Investigation of Glucose and Energy Metabolism in Eumenorrheic Female Ultra-Runners Across the Menstrual Cycle

A thesis

submitted in fulfilment

of the requirements for the degree

of

Doctor of Philosophy in Health, Sport and Human Performance

at

The University of Waikato

by

Andrew Dole

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2024

Preface

Since portions of this dissertation have already appeared in various journals and conference proceedings, some repetition is unavoidable. These publications have been incorporated into the thesis to deliver a thorough and cohesive presentation of the research. This repetition is essential to preserve the continuity and coherence of the thesis narrative, ensuring that each chapter is self-contained and contributes to a holistic view of the research.

Abstract

Female athletes, ultra-running, and the utilization of continuous glucose sensors in endurance sports are each individually underrepresented in current literature. When these areas are examined collectively, a substantial gap in knowledge becomes evident. Therefore, the purpose of this PhD study was to collect glucose and energy metabolism data in regularly menstruating female athletes within the complex sporting environment of ultra-running. Initially, a narrative review of the current study methodologies in female performance research was completed. The narrative review aided in the identification of gaps in study design and validation methodologies within female performance research, put forth a suggestive framework for standardizing terminology, and provides a comprehensive list of options for a combined methods approach to menstrual tracking.

Subsequently, a randomized, cross-over experimental design was performed with twelve experienced female ultra-runners (age 39 ± 6 y) acting as their own control across menstrual cycle phases during an ultra-marathon simulation. Participants completed a 3-hour fasted outdoor run (FASTED) followed by a one-hour treadmill run (TREAD) where three standardized oral glucose doses were provided, in the mid-follicular (FOL) and mid-luteal phases (LUT). Using a mixed linear model, the menstrual phase was identified as being statistically significant for differences in glucose measurements between CGM and capillary glucose during TREAD ($p = 0.02$) but not FASTED. Also, the CGM reported glucose levels an average of -0.43 $mmol \cdot L^{-1}$ (95% CI -0.86, -0.005) and -1.02 $mmol \cdot L^{-1}$ (95% CI -1.63, -0.42) lower in fasted and fed scenarios respectively when compared to capillary glucose. Median glucose was higher in the LUT (Capillary 0.25-0.29 $mmol \cdot L^{-1}$, CGM 0.38-0.4 $mmol \cdot L^{-1}$) both when fasted or supplemented with carbohydrate. Of note, this is the first study to show that glucose is on average higher in the mid-luteal phase as well as observe the avoidance of clinically significant non-diabetic hypoglycemia (< 3.0 $mmol \cdot L^{-1}$) during 3-hours of fasted running following an 8-hour fast among female ultra-runners. Additionally, higher individual responses of low blood sugar were observed in the follicular phase. Further, there were no significant differences in the respiratory exchange ratio (RER) between the FOL (0.805 ± 0.028) and LUT phases (0.809 ± .035); t (10) -0.401, $p = 0.697$ during comparable submaximal running intensity (57% ± 7.3% and 57.3% ±7.9% VO_{2MAX} respectively). Post-hoc analysis revealed no significant main effect of RER on glucose levels.

As a comprehensive study, the thesis expands upon the existing literature by highlighting the accentuated differences in CGM glucose data during prolonged running and expressing the need for awareness among athletes and practitioners. The requirement to emphasize the influence of individual physiological responses in female athletes across the menstrual cycle, especially when fasting or provided with exogenous carbohydrate was also apparent. The reporting of elevated glucose in mid-LUT identifies an area of further research and consideration when interpreting glucose levels. Additionally, the lack of relationship between blood glucose levels and the respiratory exchange ratio observed contributes to the current understanding of sport performance metrics. Lastly, the current body of work advocates for incorporating sex hormone perturbations in research design, standardizing menstrual cycle tracking terminology, and incorporating mixed method tracking methodologies to facilitate future female performance research.

Research Manuscripts

Published Manuscripts

Dole A, Beaven M, Sims ST. Menstrual Cycle Tracking in Sports Research: Challenges, Progress, and Future Directions. *Physiologia*. 2023; 3(4):598-610. https://doi.org/10.3390/physiologia3040044

Dole A, Sims ST, Gan H, Gill, N, Beaven M. Continuous Glucose Monitor Underreports Glucose During a Simulated Ultra-Endurance run in Eumenorrheic Female Runners. *International Journal of Sports Physiology and Performance*.

Manuscripts Currently Under Review

Dole A, Sims ST, Beaven M. Fueling in Flux: Blood Glucose Responses and Menstrual Phase Interactions in Female Runners During a Simulated Ultra Run. *Journal of Sports Sciences*.

Dole A, Sims ST, Beaven M. No Main Effect of RER on Blood Glucose in Eumenorrheic Female Runners. *Journal of Sports and Health Sciences*.

Acknowledgements

With immense gratitude I want to recognize my research advisors, Marty Beaven, Stacy Sims, and Nic Gill who guided me through the PhD process with all its twists and turns.

I must extend a massive “Thank You” to all the participants who agreed to do a physically demanding study protocol; not once, but twice! Without your determination and resilience this project could not have happened.

No words to describe the eternal patience and support of my wife who moved around the world for a few years with nothing more than three carry-on bags so I could continue chasing personal bests. Love you and thanks.

Table of Contents

List of Tables

List of Supplemental Tables

List of Figures

List of Abbreviations

(ATP)	Adenosine triphosphate
(BBT)	Basal Body Temperature
(CGM)	Continuous glucose monitor
(CHO)	Carbohydrate
(E2)	Estradiol
(EA)	Energy availability
(EEE)	Exercise energy expenditure
(EF)	Early Follicular
(EI)	Energy intake
(FASTED)	3-hour fasted outdoor run
(FDA)	Federal Drug Administration
(FFM)	Fat free mass
(FOL)	Follicular
(GI)	Gastrointestinal system
(GLUT5)	Fructose transporter
(GnRH)	Gonadotropin-releasing hormone
(GOx)	Glucose oxidase
(GPS)	Global positioning
(iCGM)	Intermittent continuous glucose monitor
(IMCL)	Intramyocellular lipid
(ISF)	Interstitial fluid

(ISO)	International Organization for Standardization
(LEA)	Low energy availability
(LF)	Late follicular
(LH)	Urinary Luteinizing Hormone
(LL)	Late luteal
(LUT)	Luteal
(MARD)	Mean absolute relative difference
(MC)	Menstrual cycle
(MF)	Mid-follicular
(ML)	Mid-luteal
(P4)	Progesterone
(RER)	Resting expiratory ratio
(SGLT-1)	Sodium-dependent glucose transporter
(TREAD)	One-hour treadmill run
(UE)	Ultra-endurance
(VO_{2MAX})	Maximal oxygen consumption

Part 1 Introduction

SECTION 1

1.1 Opening

Participation in ultra-endurance running events, defined as any event with a duration of 4-6 hours or more,[1,2] has seen steady growth since 2000 with female runners contributing to the uptick in participants as well as having a greater representation in the top overall finishers.[3,4] While men generally outperform women in sport by 10-30%[5], among ultra-running the performance gap has been reduced within 4-10%.[6] It is unclear whether female physiology, genetics, or both confer advantages in ultra-running helping close the performance gap between biological men and women or whether low participation numbers simply confound the data.[7] In addition to sex specific differences among men and women, the effects of menstrual cycle on metabolism and hydration during ultra-running has yet to be fully explored the female athlete population.

1.2 Background

1.2.1 Ultra-Endurance Running

The energetic demands required to complete ultra-running exceeds the human physiological capacity for carbohydrate storage, food consumption, fluid intakes, and nutrient processing.[8] For example estimates of caloric needs in male athletes of 13,000-16,000 kilocalories (kcal) have been made for the Western States 100 mile ultra-marathon over an average finish time of 27 hours.[9] Comparatively, the realistic endogenous storage capacity and exogenous usage rates of carbohydrate (CHO) in the human body is limited to 1,600-2,800 kcal and ~90 g/h respectively.[10,11] The sustainability of 90 g/h or more of exogeneous carbohydrate ingestion is highly individual, trainable to some extent, and potentially impractical for female athletes with smaller stomachs, and slower gastric emptying and intestinal emptying rates.[12] Such high nutritional needs combined with the physiological constraints of the human gastrointestinal system (GI) create a complex sports nutrition challenge. A primary goal of ultra-running nutrition is maximising CHO intake during the event, which can enhance performance by delaying fatigue and prolonging time to exhaustion.[11] Recommended CHO intake for events exceeding three hours is up to 90 g/h, with a commonly practiced range of 30-90 g/h based entirely on individualized tolerance of the athlete[13]

and is based on previously established physiological limitations of multiple transportable carbohydrates.[11,14–18]

Due to the variability of tolerable intake from athlete to athlete, simply eating more, or less, does not guarantee success in terms of fueling. In two separate studies the incidence of GI distress was reported to be 65%-82% with the increase directly related to distance.[19,20] Wardenaar and colleagues observed both male and female ultra runners (n= male 33; female 8) consumed only 75% of the recommended 60-90 g/kg/h of CHO during a 60 km event with a varying range of incidence moderate GI symptoms (flatulence 35%, nausea 21%; bloating 16%, loose stool 5%, diarrhea 2%).[20] Over the course of a 24-hour ultra marathon, Costa et al. found the incidence of severe GI distress to be 65% among men and women (n = male 19; female 6) with a rate of occurrence 2.5-times greater in faster runners.[19] Surprisingly, in both studies, the consumption of carbohydrates and energy did not show an association with the occurrence of GI distress. These observations are noteworthy because these factors are usually linked to typical GI distress symptoms such as nausea, vomiting, and diarrhea when consumed in large quantities during running.[8,14] Additional considerations for CHO and fluid intakes may be necessary for female athletes when recommending intakes during ultra-running due to smaller stomachs, and slower gastric emptying and intestinal emptying rates observed in females at rest.[12]

It is often thought that high rates of CHO intake or improper dilution of CHO resulting in hyperosmolar concentrations in the gut are the primary contributors to GI distress, however, GI ischemia, mechanical motion, and the position of running may predispose GI sensitivity to changes in motility, permeability, absorption.[21,22] The decrease in blood flow to the digestive system caused by the redistribution of blood to active muscles during exercise has been associated with ischemia.[23] Ischemia refers to the insufficient supply of oxygenated blood to the GI system, leading to increased permeability of the gut mucosa and the presence of endotoxins in the bloodstream. These effects can potentially lead to symptoms such as vomiting, nausea, diarrhea, and abdominal discomfort.[22,23] Additionally, the combination of upright posture and repetitive high-impact mechanics associated with running may contribute to injury of the gut and GI symptomology via the repetitive impact of the intestines with the abdominal wall.[24]

1.2.2 Female Physiology

The menstrual cycle and associated ovarian hormones distinguish biological females from males. In eumenorrheic women, the menstrual cycle (**Figure 1**) is comprised of three main phases, follicular (FOL), ovulatory, and luteal (LUT), which can be separated into six separate hormonally distinct phases: early follicular (EF), mid-follicular (MF) or late follicular (LF), ovulation, early luteal (EL), mid-luteal (ML) and late luteal (LL) phase. The FOL phase considered the first half of the menstrual cycle phase begins with a "low" hormone stage characterized by relatively low levels of 17β-estradiol (E2), the strongest of the naturally occurring estrogens (E1, E2, and E3) and progesterone (P4) the dominant form of the progestens. This stage is followed by a continuous rise and peak of E2 into the MF phase where E2 then proceeds to drop, progesterone starts to rise, and the ovulatory phase begins. Following ovulation, the second half or "high hormone" LUT phase of the menstrual cycle begins. This phase begins with rising levels of P4 and moderate E2 in the EF phase. Followed by peak P4 and a second mini peak of E2 in MF; representing the highest hormone phase of the menstrual cycle. The menstrual cycle progresses to the LF phase where E2 and progesterone return to low levels and the cycle restarts with menstruation in the EF phase.[25]

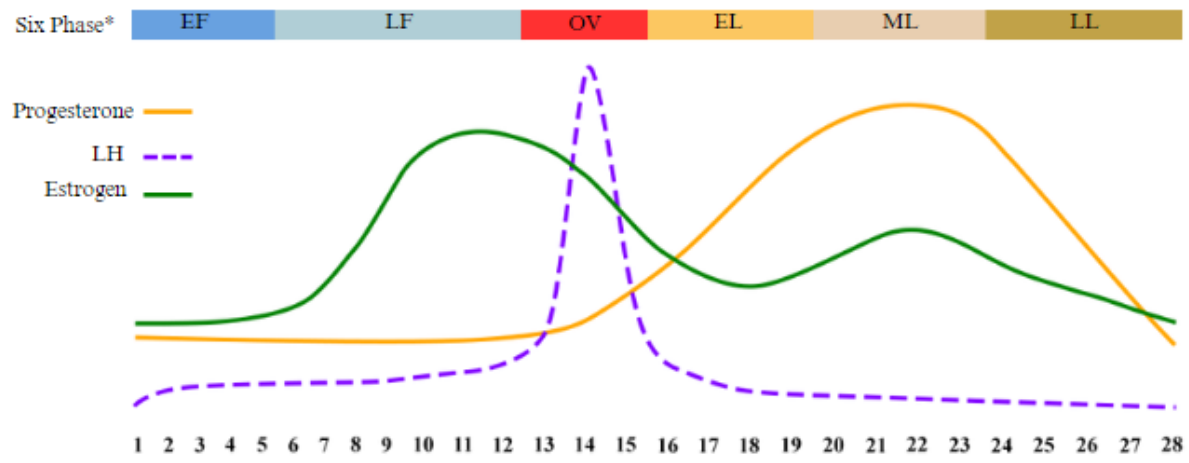


Figure 1. Overview of hormone profiles across the menstrual cycle. Adapted from McNulty and colleagues.25 * Early Follicular (EF); Late Follicular (LF); Early Luteal (EL); Mid Luteal (ML); Late Luteal (LL); Luteinizing Hormone (LH).

Female ovarian hormones, E2 and progesterone, play a vital role in the differences in substrate utilization between females and males. Specifically, E2 has been shown to impact CHO metabolism by promoting glucose uptake into type I muscle fibers while also reducing uptake into

the bloodstream.[26,27] The influence of E2 is also associated with decreased CHO and amino acid oxidation but an upregulation in fat utilization.[28] D'eon and colleagues similarly observed the effects of E2 on CHO and fat oxidation in females (n = 8) during a pharmacological suppression and replacement study where E2 and progesterone were manipulated to replicate three distinct hormone phases of the MC (E2 low + progesterone low, E2 high + progesterone low, E2 high + progesterone high) during submaximal (60% VO_{2MAX}) cycling for an hour. When E2 alone was administered during submaximal (60% VO_{2MAX}) cycling for an hour, a 17% decrease in total CHO utilization and a 50% increase in fat oxidation was observed when compared to baseline hormone levels.[27] These results have been duplicated in several studies demonstrating that eumenorrheic females, compared to males, tend to oxidize more fat and less CHO during submaximal exercise, relative to LBM, especially in the absence of CHO intake.[28–35] However, exogenous CHO availability during exercise mitigates the CHO utilization disparity between sexes, with studies showing similar substrate utilization between males and females when CHO is provided. Petterson *et al.* demonstrated in elite level cross-country skiers exercising at 70% VO_{2MAX}, that substrate oxidation was not significantly different between genders when exogenous CHO was provided (male: fat 77 g, CHO 262 g; female: fat 76 g and CHO 251 g).[36] Furthermore, these authors concluded that females can greatly increase CHO oxidation when provided exogenous CHO during endurance exercise, will utilize more exogenous CHO as a percentage of total energy than males, and oxidize less fat and more CHO during exercise when exogenous CHO is available.[36] Likewise, both Wallis *et al*.[34] and Tremblay *et al*.[35] found that during submaximal endurance (57-67% VO_{2MAX} respectively) exercise lasting ≥ 2 h, the metabolic response of females to CHO during exercise is comparable to males with no appreciable differences in RER (male 0.92; female 0.92) or rates of CHO oxidation (male 0.70; female 0.65 g/min).[34,35] These observations support the concept that exogenous CHO during exercise decreases E2's hepatic glycogen sparing effect that is responsible for the metabolic shift towards fat in the absence of CHO in females during exercise.[37,38]

Lesser known is the interaction of progesterone on CHO metabolism, although from the limited research available it appears to reverse E2's suppression of muscle glucose oxidation during submaximal exercise,[27] as well as complement E2's glucose-sparing effect in the liver.[39] Interactions of progesterone also appear to affect glucose metabolism through the upregulation of glucose transporters (GLUTs) expression to increase endometrial glycolytic metabolism,

attenuating skeletal and hepatic glycolytic pathways.[26,40,41] The impact of progesterone on energy expenditure, resulting in a 5% increase during the LUT phase while sleeping, has been observed; nevertheless, no significant effect on total daily energy expenditure was noted.[42]

1.2.3 Continuous Glucose Monitoring

It is well documented that prolonged submaximal or intermittent high-intensity exercise is impaired by low carbohydrate availability.[13,43,44] Specific strategies involving CHO intake during a race may prevent hypoglycemia, its associated clinical symptoms, and improve race outcomes, yet over the distance and duration of ultra-endurance, it has proven difficult to maintain adequate CHO intake.[20] One recent addition to monitoring fueling needs and the impacts of low carbohydrate intake is the continuous glucose monitor (CGM). Initially designed to assist in the clinical management of both insulin and noninsulin-dependent diabetes, there is now interest in the application of CGM for real-time glucose monitoring in athletic populations.[45] Current generation CGM sensors are applied to the skin using an applicator that inserts a nanofilament beneath the skin and into contact with interstitial fluid (ISF); which is the second largest volume of fluid in the human body and contains high levels of detectable glucose fluid.[46] Glucose levels are predicted using software algorithms on a reader device which analyses the enzymatic reactions of the chemically reactive filament to glucose present in the ISF.

In addition to glucose availability, various studies independently link glycemic variability to a range of health issues. A recent study by Zhou *et al.*[47] identified 12 assessment metrics for glycemic variability connected to 48 negative clinical outcomes including diabetic retinopathy, cardiovascular disease, heart failure, and metabolic syndrome. Yet a standard of reference for interpreting glycemic variability fluctuations does not exist for any population.[47–49] Therefore, interpreting the impact of chronically high or erratic blood glucose levels during exercise, for example, is problematic. There are additional concerns related to the accuracy of blood glucose readings from interstitial fluid (ISF) due to variables frequently affecting sensor reliability, such as: pH level, temporal delays in glucose changes from blood to ISF, and lower glucose concentrations in ISF compared to blood.[50–52] The temporal lag time can additionally be categorized as either physiological and technical. Physiological lag involves the delay in ISF reflecting blood glucose changes due to diffusion, which has been observed between 6-10 minutes.[51] Technological lag includes sensor hardware and software interferences, such as algorithms,

electrode membranes, and biological substances affecting the electrochemistry.[53] Earlier generation sensors had a ~15-minute delay in ISF interpretation, but recent technology advancements have minimized this gap within minutes.[52,54] However, a study in 2019 still reported a 12-minute lag during exercise using a newer generation sensor (Dexcom G5).[55]

The accuracy of CGM sensors has also been questioned when concentrations of glucose change rapidly, a common issue during exercise and ultra-running. Pleus *et al.*[56] conducted a study to assess the accuracy of two second-generation devices: a commercially available Dexcom G4 sensor and an experimental unnamed device. These researchers observed that the accuracy of the older generation G4 sensor, measured by the percentage mean absolute relative difference (MARD), was negatively affected by rapid rates of glucose changes (< 3 mg/dL /min and > 3 mg/dL/min) yet the newer generation device was far less impacted (G4 24.9% and 29.6% vs the experimental device 10.6% and 16.3%). The study concluded that newer CGM technology may overcome accuracy concerns in the presence rapidly changing glucose levels.[56] In another study utilizing the Dexcom G5 sensors, the authors also observed MARD was impacted negatively (by 6 to 22%) over prolonged exercise with significant errors during rapidly rising and declining glucose levels.[55]

Another observed limitation of CGM devices lies in their accuracy during instances of low blood sugar, which are particularly vulnerable to influence by factors such as lag time, and rapidly fluctuating glucose levels.[56] Moser and Eckstein *et al.*[57] found a lower accuracy rate (MARD 31.6%) when hypoglycemia occurred during exercise in a study involving 16 type I diabetic participants who were given a pre-exercise carbohydrate meal of 1 g/kg before 45 minutes of moderate intensity cycling. Similarly, Aberer and colleagues evaluated three CGM devices (Freestyle libre, Dexcom G4, Medtronic 640G) in type I diabetic participants under controlled conditions involving daily activities, meals, and 60 minutes of exercise over a span of 12 hours. All devices showed the highest MARD levels (poorest accuracy) during periods of low blood glucose (< 3.9 $mmol \cdot L^{-1}$).[58] Additionally, numerous studies have examined the accuracy of CGM devices in both resting and exercising conditions among populations with and without type 1 diabetes.[55,57,59–63] These studies have consistently indicated that MARD is significantly worse during exercise when compared to rest. Fabra *et al.*[64] conducted a comprehensive review of 54 studies spanning 2006-2020 confirmed this trend documenting the range of fluctuation in MARD

from negative 4.4% to 18.1% when comparing sensors at rest versus exercise. This reduced accuracy during exercise was attributed to rapidly changing glucose levels and factors related to ISF volatility. Lastly, Mian *et al.*[65] provided a comprehensive compilation of substances known to impact the accuracy of some CGM sensors. The most prevalent and pertinent to a physically active population include ibuprofen, acetaminophen, Vitamin C, and triglycerides.

While concerns of sensor convenience, cost,[54] calibration,[54,65,66] and data accessibility,[54] are less relevant with newer generations of sensors, the issue of accuracy,[54,66] primarily related to physiological and technological lag times associated with gathering glucose data from ISF, still exists. These concerns are of particular note in certain use cases such as sporting environments given that a rapid intake of CHO to fuel exercise is commonplace. Furthermore, new concerns such as the lack of data analysis standards[54] and medication interactions[65] have emerged. Finally, it is of note that use cases for CGM are hypothetical, as guidelines do not currently exist for interpreting continuous glucose data in healthy athletes during exercise, nor are there currently robust studies of efficacy in endurance or ultra-sport environments.

Within a sporting context, poor performance outcomes can be directly attributed to hypoglycemic episodes.[10] However, prior to the availability of CGM in a commercial setting, the assessment of occurrence, severity, frequency, and glycemic variability during endurance events was not feasible. Francois and colleagues examined the influence of ultra-running on glucose using CGM during the 5-day GODZone adventure race covering 326 km (n = 6 male; 2 female). Glucose data was collected from 5 out of the 8 participants, due to sensor malfunction, with no disclosure of sex. The mean glucose (4.8 $mmol \cdot L^{-1}$) over 5 days of racing remained consistent with pre-race measurements (4.9 $mmol \cdot L^{-1}$); however, a 2-fold increase in the incidence and time spent below 3.9 $mmol \cdot L^{-1}$, the lower threshold of normoglycemia in diabetics[67], was observed. Of note, there is currently no established threshold for normoglycemia in healthy individuals, but clinically significant hypoglycemia in healthy populations has been defined as < 3.0 $mmol \cdot L^{-1}$.[67,68] Nearly every participant, experienced glucose below 3.9 $mmol \cdot L^{-1}$ on each of the five days. The researchers linked the observed glucose response to suboptimal nutritional practices, which led to an inability to meet the energy requirements of the race.[69] Perhaps most importantly, they highlighted the importance of capturing glucose data during events and its potential to inform optimized fueling strategies. Ishira *et al.*[70] advanced this concept when evaluating the viability of

CGM in providing optimal CHO intakes during a 160 km ultra-running event in seven trained runners (n= 4 male; 3 female). Over the entire event, only one measure of glucose < 3.9 mmol·L^{-1} was observed with the average glucose ranging from 5.8 to 9.1 mmol·L^{-1}. The data displayed a wide range of glucose across all runners, from 3.4 to 13.3 mmol·L^{-1}, over the 160 km. There was no relationship between the highest levels of glucose and running speed (p =0.89, male; p = 0.57, female); however, the lowest level of change between low and high glucose levels among each participant was significantly related to increased running pace in males but not females (p = 0.04, male; p = 0.6, female). These data emphasize the potential importance of monitoring glucose variability and may inform future nutrition interventions aimed specifically at minimizing blood glucose fluctuations during ultra-running. Although sex differences have been observed in glucose responses, small sample sizes and a lack of standardization in menstrual phase terminology or hormonal contraceptive use indicate the need for further investigation. Additionally, Ishira *et al.*[70] demonstrated that increased CHO intake, and not PRO or fat, was associated with faster running speeds (p = 0.007) despite the reported intakes of CHO falling below current recommendations, which is consistent with existing literature and assessments of suboptimal fueling practices in endurance sport.[8,20,71]

1.3 Research Gaps

Interest in ultra-running research has increased with the rise in popularity of the sport; however, due to the complexities of biochemical changes and nutritional needs of ultra-endurance, the existing sport nutrition guidelines may not be adequate for the ultra-running population. Moreover, in line with previous biomedical research, there is an underrepresentation of female ultra-running research; primarily due to historical bias and poor methodology.[24] The application of CGM in ultra-sport environments is also not without dispute due to a lack of standardization in data interpretation, physiological and technological complications, as well as the fact that peer-reviewed research is often outpaced by advancements in sensor technology leaving assumptions and observations of accuracy and relevance in question.

Therefore, to inform guidelines, specific to female ultra-sport athletes, three specific areas were investigated for this project:

1) Assessing the accuracy and reliability of CGM sensors to inform fueling strategies during ultra-running in the field.

2) Interactions between menstrual phases and blood glucose when fasted and provided exogenous carbohydrate during simulated ultra-running.

3) Observing metabolic differences between menstrual phases during a 4-hour ultra-running simulation.

1.4 Purpose of Study

As an applied field of practice, sports nutrition relies heavily on peer-reviewed literature to inform evidence-based protocols in real-world settings. Understandably, a large percentage of that research is conducted in tightly controlled lab environments. However, in ultra-running it is the highly variable nature of the sport, including but not limited to, terrain and duration, that is a primary challenge of field research. Yet, those same characteristics directly affect mechanistic and metabolic functions in the body which are not readily replicated in lab settings and are important to real-world application. Furthermore, existing research has focused on male participants, and where females are included, typically do not account for hormonal interactions of the menstrual cycle. Therefore, the purpose of this PhD was to investigate the interaction of menstrual phase on glucose and energy metabolism in regularly menstruating female ultra-runners within the complex sporting environment of ultra-running in hopes of bridging the gap between science and practice.[72]

This was attempted by examining three specific research questions:

A). Does CGM reliably report blood glucose during a four-hour simulated ultra run?

B). Does menstrual phase impact blood sugar levels when fasted and provided exogenous carbohydrate during a 4-hour simulated ultra-run?

C). Is energy metabolism different between menstrual phases at the end of a 4-hour simulated ultra-endurance run?

1.5 Thesis Structure

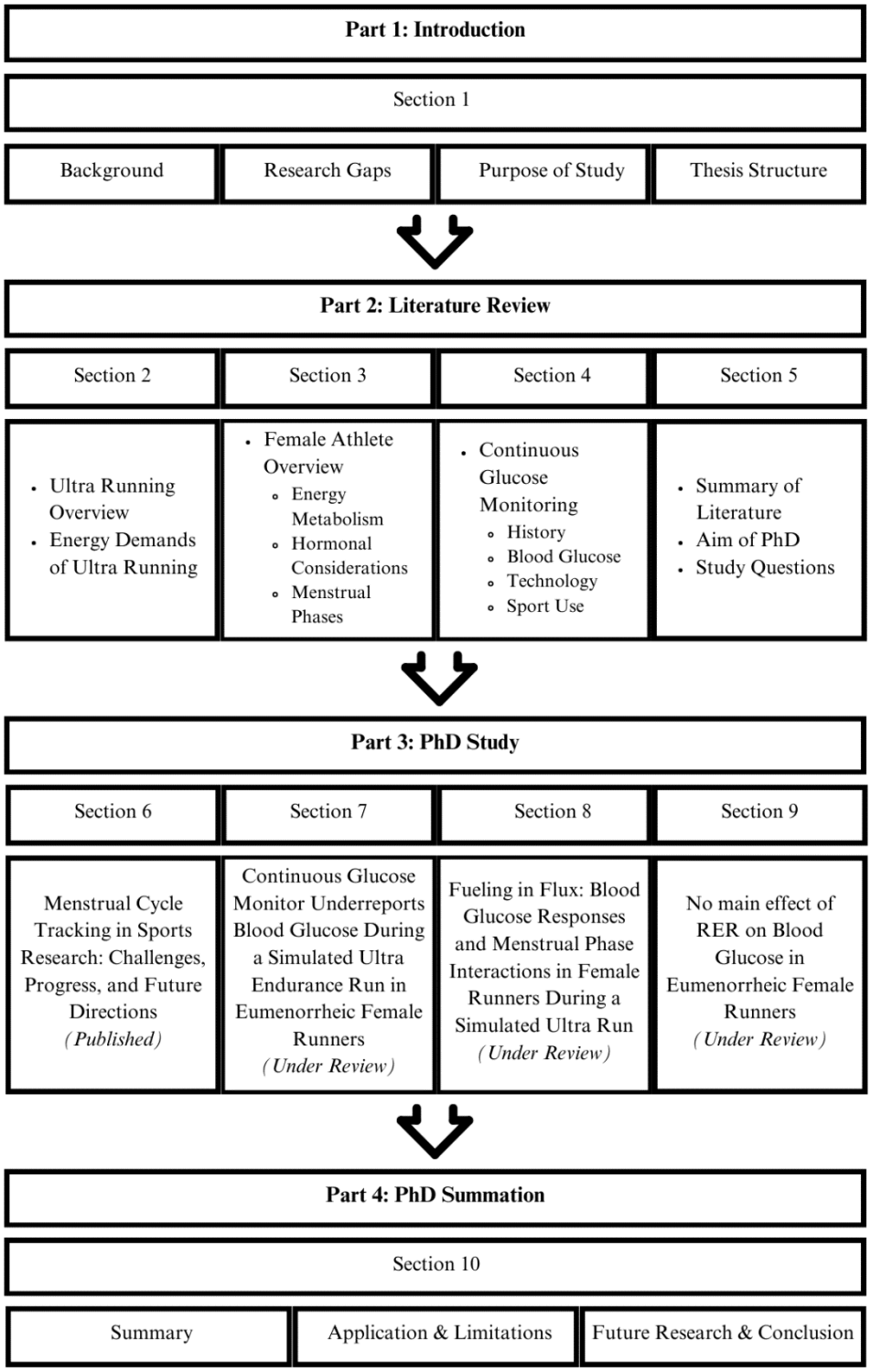


Figure 2. Thesis structure schematic.

Part 2 Literature Reviews

SECTION 2

2.1 Metabolic Demands of Ultra-Endurance Sport

Ultra-endurance events are defined as events lasting a minimum of 4-6 h[1,2] and up to several consecutive days. Swimming, cycling, running, and long-course triathlon are the most recognizable ultra-endurance events. It is common for ultra-athletes to maintain training regimens of up to 6 hours per day at exercise intensities of 50-90% of VO_{2MAX}[1] with multiple sessions occurring within a single 24-hour period.[73] Weekly training volumes of ultra-endurance runners vary between race type. The average training mileage per week for non-professional male runners in multistage, 24-hr, and 100 km events has been reported to be 85 km ± 35.8 (8.9 ± 4.4 hours per week).[74] In contrast, the average weekly training miles for the elite-level runners is ~ 117 km with a peak of 173 km (> 20 h/week).[43]

To meet the estimated daily energy demands of ultra-endurance events, current nutrition guidelines aim for individualization of macronutrient intakes to provide sufficient energy availability, (energy availability = energy intake – exercise energy expenditure / fat free mass) to support optimal health and function.[75] Recommendations for the macronutrients, CHO, fat, and protein, are provided in quantities relative to total weight (g/kg) to meet ensure adequate intake at all body sizes. For moderate to high intensity exercise lasting > 4-hours, it is advised to consume daily: CHO 8-12 g/kg[13], protein 1.2-2.0 g/kg[75], fat 0.5-1.5 g/kg[76]. Guidelines of 30-90 g/h[13] exist for exogenous CHO intakes during ultra-running and intakes of up to 120 g/h have been tolerated in male runners using a combination of maltodextrin and fructose.[14,77] These recommendations are based on the utilization of dual pathways or multiple transportable carbohydrates. Dual pathway CHO intakes rely, independently yet simultaneously, on the sodium-dependent glucose transporter (SGLT-1) for glucose, which is saturated at 60 g/h[15] and the fructose transporter (GLUT5) with a peak oxidation of ~30 g/hr[15] allowing for increased CHO uptake from the intestines into the bloodstream.[14–17] Jentjens *et al.*[15] explored the impact of glucose versus glucose + fructose beverages on peak exogenous CHO intakes in eight trained male athletes during three, independent, 150-minute cycling trials at 62% of VO_{2MAX}. Oxidation rates of fructose and glucose were captured using elemental analyzer-isotope ratio mass spectrometry. The results indicated a

44% greater CHO oxidation rate for the multiple transportable CHO solution (1.70 ± 0.07 g/min) over glucose alone (1.18 ± 0.04 g/min). Jeukendrup and colleagues found similar results comparing the exogenous CHO oxidation rates of multiple transports CHO to glucose alone during three different 5-hour cycling trials in well-trained males (n = 8) who were provided either glucose or glucose + fructose interventions. Glucose oxidation during the trials was measured using stable carbon isotopes enrichment and indirect calorimetry. The data indicated a 65-77% increase in CHO oxidation from glucose + fructose (1.4 ±0.08 g/min) as compared to the glucose-only intervention (1.24 ± 0.08 g/min).[11] In 2012, using two independent random crossover trials, Rowlands *et al.*[18] showed that ingesting a multiple transportable CHO solution of maltodextrin and fructose at a rate and ratio of 1.2 g/kg/h and 2:1 respectively, improved cycling performance among well-trained cyclists as compared to glucose-only during simulated mountain bike races in both sexes (n = 7 male; 3 female) as well as during male-only laboratory cycling trials (n = 16).

