are difficult to study due to the large intra- and inter-individual variability of phases (Allen et al., 2016; Janse et al., 2019). Mihm et al. (2011) identified several factors causing confusion among menstrual cycle lengths in females, some of which include a high individual variability between cycle length, high variability between FP and LP length, and changes in menstrual cycle length across the reproductive lifetime of females (Mihm et al., 2011). Due to the cyclical and evolving nature of the menstrual cycle, clear guidelines on phase parameters as well as typical menstrual cycle irregularities must be outlined to allow for reproducibility and transferability of results (Cole et al., 2009; Schmalenberger et al., 2021).

Eumenorrhea refers to premenopausal females who are within the "normal" 21-35-day menstrual cycle range and who are naturally ovulating a minimum of nine times per year consecutively (Elliott-Sale et al., 2021). Eumenorrheic females must also display normal hormonal profiles across all four phases, as outlined in table 1, and must not be using hormonal contraceptives.

There are several aberrations that are seen in women of menstruating age. Primary amenorrhea refers to females who do not reach menarche by the age of 15 despite displaying other secondary sex characteristics (Elliott-Sale et al., 2021). Secondary amenorrhea, similar to primary

amenorrhea, is categorized when menses does not occur in females for three consecutive cycles when they are not pregnant (Elliott-Sale et al., 2021). Amenorrhea, which will be discussed in more detail later, can be a result of several conditions such as polycystic ovarian syndrome, hyperprolactinemia, primary ovarian insufficiency, or low-energy availability (Elliott-Sale et al., 2021).

Oligomenorrhea refers to females with menstrual cycle lengths longer than 35 days and is more commonly seen in female athletes (Carlberg et al., 1983; Toriola, 1988), while polymenorrhea refers to individuals with menstrual cycle lengths shorter than 21 days (Elliott-Sale et al., 2021; Schmalenberger et al., 2021). The use of hormonal contraceptives can also vastly influence hormonal milieus by downregulating endogenous ovarian hormones, and must therefore be treated as a separate population (Elliott et al., 2005).

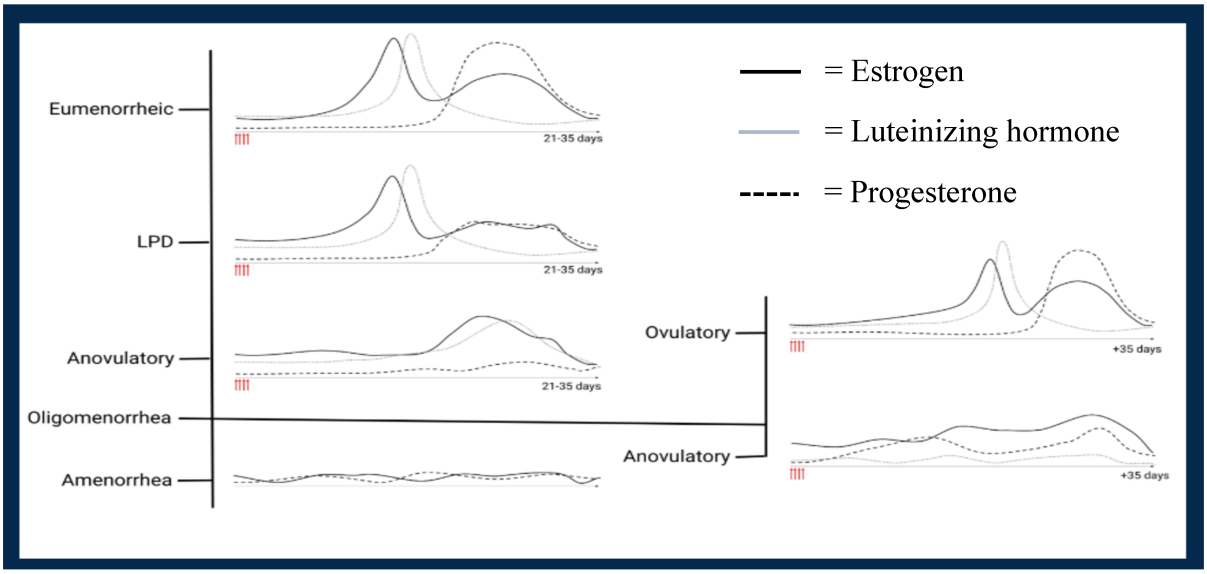

Figure 1. Hormonal profiles across various menstrual cycle status. This figure has been reproduced with permission from Flood and Elliott-Sale (n.d.).

Although females may be naturally menstruating, such as is seen in eumenorrheic individuals, with a menstrual cycle length between 21-35 days, there can still be abnormal hormone levels during ovulation and throughout the LP. The lack of a luteinizing hormone surge during ovulation is known as luteal phase deficiency (Janse et al., 2019). Luteal phase deficiency can occur as a result of impaired corpus luteum functioning and inadequate levels of estrogen,

progesterone, and luteinizing hormone (Schliep et al., 2014). Anovulation can also occur as a result of regular insufficient ovarian hormones, causing the absence of ovulation (Janse et al., 2019; Schliep et al., 2014).

Female participants with natural menstrual cycles between 21-35 days, with a positive luteinizing hormone test, without confirmed serum hormone levels are classified as naturally cycling with ovulation. If a positive luteinizing hormone test is not achieved but there is still a surge or peak of luteinizing hormone around ovulation, the athlete will be classified as naturally cycling.

Females can experience varying configurations and amplitudes of luteinizing hormone surges, as is seen in figure 2 (Direito et al., 2013). One study observing a total of 43 ovulatory menstrual cycles found luteinizing hormone surges anywhere between 12.1-104 mIU/mg of creatinine, demonstrating the large variability in luteinizing hormone surges (Park et al., 2007).

Although females can experience ovulatory cycles despite not achieving a positive luteinizing hormone test, a lack of a positive luteinizing hormone test can also be indicative of subtle menstrual disturbances (De Souza et al., 2010). One particular study conducted by De Souza et al. (2010), reported that 52.1% of exercising female participants were experiencing subtle menstrual disturbances (i.e., luteal phase deficiency and/or anovulation) with lower luteinizing hormone values, compared to only 4.2% of sedentary female participants (De Souza et al., 2010). Therefore, although a positive luteinizing hormone test or lack thereof can be indicative of ovulation or subtle menstrual disturbances, it is impossible to say for sure without serum hormone confirmation or imaging, such as a transvaginal ultrasonography.

Female participants who are naturally cycling with a luteinizing hormone surge have the same phase parameters as eumenorrheic participants and are assumed, although not guaranteed, to have the similar hormonal milieus. Table 1.0 from Smith et al. outlines tiered classifications for menstrual cycle status when conducting and evaluating menstrual cycle studies. The current study will be using tier 2 or “silver” standard for methodological purposes.

Table 1. *Tiered classifications for menstrual cycle status.*

Tier	Menstrual Cycle Methodological Considerations
Gold	Participants are eumenorrheic: 1. Have menstrual cycle lengths ≥21 days and ≤35 days resulting in nine or more consecutive periods per year. 2. Evidence of luteinizing hormone surge. 3. Correct hormonal profile (from blood sample analysis). 4. No hormonal contraceptive use 3 months prior to recruitment. Menstrual cycle characteristics are tracked for ≥2 months prior to testing. Outcome measures are repeated in a second cycle.
Silver	Participants are naturally menstruating with ovulatory cycles: 1. They experience menstruation, with menstrual cycle lengths ≥21 days and ≤35 days. 2. Confirmed ovulation (luteinizing hormone surge) but without correct hormonal profile confirmation. 3. Prior hormonal contraceptive use not stated or <3 months prior to recruitment. Menstrual cycle characteristics are tracked for 1 month prior to testing. Outcome measures are not repeated in a second cycle.
Bronze	Participants are naturally menstruating: 1. They experience menstruation, with menstrual cycle lengths ≥21 days and ≤35 days 2. Without confirmed ovulation and correct hormonal profile. 3. Prior hormonal contraceptive use not stated or <3 months prior to recruitment. No tracking of menstrual cycle characteristics prior to testing.
Ungraded	Insufficient detail to award a gold, silver, or bronze.

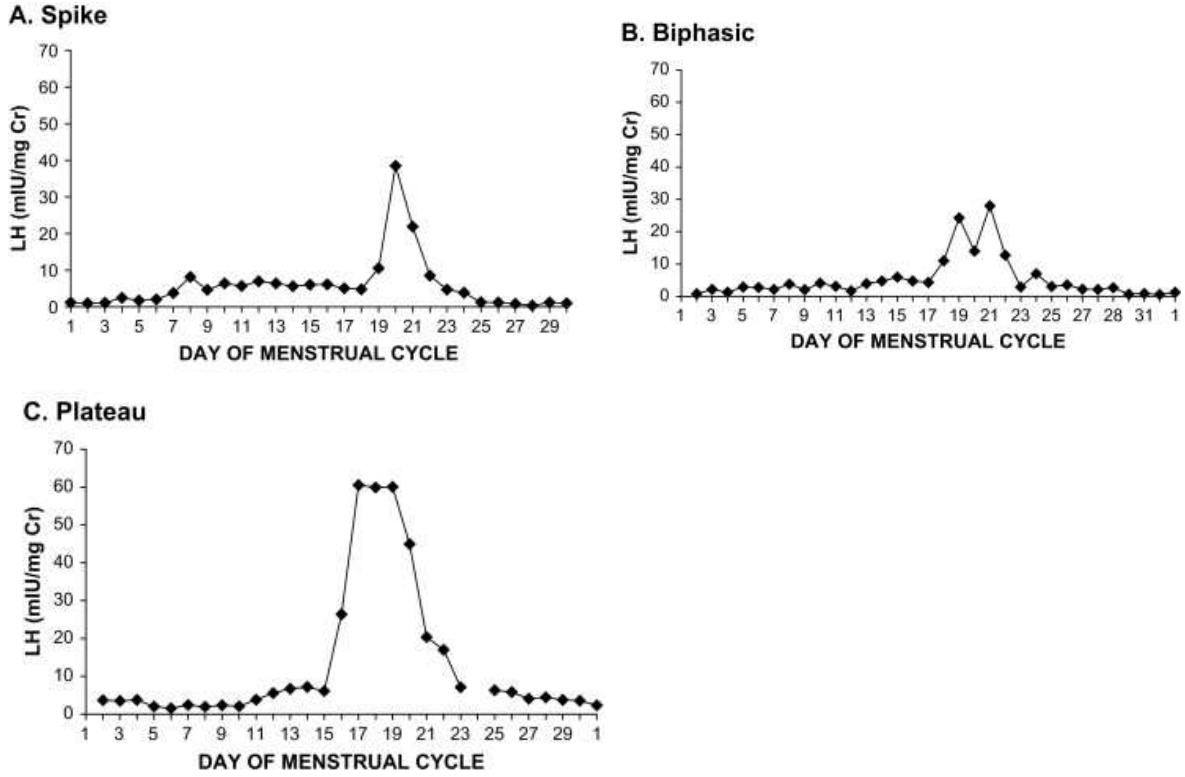

Figure 2. Varying configurations and amplitudes of luteinizing hormones surges across ovulatory menstrual cycles. This figure has been reproduced from Park et al. (2007) © Sept 1, 2007, with permission from Elsevier.

2.2 Menstrual Cycle Phases

Several studies examining the effect of the menstrual cycle phases on sport performance and various metabolic metrics have resulted in conflicting findings (Janse et al., 2019). These inconsistent findings can be due, in part, to inaccurate representation of menstrual cycle phases or insufficient phase testing (Allen et al., 2016). In order to avoid poor methodologies and invalid testing phases, it is important to define each phase clearly with appropriate hormonal profiles.

The menstrual cycle in eumenorrheic and naturally cycling females can be broadly separated into two phases: the FP and the LP. Elliot-Sale et al., (2021) suggests further breaking down the menstrual cycle phases into four phases to account for different hormonal milieus throughout the FP and the LP. Testing in the early FP (phase 1) and the mid-LP (phase 4) presents the greatest difference in hormonal profiles for menstrual cycle studies.

Phase 1 of the menstrual cycle begins on day 1 until around day 5. Phase 1 begins with menses or bleeding as a result of the shedding of the uterine lining because egg implantation has not occurred (Farage et al., 2009; Janse et al., 2019; Reed & Carr, 2000). Phase 1 is the beginning of the FP and is characterized by having the lowest ovarian hormone concentrations (Elliott-Sale et al., 2021; Yen et al., 2010). Luteinizing hormone, follicle stimulating hormone, estrogen, and progesterone are all low during this phase of the menstrual cycle (Schmalenberger et al., 2021).

Table 2. *Menstrual cycle phase definitions and rationale.*

Recommendation	Rationale (intended to...)	Pro	Con
Phase 1: indicated by the onset of bleeding until day 5 Oestrogen and progesterone levels are low	Capture the lowest concentrations of oestrogen and progesterone	Easy to determine due to obvious physical cue (i.e., bloody discharge)	Can be difficult to predict in those with variable cycle length therefore requiring reactive testing sessions (i.e., participant alerting the researcher on day 1 of bleeding and then both parties having availability for testing within the next 4 days)
Phase 2: occurs in the 14–26 h prior to ovulation and the LH surge Oestrogen higher than during phase 1, 3 and 4 and progesterone higher than during phase 1, but lower than 6.36 $nmol{\cdot}L^{-1}$	Capture the highest oestrogen concentration, while progesterone remains low	Enables the biggest difference between oestrogen and progesterone to be investigated	Difficult to predict without daily blood samples for the determination of oestrogen and progesterone
Phase 3: indicated by a positive urinary ovulation kit and lasts 24–36 h Oestrogen higher than phase 1 but lower than phase 2 and 4 and progesterone higher than phase 1 but lower than 6.4 $nmol{\cdot}L^{-1}$	Capture a medium oestrogen concentration, while progesterone remains low	Easy to establish due to the positive LH surge captured by the ovulation kit	Relies on having multiple ovulation kits available for each participant (cost) and requires reactive testing sessions (i.e., participant alerting the researcher to the positive result and then both parties having availability for testing within the next 24–36 h)
Phase 4: + 7 days after ovulation has been confirmed Oestrogen higher than phase 1 and 3 but lower than phase 2 and progesterone > 16 $nmol{\cdot}L^{-1}$	Capture the highest concentration of progesterone and a high concentration of oestrogen	Easy to establish in those with eumenorrheic cycles as it typically occurs within 7 days of confirmed ovulation	Relies on the confirmation of ovulation

This table has been reproduced with permission from Elliott-Sale et al. (2021) © (2021) CC BY 4.0.

Phase 2 of the cycle occurs approximately 24 hours prior to ovulation. Estrogen levels surge to their highest concentration (the first peak of estrogen), as the follicle develops while luteinizing hormone also begins to peak (Allen et al., 2016). Progesterone rises steadily and is higher in phase 2 than phase 1, however, is still relatively low compared to phases 3 and 4 (Elliott-Sale et al., 2021).

Phase 3 occurs at ovulation, which is around day 14 in a typical 28-day cycle. Ovulation is characterized by the highest concentrations of luteinizing hormone, moderate concentrations of estrogen (lower than phase 2 and 4), and low to moderate levels of progesterone. During the first 3 phases of the menstrual cycle, progesterone does not play a significant role in systemic bodily changes. Ovulation marks the beginning of the LP of the cycle, which is considered an overall higher hormone phase (Allen et al., 2016). Ovulation occurs when a mature egg is released from a follicle in the ovaries. The corpus luteum is formed from the follicle that released the oocyte and

will begin to produce progesterone for the following phase 4. Ovulation (phase 3) lasts around 24-36 hours and must be characterized by a surge in luteinizing hormone in order to be considered as an ovulatory menstrual cycle (Janse et al., 2019). If luteinizing hormone levels fail to peak, it may be indicative of luteal phase deficiency or an anovulatory menstrual cycle (Janse et al., 2019).

Proceeding ovulation, phase 4 begins. Phase 4 is characterized by a substantial rise in estrogen and progesterone, released from the corpus luteum. Progesterone is at its highest level (>16nmol/L), while estrogen is higher than in phase 1 and 3 but slightly lower than its peak during phase 2 (Elliott-Sale et al., 2021). Luteinizing hormone and follicle stimulating hormone levels are lower. Phase 4 is considered the luteal or the high hormone phase of the menstrual cycle. Throughout the high hormone phase, the body builds up the endometrial lining and core body temperature is slightly elevated (Reed & Carr, 2000). The cycle then begins again with phase 1 and menses. Figure 2 illustrates the fluctuating hormone levels based on a typical 28-day cycle and the various phases associated (Elliott-Sale et al., 2021).

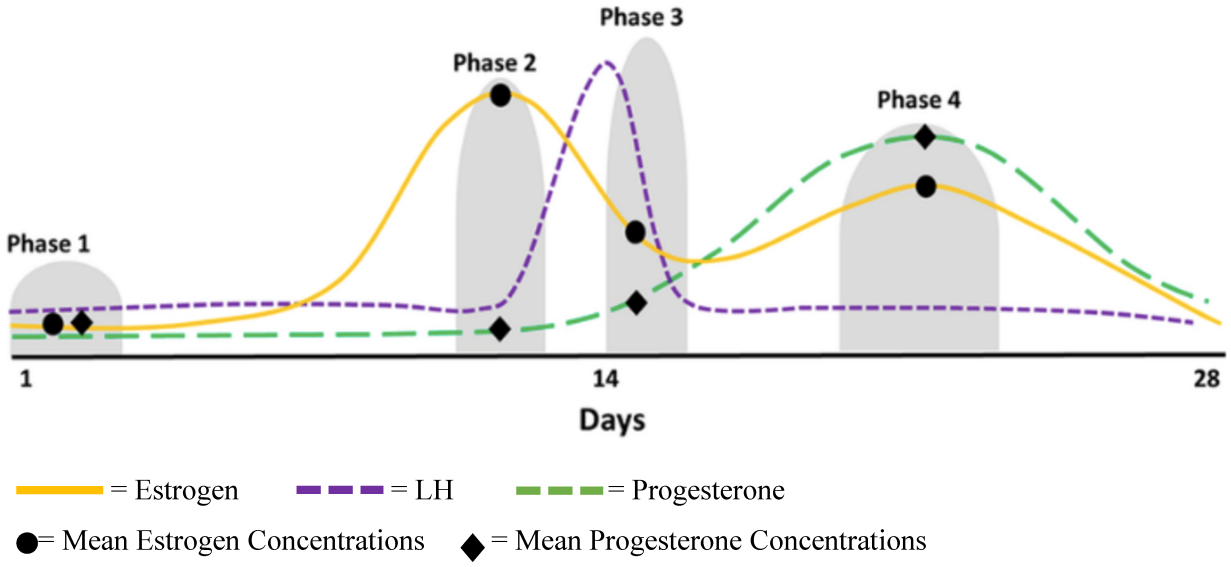

Figure 3. Ovarian hormones throughout a 28-day menstrual cycle. LH = luteinizing hormone. This figure has been adapted with permission from Elliott-Sale et al. (2021) © (2021) CC BY 4.0.