The total energy costs associated with ultra-endurance running events have been estimated in previous studies. Cuddy and colleagues measured total energy expenditures in 10 runners during the 160 km Western States 100 ultra-marathon using doubly-labelled water, and concluded the mean energy expenditure was 15,865 kcal (99 kcal/km) over the average finish time of 26.8 hours.[9] Similarly, Dumke *et al.*[78] using portable indirect calorimetry in a single runner case report over the first 64.5 km segment of the same ultra-marathon reported energy expenditure as 83.7 kcal/km. In a larger sample of female (n = 9) and male (n = 19) runners, Costa *et al.*[19] determined energy expenditure, using triaxial accelerometery, during a 24-hour ultra trail race ranged from 8,771 to 17,734 kcal with a mean of 13,145 ± 2,629 kcal across the varying distances (122 to 208 km) with no differences between sexes recognized when body mass corrections were made. In a separate case study, Cuddy and colleagues observed total energy expenditure to be 8,926 kcal (39.4 kcal/km) in a single male triathlete during the 2006 Ironman World Championships (226.3 km) using doubly-labelled water.[79] In a multi-stage ultra-marathon simulation case study, Alcock *et al.*[80], using indirect calorimetry and triaxial accelerometery simultaneously during five consecutive days of 50 km treadmill runs observed, in a single runner, energy expenditures of 7,156 ± 359 kcal per day (143 kcal/km) when provided access to full rations. As such, current research would suggest that energy requirements for ultra-endurance can range from 39 to 143 kcal/km and vary by discipline.

2.2 Contribution of Macronutrients to Energy Availability During Exercise

Similar to all endurance sports, aerobic energy metabolism is regarded as the primary energy system during ultra-running, which is characteristically performed for extended periods below 70% of VO_{2MAX} with brief but frequent requirements for higher intensity efforts.[1,81] This system generates the energy unit of muscle contraction, adenosine triphosphate (ATP), fundamental in the mechanisms of muscle fiber excitability, resulting contraction, and relaxation from the oxidation of fat and CHO.[82] At rest, and during low thresholds of VO_{2MAX} (~60-65%), fat contributes significantly to energy demands.[82] As intensity increases, the high capacity but low efficiency of fat to provide ATP (4.6 kcal/L O_2) becomes incapable of sustaining energy demands,[1] thus initiating an increase in the oxidation of CHO a more efficient (5.1 kcal/L O_2) yet limited resource to produce ATP.[1,43] Up to exercise intensities approximating 75% of VO_{2MAX}, fat and carbohydrate provide a balanced mix of fuel sources; whereas, above 80% of VO_{2MAX} there is a considerable shift away from fat oxidation to a heavy reliance on CHO to fuel working muscle.[82] Thus, during sustained, moderate intensity exercise (50-70% of VO_{2MAX}), a mixture of fuel sources contributes to total energy needs, including amino acids from protein. These sources can be endogenous or exogenous and both will vary greatly in availability. For example, endogenous stores of glycogen and fat are estimated to be 500 g[10] and ~11,000 g respectively.[82,83]

For all sports, the composition of daily diet, fasted or fed states, sport type, participant sex, intensity, duration, and level of fitness all determine the ratio of fat and CHO used during exercise.[84–87] Among these factors, intensity has the strongest impact on substrate utilization.[82] The exact breakdown of macronutrient utilization during ultra-running remains difficult to establish as it suffers from similar logistical constraints as total energy requirements with the added complexity of constantly changing aerobic and anaerobic metabolisms identified by their preferred energy substrates; fat and carbohydrate respectively, with some contribution of proteins.[88]

2.3 Carbohydrate Metabolism

Carbohydrates are widely regarded as a 'critical' fuel source for sports performance by providing significant amounts of ATP to working muscles and mitigating fatigue.[13,43] At exercise intensities above ~65% of VO_{2MAX}, CHO becomes the predominant source of energy.[88,89] To obtain CHO the body draws upon three primary sources during exercise: muscle glycogen, liver glycogen, and

exogenous intake. Endogenous stores are found as glycogen and located within two storage compartments: liver and muscle (~100 g and 400 g respectively).[10,83] At these levels muscle glycogen is sufficient for ~120-150 minutes of moderately intense activity and liver glycogen has been reported to decrease to 40-60% after 90 minutes at 70% of VO_{2MAX}.[90] Furthermore, combined muscle and hepatic glycogen contribute only 4% of the total energy stores in the body.[91]

Despite being the largest of the two endogenous CHO stores, muscle glycogen does not contribute directly to blood glucose levels. Once glucose is converted to muscle glycogen that glucose is restricted to muscle cell use only. Through anaerobic metabolism, muscle glycogen can be converted via the Cori cycle to lactate, which is then converted to glucose in the liver and returned to the working muscle.[92,93] Prolonged exercise decreases muscle glycogen stores, resulting in muscle fatigue[94] and the dependence upon the limited stores of liver glycogen rises to fuel working muscle.

Endogenous glycogen stores are limited to ~100 g in the liver[10] and, in the absence of exogenous CHO, are responsible for maintaining blood glucose levels at rest and during exercise. The reliance on liver glycogen increases as muscle glycogen is depleted. At > 3 h of exercise, liver contributions to energy supply increase and may contribute up to 0.8 g/min[11]. This increase in glucose is a result of glycogenolysis (breakdown of liver glycogen) and gluconeogenesis (creation of glucose from non-glucose molecules) performed in the liver.[83] Unlike muscle glycogen, the liver can contribute glucose to working muscle[95], but interestingly unlike muscle glycogen, hepatic glycogen stores are not improved with training.[83] Exhausting the limited supply of liver glycogen leads to fatigue and hypoglycemia.[10]

These limited stores of endogenous CHO are problematic in the context of ultra-endurance sports when exercise regularly exceeds 4-h in duration, because glycogen depletion is a well-known cause of exercise performance decline.[10,94,96–98] Therefore, there is a heavy reliance upon exogenous CHO as an additional energy source with current recommendations of 30-90 g/kg/h.[13] Unfortunately, uncapped rates of exogenous CHO intake are not possible and are limited by: 1) unmodifiable physiological barriers to intestinal absorption, which have been reported to be ~1.0-1.2 g/min from all monosaccharide sources;[14,77] and 2) gastrointestinal tolerance, a highly individualistic but trainable characteristic.[99]

To illustrate, Jeukendrup *et al.*[11] observed that exogenous CHO oxidation plateaus at ~72 to 85 g/h (1.2 to 1.4 g/min) in ultra-runners creating a gap between glucose utilization of 96-132 g/h (1.6 to 2.2 g/min)[11,77] for which limited liver glycogen contributed 0.4 to 0.8 g/m. As a result, supplemental CHO oxidation may be unable to meet the demands of prolonged exercise. It should also be noted that during exercise, exogenous CHO can prevent liver glycogen depletion but cannot replete muscle glycogen.[77]

Gastrointestinal distress, which has a high prevalence in endurance athletes[8] is an additional obstacle to consuming these upper thresholds of CHO intakes. It is estimated that between 30 and 50% of endurance athletes report a GI incident, with higher rates in triathletes and runners.[22] Two ultra-distance studies reported a 65% and 82% incidence of GI distress in 60-120 km distances[20] and a 24-h trail race respectively.[19] The symptoms of GI distress: nausea, emesis, diarrhea, flatulence, abdominal bloating or pain [71] have been attributed to dehydration, food type, genetic predisposition, heat, splanchnic hypoperfusion, and the mechanical jostling of the exercise activity[22,43,99,100]; however, a direct link to CHO intake can be made. This link is evident with concentrated sugar solutions resulting in delayed gastric emptying or osmotic shifts associated with diarrhea, nausea, bloating and flatulence.[22,99,101] A small body of literature suggests that 'gut training' may be able to alleviate GI issues related to CHO intake at the individual level through nutrition interventions aimed at improving perceived gut discomfort, enhancing CHO receptors, and reducing malabsorption.[99,102]

2.4 Fat and Protein Metabolism

Contributions of fat to energy needs during aerobic activity also vary upon intensity. Regarded as the amplest form of stored energy, endogenous fat stores comprised of fatty acids, cholesterol and intramuscular triglycerides, and are estimated to be ~11,000-15,000 g with the capacity to maintain >100 hours of marathon running.[82,103,104] The abundance of fat has led some to believe moving away from CHO and solely relying upon fat to fuel prolonged endurance exercise is an optimal fueling strategy.[105] It has been established that fat oxidation rates are modifiable through diet and training interventions[87,106–108] with observed rates of maximal fat oxidation of 1.3 g/min in trained individuals[109] and > 1.5 g/min in ketogenic-adapted individuals.[107,110] Yet, relying completely on fat has not been supported by research due to limitations of lipids during exercise. These limitations are directly related to increased O_2 cost of fat oxidation resulting in decreased

exercise economy as well as the downregulation of CHO oxidation that occurs with high-fat, low-CHO interventions being responsible for negative performance outcomes that have been reported.[107,111,112]

Protein's limited role in fueling prolonged exercise via the conversion of amino acids to glucose through gluconeogenesis is estimated to be < 10% of total energy utilized.[113] Protein is not considered a primary fuel source, however, its contribution increases as the availability of CHO decreases.[33] To mitigate endogenous protein oxidation, which impedes recovery and contributes to lean body mass loss during endurance exercise, it has been demonstrated during a 6-h trial that consuming CHO and protein together is effective for aiding in protein balance.[114]

SECTION 3

3.1 Female Energy Metabolism

We know today that gene expressions and physiological differentiations exist between biological females and males. These differences may impact areas of sport performance through a range of interactions, including fluid balance, thermoregulation, recovery, fatigue, biomechanics, mitochondrial function, and energy metabolism.[115] Additionally, within-female differences may exist when comparing normally menstruating females to those using hormonal contraception as well as over the life-cycle accounting for the hormonal changes occurring in the spectrum of menopause.[116] For this section, evidence will be presented in the context of normally menstruating females in sport.

3.2 Female Carbohydrate Metabolism

Female ovarian hormones, E2 and progesterone are regarded as the primary drivers of substrate utilization differences between female and males. The variability of these hormones, which act antagonistically on one another,[27] over the menstrual phase in regularly menstruating females may impact CHO and fat metabolism. Specifically, E2 has been observed to promote glucose uptake into type I fibers[26], while simultaneously reducing uptake of glucose into the bloodstream.[27] Additionally, when directly administered to men, E2 lowered CHO and amino acid oxidation but increased fat utilization.[28] The effects of ovarian hormones on CHO and fat utilization were duplicated in a pharmacological suppression and replacement study among eight females who had E2 and progesterone administered to mimic three distinct hormone phases of the menstrual cycle (E2 low + progesterone low, E2 high + progesterone low, E2 high + progesterone high) during submaximal (60% of VO_{2MAX}) cycling for an hour.[27] These findings illustrated the suppressive effects of E2 on CHO mobilization and underpin a common belief that females burn more fat than CHO for fuel when fasted.[117] Preferential oxidation of fat over CHO expressed in lower resting expiratory ratio (RER), in eumenorrheic females, relative to lean body mass, when fasted during submaximal endurance exercise, as compared to males has also been observed in several studies.[28–35] As recently as 2019, Pettersson *et al.* observed similar outcomes in elite-level athletes at higher effort levels (70% of VO_{2MAX}) where, in the absence of CHO, females oxidized more fat and less CHO than men over 2 hours.[36] A proposed action of E2 on CHO metabolism is the regulation of

systemic glucose homeostasis via signalling to estrogen receptors located on fat cells that reduce the liberation of free fatty acids into blood circulation.[118] However, this is at odds with the previously demonstrated concept of E2 creating an environment preferential to fat oxidation making it difficult to interpret and apply mechanisms at rest into exercise environments.

Of note, when exogenous CHO is available during exercise the differences in substrate utilization between men and women may be greatly reduced.[33–36] Three studies regarded by Kuikman *et al*.[119] in an audit of all CHO-related performance studies in women to have "sufficient methodological controls" support this observation. Petterson and colleagues also demonstrated in elite female cross-country skiers exercising at 70% of VO_{2MAX}, that total CHO and FAT oxidation were not significantly different when exogenous CHO was provided (male: fat 77 g and CHO 262 g; female fat 76 g and CHO 251 g). These researchers also found that females during endurance exercise can: 1) greatly increase CHO oxidation when provided CHO; 2) utilize more exogenous CHO as a percentage of total energy oxidized than males; and 3) oxidize less fat and more CHO during exercise when exogenous CHO is available, reducing endogenous carbohydrate utilization similar to males.[36] Likewise, both Wallis and Tremblay found that during submaximal endurance exercise (57-67% of VO_{2MAX} respectively) over 2 hours, the female metabolic response to CHO during exercise is similar between males and females with no appreciable differences in RER (M: 0.92 and F: 0.92) or rates of oxidation (M: 0.70 and F: 0.65 g/min).[34,35] Thus, it appears that exogenous CHO during exercise decreases E2's hepatic glycogen sparing effect responsible for the metabolic shift towards fat in the absence of CHO.[37,38]

Lesser known is the interaction of progesterone on CHO metabolism although, from the limited research available, it appears to reverse E2's suppression of muscle glucose oxidation during submaximal exercise[27] as well as complement E2's glucose-sparing effect in the liver.[39] Interactions of progesterone also appear to affect glucose metabolism through the upregulation of glucose transporters GLUT(s) expression to increase endometrial glycolytic metabolism, attenuating skeletal and hepatic glycolytic pathways.[26,40,41] During sleep, progesterone has been observed to increase energy expenditure up to 5% in the LUT phase; however, the total energy used over a 24-hour period was not significantly altered.[42]

Currently, the mechanisms of progesterone on metabolism continue to be poorly understood. Early impressions of progesterone on fat metabolism suggested that an increase in insulin secretion at

rest favouring fat storage occurs in addition to a suppressive effect on estrogen-mediated fat oxidation.[39] Recent studies on the effects of menstrual cycle and ovarian hormones on whole-body fat metabolism in humans are limited but suggest no interaction between them.[120–122]

However, the role of muscle fiber type and distribution may play an important role in fat utilization. There is evidence indicating a generalized difference in the make-up of muscle between males and females represented by the gradient of contribution from each fiber type (female type 1 > IIA > IIX compared to male type IIA > IIX > I) with type I representing 'slow twitch' oxidative fibers and type II considered 'fast twitch' or highly glycolytic driven fibers.[123] It should be noted that a body of research exists suggesting muscle fiber type can be adapted with chronic training stimuli[124] and this may not be accounted for in the discussion of energy substrate utilization comparisons between highly trained male and female athletes. The assumption that type I muscle fibers are more dominant in trained and untrained females, irrespective of sport discipline, is a possible explanation for the preference to oxidize fat over CHO in addition to expressing greater resistance to fatigue.[125] There are caveats to this broad belief, as sex comparisons of muscle fatigability may be more dependent on the muscle groups tested than a generalized sex dimorphism.[126]

Additionally, there is evidence showing higher concentrations of intramyocellular lipid (IMCL), stored predominately as triglyceride within the muscle for use during exercise[127], in female muscle before and after endurance exercise.[128] This storage alludes to greater availability or accessibility to alternate energy sources resulting in greater contributions to energy needs in females. In a comparison of 21 females and 21 males categorized into three groups (untrained, moderately trained, and endurance trained) Steffensen *et al.* observed a 25% decrease in intramuscular lipid concentrations in a pre-post exercise comparison at 60% of VO_{2MAX} during the mid-follicular phase compared to males.[129] These data indicate that IMCL contributes meaningfully towards total energy utilized during endurance exercise in females. Of note, the absence of menstrual cycle verification and exogenous CHO during this Danish study should be considered limitations.

3.3 Protein Metabolism

Protein is not a preferred energy source at rest or during exercise and is utilized sparingly. Protein's contribution to the glucose pool from the breakdown of amino acids is considered to be < 10%, even during prolonged endurance events when glucose levels are limited.[33,113] This contribution does not appear to differ during exercise between males and females across the menstrual cycle[33,35,36,130] despite levels of progesterone, up to 25-times higher in the LUT phase, facilitating increased amino acid oxidation.[25,27] The implications of this in a sporting context are unknown as there are no comparative studies of protein contribution to total energy usage between menstrual phase among females at peak progesterone levels during LUT phase.

3.4 Energy and the Menstrual Cycle During Exercise

Several studies have reported lower RER in women compared to men at rest and during fasted exercise indicating a greater reliance on fat over CHO.[28–35] However, when CHO is provided during exercise this difference is negated with an interesting observation that females will utilize more CHO from exogenous sources than males, thus reducing fat oxidation.[33–36] These observations imply that energy metabolism during exercise across the menstrual cycle is highly influenced by diet, fed state, and availability of CHO.[131]

When viewing the existing literature through the lens of ultra-running there are three stand-out points. First, genetic differences in muscle fiber[123] may contribute significantly to female success in the sport.[125] Second, well-established studies demonstrating that E2 enhances fat oxidation when fasted during submaximal exercise[28–35] suggest that when fueling strategies breakdown and CHO availability declines over prolonged efforts, females may realize performance advantages. Third, the influence of ovarian hormones contributing to greater efficiency in the use of exogenous carbohydrate sources toward total energy needs may offer performance benefits.[36]

SECTION 4

4.1 Continuous Glucose Monitoring Introduction

Carbohydrate is a limited resource in ultra-endurance running. Its depletion from blood glucose or muscle glycogen stores is widely recognized as a primary contributor to fatigue and performance decline.[13] In contrast, excess exogenous intakes of CHO are directly related to gastrointestinal distress which occurs at high rates among endurance sport athletes[8] and contributes to poor performance outcomes.[21,22] Therefore, it is of interest within the sports performance industry to investigate CHO fueling strategies such as the implementation of near real-time blood glucose monitoring.[132]

Improvements in hardware size, software interfaces, and mobile technology integrations have made the real-time tracking of biomarker data such as blood glucose with devices like the CGM more achievable in a variety of environments.[133] The first commercial CGM units, available in 1999-2000[134], were originally developed to improve management of type I and type II diabetes[66] and replace fingerstick management methods.[65] These CGM devices initially required transdermal implantation to obtain blood glucose data from interstitial fluid using a reactive nanofilament inserted below the skin. The filament converts glucose to hydrogen peroxide which generates an electrical signal that is relayed from sensor to reader unit. Early models also required a physical connection between the sensor and reader unit with data needing to be downloaded for analysis by a healthcare professional.[66,135,136] Wireless sensors became available in 2004 paving the way for near real-time data in 2005. Further innovations led to user-applied devices that can be applied easily to the body within minutes and can provide data wirelessly to a reader device. Additionally, up to the year 2018 CGMs required regular calibration using self-monitoring of blood glucose with capillary blood sampling readings[134]. Some current generations no longer require calibration [132,134] and provide near real-time glucose data 24/7 in intervals of 1–5 minutes providing hundreds of data points throughout the day providing a comprehensive overview of glycemic status[137] for up to 14 days.[65,132]

Continuous near real-time data derived from CGM provides previously unobtainable insights into carbohydrate intake and its effect on glucose variability. Over time, the continuously collected data can be used to construct a complete picture of historical blood glucose fluctuations while

providing acute trend and rate of change insights. When combined with established reference ranges of blood glucose control at rest[138] and during exercise[138,139] for diabetes mellitus, the benefits of CGM play a key in role in adequately managing the disease by reducing complications and slowing progression.[140] However, how this data can be used or interpreted for performance benefits in healthy individuals remains unverified as does: 1) established reference values for blood glucose levels in healthy athletes during exercise;[141] and 2) guidance on what levels of normoglycemia are.[141]

Nonetheless, a manufacturer's claims about their sport CGM device states "a glucose monitoring experience that will enable athletes to understand the efficacy of their nutrition choices during training and competition. It will therefore inform athletes about how to fuel appropriately, to fill their glycogen stores prior to a race and to know when to replenish during a race to maintain athletic performance."[142] Evidence supporting how this can be accomplished has not been elucidated in the current literature.

The following sections discuss the underlying technology and historical evolution of CGM hardware, establishing a foundation for exploring the potential applications of contemporary technologies to nutrition strategies for ultra-running.

4.2 Blood Glucose Overview

4.2.1 Blood Glucose Levels

A commonly used range for normoglycemia is a blood glucose concentration between 3.9 and 5.6 $mmol \cdot L^{-1}$.[143] with the lower threshold for normoglycemia being 3.9 $mmol \cdot L^{-1}$ and postprandial glucose levels up to 7.8 $mmol \cdot L^{-1}$ within 2-hours after eating. These guidelines aid the diagnosis and management of diabetes mellitus in clinical practice but differ significantly from the clinical assessment of hypoglycemia in healthy, non-diabetic, populations, which is assessed using Whipple's Triad; a cluster of symptoms that include: 1) anxiety, dizziness, rapid heartbeat; 2) blood glucose < 3.0 $mmol \cdot L^{-1}$; and 3) resolution of those symptoms when blood glucose levels are restored.[67,68] Low blood glucose concentrations, which can occur when awake, asleep, or during exercise is often asymptomatic until clinically significant levels are reached.[67,144]

In healthy individuals, blood glucose is managed by a complex interplay of hormones, neuropeptides, skeletal muscle, and organ systems. In the absence of disease, blood glucose is

regulated by the release of the corresponding hormone used to regulate glucose by the body. Secreted from the pancreas, the hormones insulin and glucagon are the primary regulators of glucose in the bloodstream.[145] Antagonistic to one another insulin lowers glucose levels while glucagon increases it to maintain overall blood glucose within a specific range (glucose homeostasis).[145] For example, low blood glucose is corrected endogenously by the release of glucagon from the pancreas to initiate the breakdown of liver glycogen into glucose (hepatic glycogenolysis) to be released in the bloodstream.[145] Alternatively, exogenous CHO can be consumed to correct for low blood glucose levels.

On the other hand, hyperglycemia is defined as blood glucose > 6.9 $mmol \cdot L^{-1}$ fasted or > 10 $mmol \cdot L^{-1}$ 2-hours after a 75 g CHO test.[146] In healthy populations, the liver secretes insulin in response to elevated blood glucose which supports the insulin-dependent uptake of glucose into cells via the insulin-responsive GLUT 4 glucose transporter, thus pulling glucose out of the bloodstream and reducing levels.[147]

In contrast to healthy glucose control, the inability to prevent hyperglycemia, a state of elevated blood glucose, via insulin insufficiency or resistance, is a key characteristic of diabetes mellitus with maintenance of glucose homeostasis being the primary goal of management.[141,148] Chronic hyperglycemia is associated with metabolic disorders and the development of a wide variety of cardiovascular, nervous system, renal and cerebrovascular diseases.[148,149] The three most common forms of diabetes mellitus are: Type I, an autoimmune disorder affecting insulin secreting cells of the pancreas resulting insulin insufficiency; Type 2 (or adult-onset) which accounts for 90-95% of all diabetes mellitus cases is associated with insulin resistance leading to pancreas dysfunction; and gestational diabetes mellitus, that is characterized by poor blood glucose control occurring during pregnancy.[148]

Several additional internal and external factors influence blood glucose levels: food type and quantity, physical activity, disease states, medications, stress, acute or chronic pain, menstrual cycle hormones, hydration status, and alcohol uptake.[27,150–152] A common characteristic of these factors appears to be, except for general food intake, their influence on the liver. For example, carbohydrates from food increase circulating glucose before it is taken up into the cells.[153,154] During physical activity, pain and stress-induced epinephrine are responsible for insulin resistance and subsequent increases in blood glucose levels.[155] The mechanisms of pharmacological increases

in blood glucose are beyond the scope of this review, but some induce insulin resistance as is the case with many glucocorticoids.[156] Alcohol can either raise or lower blood sugar dependent on the quantity ingested and the presence of food.[157] Dehydration decreases plasma volume, thus increasing glucose concentrations.[158] During the menstrual cycle, hyperglycemia is associated with the luteal phase and elevated progesterone levels.[150]

The connection between blood glucose levels and adverse health outcomes[159] is driving a growing interest in more tightly controlled glycemic variability in blood glucose levels in healthy populations, as well as potential applications to enhance sports performance.[160] Prior to the previously mentioned advancements in CGM technology, and its introduction commercially as a sports device, the management of blood glucose or insights into glucose variability in the absence of disease was impractical. Despite the process of data collection becoming easier, there are important unanswered questions when trying to implement therapeutic controls in a healthy athletes: 1) there is no definition of what day-to-day blood glucose control 'normoglycemia' should look like[141]; 2) no parameters exist for blood glucose ranges during activity; 3) there is no clear consensus on how transient episodes of hyperglycemia impact performance during exercise; 4) currently, minimal evidence connects blood glucose ranges to aspects of performance refueling or recovery[161]; and 5) there limited insights exist into the variability of glucose levels during real-world ultra endurance scenarios in males or females.

4.2.2 Interstitial Glucose

The convenience and enhanced accessibility of glucose data from current CGM technology, which allows for non-invasive blood glucose monitoring, is possible via interstitial glucose. Fluid within the human body is contained within two compartments: intracellular and extracellular. Intracellular fluid is enclosed within the cells. Extracellular or interstitial fluid lies under the skin between the tissues accounting for 1/3 of total body fluid and is the second largest volume of body fluid.[46] Within interstitial fluid, many biological substances such as oxygen, proteins, glucose, and other nutrients as well as waste products can be found. Most relevant is the relatively high presence of detectable glucose in interstitial fluid enabling it as a suitable surrogate for invasive blood sampling.[50] Furthermore, measuring interstitial glucose provides close to real-time data because it lacks clotting factors and remains fluid allowing for continuous monitoring.[162] There are, however, drawbacks to the use of interstitial glucose for monitoring blood glucose. Most notable is the

physiological lag time between interstitial glucose and blood glucose taken directly from the blood (~5 to 6 min).[51,52] The lag is a result of the time required for fluid to diffuse into interstitial spaces from the capillaries.[55,163] Additionally, when compared to blood, the wide variation of pH within interstitial fluid can affect the accuracy of glucose detection.[50]

4.3 Summary of Sensor Technology

First generation CGM sensors were approved by the Federal Drug Administration (FDA) and introduced commercially in 1999 for the management of diabetes mellitus.[66] Over the past 20 years of development they have successfully been integrated into the management of diabetes mellitus by: 1) replacing the painful and inconvenient practice of repeated 'fingerstick' blood testing; 2) providing 24/7 near real-time monitoring of blood glucose via interstitial glucose; 3) offering detection and warning of adverse events such as hypo/hyperglycemic episodes; and 4) providing insights into long-term blood glycemic variability.[54]

Commercial CGM sensors have three main components: sensor body (receiver, transmitter, sensor), reader device and sensor applicator. The applicator may have a spring-loaded mechanism that pierces the skin with a small needle and inserts a nanofilament beneath the dermis. In contact with subcutaneous interstitial fluid, the nanofilament sensor reacts enzymatically, via a glucose oxidase (GOx) based electrode, to the presence of glucose creating an electrical signal.[136] The signal is generated by the concentration of glucose present[164] and can be interpreted by software on the reader device.[66,136]

Advances in technology have provided significant improvements in CGM functionality. Frequent user calibration of CGM units with SMBG was a necessary step to ensure accuracy of measurement.[164] That step is no longer required in most models and is done under a controlled process during manufacturing.[132,134] Data can be transferred from sensor to reader wirelessly, unlike early models which required a hardwired connection. These advances have led to near real-time data interpretation when compared to older methods that required the download of stored data to be reviewed by a professional.[134] Application of commercial CGM units can be performed by the user without professional supervision.[165]

4.3.1 Real-time CGM

Sensors are often classified into two categories: intermittent (iCGM) or real-time (CGM). Intermittent iCGM sensor required scanning by an external reader device to obtain the collected glucose data and were unable to provide alerts or alarms. Real-time CGM wirelessly transmits continuously to a paired device and provides the benefits of alerts for blood glucose trends.[48,166] The use of the term real-time is ubiquitous in the literature but requires some clarification. Due to the combined physiological and technical delays,[52] CGMs should more accurately described as near real-time. For continuity, the use of real-time or CGM has been used throughout this document to reflect the current generation of wirelessly capable CGM sensors for use in sport.

4.3.2 CGM Sport Specific Devices

Five new generation CGM devices from three manufacturers (Abbott, Dexcom, and Medtronic) are approved for diabetes management in various countries (**Table 1**). Only Abbott devices are marketed for sporting use by multiple wellness companies and, as of 2021, the availability of the Abbott sports devices was limited to eight European countries (Austria, France, Germany, Ireland, Italy, Luxembourg, Switzerland and the United Kingdom). The FDA has not approved their use in the United States. The only notable technical difference between the Abbott Freestyle Libre, Libre Sense and Libre Freestyle 2 is the method of data transfer. Libre Sense, a CGM, provides push technology via Bluetooth sending data to mobile or reader devices. In contrast, Freestyle 2 and Libre iCGM devices both require the sensors to be scanned with a reader device to collect data. Differentiation between these devices is found in the mobile application software and the proprietary algorithms provided by respective companies.

Table 1. Overview of popular CGM systems

Device	Company	Purpose	Data	Report Rate	Life	Placement	Interactions
G6	Dexcom	DM	CGM	5 minutes	10 d	Arm	Accurate up to 1 g acetaminophen every 6 h
G7	Dexcom	DM	CGM	5 minutes	10 d	Arm	Accurate up to 1 g acetaminophen every 6 h
Guardian 3	Medtronic	DM	CGM	5 minutes	7 d	Abdomen	Acetaminophen reduces accuracy
Libre 2	Abbott	DM/ Sport	iCGM	manual scan	14 d	Arm	
Libre Sense	Abbott	DM/ Sport	CGM	1 minute	14 d	Arm	

iCGM intermittent ; CGM real-time ; DM Diabetes Mellitus Type 2

4.3.3 Methods and Standards of Measure

Reliability, accuracy, accessibility of data, and cost are considerations when assessing the viability of a glucose monitoring system within a clinical setting.[167] Among the healthy athletic population, there are no health risks associated with precision or reliability in a CGM sensor. In the same population, data accessibility and cost are issues of convenience. However, accuracy does maintain a high level of importance when trying to understand how CGM data can be applied to an athlete fueling plan.

Accuracy for CGM is typically assessed using the mean absolute relative difference (MARD)[48]; the average of the absolute error between all collected CGM values when matched with reference capillary SMBG values from study participants.[168] The MARD is a complete measure of the accuracy of the sensor and algorithm that comprise the CGM system. A small percentage value indicates that CGM sensor readings are close to the reference glucose value; whereas, a larger MARD percentage indicates greater discrepancies between the CGM and reference glucose values.[48] For example, a MARD of less than < 10% is considered good performance and viable for clinical decision making.[48,168] This threshold differs from SMBG which adhere to an International Organization for Standardization (ISO) measures. To date, no ISO performance standard exists for CGM monitoring systems.[48,168] The accuracy of CGM sensors relies greatly upon the software algorithms used to predict blood glucose from CGM data;[168] however, the

comparison of CGM sensors to alternative methods of blood glucose monitoring such as SMBG is also hampered by a lack of standardization. For example, comparing accuracy between SMBG to CGM cannot be made easily because they are held to different standards of measure. As noted, an internationally defined standard of measure (ISO 15197:2013) established by the International Organization for Standardization is in place for SMBG; while MARD is the unofficial standard of measure against which CGMs are compared.[59]

Early CGM devices from in the 2000's, prior to MARD rating, had an error rate of ±20%.[169] In 2021, devices such as the Dexcom G6 and Abbott Freestyle Libre reported MARD values of 9.9%[170] and 9.2%[171] respectively within the diabetic study populations. Approval documents from the U.S. Food and Drug Administration (FDA) do not require a MARD performance rating but instead specify accuracy ranges at specific blood glucose levels, summarized in **Table 2**.[172]

Table 2. FDA required CGM sensor accuracy at specified blood glucose ranges

Blood Glucose Range	Performance Range ±	Minimum Acceptable Accuracy	
< 3.9 mmol·L^{-1}	15%	80%	
	40%	98%	All readings ±20%
3.9-10 mmol·L^{-1}	15%	70%	over entire range
	40%	99%	
> 10 mmol·L^{-1}	15%	80%	must exceed 87%
	40%	99%	

The National Institute of Clinical Excellence, responsible for assessment of safety and efficacy of medical devices in Europe, does not have a robust set of guidelines or published criteria for approval of CGM devices. Therefore, a comparison of FDA to European reference values cannot be made. This, and other shortcomings in the assessment of CGM accuracy guidelines in Europe, were comprehensively laid out in a review by Pemberton *et al.*[173] Existing devices will retain pre-2022 approval status but will be met with increased scrutiny in subsequent years as new guidelines and approval polices are implemented. These changes in legislation leave doubt on future approval of new and existing devices when proposed changes are enacted.

4.4 Summary of Limitations and Concerns

Generalized limitations of CGM exist with a varying degree of relevance due to the rapidly changing capabilities of sensor technology which has outpaced peer-reviewed research. For example, concerns of sensor convenience, cost[54], calibration[54,65,66], and data accessibility[54], are far less relevant today. On the other hand, the issue of accuracy[54,66], which is multi-factorial and includes both physiological and technological variables persists, but the gaps reported in prior research have been narrowed.[174] Additionally, new concerns such as the lack of data analysis standards[54] and medication interactions[65] have emerged.

4.4.1 Volatility of ISF and Lag

A proposed concern is the reliability and accuracy of glucose readings taken from ISF due to frequent changes in factors that affect glucose sensor reliability: pH level, physiological lag time of glucose changes from blood to ISF, and lower concentrations of glucose in ISF that blood.[50–52]

Lag time can be separated in two categories: physiological and technical. Glucose concentrations in the blood are not reflected immediately within the ISF and a delay or lag time for diffusion occurs that represents the basis for a physiological lag. A prototype sensor study conducted in 2018, observed a 5–6-minute delay in glucose diffusion to the ISF and reflects a faster process than earlier predictions of 5-10 minutes.[51] Technological lag is comprised of the various interferences associated with the hardware and software components of the sensor. These include but not are not limited to: software algorithms, electrode membranes, electrochemical interferences from biological substances within ISF, trauma from sensor insertion, and blood flow to the sensor application area.[53] Early sensors were reported to have had a combined ~15 minute delay in interpreting interstitial glucose but advances in technology have supposedly reduced this gap within minutes.[52,54] However, in 2019 Zaharieva *et al.*[55] observed lag time to be 12±11 min during low to moderate intensity exercise (45-55% of VO_{2PEAK}) using a new generation sensor (Dexcom G5).

4.4.2 Fluctuating Glucose Concentrations

Additionally, the accuracy of CGM sensors has been questioned when concentrations of glucose change rapidly. In one study by Pleus *et al.*[56] the accuracy of two second generation sensors were tested in a non-exercise setting: a commercial Dexcom G4 sensor and an experimental unnamed device. These authors observed that the accuracy of the older generation sensor (G4), as measured by MARD, was negatively affected by rapid rates of glucose changes (< 0.2 $mmol \cdot L^{-1} \cdot min^{-1}$ and > 0.2 $mmol \cdot L^{-1} \cdot min^{-1}$); yet the newer generation device was far less impacted (G4 24.9% and 29.6% vs unnamed experimental 10.6 and 16.3%; respectively).The study concluded that newer CGM technology may overcome accuracy concerns in the presence of rapidly changing glucose levels.[56] However, despite the evidence of improved accuracy during rapidly changing glucose levels in non-exercise conditions using an experimental sensor, newer generation commercial devices continue to show inconsistency during exercise. In another study, Zaharieva and colleagues, utilizing the Dexcom G5 sensors, also observed MARD was impacted negatively (6-22%) with significant errors during rapidly rising and declining glucose levels over prolonged exercise.[55]

4.4.3 Impact of Hypoglycemia on CGM Accuracy

An observed weakness of CGM devices is accuracy during episodes of hypoglycemia related to a combination of lag time, low glucose concentrations, and rapidly changing glucose levels.[56] Clinically significant non-diabetic hypoglycemia occurs at rest or during exercise and is indicated by glucose concentrations of < 3.0 $mmol \cdot L^{-1}$.[67,68] Symptoms include rapid heart rate, sweating, anxiety, irritability, confusion, dizziness, and decreased exercise performance.[175,176] Typically during exercise, mechanisms of increased hepatic gluconeogenesis and glycogenolysis as well as elevated fatty acid contribution to energy needs offers protection against hypoglycemic episodes.[177] These mechanisms are often sufficient to maintain blood glucose in sports lasting up to 90 minutes;[177] however, prolonged endurance exercise can overwhelm compensatory mechanisms as energy needs exceed both available muscle glycogen and glucose stores.

Moser and Eckstein *et al*. reported a low rate of accuracy (MARD = 31.6%) when hypoglycemia occurred during exercise in 16 Type 1 diabetic participants who were provided a pre-exercise CHO meal of 1 g/kg bodyweight 45 minutes before moderate intensity cycling.[57] Similarly Aberer *et al.*[58] tested three CGM devices (Freestyle libre, Dexcom G4, Medtronic 640G) in Type 1 diabetic

participants under controlled conditions of daily activity, mealtimes, and 60 min of exercise, over 12 hours. In non-healthy participants, all devices experienced the highest MARD levels during low levels of blood glucose < 3.9 mmol·L^{-1}.

4.4.4 CGM Accuracy at Rest vs Exercise

A collection of studies has focused on the comparison of CGM accuracy at rest and during exercise in diabetic and non-diabetic populations.[55,57,59–63] Consensus among them is that MARD worsens during exercise when compared to rest. In a comprehensive review of 54 studies on CGM accuracy from 2006-2020, Fabra *et al.* [64] reported a wide range of accuracy (ΔMARD -4.4 to 18.1%) when comparing glucose data at rest (MARD = 13.3 ± 3.22%) to exercise (MARD 17.5 ± 5.88%). The high variability in glucose reporting from rest to exercise and the generally observed reduction in accuracy during exercise has been attributed to the previously outlined mechanisms of rapidly changing glucose concentrations as well as factors associated with interstitial fluid volatility.

It is worth noting that limited CGM accuracy studies in healthy populations exist. In 2022, Clavel[178] studied eight healthy athletes using the Freestyle Libre sensor during interval and short low-intensity running. Their findings are in agreement with the existing literature using diabetic participants: 1) the CGM was unable to detect hypoglycemia as indicated by fingerstick capillary glucose; and 2) the accuracy decreased significantly when exogenous CHO was consumed 2-hours before activity at 1 g/kg bodyweight. During exercise, MARD was 16.2% after CHO ingestion as compared to 9.4% after meal, 7.1% before exercise, and 10.1% after exercise.[178] Interestingly, in 2020, Guillot[179] indicated that the accuracy of 6th generation technology (Dexcom G6) was not "significantly impacted during aerobic, resistance, or HIIT exercise" in 25 Type 1 diabetic adults for 50-60 minutes exercise; yet MARD reported from start to end (summarized in **Table 3**), reflected a trend for decreased accuracy over time for all exercise modalities. The author also observed a tendency for the sensor to underestimate glucose at most time points.[179]

Table 3. Summary of %MARD G6 during exercise - Guillott 2020[179]

Exercise Minutes	0’	50'	60'
Aerobic MARD	10%	-	14%
Resistance MARD	9%	-	15%
HIIT MARD	17%	12%	-

MARD: Mean absolute relative difference

4.4.5 Drug Interactions

Drug and supplement interactions pose an interesting category for accuracy concerns. Mian *et al.*[65] summarized a list of substances with known interactions affecting the accuracy of some CGM sensors due to their interference with certain types of enzymatic reactions used on sensor filament to detect glucose within the ISF. The most common and relevant to a healthy athletic population are ibuprofen, acetaminophen, and Vitamin C.[65]

4.4.6 Accuracy of CGM Across Sensor Lifespan

Recently, Tsoukas *et al.*[180] assessed the accuracy of the Abbot FreeStyle Libre, which has a lifespan of 14 days, in 14 Type 1 diabetic non-athlete participants over 24 h in a closed research environment. Sensor accuracy was tested at 0-1 days, 5-7 days, and 13-14 days and compared to reference blood glucose values taken with a separate analyser. The MARD values for the sensor were least accurate on days 0-1 (14.5% and 11.2%), and days 13-14 (14.7% and 11.2%) with days 5-7 being the most accurate and ranged from 6.6 to 7.8%. Based on the current evidence, with accuracy determined by MARD, there appears to be no predictable or consistent decline in accuracy as the device ages. Of note, only one study could be found reporting CGM sensor accuracy in non-diabetic athletes. Thomas *et al*.[181] using a Medtronic Minimed subcutaneous CGM sensor found that sensor accuracy decayed over time during cycling in 10 sub-elite endurance athletes (7 male; 3 female). During cycling MARD was 11% at 30 minutes, while at 60-150 minutes accuracy was decreased to 20%.[181] This decrease indicates the potential accuracy concerns with use in prolonged endurance sport. However, this study used older technology and may not be

representative of newer generations of CGM sensors technology due to improvements in algorithm predictions and nanotechnology sensitivity.

4.5 Challenges for Use in Sport

While not life threatening to non-diabetic athletes, the data to date suggests that sensor accuracy may suffer in the presence of just one of these variables: high rates of glucose change, low glucose concentrations, prolonged use, and in the presence of commonly used NSAIDs or supplements.[56,65] These limitations foreshadow potential obstacles for use in both short duration and endurance sports that experience these situations frequently and in combination. Specific to ultra-running, characterized as extremely protracted, moderately intense exercise, with a high frequency of bolus CHO intakes, and high rate of change in available CHO stores, the confluence of many known variables known to impact CGM accuracy (**Figure 3**) indicates that it may be difficult to rely on CGM to inform fueling decisions.

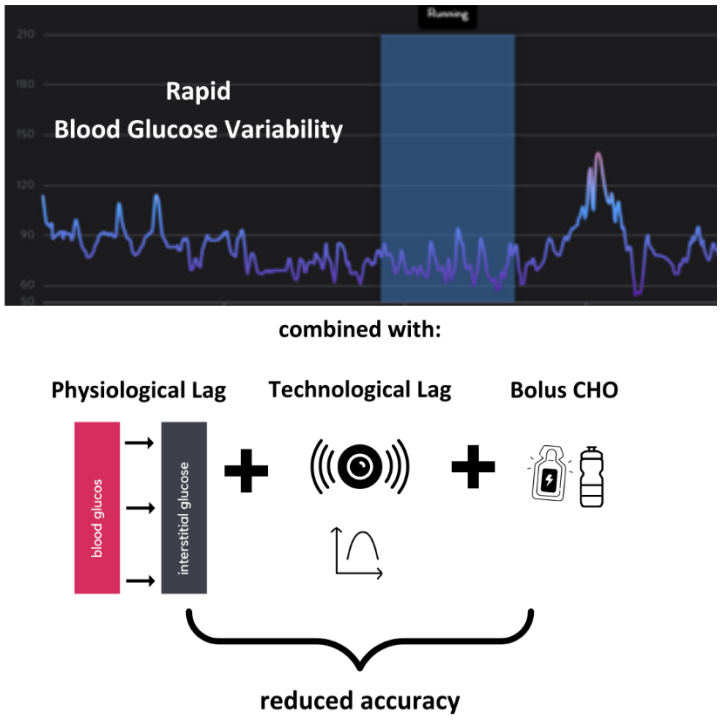


Figure 3. Variables affecting continuous glucose monitoring accuracy during running.

From a convenience perspective, a lack of wearable reader devices was an early obstacle to using CGM in the field. Accessing data from mobile phone devices was a prohibitive inconvenience. To solve this problem wearable wrist band and possible output to sport watches have been either proposed or brought to market; making it easier to view the glucose data during activity. Despite the ubiquity of Bluetooth™, however, the wireless connection between devices is not 100% reliable and this can directly impact data collection as well as software projections of glucose. Blocks of glucose data can be erroneous due to sensor error or be missing entirely for meaningful periods of time lasting 5-10 minutes. This issue was observed during a review of data from this PhD study following 12 adult regularly menstruating females during prolonged running while wearing the Abbot Libre Sense CGM sensor; whereby 25% of the data points at specified times were not captured. Additionally, it is noted that the sport sensors are self-calibrating with no SMBG verification requirements. In the event of a faulty sensor, a runner would be unaware of poor connectivity, missing data, and potentially make fueling decisions with an inaccurate representation of CGM-estimated blood glucose.

4.6 Existing CGM Research in Ultra Running

Existing research on the use of CGM in an ultra-running environment is limited to three studies. The first is an observational study by Ishihara *et al.*[70] which collected glucose data from four males and three females during a 165 km ultra trail running event using the Freestyle Libre iCGM sensor. Three notable outcomes from this study were observed. First, that a significant correlation between higher CHO intake and higher sustained running pace existed, but no relationship was seen with higher glucose levels. Second, on average, runners maintained a blood glucose range between 4.0 $mmol \cdot L^{-1}$ to 14 $mmol \cdot L^{-1}$ over 165 km. The lowest recorded glucose level during the event was 3.4 $mmol \cdot L^{-1}$ obtained from a male participant as compared to the lowest female measure of 4.4 $mmol \cdot L^{-1}$. Third, female running speed was less impacted by large changes in glycemic variability (difference between resting and highest blood glucose values).

An additional observational study from 2021, Ishihara[182] was limited to observing glucose collected via CGM and its comparison to researcher documented food intake in a single female ultra-runner over a multiday 438 km trail run. Nonetheless, some interesting occurrences were reported: 1) macronutrient type did not significantly impact glucose levels; 2) the runner avoided glucose below < 3.9 $mmol \cdot L^{-1}$ for the duration of the event; and 3) running pace was correlated

with CHO intake. The authors acknowledged potential limitations of the CGM, specifically the sensors inability to detect low blood glucose events due to low accuracy as previously discussed.

A third observational study, completed by Sengoku *et al.*[183], of two male ultra-runners wearing iCGM during a 100-km ultra run, found that higher rates of exogenous CHO did not guarantee prevention of low glucose events of < 3.9 mmol·L^{-1}. Despite a higher total intake of energy and grams of CHO runner B (1,599 kcal, 366 g CHO) recorded a rapid decline in blood glucose (5.6 mmol·L^{-1} to < 3.3 mmol·L^{-1}) over the last 70 min of the event; whereas runner A (1,125 kcal. 249 g CHO) remained normoglycemic.

There are notable limitations for these three studies. Combined there are a total of 10 participants, none of the studies provided an experimental component or simultaneous methods of SMBG verification to confirm the accuracy of CGM glucose data during the event, and no controls for menstrual phase or hormonal contraception for female participants were implemented.

4.7 Potential of CGM in Endurance Sport

The application of CGM technology for sport performance is novel and the few proposed uses for enhanced performance remain untested. The most promising ideas appear to be focused on the optimization of CHO intake strategies: enhanced glycogen storage, improved timing of carbohydrate intake during exercise, individualized carbohydrate strategies, and reduced inflammation.

4.7.1 Individualized Fueling Strategies

It is well established that CHO intake improves sport performance.[13,16,184,185] Additionally, the use of exogenous CHO during prolonged exercise is believed to enhance performance by preventing or delaying hypoglycemia;[77,89] however, the ability for the human gut to tolerate CHO is limited by physiology as well as individual tolerance.[11,14,77] In particular, GI distress related to CHO intake is a common occurrence during endurance events.[19,20,22,186,187] To minimize the occurrence of GI issues, the CGM has been proposed to potentially inform a more strategic intake of CHO during exercise in lieu of following generic intake recommendations.

Implementing daily nutritional approaches that ensure adequate energy availability (EA), expressed as energy intake (EI) minus exercise energy expenditure (EEE) divided by fat-free mass

(FFM), is also essential for sports performance. These strategies play a central role in minimizing the risk of low energy availability (LEA), a condition to which athletes are particularly susceptible.[188,189] Characterized by an intake of less than 30 kcal/kg of FFM for four or more consecutive days in females,[188] LEA is associated with detrimental effects on athletic performance. It has been asserted by Bowler *et al.*[132] that CGM may have potential as a screening tool for LEA[132] based on observations of low blood glucose and more frequent excursions into hypoglycemia during times of low energy intake.[190]

4.7.2 Enhanced recovery

There are claims, based upon established interactions between chronic hyperglycemia and the activation of an inflammatory cascade involving cytokines, tumour necrosis factor-alpha, and interleukin-6 within the body, suggesting that information derived from CGMs may be useful to enhance sport recovery. The enhancement in recovery would be achieved by offering continuous insights into glucose levels, informing dietary interventions to reduce excessive glucose levels and subsequently minimizing the frequency of inflammatory events.[191] The mechanism is a complicated positive feedback loop whereby glucose and hyperglycemia cause inflammatory responses, while inflammation causes increased glucose to be released into the bloodstream, thus perpetuating hyperglycemia. Ultimately, this feedback loop is controlled by the introduction of insulin and subsequent reduction in blood glucose.[191] Of note, these observations are typically only associated with unhealthy populations or the critically ill; however, their extrapolation into athletes fueling with high sugar and CHO strategies or performing exhaustive endurance exercise are reasonable considerations for use of CGM as a performance aid.

The implications of energy requirements on blood glucose control were observed by Thomas *et al.*[181] in 10 healthy sub-elite male athletes over six days using CGM. It was originally hypothesized that hypoglycemia would be a frequent occurrence. However, their data suggested that adequate energy intake results in hyperglycemia with 4/10 subjects having blood glucose > 6.0 $mmol \cdot L^{-1}$ for 70% of the trials. Interestingly, they also attributed one participant's frequent hypoglycemic episodes to inadequate energy intake.

In a later study, Kulawiec *et al.*[161] observed a persistence in elevated blood glucose during overnight periods for 3 days after a time trial to exhaustion in 8 of 10 participants using a CGM in

healthy sub-elite athletes that performed indoor cycling. These authors attributed the disruption in glucose control to the systemic inflammation resulting from exhaustive endurance exercise. Both studies lend credibility to the potential application of CGM in sport performance, as the sensors can provide continuous insight into glucose levels. This information could inform athletes and practitioners about the frequency and duration of inflammatory episodes, allowing for the implementation of appropriate training and nutrition interventions.

4.8 Future Directions

The potential of CGM to enhance endurance performance is unclear despite a robust set of features and capabilities. This lack of clarity is partly due to rapidly moving technology outpacing literature resulting in outdated assessments of accuracy and efficacy being the only source of evidence to inform new ideas. Additionally, the advances in technology have uncovered new concerns and potential use cases Unfortunately, existing research is extremely limited leaving gaps for general nutrition use as well as sport specificity. This dearth of information clearly reflects a need for more research using current generation technology in applied sports settings.

SECTION 5

5.1 Summary of Literature

Studies have estimated the energy costs of various ultra-endurance events, lasting a minimum of 4-6 hours[1,2] to range between approximately 9,000 to 18,000[9,19,78–80] kcal for single day events. Nutrition guidelines aim to individualize macronutrient intakes to meet these energy demands. Of the micronutrients, CHO are the primary fuel source for sports, including ultra-endurance, but are limited to <3000 kcal of endogenous storage capacity.[10,83] Therefore, nutrition strategies aim to extend endogenous muscle glycogen stores, enhance exogenous CHO intakes, and reduce instances of GI distress associated with high rates of CHO intake.

An emerging strategy to enhance CHO fueling decisions is the monitoring of glucose levels in near-real time using CGM sensors. Due to technological advancements consumer-based wearable CGM devices have emerged allowing for continuous glucose monitoring over extended periods in outdoor environments offering the potential to inform CHO intake throughout the day and during ultra-endurance events. However, the reliability and validity of these devices in real-world sporting conditions are yet to be established.

The assessment of emerging technologies such as CGM and the utilization of glucose as a metric for sports performance warrants thorough examination, particularly within sex-specific cohorts, taking into account the nuances of female physiology. More specifically, the influence of the menstrual cycle on glucose management remains understudied, especially in ultra-running environments. Additionally, concerns have been raised regarding the validity of data from existing studies due to poor study design and controls during the menstrual cycle. This issue may be attributed to the absence of a universally adopted structure for testing during different hormone phases, given the complexity of the menstrual cycle.

5.2 Study and Questions

To address these gaps in knowledge, it is crucial to examine a subset of regularly menstruating, ultra-distance trained women in a prolonged testing environment. By doing so, we can strive to find answers to the unanswered questions regarding the performance of female athletes in ultra-distance events:

A) Does CGM reliably report blood glucose during a 4-hour simulated ultra run?

B) How does menstrual phase impact blood glucose fasted and fed during a 4-hour simulated ultra run?

C) Is energy metabolism different between menstrual phases at the end of a 4-hour simulated ultra run?

5.3 Aim of Study

Current literature suggests that the fluctuating levels of estrogen and progesterone throughout the menstrual cycle can influence energy metabolism during rest and exercise, favouring fat oxidation over carbohydrates. This metabolic difference between males and females, driven by estrogen's decrease of liver glucose production, has been reported to be mitigated when exogenous carbohydrates are provided via a lessening of estrogen's hepatic glycogen sparing effects. However, there is a lack of research on energy metabolism during the luteal phase and comparisons between menstrual cycle phases among females themselves. Additionally, the importance of blood glucose levels in performance has gained attention, but validation of tracking methods and guidelines for exercise are lacking. Criticism of existing female performance research methodologies also raises questions about fueling strategies. Thus, further investigation is needed to understand the impact of menstrual phase on blood glucose, interactions between menstrual phase and blood glucose with carbohydrate intake, energy metabolism during prolonged endurance exercise, and the reliability of continuous glucose monitors in female athletes.

Part 3 PhD Study

SECTION 6

6.1 Menstrual Cycle Tracking in Sports Research: Challenges, Progress, and Future Directions Manuscript 1 - published

Dole A, Beaven M, Sims ST. Menstrual Cycle Tracking in Sports Research: Challenges, Progress, and Future Directions. *Physiologia*. 2023; 3(4):598-610.
https://doi.org/10.3390/physiologia3040044

6.2 Abstract:

The roles of 17β estradiol (E2) and progesterone, the primary female sex hormones, are pivotal in regulating various aspects of metabolism. E2 influences food intake, energy expenditure, adipose tissue distribution, and insulin sensitivity across multiple tissues. Meanwhile, progesterone impacts energy expenditure, electrolyte balance, amino acid oxidation, muscle protein synthesis, and glucose metabolism. The interactions between these hormones affect macronutrient utilization, both at rest and during exercise. Acknowledging the need to incorporate sex hormone perturbations in research, this paper explores the current landscape of study design and menstrual cycle tracking for female-specific sport research. It emphasizes the importance of standardization in terminology, hormone phases, reference values, and affordable hormone detection methods to advance our understanding of how the menstrual cycle influences female athletes.

6.3 Introduction

The primary female sex hormones are 17B estradiol (E2), the predominant endogenous estrogen in humans, and progesterone. E2 actions in hypothalamic nuclei differentially control food intake, energy expenditure, and white adipose tissue distribution.[118,192–194] E2 actions in skeletal muscle, liver, adipose tissue, and immune cells are involved in insulin sensitivity as well as prevention of lipid accumulation and inflammation.[194–197] E2 actions in pancreatic islet β-cells also regulate insulin secretion, nutrient homeostasis, and survival.[118,198,199] Less is known about the specific mechanisms whereby progesterone exerts its metabolic influences; however, direct effects on energy expenditure through a progesterone-mediated increases in metabolic rate have been observed.[42,200,201] Additionally, progesterone alters serum electrolyte balance through

progesterone-mediated increases in aldosterone,[202–205] functions catabolically to increase amino acid oxidation and decreases muscle protein synthesis,[206–209] and affects glucose metabolism through the upregulation of the facilitated diffusion glucose transporter 1 (GLUT1) to increase endometrial glycolytic metabolism, attenuating skeletal and hepatic glycolytic pathways.[26,40,41,210] Consequently, both hormones exert agonistic and antagonistic effects on metabolism and nutrient needs whereby the ratios and levels of E2 and progesterone affect the proportions of macronutrients used as fuel, not only at rest but also during exercise.[122]

Therefore, to effectively conduct research in pre-menopausal eumenorrheic female athletes, the perturbation of the sex hormones should be included in the methodology.[211,212] Yet due to the historical perspective and dogma around scientific design, as well as sociocultural discrepancies around funding, leadership positions, and the overall gender-data gap, female-specific research has not been performed or has not been executed well. Specifically, in sport and biomedical research, only 6% of human performance research focuses on women, often with methodology applied through the male lens, with female participation and outcomes investigated *post-hoc*.[213,214]

Early research using female participants, intentionally or unintentionally, often neglected to account for the effect of fluctuating hormone profiles (E2 and progesterone)[215] over the course of the menstrual cycle (MC) on exercise or substrate metabolism.[29,30,37,216–221] Sport performance studies would include females with males regardless of the female's MC phase or combine eumenorrheic participants with hormonal contraceptive users.[216,222] In instances where MC phase validation was attempted, the method(s) such as: counting cycle days, luteinising hormone testing, and body temperature, in retrospect, may potentially have been inadequate when used as the only method of verification.

A better awareness of the female hormone profile throughout the MC and greater participation in high performance sports [223] has driven an emphasis to better understand how the menstrual phase influences biological females during exercise. As a result, an attempt to develop improved research methods for female athletes, including validation and standardization of menstrual cycle tracking [224], has followed. The purpose of this review is to evaluate the current practices and obstacles of menstrual cycle tracking in sports research.

6.4 The Menstrual Cycle

The menstrual cycle (MC) and its systemic effect on the body is a crucial area for research as it has been found that women frequently experience different adaptations and stress responses to their male counterparts.[225,226] For eumenorrheic women, the MC is characterized by fluctuations in several hormones, most notably the gonadal steroids, E2 and progesterone, and can be partitioned into the following six phases: early follicular, late follicular ovulation, early luteal, mid-luteal and late luteal phases. Throughout each phase, fluctuations in hormones trigger not only changes in the reproductive system, but also across a range of tissues throughout the body, which can have a direct effect on stress resilience, metabolism, and adaptations.[227] As a brief review, the length of a normal menstrual cycle is 21 to 40 days.[228] However, the length of a complete MC is not consistent. Its duration can be shortened or lengthened by a variety of factors such as: energy balance, diet, exercise, disease state, pregnancy, stress, hormonal contraceptives, hormone therapies, and medication. The first half of the MC is comprised of the menstrual and follicular phases during which time E2 levels are low (early follicular/menstrual) then rise (mid follicular), and peak (late follicular), ending with the periovulatory phase in which follicular-stimulating hormone and luteinizing hormone reach peak concentrations. After ovulation, the second half of the cycle is comprised of the early luteal (during which time E2 level drops and then rises while progesterone rises), the mid-luteal (during which time E2 and progesterone levels peak), and finally, the late luteal phase (during which time E2 and progesterone levels fall). These cyclic hormone changes can affect several physical and psychological attributes and may ultimately influence sports performance, although the effects are highly individual.[227,229,230]

6.5 Female Hormone Impact on Sport Physiology

A complete review of endogenous hormonal effects on female physiology is beyond the scope of this article. Comprehensive reviews on the full scope of endogenous hormone effects are available.[231,232] However, their effect on key aspects of sport physiology is the primary assertion for menstrual cycle tracking and phase verification when conducting sport research in female participants.

Unfortunately, existing research in female applied sport and exercise sciences has often neglected to address the status of ovarian hormones on observed outcomes. These shortcomings are rooted

in insufficient methodologies that fail to consider the impact of hormone fluctuations across distinct phases of the menstrual cycle. Such oversights can include but are not limited to, the inclusion of participants based solely on the presence of a regularly occurring menstrual cycle, failure to document the menstrual phase, use of only one method of menstrual phase verification, and failing to control for participants with menstrual dysfunction. To improve the quality of studies focused on female athletes it is important to acknowledge the impact ovarian hormones have on physiological function, as well as provide a reasonable framework for future study design within lab and applied settings.

It has been suggested that most of the current research has been limited to comparisons of the distinct hormonal phases follicular (FOL) and luteal (LUT) without taking into consideration moments of peak hormone levels found in the late FOL and mid LUT phases.[215] To improve the accuracy of study design, an optimal testing strategy consisting of repeated serum assessments of E2 and progesterone based on timings derived from LH hormone and calendar tracking has been proposed.[233] Additionally, it is recommended that luteal phase testing take place 7-9 days post LH surge detection to capture adequate progesterone levels to ensure the exclusion of anovulatory participants.[233]

The introduction of this framework provides a gold standard for testing, however, its implementation in many settings is problematic. Here we present a scoping review of existing phase verification techniques and their combined use to inform future methodologies in a wider variety of applied sporting environments.

6.5.1 Energy Metabolism

Energy metabolism during submaximal intensities when fasted is different between the sexes as evidenced by lower resting expiratory ratios (RER). Tarnopolsky and colleagues[216] observed a 7% difference in mean RER between males and females (0.94 and 0.87 respectively) during 15 km of running at 65% of maximal aerobic capacity (VO_{2MAX}). Similar were the observations of Carter and colleagues [37] during 90 minutes of cycling at 60% peak O_2. These differences are attributed to E2's possible upregulation of fat breakdown through alterations in hormone and enzyme levels [234]. The exact mechanism is unknown; however, it is widely accepted that E2's impact on liver function is directly responsible for females preferentially burning more fat (~7%) as a total

percentage of energy expenditure than males at submaximal intensities (40-70%) VO_{2MAX}.[30,37,117,216,218] Further distinctions in energy metabolism exist among just females across the MC phases as evidenced by greater carbohydrate (CHO) utilization (~25%) during fasted submaximal efforts in the FOLL phase.[30,219,220,235] However, these differences in CHO oxidation between the phases do not appear to exist in a fed state and are negated when exogenous CHO is introduced.[235]

Less clear is the role of progesterone on energy metabolism. Observations in the literature allude to inconsistencies in the role of progesterone in energy metabolism and how its influence is manifested singularly or in combination with E2.[27,117] For example, Oosthuyse *et al.*[122] asserted, citing rat model studies, that E2 co-administered with progesterone suppressed the availability of glucose impacted substrate metabolism during exercise. This observation was duplicated in human studies by Hackney and Devries who reported that muscle glycogen utilization was lower during exercise in the LUT phase despite progesterone dominance in the hormone profile.[30,219] In contrast, D'Eon *et al.*[27] demonstrated via exogenous hormone manipulation in exercising females that high levels progesterone added to a high E2 environment reversed the glycogen sparing effect of E2, which may have resulted from the differences in hormone ratios created[236] and/or the influence of synthetic exogenous hormones acting differently than naturally occurring endogenous forms.

While differences between male and female energy metabolism are well documented, a collection of studies shows no differences in energy metabolism between the MC phases. Casazza 2004 and Suh 2002 reported similar RER values in the FOL and LUT phases during submaximal 45 and 65% VO_{2MAX} efforts. These reports independently concluded that substrate utilization is less determined by ovarian hormone profiles over the MC and is affected to a greater extent by exercise intensity and CHO availability.[237,238] Similarly, a comprehensive review of substrate metabolism by Boisseau *et al.*[239] in 2021 reached a consensus that most studies do not detect a difference in energy metabolism between the menstrual phases; however, they did question the study environments from which the current literature is based. Specifically, the authors of the review asserted that the literature was potentially biased to high-intensity efforts that negate fat oxidation benefits found in mid-LUT phases during protracted sporting disciplines (ultra-endurance) performed predominantly below < 75% of VO_{2MAX}.

6.5.2 Hydration and Fluid Balance

Fluctuating levels of E2 and progesterone may exert an influence on the complex matrix of organ systems, hormone messengers, and neural triggers responsible for the management of fluid balance.[240] The exact impact these hormones is yet to be elucidated. Early studies were unable to isolate E2 or progesterone to assess individual effects and relied upon oral contraceptives which provided hormone at levels 6-10-times that of regular endogenous production, but did observe lowered thirst stimulation and decreased osmotic threshold of the free water regulating hormone arginine vasopressin when E2 was administered.[241] More recent studies utilizing gonadotropin-releasing hormone (GnRH) to manipulate hormone profiles have allowed MC sex hormones to be observed in isolation.[241] The results of these studies on fluid compartment distribution at rest indicate that plasma volume is increased with E2 and decreased by progesterone[241] through oncotic (protein colloid concentrations) and hydrostatic (fluid diffusion via pressure gradients) regulatory mechanisms.[242,243] These mechanisms result in shifts of fluid between the intravascular and interstitial ECF compartments, but not an overall volume increase. These hormonally-driven fluid shifts may be superseded by fluid balance mechanisms associated with exercise that drive fluid out of intravascular spaces into working muscle.[244] Interestingly, repeated observations by Stachenfeld *et al.* indicate MC hormones have only a small effect on total body water.[241,245]

Not all studies detected changes in fluid balance. A blood lactate study conducted by McCracken, Ainsworth, and Hackney in 1994 indirectly observed no differences in hematocrit between mid-follicular and mid-luteal phases among nine eumenorrheic physically active females exercising between 20-60% VO_{2MAX} during a continuous incremental treadmill challenge to exhaustion.[246] These data would indicate no significant changes in plasma volume associated with hormone profile and agrees with a larger review by Rodriguez-Giustiniani, Rodriguez-Sanchez, & Galloway.[247] More controlled testing and comparisons between distinct phases of the MC, such as late FOL phase where plasma volume is elevated,[248] may be warranted due to the changing concentrations of protein colloids between phases and their relationship in human fluid dynamics.[249,250]

Since most of the current sports hydration recommendations are derived from male studies, these hormonal influences may be relevant in a sport setting because women tend to have lower plasma volume, less extracellular fluid, and lower levels of absolute body water when compared to men.[251]

6.5.3 Menstrual Phase Terminology

As illustrated earlier, leaders in the field of female research agree that verifying MC phase is critical to study design and the interpretation of results.[211,212,252] This verification becomes especially relevant when attempting to compare differences between the phases in any experimental study. One potential barrier to research lies in the varying interpretations of the distinctly different hormonal profiles by researchers. For example, three to six phases have been used by various authors to differentiate distinct hormone profiles of the MC, **Table 4**. When compared, various phase nomenclatures can encompass one or more of the suggested hormone profiles causing inconsistencies in reporting and confusion in the literature, **Figure 4**.

Table 4. Menstrual phase terminology & alignment

			Alignment Days to Phase	
Three Phase	Four Phase	Six Phase	Days	Days
Early Follicular	Early Follicular	Early Follicular	1-5	2-7
Late Follicular	Late Follicular	Late Follicular	6-12	9-13
		Ovulation	13-15	
		Early Luteal	16-19	
Mid Luteal	Mid Luteal	Mid Luteal	20-23	18-24
	Late Luteal	Late Luteal	24-28	

note: based on 28 day "average" cycle

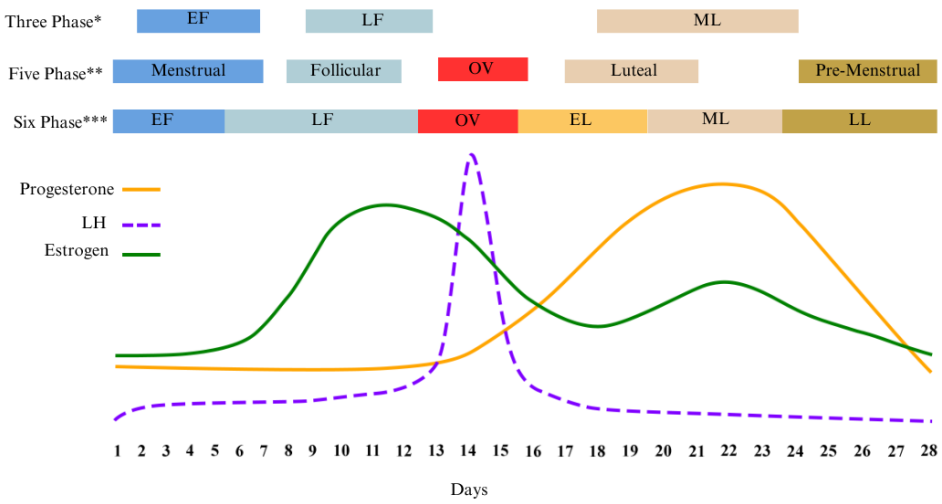


Figure 4. Diagram of the different menstrual cycle phases found in the literature and overlaid with corresponding ovarian hormone levels. Adapted from McNulty et al.[253]. *[212] ; **[207] ; ***[253]. Early Follicular (EF); Late Follicular (LF); Early Luteal (EL); Mid Luteal (ML); Late Luteal (LL); Luteinizing Hormone (LH).