2.3 Methods for Verification of Menstrual Cycle Phase

Over the past few decades, studies performed on female athletes evaluating the impact of menstrual cycle phase on performance have increased, benefitting clinicians, coaches, and female athletes (Sims & Heather, 2018). Unfortunately, many studies performed on females have come up with conflicting results due to potentially poor methodological design and transparency and/or lack of confirmation of menstrual cycle phase (Allen et al., 2016; Elliott-Sale et al., 2021; Ikeda, 2022; Janse et al., 2019; McNulty et al., 2020; Schmalenberger et al., 2021). There are several methods used for verification of menstrual cycle phase including serial follicular scanning or sonography, calendar-based counting, basal body temperature, salivary ferning, urinary luteinizing hormone measurements, salivary hormone analysis, and serum hormone analysis (Allen et al., 2016; Janse et al., 2019). Janse et al., (2019) outline each method in their review on *Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise*. Each method of testing contains some limitations and therefore ideal determination of menstrual cycle phase would be determined from a combination of methods.

Serial Follicular Scanning and Sonography

A serial follicular scanning and sonography with measures of ovarian hormone concentrations would be an ideal method of determining all four menstrual cycle phases, however it is often not feasible for research purposes due to the high cost, invasive nature, and burden involved in conducting these techniques (Allen et al., 2016; Janse et al., 2019).

Calendar-Based Counting

Calendar-based counting or self reporting is a commonly-used method in previous research to determine menstrual cycle phase length (Janse et al., 2019). This method involves participants self reporting days of menses and researchers using approximate time frames for each phase in eumenorrheic women to determine the phase of the menstrual cycle. Self reporting and calendar-based counting is very feasible due to the low cost, low burden, and low technical skill involved. It does however contain many limitations, including the inability to identify women with luteal phase deficiency or with anovulation and the lack of knowledge of ovarian hormone concentrations (Janse et al., 2019). This method also presumes that menstrual cycle phases are standard lengths, which, as discussed earlier has caused confusion and inaccurate results among studies. Furthermore, this method relies heavily on the accurate reporting of menses by participants.

Basal Body Temperature

Basal body temperature is another feasible method due to the ease of procedure and the relatively low cost (Janse et al., 2019). For most menstruating women, basal body temperature increases by approximately 0.3 degrees Celsius during the LP (phase 4) of the cycle (Janse et al., 2019). Thus, by measuring the basal body temperature, researchers can determine when the LP begins and the approximate day of ovulation. Some limitations of this method include the inability to determine precise hormone concentrations, participant error in collecting body temperature at consistent times, and confounding variables in determining basal body temperature, including stress, sleep, and alcohol. (Allen et al., 2016; Janse et al., 2019).

Salivary Ferning

Salivary Ferning is a technique used to determine menstrual cycle phase by observing the crystallization of saliva in a microscope (Janse et al., 2019). This technique is only 53% reliable in confirming ovulation compared to sonography techniques, demonstrating a lack of reliability for research purposes (Janse et al., 2019). Salivary ferning is also relatively costly and involves some burden and technique on the researchers involved (Janse et al., 2019).

Urinary Luteinizing Hormone Test

Urinary luteinizing hormone measurements are a feasible and more direct method of determining menstrual cycle phase and specifically ovulation. Using ovulation predictor kits allows for an identification of a luteinizing surge. This method allows for identification and proper considerations of anovulatory female participants. There is some participant burden involved, as the participant must collect urine samples starting on day 8 of the menstrual cycle and insert a test strip in the urine until a positive test occurs. In most cases, ovulation occurs within 14-26 hours post positive test result (Janse et al., 2019). Although this test allows for identification of anovulatory participants, it does not allow for identification of luteal phase deficient participants. Furthermore, a lack of a positive luteinizing hormone test does not always indicate anovulation. As mentioned previously, females can have varying amplitudes and configuration of luteinizing hormone surges (Park et al., 2007). The most commonly used threshold that luteinizing hormone must reach in order to result in a positive test reading and in ovulation occurring within 24 hours

of the test is between 25 and 30mIU/mL (Leiva et al., 2017). Although most ovulation predictor tests use a threshold value of 25mIU/mL, not all ovulatory cycles achieve this threshold (Park et al., 2007). Thus, this method is accurate at estimating when ovulation is likely to occur, but is limited in identifying normal hormone concentrations post ovulation.

Salivary Hormone Analysis

Salivary hormone analysis allows for detection of estrogen and progesterone through non-invasive techniques. Salivary hormone analysis is relatively low cost and low burden. There are some limitations, however. Salivary hormone levels are much lower than serum hormone levels and therefore do not depict actual blood hormone concentrations (Janse et al., 2019). Although salivary hormone analysis is feasible, it is not as accurate at predicting hormone levels as serum analysis.

Serum Hormone Analysis

Serum hormone analysis is considered to be a gold standard method of determining menstrual cycle phase (Janse et al., 2019). Serum hormone analysis allows for researchers to determine estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone levels in menstruating females, while also allowing for identification of any deviations from eumenorrheic hormone levels. This method helps prevent the inclusion of anovulatory and luteal phase deficient participants without appropriate consideration.

According to Janse et al. (2019), using a combination of serum hormone analysis along with urinary luteinizing hormone measurements and the calendar-based counting approach is the gold standard for the determination of menstrual cycle phase for research purposes. Table 2 demonstrates proposed menstrual cycle phase parameters to allow for reproducibility and consistency in studies.

There has been a large number of studies performed on the menstrual cycle and its impact on exercise performance over the past few decades that contain inconsistent and unreliable methods of determining menstrual cycle phase, thus causing confusion in literature (Schmalenberger et al., 2021). Only seven out of eighteen research articles examining menstrual cycle differences in, so called, eumenorrheic females reviewed by Janse et al. (2019) and published in the last few years have included serum hormone measurements, which according to Elliot-Scale

et al. (2021), could contribute to the lack of homogeneity and reproducibility of results. Although female participants may experience a naturally occurring menstrual cycle, estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone levels may remain below typical levels, thus causing mixed results. Without analyzing serum or saliva hormonal concentrations, luteal phase-deficient females may accidentally be included in studies and labelled as eumenorrheic without proper considerations, thus skewing the results (Janse et al., 2019). Of the seven studies that included serum hormone verification, only four studies verified a progesterone peak in the mid-LP (phase 4) of the menstrual cycle (Janse et al., 2019). Three of the four studies finding a progesterone peak also found statistically significant differences between performance metrics in the FP of the menstrual cycle versus the LP. Furthermore, one out of the four studies that failed to discover statistically significant differences had a lower progesterone peak of 9.5 nmol/L rather than the suggested 16.0 nmol/L, further suggesting that verifying that hormone levels and thresholds are met in eumenorrheic females is essential in menstrual cycle research (Janse et al., 2019). Janse et al. (2019) suggest that up to 78% of the studies on "eumenorrheic" females from the past 10 years could have included anovulatory or luteal phase-deficient participants. It is evident from systematic reviews on the menstrual cycle and exercise science research that appropriate testing, terminology, and verification must be conducted going forward in order to produce reliable and valid results.

2.4 Amenorrhea, Relative Energy Deficiency, and Low Energy Availability

The term amenorrhea as mentioned above, describes women who are not menstruating, despite attaining a biological age where menstruation ought to occur. Amenorrhea can be a result of several conditions including polycystic ovarian syndrome, hyperprolactinemia, primary ovarian insufficiency, or low-energy availability. Amenorrhea or other menstrual irregularities, such as oligomenorrhea, luteal phase-deficiency, and anovulation, as a result of low energy availability is more common in female athletes (De Souza et al., 2010).

Amenorrhea occurs as a result of insufficient ovarian hormone levels and failure to initiate the menstrual cycle. Menstrual dysfunction, such as amenorrhea, is related to negative energy balance, high training loads, inadequate fat stores and abnormal hormone levels (Mountjoy et al., 2014). Adipose tissue produces and preserves estrogen (Tornberg et al., 2017). Therefore a lack of adipose tissue can contribute to a downregulation of ovarian hormones (Tornberg et al., 2017).

Low energy availability can also cause a decrease in resting metabolism and BG over time (Tornberg et al., 2017). Absent or irregular menstrual cycles can be indicative of low energy availability and can pose a variety of health and performance problems associated with an energy deficiency. Negative energy balance can stem from either purposeful or inadvertent undereating, over-exercising, food insecurity, knowledge deficit, restrictive dietary practices, and/or eating disorders (Holtzman & Ackerman, 2019). Sufficient energy availability is required not only to ensure appropriate endocrinological health, indicated by the presence of ovarian hormones and the menstrual cycle, but also to ensure immunological, gastrointestinal, cardiovascular, metabolic, and psychological health (Fahrenholtz, 2018; Mountjoy et al., 2015).

Relative energy deficiency in sport (RED-S), formerly known as the female athlete triad is one of the few topics within the sports science and exercise physiology realm that has been studied with a female-centric lens because it is so common in female athletes (Holtzman & Ackerman, 2021). The female athlete triad was first conceptualized in 1993 by the American College of Sport Medicine as pertaining to female athletes experiencing low bone mineral density, menstrual cycle dysfunction, and low energy availability (Yeager et al., 1993). Since then, there has been a shift towards using RED-S terminology as a more inclusive and comprehensive understanding of low energy availability and its corresponding complications. RED-S refers to an energy imbalance in athletes. Energy imbalance can occur as a result of consuming an insufficient number of calories to maintain exercise metabolism in addition to resting metabolic functioning, resulting in low energy availability and energy deficits (Dipla et al., 2021). This energy deficit can cause several physiological and psychological health risks for individuals, including decreased resting metabolic rate, increased irritability and depression, decreased coordination, concentration and judgment, decreased muscular strength and muscle glycogen storage, increased risk of injury (especially stress fractures), along with decreased training response and overall performance (Mountjoy et al., 2023).

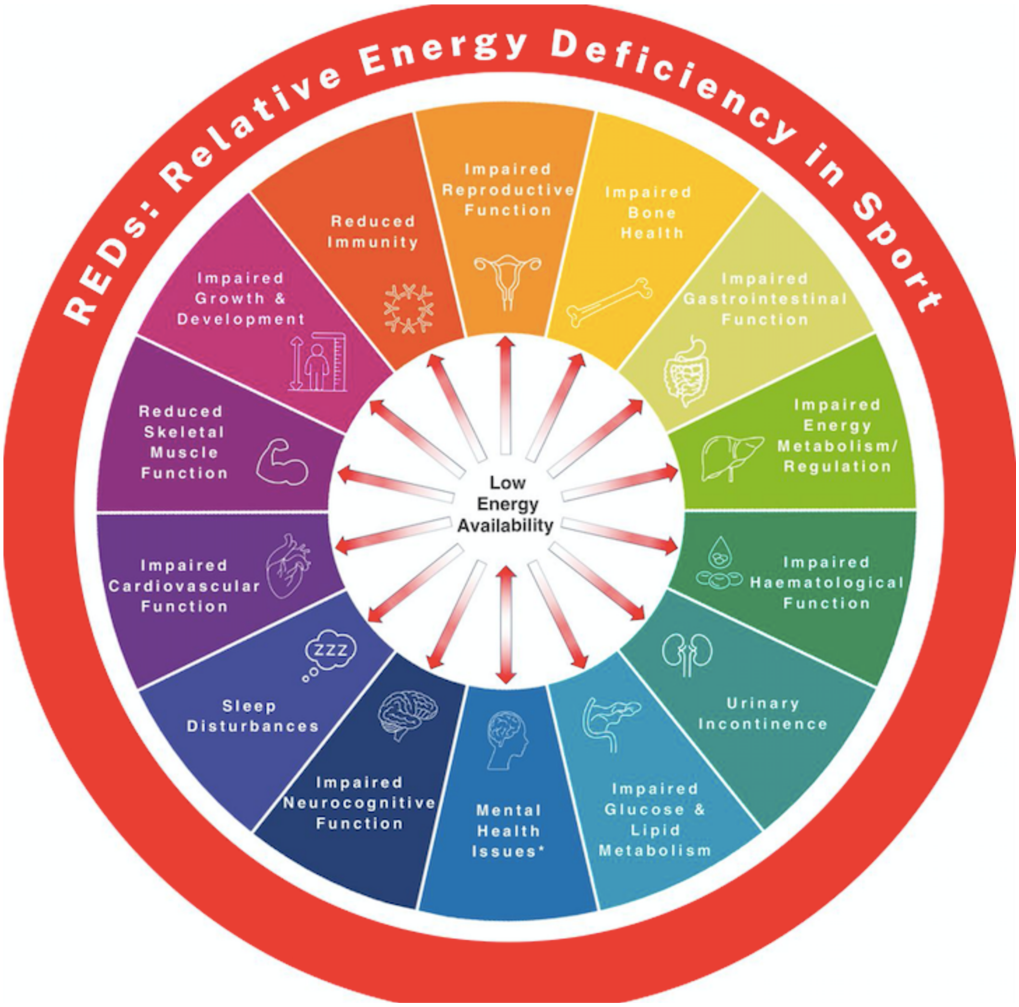

Figure 4. Health Consequences of Relative Energy Deficiency in Sport (RED-S). This figure has been reproduced from Mountjoy et al. (2023) © Sept 1, 2023, with permission from BMJ Publishing Group Ltd.

Low energy availability is extremely prevalent for athletes in endurance sports, aesthetic-based sports, and weight class sports (Holtzman & Ackerman, 2021; Kong & Harris, 2015; Torstveit & Sundgot-Borgen, 2005). The prevalence of this condition is evident in one particular study showing that over 80% of elite female endurance runners show low energy availability risk (Jesus et al., 2021). Another study found that 96% of collegiate, female ballet dancers had low energy availability, while 80% of all female athletes in the study had low energy availability (Torres-McGehee et al., 2021). Eighty-eight percent of professional female soccer players had low energy availability, further demonstrating that energy imbalances pose a problem for both recreational and elite athletes alike (Morehen et al., 2022). Furthermore, about one out of three

female athletes have self-reported menstrual cycle dysfunction which can be directly linked to energy imbalances and low energy availability (Ravi et al., 2021)

Low BG levels may occur as a result of chronic low energy availability and menstrual cycle dysfunction (Melin et al., 2015; Tornberg et al., 2017). Because of the high prevalence for low energy availability in endurance athletes, and because of the impact that low energy availability has on overall health and metabolism, screening questionnaires must be used to assess athletes for low energy availability and to take into account menstrual cycle status. The menstrual cycle or lack thereof is tied to energy availability, metabolism, and regulation of substrates and must therefore be examined in relation to BG levels and performance.

2.5 Substrate Utilization

Relative substrate metabolism and use is important to note when looking at interstitial glucose levels for female athletes because oxidation of carbohydrates can directly impact BG levels. Sex differences in substrate selection exist during exercise and at rest (Devries et al., 2006; Hackney et al., 2000; Horton et al., 2006; Horton et al., 1998; Tarnopolsky et al., 1990; Venables et al., 2005; Wismann & Willoughby, 2006). The sex differences in metabolism are not seen until post puberty, which suggest that ovarian hormones play a role in oxidation, transportation, and use of substrates (Aucouturier et al., 2008). Furthermore, although evidence is a little conflicting, substrate metabolism and oxidation have been suggested by some to fluctuate according to the ovarian hormones present (Campbell et al., 2001; Hackney et al., 2000).

Although there are inconsistencies throughout literature, some studies found that estrogen promotes a glycogen-sparing effect during exercise and creates a greater reliance on fat as a substrate over carbohydrates (D'Eon et al., 2002; Devries et al., 2006), while progesterone may work antagonistically by promoting insulin resistance and decreasing glycogen storing (D'Eon et al., 2002; McLay et al., 2007). In one particular study involving ovariectomized rats, the authors found that the rats injected with estrogen before exercise oxidized more fatty acids compared to rats injected with both estrogen and progesterone when performing endurance exercise (Hatta et al., 1988).

Estrogen concentrations have been associated with an increased use of fat for energy production over carbohydrates both at rest and during exercise (Campbell et al., 2001; Campbell & Febbraio, 2001; D'Eon et al., 2002; Devries et al., 2006; Hackney et al., 2000; Kendrick & Ellis,

1991). This estrogenic shift in substrate oxidation is further established in a study performed on amenorrheic participants with medicated transdermal estrogen infusions. The study found that glucose metabolism at rest and during exercise significantly decreased after estrogen infusions compared to initial low estrogen values, while free fatty acid appearance increased after estrogen infusions (Ruby et al., 1997).

Furthermore, the rate of appearance and disappearance of glucose during exercise decreases with estrogen present, while muscle glycogen storage capacity is augmented (Campbell et al., 2001; Carter et al., 2001). Another study evaluating menstrual cycle phase differences in glucose kinetics during 90 minutes of moderate cycling found lower rates of glucose appearance and disappearance for females during the LP compared to women in the mid-FP when undergoing constant glucose infusions (Devries et al., 2006). This is similar to findings of another study performed on male participants taking estrogen supplements for 8 days, followed by a 90-minute cycling intervention at 65% VO_2max. The participants taking estrogen supplementation showed a significantly lower respiratory exchange ratio (RER) indicating a greater reliance on fat as a substrate over carbohydrates, rate of glucose appearance, and rate of glucose disappearance compared to controls (Devries et al., 2005).

Estrogen promotes muscle-contraction mediated uptake of glucose into cells in rats (Campbell & Febbraio, 2002) and increases insulin sensitivity in post-menopausal women receiving estrogen infusions (Van Pelt et al., 2003). Estrogen also increases lipolysis by promoting lipoprotein lipase activity thus increasing the amount of plasma free fatty acids available for oxidation during endurance exercise (Kendrick & Ellis, 1991).

Very few studies show a trend toward lower RER in the FP compared to the LP, which indicates greater carbohydrate oxidation during the LP compared to the FP, and of the few that suggest this difference, none have reached statistical significance (Dokumaci & Hazir, 2019; Oosthuyse et al., 2003; Vaiksaar et al., 2011b). One such study performed on competitive rowers doing hour-long submaximal rowing at 70% of their VO_2 max, showed a slightly higher RER during the LP compared to the FP (FP=0.88, LP=0.91, $p > 0.05$) in recreationally trained athletes, although statistical significance was not reached (Vaiksaar et al., 2011b). Similarly, female athletes performing submaximal running at 60% of their VO_2 peak, had a mean RER value of 0.87 in the LP of the cycle compared to the 0.83 in the FP, indicating greater carbohydrate oxidation during the LP compared to the FP, however these results did not reach statistical significance (Oosthuyse

et al., 2003). Another study performed on 11 eumenorrheic female athletes found that mean RER was 0.99 ± 0.027 in the LP compared to 0.96 ± 0.055 in the FP while running at a speed corresponding to 95% of individual lactate threshold. Once again, these results did not reach statistical significance (Dokumaci & Hazir, 2019).

Conversely, other studies suggest the opposite, with lower RER values occurring in the LP compared to the FP. One such study using constant infusion of glucose 90 minutes prior to exercise demonstrated that RER was lower during the LP compared to the FP during exercise corresponding to 90% of individual lactate threshold (FP=0.87 ± 0.015, LP=0.84 ± 0.017) (Zderic et al., 2001). Another study performed on eumenorrheic participants found that at 35% and 65% of maximal running intensity, carbohydrate oxidation rates were significantly lower, while lipid oxidation rates were significantly higher during the mid-LP compared to the mid-FP (Hackney et al., 1994). Redman et al. (2003) also found that RER was significantly lower during the LP compared to the FP in female athletes cycling at their individual VO_2 peak level ($p = 0.03$) (Redman et al., 2003).