Low hormone or high hormone characterizations are also commonly used to differentiate the phases of the menstrual cycle. In comparative studies, these low and high phases are most often the target windows for study protocols as they represent the most noticeable differences in hormone profiles. Early FOL is associated with low E2 and P4 "low phase" while mid-LUT exhibits high E2 and P4 "high phase".[211,233] More recently, Elliot-Sale and colleagues deviated from the conventional utilization of FOL and LUT for describing the menstrual cycle. Their aim was to move away from what the author characterizes as frequently ambiguous, wherein the phases are connected to indistinct or undefined ovarian steroid profiles. Instead, they recognized discrete phases within the menstrual cycle that correspond to average concentrations of E2 and P, designating these phases as 1-4.[211]

Alignment of specific MC phases to set days in a 28-day period of time is unlikely in the real world. This is partly due to the variability of the MC across individuals. Two extensive studies, observing 1060 and 612,613 total cycles respectively both concluded that the average MC lasts a total of 28 days. Furthermore, they identified the follicular phase to be the most volatile phase and responsible for the high degree of MC variability.[228,254] The average length of the FOL phase was 16.5 ± 3.4 and 16.9 ± 5.3 respectively as compared to LUT 12.4 ± 2 and 12.4 ± 2.4.[228,254] In both

studies, the majority of all cycles were reported to last between 22-36 (28.9 ± 3.9) [228] or 25-30 (29.3 ± 5.2) [254] days in length.

It should also be noted that ovarian hormone concentrations also display high levels of inter-individual variability within each distinct phase. Dam and colleagues collected serum hormone levels in 40 females (n=10 contraceptive users; n=30 eumenorrheic) across six phases of the menstrual cycle. The median and interquartile data for E2 and P4 illustrate the considerable inter-individual differences in hormone levels within each distinct phase, **Figure 5**.[255] Similar to the variation of hormone levels across the MC is the variability among individuals for which no clear set of reference value has been established at each phase of the MC for specific study populations such as athletes who may have different hormone profiles than the average female. This variability is indicated by observations of De Souza et al. that P4 values are lower and LUT phase length shorter in female runners when compared to sedentary populations.[256]

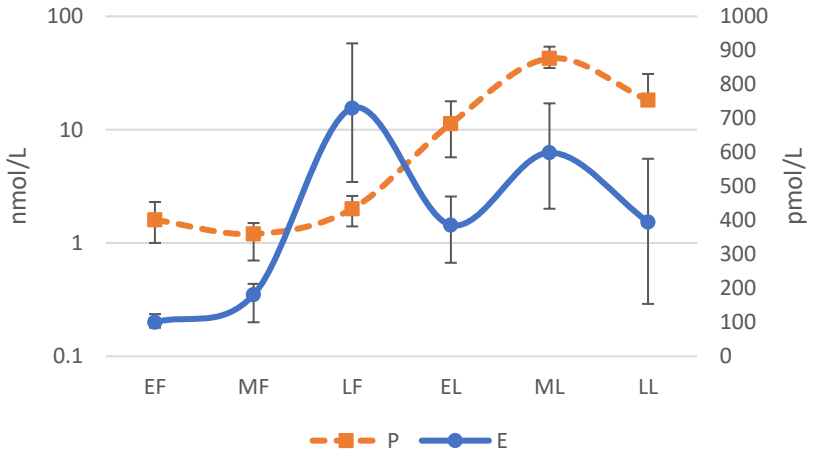


Figure 5. Median and interquartile levels for estrogen and progesterone across six phase menstrual cycle.[255]. Early Follicular (EF); Mid Follicular (MF); Late Follicular (LF); Early Luteal (EL); Mid Luteal (ML); Late Luteal (LL); Progesterone (P); Estrogen (E). Log-10 applied to y-axis and demonstrates a sinusoidal wave pattern.

6.6 Methodologies Past & Present

The impact of the MC on sport performance is unclear. Inconsistencies in testing methodology, specifically MC phase verification, may be contributing to the lack of clarity.[224] Methods of validation can be divided into two group types (hormone profile or point of ovulation), providing a spectrum of effectiveness and practicality summarized in **Table 5**.

Table 5. Summary of Menstrual Cycle Verification Methods

Method	Verifies	Data Collected	Process	Accuracy	Limitations	Benefits
Point of Ovulation						
Cervical Mucus	Point of ovulation	changes in vaginal mucus consistency	physical inspection of fluids	48–76% moderate	wide range of accuracy	low cost, no expertise required, may benefit from pairing with BBT
Counting Days	Point of ovulation	start and end of menses	record keeping, estimation	low to moderate 18–59%	excludes anovulatory cycles, human error in recall	free, convenient access, benefits from pairing with additional methods
Menstrual Cycle Tracking Apps	Point of ovulation	start and end of menses	record keeping, predictive algorithm	low 21%	no secondary verification used for markers of ovulation	free, convenient access, may benefit from pairing with additional methods
Ultrasonography	Point of ovulation	tracks growth of follicles in ovary	transvaginal ultrasound	high 80% (gold standard)	5–6 scans over 3–8 days, expensive, high level of expertise required, costly equipment	high level of accuracy
Basal Body Temperature	End of ovulation/start of LUT phase	changes in resting body temperature	daily recording of oral, vaginal or rectal body temperature	low 22%	low accuracy in detecting ovulation window	low cost, may benefit from pairing with additional methods
Salivary Ferning	Point of ovulation	saliva	viewing of crystalized saliva patterns	moderate 42–53%	requires equipment, moderate accuracy	low cost may benefit from pairing with additional methods
Urinary Luteinizing Hormone	Point of ovulation	LH levels from urine	urine applied to test strips	high 80% based on 100% correlation with Ultrasonography	excludes LUT deficiency disorders, false positives	low cost, convenient, no technical expertise, reliably with high accuracy
Hormone						
Serum Progesterone	hormone level	serum Progesterone	blood draw	high 89% (gold standard)	cost, expertise, infection risk in field	high accuracy

Key: basal body temperature BBT; Luteal LUT, Luteinizing Hormone LH

6.6.1 Hormone Profile Validation

Venous hormone verification, the measure of blood serum E2 and progesterone, omits any predictive element and is the gold standard for determining hormone status by providing an exact snapshot in time.[233,257] Reference levels of E2 and progesterone have been established for phases of the MC in a recent study by Stricker et al.[258] yet no consensus exists on minimum values for inclusion.[224] A wide range for the minimum level of progesterone (2-16 nmol/L) has been recommended to properly exclude menstrual abnormalities such as LPD or anovulation.[233]

6.6.2 Point of Ovulation Validation

In contrast, point of ovulation methods (PO) require some form of prediction or estimation by counting forwards of backwards from ovulation. Different methods of PO provide varying levels of accuracy, but all are subject to the variability of phase length.

6.6.3 Follicular Monitoring

The use of ultrasound to track the development of follicle size is a direct method of follicular monitoring, and with 80% accuracy is considered the gold standard of detection of ovulation.[259–262]

6.6.4 Salivary Ferning

Crystallization of NaCl in dried saliva occurs during the ovulation period creating a distinct pattern referred to as “ferning”. This pattern is created by the increase in E2 and directly mirrors ferning found with cervical smears. Less invasive and readily available, saliva samples present a low-cost detection method for ovulation but does require the use of a microscope.[263] An overall accuracy of 42-53% indicates the need to pair with a secondary method.[260]

6.6.5 Counting Days

Establishes Day One of the MC and the beginning of the FOL phase with the appearance of bleeding. It retrospectively indicates the end of a MC with the start of the following cycle’s menses. Relies upon estimation to determine intra-phase lengths. Useful tool for determining total cycle length but varies widely in accuracy precluding the prediction of ovulation 18-59%.[233,257]

6.6.6 Menstrual Cycle Tracking Apps

Most applications only provide a window of fertility with large variability between applications (4-12 days) and do predict an exact ovulation date. As expected, the probability of ovulation occurring in the fertility window increased with window length. Those applications providing ovulation dates did so at an accuracy of 21% or less.[264]

6.6.7 Basal Body Temperature (BBT)

A temperature increase of 0.3 °C can occur during the LUT phase but does not happen in all females.[233] This rise in temperature can be used to determine the end of ovulation/fertility window; however, menstrual irregularity can lower the accuracy of body temperature changes.[260] Additional confounding factors such as alcohol, stress, and climate can also influence BBT.[260] Low cost with no technical expertise required this method of PO is widely used. However, it is not viewed by all as a reliable predictor of ovulation with a reported 22% rate of accuracy.[265]

6.6.8 Urinary Luteinizing Hormone (LH)

LH is a glycoprotein hormone that increases in the blood serum 35-44 hours prior to ovulation with peak levels occurring 10-12 h prior to ovulation.[260] It can be measured in urine with low-cost strips, has high usability outside lab environments, and requires no technical knowledge. This method is highly accurate at predicting ovulation within a 48-hour window;[266] however, there is potential for a LH surge to be detected without ovulation in infertile women.[267] Described as "the most validated method for estimating ovulation"[268] it has demonstrated 100% congruence with ultrasonography, the gold standard.[269]

6.6.9 Cervical Mucus

Cervical mucus can be used to determine ovulation with a 48.3-75.9% accuracy.[260] This technique involves the visual and tactile inspection of secreted vaginal fluid, from the lower part of the uterus, for consistency and color changes that occur near ovulation in response to changing levels of E2. Most notably the rise in E2 before ovulation stimulates peak-type mucus characterized as clear, stretchy, and slippery indicating a strong probability of successful ovulation.[270]

6.6.10 Combined Methods

Due to the variability of hormonal profiles across the female lifespan, no current single detection method, except for serum hormone testing, is adequate to verify where in the MC a female lies.[211] Due to the cost of serum hormone testing, strategies to minimize blood draws have been introduced. Schaumberg *et al.*[224] have suggested a validation method for the "high hormone" mid-LUT phase with a reported accuracy of 90% in normally menstruating, physically active females. This method utilized three different validation modalities. First, calendar tracking was implemented for three months to establish menstrual patterning i.e., average total MC length from which a day counting strategy could be structured. Second, LH hormone testing for seven consecutive days was completed using urinary strips to establish ovulation, which was approximated at two days after the LH surge. The mid-LUT phase was then estimated to start 6-8 days after ovulation. Third, a venous serum hormone assay was taken 6-12 days post confirmed LH surge. The Mid-LUT phase was validated through P4 serum levels of > 6 ng/ml to exclude luteal phase deficiency.[224]

Various studies have utilized combined methods of menstrual tracking which did not employ serum hormone testing as part of the protocol. Specific to research in female sports, De Jonge *et al.* compiled a comprehensive list of these studies showing basal body temperature, urinary ovulation kits, and calendar-based counting as the most common.[233] Rogan and Black, while researching dietary intakes across the MC, similarly found several female studies verifying MC phase through a combination of methods that did include hormone testing.[271] Both groups of authors, in line with others,[211,257] concluded that the absence of serum hormone testing allowed for the inclusion of study participants with deficient levels of P4 in the luteal phase which could skew results.

6.7 Obstacles to Phase Validation

Repeated venous blood sampling of endogenous E2 and progesterone, the costliest approach, appears to be the only method of pin-pointing the exact hormone profile of female study participants and at this time, it is also the only method that can be used independently. Therefore, the implementation of multiple repeated PO validation methods is required to limit the required blood draws as validated by Schaumberg;[224] however, this method still requires costly serum hormone testing.

Testing procedures aside, existing inconsistencies in phase terminology can confuse study design and data comparisons across studies. Elliot-Sale and colleagues have recommended moving away from debating phase nomenclature to focus on the adoption of specific time points that represent hormone profiles that can be reliably studied and compared.[211] With the obvious variability of MC, particularly the early follicular phase length, it would seem prudent to focus on stability where it exists. Recent work by Francis *et al.*[236] alludes to such windows of hormone stability, identified as the late follicular, early luteal, and late luteal, which may provide optimal study opportunities. Briefly, the MC would be divided into two primary phases (FOL and LUT, differentiated by the signature LH peak and comprised of three or more distinct hormone profiles characterized by physiologically relevant hormone levels with relative stability. However, it's important to note that the phases with more consistent lengths don't correspond to the periods of highest or lowest hormone levels, nor do they include the phases where the difference between hormones E2 and progesterone is most pronounced. These phases, like the late follicular and mid-luteal phases, are the ones that may possess the most physiological significance.

Furthermore, beyond the > 6 ng/ml criteria for progesterone used to confirm ovulation [224], there is an absence of similar relevant sports physiology reference values for E2 and P4 among the general population or sport-specific cohorts. This gap or lack of comprehensive benchmarks hinders the understanding of what hormone levels impart an influence on sports physiology. Additionally, as women experience physiological changes linked to training, the consequent effects on hormone levels remain challenging to monitor consistently across time without baseline reference values.

6.8 Future of Menstrual Cycle Tracking

Identification of gaps in study design and validation methodologies within the existing body of female performance research highlights the need for standardization. Further research would benefit from the creation of universal terminology as well as: standardization of distinct hormone phases, the establishment of hormone reference values, and the creation of low-cost, point-of-care detection devices for E2 and progesterone which would greatly improve the validity of MC phase comparisons. A possible solution may come from a combination of Francis *et al.* [236] summary of hormone phase stability and the phase classification system presented by Elliot-Sale and colleagues.[211] This additional structure could help researchers navigate the variability within an individual's entire MC as well as intra-phase durations. Until that time arrives, the requirement for ongoing female-focused research remains crucial. Thoughtfully constructed experiments that uphold rigorous population controls and inclusion/exclusion parameters, while utilizing a variety and/or combination of predictive outcome techniques such as basal body temperature tracking, urine LH strips, and calendar monitoring, ought to be acknowledged, especially in field studies: an area lacking adequate representation in the literature. However, it is advisable to approach their findings with consideration.

SECTION 7

7.1 Continuous Glucose Monitor Underreports Blood Glucose During a Simulated Ultra Endurance Run in Eumenorrheic Female Runners – Manuscript 2

Authors: Andrew Dole, Stacy Sims, Han Gan, Nic Gill, Martyn Beaven

Currently in second round of review with the *International Journal of Sports Physiology and Performance*

7.2 Abstract

Purpose: Continuous glucose monitoring sensors (CGM) provide near real-time glucose data and have been introduced commercially as a tool to inform nutrition decisions. The aim of this study was to explore how factors such as menstrual phase, extended running duration, and carbohydrates affect CGM outcomes among trained eumenorrheic females in an outdoor simulated ultra-endurance running event. **Methods:** Twelve experienced female ultra-runners (age 39 ± 6 y) participated in this cross-over study. Participants completed an ultra-endurance simulation run of 4 hours in the mid-follicular (FOL) and mid-luteal phases (LUT) of their menstrual cycle which consisted of a 3-hour fasted outdoor run (FASTED) followed by a one-hour treadmill run (TREAD) where three standardized 20 g oral glucose doses were provided. **Results:** Using a mixed linear model, menstrual phase was statistically significant for differences in glucose measurements from CGM as compared to capillary glucose during TREAD ($p = 0.02$) but not FASTED. Additionally, the CGM sensor reported glucose levels an average of -0.43 mmol·L-1 (95% CI -0.86, -0.005) and -1.02 $mmol \cdot L^{-1}$ (95% CI -1.63, -0.42) lower in fasted and fed scenarios respectively when compared to capillary glucose. Bland Altman plots displayed a discernible trend in the reduction of CGM underreporting as glucose values increased when compared to capillary samples during the FOL phases of FASTED -0.37 $mmol \cdot L^{-1}$ (95% CI -0.70, -0.04) and TREAD -1.0 $mmol \cdot L^{-1}$ (95% CI -1.32, -0.58). Conversely, during LUT, the trend for the CGM to underreport as compared to capillary worsened when glucose concentration increased in FASTED -0.58 $mmol \cdot L^{-1}$ (95% CI -0.95, -0.22) and TREAD -1.05 $mmol \cdot L^{-1}$ (95% CI -1.41, -0.68). **Conclusion:** Athletes and practitioners should be aware of these accentuated differences in CGM glucose data when used as a tool to inform nutrition interventions during UE running.

7.3 Introduction

Ultra-endurance (UE) events are defined as events lasting a minimum of 4-6 h and up to several consecutive days.[1,2] The energy demands of these events, estimated between 8,000-18,000 kcal/day, rely on the utilization of endogenous stores of fat, protein, and crucially, carbohydrates.[9,19,78,80] Stored as glycogen in the liver and muscles, carbohydrate plays a critical role in sustaining performance by providing ATP to working muscles and mitigating fatigue.[13,43] However, the limited capacity of these endogenous stores of glycogen, estimated to be 500 g.[10] becomes a significant concern during prolonged exercise where muscle glycogen depletion can lead to performance decline and dependence on exogenous carbohydrate sources.[94] To minimize performance decline, athletes adhere to exogenous carbohydrate intake recommendations of 30-90 $g{\cdot}h^{-1}$ during ultra-endurance events,[13] emphasizing the importance of managing carbohydrate availability, avoiding low blood glucose "hypoglycemia",[10] and supports the emerging interest in continuous glucose monitor (CGM) as an endurance performance tool to monitor and manage glucose.

The importance of glucose management, beyond the avoidance of hypoglycemic levels, in athletic performance is poorly understood. There are six identified issues precluding implementation of therapeutic norms in healthy athletes. First, there is no definition of what day-to-day glucose control "normoglycemia" should look like.[141] Second, no parameters exist for normative glucose ranges during physical activity. Third, a clear consensus on how transient episodes of hyperglycemia impact performance during exercise has not been made. Fourth, minimal evidence connects glucose ranges to aspects of performance, refueling, or recovery.[161] Fifth, few insights are available into the variability of glucose during real-world performance scenarios among males or females. Finally, the primary methods of glucose tracking, capillary glucose and the rapid glucometer monitoring of the glucose oxidase reaction in interstitial fluid (ISF), both have limitations. For example, continuous capillary monitoring is impractical in an applied setting. While CGM, initially developed for diabetes management, now offers near real-time glucose data 24/7 in intervals of 1–5-minutes for up to 14 days in a commercially available format,[65,132] it faces challenges in accuracy, particularly during exercise. The use of ISF for monitoring glucose has drawbacks, primarily due to a physiological lag time of about 5-6 minutes[51,52] between ISF and blood glucose levels, caused by fluid diffusion from capillaries into interstitial spaces.[55,163]

Additionally, there is a technological lag related to the sensor's detection capabilities and software algorithms. Additionally, when compared to blood the wide variation of pH within ISF, which can affect the accuracy of glucose detection, is a potential concern.[50]

Prior studies have indicated a decrease in CGM accuracy during physical activity[55,57,59–63] determining that the mean absolute relative difference (%MARD) is significantly worse during exercise when compared to rest. A comprehensive review by Fabra *et al.*[64], on CGM accuracy from 2006-2020, identified a wide range of accuracy (%MARD from -4.4 to 18.1%) at rest compared to exercise agreeing with the consensus of individual studies of the reduced accuracy attributed to rapidly changing glucose concentrations and ISF volatility. Of the few CGM accuracy studies in athletes, there is consistent agreement that the accuracy of CGM decreases for episodes of hypoglycemia[178] and deteriorates over time.[179]

Furthermore, the influence of ovarian hormones, estrogen (E2) and progesterone (P4), on blood glucose across the menstrual cycle during prolonged endurance has yet to be elucidated despite well-known influences on carbohydrate metabolism. For example, E2 has been observed to promote glucose uptake into type I fibers[26], while simultaneously reducing uptake of glucose into the bloodstream.[27] Conversely, P4 appears to reverse E2's suppression of muscle glucose oxidation during submaximal exercise[27] as well as complement E2's glucose-sparing effect in the liver.[39] The lack of clarity in mechanisms may be due to the historical gender-data gap in female-specific sport and biomedical research with only 6% of human performance research conducted on women and often completed with methodology applied through the male lens and/or with female participation and outcomes investigated *post-hoc*.[213,214]

The existing body of CGM research in endurance sports, specifically UE, is limited and lacks well-controlled studies involving females. One observational study by Ishihara *et al.*[70] examined blood glucose levels in seven participants (female 3; male 4) during a 165 km ultra trail run, found no correlation between running speed and the highest blood glucose levels. However, the large ranges of glucose variability impacted female running speed less than males ($r^2 = 0.01$, $p = 0.60$; $r^2 = 0.11$, $p = 0.01$ respectively).[70] Another case study by the same researchers[182] focused on a single female ultra endurance runner during a 438 km trail run and observed that running pace had no relationship with glucose level or glucose variability. Notably, these observational field-based studies conducted in real-time race situations collectively included only eight participants (4-female; 4-

male) and did not verify CGM data accuracy with a secondary method. Additionally, there was no reporting of menstrual cycle or hormonal contraceptive use of the female participants; influences of which have yet to be elucidated on exercise metabolism and may or may not have an impact on energy metabolism during exercise, as suggested by previous studies.[26,28,33,39,211,272]

Therefore, the objective of this study was to simulate an ultra-endurance run and explore how variables such as menstrual phase, extended running duration, and carbohydrate intake may influence CGM in real-world situations. Additionally, this research may to offer insights for potential applications of CGM technology in endurance sports performance and for a better understanding of female physiology during long-duration exercise in fasted and exogenously supplemented states.

7.4 Methods

7.4.1 Experimental Design

A randomized, cross-over design with participants acting as their own control across menstrual cycle phases was used during an ultra-marathon simulation consisting of a standardized outdoor 3-hour fasted depletion run (FASTED) followed by a 60-minute treadmill run (TREAD) with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). Participants completed the run protocols (FASTED and TREAD) once during the mid-follicular (4-8 days after the start of menses) and mid-luteal (5-8 days post ovulation) phases of the menstrual cycle. Ethics approval was obtained from the University of Waikato's Human Research and Ethics Committee (HREC(Health)2021#73). Written informed consent was obtained from each participant. Recruitment and data collection occurred from December 2021 through November 2022.

7.4.2 Participants

Eighteen recreational female ultra-endurance runners, recruited from endurance sports clubs located in New Zealand, agreed to participate in this study. Six were excluded from the initial sample group due to injury or disruptions in hormone profile (pregnancy; hormone therapy). Twelve participants were included in the data analysis unless otherwise specified (mean ± (SD), age: 39.3 ± 6.3 y, height: 165.3 ± 5.6 cm, body mass: 62 ± 8.0 kg, VO_{2MAX}: 45.9 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$).

An electronic questionnaire was administered to all participants to screen for established inclusion criteria: 1) eumenorrheic experiencing regular cycles (28-40 d), 2) not using hormonal contraception for at least 12 months except for Mirena IUDs implanted for a minimum of 9 months, 3) at least one year of ultra-endurance experience, 4) and no current injuries that would impact performance. Additional exclusion criteria included 1) a history of cardiovascular, renal, or metabolic disease, 2) insulin or glycemic disorders, 3) the use of any exogenous hormones, or 4) confirmed anovulatory cycles.

7.4.3 Hormone Control

Prior to familiarization, participants were required to provide photo documentation to the lead researcher of menstrual tracking for three consecutive cycles using a commercial mobile menstrual tracking application (Garmin Connect, Olathe, KS, USA; FITRwoman, Encino, CA, USA; Wild.AI, San Francisco, CA, USA). In-home urine ovulation test strip predictor kits (Pregmate, Fort Lauderdale, FL USA) were then used to detect a luteinizing hormone surge, confirm ovulatory function for two consecutive months, and establish consistency in calendar tracking of MC length. Participants were instructed to begin strip testing for seven consecutive days prior to their predicted ovulation. A count forward method was used to predict the early follicular (days 4-8 from the start of menses) and mid-luteal phases (days 5-8 post-ovulation) for testing.

7.4.4 Metabolic Data

Respiratory exchange ratio (RER) was obtained during the treadmill portions of the run protocol using a TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT USA). A mouthpiece and nose covering were worn by participants to collect expired gases for most of the time trial; being removed for carbohydrate and fluid intake which was recorded and time-stamped for data collection integrity. Heart rate was obtained using a telemetry system (Polar Electro, Inc., Lake Success, NY, USA). Fingerstick capillary blood glucose (Capillary) samples were taken using a CareSens lancing device, lancets, and testing strips (i-SENS Global, Gordon NSW, AU) three times during the 3-h depletion run: prior to start, after 90 minutes of exercise, and at the completion of the three-hour fasted run. During the 1-h treadmill run, capillary samples were taken just before start, and then at 10-min intervals (7-time points; **Figure 6**). Continuous glucose was measured during the entire 4-h run protocol at 1-min intervals using the Abbott Libre Sense continuous glucose monitor (Abbott Laboratories, Abbott Park, IL USA) worn on the back of the upper arm for a minimum of 24 hours and a maximum of 13 days before run protocol testing. Interstitial glucose data was collected by the CGM via a thin, flexible amperometric sensor filament inserted subcutaneously. The stored data from the CGM was uploaded by pairing the sensor to a mobile device which wirelessly transmitted to cloud-based storage and was retrieved onto PC using the cloud-based coach dashboard features (Supersapiens, Atlanta, GA USA). When necessary, glucose in $mg \cdot dL^{-1}$ was converted to $mmol \cdot L^{-1}$ by multiplying by 0.0555.

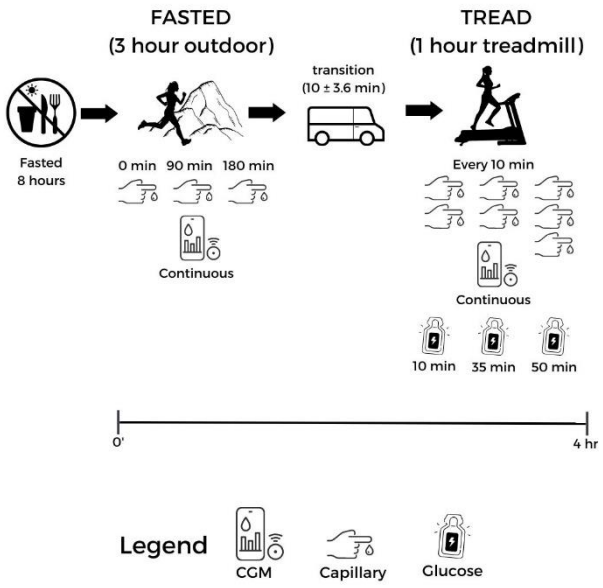


Figure 6. Schematic of study design.

7.4.5 Familiarization

Participants reported to the laboratory to complete VO_{2MAX} testing, receive study instructions, and familiarize participants with testing procedures/equipment. An instructional video and walk-through of the continuous glucose sensor (CGM) application were provided. Depletion run instructions and outdoor route were reviewed. A combination ramp and graded VO_{2MAX} test protocol was conducted on a motorized treadmill with a stiffness of 365 $kN \cdot m^{-1}$ (Steelflex PT10 Fitness, Steelflex Fitness, Taipei, Taiwan) with the collection of pulmonary gas exchange using a TrueOne 2400 metabolic cart[273]. Each participant was instructed to begin with a self-paced 5-min warm-up. Following warm-up, the testing began with an initial stage running on a 0% grade at 8.5 $km \cdot h^{-1}$ for two minutes. This stage was followed by increases in speed of 1.4 $km \cdot h^{-1}$ every 30 seconds until 14.1 $km \cdot h^{-1}$ was reached. After 30 seconds at 14.1 $km \cdot h^{-1}$ the grade was increased from 0% to 2% for two minutes followed by a 2% increase in grade every two minutes to volitional exhaustion. Maximum effort was determined when both conditions were met: 1) failure to continue test and 2) respiratory exchange ratio (RER) > 1.1.

7.4.6 Outdoor Depletion Run

Participants were instructed to apply the CGM sensor 24-48 h before their scheduled run day, arrive at the lab in a fasted state of 8 hours, and have avoided caffeine, alcohol, and strenuous activity for 24 hours preceding the run. Upon arriving at the lab on the morning of the (FASTED) protocol, a urine sample was taken and urine specific gravity was measured using a hand-held refractometer (Atago CO LTD, Tokyo Japan). Body weight was documented using a Wedderburn WM202 patient scale (Wedderburn, Ingleburn, NSW, Australia). Capillary glucose was recorded immediately (< 1 min) before the start of the depletion run. Participants ran at a self-selected pace along a pre-determined, two loop, outdoor route, chosen to simulate an ultra-endurance run course for 3-hours. Starting from the laboratory participants ran a flat 2.6 km stretch to the offroad course where they directly began the first loop of 2.65 km consisting of 133 m of elevation gain/loss followed by the second loop of 3.47 km and 48 m of elevation gain/loss. These loops were repeated for three consecutive hours. Participants checked in with the researcher at an aid station located along the route after the completion of each loop to receive, *ad libitum*, a standardized non-caloric electrolyte solution containing 600 mg sodium (Na^+) and 100 mg K potassium (K^+) per Litre. Participants were instructed to drink no other fluids than those provided by the researcher and to eat nothing during the duration of the depletion run. Intake of all fluid was measured and documented. Capillary glucose was recorded two additional times at 90- and 180-minutes (**Figure 6**). Upon completion of the depletion run, a rest aid station was simulated where the runner was allowed to sit and hydrate while being driven back to the lab to begin the 1-h treadmill trial. The average transition time from the depletion run's end to the treadmill trial's start was 10 ± 3 min. Run data (distance, elevation, pace) was tracked using a runner-provided watch with global positioning (GPS) and documented with screenshots of Strava (Strava, San Francisco, CA, USA) or Garmin Connect (Garmin Connect, Olathe, KS, USA) mobile applications. Outdoor runs were completed between 4 a.m. and 12 noon to minimize variations in weather and temperature (13.5 ± 6.6 °C).

7.4.7 Indoor Treadmill Trial

Following FASTED and brief transition described above, participants were instructed to run at a self-selected pace for an additional 60 minutes on the motorized treadmill at 0% grade while having expiratory gases and heart rate continuously monitored with a TrueOne 2400 metabolic cart in a temperature-controlled environment (19 °C). Blood glucose was taken via capillary seven times, starting at 0 minutes and again every 10 minutes until completion. A 20 g bolus of glucose was provided at three time points (15, 35 and 50 minutes; **Figure 6**). A standardized non-caloric electrolyte solution containing 600 mg Na and 100 mg K per Litre was provided *ad libitum* with intakes recorded. Interruptions to the expiratory gas collection were time stamped.

7.4.8 Statistical Analysis

A linear mixed model was fit, using RStudio statistical package, with the difference between CGM and Fingerstick (Difference = CGM – capillary blood) measurements as the response variable and *Time*, *Person,* and *Phase* as explanatory variables, where *Person* was a random effect. Data was then separated into two different scenarios and modelled independently: one for the first 3 hours of the depletion phase (FASTED), and a second model for the final hour (TREAD). Data are presented as estimated coefficient averages and were checked for normality using a normal probability chart. A marginal fitted equation of the coefficients, provided in supplementary materials, was used to determine additional effects of time at each time point in both FASTED and TREAD. Blood glucose from the CGM sensor was compared to capillary blood samples using Bland-Altman plots for visual inspection of bias and precision.

Marginal Fitted Equation:

Glucose at time point = Average Overall Coefficient + Time X

Depletion (FASTED) Phase

The linear mixed model for the FASTED run was fit with *Phase* nested in *Person* and including interaction terms. As a result of menstrual phase being statistically insignificant, the final model included only *Time* and *Person* as fixed and random effects, respectively. *Time* was used as a categorical variable as there are limited levels in the first three hours.

Treadmill (TREAD) Phase

The TREAD linear mixed model was fit to examine the relationship between blood glucose levels and various explanatory variables. The response variable was blood glucose level, *Time* was measured every 10 minutes and was fit as a categorical main effect, *Phase* was fit as a categorical main effect, and the effect of each *Person* was fit as a random effect, which included interaction terms with *Phase*. In addition to this model, as normality of the residuals was not met, we fit a model with the same explanatory terms using the log transformation of the response variables. *Time, Person,* and *Phase* were included as explanatory variables, with *Person* treated as a random effect. One participant was excluded from this analysis due to equipment failure.

7.5 Results

Blood glucose levels, reported by the Abbott Libre Sense CGM, were on average -0.43 mmol·L^{-1} (p = 0.048, 95% CI -0.86, -0.005 mmol·L^{-1}) lower than the corresponding capillary measurements taken at the three time points (0, 90,180 min) over the FASTED run in regularly menstruating females in both phases of the menstrual cycle (**Figure 7** and **Figure 8**). An effect of the individual *Person* was significant (p = 0.043) for the differences between CGM and capillary samples during the FASTED run, as was *Time* (p < 0.01). The extra effect of *Time* between average difference of CGM and capillary glucose (-0.43) at the specific time points was -0.52 mmol·L^{-1} (0 min), -0.018 mmol·L^{-1} (90 min), and -0.755 mmol·L^{-1} (180 min), (**Figure 7** and **Figure 8**).

During TREAD, the CGM sensor reported glucose levels on average -1.02 mmol·L^{-1} (p = 0.004, 95% CI -1.63, -0.42) lower than the matching time-stamped capillary samples taken every ten minutes in both phases of the menstrual cycle (**Figure 2** and **Figure 3**). Only at 10 min was the extra effect of time (-0.18 mmol·L^{-1}) significantly different (p = < 0.01) than the average difference (-1.02) between CGM and capillary samples. The influence of *Phase* was statistically significant for the differences in glucose measurements between CGM and capillary only during TREAD (p = 0.02).

Parity of glucose values from CGM and capillary samples were also assessed using Bland-Altman plots (**Figures** 4 - 7). The tendency of the CGM to underreport was visually displayed, showing similar bias (95% LoA) in FOL and LUT phases during FASTED of -0.37 mmol·L^{-1} (1.36 to -2.10 mmol·L^{-1}) and -0.58 mmol·L^{-1} (1.29 to -2.45 mmol·L^{-1}), respectively. A bias (95% LoA) of -1.0

mmol·L^{-1} in TREAD was observed for FOL (1.75 to -3.65) and LUT (-1.76 to -3.86). When CGM and capillary glucose data were combined for FASTED and TREAD the following biases (95% LoA) emerged -0.48 mmol·L^{-1} (-2.27 to 1.32) and -1.08 mmol·L^{-1} (-4.24 to 2.09), respectively.