Although several of the studies mentioned above demonstrate potential ovarian hormone-related effects on relative substrate oxidation, the majority of studies suggest no menstrual phase effect on RER and substrate oxidation (Ekberg et al., 2023; Jurimae et al., 2016; Rael et al., 2021; Williams et al., 2023). One such study performed on ten endurance-trained female athletes running or cycling at 70%, 80%, 90%, and 100% of their individual VO_2 peak, found that there were no statistically significant differences in RER during three different menstrual cycle phases (early FP, late FP, mid-LP) (Ekberg et al., 2023). In addition, a recent study evaluating substrate oxidation at rest and during submaximal cycling at 40% and 65% of VO_2 peak, found no impact of menstrual cycle phase on RER and substrate oxidation in naturally cycling female participants. (Williams et al., 2023). Another study performed on endurance trained, eumenorrheic female athletes found no statistically significant difference in RER during interval exercise in three different phases of the menstrual cycle (Rael et al., 2021).

As a result of conflicting findings in the current body of literature, a recent systematic review concluded that overall, there is no phase-related difference in relative substrate oxidation during exercise (D'Souza et al., 2023). This review illustrates the high levels of intra- and inter-individual variability of ovarian hormone effects on metabolism. Figure 5, taken from D'Souza et al.'s (2023) meta-analysis on various changes throughout the menstrual cycle, outlines differences in RER in the FP compared to the LP from several studies from the past 30 years.

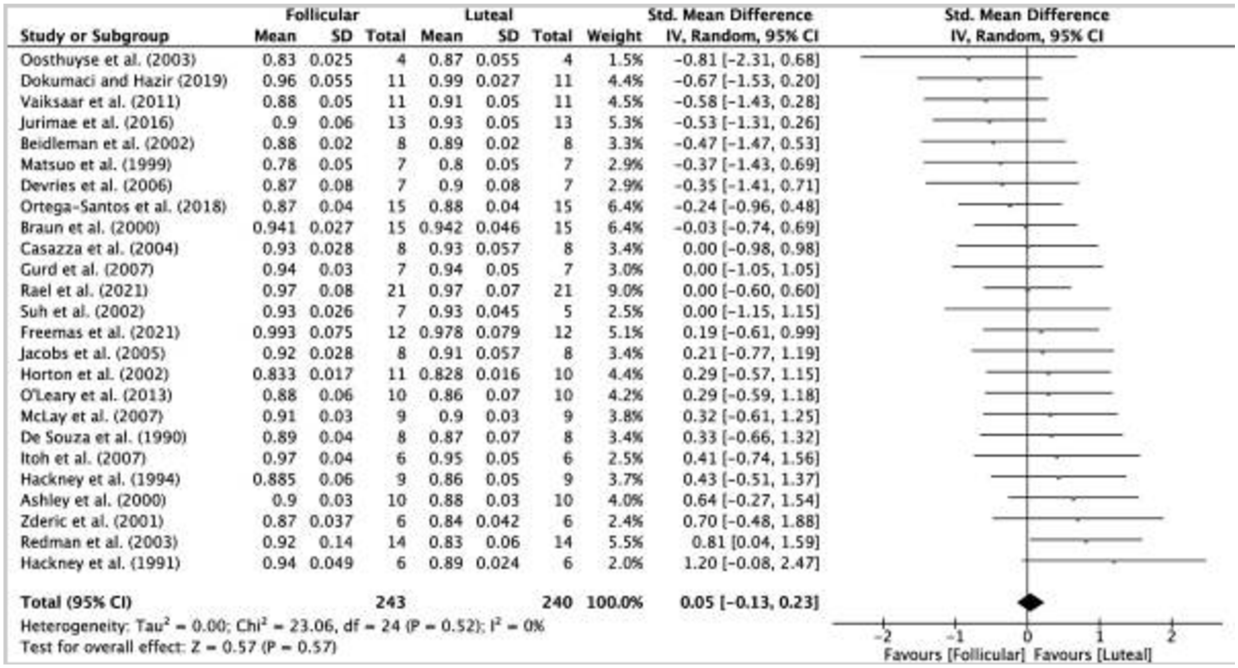

Figure 5. A forest plot comparing RER during moderate exercise in the FP compared to the LP of the menstrual cycle. This figure has been reproduced with permission from D'Souza et al. (2023) © (2023) CC BY 4.0.

When endurance exercise is performed at moderate intensities, the menstrual phase could have an influence on substrate metabolism; however, there is no consistent evidence of this. Potential factors contributing to a lack of consistency regarding menstrual cycle phases on relative substrate metabolism can include: dietary intake and carbohydrate supplementation during exercise, hydration status, exercise timing (postprandial or fasted), exercise duration and intensity, muscle fiber type, menstrual irregularities, sleep, and illness (Rothschild et al., 2021; Rothschild et al., 2022; Stoa et al., 2016). One review article performing a modeling analysis on 434 different studies demonstrated how daily carbohydrate and fat intake can influence exercising RER more so than acute carbohydrate supplementation during exercise (Rothschild et al., 2022). Changes in metabolism due to fluctuating ovarian hormones may be negligible compared to these other factors (Campbell et al., 2001; Devries et al., 2006; Hackney et al., 1994; Hulton et al., 2021).

Furthermore, since progesterone has been found to act antagonistically to estrogen in terms of relative substrate oxidation (D'Eon et al., 2002), the ratio between estrogen and progesterone seems to be an important indicator whether RER is affected based on menstrual cycle phase or not.

If the ratio between estrogen and progesterone is different between individuals and between cycles then finding consistent results would be difficult. There are also slightly different time frames used to schedule menstrual cycle phases and hormone concentrations between studies which can also contribute to a lack of homogeneity in results. If estrogen and progesterone levels do not meet threshold criteria, as seen in females with luteal phase-deficiency, results of their impact on metabolism may be skewed further (Oosthuyse & Bosch, 2010). Finally, as is the case for many symptoms of the female menstrual cycle, such as cramps, fatigue, and perception of exercise performance, individuals can have different responses and reactions to hormones. Individual differences cannot be overlooked and disregarded.

Furthermore, with approximately half of the female athlete population using hormonal contraceptives (Martin et al., 2018), it is important to examine the effect that exogenous hormones may have on substrate metabolism. Some studies suggest that exogenous progestins from monophasic oral contraceptive pills may enhance the increased reliance on fat as a substrate over carbohydrates during exercise in females (Bemben et al., 1992; Redman et al., 2005). Other studies suggest that although an increase in lipolysis and plasma free fatty acids availability occurs with high exogenous hormones, relative oxidation rates did not change significantly (Casazza et al., 2004; Jacobs et al., 2005). While some studies demonstrate a difference in relative substrate oxidation as a result of exogenous hormones, there are still others that find no significant difference in substrate oxidation between endogenous and exogenous hormones (Isacco, Thivel, et al., 2012). With different forms of contraception being so widespread, it is important to take into account the use of contraceptives and their potential confounding effects.

Table 3. *Summary table of the effect of estrogen only on substrate oxidation in human and rodent participants. A lower RER value indicates a greater reliance on fat as a substrate.*

Population	Effects	Reference
Females	↓ RER during exercise	(Devries et al., 2005; Hackney et al., 2000; Ruby et al., 1997)
Males	↓ RER during exercise	(Carter et al., 2001; Devries et al., 2005; Hamadeh et al., 2005)
Both Females and Males	↓ CHO oxidation during exercise	(D'Eon et al., 2002; Hackney et al., 2000; Hamadeh et al., 2005; Ruby et al., 1997)
Both Females and Males	↑ Fat oxidation during exercise	(Hackney et al., 2000; Hamadeh et al., 2005; Ruby et al., 1997)
Both Females and Males	↑ Circulating free fatty acid during exercise	(Ruby et al., 1997)
Both Females and Males	↓ Rate of appearance of glucose during exercise	(Carter et al., 2001; Devries et al., 2005; Ruby et al., 1997)
Both Females and Males	↓ Rate of disappearance of glucose during exercise	(Carter et al., 2001; D'Eon et al., 2002; Devries et al., 2005)
Rats	↑ Lipoprotein lipase activity in muscle during exercise	(Ellis et al., 1994; Kendrick & Ellis, 1991)
Rats	↑ Fat oxidation during exercise	(Campbell & Febbraio, 2001; Hatta et al., 1988)

CHO = carbohydrate. This table has been adapted from Isacco and Boisseau (2017). © Jan. 1, 2017, Springer Nature with permission from Elsevier.

2.6 Energy Intake Across the Menstrual Cycle

Perturbations in hormone levels across the cycle can influence overall caloric intake and food preferences (Johnson et al., 1994). In fact, in a recent review on dietary intake across the menstrual cycle, food intake was found to vary by 159 kcal/day–529 kcal/day depending on menstrual cycle phase (Rogan & Black, 2023). Changes in food intake behavior must be noted when looking at BG levels because of the large and direct impact that food intake has on BG. Therefore, any changes in glucose across menstrual cycle phases could be due in part to changes in food seeking behavior and intake.

Food preferences change throughout the menstrual cycle (Krishnan et al., 2016; Yen et al., 2010). Food cravings commonly occur premenstrually and have been well-studied over the past three decades (Michener et al., 1999). The LP (phase 4) of the cycle has been positively associated with an overall increase in energy intake, particularly from foods rich in carbohydrates (Cohen et al., 1987; Krishnan et al., 2016; Rozin et al., 1991). According to Krishnan, et. al. (2016), total fat intake also increases during the LP. Since many simple carbohydrate foods are also high in fat, it follows naturally that there is an overall increase in both macronutrients during the LP (Krishnan et al., 2016). Dalvit-McPhillips however, found that fat and protein intake remained relatively constant independent of the phase of cycle, and only carbohydrate intake significantly increased in the LP (Dalvit-McPhillips, 1983). Gorczyca et al. (2016) found an increase in total caloric intake and an increase in protein intake in the LP. There is a common consensus, however, that total caloric intake and carbohydrate intake increases in the LP compared to the FP of the menstrual cycle (Barr et al., 1995; Chung et al., 2010; Gil et al., 2009; Krishnan et al., 2016; Lissner et al., 1988).

Many studies have found an increase in cravings and intake of all sweet foods, specifically chocolate and chocolate chip cookies in the LP of the cycle (Bowen & Grunberg, 1990; Cohen et al., 1987; Malo-Vintimilla et al., 2024; Michener et al., 1999; Rozin et al., 1991). One particular study by Michener et al. (1999) found that 48% of women with premenstrual syndrome craved chocolate and sweets in a cyclical manner reflective of the menstrual cycle. Furthermore, cravings for salty snacks and overall appetite scores increased during the LP (Gorczyca et al., 2016).

Several theories have emerged as to why there is an increase in food preferences of sweet and high fat food sources during the LP of the cycle. One such theory is that high carbohydrate and fat food sources help to increase serotonin production, which can increase feelings of satisfaction and happiness (Dye & Blundell, 1997; Nowak et al., 2020). Another theory for an increase in carbohydrate rich food cravings is that progesterone may increase cravings, thus increasing food cravings during the mid-LP when it is at its peak concentration (Fox et al., 2013). Basal metabolic rate also increases during the LP, potentially triggering an increase in food seeking behavior through centrally-regulated mechanisms (Dalvit-McPhillips, 1983).

Since eumenorrheic and naturally cycling females tend to increase their total caloric intake and particularly their intake of carbohydrate rich food sources during the LP (phase 4) of the menstrual cycle, it follows accordingly that BG could be affected.

2.7 Glucose Regulation

Glucose is the body's primary substrate for energy metabolism during most forms of exercise (Brun et al., 2001; Isacco, Duche, et al., 2012; Kondo et al., 2019; Mathew & Thoppil, 2023). As such, the body usually maintains strict control over BG levels. Blood glucose levels in healthy individuals usually remain between 4 mmol/L and 7.8 mmol/L (Shah et al., 2019). Large perturbations, both high and low, from this glycemic range, such as is seen in people with type 1 and type 2 diabetes, can cause irreversible and severe damage to several organs in the body over time (Daryabor et al., 2020). Although life-threatening glycemic events are unlikely to occur in this female athlete population, episodes of hypoglycemia can still severely hinder athletic performance and recovery (Amiel, 1998; Gleeson et al., 1998; Nybo, 2003; Rustad et al., 2016; Smith et al., 2016; Tornberg et al., 2017). Hypoglycemia, defined by the International Continuous Glucose Monitoring Consensus Statement as being BG levels <3.9 mmol/L (American Diabetes Association, 2022; Battelino et al., 2023), can result in a myriad of problems including decreased neuromuscular performance (Tornberg et al., 2017), weakened immune function (Gleeson et al., 1998), reduced overall cognitive and motor performance (Nybo, 2003; Rustad et al., 2016), and less overall recovery (Gleeson et al., 1998; Ivy et al., 1988).

Blood glucose levels are primarily influenced by exogenous glucose or carbohydrate consumption, endogenous glucose production by means of gluconeogenesis and glycogenolysis, insulin secretion or sensitivity, and exercise mediated GLUT4 transport (Kondo et al., 2019). More specifically, glucose rate of appearance is regulated by endogenous glucose production and exogenous glucose absorption, while glucose rate of disappearance is regulated by insulin and uptake of glucose by the cells (Kondo et al., 2019). Therefore, although BG levels can be low, high or regular, determining the mechanisms behind glycemic events can be difficult (Bowler et al., 2022).

Although glucose regulation is often studied in the context of disease such as diabetes, the purpose of this study is to look at glucose levels in female athletes and how the presence of ovarian hormones or lack thereof can influence BG levels and potential glycemic events, specifically post-exercise and in recovery. Studies thus far have identified greater resting BG levels during the LP compared to the early FP of the menstrual cycle (Dey et al., 2019; Lin et al., 2023; Nandimath & Bindu, 2015; Zarei et al., 2013). Furthermore, some studies have identified greater BG in the LP

compared to the FP during exercise but these have been limited to within-laboratory blood tests and do not account for longer recovery periods (Zderic et al., 2001). To our knowledge, the proposed study described in chapter 3 would be the first study to use continuous glucose monitors to determine possible phase effects on BG across the entire menstrual cycle in aerobically trained athletes.

2.8 Glucose Uptake

Hypoglycemia can occur when the body's cells take up more glucose from the blood than what is available or being replenished (Evans et al., 2019; Jeukendrup & Killer, 2010; Zignoli et al., 2023). Blood glucose can be taken up by the muscle cells by means of a GLUT4 transporter. GLUT4 transporters are translocated to the cell membrane as a result of two mechanisms, (1) insulin binding and action and (2) muscle contraction (Douen et al., 1990; Evans et al., 2019). The presence of GLUT4 transporters postprandially with the addition of performing exercise, can lead to a large uptake of glucose by the cells and a resultant drop in BG levels, also known as reactive or transient hypoglycemia (Kondo et al., 2019; Moseley et al., 2003; Zignoli et al., 2023). Hyperinsulinemia, a condition where high amounts of insulin are released postprandially, often in response to a high glucose meal, causes a large translocation of GLUT4 transporters. This situation is exacerbated by exercise, as muscle contraction also contributes to an increase in GLUT4 transporter availability, thus resulting in possible hypoglycemia during exercise (Kondo et al., 2019). Post exercise, muscle glucose uptake tends to increase, because of an increase in insulin sensitivity, in order to replenish muscle glycogen stores. This increase in insulin sensitivity can remain elevated for 16-48 hours post exercise (Brun et al., 2001; Jensen & Richter, 2012; Mikines et al., 1988).

Exercise and sports that involve high intensity interval training, prolonged aerobic training, or neuromuscular activation and reaction time can be impaired by low BG and low muscle glycogen levels (Tornberg et al., 2017; Widrick et al., 1993). Although the relationship between carbohydrate consumption, BG, muscle glycogen, and actual use of cellular glucose is complex, chronic low carbohydrate intake and low energy availability have generally been associated with an increase in hypoglycemic events (Bowler et al., 2022; Melin et al., 2015; Tornberg et al., 2017). Furthermore, Kuipers et al. (1999) found that just over 30% of well-trained cyclists in their study experienced hypoglycemia (which they defined as BG <3.0 mmol/L) due to hyperinsulinemia,

during 40 minutes of moderate exercise, when beginning exercise 30 minutes after consuming 50 grams of glucose, proceeding a 4-hour fast (Kuipers et al., 1999).

Furthermore, a recent study performed on regularly menstruating female participants found that BG levels varied in a biphasic manner according to the menstrual cycle, with highest BG levels occurring during the LP and lowest BG levels occurring during the late FP (Lin et al., 2023). Another study performed on females experiencing premenstrual syndrome found that BG levels and insulin resistance were significantly lower during both the FP and the LP in female participants experiencing severe premenstrual syndrome compared to control groups, further demonstrating a link between the menstrual cycle and BG (Zarei et al., 2013).

Since estrogen increases cell sensitivity to insulin (Yan et al., 2019) and increases contraction-stimulated glucose uptake (Campbell & Febbraio, 2002), there is reason to explore the potential impact that the menstrual cycle has on BG levels (Kendrick & Ellis, 1991). Furthermore, since many female athletes may be at risk for low energy availability and low BG as a result of low energy availability, exploring BG levels throughout the menstrual cycle would be an important contribution to existing literature.

2.9 Continuous Glucose Monitors

Continuous glucose monitors are devices that are commonly used in people living with diabetes to measure interstitial glucose levels, thus informing the user of potential glucose variability and deviations from euglycemia (Thomas, 2017). The use of CGMs in people living with diabetes has become more common in recent years due to their less invasive and user-friendly features, their decrease in price and greater accessibility, their frequency of BG detection, and their improvement in accuracy (Battelino et al., 2019; Thomas, 2017). Continuous glucose monitors use microneedle technology, typically inserted into the subcutaneous layer of the upper arm, upper gluteus, or abdomen, to detect glucose concentrations in surrounding interstitial fluid (Holzer et al., 2022; Thomas, 2017). Continuous glucose monitors contain a small glucose sensor, a transmitter, and a data display device, which can be a receiver or another compatible smart device (Dexcom, n.d.). Sensors use electrochemical glucose oxidation reactions, combined with the use of calibration algorithms to convert interstitial glucose readings to BG values (FDA, 2018).

2.10 Applications for Continuous Glucose Monitors in Sport and Performance Contexts

The use of CGMs has been studied relatively thoroughly in clinical populations, however the use of CGM technology by athletes has gained popularity and has garnered more research in recent years (Holzer et al., 2022; Kulawiec et al., 2021; Skroce et al., 2024; F. Thomas et al., 2016; Thomas, 2017). Some reviews suggest that CGM technology has the potential to aid in performance and recovery by detecting BG levels and informing potential nutritional strategies and interventions (Holzer et al., 2022; Thomas, 2017). Glucose regulation and oxidation is a complex process, however increasing BG availability can contribute to an increase in muscle glycogen storage (Ivy et al., 1988) and ultimately delay the onset of fatigue (Schabort et al., 1999; Widrick et al., 1993). Furthermore, low BG levels and limited carbohydrate availability can hinder performance, recovery, and immunological health (Hashimoto et al., 2014; Tsintzas et al., 1996).

One particular study demonstrated a link between menstrual cycle phase, carbohydrate consumption, and immunological health. Female athletes performing prolonged exercise were found to have greater immunological disturbance when they did not consume carbohydrate beverages during exercise, particularly in the LP of the menstrual cycle compared to female participants who did consume carbohydrates (Hashimoto et al., 2014). This study demonstrates the potential impact that low carbohydrate consumption can have on immunological health, identifying a potential application of CGM use in exercising individuals.

Another study examining sex differences in substrate oxidation by using indirect calorimetry, demonstrated the impact that food selection and timing has on substrate oxidation (Tremblay et al., 2010). Consistent with other studies, male participants used more carbohydrate as fuel for moderate exercise compared to female participants who used more fat when dietary intake was the same. When female participants increased carbohydrate intake and supplementation during exercise however, these sex differences disappeared, demonstrating the influence that fuel selection and timing can have on substrate oxidation (Tremblay et al., 2010). Depending on the activity being performed, athletes could potentially inform their fueling selection based on feedback from a CGM.

As mentioned in a review by Bowler et al., (2022) it has been assumed that maintaining adequate levels of BG can help replenish hepatic and muscle glycogen stores thus preventing substrate-limiting fatigue (Karelis et al., 2010). A few pilot studies have emerged demonstrating the potential applications of CGMs to endurance athletes. Bowler et al., (2022) present a summary table of studies performed on endurance athletes using CGMs. One such study found that during

a 165 km ultra-trail race, athletes who had lower interstitial glucose levels and consumed less carbohydrates during the race took longer to complete the race (Ishihara et al., 2020).

Another case study found that a trained, competitive endurance runner demonstrated less glucose variability throughout an ultramarathon compared to a recreational runner who experienced greater fluctuations in BG levels during the ultramarathon and then experienced hypoglycemia in later stages of the race, despite consuming more carbohydrates than the competitive runner (Sengoku et al., 2015). One could speculate that with a greater reliance on carbohydrates as a substrate, individuals might also experience greater variability in blood glucose levels.

A few larger studies using CGMs in athletic populations have begun to emerge as well. Recently, an observational study, using CGMs in 12,504 physically active adults, was conducted. Participants self-identified as athletes and used a software platform entitled Supersapians to log exercise, sleep, food intake, and CGM data in a free-living scenario. This study found that participants spent 10.3 ± 16.7% of time during exercise events above 7.8 mmol/L and 11.9 ± 11.6% of exercising time below 3.9 mmol/L. Time below 3.9 mmol/L occurred more often at night than during the day and was more common in female participants compared to males. This study also found that female participants had lower overall BG compared to male participants during exercise, meals, and sleep (Skroce et al., 2024).

As opposed to the free-living study mentioned above, Bowler et al. (2024) used a standardized diet and exercise approach to control for differences in exogenous glucose consumption. The amount of time spent with low and high BG was lower compared to other free-living studies on elite endurance athletes (Flockhart et al., 2021; Skroce et al., 2024), however similar to the study by Skroce et. al. (2024), the authors once again determined that female participants had lower mean glucose values compared to male participants (Bowler et al., 2024). The study performed by Bowler et al. found that elite racewalkers spent 1.3 ± 2.4% of time per 24 hours below 4mmol/L and 2.4 ± 1.6% of time per 24 hours above 8mmol/L; compared to findings from Flockhart et al. (2021), where time spent below 4mmol/L was 8.0% per 24 hours and time spent above 8mmol/L was 2.8% per 24 hours.

Furthermore, CGMs may serve as an indicator for athletes with LEA who may be experiencing metabolic disruptions and concurrent episodes of hypoglycemia (Bowler et al., 2022). Individuals experiencing LEA or eating disorders are at risk for experiencing greater

glycemic disruptions and more time with low BG (Germain et al., 2022), therefore a CGM could be used as a tool for practitioners to help identify athletes at risk for LEA and/or eating restrictions.

Unlike having to take venous glucose measurements in a lab, CGMs allow researchers to take consistent and frequent interstitial glucose measurements, allowing for more robust examination of glucose during periods that would normally have not been viable, such as during sleep and daily activities. There are several potential applications for CGM use in sport and performance, however it is important to note that there is currently no evidence that CGM data can inform the user of appropriate carbohydrate selection and timing to promote optimal performance. The hypothesis that glucose data from CGMs can provide information on specific fueling interventions for sports performance is, as of yet untested (Bowler et al., 2022).

2.11 Glucose Monitoring and the Menstrual Cycle

Studies measuring BG levels throughout the menstrual cycle have been undertaken in diabetic populations. Preliminary results show that females with type 1 diabetes may be more susceptible to high BG during the LP due to decreased insulin sensitivity (Brown et al., 2015). An increase in time spent in hyperglycemia during the LP of the menstrual cycle has been observed in several studies (Barata et al., 2013; Brown et al., 2015; Goldner et al., 2004; Li et al., 2024; Tatulashvili et al., 2022). These findings are consistent with studies on participants without diabetes, where female participants had higher glucose levels when blood samples were taken at rest during the LP compared to the FP (Dey et al., 2019; Lin et al., 2023; Zarei et al., 2013).

2.12 Limitations of Continuous Glucose Monitoring Technology

There are limitations of using CGM in active living contexts. CGM sensors can be displaced or removed because of possible contact in combative or contact sports, large amounts of sweat, water exposure or immersion, or accidental displacement while sleeping (Bowler et al., 2022). Furthermore, because CGMs use interstitial glucose measurements to estimate BG, there is a time lag before changes in BG are reflected in interstitial fluid (Moser et al., 2018; Schmelzeisen-Redeker et al., 2015). This time lag may cause values to be inaccurate during exercise or during other times where glucose can fluctuate rapidly. One review suggests that several other factors such as interstitial fluid volume, redistribution of blood, lymphatic, and interstitial fluid, and the rate of production and absorption of glucose by cells can also be altered during exercise (Moser et

al., 2018). These physiological changes that occur during various types and intensities of exercise can contribute to an decrease in reliability of CGM results during exercise (Moser et al., 2018).

Although BG fluctuations can have impacts on overall health, performance, and recovery, continuous glucose profiles during exercise and recovery are still relatively unknown in female athletes and especially with menstrual cycle considerations. As outlined in the above literature review, prior studies have demonstrated that many physiological systems, including BG regulation, can be affected by the menstrual cycle (or its absence). Studies to date have not examined the prevalence of hypoglycemic events nor the regulation of BG during and in the 24 hours post exercise in different phases of the menstrual cycle. Furthermore, prior studies have demonstrated an increase in time outside of the normoglycemic range in endurance athletes compared to healthy, sedentary individuals, however no consideration was given to menstrual cycle phase (Flockhart et al., 2021). Elite endurance athletes are susceptible to disturbed glucose regulation and decreased glucose tolerance especially in response to excessive training (Flockhart et al., 2021). As a result, this study seeks to examine the changes in BG throughout the menstrual cycle, including the prevalence of hypoglycemia during exercise and in exercise recovery in female endurance athletes. The findings of this study can provide researchers, coaches, and trainers with further considerations on nutrition, training, and recovery for female endurance athletes.

Chapter 3: Research Methodology

3.1 Population

The population recruited for this study was aerobically fit female athletes who are naturally cycling with a visible luteinizing hormone surge. Data from naturally cycling participants was used for the primary objectives of this study. We used the terminology of naturally cycling with an luteinizing hormone surge, as recommended by Smith et al. (2022), as opposed to eumenorrheic female participants because the current study did not perform serum hormone collection and analysis and therefore cannot determine actual ovarian hormone concentrations. Testing females who are naturally cycling is considered silver standard if blood tests are unable to be collected (Smith et al., 2022). This study defined aerobically fit athletes as participants competing in university athletics and/or competitive clubs and/or individual training programs, with a $VO_{2peak} \geq$ 45ml/kg/min and who are training $\geq$5 hours per week. Although athletes competing in non-endurance sports were welcome to participate, as aforementioned the inclusion criteria ensures athletes with high aerobic fitness.

***Inclusion Criteria*:** We recruited 11 female athletes with the following inclusion criteria:

- 18-40 years of age.
- Naturally cycling with a visible luteinizing hormone surge.
- Self-reported menstrual cycle lengths $\geq$ 21 days and $\leq$ 35 days.
- Confirmed luteinizing hormone surge.
- No hormonal contraceptive use within 3 months prior to recruitment and during the study.
- Menstrual cycle characteristics tracked for 1 month prior to testing.
- Competing in university athletics and/or competitive clubs and/or individual training programs with a total training time above or equal to 5 hours per week.
- Peak oxygen consumption (VO_{2peak}) equal to, or above 45ml/kg/min. A relative $VO_2max \geq$ 45ml/kg/min is categorized as a "very good" to "excellent" standards according to the Canadian Society for Exercise Physiology standards (CSEP, n.d.). Furthermore, an average $VO_2max \geq$ 45 ml/kg/min corresponds to above the 95th percentile for cardiorespiratory fitness in Canadian women between the ages of 18 and 40 (Matt D. Hoffmann & Lang, 2019).

- Able to run on a treadmill for 60 minutes of combined continuous and interval work.

Exclusion Criteria:

- Individuals with acute or chronic diseases, such as type 1 diabetes, cardiovascular conditions, hyper/hypothyroidism, polycystic ovary syndrome, metrorrhagia, inflammatory or autoimmune disease, or with any other medical history affecting to the ability to exercise safely will be excluded from the study.
- Diagnosed eating disorders, such as anorexia nervosa, anorexia bulimia, and binge-eating disorder.
- Individuals who are pregnant or who are less than 1 year postpartum.
- Individuals who are breastfeeding and/or who are within 2 months of ending breastfeeding.
- Individuals who smoke.
- Individuals who are taking any medication that could directly interfere with the test results.

3.2 Pre-testing Measures

Once participants were deemed eligible for the study and informed consent forms were signed, they underwent a series of baseline testing measurements including bioelectrical impedance to determine body composition (InBody 770 Body Composition Analyzer, InBody Co. Ltd., Ottawa, Canada) and a maximal oxygen consumption (VO_{2peak}) test on the treadmill. The VO_{2peak} graded exercise test that was be used was adapted from the McConnell & Clark (1988) Treadmill Protocol 4. Metabolic data were collected using a metabolic cart (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah, USA). Peak oxygen consumption was determined once the athletes reached volitional exhaustion and ventilatory threshold was determined using the v-slope method. The test began with a 5-minute warm-up at a 0% grade and a self-selected pace. Following the warm-up, the participants were instructed to select a running pace that was comfortable yet challenging, somewhere between 5.5-8.5 miles per hour (mph). The speed remained the same for the remainder of the test while the incline of the treadmill was increased by 2% every 2 minute until volitional exhaustion (McConnell & Clark, 1988). In order to achieve the highest VO_{2peak} and to accommodate for participants who chose a speed that was too slow, some participants had the speed increased once during the test. While participants were

required to achieve a $VO_{2peak} \geq 45$ ml/kg/min to participate in the present study, if they did not achieve this threshold on the first attempt but were close to achieving the threshold score, they were permitted try a second attempt a few days later. We did not, however, have any participants re-attempt the VO_{2peak} test after performing their first baseline.

During the initial meeting, participants were also asked to fill out the Canadian Society for Exercise Physiology's Get Active questionnaire to ensure participants could safely exercise, and the Low Energy Availability in Females Questionnaires (LEAF-Q) to assess energy availability, menstrual history, and ensure inclusion criteria was met. LEAF-Qs have been validated for use in female endurance athletes (Witkos et al., 2023). Menstrual cycles were tracked for a minimum of 1 month prior to beginning the testing protocol.

3.3 Protocol

Continuous glucose monitors (Dexcom G6 CGM) were inserted on three separate occasions throughout the menstrual cycle. Each CGM can be worn for 10 days, which allowed BG to be monitored for the entire duration of most menstrual cycles (~30 days). All participants were asked to wear a minimum of three different CGMs individually, (sometimes more if a CGM failed before the expected 10 days), which were inserted in succession. Figure 4 provides an outline of CGM insertion days given a typical 28-day menstrual cycle model with testing in the early-FP first. Continuous glucose monitors (Dexcom G6) have previously been validated and approved for use in individuals with or without diabetes (Merino et al., 2022; Roze et al., 2021). If Dexcom G6 sensors failed, a new CGM was inserted as early as possible. Food logs were collected for 24 hours, beginning at 12:00 am and ending at 11:59 pm the day before testing, the day of testing, and the day after testing. Food logs were analyzed using the MyFitnessPal application and total caloric intake as well as macronutrient distribution were used for comparisons between menstrual cycle phases. Capillary BG measurements were taken immediately before and after the standardized exercise sessions to ensure CGM accuracy.

The training intervention included two, 60-minute treadmill sessions. The exercise sessions involved a continuous portion and an interval portion. There was a 5-minute warm-up at a comfortable, self-selected pace, followed by 35 minutes of continuous effort at 10% below individual ventilatory threshold, followed by 18 minutes of aerobic interval training, based on the general guidelines for submaximal aerobic high intensity interval training (3min on 90% VO_2peak

and 3 min off at 40% VO_2peak) x three sets (Dolci et al., 2020). The treadmill exercise protocol finished with a 2-minute cool down of walking. The treadmill speed and grade from the first session were adjusted if necessary and were replicated during the second session. Metabolic and CGM data were collected and analyzed during exercise and CGM data continued to be monitored in the 24 hours post exercise intervention.

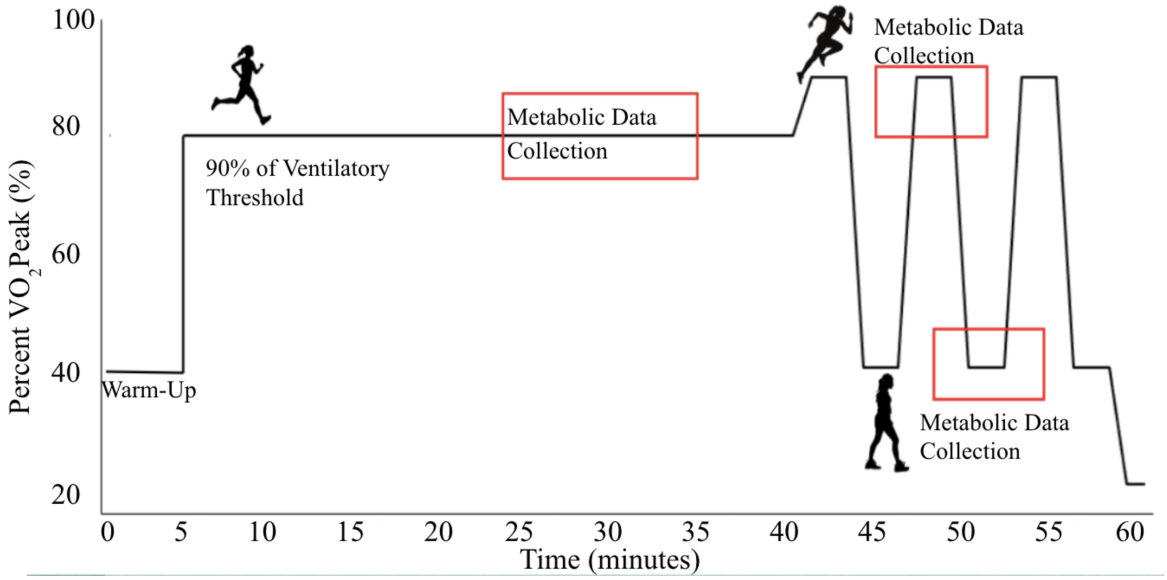

Figure 6. A graphical representation of the exercise intervention ran on a treadmill with data collection points outlined.

One exercise session occurred during phase 1 (early FP) and one during phase 4 (mid-LP). The early FP and the mid-LP present the greatest difference in hormonal milieus and were therefore used as testing phases. Phases were determined using a calendar-based counting approach, which changed slightly depending on reported length of menstrual cycle, basal body temperature, and ovulation predictor kits (Easy@Home, Easy Healthcare Corporation, Illinois, USA) used to ensure an appropriate luteinizing hormone surge during ovulation. A test to control ratio of 1 or more for the luteinizing hormone test strip is equivalent to a positive test. This particular luteinizing hormone test strip that was used for the study uses 25mIU/mL as a threshold value for attaining a positive test (test to control ratio >1.0). Participants were asked to track their menstrual cycle, luteinizing hormone status, and basal body temperature using a third-party mobile

application (app) created by Easy Healthcare Corporation entitled “Premom.” This app allowed participants to organize and view their menstrual cycle phases, ensured correct interpretation of luteinizing hormone test strips, and allowed researchers to schedule participants accordingly. Testing in phase 1 occurred between days 2-5 after the onset of menses, in order to capture the lowest endogenous hormone concentrations. Testing in phase 4 occurred 6-9 days post ovulation (confirmed by the luteinizing hormone peak) while considering that the session should be ~5-8 days before the onset of the predicted menses in a typical 28-day menstrual cycle. Participants were trained on using ovulation predictor kits and tested luteinizing hormone levels at home from ~day 8 until a luteinizing hormone surge. Basal body temperature measurements were taken using an oral thermometer from ~days 8 to days 20 to further corroborate the beginning of the LP. The highest luteinizing hormone test day was used as an indicator of ovulation for scheduling testing dates, regardless of whether or not a positive test was achieved. Figure 7 outlines a visual representation of the testing phases, phase determination methods, and CGM installation days for participants with a typical 28-day menstrual cycle. The figure was adjusted for participants with shorter and longer cycle lengths, and for testing occurring in phase 4 first.

3.4 Outcomes

Based on the glycemic targets from the American Diabetes Association, primary and secondary outcome measurements were as follows:

Primary: Time <3.9 mmol/L during the intervention and in the 24 hours after exercise.

Secondary: Time in normal BG range (3.9-8.0 mmol/L), 0-6 am mean glucose, mean glucose, time >8.0 mmol/L, change in glucose from pre to post exercise, (American Diabetes Association, 2022). Total energy expenditure, heart rate and respiratory exchange ratio during exercise were compared between sessions. In addition, total energy intake and the proportion of energy from each macronutrient was compared between the early FP and the mid-LP.

3.5 Study Design

This pilot study was a repeated measures study design with two testing sessions performed in random order (phase 1 or early FP and phase 4 or mid-LP).

3.6 Randomization

The order of testing sessions between early FP versus the mid-LP was determined by an online randomization program (Sealedenvelope™ V1.23.0) This program was used by someone who was not involved in baseline assessments.

3.7 Statistics

A paired T-test for repeated measures was used to compare the amount of time <3.9 mmol/L, 24 hour mean glucose, the amount time >8.0 mmol/L, 0-6 am mean glucose, and mean glucose during exercise between phases 1 and 4. A 2x2 factorial ANOVA was used to compare menstrual cycle phase differences (FP vs LP) in glucose pre vs post exercise. A 2x3 factorial ANOVA was used to compare to FP vs LP RER, heart rate, VO_2, and energy expenditure at three timepoints (continuous exercise, high intensity interval, and recovery interval). Similarly, FP and LP were compared for energy consumption the day before, day of, and day after each exercise sessions.

Although research on time spent outside of normoglycemia has not been studied in female endurance athletes at different phases of the menstrual cycle, based on previous studies examining resting BG levels in the FP of the cycle compared to the LP, the power to detect a similar effect size was estimated. In the study of Zderic et al, it was found that the mean difference between the FP and the LP would be 0.2 mmol/L with a standard deviation of 0.2 mmol/L, which corresponds to a large effect size (1.0) (Zderic et al., 2001). The sample population that we recruited was 11 naturally cycling participants. With an alpha value of 0.05, 10 participants would have provided power of 0.8 to detect differences between phases of the menstrual cycle. We strived to exceed this target to account for missing data that occurred with the challenges of coming to the laboratory in the required menstrual cycle windows (see below).