Differences in glucose across the menstrual cycle were observed but the scope of discussion lies beyond the purpose and narrative of this study aimed at exploring the use of CGM as a valid tool in ultra-running across the menstrual cycle. A summary of results for run data and glucose comparisons across the menstrual cycle are provided as supplementary materials.

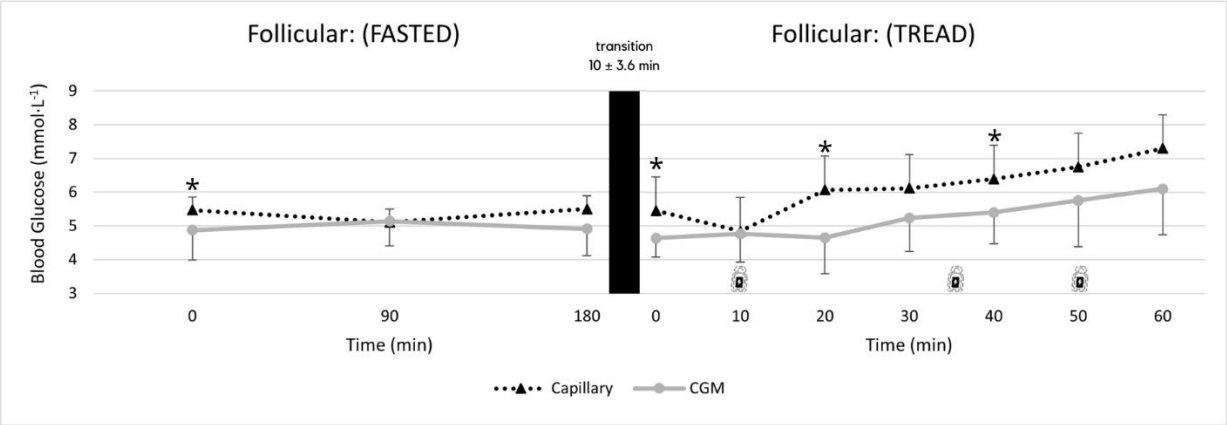


Figure 7. Comparison of the estimated means of blood glucose from continuous glucose monitor (CGM) to capillary during follicular phase. FASTED, a standardized outdoor 3-hour fasted depletion run. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). Values are means ± SD. * Statistically significant differences ($p < .05$) in glucose between CGM and capillary methods using individual paired t-tests at specific time points.

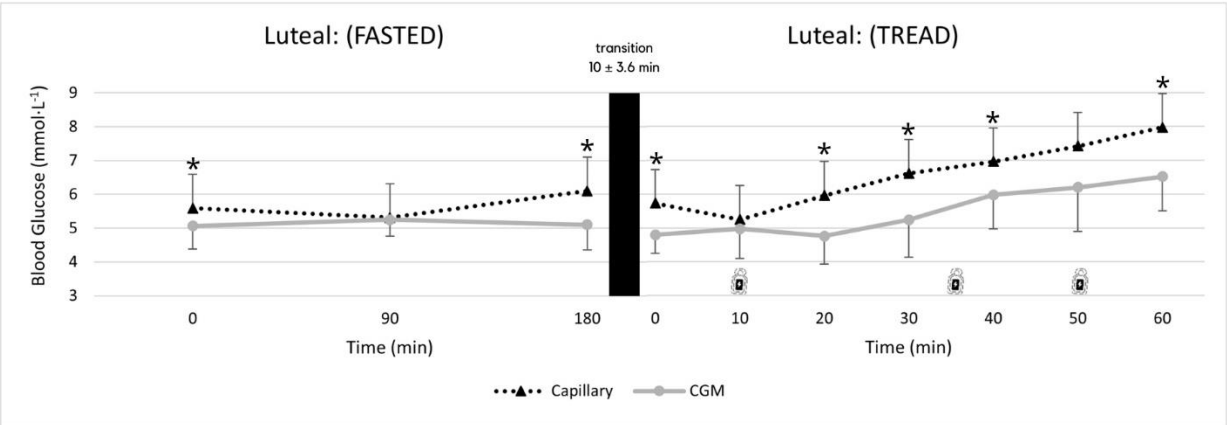


Figure 8. Comparison of the estimated means (mean ± SD) of blood glucose from continuous glucose monitor (CGM) to capillary during luteal phase. FASTED, a standardized outdoor 3-hour fasted depletion run. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention

provided at 10, 35, and 50 minutes). Values are means ± SD. * Statistically significant differences ($p < .05$) in glucose between CGM and capillary methods using individual paired t-tests at specific time points.

During TREAD, the CGM sensor reported glucose levels on average -1.02 mmol·L^{-1} ($p = 0.004$, 95% CI -1.63, -0.42) lower than the matching time-stamped capillary samples taken every ten minutes in both phases of the menstrual cycle (**Figure 7** and **Figure 8**). Only at 10 min was the extra effect of time (-0.18 mmol·L^{-1}) significantly different ($p = < 0.01$) than the average difference (-1.02) between CGM and capillary samples. The influence of *Phase* was statistically significant for the differences in glucose measurements between CGM and capillary only during TREAD ($p = 0.02$).

Parity of glucose values from CGM and capillary samples were also assessed using Bland-Altman plots. The tendency of the CGM to underreport was visually displayed, showing similar bias (95% LoA) in FOL and LUT phases during FASTED of -0.37 mmol·L^{-1} (1.36 to -2.10 mmol·L^{-1}) and -0.58 mmol·L^{-1} (1.29 to -2.45 mmol·L^{-1}), respectively (**Figure 9**). A bias (95% LoA) of -1.0 mmol·L^{-1} in TREAD was observed for FOL (1.75 to -3.65) and LUT (-1.76 to -3.86), (**Figure 10**). When CGM and capillary glucose data were combined for FASTED and TREAD the following biases (95% LoA) emerged -0.48 mmol·L^{-1} (-2.27 to 1.32) and -1.08 mmol·L^{-1} (-4.24 to 2.09), respectively, (**Figure 11** and **Figure 12**).

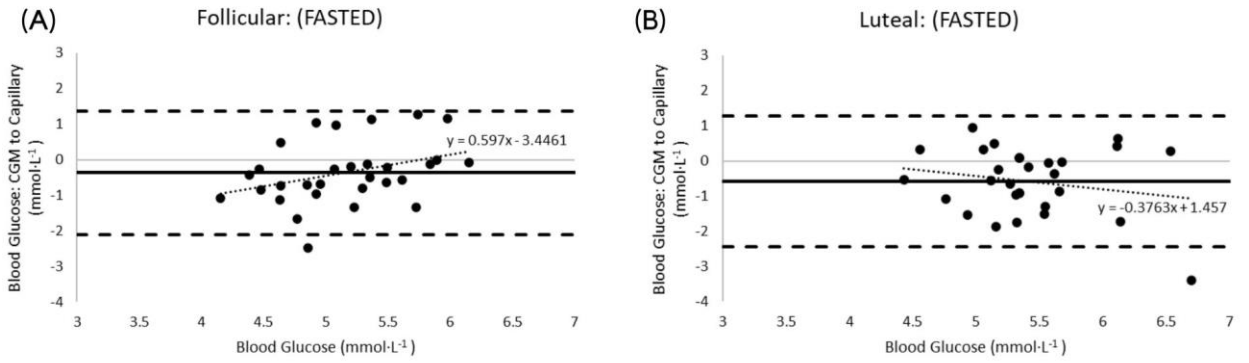


Figure 9. Comparison of reference capillary blood glucose to continuous glucose monitor (CGM) using Bland-Altman during FASTED protocol. Displayed as 95% levels of agreement (95% LoA). A) Follicular phase -0.37 mmol·L-1 (1.36 to -2.10 mmol·L-1). B) Luteal phase -0.58 mmol·L-1 (1.29 to -2.45 mmol·L-1). FASTED: a standardized outdoor 3-hour fasted depletion run.

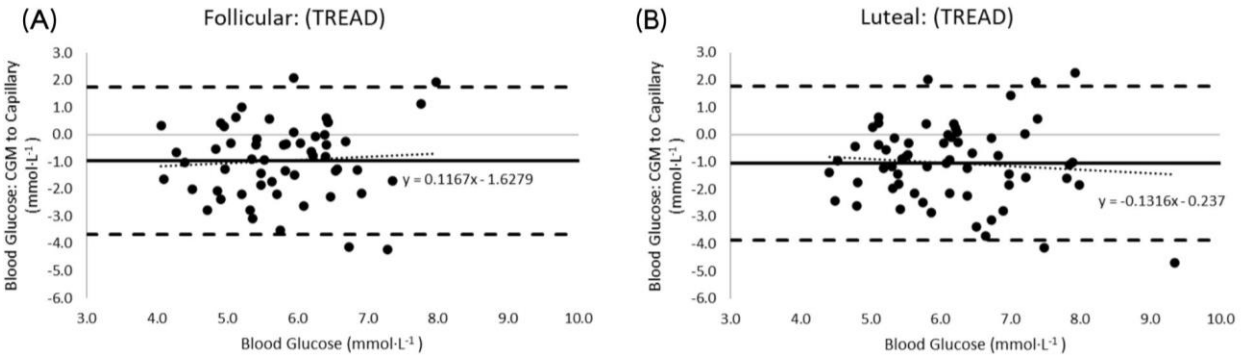


Figure 10. Comparison of reference capillary blood glucose to continuous glucose monitor (CGM) using Bland-Altman during TREAD protocol. Displayed as 95% levels of agreement (95% LoA). A) Follicular phase -1.0 mmol·L-1 (1.75 to -3.65 mmol·L-1). B) Luteal phase -1.0 mmol·L-1 (-1.76 to -3.86 mmol·L-1). TREAD: a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

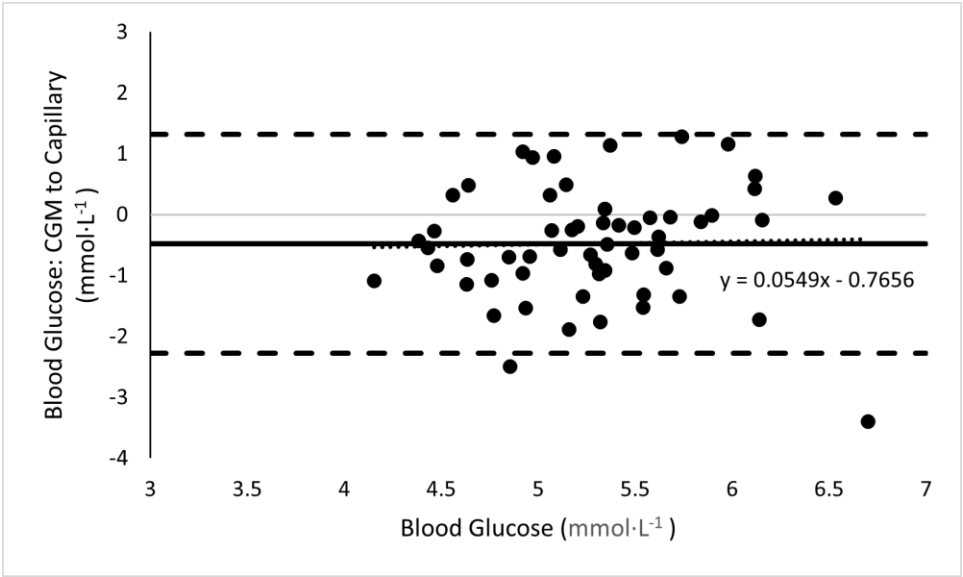


Figure 11. Comparison of combined follicular and luteal phase capillary blood glucose to continuous glucose monitor (CGM) using Bland-Altman during FASTED protocol. Displayed as 95% levels of agreement (95% LoA) -0.48 mmol·L^{-1} (-2.27 to 1.32 mmol·L^{-1}). FASTED: a standardized outdoor 3-hour fasted depletion run.

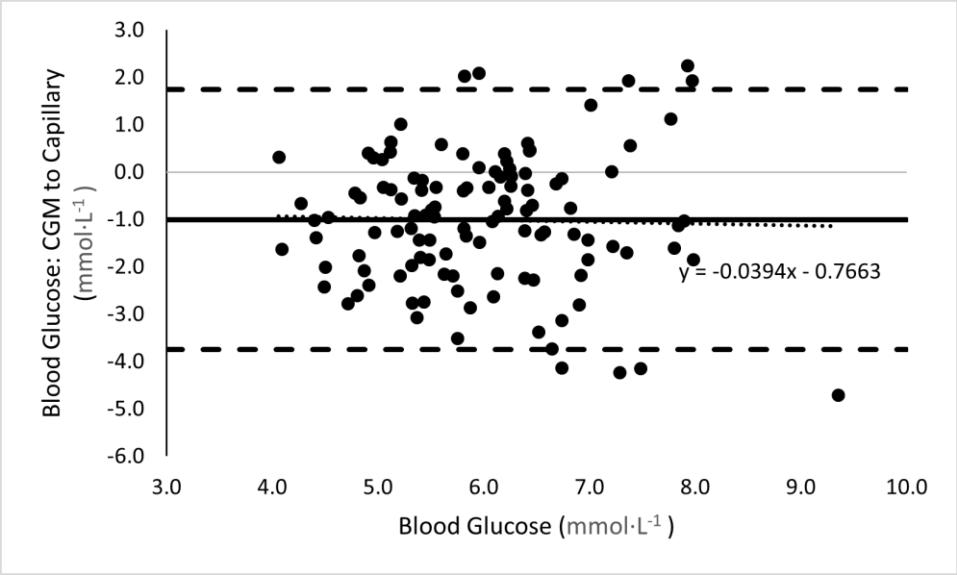


Figure 12. Comparison of combined follicular and luteal phase capillary blood glucose to continuous glucose monitor (CGM) using Bland-Altman during TREAD protocol. Displayed as 95% levels of agreement (95% LoA) -1.08 mmol·L-1 (-4.24 to 2.09 mmol·L-1). TREAD: a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

7.6 Discussion

This study examined the influence of menstrual phase, prolonged running, and carbohydrate intakes on glucose and investigated the validity of CGM as a reliable tool for assessing glucose during fasted and glucose supplemented exercise. Specifically, data from this study demonstrated that on average, the CGM reports glucose levels lower than capillary glucose during a 3-hour fasted run by -0.43 mmol·L^{-1} with a variability between CGM and capillary blood glucose of -0.86 to -0.005 mmol·L^{-1}. With the 20 g of oral glucose (administered to the participants at three different times points during the fourth hour of running) the CGM sensor continued to report lower glucose levels by an average of -1.02 mmol·L^{-1} as compared to capillary glucose sampled concurrently. Variability between CGM and capillary blood glucose was -1.63 to -0.42 mmol·L^{-1} during the TREAD run. These findings concur with previous studies showing that CGM accuracy can be negatively impacted by rapidly changing glucose levels.[56] An additional effect of time was observed on CGM, as compared to capillary blood glucose, at 180 min (-0.755 mmol·L^{-1}) during the FASTED run.

The accentuated differences of glucose levels from CGM and a tendency towards reduced accuracy over time have previously been observed by Guillot *et al.* in exercising adults with type 1 diabetes using a similar generation of sensor.[179] Novel to the present study was the observation that menstrual cycle influenced the relationship between CGM and capillary glucose data. On average, whether fasted or provided supplemental CHO, as glucose levels increased in the FOL phase the tendency of CGM to report lower glucose levels lessened and moved closer to capillary samples. This would concur with previous studies expressing CGM accuracy concerns during times of low blood sugar levels.[56,58] The opposite was observed in the LUT phase as the trend in CGM values moved away from capillary samples as glucose increased. The magnitude of the tendency for CGM to move away from capillary was greater when fasted and less pronounced in the presence of exogenous CHO (**Figure 9** and **Figure 10**), which is surprising and in contrast with previous observations of this study and others.[56] It is possible that the larger glucose sample size in TREAD (n = 77) explains the less dramatic shift than was observed during FASTED (n = 33). When glucose data from capillary blood glucose and CGM are combined from both phases (FOL + LUT) during FASTED and TREAD (**Figure 11** and **Figure 12**) the glucose trends observed in the FOL phase to move closer to capillary glucose samples at higher glucose concentrations become diminished in FASTED and unrecognizable in TREAD. This may indicate that CGM validity data presented in female populations without consideration of menstrual phase may be inconsistent and flawed. Also, the need for CGM sensor algorithms used to calculate glucose from ISF may potentially need tuning or calibration for specific female populations.

In the current study, the individual but not menstrual phase emerged as a statistically significant influence for why CGM glucose data was different than capillary measurements ($p = 0.043$), in FASTED. This observation may be attributed to a combination of inherent, yet undefined, biological differences among individuals and implies that glucose data from CGM seems to vary by person during fasted exercise. Differences of insulin action in muscle and liver among individuals during prolonged submaximal exercise[274] may influence ISF levels in subcutaneous tissue which could impact ISF glucose concentrations. Varying levels of adiposity, either whole body or at sensor application site, may also explain the tendency for individual differences in glucose values.[275] Lastly, other glucose modulating hormones such as insulin, cortisol and epinephrine associated with exercise[276] may affect glucose diffusion across the ISF compartment.

During TREAD, *Phase* nested with *Person* was a significant contributor to the differences between glucose values from CGM and capillary blood glucose. This observation demonstrates that menstrual phase may also influence CGM measurements, the extent of the difference will vary from person to person, and that the influence is dependent on the availability of carbohydrates. A possible explanation for this observation could be multifactorial and include: 1) the variation of ovarian hormone ratios E2 and P4 between individuals at any given time point in the menstrual cycle[258] and 2) the cyclic nature of glucose throughout the menstrual cycle characterized by lower daily levels of glucose in the mid FOL phase due to elevated E2 and subsequent elevated daily glucose levels in the ovulatory and luteal phases when E2 is lower.[277] The exact mechanisms behind a hormonal influence of glucose in the ISF glucose compartment is unclear, however ovarian hormones may contribute to the difference between CGM and capillary glucose indirectly through actions associated with the metabolic rates of surrounding organ systems, interactions with insulin, glucose uptakes[275] and brain mediated insulin sensitivity processes across the menstrual cycle.[278]

In addition to the hypothesized biological differences and ovarian hormonal influences that may contribute to the differences between CGM and capillary glucose measures, there is potential for high training loads, independent of energy balance, to lower glucose levels during exercise, particularly when the athlete is overreached.[279,280] This proposed alteration in CHO metabolism associated with impaired mitochondrial function could affect the concentration of glucose within ISF impacting the reliability of glucose data from CGM. Considering the high training volumes of ultra-endurance athletes and the variations in annual training plans among the study participants, it is plausible that some of the discrepancies in glucose measurements between CGM and capillary measurements can be attributed to the variability of individual training loads prior to testing.

Caution is required when interpreting this data due to unanswered questions when trying to disseminate glucose data healthy athletes. Primarily, there are no established parameters for glucose ranges during physical activity and a lack of consensus on how transient episodes of hyperglycemia impact performance during exercise. There is also minimal empirical support linking a specific range of glucose exceeding 3.0 $mmol \cdot L^{-1}$, which is considered clinically significant and indicative of symptomatic hypoglycemia[67,68] in healthy individuals, to aspects of performance refueling or recovery.[161] The confounding nature of glucose on endurance

performance is evidenced in the findings of [70] who observed a positive correlation (p = 0.002) between higher running speeds and low to average overall changes in glucose from pre-race resting levels in men but not women (male 4; female 3), using CGM during a 160-km ultra-marathon. Concurrently, these authors observed no association between running speed and the highest levels of glucose. Perhaps, the most interesting observation made was the negative impact large changes in blood glucose variability (difference between highest and lowest glucose values from resting) had on running speed for male runners (p = 0.04) but not females (p = 0.6). This sex difference could be partly attributed to the lesser-understood physiological differences in females, such as intramyocellular lipid density and gene expressions highlighted by Devries *et al.*[128] and Maher *et al.*[281] Specifically, women have increased concentrations of the enzymes associated with long and medium chain fatty acid oxidation as well as increased intramuscular lipid in contact with mitochondria that may infer a performance benefit during prolonged endurance exercise.

The present study and previous research by Ishihara and colleagues[70] may imply that sexual dimorphisms exist in how glucose is managed during endurance sport and may also indicate that glucose and its variability can play a role in guiding nutritional strategies during endurance exercise. Future studies in larger populations to assess the impact of glucose variability in females during endurance exercise is indicated. Moreover, as evidenced previously,[70] the importance of CGM accuracy during non-fasted exercise to report absolute glucose levels may be less important than its ability to reliably report changes in glucose variability if it is to be used as a performance tool. Collectively, this data suggests that the CGM device used in the current study may not presently offer an accurate depiction of blood glucose levels during ultra-endurance running in either the fasted or fed state and advise this be a consideration when using CGM to inform nutrition strategies for endurance sport.

A limitation of this research includes the small sample size. Like numerous longitudinal intervention studies, limitations related to participant selection criteria and attrition rates were encountered. The primary challenge lay in identifying and retaining a cohort of eumenorrheic ultra-running athletes over an extended period. We do note, however, that the final sample size exceeded the entirety of all research in the area to date. As this was an applied, infield study, blood serum hormone testing was not feasible in the absence of low-cost detection methods; however, this study was thoughtfully constructed, upheld rigorous population controls and utilized a

combined method[282] of menstrual cycle phase verification comprised of self-reported menstrual tracking and ovulation predictor urine dipsticks across three full menstrual cycles. Unfortunately, standardization of training load was unmanageable given the demands of the testing protocol, participant availability, and alignment of menstrual cycles. Standardization of diet prior to testing was limited to an 8-hour fast as well as controlled energy intakes when comparing exercise between phases. Lastly, limited resources prevented the use of multiple independent CGM sensors to collect multiple data sets simultaneously to assess inter-device reliability and any variability inherent to the sensor application site on the body. Future research could improve upon these limitations.

Here, we report the first study to focus on and describe the glucose response across the menstrual cycle in a group of endurance-trained, eumenorrheic female athletes. Data from the present study demonstrated that the sport CGM device used in this study tends to, on average, underreport glucose levels by varying degrees during fasted and unfasted exercise when compared to capillary glucose. Furthermore, as glucose levels increased, during fasted or fed states, the tendency for the CGM to underreport lessened in the FOL phase but worsened in LUT. This study also demonstrated that individual physiological characteristics were significant for influencing glucose measurements of the CGM when compared to capillary glucose values during fasted and CHO supplemented ultra-endurance running. Of note, menstrual phase emerged as an influence on the glucose measures of the CGM, but only when exogenous CHO was available and not during fasted prolonged running. The extent of this difference between CGM and capillary measurements between menstrual phases was similarly dependent on differences of the individual, which may include ovarian hormone ratios, brain insulin sensitivity fluctuations or other glucose modulating hormones such as insulin, cortisol and epinephrine. It is possible that a combination of these factors is contributing to glucose concentrations in the ISF and indicates another area of future research. Consequently, athletes and practitioners should be aware of these accentuated differences in CGM glucose data when used as a tool to inform nutrition interventions during UE running. The sensor could provide useful snapshots into glucose variability and general trends if calibrated to the individual, however the application of this data to sport performance requires additional study. The CGM device used in this study is factory calibrated; however, a reversion to user calibration maybe more appropriate for the female athlete population. Further research is indicated to understand the role of glucose variability as a metric in female endurance performance as well as the impact of

female biological diversity on the accuracy of CGM across the menstrual cycle in both fasted and in present of supplemented CHO.

The present study and previous research by Ishihara and colleagues[70] may imply that sexual dimorphisms exist in how glucose is managed during endurance sport and may also indicate that glucose and its variability can play a role in guiding nutritional strategies during endurance exercise. Future studies in larger populations to assess the impact of glucose variability in females during endurance exercise is indicated. Moreover, as evidenced previously,[70] the importance of CGM accuracy during non-fasted exercise to report absolute glucose levels may be less important than its ability to reliably report changes in glucose variability if it is to be used as a performance tool. Collectively, this data suggests that the CGM device used in the current study may not presently offer an accurate depiction of glucose levels during UE running in either the fasted or fed state and advise this be a consideration when using CGM to inform nutrition strategies for endurance sport.

A limitation of this research includes the small sample size. Like numerous longitudinal intervention studies, limitations related to participant selection criteria and attrition rates were encountered. The primary challenge lay in identifying and retaining a cohort of eumenorrheic ultra-running athletes over an extended period. We do note, however, that the final sample size exceeded the entirety of all research in the area to date. As this was an applied, in-field study, blood serum hormone testing was not feasible; however, menstrual cycle phase verification was confirmed with self-reported menstrual tracking and ovulation predictor urine dipsticks across three full menstrual cycles. Lastly, limited resources prevented the use of multiple independent CGM sensors to collect multiple data sets simultaneously to assess inter-device reliability and any variability inherent to the sensor application site on the body. Future research could improve upon these limitations.

Here, we report the first study to focus on and describe the glucose response across the menstrual cycle in a group of endurance-trained, eumenorrheic female athletes. Data from the present study demonstrated that the sport CGM device used in this study tends to, on average, underreport glucose levels by varying degrees during fasted and unfasted exercise when compared to capillary glucose. Furthermore, as glucose levels increased, during fasted or fed states, the tendency for the CGM to underreport lessened in the FOL phase but worsened in LUT. This study also

demonstrated that individual physiological characteristics were significant for influencing glucose measurements of the CGM when compared to capillary glucose values during fasted and fed UE running. Of note, menstrual phase emerged as an influence on the glucose measures of the CGM, but only when exogenous CHO were available and not during fasted prolonged running. The extent of this difference between CGM and capillary measurements between menstrual phases was dependent on unknown biological differences of the individual. Consequently, athletes and practitioners should be aware of these accentuated differences in CGM glucose data when used as a tool to inform nutrition interventions during UE running. The sensor could provide useful snapshots into glucose variability and general trends if calibrated to the individual, however the application of this data to sport performance requires additional study. The CGM device used in this study is factory calibrated; however, a reversion to user calibration maybe more appropriate for the female athlete population. Further research is indicated to understand the role of glucose variability as a metric in female endurance performance as well as the impact of female biological diversity on the accuracy of CGM across the menstrual cycle in both fasted and fed states.

7.7 Supplementary Materials

Supplemental Table 1. Summary of mean glucose.

	Phase	***Capillary*** *(mmol)*	***CGM*** *(mmol)*
Protocol			
(FASTED)	Follicular	5.38 ± 0.58	5.16 ± 0.95
	Luteal	5.62 ± 0.77	5.44 ± 0.89
	Δ LUT	0.24	0.28
(TREAD)	Follicular	6.16 ± 1.08	5.16 ± 1.09
	Luteal	6.56 ± 1.27	5.54 ± 1.16
	Δ LUT	0.4	0.38

Continuous glucose monitor (CGM); TREAD: a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes); FASTED: a standardized outdoor 3-hour fasted depletion run. Data presented as mean and SD.

Supplemental Table 2. Summary of run data across the menstrual cycle.

Run Summary	**Unit**	**FOL *Phase***	**LUT *Phase***
Distance completed FASTED	km	23.8 ± 2.6	23.82 ± 2.90
Vertical ascent FASTED	m	628.18 ± 75.82	629.09 ± 180.23
Distance completed TREAD	km	7.75 ± 1.23	7.93 ± 1.19
TREAD %VO_{2MAX}	%	57 ± 3.99	57.5 ± 4.65
Distance completed total (4hrs)	km	31.59 ± 3.62	31.76 ± 3.82
Energy Contribution (RER)			
Fat	%	64.4 ± 0.09	63.4 ± 0.12
Carbohydrate	%	35.9 ± 0.09	36.9 ± 0.12
Urine Specific Gravity (USG)			
USG after completed trial (4hrs)	*USG*	1.018	1.017

TREAD: a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes); FASTED: a standardized outdoor 3-hour fasted depletion run. Respiratory exchange ratio (RER). USG: Urine specific gravity. Data presented as mean and SD.

Supplemental Table 3. Marginal fitted equation summary for differences between CGM and Fingerstick glucose during FASTED

Term	*Coefficient (mmol/L)*	*SE Coef*	*DF*	*T-Value*	*P-Value*
Constant (mmol/L)	-0.431	0.191	10.04	-2.256	0.048
Time (minutes')					
0'	-0.089	0.126	46.82	-0.706	0.483
extra effect diff	*-0.52*	*(-0.432 + -0.089 = -0.52)*			
90'	0.413	0.13	46.72	3.16	0.003
extra effect diff	*-0.018*	*(0.431 + 0.413 = -0.018)*			
180'	-0.324	n/a	n/a	n/a	n/a
extra effect diff	*-0.755*	*(-0.431 + -0.324 = -0.755)*			

FASTED: a standardized outdoor 3-hour fasted depletion run. Data presented as estimated mean.

Supplemental Table 4. Marginal fitted equation summary for differences between CGM and Fingerstick glucose during TREAD

Term	*Coefficient*	*SE Coef*	*DF*	*T-Value*	*P-Value*
Constant (mmol/L)	-1.02	0.271	9.99	-3.755	0.004
Time (minutes')					
0'	-0.021	0.152	108.61	-0.137	0.891
extra effect diff	*-1.04*				
10'	0.843	0.148	110.7	5.687	<0.01
extra effect diff	*-0.18*				
20'	-0.248	0.156	108.76	-1.595	0.114
extra effect diff	*-1.27*				
30'	-0.045	0.149	108.61	-0.298	0.766
extra effect diff	*-1.06*				
40'	-0.059	0.149	108.65	-0.396	0.693
extra effect diff	*-1.07*				
50'	-0.129	0.156	108.76	-0.825	0.411
extra effect diff	-1.15				
60'	-0.341	n/a	n/a	n/a	n/a
extra effect diff	-1.36				

TREAD: a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). Data presented as estimated mean.

SECTION 8

8.1 Fueling in Flux: Glucose Responses and Menstrual Phase Interactions in Female Runners During a Simulated Ultra Run – Manuscript 3

Authors: Andrew Dole, Stacy Sims, Martyn Beaven

Under initial review with *Journal of Sports Sciences*

8.2 Abstract

Blood glucose, a key metric in endurance sports, is underexplored in its dynamic interplay with the menstrual cycle. This cross-over study aimed to investigate the influence of menstrual cycle phase on blood glucose while fasted and when provided exogenous carbohydrate during a 4-hour simulated ultra-endurance run in twelve, experienced, eumenorrheic, female ultra-runners (age 39 ± 6 y). Participants completed the protocol, which consisted of a 3-hour fasted outdoor run (FASTED) followed by a one-hour treadmill run (TREAD) where three standardized oral glucose doses were provided, in the mid-follicular (FOL) and mid-luteal phases (LUT). Median glucose was higher in the LUT (Capillary 0.25-0.29 $mmol \cdot L^{-1}$, CGM 0.38-0.4 $mmol \cdot L^{-1}$) when fasted or provided carbohydrate. Menstrual phase was significant for differences (0.46 $mmol \cdot L^{-1}$) in capillary glucose at 180 minutes of fasted running ($p = 0.046$). Incidence of glucose < 3.9 $mmol \cdot L^{-1}$ was greater in FOL among specific individuals. This is the first study to show that glucose is on average higher in the mid-luteal phase and to observe the avoidance of clinically significant non-diabetic hypoglycemia (< 3.0 $mmol \cdot L^{-1}$) during 3-hours of fasted running following an 8-hour fast among female ultra-runners with higher individual responses of low blood sugar in the follicular phase.

8.3 Introduction

Ultra-endurance events are defined as events lasting a minimum of 4-6 h[1,2] and up to several consecutive days. The aerobic energy metabolism is regarded as the primary energy system during ultra-running, which is characteristically performed for extended periods below 70% maximal volume of oxygen uptake VO_2max with brief but frequent need for higher intensity efforts.[1,81] The energy demands associated with ultra running have been estimated in males and females to average 8700-17000 kcal over varying distances of a 24-hour race period with no differences attributable to sex.[19] To meet these energy demands a combination of exogenous and endogenous fuel sources, predominately carbohydrate (CHO) and fat, are utilized.[82] At a modest 65% of VO_2max, CHO is the primary source of energy;[88,89] however, it is a limited resource and the human body's capacity for CHO storage cannot meet the energetic demands of prolonged endurance exercise. The depletion of the approximately 2,000 kcal of available glycogen stores (liver 100 g and muscle glycogen 400 g)[10,83], is widely recognized as a primary contributor to fatigue and performance decline.[13] When compared to the estimated energy demands of a 100 mile ultra-run (13,000-16,000 kcal)[9,78] CHO is insufficient even with the contribution of fat oxidation; as fat oxidation is unable to entirely compensate for the energy demand deficit observed between CHO oxidation and energy utilization rates during requisite intensities for ultra-sport.[11,77,283]

The recommended exogenous CHO intakes to meet the energy demands of ultra-sport are 90 $g \cdot h^{-1}$ and up to 120 $g \cdot h^{-1}$ in males.[16] These intakes are typically achieved through a combination of maltodextrin and fructose[14,77] but tax the physiological limits of the gastrointestinal system's ability to absorb and utilise this mixed energy source, especially under prolonged duress. The result is often gastrointestinal distress which is prevalent among runners[186] with observed rates of 65-82% in ultra-running events[19,20] This distress is often associated with high CHO intakes[22] as a result of blood being diverted away from the digestive system to working muscles[284] as well as the current understanding that saturation of primary glucose (SLGT1) and fructose (GLUT5) absorption pathways in the intestine occur at 60 $g \cdot h^{-1}$ and 30 $g \cdot h^{-1}$ respectively.[14,16,17]

Therefore, it is of interest within the sports performance industry to investigate CHO fueling strategies and inform individualized CHO recommendations for recovery, daily nutrition, and race day intakes.[132] Near real-time glucose monitoring through interstitial fluid is now more achievable in a variety of sporting environments through the use of continuous glucose monitors (CGM).[133]

However, the blood glucose research data in ultra-endurance sports, to inform fueling strategies is limited and lacks well-controlled female studies. An observational study by Ishihara *et al.* (2020)[70] examined blood glucose levels in seven participants (female 3; male 4) during a 165 km ultra trail run. They found no relationship between running speed and the highest glucose levels with the difference between rested glucose and highest recorded levels during the event, impacting female running speed less than males.[70]An additional case study by the same researchers observed that running pace had no relationship with glucose level or variability in a single female ultra runner over a 438 km trail run.[182]

Collectively, these studies included only 4 female participants, lacked an experimental component, and did not verify CGM data accuracy during the events. Furthermore, the four female participants were not assessed for hormonal contraceptive usage or menstrual phase to account for potential variations in ovarian hormone profiles. The impact of ovarian hormones, estrogen and progesterone, on blood glucose across the menstrual cycle during prolonged endurance has yet to be elucidated despite their well-known influences on carbohydrate metabolism. For example, E2 has been observed to promote glucose uptake into type I fibers,[26] while simultaneously reducing uptake of glucose into the bloodstream.[27] Conversely, progesterone appears to reverse E2's suppression of muscle glucose oxidation during submaximal exercise[27] as well as complement E2's glucose-sparing effect in the liver [39] Therefore, the present study aimed to investigate the influence of menstrual cycle phase on blood glucose while fasted and provided exogenous CHO during a simulated ultra-endurance run in regularly menstruating females.

8.4 Methods

8.4.1 Experimental Design

A randomized, cross-over design with participants acting as their own control across menstrual cycle phases was used during an ultra-marathon simulation consisting of a standardized outdoor 3-hour fasted depletion run (FASTED) followed by a 60-minute treadmill run (TREAD) with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). Participants completed the run protocols (FASTED and TREAD) once during the mid-follicular (FOL: 4-8 days after the start of menses) and mid-luteal (LUT: 5-8 days post ovulation) phases of the menstrual cycle. Ethics approval was obtained from the University of

Waikato's Human Research and Ethics Committee (HREC(Health)2021#73). Written informed consent was obtained from each participant. Recruitment and data collection occurred from December 2021 through November 2022.

8.4.2 Participants

Eighteen recreational female ultra-endurance runners, recruited from endurance sports clubs located in New Zealand, agreed to participate in this study. Six were excluded from the initial sample group due to injury or disruptions in hormone profile (pregnancy; hormone therapy). Twelve participants were included in the data analysis unless otherwise specified (age: 39.3 ± 6.3 y, height: 165.3 ± 5.6 cm, body mass: 62 ± 8.0 kg, VO_{2MAX}: 45.9 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$). An electronic questionnaire was administered to all participants to screen for established inclusion criteria: 1) eumenorrheic experiencing regular cycles (28-40 d), 2) not using hormonal contraception for at least 12 months except for Mirena IUDs implanted for a minimum of 9 months, 3) at least one year of ultra-endurance experience, 4) and no current injuries that would impact performance. Additional exclusion criteria included 1) a history of cardiovascular, renal, or metabolic disease, 2) insulin or glycemic disorders, 3) the use of any exogenous hormones, or 4) confirmed anovulatory cycles.

8.4.3 Hormone Control

Prior to familiarization, participants were required to provide photo documentation to the lead researcher of menstrual tracking for three consecutive cycles using a commercial mobile menstrual tracking application (Garmin Connect, Olathe, KS, USA; FITRwoman, Encino, CA, USA; Wild.AI, San Francisco, CA, USA). In-home urine ovulation test strip predictor kits (Pregmate, Fort Lauderdale, FL USA) were then used to detect a luteinizing hormone surge, confirm ovulatory function for two consecutive months, and establish consistency in calendar tracking of MC length. Participants were instructed to begin strip testing for seven consecutive days prior to their predicted ovulation. A count forward method was used to predict the early follicular (days 4-8 from the start of menses) and mid-luteal phases (days 5-8 post-ovulation) for testing.

8.4.4 Metabolic Data

Respiratory exchange ratio (RER) was obtained during the treadmill portions of the run protocol using a TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT USA). A mouthpiece and nose covering were worn by participants to collect expired gases for most of the time trial; being removed for carbohydrate and fluid intake which was recorded and time-stamped for data collection integrity. Heart rate was obtained using a telemetry system (Polar Electro, Inc., Lake Success, NY, USA). Capillary blood glucose samples were taken using a CareSens lancing device, lancets, and testing strips (i-SENS Global, Gordon NSW, AU) three times during the 3-h depletion run: prior to start, after 90 minutes of exercise, and at the completion of the three-hour fasted run. During the 1-h treadmill run, capillary samples were taken just before start, and then at 10-min intervals (7-time points). Interstitial glucose was measured during the entire 4-h run protocol at 1-min intervals using the Abbott Libre Sense continuous glucose monitor (Abbott Laboratories, Abbott Park, IL USA) worn on the back of the upper arm for a minimum of 24 hours and a maximum of 13 days before run protocol testing. Interstitial glucose data was collected by the CGM via a thin, flexible amperometric sensor filament inserted subcutaneously. The stored data from the CGM was uploaded by pairing the sensor to a mobile device which wirelessly transmitted to cloud-based storage and was retrieved onto PC using the cloud-based coach dashboard features (Supersapiens, Atlanta, GA USA). When necessary, glucose in $mg \cdot dL^{-1}$ was converted to $mmol \cdot L^{-1}$ by multiplying by 0.0555.

8.4.5 Familiarization

Participants reported to the laboratory to complete VO_{2MAX} testing, receive study instructions, and familiarize participants with testing procedures and equipment. An instructional video and walk-through of the continuous glucose sensor (CGM) application were provided. Depletion run instructions and outdoor route were reviewed. A combination ramp and graded VO_{2MAX} test protocol was conducted on a motorized treadmill with a stiffness of 365 $kN \cdot m^{-1}$ (Steelflex PT10 Fitness, Steelflex Fitness, Taipei, Taiwan) with the collection of pulmonary gas exchange using a TrueOne 2400 metabolic cart.[273] Each participant was instructed to begin with a self-paced 5-min warm-up. Following warm-up, the testing began with an initial stage running on a 0% grade at 8.5 $km \cdot h^{-1}$ for two minutes. This stage was followed by increases in speed of 1.4 $km \cdot h^{-1}$ every 30 seconds until 14.1 $km \cdot h^{-1}$ was reached. After 30 seconds at 14.1 $km \cdot h^{-1}$ the grade was increased

from 0% to 2% for two minutes followed by a 2% increase in grade every two minutes to volitional exhaustion. Maximum effort was determined when both conditions were met: 1) failure to continue test and 2) RER > 1.1.

8.4.6 Outdoor Depletion Run

Participants were instructed to apply the CGM sensor 24-48 h before their scheduled run day, arrive at the lab in a fasted state of 8 hours, and have avoided caffeine, alcohol, and strenuous activity for 24 hours preceding the run. Upon arriving at the lab on the morning of the protocol, a urine sample was taken, and urine specific gravity was measured using a hand-held refractometer (Atago CO LTD, Tokyo Japan). Body weight was documented using a Wedderburn WM202 patient scale (Wedderburn, Ingleburn, NSW, Australia). Capillary blood glucose was recorded immediately (< 1 min) before the start of the depletion run. Participants ran at a self-selected pace along a pre-determined, two loop, outdoor route, chosen to simulate an ultra-endurance run course for 3-hours. Starting from the laboratory participants ran a flat 2.6 km stretch to the offroad course where they directly began the first loop of 2.65 km consisting of 133 m of elevation gain/loss followed by the second loop of 3.47 km and 48 m of elevation gain/loss (**Figure 13**). These loops were repeated for three consecutive hours. Participants checked in with the researcher at an aid station located along the route after the completion of each loop to receive, *ad libitum*, a standardized non-caloric electrolyte solution containing 600 mg sodium (Na^+) and 100 mg K potassium (K^+) per Litre. Participants were instructed to drink no other fluids than those provided by the researcher and to eat nothing during the duration of the depletion run. Intake of all fluid was measured and documented. Capillary glucose was recorded two additional times at 90- and 180-minutes (**Figure 14**). Upon completion of the depletion run, a rest aid station was simulated where the runner was allowed to sit and hydrate while being driven back to the lab to begin the 1-h treadmill trial. The average transition time from the depletion run's end to the treadmill trial's start was 10 ± 3 min. Run data (distance, elevation, pace) was tracked using a runner-provided watch with global positioning (GPS) and documented with screenshots of Strava (Strava, San Francisco, CA, USA) or Garmin Connect (Garmin Connect, Olathe, KS, USA) mobile applications. Outdoor runs were completed between 4 a.m. and 12 noon to minimize variations in weather and temperature (13.5 ± 6.6 °C).

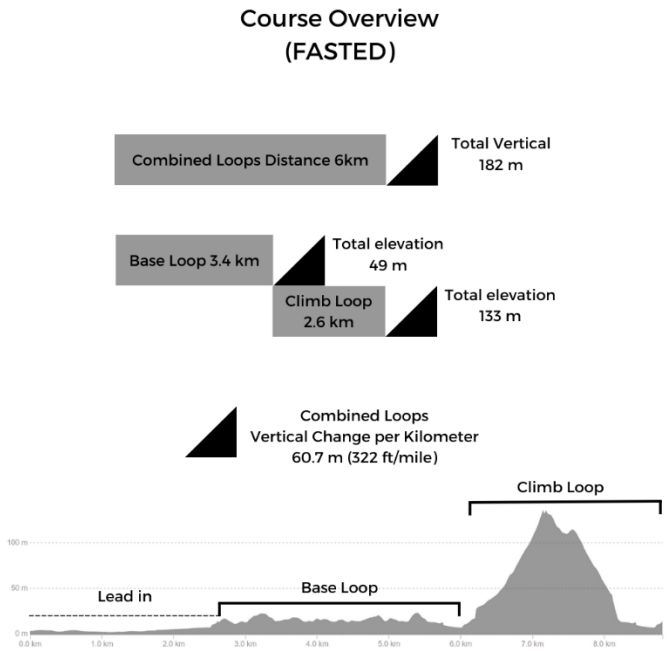


Figure 13. Overview of outdoor FASTED run course. FASTED, a standardized outdoor 3-hour fasted depletion run.

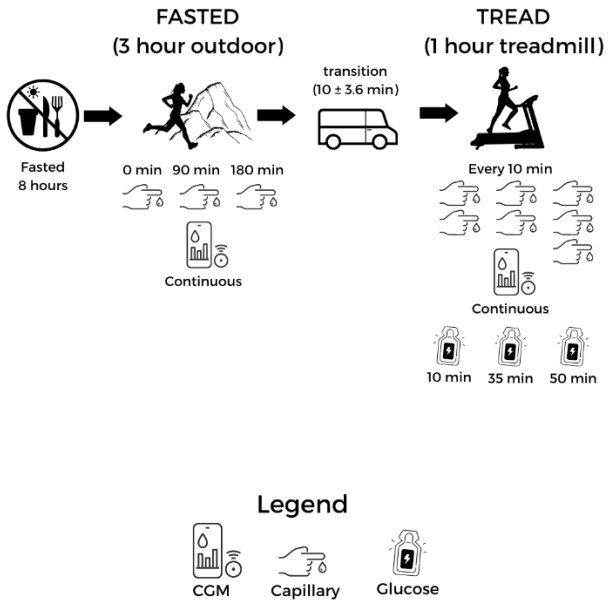


Figure 14. Schematic of study design.

8.4.7 Indoor Treadmill Trial

Following the FASTED protocol and the brief transition described above, participants were instructed to run at a self-selected pace for an additional 60 minutes on the motorized treadmill at 0% grade while having expiratory gases and heart rate continuously monitored with a TrueOne 2400 metabolic cart in a temperature-controlled environment (19 °C). Blood glucose was taken via capillary seven times, starting at 0 minutes and again every 10 minutes until completion. A 20 g bolus of glucose was provided at three time points (15, 35 and 50 minutes; **Figure 14**). A standardized non-caloric electrolyte solution containing 600 mg Na and 100 mg K per Litre was provided *ad libitum* with intakes recorded. Interruptions to the expiratory gas collection were time stamped.

8.5 Statistical Analysis

8.5.1 Depletion

A linear mixed model was used to assess the main effects of *Time* and menstrual cycle *Phase* with the biological variability of the *Person* as a random effect on blood glucose levels collected with capillary fingerstick at three points (0 min, 90 min, 180 min) and simultaneously using a CGM over the FASTED protocol for all participants ($n = 12$). Statistical significance was set at $P \leq 0.05$. Data are presented as estimated coefficient averages of blood glucose measures ($mmol \cdot L^{-1}$). Normality was tested using Shapiro Wilk's. Capillary data was normally distributed, however, CGM data was non-normally distributed and response variables were log transformed with a *post-hoc* Bonferroni analysis was applied to capillary and CGM data.

8.5.2 Treadmill

A similar linear mixed model was for fit for *Time* and *Phase* with *Person* as a random effect to examine repeated measures of blood glucose data collected via capillary fingerstick during the TREAD protocol every 10 minutes and continuously with CGM. Due to sensor malfunction one participant's data was excluded from this analysis ($n = 11$). Normality of the residuals was not met for the response variables; therefore, a model with the same explanatory terms using the log transformation of the response variables was fitted. A Bonferroni *post-hoc* analysis was applied.

8.6 Results

8.6.1 Outdoor Depletion Run (FASTED)

Capillary Blood Glucose

Mean glucose for capillary samples over the three hours were FOL 5.38 ± 0.58 mmol·L^{-1} and LUT 5.63 ± 0.77 mmol·L^{-1} (**Table 6**). Independently, *Phase* (p = 0.046) was statistically significant for the 0.46 mmol·L^{-1} difference in glucose during LUT as compared to FOL at 180 min when sampled from capillary blood, (**Figure 15**). Within phase, *Time* was also independently significant for the difference in capillary glucose between 90-180 min; FOL 0.49 mmol·L^{-1} (p = 0.0475) and LUT 0.73 mmol·L^{-1} (p = 0.002).

Table 6. Comparison of Mean Glucose

Protocol	*Phase*	*Capillary*	*CGM*
(FASTED)	Follicular	5.38 ± 0.58	5.16 ± 0.95
	Luteal	5.62 ± 0.77	5.44 ± 0.89
(TREAD)	Follicular	6.16 ± 1.0	5.16 ± 1.09
	Luteal	6.56 ± 1.27	5.54 ± 1.16

Data presented in means and SD.

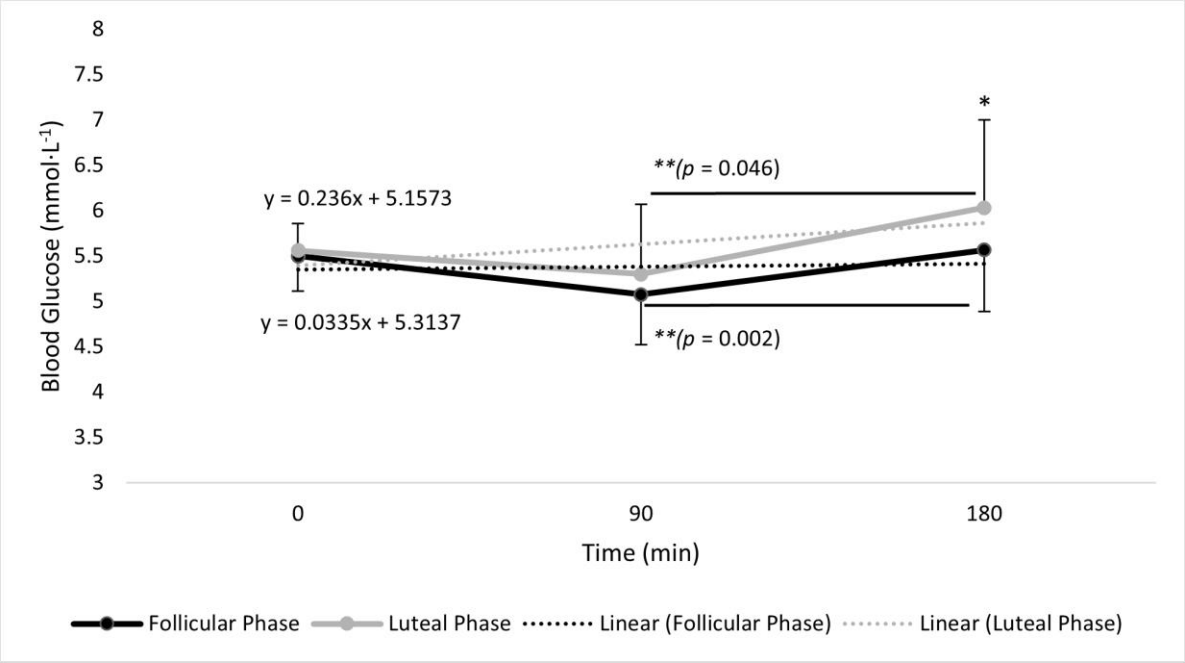


Figure 15. Comparison of the estimated means of capillary glucose during follicular and luteal phases in FASTED protocol. FASTED, a standardized outdoor 3-hour fasted depletion run. * Statistically significant differences in glucose between phases at specific time point. ** Statistically significant differences in glucose between time points within phase. Values are means ± SD.

CGM Blood Glucose

Mean glucose from CGM measures were FOL 5.16 ± 0.95 mmol·L^{-1} and LUT 5.44 ± 0.89 mmol·L^{-1} (**Table 6**). Similar differences and interactions were not observed in CGM measurements when matched for capillary time points. Measurements from CGM (**Figure 16**), assessed at 5 min intervals, indicated that glucose was significantly different between the FOL and LUT phases at only two time points: 5 min (p = 0.0147) and 35 min (p = 0.028). Within phase, CGM measures of glucose between consecutive time points were significantly different only between 0 min and 5 min in both the FOL (p = 0.042) and LUT (p = < 0.01) phases.

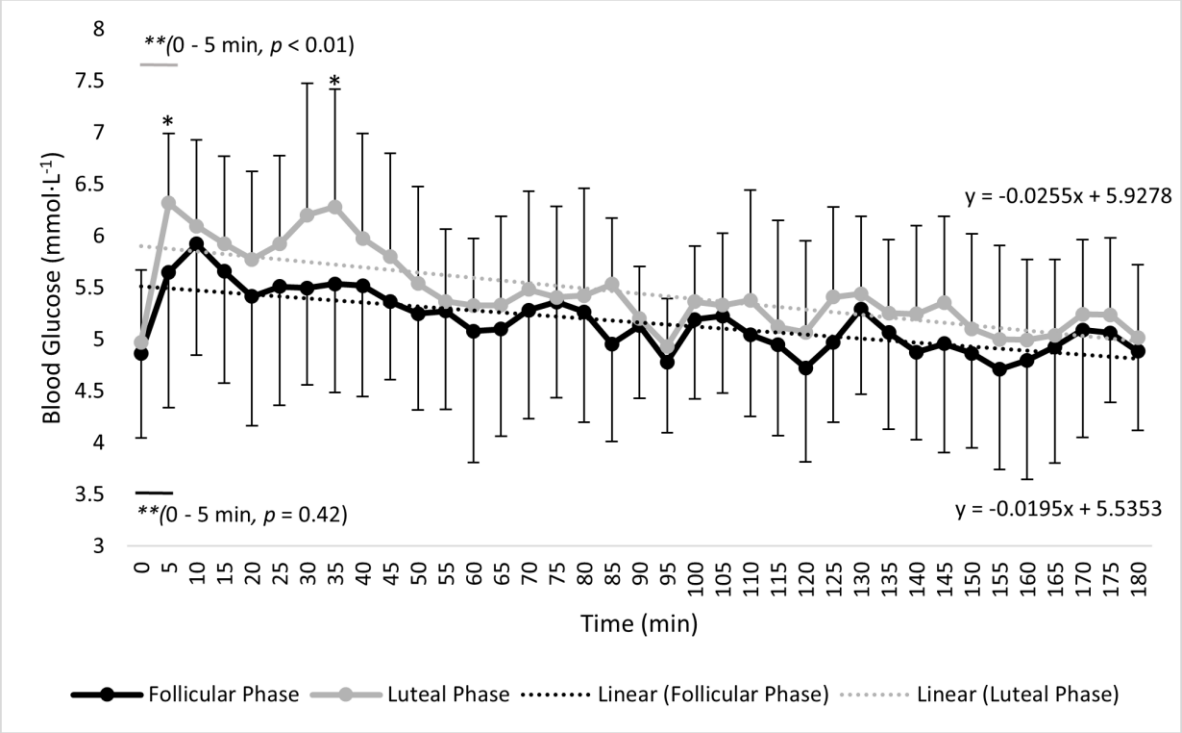


Figure 16. Comparison of the estimated means of continuous glucose monitor (CGM) glucose at 5-minute intervals during follicular and luteal phases in FASTED protocol.FASTED, a standardized outdoor 3-hour fasted depletion run. * Statistically significant differences in glucose between phases at specific time point. ** Statistically significant differences in glucose between time points within phase. Values are means ± SD.

Glycemia

Across the FASTED protocol, for both capillary and CGM measures, no clinically relevant hypoglycemic episodes (< 3.0 mmol·L^{-1})[67,68] were observed for any of the participants despite 3-hour of fasted running. The minimum recorded glucose measures for capillary were 4.4 mmol·L^{-1} FOL, 3.9 mmol·L^{-1} LUT, and for CGM were 3.1 mmol·L^{-1} FOL phase and 3.94 mmol·L^{-1} LUT, respectively. The incidence of glucose from CGM data below 3.9 mmol·L^{-1}, the lowest desirable level of normoglycemia in diabetics[67] often used as indicator of hypoglycemia in healthy adults, occurred 36 times in FOL (FOL: 36; LUT: 0) as compared none in capillary (FOL: 0; LUT: 0). Of these low CGM glucose values, < 3.9 mmol·L^{-1}, 88% were attributable to two participants.

8.6.2 Indoor Treadmill Trial (TREAD)

Capillary Blood Glucose

Mean glucose for capillary samples over the 1-hour TREAD were FOL 6.16 ± 1.00 mmol·L^{-1} and LUT 6.56 ± 1.27 mmol·L^{-1}. During TREAD, glucose was not significantly different between FOL and LUT phases at any of the seven capillary measurements. Within phase, *Time* was statistically significant for the changes in capillary glucose ($p < 0.001$) over the 1-hour. Additionally, glucose between 10 and 20 min in both the FOL (1.18 mmol·L^{-1} (t(120) = 5.48, < 0.001)) and LUT (0.71 mmol·L^{-1} (t(120) = 3.28, 0.008)) phases were significantly different.

CGM Blood Glucose

Mean glucose from CGM measures were FOL 5.16 ± 1.09 mmol·L^{-1} and LUT 5.54 ± 1.16 mmol·L^{-1}. Menstrual phase was significant for differences in glucose at time points 45 min ($p = 0.0436$) and 55 min ($p = 0.0391$) (**Figure 17**). Within each phase, *Time* was significant for changes in glucose over the hour ($p < 0.001$) but was not statistically different between any of the consecutive time points.

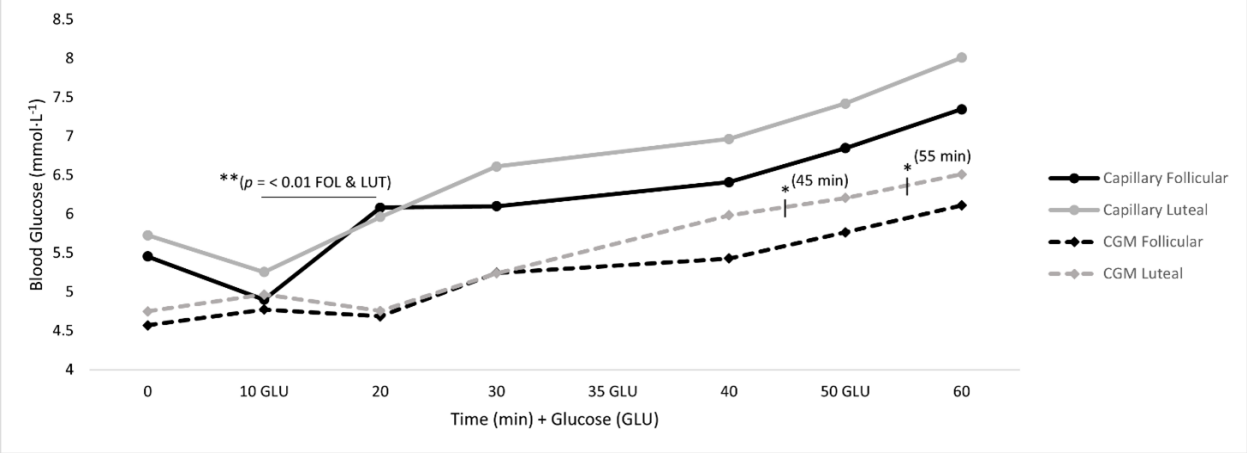


Figure 17. Comparison of the estimated means of capillary and continuous glucose monitor (CGM) glucose at matched time points during follicular and luteal phases in TREAD protocol.TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). * Statistically significant differences in glucose between phases at specific time point. ** Statistically significant differences in glucose between time points within phase. Values are means.

Glycemia

No incidents of clinically relevant hypoglycemic episodes (< 3.0 mmol·L^{-1})[67,68] were detected in the FOL (capillary 3.9 mmol·L^{-1}; CGM 3.28 mmol·L^{-1}) or LUT (capillary 4.7 mmol·L^{-1}; CGM 3.28 mmol·L^{-1}) phases during TREAD. No glucose values below 3.9 mmol·L^{-1} were reported in capillary blood samples. However, the incidence from CGM data was 2.29 times greater in FOL (FOL: 16; LUT: 7). Of these CGM values, < 3.9 mmol·L^{-1}, 86% were attributable to a single participant.

8.7 Discussion

The aim of the present study was to observe the impact of menstrual cycle on blood glucose when fasted or supplemented with CHO during a simulated ultra-run. There were two key observations made. First, glucose was consistently higher in the LUT phase over 4-hours of prolonged running in both fasted and CHO supplemented states. Second, clinically significant levels of hypoglycemia in non-diabetics (< 3.0 mmol·L^{-1})[67,68] were not observed during a 3-hour fasted run preceded by an 8-hour fast.

Whether fasted or provided with 60 g exogenous CHO within the recommended range of 60-90 g per hour [16], glucose was higher in the LUT phase at almost all time points. This difference is reflected in the mean glucose values for capillary and CGM during both FASTED and TREAD (**Table 6**) and may be explained by the cyclic nature of blood glucose throughout the menstrual cycle caused by the inverse relationship between estrogen and glucose as well as progesterone's increase in insulin resistance.[285] This inverse relationship is characterized by lower daily levels of glucose in the FOL phase when estrogen levels are highest and progesterone is lowest.

Subsequently, this is followed by elevated daily glucose levels in the ovulatory and luteal phases when estrogen levels dip and progesterone peaks.[277] No guidelines have been established for blood glucose levels > 3.0 mmol·L^{-1} during exercise making clinical significance of this difference difficult to ascertain, but there are unlikely to be any relevant performance or health implications from such small variances.

Furthermore, despite differences in reported mean glucose values and visual variances in graphed glucose data between the phases, for both FASTED and TREAD, statistical analysis indicated statistical significance was isolated to specific time points within FASTED (capillary 180 min;

CGM 5 min & 35 min). While the mean glucose difference of 0.46 mmol·L^{-1} between FOL (5.57 mmol·L^{-1}) and LUT (6.03 mmol·L^{-1}) at 180 min, measured by capillary sampling, was not surprising, the contrasting nature of where significance was observed in CGM was unexpected (early in CGM versus late in capillary). The FASTED protocol, designed to capture glucose patterns under fasted, prolonged running conditions, using both capillary and CGM methods, surprisingly yielded inconsistent glucose patterns between the two sampling methods, thus raising concerns about the reliability of CGM in endurance sport settings.

During TREAD, menstrual cycle did not influence the differences in glucose values between the FOL and LUT phases when capillary data was examined. Post-hoc analysis of CGM glucose data detected statistically significant differences in glucose between FOL and LUT at time points 45 min ($p = 0.0436$) and 55 min ($p = 0.0391$) (**Figure 17**). The inconsistency of statistical significance in the later stages of TREAD, evidenced by significance of the time stamps at 45 min and 55 min but not 50 min and 60 min, likely points to accuracy concerns of the CGM related to fluctuating glucose levels which has been observed by others[56] and seems to be exacerbated by prolonged exercise.[55] Additionally, when TREAD capillary data is compared directly to CGM it appears the CGM consistently underreports glucose (**Figure 17**). Nevertheless, our data suggests that menstrual phase likely had no impact on blood glucose in the presence of exogenous carbohydrate because glucose values were not significantly different between FOL and LUT phases at any of the seven capillary measurements. However, further investigation with larger sample sizes is warranted as this conflicts with the observation of the present study that mean glucose levels were consistently higher in the LUT phase across all phases and interventions, (**Table 6**). A possible explanation may lie in the variability of hormone ratios that could be statistically significant within the individual but is reduced in significance viewed as an average. Therefore, perhaps it is not how much glucose varies between the phases on average, but the difference between the individual and biological variability.

Accuracy of CGM sensors in sport among healthy participants is not well documented and has been questioned in existing literature.[54,66] More specifically, the reliability and accuracy of glucose readings taken from ISF can be impacted by frequent changes in variables that affect glucose sensor reliability, such as pH level, physiological lag time of glucose changes from blood to ISF, and lower concentrations of glucose in ISF than blood.[50–52] Additionally, consensus among a

collection of studies focused on the comparison of CGM accuracy at rest and during exercise DM1 and non-diabetic populations [55,57,59–63] is that accuracy is significantly worse during exercise when compared to rest. Of the two sampling methods used in this study, capillary glucose is the standard to which CGM sensor accuracy has been compared.[168]

Of note, no episodes of clinically significant non-diabetic hypoglycemia (< 3.0 mmol·L^{-1})[67,68] were detected in either capillary or CGM measures during FASTED despite participants fasting for a minimum of 8 hours before the fasted running protocol that lasted for 3 hours over undulating terrain with a repeated sustained climb of 136 m over 1.14 m distance at 4.2 to 11.2% gradient. During FASTED, the lowest observed glucose levels from capillary were FOL 4.4 mmol·L^{-1}; LUT 3.9 mmol·L^{-1} and for the CGM measures were FOL 3.1 mmol·L^{-1}; LUT 3.9. The avoidance of clinically significant non-diabetic hypoglycemia (< 3.0 mmol·L^{-1})[67,68] during FASTED is noteworthy; however, observances of less than 3.2 mmol·L^{-1} (3.16, 3.16, 3.11 mmol·L^{-1}) were recorded with CGM of which two were attributed to a single participant in consecutive measurements and all occurred between 155-160 minutes in the FOL phase. Likewise, clinically significant non-diabetic hypoglycemia was also avoided during TREAD and the lowest recorded measures were not remarkable with two incidents of 3.28 mmol·L^{-1} occurring in different participants at different menstrual phases. However, although we didn't make any direct observations of a protective effect of the LUT phase against low blood glucose, the likelihood of glucose reaching below 3.9 mmol·L^{-1}, the lowest desirable level of normoglycemia in diabetics[67] as no standard is in place for healthy persons, is greater in the FOL phase and risk was largely attributed to the individual.

The mechanisms behind the maintenance of normoglycemia during prolonged running under fasted conditions may be multifactorial. There is evidence indicating a generalized difference in the make-up of muscle between males and females represented by the gradient of contribution from each fiber type (female type 1 > IIA > IIX compared to male type IIA > IIX > I) with type I representing "slow twitch" oxidative fibers and type II considered "fast twitch" or highly glycolytic driven fibers.[123] The assumption that type I muscle fibers are more dominant in trained and untrained females irrespective of sport discipline is a possible explanation for the preference to oxidize fat over CHO in addition to expressing greater resistance to fatigue.[125] There are, however, caveats to this broad belief as sex comparisons of muscle fatigability may be more

dependent on the muscle groups tested than a generalized sex dimorphism.[126] Also, there is evidence showing higher concentrations of intramyocellular lipid (IMCL) in females, stored predominately as triglyceride within the muscle for use during excecise.[127,128] This observation alludes to greater availability or accessibility to alternate fuel sources resulting in greater contributions to energy needs and a sparing of endogenous CHO stores. Lastly, in combination with increased IMCL, differences in female gene expressions that increase concentrations of the enzymes associated with long and medium-chain fatty acid oxidation may suggest a performance benefit during prolonged endurance exercise.[281]

An ability to delay the onset of hypoglycemia during prolonged running without exogenous fueling preceded by an extended fast would be advantageous in the sport of ultra-endurance and may explain the capacity of female ultra-runners to narrow the gap between their male counterparts. For instance, men tend to complete a standard marathon 10% faster than women, but the difference decreases to 9% in 50-mile ultra marathons and 4% in 100-mile distances.[6] While this is not an endorsement for fasted or keto-adapted fueling strategies, in combination with the female tendency to perform at greater submaximal efforts at maximal fat oxidation rates[286], it may provide additional insights into the resilience of females in longer distance races when CHO fueling strategies are incapable of feasibly and physiologically maintaining energy requirements. No fasted running studies with comparable distance or duration were available, even among Ramadan research, however, Zinker and colleagues[287] observed the maintenance of normoglycemia in seven untrained males cycling to exhaustion (~90 min) at 50% of VO_{2MAX} after a 36 hour fast. They concluded that while blood glucose played a role, other factors contributed more significantly to fatigue during submaximal exercise.

Future studies should include a subjective diagnostic to allow participants to self-report signs and symptoms of hypoglycemia based on their interpretation of biofeedback or performance and possibly male participants acting as a control for comparison of outcomes by sex. Additional research should also be directed into assessing the accuracy of CGM as comparted to capillary during exercise. Limitations of this study, like many longitudinal intervention studies, were related to participant selection criteria and attrition rates. A major challenge was the recruitment and retention of eumenorrheic ultra-running athletes over an extended period who were able to commit to the repeat nature of the demanding testing protocols. Also, limited resources prevented the

repeated testing of capillary blood glucose, access to multiple independent CGM sensors to collect data sets simultaneously, and blood serum hormone testing to assess menstrual phase. However, menstrual cycle phase verification was confirmed with self-reported menstrual tracking and ovulation predictor urine dipsticks across three full menstrual cycles.