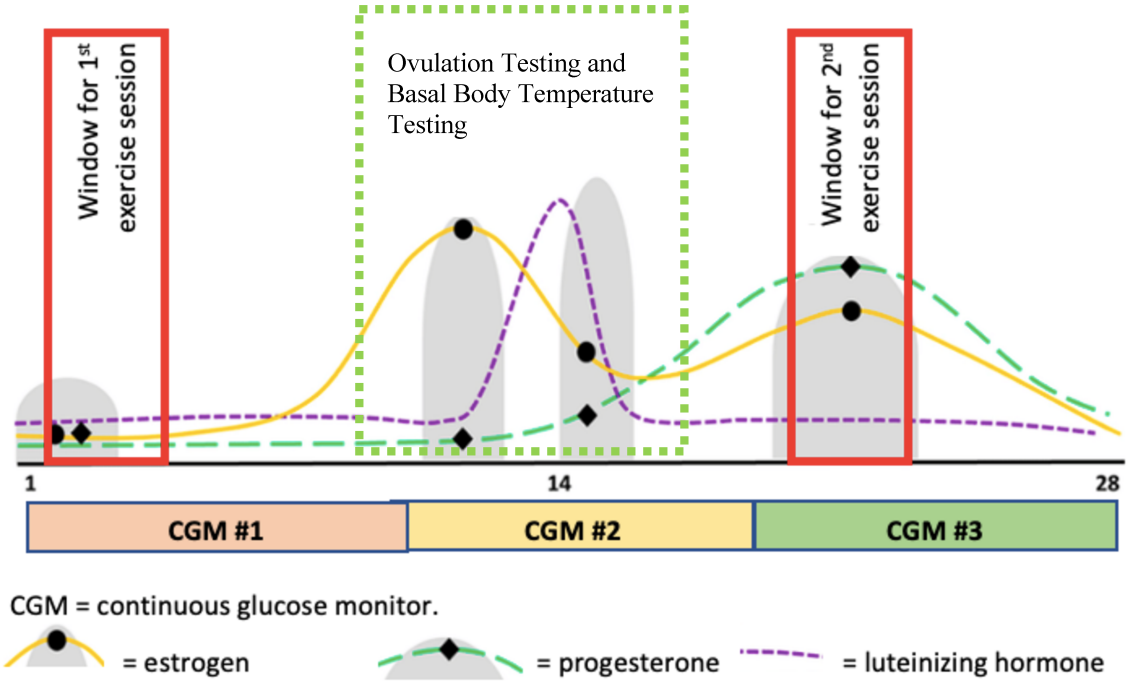

Figure 7. Summary of Testing Timeline for Participants Beginning with Early Follicular Testing. This figure has been adapted with permission from (Elliott-Sale et al., 2021) © (2021) CC BY 4.0.

Chapter 4: Results

Fourteen naturally cycling participants underwent baseline testing for the study. Of the 14 individuals who completed the baseline, 11 participants completed the study protocol. The 3 participants who were unable to continue with the study after completing the baseline were deemed ineligible based upon not reaching the inclusion criteria of achieving a VO_2 peak $\geq$ 45 ml/kg/min.

Descriptive characteristics for participants are presented in table 4. Participants had a mean age of 29 ± 8 yrs and a mean weight of 62.5 ± 10.6 kg. The mean percent body fat was 20.2 ± 5.2%. Naturally cycling participants had a mean cycle length of 28 ± 3 days. Participants had a mean luteinizing hormone surge on day 15 ± 2 after the onset of menstruation with the mean test to control ratio being 0.74 ± 0.45. The majority of participants (n=8) did not achieve a positive luteinizing hormone test according the Easy Healthcare Corporation application that was used. Basal body temperature was measured by participants however statistics were not run on change in temperature post luteinizing hormone surge because there was a lack of confidence in the accuracy of the data. Higher luteinizing hormone test to control ratios were not positively correlated with higher percent body fat for all participants (r = 0.32). However, when a potential outlier was removed, the correlation between percent body fat and luteinizing hormone test to control ratio increased to r = 0.82. No participant showed significant risk for low energy availability based on the LEAF-Q questionnaire administered (total score <8; the LEAF-Q scoring key is available in the appendix). Participants enrolled in the study were exercising a minimum of 5 hours per week, with the mean amount of exercise being 6 hours per week and the range was recorded from 5.0-13.5 hours per week. Primary activities listed were running and strength training. The VO_2 peak ranged from 44.5-62.7 ml/kg/min with a mean value of 49.1 ± 5.7 ml/kg/min. Ventilatory threshold occurred between 72.6-91.6% of VO_2 peak with a mean value of 84.8 ± 5.9%.

Table 4. *Subject Characteristics Table. Values are sample means from the baseline visit.*

	Naturally cycling participants (n = 11)
Age (yrs)	29 ± 8
BMI (kg/m^2)	22.3 ± 2.5
Weight (kg)	62.5 ± 10.6

VO_2peak (ml/kg/min)	49.1 ± 5.7
HR max (bpm)	189 ± 9
VT (ml/kg/min)	41.4 ± 3.4
VT (as % VO_2peak)	84.8 ± 5.9
% BF	20.2 ± 5.2
Mean MC length (days)	28 ± 3
Activity (hours/week)	6 [5.0 - 13.5]
LH surge day	15 ± 2
LH test to control ratio on surge day	0.74 ± 0.45

Data shown as mean ± standard deviation or median [range], BMI = body mass index, VO_2max = maximum volume of oxygen consumption, HR = heart rate, VT= ventilatory threshold, %BF = percent body fat, MC = menstrual cycle, LH = luteinizing hormone.

4.1 Standardized Exercise During Follicular and Luteal Phases.

Four participants completed their first exercise session in the FP and 7 participants completed it in the LP. Exercise sessions were completed at approximately the same time in both phases for consistency, with the exception of one participant (n=1) who completed one exercise test in the morning and one in the afternoon due to scheduling difficulties. The intensity for the exercise protocol was predetermined based on individual VO_2 peak test scores. Intensities were predetermined using the v-slope method for determining ventilatory threshold and 30 second means for VO_2 max. Two participants were unable to complete the computed intensities during their first session and therefore ran at a lower incline compared to what had originally been calculated from their VO_2 peak baseline results. The same protocol was followed for both exercise tests including any speed/incline modifications that occurred. Participants had significantly higher RER values during the mid-LP compared to the early FP (main effect of phase: $p = 0.04$). Mean metabolic data during exercise can be found in table 5.

Table 5. *Mean metabolic data during three different running intensities on a treadmill.*

N=11	FP	LP	Main effect of phase p =	Main effect of intensity p =	Phase by intensity interaction p =
RER					
Submaximal	0.94 ± 0.04	0.96 ± 0.02			
High interval	0.96 ± 0.04	0.97 ± 0.03	0.04*	0.27	0.99
Low interval	0.96 ± 0.06	0.98 ± 0.04			
VO_2 (ml/kg/min)					
Submaximal	36.8 ± 3.2	36.9 ± 2.9			
High interval	43.1 ± 3.7	43.0 ± 3.4	0.98	<0.001	0.94
Low interval	19.8 ± 3.3	19.8 ± 3.3			
HR (bpm)					
Submaximal	168 ± 12	170 ± 12			
High interval	179 ± 9	182 ± 8	0.10	<0.001	0.97
Low interval	139 ± 14	141 ± 13			
Energy Expenditure (kcal/min)					
Submaximal	11.4 ± 2.1	11.5 ± 2.1			
High interval	13.3 ± 1.5	13.4 ± 1.7	0.39	<0.001	1.00
Low interval	6.1 ± 1.3	6.2 ± 1.3			
Body weight (kg)	62.4 ± 10.6	62.5 ± 10.5	0.73	NA	NA

FP= follicular phase, LP = luteal phase, RER = respiratory exchange ratio, bpm = beats per minute. Values were averaged during a middle ten minutes of the submaximal portion and the last minute of a high intensity and low intensity interval. The submaximal intensity is 35 minutes of continuous exercise at a workload corresponding to 10% below individual ventilatory threshold. The high intensity interval is at a workload corresponding to 10% below individual VO_2 max. The low intensity interval is at a workload corresponding to 40% of individual VO_2 max. Metabolic data were collected during minutes 25-35 of the submaximal intensity exercise and during minutes 46-52 of the interval portion of exercise.

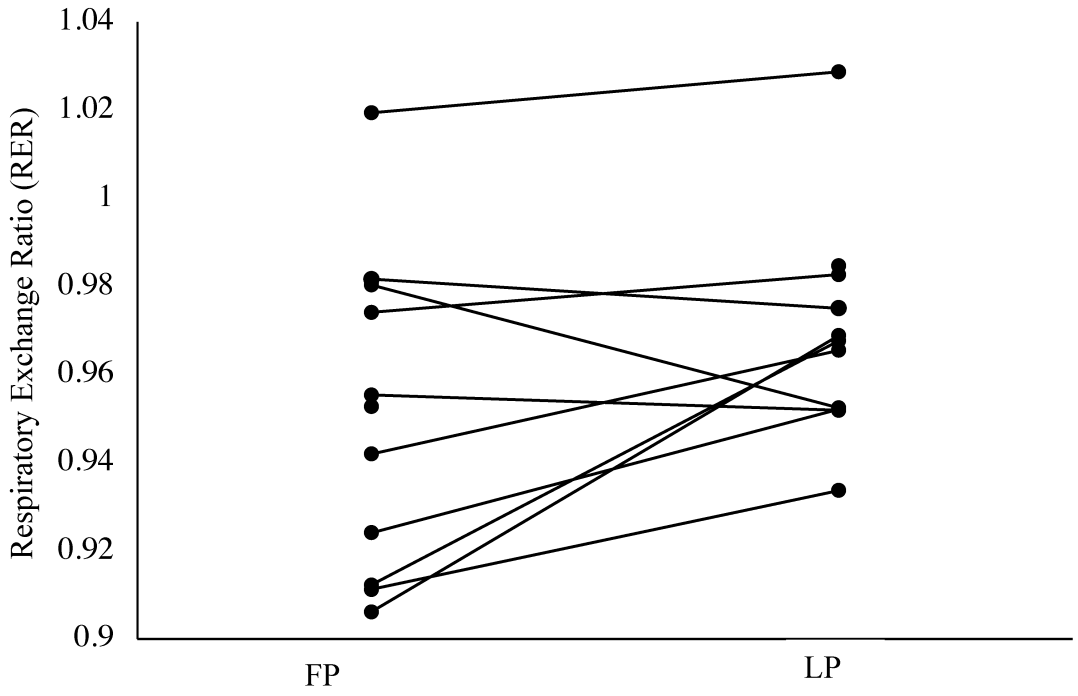

Figure 8. Individual mean exercise RER (mean of submaximal, high intensity, and low intensity intervals) during the early follicular phase (FP) and the mid-luteal (LP) of the menstrual cycle. The submaximal intensity is at a workload corresponding to 10% below individual ventilatory threshold. The high intensity interval is at a workload corresponding to 10% below individual VO_2 max. The low intensity interval is at a workload corresponding to 40% of individual VO_2 max. RER was collected during minutes 25-35 of the submaximal intensity exercise and during minutes 46-52 of the interval portion of exercise.

Capillary BG measurements and CGM glucose were taken before and after each exercise session. Neither capillary glucose nor CGM glucose had statistically significant differences between menstrual cycle phases (Capillary glucose main effect of phase: $p = 0.19$; CGM glucose main effect of phase: $p = 0.28$). Capillary glucose and CGM glucose pre and post-exercise intervention did not differ significantly (pre-exercise $p = 0.86$, post-exercise $p = 0.19$). Table 6 represents glucose data pre and post exercise intervention using two different measuring methods (capillary BG and CGM glucose). Figures 7 illustrates glucose changes pre, during, and post exercise intervention in the FP and the LP phase respectively. Mean glucose during exercise was 5.8 ± 0.9 mmol/L in the mid-LP compared to 5.5 ± 1.2 mmol/L in the early FP, no statistically significant difference was found ($p = 0.44$).

Table 6. *Pre and post exercise glucose measurements using two different methods.*

	FP	LP	Main effect of phase p =	Main effect of time p =	Phase by time interaction p =
Capillary Glucose (mmol/L) †					
Pre-exercise	5.4 ± 0.5	5.5 ± 0.5	0.19	0.38	0.5
Post-exercise	5.6 ± 1.2	6.0 ± 1.1			
CGM Glucose (mmol/L) †					
Pre-exercise	5.4 ± 0.92	5.4 ± 0.39	0.28	0.32	0.09
Post exercise	6.0 ± 1.9	6.6 ± 1.9			
Mean glucose during exercise†	5.5 ± 1.2	5.8 ± 0.9	0.44	-	-
Capillary Glucose (mmol/l) *					
Pre-exercise	5.6 ± 0.7	5.8 ± 0.5	0.28	0.80	0.88
Post-exercise	5.7 ± 1.0	5.9 ± 1.0			

CGM = continuous glucose monitor, FP = follicular phase, LP = luteal phase. Capillary blood glucose was taken for all 11 participants, however n=8 was used for the glucose analysis due to inaccurate CGM readings for three participants. *n=11, †n=8.

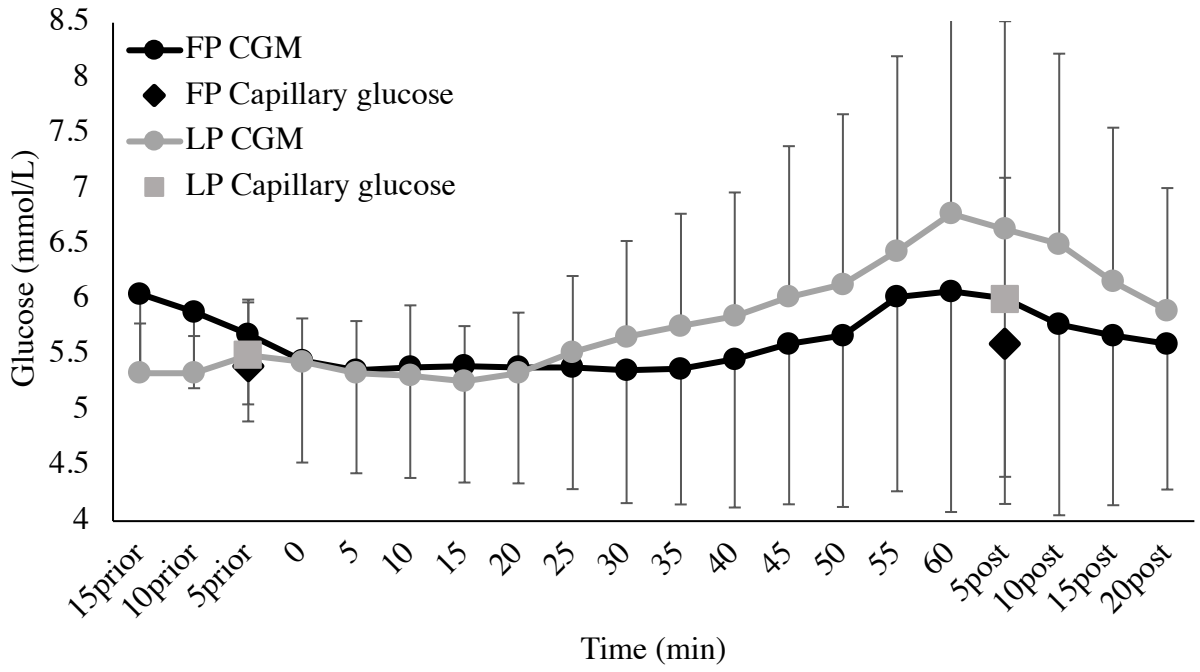

Figure 9. Continuous glucose monitor (CGM) and capillary blood glucose data 15 minutes prior, during, and 20 minutes after the exercise session in the early follicular phase and the mid-luteal phase of the menstrual cycle (n = 8). Standard error bars are shown below for the follicular phase and above for the luteal phase. P = follicular phase, LP = luteal phase

4.2 24-Hour Continuous Glucose Monitor Data

Of the 11 participants who completed the study three participants were unable to be included in the full glucose analysis due to failed sensors and inaccurate glucose readings. They were therefore excluded from the 24-hour glucose analysis represented in (table 4). No significant difference was found in 24-hour mean glucose between the early FP and mid-LP (FP 5.5 ± 0.6 mmol/L, LP 5.4 ± 0.5 mmol/L, p = 0.58). Time spent in range (3.9-8.0 mmol/L), below 3.9 mmol/L and equal to or above 8.0 mmol/L were unchanged by phase as well (p = 0.81, p = 0.82, p = 0.86 respectively). Figure 10 demonstrates individual time spent with low blood glucose between the early FP and the mid-LP of the menstrual cycle. Five out of eight participants had a greater amount of time spent below 3.9 mmol/L in the FP compared to the LP, while two out of eight participants had greater amount of time spent below 3.9 mmol/L in the LP. One participant had no time spent below 3.9 mmol/L in either phase. Nighttime glucose (0-6 am) did not change from menstrual

cycle phase (p = 0.3). Twenty-four hour glucose and exercise CGM glucose data are presented in table 7.

Table 7. *Glucose data from CGM during exercise and in the 24 hours post-exercise.*

N=8	FP	LP	p =
24-hour mean glucose (mmol/L)	5.5 ± 0.6	5.4 ± 0.5	0.58
Percent of time in range (3.9-8 mmol/L)	95 ± 7	95 ± 5	0.81
Percent of time spent <3.9 mmol/l	4 ± 6	3 ± 4	0.82
Percent of time spent >8.0 mmol/L	2 ± 4	1± 2	0.86
0-6am mean glucose (mmol/L)	5.5 ± 0.8	5.2 ± 0.7	0.30

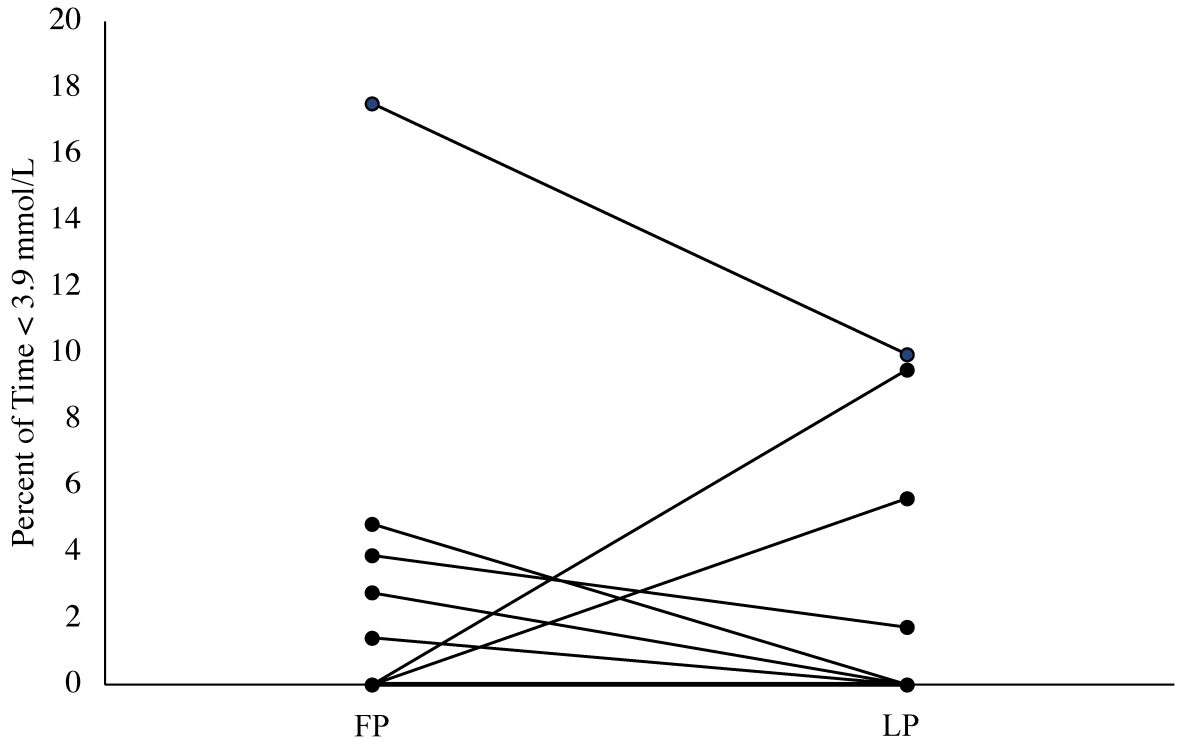

Figure 10. Individual percent of time below 3.9 mmol/L during exercise and in the 24-hour recovery period in the early follicular phase (FP) and the mid-luteal phase (LP) of the menstrual cycle (n = 8).

4.3 Energy Intake

Out of 11 participants, two participants were unable to complete the food logs accurately and were therefore excluded from the energy intake analysis (n=9). Energy intake the day of exercise was 2194 ± 708 kcal and 2472 ± 538 kcal in the FP and the LP respectively and varied widely between participants, with standard deviation ranging from 441 to 708 kcal/day across all 3 days. No statistically significant differences existed in overall caloric intake between menstrual cycle phases ($p = 0.16$). No significant difference was found in percent of total energy from carbohydrate, fat, or protein between the early FP and the mid-LP (CHO $p = 0.86$, FAT $p = 0.91$, PRO $p = 0.82$). Energy intake and percent contribution of each macronutrient can be found in table 8. Figure 11 illustrates overall energy intake in the early FP and the mid-LP the day before, of, and after exercise.

Table 8. *Energy intake and percent of energy contribution during the day prior to, the day of, and the day post exercise session.*

N=9	FP	LP	Main effect of phase p =	Main effect of day p =	Phase by day interaction p =
Energy intake (kcal)					
Day before exercise	2121 ± 579	2241 ± 571			
Day of exercise	2194 ± 708	2472 ± 538	0.16	0.25	0.70
Day after exercise	2021 ± 579	2185 ± 441			
Percent energy CHO					
Day before exercise	45.8 ± 9.2	47.9 ± 8.8			
Day of exercise	48.4 ± 9.6	46.1 ± 7.5	0.86	0.68	0.30
Day after exercise	45.6 ± 5.5	44.4 ± 8.8			
Percent energy FAT					
Day before exercise	37.1 ± 7.3	34.1 ± 7.0			
Day of exercise	35.0 ± 6.7	35.3 ± 6.4	0.91	0.75	0.27

Day after exercise	36.0 ± 8.5	37.7 ± 8.0			
Percent energy PRO					
Day before exercise	17.4 ± 5.0	17.9 ± 4.4			
Day of exercise	17.9 ± 5.8	19.5 ± 3.8	0.82	0.39	0.68
Day after exercise	19.9 ± 6.0	18.8 ± 4.0			

CHO= carbohydrate, FAT = fat, PRO = protein.

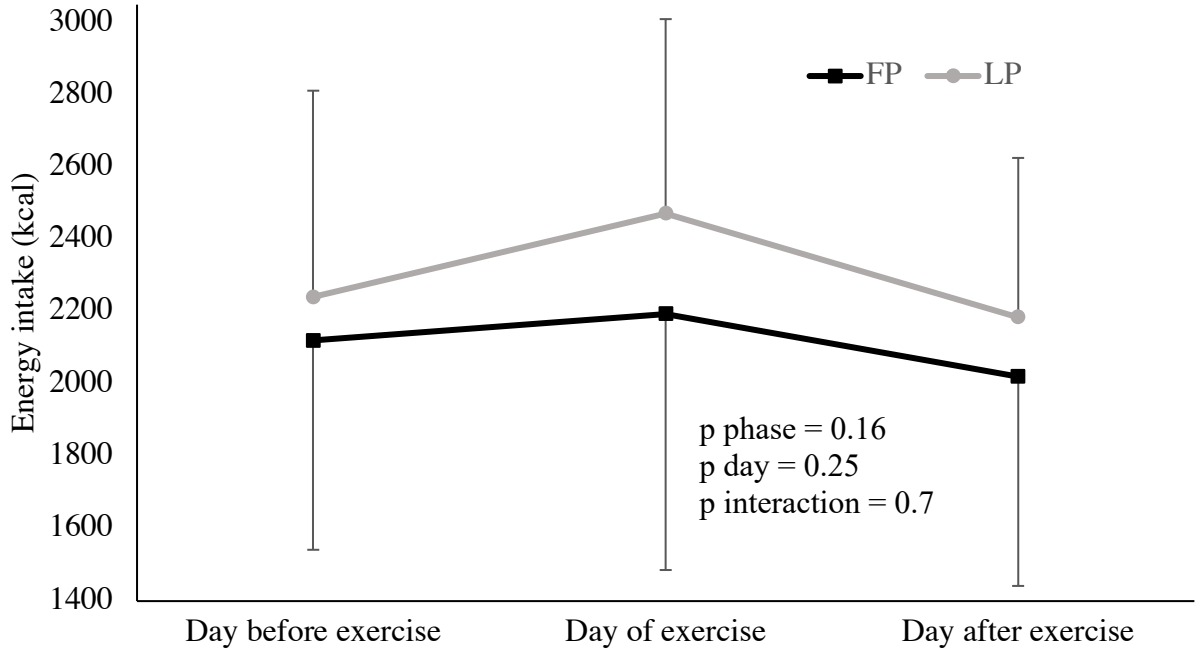

Figure 11. Energy intake the day before, of, and after exercise sessions in the early follicular (FP) and the mid-luteal (LP) phases. P values for main effect of phase, main effect of day, and main effect of interaction are provided.

Chapter 5: Discussion

5.1 Glucose Outcomes

This is the first study to use CGMs in female athletes during standardized exercise and at different phases of the menstrual cycle. Previous studies have identified higher BG during the LP compared to the FP of the menstrual cycle but with no exercise and recovery considerations for athletes (Lin et al., 2023; Zarei et al., 2013). Since athletes with high training loads may have a higher likelihood of experiencing glycemic excursions compared to healthy controls (Flockhart et al., 2021) and since low BG levels are correlated with decrements in performance (Tornberg et al., 2017), this study aimed to identify whether glucose levels changed between menstrual cycle phases during exercise and in recovery.

The primary outcome of the present study, was to identify time spent below 3.9 mmol/L during exercise and in the 24 hours of recovery during the FP and the LP of the menstrual cycle. Contrary to our hypothesis and to previous literature, the MCG study did not identify any significant difference in time spent below 3.9 mmol/L during exercise and in the 24 hours of recovery in the early FP compared to the mid-LP. This finding suggests that, on average, female athletes do not need to be concerned for experiencing low BG at these two different times throughout their menstrual cycle.

Participants spent 95% of the 24-hour time in range (between 3.9-8.0 mmol/L) in both menstrual cycle phases. This amount of time spent in range is similar to findings from Bowler et al. (2024), who found that athletes spent 96% of the time in euglycemia. Flockhart et al., (2021) found that athletes with high training loads spent closer to 89% of time per day in euglycemia and had more excursions from this normoglycemic range, however no consideration was given to timing of the menstrual cycle. Interestingly, the pool of participants in the present study are considered aerobically trained athletes, exercising a minimum of 5 hours per week, while in the study by Flockhart et al. (2021), the athletes were elite endurance athletes, competitive at a national team level. One could speculate that this difference in activity level, training loads, and training type (high intensity intervals, prolonged submaximal, etc.) could contribute to the difference in findings for the amount of time spent outside normoglycemia, in spite of both groups being aerobically fit. It is possible that a higher training volume in the Flockhart study contributed to greater glycemic excursions compared to the present study.

Bowler et al. (2024), however, used elite racewalkers and a standardized meal and exercise protocol and identified similar amounts of time in normoglycemia as the present study, however showed less time spent below 3.9 mmol/L (1.3 ± 2.4%) compared to this study (FP 4 ± 6%, LP 3 ± 4%). Contrary to Flockhart et al. (2021), perhaps the meal standardization during the Bowler et al. (2024) study prevented further excursions from normoglycemia.

A similar, less standardized, study identifying similar amounts of time spent with low BG as the present study was done on a large cohort using CGMs in a free-living scenario. Skroce et al. (2024) identified 3.4% of time spent below 3.9 mmol/L across a 24-hour period in recreationally active individuals. The free living scenario in the present study is similar to the study by Skroce and colleagues and yielded similar results, however the present study incorporated standardized exercise and diet tracking, unlike Skroce et al. (2024). Skroce et al. (2024), did however see lower overall glucose in female participants compared to male participants further demonstrating sex differences in glucose regulation despite the lack of change throughout the menstrual cycle that is seen in the present study.

Unlike previous studies using CGMs throughout the menstrual cycle and containing larger sample sizes (Lin et al., 2023; Zarei et al., 2013), the present study did not identify significant differences between menstrual cycle phases in mean glucose, time below 3.9 mmol/L, time above 8.0 mmol/L, time in range (3.9-8.0 mmol/L), nor nighttime mean glucose (0-6 am). Unlike other studies cited above, the present study did not collect serum hormone tests and could therefore be identifying different hormonal milieus when examining BG. Interestingly, in the study by Lin et al. (2023), the lowest median glucose level was achieved around day 13.6 ± 3.4 (late FP) with a value of 5.8 ± 0.7 mmol/L, while the highest median glucose level peaked around day 24.5 ± 8.0 (mid-LP) with a value of 6.1 ± 0.8 mmol/L. These results are at odds with findings of the current study, as is seen in figure 9 and tables 6 and 7, suggesting no significant difference in BG between the early FP and mid-LP.

Several differences exist in the protocol from this study compared to previous literature identifying greater mean glucose in the LP compared to the FP. The study by Lin et al. (2023), examining BG throughout the menstrual cycle used the same CGM as the present study (DexcomG6) and had a similar free-living protocol, however did not introduce standardized exercise sessions. In the Lin et al. (2023) study, participants logged daily food and step counts as well as perception of fatigue, but the researchers did not control for confounding variables.

Similarly, the present study used food logs, however standardized the exercise sessions and used CGM data for specific time frames surrounding exercise rather than an entire menstrual phase. Lin et al. (2023) accounted for glucose throughout the entire menstrual cycle as opposed to 24 hours in the FP and 24 hours in the LP.

The main outcome of this study, although not significant, focused on glucose in the context of exercise and recovery rather than overall daily glucose. One could speculate that the exercise stimulus overrides any menstrual cycle phase-based differences that could be seen in glucose at rest. This speculation has been echoed by previous review authors hazarding that exercise intensity can override changes sometimes seen in metabolism across the menstrual cycle (Oosthuyse & Bosch, 2010). One could speculate that with a higher sample size differences in glucose from the FP to the LP, as is seen in table 6 and figure 9 could become significant.

Time spent above 8.0 mmol/L did not change significantly from the FP to the LP and also was not as common of an occurrence as time spent below 3.9 mmol/L. Similar to previous research, this finding suggests that healthy individuals who are exercising regularly and do not have diabetes maintain a tight range of BG (Adams, 2013; Borghouts & Keizer, 2000). In contrast, Flockhart et al., (2023), discovered that endurance athletes exercising for 3 hours at 65% of individual VO_2 max, experienced decreased glucose tolerance and higher insulin resistance post exercise compared to controls, but did not experience time above 8.0 mmol/L. This finding could demonstrate the potential adaptation of endurance athletes to use higher amounts of fatty acids compared to glucose in recovery of endurance events (Flockhart et al., 2023).

An additional study by Flockhart et al. (2021), showed an unexpectedly high amount of time spent in hyperglycemia in elite endurance athletes compared to healthy controls during two weeks of free-living. Elite endurance athletes experienced 41 minutes per day spent in hyperglycemia compared to 22 minutes per day spent in hyperglycemia for recreationally active, healthy controls (Flockhart et al., 2021). The present study did not show increased levels of glucose post exercise nor significant amounts of time spent in hyperglycemia; this could be due in part to the difference in length of exercise sessions between studies, the difference in training status of participants, or any differences in fueling pre and post exercise. Athletes consuming high glycemic index carbohydrates after exercise will naturally experience a corresponding rise in BG post exercise.

Mean BG during exercise and in the 24 hours of recovery did not change significantly between two different menstrual cycle phases. The lack of significance in glucose differences between the FP to the LP suggest that female athletes do need to be concerned for experiencing abnormal glucose regulation at different time points of their menstrual cycle. Time spent outside normoglycemia although not high enough to warrant clinical concern, could be higher than previously thought (Borghouts & Keizer, 2000). Some studies suggest that excessive training loads (more than 152 minutes of high intensity interval training per week in one particular study) can lead to mitochondrial damage and cause disturbance in glucose metabolism and regulation as a result (Flockhart et al., 2021). Although there is some time spent outside the normoglycemic range in the present study, it does not warrant recommendations to make further training or lifestyle adaptations. The time spent outside of normoglycemia for this study is similar to the healthy controls from previous studies and demonstrates healthy glucoregulatory mechanisms in this population (Flockhart et al., 2021; Shah et al., 2019).

Previous literature has identified increased BG levels positively associated with greater food intake and cravings (Lin et al., 2023). In the present study, energy intake did not change significantly during the three days surrounding the exercise session in the early FP compared the mid-LP. This lack of difference is contrary to other articles finding significant increases in cravings and energy intake during the LP of the menstrual cycle (Chung et al., 2010; Krishnan et al., 2016), but our study may have been underpowered to detect differences.

5.2 Substrate Oxidation during Exercise

Substrate oxidation throughout the menstrual cycle is an area that has yielded very inconsistent findings. Despite several studies examining RER at different phases of the menstrual cycle in exercising female participants, a recent meta-analysis concluded that overall, no significant differences in substrate oxidation exist throughout the menstrual cycle (D'Souza et al., 2023). Interestingly, the findings of the current study are at odds with this conclusion. As demonstrated in table 5.0, mean RER is higher in the mid-luteal phase compared to the early follicular phase ($p = 0.04$). This change in RER from the FP to the LP of the menstrual cycle, suggests that female athletes may oxidize higher amounts of carbohydrate compared to fats while exercising at moderate intensities in the LP compared to the FP. This finding contrasts with much

of the previous literature suggesting no difference or a slightly higher RER in the FP compared to the LP (Rael et al., 2021; Zderic et al., 2001).

When comparing the protocol from the MCG study to others, reporting no difference in substrate oxidation throughout the menstrual cycle, there are a few key differences to keep in mind. First, the present study is a free-living scenario and does not control or standardize dietary intake. As a result, if participants are inadvertently consuming higher amounts of carbohydrate particularly prior to the exercise session, RER will likely exhibit a corresponding increase. As a recent modelling analysis demonstrates, a diet high in carbohydrate and lower in fat can increase RER regardless of phase of cycle (Rothschild et al., 2022). Several studies showing no change or the opposite effect of the MCG study either standardized diet between the two exercise testing sessions or used primed constant glucose infusions in order to control for dietary confounders (Dokumaci & Hazir, 2019; Hackney et al., 1991; Oosthuyse et al., 2003; Redman et al., 2003; Vaiksaar et al., 2011b; Zderic et al., 2001).

Although it is impossible to pinpoint the exact reason why RER is higher in the mid-LP compared to the early FP as a result of not standardizing dietary intake, part of the motivation for avoiding controlling food intake was because females tend to increase their total caloric intake and specifically carbohydrate rich foods during the LP of the menstrual cycle (Krishnan et al., 2016). In order to account for a real-life scenario in which females could be consuming more simple carbohydrates during the LP compared to the early FP, dietary intake was not controlled for, however it was tracked.

Total caloric intake did not change significantly between the early FP compared to the mid-LP. Similarly, the percent of energy contribution from carbohydrate also did not increase from the FP to the LP. Once again, this is opposite of several findings demonstrating increases in total caloric intake and specifically carbohydrate rich food sources in a cyclic matter according to the menstrual cycle (Gorczyca et al., 2016; Krishnan et al., 2016). The variability in caloric intake between participants was quite large, with some participants consuming less than 1000 total calories in a day, while others consumed over 3000 calories in a day. As a result, the standard deviation for total caloric intake is quite large and could be contributing to the lack of significance. This discrepancy in caloric intake between participants could also demonstrate potential errors in reporting, specifically errors in underreporting food intake, as less than 1000 calories per day is unsustainable for athletes.

Previous studies and review articles also demonstrate that food logs are known to be imprecise (Kipnis et al., 2003; Livingstone et al., 1990; Ravelli & Schoeller, 2020; Weber et al., 2001). The percent contribution of carbohydrate, protein, and fat were within the acceptable macronutrient distribution ranges of 45-65% carbohydrate, 10-35% protein, and 20-35% fat, with the exception of fat being up to approximately 3% higher at two different time points (day before exercise in the FP and day after exercise in the LP). Percent contribution of energy from carbohydrate, protein, and fat did not change significantly across phases or days surrounding exercise. Body weight also did not change significantly between menstrual cycle phases. Regardless of the lack of significance in food intake, the lack of difference in body weight, and previous literature on substrate oxidation throughout the menstrual cycle, curiously, RER remains significantly higher in the LP compared to the FP.

Menstrual cycle testing days are another important factor of differentiation from previous studies on RER during exercise to consider. The present study identified higher RER values during the mid-LP, which was identified as the 6-9 days post luteinizing hormone surge and between day 19-24 of the menstrual cycle, compared to the early FP which was identified as day 2-5 of the menstrual cycle. Previous literature uses slightly different time frames for testing, especially when testing in the FP (Dokumaci & Hazir, 2019). Most studies in a recent review on RER throughout the menstrual cycle used the mid-FP (days 6-10) as testing in the low hormone phase of the menstrual cycle (D'Souza et al., 2023). Although the previous studies confirmed lower estrogen and progesterone levels compared to their LP testing session via serum analysis, estrogen could have been slightly elevated compared to a couple days earlier during the early FP (Hackney et al., 1991; Vaiksaar et al., 2011b). Although it is unlikely to cause a great change, one could speculate that using slightly different testing days plays a role in the inconsistency of results.

Finally, the present study does not use serum hormone analysis, therefore it is impossible to know for sure if the participants had regularly fluctuating ovarian hormone levels in all phases of the menstrual cycle. The participants in the present study were naturally cycling, whereas participants in previous studies were eumenorrheic (confirmed hormone levels with blood tests). Since the majority of the participants did not experience a positive luteinizing hormone test, it follows that some could have experienced anovulation or luteal phase deficiency as a result. Consequentially, both anovulation and luteal phase deficiency could impact hormone levels and our results. Table 9 presents a variety of study protocols yielding either no significant differences

in RER throughout the menstrual cycle or opposite findings of the MCG study to demonstrate the discrepancy in protocols and potential reasons for obtaining conflicting findings. The six studies presented in table 9 are taken from the recent meta-analysis by D'Souza et al. (2023), and were selected because their results exhibited the greatest change in glucose from the LP to the FP.