8.8 Conclusion

This applied field study is the first to observe the dynamics of blood glucose in a cohort of eumenorrheic ultra-running athletes during a simulated 4-hour ultra-run, replicating real-world conditions when fasted and or supplemented with exogenous CHO, while accounting for the interactions of menstrual phase. Data from the present study demonstrated that glucose in the mid-luteal phase is consistently higher than the mid-follicular under fasted or CHO supplemented conditions. The difference (Capillary 0.25-0.29 $mmol \cdot L^{-1}$, CGM 0.38-0.4 $mmol \cdot L^{-1}$) is unlikely to impact performance or inform carbohydrate fueling practices when blood glucose is above clinically significant levels (> 3.0 $mmol \cdot L^{-1}$)[67,68] in healthy adults and the data does not imply the LUT phase infers a protective effect against hypoglycemia. Furthermore, under normal prolonged running conditions with consistent carbohydrate availability, menstrual phase does not appear to influence blood glucose and would imply that no unique carbohydrate fueling interventions are indicated for long duration, submaximal, exercise across the menstrual cycle. Yet, during prolonged running under fasted conditions, the influence of menstrual phase is unclear as evidenced by the conflicting observations from capillary and CGM methods indicating the need for future research. Unexpected observations from this study were the confounding nature of the glucose data derived from the CGM sensor when used in an ultra-endurance environment and the absence of clinically significant non-diabetic hypoglycemic levels despite 3-hours of fasted running preceded by an 8+ hour fast. Also, the lack of statistical significance in glucose levels between phases, despite being consistently elevated in the LUT phase is confounding but may imply the influence of ovarian hormones on glucose is present, but the extent of the influence is largely based on individual hormone levels and responses that become homogenized in aggregated analysis. Future testing of CGM sensors is recommended before relying on them as a tool to inform fueling strategies in real-time and is supported by previous research questioning their accuracy in field settings. Regarding the absence of hypoglycemia, without male controls for direct sex comparisons, it is premature to assert that regularly menstruating females definitively have a

performance advantage during prolonged fasted running; however, this study indicates, regardless of menstrual phase, that regularly menstruating females are capable of avoiding clinically significant non-diabetic hypoglycemia when performing prolonged running without exogenous CHO fueling.

SECTION 9

9.1 No Main Effect of RER on Glucose in Eumenorrheic Female Ultra-Runners

Authors: Andrew Dole, Stacy Sims, Martyn Beaven

Under initital review *Journal of Sports and Health Sciences*

9.2 Abstract

The interest in ultra endurance running, female physiology and sport performance indicators has grown rapidly. Sexual dimorphism has been a focus of human performance research of late, within which carbohydrate (CHO) and fat metabolism has been emphasized. However, the effects of female sex hormones across a natural menstrual cycle and any impact they may exert on exercise metabolism, especially during ultra endurance exercise, has yet to be elucidated. The purpose of this study was to observe the influence of menstrual cycle on CHO and fat utilization, as well as blood glucose, in eumenorrheic female ultra endurance runners in a performance context. In this randomized design, 11 experienced and regularly menstruating female ultra-endurance runners participated in an ultra-marathon simulation consisting of a standardized outdoor 3-hour fasted depletion run followed by a 60 min treadmill run with standardized exogenous glucose supplementation in both the mid-follicular (FOL) and mid-luteal phase (LUT) of their menstrual cycle. During the treadmill run, blood glucose (BG) was measured simultaneously using capillary blood sampling at 10 min intervals. Respiratory exchange ratio (RER) was monitored throughout the treadmill run with fat and CHO oxidation rates calculated from gas exchange. A paired-samples t-test was conducted to assess the differences in respiratory exchange ratio RER between the FOL and LUT phases. There was no significant difference in RER between the FOL the FOL (0.805, ± .028) and LUT phases (0.809, ± .035); t (10) -.401, p = .697 during comparable submaximal running intensity (57% ± 7.3% and 57.3% ±7.9% VO_{2MAX} respectively). Post-hoc analysis observed no significant main effect of RER on glucose levels; however, on average, levels of glucose were 0.43 $mmol \cdot L^{-1}$ higher in LUT ($p < .001$). Menstrual cycle does not appear to influence substrate oxidation or BG in the presence of exogenous CHO over time, nor does there seem to be an association between BG levels and RER.

9.3 Introduction

Research on energy metabolism in females indicates a tendency for prioritizing fat utilization over carbohydrates during submaximal exercise.[29,35,36] This preferential utilization may contribute to inherent advantages over males in ultra-endurance sports. Mechanisms for this difference are thought to relate to the female ovarian hormones 17B estradiol (E2) and progesterone which act on CHO and fat metabolism.[26,28,39] as well as sexual dimorphisms in mitochondrial density and gene expressions[281] influencing glucose metabolism.[128,288] However, it is possible that muscle fiber type distribution[123] or other genetic expressions and physiological differentiations between males and females, such as fluid balance, thermoregulation, recovery, fatigue, biomechanics, mitochondrial function, and energy metabolism[115] could be responsible for differences in metabolism impacting sport performance.[25,28–35,117]

Unique to the female athlete, differences on substrate metabolism may also exist within a spectrum across the menstrual among regularly menstruating females themselves. Nicklas *et al.*[31] observed the respiratory exchange ratio (RER) to be higher in the follicular (FOL) phase among 6 eumenorrheic females while cycling to exhaustion at 70% of VO_{2MAX}. In contrast, Devries and colleagues found no difference in RER between menstrual phases, but utilized 65% of VO_2peak as their intensity making absolute comparisons difficult.[30] The importance of exercise intensity when evaluating RER across the menstrual cycle can be evidenced by Hackney and colleagues who observed that carbohydrate (CHO) utilization was significantly lower and fat higher in the mid-LUT as compared to mid-FOL phase in low and moderate intensities (VO_{2MAX} of 35% and 60% respectively) while fasted, but were comparable with no significant differences at 75% of VO_{2MAX}.[29] Williams *et al.* compared fasted RER of regularly menstruating women to those using second-generation hormonal contraceptives during eight-minute bouts of submaximal cycling (40% and 65% of VO_{2PEAK}) across the menstrual phase and found no difference among them.

The previously mentioned research was conducted while participants were fasted; however there is evidence that the presence of exogenous CHO is key in determining female macronutrient utilization with the potential to mitigate differences in CHO utilization rates between sexes and possibly within menstrual phases during exercise.[33,36] Additionally, blood glucose (BG) is known to play a role in sport performance[10,70] but it's relationship with RER or how the menstrual cycle may influence levels is not well known. Riddell and colleagues compared RER, BG, and

exogenous CHO utilization over 90 min of cycling at 60% of VO_{2PEAK} between males and females in the mid-FOL phase of their menstrual cycle. They found women had the capacity to oxidize a larger percentage of the exogenous CHO than males at the later stages of prolonged running which reduced the reliance upon endogenous stores of CHO. When CHO was provided BG was higher in females than men, but similar when fasted. No comparison was made across the menstrual cycle and no verification of MC phase was documented.[33] Similarly, in a comparison of male (n =6) to female (n = 6) elite cross-country skiers, Petterson *et al.* observed that sex differences in RER and substrate preference were mitigated when exogenous CHO was provided during exercise. However, this study did not control for MC phase among the naturally menstruating participants and included a mix of hormonal profiles with the inclusion of hormonal contraceptive users (2 monophasic, 1 hormonal coil, 3 regularly menstruating).[36]

Thus, to build upon these existing works and provide a direct comparison of females across the menstrual cycle, this present study aimed to provide novel insight into substrate utilization and BG in female endurance athletes across the menstrual phases when CHO was provided. Specifically, RER and BG were assessed in eumenorrheic trained ultra-endurance athletes during the follicular and luteal phases of the menstrual cycle. We hypothesized that glucose levels would be influenced by the contribution of energy substrates during prolonged running.

9.4 Methods

9.4.1 Experimental Design

A randomized, cross-over design with participants acting as their own control across menstrual cycle phases was used during an ultra-marathon simulation consisting of a standardized outdoor 3-hour fasted depletion run (FASTED) followed by a 60-minute treadmill run (TREAD) with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 min). Participants completed the run protocols (FASTED and TREAD) once during the mid-follicular (4-8 days after the start of menses) and mid-luteal (5-8 days post ovulation) phases of the menstrual cycle. Ethics approval was obtained from the University of Waikato's Human Research and Ethics Committee (HREC(Health)2021#73). Written informed consent was obtained from each participant. Recruitment and data collection occurred from December 2021 through November 2022.

9.4.2 Participants

Eighteen recreational female ultra-endurance runners, recruited from endurance sports clubs located in New Zealand, agreed to participate in this study. Six were excluded from the initial sample group due to injury or disruptions in hormone profile (pregnancy; hormone therapy). Twelve participants were included in the data analysis unless otherwise specified (mean ± (SD), age: 39.3 ± 6.3 y, height: 165.3 ± 5.6 cm, body mass: 62 ± 8.0 kg, VO_{2MAX}: 45.9 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$).

An electronic questionnaire was administered to all participants to screen for established inclusion criteria: 1) eumenorrheic experiencing regular cycles (28-40 d), 2) not using hormonal contraception for at least 12 months except for Mirena IUDs implanted for a minimum of 9 months, 3) at least one year of ultra-endurance experience, 4) and no current injuries that would impact performance. Additional exclusion criteria included 1) a history of cardiovascular, renal, or metabolic disease, 2) insulin or glycemic disorders, 3) the use of any exogenous hormones, or 4) confirmed anovulatory cycles.

9.4.3 Hormone Control

Prior to familiarization, participants were required to provide photo documentation to the lead researcher of menstrual tracking for three consecutive cycles using a commercial mobile menstrual tracking application (Garmin Connect, Olathe, KS, USA; FITRwoman, Encino, CA, USA; Wild.AI, San Francisco, CA, USA). In-home urine ovulation test strip predictor kits (Pregmate, Fort Lauderdale, FL USA) were then used to detect a luteinizing hormone surge, confirm ovulatory function for two consecutive months, and establish consistency in calendar tracking of MC length. Participants were instructed to begin strip testing for seven consecutive days prior to their predicted ovulation. A count forward method was used to predict the early follicular (days 4-8 from the start of menses) and mid-luteal phases (days 5-8 post-ovulation) for testing.

9.4.4 Metabolic Data

Respiratory exchange ratio (RER) was obtained during the treadmill portions of the run protocol using a TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT USA). A mouthpiece and nose covering were worn by participants to collect expired gases for most of the time trial; being

removed only for carbohydrate and fluid intake which was recorded and time-stamped for data collection integrity. Heart rate was obtained using a telemetry system (Polar Electro, Inc., Lake Success, NY, USA). Fingerstick capillary blood glucose (Capillary) samples were taken using a CareSens lancing device, lancets, and testing strips (i-SENS Global, Gordon NSW, AU) three times during the 3-h depletion run: prior to start, after 90 minutes of exercise, and at the completion of the three-hour fasted run. During the 1-h treadmill run, capillary samples were taken just before the start, and then at 10-min intervals (7-time points; **Figure 18**). Continuous blood glucose was measured during the entire 4-h run protocol at 1-min intervals using the Abbott Libre Sense continuous glucose monitor (Abbott Laboratories, Abbott Park, IL USA) worn on the back of the upper arm for a minimum of 24 hours and a maximum of 13 days before run protocol testing. Interstitial glucose data was collected by the CGM via a thin, flexible amperometric sensor filament inserted subcutaneously. The stored data from the CGM was uploaded by pairing the sensor to a mobile device which wirelessly transmitted to cloud-based storage and was retrieved onto PC using the cloud-based coach dashboard features (Supersapiens, Atlanta, GA USA). When necessary, glucose in $mg \cdot dL^{-1}$ was converted to $mmol \cdot L^{-1}$ by multiplying by 0.0555.

9.4.5 Familiarization

Participants reported to the laboratory to complete VO_{2MAX} testing, receive study instructions, and familiarize participants with testing procedures/equipment. An instructional video and walk-through of the continuous glucose sensor (CGM) application were provided. Depletion run instructions and outdoor route were reviewed. A combination ramp and graded VO_{2MAX} test protocol was conducted on a motorized treadmill with a stiffness of 365 $kN \cdot m^{-1}$ (Steelflex PT10 Fitness, Steelflex Fitness, Taipei, Taiwan) with the collection of pulmonary gas exchange using a TrueOne 2400 metabolic cart[273]. Each participant was instructed to begin with a self-paced 5-min warm-up. Following the warm-up, the testing began with an initial stage running on a 0% grade at 8.5 $km \cdot h^{-1}$ for two minutes. This stage was followed by increases in speed of 1.4 $km \cdot h^{-1}$ every 30 seconds until 14.1 $km \cdot h^{-1}$ was reached. After 30 seconds at 14.1 $km \cdot h^{-1}$ the grade was increased from 0% to 2% for two minutes followed by a 2% increase in grade every two minutes to volitional exhaustion. Maximum effort was determined when both conditions were met: 1) failure to continue test and 2) respiratory exchange ratio (RER) > 1.1.

9.4.6 Outdoor Depletion Run

Participants were instructed to apply the CGM sensor 24-48 h before their scheduled run day, arrive at the lab in a fasted state of 8 hours, and have avoided caffeine, alcohol, and strenuous activity for 24 hours preceding the run. Upon arriving at the lab on the morning of the (FASTED) protocol, a urine sample was taken and urine specific gravity was measured using a hand-held refractometer (Atago CO LTD, Tokyo Japan). Body weight was documented using a Wedderburn WM202 patient scale (Wedderburn, Ingleburn, NSW, Australia). Capillary blood glucose was recorded immediately (< 1 min) before the start of the depletion run. Participants ran at a self-selected pace along a pre-determined, two loop, outdoor route, chosen to simulate an ultra-endurance run course for 3-hours. Starting from the laboratory participants ran a flat 2.6 km stretch to the offroad course where they directly began the first loop of 2.65 km consisting of 133 m of elevation gain/loss followed by the second loop of 3.47 km and 48 m of elevation gain/loss. These loops were repeated for three consecutive hours. Participants checked in with the researcher at an aid station located along the route after the completion of each loop to receive, *ad libitum*, a standardized non-caloric electrolyte solution containing 600 mg sodium (Na^+) and 100 mg K potassium (K^+) per Litre. Participants were instructed to drink no other fluids than those provided by the researcher and to eat nothing during the duration of the depletion run. Intake of all fluid was measured and documented. Capillary glucose was recorded two additional times at 90- and 180-min (**Figure 18**). Upon completion of the depletion run, a rest aid station was simulated where the runner was allowed to sit and hydrate while being driven back to the lab to begin the 1-h treadmill trial. The average transition time from the depletion run's end to the treadmill trial's start was 10 ± 3 min. Run data (distance, elevation, pace) was tracked using a runner-provided watch with global positioning (GPS) and documented with screenshots of Strava (Strava, San Francisco, CA, USA) or Garmin Connect (Garmin Connect, Olathe, KS, USA) mobile applications. Outdoor runs were completed between 4 a.m. and 12 noon to minimize variations in weather and temperature (13.5 ± 6.6 °C).

9.4.7 Indoor Treadmill Trial

Following FASTED and the brief transition described above, participants were instructed to run at a self-selected pace for an additional 60 minutes on the motorized treadmill at 0% grade while having expiratory gases and heart rate continuously monitored with a TrueOne 2400 metabolic cart in a temperature-controlled environment (19 °C). Blood glucose was taken via capillary seven times, starting at 0 minutes and again every 10 minutes until completion. A 20 g bolus of glucose was provided at three time points (15, 35 and 50 minutes; **Figure 18**). A standardized non-caloric electrolyte solution containing 600 mg Na and 100 mg K per Litre was provided *ad libitum* with intakes recorded. Interruptions to the expiratory gas collection were time stamped.

9.4.8 Statistical Analysis

A paired samples t-test was performed to compare RER between the FOL and LUT phases in regularly menstruating females during the last hour of a 4-hour ultra-run simulation when CHO was provided following 3 hours of fasted running. Data are presented as mean values with SD and were checked for normality using the Shapiro-Wilk test. Post-hoc analysis for interactions of RER and glucose was completed using a linear mixed model fit with *Time*, *RER*, and *Phase* as fixed variables with *Person* as a random effect.

9.5 Results

Due to complications with testing equipment during one participant's test they could not be included in the final analysis, resulting in 11 participants being included in the final analyses. Results indicate RER was not significantly different (-0.004 ± 0.316) between the LUT and FOL phases of the menstrual cycle in regularly menstruating female runners FOL (0.805, ± .028) and LUT phases (0.809, ± .035); t(10) -.401, p = .697, **Figure 18**. Participants completed the fourth hour of running at a mean submaximal running intensity of 57% ± 7.3% and 57.3% ±7.9% VO_{2MAX} for the LUT and FOL phases respectively. The %VO_{2MAX} during TREAD varied by participant and ranged from 44% to 70% in FOL and 44% to 72% in LUT. Fat and CHO contributed similarly to the total energy utilized during the fourth hour of running in the phases of the menstrual cycle, **Figure 19**. Post-hoc analysis observed no significant main effect of RER on capillary glucose levels, **Figure 20**. On average, the levels of glucose were observed to be 0.40 $mmol \cdot L^{-1}$ higher in LUT 6.56 ± 0.98 than FOL 6.16 ± 0.82 (p <.001), **Figure 21**.

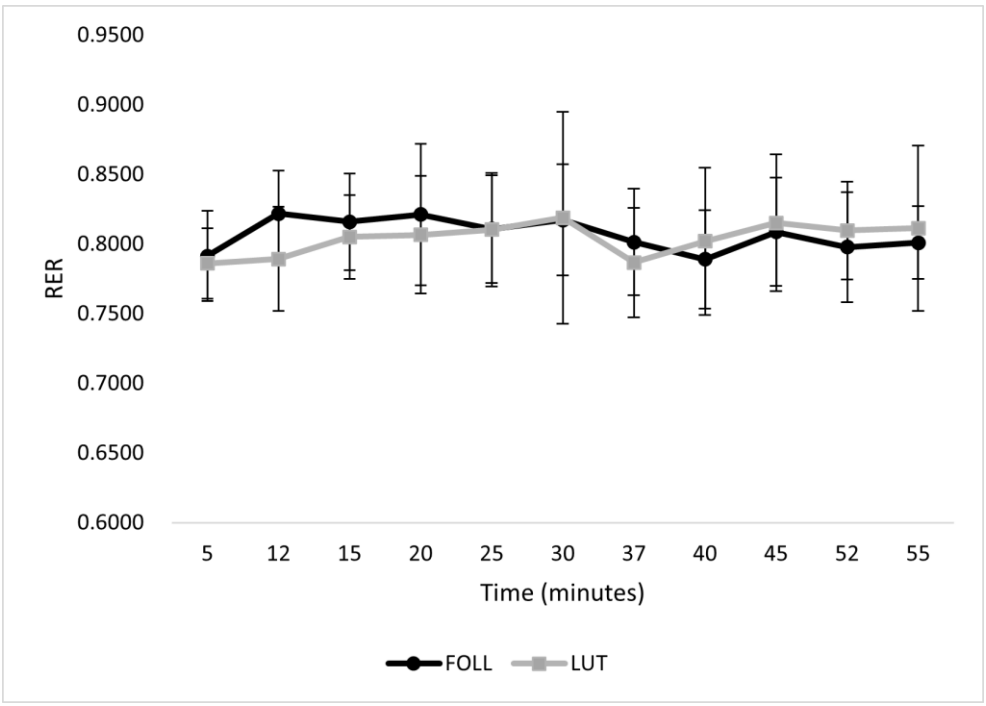


Figure 18. Comparison of RER in the mid-follicular and mid-luteal phases during TREAD. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

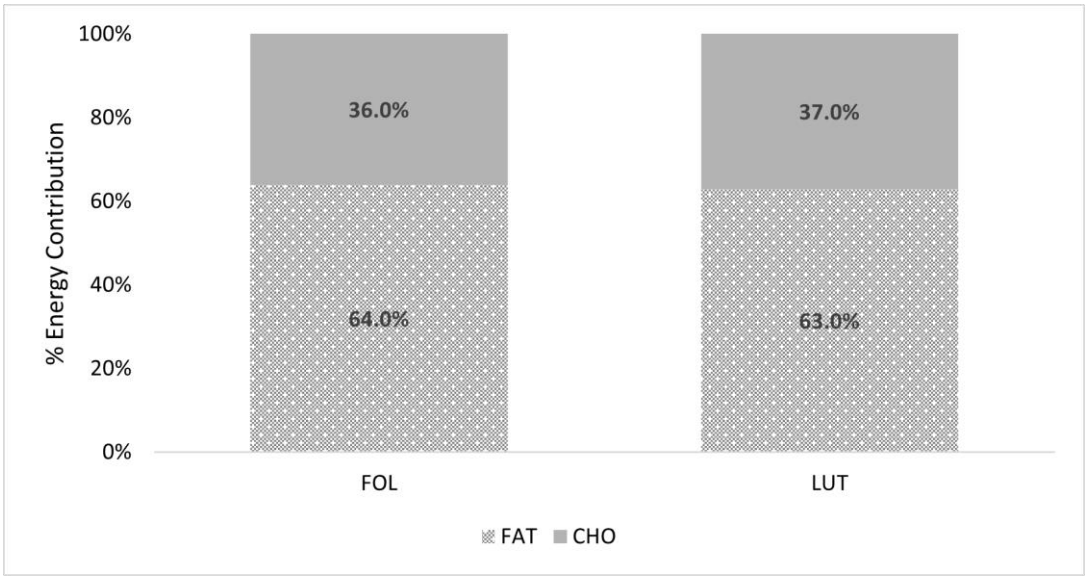


Figure 19. Contribution of fat and carbohydrate toward total energy during TREAD in the mid-follicular and mid-luteal phases. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

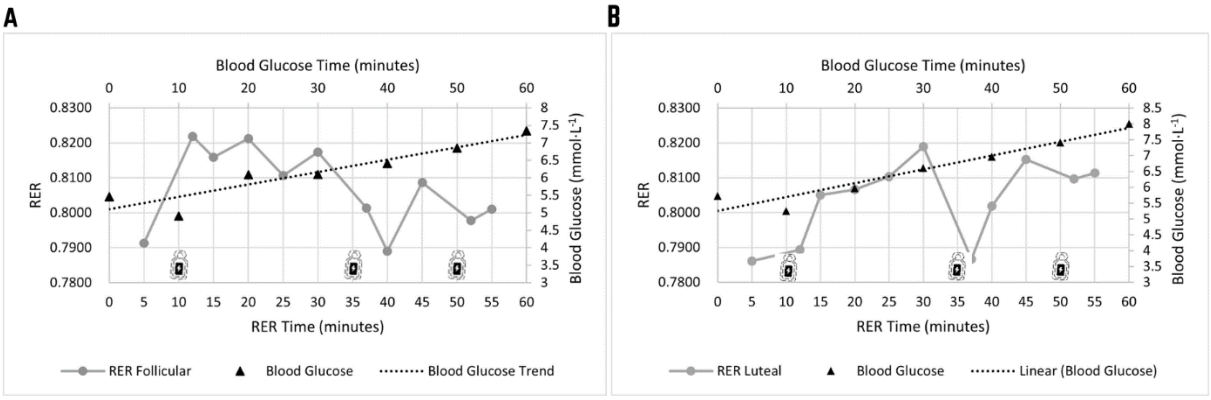


Figure 20. A) Relationship of capillary glucose to RER during TREAD in mid-follicular phase. B) Relationship of capillary glucose to RER during TREAD in mid-luteal phase. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

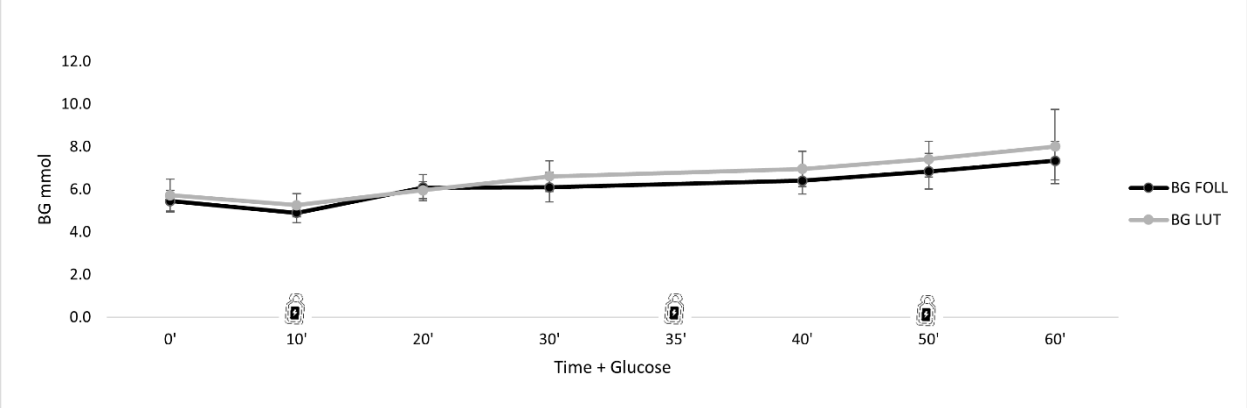


Figure 21. Mean capillary blood sugar comparison between mid-follicular and mid-luteal phases of the menstrual cycle during TREAD. TREAD, a 60-minute treadmill run with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes).

9.6 Discussion

To date, this is the first study to investigate the relationship of BG and RER during prolonged running in eumenorrheic females. The findings of this study suggest that RER is not different between phases when exogenous CHO is provided among regularly menstruating females at running intensities between 44-72% of VO_{2MAX}. Devries and colleagues found similar results in a mixed cohort of females, regularly menstruating or using oral contraceptives, during 90-min of fasted cycling at 65% of VO_{2PEAK}.[30] These similarities, could indicate that that differences in RER are isolated to male vs female comparisons[33,35] and that the spectrum of hormone changes across

the FOL and LUT phases do not impact energy preferences within the female population regardless of fasted states or availability of exogenous CHO.

The underlying cause of these sex differences in energy metabolism are evolving beyond the influence of just ovarian hormones. The assumption that type I muscle fibers are more dominant in trained and untrained females irrespective of sport discipline is a possible explanation for the preference to oxidize fat over CHO when compared to men. Additionally, there is evidence showing higher concentrations of intramyocellular lipid (IMCL), stored predominately as triglyceride within the muscle for use during excecise,[127] in female muscle before and after endurance exercise.[128] This availability alludes to greater accessibility resulting in greater contributions of IMCL to energy needs. Furthermore, emerging differences in skeletal muscle at the cellular level such as a greater quantity of mRNA and fatty acid transporters implicated in lipid metabolism and mitochondrial function have been observed between in females; suggesting they are capable of enhanced fatty acid transport in working muscle.[281]

In contrast, are findings by Nicklas *et al.*[31], who reported that RER was higher in the FOL phase during fasted cycling at 70% of VO_{2MAX}, which was attributed to low levels of E2, and a reduction in that hormone's mobilization of fatty free acids in combination with a down regulation of muscle glycogenolysis.[131] Hackney and colleagues also observed differences in RER across the menstrual phase, but their data suggests the potential of exercise intensity as a primary driver of substrate preference. These authors reported that during the mid-LUT phase a high percentage of fat, and not CHO, was utilized at moderate intensities (35% and 60% of VO_{2MAX}) during fasted cycling. However, at 75% of VO_{2MAX}, there were no differences in RER between the FOL and LUT phases.[29] Possible mechanisms for this difference could be that high intensity exercise, acting as a larger influence on metabolism[238] increases hepatic glucose output to meet activity demands, which may mitigate the combined hepatic glucose-sparing effect[39,131] associated with E2 and progesterone. The difference in observed outcomes is attributable to the fasting protocol followed by Hackey *et al.*[29] and Nicklas *et al.*[31] and contrasts the provision of CHO in the present study.

When exogenous CHO is available the difference in CHO utilization at low to moderate intensities between males and females is negated.[33,35] These observations also appear to occur when comparing females across the menstrual phase and are supported by the findings of the current study. It is probable that, in contrast to fasted states, the mechanisms of exercise metabolism

override the hormonal influences of substrate metabolism and are largely responsible for the increased CHO utilization. Exogenous CHO during exercise may simply be providing an alternative to endogenous CHO stores, which are rate limited by the presence of E2.[131]

Additionally, data indicates no relationship between RER and BG across the menstrual cycle in regularly menstruating females over the final hour of a 4-hour simulated ultra-endurance run when CHO is available. This observation may be a result of exercise intensity, fat adaptation of the athlete, availability of CHO, the prior 3 h of running following an 8-hour fast, or a combination of these factors. In males, during low energy availability exercise, RER was reduced and exogenous CHO utilization, while not enhanced, was not negatively impacted.[289] The authors postulated that exogenous CHO uptake may be directed to glycogen resynthesis and not utilized during exercise. Future research using substrate tracing methods to assess female glucose kinetics are indicated, as this would directly inform fueling goals to avoid low energy states and maximize exogenous CHO for performance.

In the present study we observed that BG in LUT was consistently higher than FOL. During physical activity, pain, and stress epinephrine can mediate insulin resistance and subsequently increase BG levels.[155] Also, the cyclic nature of BG throughout the menstrual cycle is caused by the inverse relationship between estrogen and BG, as well as progesterone's increase in insulin resistance.[285] This inverse relationship is characterized by lower daily levels of BG in the FOL phase when estrogen levels are highest and progesterone is lowest. Subsequently, this is followed by elevated daily BG levels in the ovulatory and luteal phases when estrogen levels dip and progesterone peaks.[277] The magnitude of the difference observed in the current study is unlikely to impact sport performance; however, follow-up studies should explore the possibility of a protective effect against symptoms of low blood sugar such as dizziness, nausea, headache, fatigue, and associated performance declines.

A limitation of the present study, like many longitudinal intervention studies, was a small sample size resulting from inclusion and exclusion criteria for the targeting population. This study may also have benefited from controlling the participants' $\%VO_{2MAX}$ over the course of the treadmill run. Additionally, the standardized 60 g of total exogenous CHO provided in three 20 g boluses was chosen to replicate current ultra-endurance fueling recommendations, to avoid GI distress associated with excess CHO intakes related to body weight calculations and minimize the

influence of CHO rate on BG readings; however, an alternative method could have been the individualization by using a g/kg of body weight protocol.

Based on the results of this study and the findings of others we conclude that menstrual phase does not influence energy metabolism when exogenous CHO are available during submaximal running intensities (~ 57% of VO_{2MAX}) following 3-hours of fasted running. A relevant consideration for female athletes would be the timing and availability of CHO around periods of exercise to enhance the contribution of CHO to total energy needs throughout the menstrual cycle. We observed no relationship between RER and BG within the study conditions and assert that BG reacts independently from energy preferences during exercise. Future research with access to a larger sample size as well as blood hormone data to assess hormone ratios are necessary to advance our understanding of the mechanisms behind the influence exogenous CHO on energy metabolism. Additionally, future studies should explore the influence of low energy availability on exogenous CHO utilization in female athletes.

Part 4 PhD Summation

SECTION 10

10.1 Summary

The purpose of this PhD study was to collect metabolic data such as glucose and energy metabolism in regularly menstruating female ultra runners within the complex sporting environment of ultra-running in hopes of bridging the gap between science and practice. This research aimed to provide new insights into female metabolism during ultra-endurance running, addressing gaps in the existing literature. A combination of literature reviews covered a wide scope of relevant topics such as: energy demands of ultra-running, metabolism of energy substrates, current carbohydrate fueling practices for endurance sport, key aspects of female physiology impacting energy metabolism, research methodologies affecting female sport research, and technical aspects of CGM sensors which frame the multifactorial study environment. The three precise questions this study investigated were:

A) Does CGM reliably report blood glucose during a 4-hour simulated ultra run?

B) How does menstrual phase impact blood glucose fasted and fed during a 4-hour simulated ultra run?

C) Is energy metabolism different between menstrual phases at the end of a 4-hour simulated ultra run?

The investigation of these questions began with an analysis of menstrual cycle tracking considerations for study design in female performance research, presented in a narrative review. This was followed by a randomized, cross-over study design with participants acting as their own control across the menstrual cycle phases. This methodological approach aimed to evaluate the efficacy of the CGM sensor in an applied setting, while simultaneously collecting both glucose data and metabolic data to assess the influence of menstrual cycle on glucose levels.

10.1.1 Menstrual Tracking in Sports Research

Across the menstrual cycle ovarian hormone profiles change; these fluctuations in estrogen and progesterone influence aspects of sports performance such as energy metabolism.[122] Therefore, to effectively conduct research in female athletes the perturbation of sex hormones inherent in the menstrual phase should be accounted for in study design.[211,212,233] To achieve this, menstrual phase tracking is necessary to align target hormone profiles with study interventions. Yet, a lack of standardization in menstrual cycle tracking practices exists. In *Section 6* a narrative review was used to evaluate the current practices and obstacles of menstrual cycle tracking in sports research. From this assessment key challenges to menstrual phase detection were identified and attributed to the lack of standardization of terminology, hormone phases, and reference values, as well as the absence of low-cost, point-of-care hormone detection methods. These challenges in study design and validation methodologies when conducting female performance research are in agreement with the existing literature which suggests that future research would benefit from a universal structure and improve the validity of study comparisons.

Separating this narrative review from existing publications was the compilation of the various menstrual phase taxonomies highlighting the confusion created by the variable length and/or overlapping phase classifications.[207,212,253] Additionally, building on the existing literature it was possible to suggest a framework for the standardization of menstrual phase terminology by integrating the concepts of hormone rhythmicity[207], hormone variability[258], and phase classification[211]. The goal of this structure was to aid researchers in navigating the hormone variability throughout an individual's entire menstrual phase, including intra-phase durations as well as improve the reliability of research outcomes and cross-study comparisons. This attempt at best practices also aims to address three fundamental criticisms of existing female research: 1) that most have been limited to comparisons of two distinct, but broad hormonal phases, follicular and luteal, without taking into consideration of peak hormone levels and intra-phase hormonal fluctuations found in the late follicular or mid-luteal phases,[290] 2) the ambiguous nature of menstrual phase nomenclature, which is often associated with indistinct or undefined ovarian hormone profiles[211], and 3) varying interpretations of the distinctly different hormonal profiles by researchers.