Interestingly, RER increased with higher exercise intensity from submaximal to interval running, it did not, however, decrease during the low intensity interval as one might have expected. One can speculate that the lack of change in RER during the interval portion of exercise was due to the short length of intervals and inability to reach steady state. As a result, when looking at the p value for the main effect of intensity on RER during exercise, it is much higher than would typically be expected based on previous research ($p = 0.27$) (Ramos-Jiménez et al., 2008; Rothschild et al., 2022).

Table 9. Comparisons of the current MCG study to other studies done on substrate oxidation during exercise.

Study	**Participants**	**Exercise**	**Finding**	**RER-FP**	**RER-LP**	**Food**
MCG (present study)	11 Naturally Cycling Female athletes, no confirmed ovarian hormone blood test	Running @90% of VT and intervals @90% of VO_2 peak	Significant difference between phases	0.94 ± 0.04 0.96 ± 0.02 0.96 ± 0.04 Testing window: Days 2-5	0.97 ± 0.03 0.96 ± 0.06 0.98 ± 0.04 Testing window: Days 19-24	Not controlled. Food logs used.
Higher RER in the LP compared to the FP						
(Oosthuyse et al., 2003)	8 Eumenorrheic females with confirmed ovarian hormone blood test	Running at 60% VO_2 max	No significant difference between phases	0.83 Testing window: Days 2-7	0.87 Testing window: Days 4-10 post ovulation	Asked to eat the same thing 48 hrs before exercise and standardized meal 3 hours before the exercise

(Dokumaci & Hazir, 2019)	11 Eumenorrheic female athletes with confirmed ovarian hormone blood test	Running @ 95% of lactate threshold	No significant difference between phases	0.96 Testing window: Days 7-9	0.99 Testing window: Days 21-23	Standardized breakfast 3 hrs before exercise
(Vaiksaar et al., 2011b)	11 Recreational and competitive eumenorrheic rowers with confirmed ovarian hormone blood test	Rowing at 70% of VO_{2max}	No significant difference between phases	0.88 Testing window: Days 8 ± 3	0.91 Testing window: Days 20 ± 2	Participants are asked to eat the same thing 24 hrs before exercise
Higher RER in the FP compared to the LP						
(Hackney et al., 1991)	6 Eumenorrheic females with confirmed ovarian hormone blood test	Cycled at 60 minutes of submaximal graded exercise	No significant differences however approaching (P = 0.07).	0.94 Testing window: Days 7-8	0.89 Testing window: Days 22-23	Participants asked to replicate diets 36 hrs before exercise
(Redman et al., 2003)	14 Eumenorrheic sedentary females with confirmed ovarian hormone blood test	Cycled at 75% VO_2 peak and at VO_2 peak	*Significant difference in RER (p=0.01) at VO2 peak	0.92 Testing window: Days 5-7	0.83 Testing window: Days 21-23	Recorded dietary intake and asked to replicate
(Zderic et al., 2001)	6 Eumenorrheic moderately active females. Only serum	Cycled at 90% VO_2 max	*Significant difference in RER at 90% VO2 peak	0.87 Testing window: Days 4-6	0.84 Testing window: Days 22-27	10 hrs post fast, participants had a constant glucose infusion

	estrogen confirmed					through a catheter during exercise

RER = respiratory exchange ratio, hrs = hours, VO_2 = volume of oxygen consumption.

5.3 Heart Rate

Mean heart rate during exercise did not change significantly from the FP to the LP ($p = 0.1$). Many previous studies have demonstrated increased pre-exercise mean heart rate, mean peak heart rate during exercise, and mean heart rate during exercise in the LP compared to the FP (Bandyopadhyay & Dalui, 2012; Barba-Moreno et al., 2022; Godbole et al., 2016). One study suggests greater sympathetic activity occurring in the LP, leading to an increase in heart rate at rest compared to the FP (Brar et al., 2015). Speculations as to why mean heart rate did not increase significantly during exercise in the LP in the present study could be due to the large variability in heart rate measures taken, along with a smaller sample size used. It is also important to note that some studies have not found significant differences in heart rate during exercise throughout the menstrual cycle (Gordon et al., 2018; Vaiksaar et al., 2011a). One study in particular looking at female rowers performing incremental tests to exhaustion on a rowing ergometer did not find a significant difference in heart rate between the FP and the LP during the exercise test (Vaiksaar et al., 2011a).

5.4 Energy Expenditure

Energy expenditure did not change significantly during exercise between the early FP and the mid-LP in the present study. This finding is similar to previous literature reporting no significant changes in energy expenditure during 90 minutes of exercise across the menstrual cycle (Oosthuyse et al., 2003). Similarly, another study involving recreational and competitive rowers, rowing at 70% of individual VO_2 max found no significant difference in energy expenditure between the FP and the LP (Vaiksaar et al., 2011b). These findings are further supported by other articles examining energy expenditure during exercise and at rest in the FP and the LP (Beidleman et al., 2002; Bisdee et al., 1989; Rael et al., 2021).

Contrary to the findings of the MCG study, other studies suggest an increase in energy expenditure both at rest and during exercise in the LP compared to the FP. One such study looking at energy expenditure and metabolic rate during 15 minutes of exercise at 122 watts of cycling in

the FP and the LP, found that metabolic rate increased by 5.6% in the LP (Hessemer & Bruck, 1985). Additionally Matsuo et al. (1999) found excess post exercise oxygen consumption to be higher after cycling at 60% of individual VO_2 max in the LP compared to the FP (Matsuo et al., 1999). Furthermore, other articles have found a significant increase in energy expenditure for sedentary women at rest in the LP. Using whole body calorimetry and standardized meals, it was concluded that there was a 9% increase in energy expenditure post ovulation in female participants (Webb, 1986). More recently, a study examining energy expenditure at rest during different phases of the menstrual cycle, found that there was a significant increase in mean resting metabolic rate in the LP compared to the FP in healthy women (Malo-Vintimilla et al., 2024).

As is the case with many physiological variables studied across the menstrual cycle, the topic of energy expenditure has provided conflicting results. Although the present study did not find any significant differences in mean energy expenditure during exercise in the FP compared to the LP, several studies mentioned above have found different findings. Once again, this lack of homogeneity in results demonstrates the individuality of responses to ovarian hormones in females.

5.5 Limitations

Limitations to this study include a small sample size which may have limited the ability to detect meaningful differences in BG levels across the menstrual cycle, including the prevalence of low BG during exercise and in recovery in the FP and the LP. Based on previous studies examining BG during exercise at two different phases of the menstrual cycle, the MCG study was powered to detect 80% of change of the main effect of phase on time spent below 3.9 mmol/L, with an alpha value of 0.05. The effect size of the MCG study was 0.1 corresponding to a small effect size. The sample size for the exercise sessions of the current study was n=11, however with the full 24-hour CGM analysis, the sample size decreased to n=8 due to inaccurate CGM readings, thus the power decreased as a result. A small sample size fails to detect minute changes in glucose from the FP to the LP. Glucose changes in people without diabetes are likely to be small in nature due to the body's healthy glucoregulatory responses, therefore small sample sizes may miss variations in BG. Glucose values were quite similar between menstrual cycle phases and it seems likely that there would not be any meaningful change between the FP and the LP regardless of sample size. Due to the free living conditions of the MCG study, the results are generalizable to most female athletes.

Another limitation that exists in this study is the use of CGMs in people without diabetes. Continuous glucose monitors were originally designed for people with diabetes and as a result can respond differently for people without diabetes (Shah et al., 2023). While CGMs have been validated for accuracy in people with diabetes, there is less known about their accuracy in people without diabetes (Shah et al., 2018). The Dexcom G6 CGM is clinically accurate for people with diabetes and lists up to 20% error on both sides of the CGM value given (Shah et al., 2018). The mean absolute relative difference for the Dexcom G6 CGM was found to be 9.3% and was maintained throughout the 10 days of use in people with diabetes (Shah et al., 2018). This is comparable to several different brands of capillary BG tests having a mean absolute relative difference between 5-10% (Cappon et al., 2019; Freckmann et al., 2015). The present study did not find a significant difference in CGM values compared to capillary BG values pre or post-exercise.

With the growing popularity of CGM use in people without diabetes, a recent study looked to evaluate the accuracy of CGM use in populations without diabetes compared to previous studies on CGM accuracy in people with diabetes (Shah et al., 2023). The study compared the predicted hemoglobin A1c value from CGMs to a serum analysis of hemoglobin A1c. Out of 153 participants, 71% had CGM predicted hemoglobin A1c values greater than 0.4% difference from the laboratory hemoglobin A1c values, compared to people with diabetes where only 39% showed a disparity greater than 0.4% between the two metrics. Furthermore, the CGM predicted hemoglobin A1c was 0.59% higher than the hemoglobin A1c in people without diabetes (Shah et al., 2023). These findings suggest that although CGMs can be used in populations without diabetes, the values may not be as accurate as in those with diabetes.

When looking at CGM accuracy during exercise in people without diabetes, one study performed on 10 sub-elite athletes doing fasted exercise, found that CGMs were highly accurate during steady state exercise however once a bolus of glucose was consumed at the 30th and 90th minute of exercise, CGM accuracy differed from BG draws (Thomas, 2017). This finding reflects the notion that CGMs may contain more error post-prandially or when glucose changes rapidly, such as can be the case during exercise. In the case of exercise, BG will likely see faster changes than interstitial glucose due to the time lag that exists (Schmelzeisen-Redeker et al., 2015). Some other sources of error that may exist in CGM use in populations with or without diabetes include displacement of the sensor due to high activity, high levels of sweat, prolonged pressure on the

sensor (sleeping on the sensor), and changes in interstitial volume and other bodily fluids (blood flow, lymphatic fluid) (Bowler et al., 2022; Moser et al., 2018).

Interestingly, the present study identified several participants who experienced inaccurate CGM readings during at least one time point throughout their menstrual cycle. The population for the MCG study were mostly lean individuals with low percent body fat, raising the issue on whether body composition, or less fat mass can impact CGM results and accuracy. Despite previous studies indicating no correlation between body composition and CGM result accuracy, the number of CGM inaccuracies and failures in the present study make one speculate otherwise (Abraham et al., 2023; Ling et al., 2024). One study did demonstrate an underestimation of plasma glucose associated with overweight or obese youths without diabetes (Ghane et al., 2019); however, there is little known surrounding low percent body fat and CGM accuracy.

Furthermore, other studies, including a comprehensive review on CGM accuracy during exercise, suggest that CGM accuracy decreases during exercise, with an increase in mean absolute relative difference during exercise compared to rest (Da Prato et al., 2022; Moser et al., 2018; Munoz Fabra et al., 2021). Since the present study revolved around exercise, it is important to note that CGM accuracy may be impacted at this time. A review article on the physiological changes that occur during exercise and the corresponding technological issues with CGM prediction values, suggests that changes in interstitial fluid volume can be impacted by factors such as changes in blood and lymphatic fluid flow, changes in sweat rate, and muscular contraction, all of which occur during exercise (Moser et al., 2018). The change in interstitial fluid volume during exercise can alter glucose concentrations in interstitial fluid and as a result, cause changes to predicted BG readings from CGMs (Moser et al., 2018). The rate of glucose uptake into cells and of endogenous glucose production during exercise can also impact interstitial glucose levels. The amount of time it takes for glucose to enter interstitial fluid is impacted by exercise type and intensity (Moser et al., 2018). Although the accuracy of CGM technology during exercise has greatly increased over the past few years, authors continue to recommend awareness for potential sources of error during exercise (Da Prato et al., 2022; Moser et al., 2018).

Food logs also pose a limitation to the strength of the present study. Food logs involve high participant compliance and knowledge. Two participants could not recall food intake with adequate accuracy, but most participants (n=9) in this study were able to complete the foods logs, however, without standardized meals, it is impossible to determine whether the food logs provided

were 100% accurate. As such, mistakes in energy intake throughout the menstrual cycle may be present.

This study also incorporated a free-living protocol. This protocol means that hydration status, sleep, stress levels, additional exercise, and other activities were not monitored or controlled for during the study. While a free-living protocol emulates real-life, applicable scenarios, it also provides several confounding variables that could skew results in one way or another. For example, it might be tempting to standardize food intake over the 24-hour period, but if energy intakes does actually change throughout the menstrual cycle, such standardization may affect real-world differences.

Menstrual cycle research often leads to conflicting results and is difficult to standardize. Although authors suggest not excluding females from sports research, it is not always feasible to perform blood hormone analysis when doing menstrual cycle research. As a result, clear language is important to distinguish characteristics of participants. This study used the term "naturally cycling" for participants who did not have confirmed serum hormone analysis however did experience an luteinizing hormone surge. Interestingly, the majority of the participants in this study, although they experienced an luteinizing hormone surge, did not experience a positive luteinizing hormone test. Females can experience varying amplitudes and configurations of luteinizing hormone surges that can still result in ovulation despite not achieving a positive luteinizing hormone test (Park et al., 2007). However, it is also known that a positive luteinizing hormone test is more commonly associated with ovulation compared to a negative luteinizing hormone test (Leiva et al., 2017).

As demonstrated by previous studies, female athletes are at an increased risk of experiencing low energy availability and severe and subtle menstrual disturbances compared to healthy controls (De Souza et al., 2010). Subtle menstrual disturbances can include anovulation and luteal phase deficiency which can result from a lack of a sufficient luteinizing hormone surge. Percent body fat was not positively correlated to a higher luteinizing hormone test to control ratio when all participants were included in the analysis. When an outlier with the highest percent body fat but a low luteinizing hormone test to control ratio was excluded, the correlation increased to 0.82, demonstrating the potential for percent body fat to impact luteinizing hormone levels. Eight out of eleven participants did not experience a positive ovulation test and thus could be experiencing subtle menstrual irregularities. These subtle menstrual irregularities could result in

lower ovarian hormone concentrations at specific time points throughout the menstrual cycle which could then skew the results. Since the comparison in all outcomes is being done in the early FP (low estrogen and progesterone) and the mid-LP (supposedly higher estrogen and progesterone), a lower concentration of estrogen and progesterone in the LP could minimize the impact that ovarian hormones have on BG. Furthermore, some studies and reviews have suggested that the ratio between estrogen and progesterone may be important in predicting certain outcomes throughout the menstrual cycle, for which this study does not account (D'Eon et al., 2002; Oosthuyse et al., 2023). Without serum hormone analysis, it is impossible to say whether the female participants in this study experienced typical hormone levels throughout their cycle or not. Future studies may wish to confirm serum hormone analysis when comparing glucose outcomes in the FP and the LP.

Future studies should attempt to focus on a larger sample size with eumenorrheic female athletes. Stratifying groups based on activity level and training loads may also be of interest. Based on data from previous studies, increased training loads may impact glucose regulation, therefore identifying high loads of high-intensity training in eumenorrheic female athletes could be of interest (Flockhart et al., 2021). Furthermore, amenorrheic female athletes have lower resting BG levels compared to eumenorrheic athletes and warrant further research (Tornberg et al., 2017). Athletes in general can experience chronic and/or acute periods of high and low energy availability and changes in BG as a result (Loucks & Thuma, 2003). Therefore, looking at changes in BG in athletes may be helpful in developing nutritional strategies and advice specific for athletes.

Conclusion

Recently, monitoring glucose in athletic populations has gained popularity as a way of optimizing nutrition and health (Nutrisense, n.d.; Ultrahuman, n.d.). Although certain platforms market CGM use towards populations without diabetes, the practical utility and accuracy of these devices is still uncertain. Some studies using CGMs in athletes have found higher excursions from normoglycemia with higher training loads (Flockhart et al., 2021), while others have found similar time spent outside normoglycemia as healthy controls (Shah et al., 2019). Females specifically, contend with various cyclical changes throughout the menstrual cycle. Changing ovarian hormone levels impact several biological systems including glucose regulation (Dey et al., 2019; Lin et al., 2023). A few studies have noted higher BG levels during the LP compared to the FP in healthy

female participants (Dey et al., 2019; Lin et al., 2023; Zarei et al., 2013) demonstrating the importance of recognizing sex-specific differences that could exist in health and research contexts.

The aim of this study was to identify whether differences exist in glucose levels in female athletes in the FP compared to the LP during exercise and in recovery, particularly the prevalence of low BG between phases. The present study did not find significant differences in glucose levels throughout the menstrual cycle during exercise and in recovery in female athletes. Time spent with low BG during exercise and in the 24-hour recovery window was minimal in both menstrual cycle phases, with the percent of time below 3.9 mmol/L being only 4% and 3% in the FP and the LP respectively. Exercise glucose also did not change significantly between menstrual cycle phases despite observed changes in RER, with mean glucose values of 5.5 and 5.8 mmol/L in the FP and the LP respectively. The results from this study are contrary to other findings, suggesting higher mean BG at rest in the LP compared to the FP. This raises the question on whether exercise stimulus can override potential changes that have previously been seen in BG throughout the menstrual cycle or if responses to ovarian hormones are simply variable between individuals. This contrary finding further corroborates the notion of taking an individual approach to health recommendations. Overall, female athletes did not experience significant changes in BG outcomes throughout the menstrual cycle during exercise and in recovery and do not need to have an increased concern of experiencing more time in hypoglycemia during the FP compared to the LP.

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Appendix

Informed Consent Form

UNIVERSITY OF ALBERTA

Information Sheet and Consent Form

Title of Research Study. Effects of the Menstrual Cycle (or its absence) on Blood Glucose During Exercise and in Recovery in Aerobically Fit Athletes.

Principal Investigator. Normand Boulé, PhD (780-492-4695).
Research Coordinator. Janai Martens, MSc student (780-492-8079).

Why am I being asked to take part in this research study?

You are being asked to take part in a research study on the effects of the menstrual cycle on blood glucose. Endurance athletes are being recruited to study exercise and recovery. Blood glucose levels impact overall health and performance. The menstrual cycle can impact which fuels you use for energy. We don't know if changes throughout the cycle affect the potential of experiencing low blood glucose (hypoglycemia) during and after exercise.

This form contains information about the study. Before you read it, a member of the study team will explain the study to you in detail. You are free to ask questions if there is anything you do not understand. You will be given a copy of this form for your records.

What is the reason for doing the study?

This study is looking at whether the menstrual cycle affects low blood glucose during and after endurance exercise. For athletes with a regular menstrual cycle, we will look at blood glucose during and after exercise on two different days. Once, 2-5 days after the start of your period and the other about a week before your period. The first is known as the follicular phase and the second as the luteal phase of your cycle. If you are using oral contraceptives or do not have a regular menstrual cycle, the timing of the sessions will be adapted.

What will happen in the study?

The total time commitment for the duration of the study is expected to be 1-2 months. During this time, we expect you to come to the University of Alberta ~3 times, for a total of about 5 hours.

Step 1. Determining if you are eligible.

Initial Meeting. You will come to the Physical Activity and Diabetes Laboratory (**PADL**) on University of Alberta main campus. We will address any questions/concerns you may have and ask you to fill out this consent form. You will also fill out questionnaires to check your eligibility. These include information about your health, your ability to exercise, and your menstrual cycle history. Height, weight, body composition, fitness test, and blood pressure will also be measured. This initial meeting will take ~1.5 hours.

- **Determining the Menstrual Cycle.** We will ask you to fill out a menstrual cycle history questionnaire. If you have a regular menstrual cycle and are not using hormonal contraceptives, we will also provide you with a digital oral thermometer and an ovulation kit. You will monitor your body temperature and urine test strip until you are scheduled for the second testing session. We will ask you to track your menstrual cycle, body temperature, as well as your luteinizing hormone (LH) using a urine test strip. Optionally, you can use a LH mobile application (app) called "Premom" (www.premom.com). This app will allow you to view your menstrual cycle phases and help you interpret the urine test strips. This will allow us to assess when you ovulate, and to schedule your testing sessions accordingly. For those using hormonal contraceptives, we will use the active/non-active pills in your pack to determine your testing dates. If you do not have a menstrual cycle, you will be tested once, scheduled at your convenience.
- **Baseline Exercise Fitness Test.** If you are eligible for the study, we will have you perform an exercise test. This test will involve running on the treadmill with a gradual increase incline until you are going as hard as you can. You will likely perform ~8-12 minutes of exercise total. The visit may take ~1.5 hours with explanation, questionnaires, preparation, and some pre- and post-exercise measures.
- **Continuous Glucose Monitor (CGM).** Prior to leaving the lab, we will insert a sensor for a continuous glucose monitor (CGM). A CGM is a small device (~ 2 x 3 cm) that measures your blood glucose every 15 minutes for up to 10 days. A small and flexible filament connected to this sensor (<1 cm long, <0.1 mm wide) will be inserted under the skin of your upper arm. The CGM will then be attached to the sensor. Tape will be placed over the CGM to hold it in place. Inserting the sensor sometimes causes minor bleeding and there is a small risk of infection. The risk of infection will be minimized with proper procedures including disinfection of the area by alcohol swabs. The CGM insertion will take no more than 5 minutes. The CGM is normally inserted in the back of the arm, but other options are available. It will be replaced on day 10 and day 20 of your cycle.

Step 2. Test days.

We will ask you to take part in two identical testing sessions. These sessions involve a total of 60 minutes of treadmill exercise. The order of the testing sessions (follicular phase vs. luteal phase) will be decided based on the toss of a coin and scheduled accordingly.

- **Exercise testing session.** For these sessions we will ask you to run on a treadmill for a total of 60 minutes. This includes 5 minutes of warm-up, 35 minutes of continuous running at a moderate intensity. This will be followed by 18 minutes of interval running (3x3 minute intervals at 90% of the intensity reached in your fitness test). Finally, you will finish with a and a 2-minute cool-down. You will wear a heart rate monitor and a CGM throughout exercise. We will collect the air that you expire before, during, and after exercise (each measurement lasting for 5-10 minutes). We will collect a drop of blood from your fingertip before and after the exercise session. We will measure glucose from this drop of blood and compare it to the CGM. This procedure will be followed during both sessions.

- **After leaving the lab**. On the day before, day of, and day after the first exercise test, you will be asked to record in detail your food intake. We will have you repeat the same pattern as closely as possible for the second test. We will request that you avoid exercise and alcohol intake during the testing days.

What are the risks and discomforts?

It is possible that you experience light headedness, muscle cramps, fatigue, nausea, joint pain, and in rare cases, heart attack from participating in exercise. Personnel certified in CPR will supervise the exercise sessions to minimize the risks. However, you are free to stop exercising if you feel any discomfort or do not wish to continue exercising.

The CGM sensor inserted in your arm and the fingertip blood sample may cause bruising and poses a small risk of infection. The risk will be minimized through alcohol cleaning of the insertion area. Adhesive tape that covers the CGM may also cause minor irritation.

There is no agreement between the "Premom" app and the University of Alberta. Premom will have access to you LH data and may send you advertising. We do not know what Premom does with the LH data or how long they keep it. We suggest you do not enter your real name and date of birth.

Travel to the lab, and time spent in the lab with the research team may also be associated with an increased risk of Covid-19 transmission. During your time in the lab, physical distancing measures will be observed where possible. Hand sanitizer will be available for both personnel and participants.

What are the benefits to me?

We hope that this study will help us better understand the effect of the menstrual cycle (or its absence) on blood glucose levels. We will provide you with data on your current level of physical fitness and your CGM results. You are not otherwise expected to get benefits from partaking in this research study.

What happens if I am injured because of this research?

If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form, you are not giving up any of your legal rights or releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

Do I have to take part in the study?

This section should stress the voluntary nature of the study. Being in this study is your choice. If you decide to be in the study, you can change your mind and stop being in the study at any time, and it will in no way affect the care or treatment that you are entitled to.

Can my participation in the study end early?
Your participation is completely voluntary. You are free to withdraw from the study at any time. You can withdraw from the study by contacting the principal investigator listed on the first page. Also, if you decide not to show up and we lose contact with you, we will consider that you have withdrawn from this research study.

What will it cost me to participate?

We will provide all the CGM and menstrual cycle tracking equipment required for the study. Due to a very limited budget, we are unable to reimburse parking costs for this study.

Privacy and Confidentiality

During this study we will be collecting information about you. We will use the data to help answer research questions and we will share your information with others such as the study sponsor and other researchers.

Below we describe in more detail how your data will be collected, stored, used and disclosed.

What data will we be collecting?

During this study we will be collecting data about you. Examples of the types of data we may collect includes your name, where you live, your ethnic background, your date of birth, your age, your health conditions, your health history, your medications and results of tests or procedures that you may have had. We will only look for and collect the information that we need do the research. We will get this information by asking you questions and doing the tests outlined in this form.

How will the study data be stored?

The study data we collect which will include your name will be securely stored by the study investigators during and after the study. We will also put a copy of this consent form in your record. In Canada, the law says we have to keep the study data stored for at least 15 years after the end of the study. The study investigators will not release your name to anyone unless the law says that they have to.

The "Premom" app that helps us interpret the ovulation test strips is operated by Easy Healthcare Corporation. Data submitted to this app is housed outside of Canada and is not under our control. We encourage you not to enter your real name and date of birth in the app. It is also acceptable if you prefer not to use the app.

How will the study data be used?

Your study data will be coded (with a number) so that it no longer contains your name, address or anything else that could identify you. Only your study investigator will be able to link your coded study data to you.

This coded study data may also be shared with people who work with the Sponsor and with regulatory authorities. The Sponsor and/or the people they work with may be located outside of Canada, in countries that do not have the same privacy laws as in Canada. However, because nothing that is sent to the Sponsor will contain your name, no one who uses this information in the future will be able to know it came from you. The risk to your privacy, then, should be very small.

When the study is done, the Sponsor may place your coded study data into a secure database. The coded data may then be used to answer other research questions in the future. Only researchers who have the training and experience to do the research (also known as "qualified researchers") will be allowed to use the data. Data will be anonymized (i.e., no way to link back to individual ever) but will be kept indefinably for future use.

Who will be able to look at my health data?

During research studies it is important that the data we get is accurate. Therefore, your study data and original medical records may also be looked at by people from the University of Alberta auditors and members of the Research Ethics Board.

By signing this consent form, you are saying it is ok for the study staff to collect, use and disclose information from your records and your study data as described above.

If you would like to see the study data collected about you, please ask the study investigator. You will be able to look at the study data about you and you can ask for any mistakes to be corrected. The study doctor may not be able to show you your study data right away and you may have to wait until the study is completed or another time in the future before you can see your study data.

The CGM data will be uploaded to a secure website. This is a centralized, web-based software used by health care professionals and researchers to upload, store and analyze glucose readings from people who have worn a device. No identifying information will be uploaded to this site; only your study ID, blood glucose readings, and logbook information will be uploaded. Dexcom is responsible for hosting and maintaining the website. Dexcom will have access to the non-identifying data uploaded to their website. If you leave the study, we will not collect new health information about you, but we will need to keep the data that we have already collected.

What if I have questions?

If you have any questions about the research now or later, or if you experience any adverse effects, or think that you have suffered a research related injury please contact:

Principal Investigator. Normand Boulé, PhD: 780-492-4695.
Research Coordinator. Janai Martens, MSc student: 780-492-8079.

If you have any questions regarding your rights as a research participant, you may contact the University of Alberta Research Ethics Office at reoffice@ualberta.ca. This office is independent of the study investigators

How do I indicate my agreement to be in this study?

By signing below, you understand:

- That you have read the above information and have had anything that you do not understand explained to you to your satisfaction.
- That you will be taking part in a research study.
- That you may freely leave the research study at any time
- That you do not waive your legal rights by being in the study.
- That the legal and professional obligations of the investigators and involved institutions are not changed by your taking part in this study.

SIGNATURE OF STUDY PARTICIPANT

_____
Signature of Participant

_____ _____
Name of Participant Date

SIGNATURE OF PERSON OBTAINING CONSENT

_____
Signature of Person Obtaining Consent

_____ _____
Name of Person Obtaining Consent Date

A signed copy of this consent form has been given to you to keep for your records and reference.

Low Energy Availability in Females Questionnaire

UNIVERSITY OF ALBERTA

Effects of the Menstrual Cycle on Blood Glucose During Exercise and in Recovery in Endurance Athletes.

(Supplemental Digital Content 1)

The LEAF-Q

A questionnaire for female athletes

Department of Nutrition, Exercise and Sports
Life Science
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Denmark
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The low energy availability in females questionnaire (LEAF –Q), focuses on physiological symptoms of insufficient energy intake. The following pages contain questions regarding injuries, gastrointestinal and reproductive function. We appreciate you taking the time to fill out the LEAF-Q and the reply will be treated as confidential.

Participant ID: _____

Age: _____

Are you or could you be pregnant at this time? Yes No

Do you track your menstrual cycle? If yes, how long is your typical

menstrual cycle? Yes No _____

Do you use any medication (excluding oral contraceptives)? Yes No

If yes, what kind of medication?_____

Have you ever been diagnosed with an acute or chronic disease? (Ex: type 1 diabetes, cardiovascular conditions, hyper/hypothyroidism, polycystic ovary syndrome, inflammatory or autoimmune disease) ?
Yes No
If yes, please explain:

_____

Do you smoke? Yes No

Your normal amount of training (average) – number of hours per week and what kind of exercise, such as running, swimming, bicycling, strength training, technique training etc.:

_____

_____

Comments or further information regarding exercise:_____

_____

_____

Have you ever been diagnosed with an eating disorder? Yes No

Your highest weight with your present height: _____(kg)
(excluding pregnancy)

Your lowest weight with your present height: _____(kg)

1. Injuries **Mark the response that most accurately describes your situation**

A: Have you had absences from your training, or participation in competitions during the last year due to injuries?

☐ No, not at all ☐ Yes, once or twice ☐ Yes, three or four times ☐ Yes, five times or more

A1: If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

☐ 1-7 ☐ 8-14 days ☐ 15-21 ☐ 22 days or more

A2: If yes, what kind of injuries have you had in the last year? _____

_____

Comments or further information regarding injuries:_____

_____

_____

2. Gastro intestinal function

A: Do you feel gaseous or bloated in the abdomen, also when you do not have your period?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B: Do you get cramps or stomach ache which cannot be related to your menstruation?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

C: How often d0 you have bowel movements on average?

☐ Several times a day ☐ Once a day ☐ Every second day

☐ Twice a week ☐ Once a week or more rarely

D: How would you describe your normal stool?

☐ Normal (soft) ☐ Diarrhoea-like (watery) ☐ Hard and dry

Comments regarding gastrointestinal function: _____

_____

3. Menstrual function and use of contraceptives

3.1 Contraceptives

Mark the response that most accurately describes your situation

A: Do you use oral contraceptives?

☐ Yes ☐ No

A1: If yes, why do you use oral contraceptives?

☐ Contraception ☐ Reduction of menstruation pains ☐ Reduction of bleeding

☐ To regulate the menstrual cycle in relation to performances etc..

☐ Otherwise menstruation stops

☐ Other_____

_____

A2: If no, have you used oral contraceptives earlier?

☐ Yes ☐ No

A2:1 If yes, when and for how long?_____

_____

_____

B: Do you use any other kind of hormonal contraceptives? (e.g. hormonal implant or coil)

☐ Yes ☐ No

B1: If yes, what kind? Please list the brand name below.

☐ Hormonal patches ☐ Hormonal ring ☐ Hormonal coil ☐ Hormonal implant ☐ Other

_____

3.2 Menstrual function **Mark the response that most accurately describes your situation**

A: How old were when you had your first period?

☐ 11 years or younger ☐ 12-14 years ☐ 15 years or older ☐ I don't remember

☐ I have never menstruated

(If you have answered "I have never menstruated" there are no further questions to answer)

B: Did your first menstruation come naturally (by itself)?

☐ Yes ☐ No ☐ I don't remember

B1: If no, what kind of treatment was used to start your menstrual cycle?

☐ Hormonal treatment ☐ Weight gain

☐ Reduced amount of exercise ☐ Other

C: Do you have normal menstruation?

☐ Yes ☐ No **(go to question C6)** ☐ I don't know **(go to question C6)**

C1: If yes, when was your last period?

☐ 0-4 weeks ago ☐ 1-2 months ago ☐ 3-4 months ago ☐ 5 months ago or more

C2: If yes, are your periods regular? (Every 28th to 34th day)

☐ Yes, most of the time ☐ No, mostly not

C3: If yes, for how many days do you normally bleed?

☐ 1-2 days ☐ 3-4 days ☐ 5-6 days ☐ 7-8 days ☐ 9 days or more

C4: If yes, have you ever had problems with heavy menstrual bleeding?

☐ Yes ☐ No

C5: If yes, how many periods have you had during the last year?

☐ 12 or more ☐ 9-11 ☐ 6-8 ☐ 3-5 ☐ 0-2

3.2 Menstrual function **Mark the response that most accurately describes your situation**

C6: If no or "I don't remember", when did you have your last period?

☐ 2-3 months ago ☐ 4-5 months ago ☐ 6 months ago or more

☐ I'm pregnant and therefore do not menstruate

D: Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?

☐ No, never ☐ Yes, it has happened before ☐ Yes, that's the situation now

---

E: Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?

☐ Yes ☐ No

E1: If yes, how? (Check one or more options)

☐ I bleed less ☐ I bleed fewer days ☐ My menstruations stops

☐ I bleed more ☐ I bleed more days

Low Energy Availability in Females Questionnaire Key

(Supplemental Digital Content 2)

The LEAF-Q Scoring key

A total score ≥8 is to be considered at risk for the Triad

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1. A: **0** No, not at all, **1** Yes, once or twice, **2** Yes, three or four times, **3** Yes, five times or more

1. A1: **1** 1-7 days, **2** 8-14 days, **3** 15-21 days, **4** 22 days or more

2. A: **3** Yes, several times a day, **2** Yes, several times a week, **1** Yes, once or twice a week or more seldom, **0** Rarely or never

2. B: **3** Yes, several times a day, **2** Yes, several times a week, **1** Yes, once or twice a week or more seldom, **0** Rarely or never

2. C: **1** Several times a day, **0** Once a day, **2** Every second day, **3** Twice a week, **4** Once a week or more rarely

2. D: **0** Normal, **1** Diarrhoea-like, **2** Hard and dry

3.1 A1: **0** Contraception, **0** Reduction of menstruation pains, **0** Reduction of bleeding, **0** To regulate the menstrual cycle in relation to performances etc.., **1** Otherwise menstruation stops

3.2 A: **0** 11 years or younger, **0** 12-14 years, **1** 15 years or older, **0** I don't remember, **8** I have never menstruated

3.2 B: **0**Yes, **1** No, **1** I don't remember

3.2 B1: **1** Hormonal treatment, **1** Weight gain, **1** Reduced amount of exercise, **1** Other

3.2 C: **0** Yes, **2** No (go to question 3.2 C6), **1** I don't know (go to question 3.2 C6)

3.2 C1: **0** 0-4 weeks ago, **1** 1-2 months ago, **2** 3-4 months ago, **3** 5 months ago or more

3.2 C2: **0** Yes, most of the time, **1** No, mostly not

3.2 C3: **1** 1-2 days, **0** 3-4 days, **0** 5-6 days, **0** 7-8 days, **0** 9 days or more

3.2 C4: **0** Yes, **0** No

3.2 C5: **0** 12 or more, **1** 9-11, **2** 6-8, **3** 3-5, **4** 0-2

3.2 C6: **1** 2-3 months ago, **2** 4-5 months ago, **3** 6 months ago or more

0 I'm pregnant and therefore do not menstruate

3.2 D: **0** No, never, **1** Yes, it has happened before, **2** Yes, that's the situation now

3.2 E: **1** Yes, **0** No

3.2 E1: **1** I bleed less, **1** I bleed fewer days, **2** My menstruations stops, **0** I bleed more, **0** I bleed more days

Get Active Questionnaire

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer YES or NO to each question before you become more physically active. If you are unsure about any question, answer YES.

YES	NO	
		1 Have you experienced **ANY** of the following (A to F) **within the past six months?**
●	●	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
●	●	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
●	●	**C** Dizziness or lightheadedness during physical activity?
●	●	**D** Shortness of breath at rest?
●	●	**E** Loss of consciousness/fainting for any reason?
●	●	**F** Concussion?
●	●	2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
●	●	3 Has a health care provider told you that you should avoid or modify certain types of physical activity?
●	●	4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

➤ **NO** to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY ➤

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE ➤➤

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ☐ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ☐ MINUTES/DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ☐ MINUTES/WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print]	Signature (or Signature of Parent/Guardian if applicable)	Date of Birth

Date	Email (optional)	Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

Baseline Exercise Collection Form

UNIVERSITY OF ALBERTA

Faculty of Physical Education and Recreation

1-052 Li Ka Shing Center

Edmonton, AB, Canada T6G2H9

1

ID :

Date:

Weight: kg Height: cm Age:

Resting HR: bpm Resting BP:

BIA:

Time (min)	Speed (mph)	Grade (%)	HR (bpm)	RER	VO_2 (L/min)	RPE	Comments
0-1							
1-2							
2-3							
3-4							
4-5							
5-6							
6-7							
7-8							
8-9							
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24-25							
25-26							

Immediately Post-ex HR:_____

UNIVERSITY OF ALBERTA
Faculty of Physical Education and Recreation
1-052 Li Ka Shing Center
Edmonton, AB, Canada T6G2H9

Immediately Post-ex BP:_____

Reason for stopping:

- ❑ Fatigue
- ❑ Test is terminated by the cardiac nurse or exercise physiologist
- ❑ Leg muscle fatigue/pain
- ❑ Hip/Knee pain
- ❑ Angina
- ❑ ECG changes
- ❑ Short of breath
- ❑ Dry mouth
- ❑ Incline too steep
- ❑ Other: _____

Were there any unexpected findings during the test? □ Yes □ No

If yes, please provide a brief overview of the event and the action taken:

Absolute VO_2peak:

Relative VO_2peak:

Ventilatory Threshold:

Exercise Intervention Collection Form

UNIVERSITY OF ALBERTA
Faculty of Physical Education and Recreation 1
1-052 Li Ka Shing Center
Edmonton, AB, Canada T6G2H9

ID : _____ Date: _____

Exercise Session: _____

Resting HR: _____bpm Resting BP: _____ Capillary Blood Glucose: _____

Menstrual Cycle Phase: _____

Time (min)	Speed (mph)	HR (bpm)	RER	VO_2 (L/min)	VCO_2 (L/min)	Comments
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UNIVERSITY OF ALBERTA
Faculty of Physical Education and Recreation 2
1-052 Li Ka Shing Center
Edmonton, AB, Canada T6G2H9

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Immediately Post-ex HR:_____

Immediately Post-ex BP:_____

Capillary Blood Glucose:_____

RPE:_____