Contributions of previous authors have also provided valuable guidance on best practices when considering appropriate study design in female performance research. Unfortunately, it is this author's opinion, that these guidelines and recommendations for high quality female sport research, predominately based on blood hormone collection, minimize the already fleeting windows of testing availability, and would rely heavily on the obtainability of low-cost detection methods to expand research opportunities to large cohort and field studies. Nevertheless, the necessity for continued female-focused research remains paramount; particularly in field studies which are underrepresented in the literature and pose logistical as well as safety concerns due to blood hormone testing. To continue meaningful contributions to female sport research in the absence of affordable, point-of-care hormone testing solutions, researchers should employ a combined-methods approach, utilizing a mixture of high to moderate accuracy techniques, as catalogued in our review, to ensure quality data is collected. As an example, this PhD study employed urine luteinizing hormone strips, calendar/mobile application monitoring, and rigorous participant inclusion/exclusion parameters to target the desired windows of menstrual testing. Although, it should be noted that the concept of 'menstrual phases' may be beyond usefulness in research. The menstrual phases themselves have been characterized as ambiguous even when dissected into distinct subphases due to their inability to accurately describe hormone ratios of an individual at any specific time. Thus, adopting hormone profiles that reflect predetermined hormone levels or ratios may represent the next progressive step. However, the introduction of such best practices could potentially lead to unintended consequences, particularly regarding budget and logistical considerations, resulting in prohibitive research constraints. For example, the present study was designed to observe sensor function and menstrual cycle influence on glucose during the specific 4-hour ultra-endurance stimulus. When considering the time commitment, physical impact of the 4-hour trials, and alignments of menstrual cycle with participant availability (work, travel, recovery, annual training/racing), it is understandable that data collection occurred over a two-year period. Therefore, targeting a very specific hormone window without a participant administered POC hormone testing method, which currently does not exist, would have been unmanageable.

As a whole, this narrative review aimed to highlight the significance of female sex hormones on athlete research outcomes and advocate for standardized terminology regarding menstrual cycle phases. Additionally, the review aided in the identification of gaps in study design and validation

methodologies within female performance research. The results of this effort were the publication of a narrative review to be used as a tool for selecting suitable study methods to effectively track menstrual phases in female sports research and a suggestive framework for standardizing terminology.

10.1.2 Efficacy of CGM in Ultra-Endurance

The existing body of literature exploring CGM devices in ultra-running is limited and lacks well-controlled studies involving females. This study, presented in *Section 7*, aimed to explore how factors such as menstrual phase, extended running duration, and carbohydrates affect CGM sensor outcomes among eumenorrheic females in a simulated ultra-endurance run. To our knowledge this study is the first to observe and report on glucose responses across the menstrual cycle in endurance-trained eumenorrheic female athletes. The principal finding, which addressed the primary objective, was that the CGM sensor used in this study tends to, on average, underreport glucose levels by varying degrees during fasted and unfasted exercise when compared to capillary glucose controls. Analysis of the data identified two additional novel findings. First, it was observed that menstrual phase was an influence on the glucose measures of the CGM, but only when exogenous CHO were available and not during fasted prolonged running. Bland-Altman analysis revealed a bias in glucose trends and indicates that menstrual phase can influence the validity of CGM glucose measures. Consequently, prior research into the validity of CGMs in female participants that has not accounted for menstrual phase should be interpreted cautiously. Second, that individual physiological characteristics were statistically significant for influencing glucose measurements of the CGM when fasted and provided exogenous carbohydrate.

Collectively, the existing literature using CGM in ultra-running settings with female athletes consists of only two studies[70,182] with a combined total of four female participants. The purpose of these studies was to evaluate the effectiveness of CGM to report blood glucose levels with intent to inform carbohydrate intakes and observe potential relationships between food intakes and glucose variability during ultra-endurance running. When reviewing these studies, two observations can be made regarding study design. First, no supplementary control method of glucose monitoring was implemented to verify CGM glucose data accuracy. Second, neither study controlled for the menstrual cycle or hormone profiles of the female participants.

It is understandable that the above-mentioned studies would not have implemented a second form of glucose collection because they were conducted in race settings. However, it is interesting that verifying the accuracy or reliability of the device used to collect the primary data set (glucose) was not a study design consideration. It is likely that the authors made assumptions regarding the device's reliability based upon their use in healthcare settings to make medical decisions related to glucose management. Nonetheless, prior studies demonstrated accuracy concerns of CGM devices during exercise and when carbohydrate intakes rapidly increase or decrease glucose levels.[55,56] The lack of secondary glucose collection methods in the existing ultra-running literature however, is reasonably important, because the present study implemented both CGM sensors and capillary glucose collection methods which resulted in the observation that the CGM sensor consistently reported lower glucose levels than corresponding capillary glucose samples. Furthermore, the extent of CGM underreporting, when compared to capillary glucose samples varied noticeably between fasted (-0.43 $mmol \cdot L^{-1}$) and carbohydrate supplemented conditions (-1.02 $mmol \cdot L^{-1}$). These data suggest that study design can directly influence the interpretation of glucose data from CGM in female populations.

Moreover, with no baselines for comparison, there is no clinical significance of underreporting by 1 $mmol \cdot L^{-1}$ so long as glucose stays within the range. For example, there is no evidence to support managing glucose above 5.5 $mmol \cdot L^{-1}$ during exercise. Similarly, there is no indication for intervention with glucose levels of 4 to 4.5 $mmol \cdot L^{-1}$, which sit between a rested maintenance range. However, if during exercise the CGM underreports consistently by 1.0 $mmol \cdot L^{-1}$ and relays glucose data of 3.6 $mmol \cdot L^{-1}$ when the actual glucose level is 4.6 $mmol \cdot L^{-1}$, we can begin to see where a problem may develop. Interestingly, there does not seem to be an issue where the sensor reports BG less than 3.0 $mmol \cdot L^{-1}$, potentially due to the sensor's inability to accurately detect low glucose levels as evidenced in existing literature. However, the lack of glucose reported below 3.1 $mmol \cdot L^{-1}$ in our data appears to be consistent across all the participants and sensors used; suggesting a hardware or algorithm function outside of the scope of this thesis to assess.

Based on this key finding from the present study, which agrees with previous literature suggesting CGM accuracy suffers in the presence of just one of these variables: high rates of glucose change, low glucose concentrations, prolonged use, and in the presence of commonly used NSAIDs or supplements[56,65] all which can occur frequently and in combination during ultra-endurance

running, it would be advisable to not assume CGM sensor accuracy and study design should incorporate a second form of non-CGM glucose sampling.

When addressing the lack of menstrual phase controls and considerations for ovarian hormone influences on glucose in previous studies the present study is also the first to observe two novel considerations, specific to female athletes, when implementing CGM in endurance running. To begin, the present study observed that the individual and menstrual phase were statistically significant for differences in CGM glucose data when exogenous carbohydrate was provided but not when fasted. This was unexpected because existing literature has repeatedly shown that exogenous carbohydrate mitigates the differences in energy metabolism between sexes.[34–36] An expected result would have been a closer alignment of CGM and capillary glucose data. Data suggests that menstrual phase may influence CGM measurements, and the extent of that influence will be dependent on the individual's physiological response to carbohydrate. Piecing together the emerging knowledge of female physiology leads to the hypothesis that a confluence of factors such as: 1) the variation in ovarian hormone ratios of estrogen and progesterone between individuals at any given time point in the menstrual cycle;[258] and 2) the cyclic nature of glucose throughout the menstrual cycle. This pattern in ovarian hormones, which is responsible for the observed differences in glucose levels across the menstrual cycle, can be characterized by lower daily levels of glucose in the mid-follicular phase due to elevated estrogen and subsequent elevated daily glucose levels in the ovulatory and luteal phases when estrogen is lower.[277]

However, capillary glucose data did not present a similar interaction with menstrual phase and was observed to be influenced by the individual only. We can speculate that under fasted conditions the glucose concentration within interstitial fluid, from which the CGM obtains glucose data, may be impacted by individual insulin responses to prolonged submaximal exercise.[274] An additional consideration for fasted scenarios is this difference between levels of adiposity, either whole body or at sensor application site, affecting sensor interaction with the interstitial compartment and would explain the tendency for individual differences in glucose values.[275]

Finally, building on the interactions introduced above, the data supports the need to consider individual physiological responses to when implementing CGM to collect and interpret glucose in female athletes due to the consistent influence on glucose measurements across the menstrual cycle when fasted and provided exogenous carbohydrate.

10.1.3 Menstrual Phase Interaction on Glucose Responses

This applied field study, presented in *Section 8*, is the first to observe the dynamics of blood glucose in a cohort of eumenorrheic ultra-running athletes during a simulated 4-hour ultra-run, replicating real-world conditions when fasted and or supplemented with exogenous CHO, while accounting for the interactions of menstrual phase. This was achieved through a randomized, cross-over design with participants acting as their own control across menstrual cycle phases was used during an ultra-marathon simulation consisting of a standardized outdoor 3-hour fasted depletion run (FASTED) followed by a 60-minute treadmill run (TREAD) with standardized exogenous glucose supplementation (20 g oral glucose intervention provided at 10, 35, and 50 minutes). Participants completed the run protocols (FASTED and TREAD) once during the mid-follicular (FOL: 4-8 days after the start of menses) and mid-luteal (LUT: 5-8 days post-ovulation) phases of the menstrual cycle. The main findings of this study were that during prolonged running, there were consistently higher glucose levels in the mid-luteal phase compared to the mid-follicular phase under both fasted and carbohydrate-supplemented conditions. Also, despite a minimum 8-hour fast followed by a 3-hour fasted outdoor run, there were no observed incidents of clinically significant non-diabetic hypoglycemia during the fasted running protocol.

Previous research has established the effects of menstrual cycle on glucose utilization and carbohydrate metabolism in variety of exercise and non-exercise settings. For example, our current understanding is that estrogen promotes glucose uptake into type I fibers,[26] while simultaneously reducing uptake of glucose into the bloodstream.[27] On the other hand, progesterone works antagonistically to reverse the suppression effect of estrogen on muscle glucose oxidation during submaximal exercise[27] as well as complement estrogen's glucose-sparing effect in the liver.[39] However, the influence of these functions on female endurance running performance, as they relate to glucose management, prior to this study have not been explored. Therefore, our observation that regardless of glucose collection method (CGM or capillary) and whether fasted or provided carbohydrate, glucose levels were higher in the mid-luteal phase by approximately 0.24 to 0.40 $mmol \cdot L^{-1}$ potentially contributes to our understanding of the inverse relationship between estrogen and glucose as well as progesterone's increase in insulin resistance[285] under prolonged running conditions.

More specifically, it appears that while a nominal average difference in glucose existed between the menstrual phases in this study, which can be explained by established mechanisms, the difference was only statistically significant at select time points within the study; 180 min during FASTED in capillary samples and inconsistently from CGM samples appearing at 45 min and 55 min during TREAD. At face value, the presence of consistently elevated average glucose level in LUT across the study without any statistical significance of menstrual phase being detected to account for differences in glucose in either the fasted or carbohydrate intervention, appear to be in conflict. Intuitively, the analysis and underlying statistical method used would present as a likely cause for this disagreement. However, the linear-mixed model chosen was capable of being fit for random effects of the individual to account for biological variability. Using this model, an alternative interpretation of the data could be that the variability of hormone ratios within the individual are statistically significant, but the effect is reduced when viewed as an average. Therefore, perhaps it is not how much glucose varies between the phases on average, but the difference between the individual and biological variability. This perspective highlights the importance of incorporating menstrual phase considerations, particularly the statistical methods used, when conducting future female endurance research and possibly across all domains.

The final observation from the *Section 8* was the absence of any clinically significant non-diabetic hypoglycemia during the fasted running protocol despite a minimum 8-hour fast followed by a 3-hour fasted outdoor run. The terminology used to describe hypoglycemia is specific: "clinically significant non-diabetic hypoglycemia". This was chosen because the ubiquitous term hypoglycemia found in sports performance research describes a state of low blood sugar, < 3.9 $mmol \cdot L^{-1}$, indicating blood sugar low enough to cause performance decline. However, the significance of blood sugar < 3.9 $mmol \cdot L^{-1}$ is derived from the lowest level of acceptable blood sugar in diabetics and represents a blood sugar trend associated with undesirable symptoms and informs the need for corrective interventions.[67] Conversely, in non-diabetics, blood sugar levels below 3.9 $mmol \cdot L^{-1}$ are often asymptomatic and hypoglycemia is characterized differently using Whipple's Triad; a cluster of symptoms which include: 1) anxiety, dizziness, rapid heartbeat, 2) blood glucose < 3.0 $mmol \cdot L^{-1}$, and 3) resolution of those symptoms when blood glucose levels are restored.[67,68] The effort to make this distinction is not pedantic and was rooted in this researcher's interaction with the participants who independently reported the surprising absence of hypoglycemic symptoms following trials despite verbal concerns on the initial run.

Interestingly, based on the non-diabetic assessment of hypoglycemia the avoidance of glucose levels below 3.0 mmol·L^{-1} during FASTED is noteworthy. Additionally, the three observances of glucose less than 3.2 mmol·L^{-1} (3.16, 3.16, 3.11 mmol·L^{-1}), recorded using CGM, were attributed to two participants and occurred between 155-160 minutes in the FOL phase. Likewise, the lowest recorded measures during TREAD were limited to two incidents of 3.28 mmol·L^{-1} occurring in different participants in different menstrual phases. While no direct claims can be made for a protective effect in either menstrual phase, the potential to delay the onset of symptomatic low glucose levels during prolonged running would be valuable. Furthermore, existing insights into female physiology, beyond ovarian hormones, such as increased intramyocellular lipid concentrations near muscle providing greater access to non-glucose energy sources [127,128] and female gene expressions that facilitate fat oxidation through increased concentrations of fatty acid enzymes [281], lend support for how this could occur.

10.1.4 Associations of Energy Metabolism and Glucose Response

Section 9 concluded the study portion of this PhD and utilized glucose as well as respiratory exchange ratio data, collected during the fourth hour of the study protocol, to investigate whether either energy substrate preferences or glucose levels differ between menstrual phases at the end of a 4-hour simulated ultra run. Results from this study indicated that, while a difference between males and females can exist under fasted conditions, among regularly menstruating female runners themselves, RER was not significantly different between the LUT and FOL phases when exogenous CHO are available. Furthermore, no appreciable difference in running intensity was observed between the menstrual phases. Most notably, and to our knowledge, this is the first study to report that RER had no main effect capillary glucose levels.

These findings contribute to the existing body of literature because historically, respiratory exchange ratio data has been used to define differences in energy metabolism during exercise between sexes. Current consensus is that preferential oxidation of fat over carbohydrate expressed in lower resting expiratory ratios, occurs among eumenorrheic females, relative to lean body mass, when fasted during submaximal endurance exercise, as compared to males and has been observed in several studies.[28–35] However, the difference in energy preference is mitigated when exogenous carbohydrate is provided.[30,33–36] Thus, the presence of differences in energy metabolism between females themselves across the menstrual cycle when fasted or provided exogenous carbohydrate

during a prolonged ultra-endurance running environment was an unexplored question. Additionally, no existing studies had investigated glucose levels in female athletes in relationship to RER with an appropriate study design controlling for menstrual phase profiles.

Based on the outcomes of this study, it is reasonable to assert that RER and blood glucose are not associated. Therefore, practitioners attempting to use blood glucose to inform nutrition related performance interventions should not be concerned with energy substrate preferences of the athlete when exogenous CHO are available.

10.2 Key Outcomes

1) The lack of standardization in terminology, distinct hormone phases, and reference hormone values, as well as the absence of low-cost, point-of-care hormone detection methods contribute to the challenges in female sport research design and study comparisons.

2) Publication of a narrative review to be used as a tool for selecting suitable study methods to effectively track menstrual phases in female sports research.

3) Synthesis of a suggestive framework for standardization of terminology and hormone phase from existing authors contributions.

4) First to observe and report on glucose responses across the menstrual cycle in endurance-trained eumenorrheic female athletes.

5) Observation that the CGM sensor used in this study tends to, on average, underreport glucose levels by varying degrees during fasted and unfasted ultra-endurance exercise when compared to capillary glucose controls.

6) Among eumenorrheic female runners the LUT phase was observed to have a significant influence on how closely CGM-derived glucose corresponded with benchmark capillary glucose samples. This observation brings into question the validity of CGM in female populations when menstrual phase is not accounted for.

7) Observation that individual physiological characteristics need to be considered when implementing and interpreting CGM glucose data in female athlete populations.

8) During prolonged running, there were consistently higher glucose levels in the mid-luteal phase compared to the mid-follicular phase, under both fasted and carbohydrate-supplemented conditions.

9) Observation that trained female ultra-runners, despite a minimum 8-hour fast, were able to avoid non-diabetic hypoglycemic glucose levels during a 3-hour fasted outdoor run.

10) Confirmatory observation that energy preferences in eumenorrheic female ultra-runners is similar across the menstrual during prolonged running when carbohydrate is available, which agrees with the existing literature conducted during shorter exercise durations.

11) Novel finding that there is no relationship between respiratory exchange ratio and glucose levels across the menstrual cycle in regularly menstruating females over 4-hours of running when CHO is available.

10.3 Practical Applications

The landscape of athletic performance is in a constant state of evolution, driven by the pursuit of human potential through advancements in research and technology. However, this progress isn't always balanced, as evidenced by scenarios where the rapid advancement of wearable technology, such as CGM, outpaces research, particularly in underrepresented demographics like female athletes in sports such as ultra-running. This discrepancy highlights the necessity for further research on female athletes, leading to evidence-based interventions. It also serves as the foundation for the current PhD project, along with subsequent recommendations for applying the key findings.

First, the importance of menstrual phase tracking in study design to accurately describe hormone variability within female cohorts is well established. However, the suggested best practices, such as hormone blood testing, the progression away from menstrual phase to standardized hormone levels, or both, could represent gold standards of practices. Yet, in the absence of reliable, inexpensive, point-of-care, blood hormone testing methods, these guidelines present obstacles to future female sport research. The aphorism, “Better is the enemy of good”, embodies this current situation in female sport research. Therefore, until such hormone testing methods are available researchers need to employ a reliable arsenal of testing methods, appropriate to their study design

needs, that can be utilized in a combined method of menstrual phase tracking to achieve the closest approximation to a desired hormone profile.

Second, practitioners and athletes should not assume the accuracy of CGM data during exercise, regardless of the sensor's application in a medical setting and that the use of near-real time glucose data to inform sport fueling practices during endurance running is questionable. Data presented in *Section 7* demonstrated that the sport CGM device used in this study tends to, on average, underreport glucose levels by varying degrees during fasted and unfasted exercise when compared to capillary glucose controls. Additionally, this variability was also influenced by the menstrual phase and by individual biological differences. Also, the accuracy of CGM will vary based on whether the athlete is fasted or has access to carbohydrates. Consequently, the results of CGM glucose data should be interpreted cautiously to minimize improper judgements in carbohydrate intake leading to gastrointestinal distress or the athlete anticipating or psychosomatically experiencing undesirable symptoms, commonly associated with low blood sugar, leading to negative performance outcomes, when those symptoms would otherwise not be indicated.

Third, there are no guidelines for blood sugar levels above 3.0 mmol·L^{-1} in healthy athletes during exercise. Thus, any glucose data derived from CGM during exercise seems limited to glucose trend identification. Perhaps most intriguing was the ubiquitous use of glucose < 3.9 mmol·L^{-1} throughout the existing body of sports research to designate low blood glucose leading to decreased performance and adverse symptoms. However, observations from study data in *Section 8*, that blood sugar levels between 3.1 mmol·L-1 and 3.9 mmol·L^{-1} were asymptomatic among the cohort of trained, eumenorrheic, female, ultra-runners who participated in this PhD project, prompted a more detailed review of the < 3.9 mmol·L^{-1} practice in endurance sport. The result of this was the identification of < 3.9 mmol·L^{-1} as the lowest level of desirable blood glucose in diabetics and suggests corrective interventions. However, in healthy individuals' non-diabetic hypoglycemia is characterized by glucose < 3.0 mmol·L^{-1} and accompanied by symptoms of hypoglycemia. This suggests that practitioners and athletes choosing to use CGM should assess the individual's response to glucose levels before assuming corrective actions are required. It may also suggest that ultra-runners are desensitized to the effects of low blood sugar from frequent exposure related to under-fueling during prolonged training session, which has negative consequences if the physiological warning mechanisms are ineffective.

10.4 Limitations

Each manuscript presented in Chapter 3 recognized constraints specific to the individual studies. Nonetheless, throughout the entire project, there are underlying limitations. To begin with, this study was unable to verify hormone status using blood samples. As an alternative verification process, a combined method was used to predict the target menstrual phases, but any method outside of blood sampling is incapable of detecting luteal phase deficiency or exact hormonal phase, both of which can impact the interpretation of female research data. Next, the running durations (4+ hours) required to recreate an ultra-running environment were difficult to align with exact menstrual phases, participant availability, training plans, and race calendars. This led to a variable length of time, 6 weeks to 8 months, between FASTED and TREAD protocols among the participants. Such differences in time could have positively or negatively impacted the training or underlying hormone status of the participants and impacted the results.

Furthermore, while the specificity of the study population, comprised of trained eumenorrheic female ultra-runners, stands as a notable strength of the project, it also presents a limitation by restricting the generalizability of menstrual phase findings to the broader subset of female athletes, particularly those utilizing hormonal contraception. Moreover, an opportunity to track perceived blood sugar symptoms and compare them to actual glucose data was not incorporated into the study design and could have provided additional insight. The inclusion of this process would have allowed for a more robust interpretation of the glucose data and interactions with ultra-endurance performance. Similarly, the primary study did not implement multiple or redundant CGM sensors during data collection to mitigate technological failures or as a secondary method of CGM validation. In hindsight the implementation of redundant CGM sensors in any study, as well as capillary of venous benchmarks, cannot be over-stated. The 25% error rate in during specific time-stamped collection periods combined with the sporadic nature of sensor failure across an entire span of time was unexpected from devices approved for use in the management of diabetes.

10.5 Future Research

Future investigations into glucose as a performance metric in ultra-running, stemming from this thesis, should consider the findings of our CGM feasibility study. The consensus within the existing literature is that CGM becomes less accurate in times of rapid glucose changes associated with glucose availability or lack thereof. Our glucose data from CGM, when compared to capillary glucose controls, supports this. Also, the consistent underreporting of the CGM device by ~1.0 $mmol \cdot L^{-1}$ compared to capillary control measures with as little as 60 g of carbohydrate per hour were provided in 20 g boluses, a common occurrence in endurance sport, is concerning. All of which indicates that additional work is needed to successfully validate the devices under prolonged running conditions.

Larger sample sizes to confirm the consistently elevated glucose levels within the mid-luteal phase are also indicated. These follow up studies could specifically aim to elucidate whether there is a generalized protective effect of female physiology against hypoglycemia or, if such an effect exists, is that effect isolated to the distinct hormone phase found within the mid-luteal phase. The introduction of male participants for comparison is also indicated for this area of study.

Lastly, our data suggests that the biological differences observed among individuals are partly responsible for the differences between CGM and capillary glucose data. These biological differences also appear to influence the magnitude of the variability in glucose between the menstrual phases when fasted or provided glucose. Consequently, future research should aim to fully investigate individual metabolic diversity as it relates to glucose fluctuations across menstrual cycle. These observations also emphasize the need for a standardization in menstrual phase terminology, clinical based hormone guidelines, and the advancement of hormone detection methods for use in future study design. For example, additional research into hormone ratios to determine what ratios are sufficient to elicit changes in metabolism as well as the development of an accessible point-of-care hormone testing method would both allow for a more consistent application of best-practices in female performance research.

10.6 Conclusion

This study set out to investigate metabolic data such as glucose and energy metabolism in regularly menstruating female ultra runners. This aim was achieved through a randomized, cross-over design with participants acting as their own control across menstrual cycle phases during an ultra-marathon simulation. Individually, female athletes, ultra-running, and the use of continuous glucose sensors in endurance sport, are underrepresented in the existing literature. When combined, a significant gap in knowledge is apparent as highlighted throughout this thesis. However, as interest in all three aspects grows the contributions of this thesis will hopefully act as a foundation for future study or field application by athletes and practitioners. Specifically, the awareness of how CGM glucose data is influenced during prolonged running within the female population, as well as the reporting of elevated glucose in mid-luteal phase, identifies an area of consideration when interpreting glucose levels and future research. Also, the lack of observed hypoglycemia in female ultra-runners did not fall within the current understanding or guidelines, and suggests more clarity is needed. Moreover, researchers choosing to utilize female participants should be aware of the current methodological landscape, including challenges and solutions.

References

1. Kreider RB. Physiological considerations of ultraendurance performance. *Int J Sport Nutr*. 1991;1(1):3-27. doi:10.1123/IJSN.1.1.3

2. Zaryski C, Smith DJ. Training principles and issues for ultra-endurance athletes. *Curr Sports Med Rep*. 2005;4(3):165-170. doi:10.1097/01.CSMR.0000306201.49315.73

3. HOFFMAN MD, WEGELIN JA. The Western States 100-Mile Endurance Run. *Med Sci Sports Exerc*. 2009;41(12):2191-2198. doi:10.1249/MSS.0b013e3181a8d553

4. Western State 100: Women Set the Pace - The New York Times. Accessed April 27, 2022. https://www.nytimes.com/2021/07/11/sports/western-states-women.html

5. Hunter SK, Angadi SS, Bhargava A, et al. The Biological Basis of Sex Differences in Athletic Performance: Consensus Statement for the American College of Sports Medicine. *Med Sci Sports Exerc*. 2023;55(12):2328-2360. doi:10.1249/MSS.0000000000003300

6. Waldvogel KJ, Nikolaidis PT, Di Gangi S, Rosemann T, Knechtle B. Women Reduce the Performance Difference to Men with Increasing Age in Ultra-Marathon Running. *Int J Environ Res Public Health*. 2019;16(13). doi:10.3390/IJERPH16132377

7. Tiller NB, Elliott-Sale KJ, Knechtle B, Wilson PB, Roberts JD, Millet GY. Do Sex Differences in Physiology Confer a Female Advantage in Ultra-Endurance Sport? *Sports Med*. 2021;51(5):895-915. doi:10.1007/S40279-020-01417-2

8. Pfeiffer B, Stellingwerff T, Hodgson AB, et al. Nutritional intake and gastrointestinal problems during competitive endurance events. *Med Sci Sports Exerc*. 2012;44(2):344-351. doi:10.1249/MSS.0b013e31822dc809

9. Cuddy J, Slivka D, Hailes W, Dumke C, Ruby BC. Total energy expenditure, body water turnover, hydration status, and blood composition during the western states 100. *Med Sci Sports Exerc*. 2009;41(5):189. doi:10.1249/01.mss.0000355133.78976.4a

10. Hearris MA, Hammond KM, Fell JM, Morton JP. Regulation of muscle glycogen metabolism during exercise: Implications for endurance performance and training adaptations. *Nutrients*. Published online 2018. doi:10.3390/nu10030298

11. Jeukendrup AE, Moseley L, Mainwaring GI, Samuels S, Perry S, Mann CH. Exogenous carbohydrate oxidation during ultraendurance exercise. *J Appl Physiol (1985)*. 2006;100(4):1134-1141. doi:10.1152/japplphysiol.00981.2004

12. Graff J, Brinch K, Madsen JL. Gastrointestinal mean transit times in young and middle-aged healthy subjects. *Clin Physiol*. 2001;21(2):253-259. doi:10.1046/J.1365-2281.2001.00308.X

13. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE. Carbohydrates for training and competition. *J Sports Sci*. 2011;29 Suppl 1(SUPPL. 1):S17-27. doi:10.1080/02640414.2011.585473

14. Rowlands DS, Houltham S, Musa-Veloso K, et al. Fructose-Glucose Composite Carbohydrates and Endurance Performance: Critical Review and Future Perspectives. *Sports Medicine*. 2015;45:1561-1576. doi:10.1007/s40279-015-0381-0

15. Jentjens RLPG, Achten J, Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. *Med Sci Sports Exerc*. 2004;36(9):1551-1558. doi:10.1249/01.MSS.0000139796.07843.1D

16. Jeukendrup A. A Step Towards Personalized Sports Nutrition: Carbohydrate Intake During Exercise. *Sports Med*. 2014;44(Suppl 1):25. doi:10.1007/S40279-014-0148-Z

17. Burelle Y, Lamoureux MC, Pèronnet F, Massicotte D, Lavoie C. Comparison of exogenous glucose, fructose and galactose oxidation during exercise using 13C-labelling. *Br J Nutr*. 2006;96(1):56. doi:10.1079/BJN20061799

18. Rowlands DS, Swift M, Ros M, Green JG. Composite versus single transportable carbohydrate solution enhances race and laboratory cycling performance. *Appl Physiol Nutr Metab*. 2012;37(3):425-436. doi:10.1139/H2012-013

19. Costa RJS, Gill SK, Hankey J, Wright A, Marczak S. Perturbed energy balance and hydration status in ultra-endurance runners during a 24 h ultra-marathon. *British Journal of Nutrition*. 2014;112(3):428-437. doi:10.1017/S0007114514000907

20. Wardenaar FC, Dijkhuizen R, Ceelen IJM, et al. Nutrient intake by ultramarathon runners: Can they meet recommendations? *Int J Sport Nutr Exerc Metab*. 2015;25(4):375-386. doi:10.1123/ijsnem.2014-0199

21. de Oliveira EP, Burini RC. Carbohydrate-dependent, exercise-induced gastrointestinal distress. *Nutrients*. 2014;6(10):4191-4199. doi:10.3390/NU6104191

22. De Oliveira EP, Burini RC, Jeukendrup A. Gastrointestinal complaints during exercise: Prevalence, etiology, and nutritional recommendations. *Sports Medicine*. 2014;44(SUPPL.1). doi:10.1007/s40279-014-0153-2

23. Ter Steege RWF, Kolkman JJ. Review article: The pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Aliment Pharmacol Ther*. 2012;35(5):516-528. doi:10.1111/j.1365-2036.2011.04980.x

24. Porter AM. Marathon running and the caecal slap syndrome. *Br J Sports Med*. 1982;16(3):178. doi:10.1136/BJSM.16.3.178

25. Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. *Endotext*. Published online August 5, 2018. Accessed April 10, 2023. https://www.ncbi.nlm.nih.gov/books/NBK279054/

26. Campbell SE, Febbraio MA. Effect of the ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake. *Am J Physiol Endocrinol Metab*. 2002;282(5 45-5):E1139-E1146. doi:10.1152/ajpendo.00184.2001

27. D'eon TM, Sharoff C, Chipkin SR, et al. Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women. *Am J Physiol Endocrinol Metab*. 2002;283(5 46-5):1046-1055. doi:10.1152/ajpendo.00271.2002.-To

28. Tarnopolsky MA. Sex differences in exercise metabolism and the role of 17-beta estradiol. *Med Sci Sports Exerc*. 2008;40(4):648-654. doi:10.1249/MSS.0B013E31816212FF

29. Hackney AC, McCracken-Compton MA, Ainsworth B. Substrate responses to submaximal exercise in the midfollicular and midluteal phases of the menstrual cycle. *Int J Sport Nutr*. 1994;4(3):299-308. doi:10.1123/ijsn.4.3.299

30. Devries MC, Hamadeh MJ, Phillips SM, Tarnopolsky MA. Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *Am J Physiol Regul Integr Comp Physiol*. 2006;291(4):1120-1128. doi:10.1152/ajpregu.00700.2005

31. Nicklas BJ, Hackney AC, Sharp RL. The menstrual cycle and exercise: performance, muscle glycogen, and substrate responses. *Int J Sports Med*. 1989;10(4):264-269. doi:10.1055/S-2007-1024913

32. Roepstorff C, Steffensen CH, Madsen M, et al. Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. *Am J Physiol Endocrinol Metab*. 2002;282(2 45-2). doi:10.1152/ajpendo.00266.2001

33. Riddell MC, Partington SL, Stupka N, Armstrong D, Rennie C, Tarnopolsky MA. Substrate Utilization during Exercise Performed with and Without Glucose Ingestion in Female and Male Endurance-Trained Athletes. *Int J Sport Nutr Exerc Metab*. 2003;13(4):407-421. doi:10.1123/ijsnem.13.4.407

34. Wallis GA, Dawson R, Achten J, Webber J, Jeukendrup AE. Metabolic response to carbohydrate ingestion during exercise in males and females. *Am J Physiol Endocrinol Metab*. 2006;290(4). doi:10.1152/ajpendo.00357.2005

35. Tremblay J, Peronnet F, Massicotte D, Lavoie C. Carbohydrate supplementation and sex differences in fuel selection during exercise. *Med Sci Sports Exerc*. 2010;42(7):1314-1323. doi:10.1249/MSS.0b013e3181cbba0b

36. Pettersson S, Edin F, Bakkman L, McGawley K. Effects of supplementing with an 18% carbohydrate-hydrogel drink versus a placebo during whole-body exercise in-5 °c with elite cross-country ski athletes: A crossover study. *J Int Soc Sports Nutr*. 2019;16(1):1-14. doi:10.1186/S12970-019-0317-4/FIGURES/5

37. Carter SL, Rennie C, Tarnopolsky MA. Substrate utilization during endurance exercise in men and women after endurance training. *Am J Physiol Endocrinol Metab*. 2001;280(6 43-6):898-907. doi:10.1152/ajpendo.2001.280.6.e898

38. Friedlander AL, Casazza GA, Horning MA, et al. Training-induced alterations of carbohydrate metabolism in women: women respond differently from men. *https://doi-org.ezproxy.waikato.ac.nz/101152/jappl19988531175*. 1998;85(3):1175-1186. doi:10.1152/JAPPL.1998.85.3.1175

39. Kalkhoff RK. Metabolic effects of progesterone. *Am J Obstet Gynecol*. 1982;142(6 Pt 2):735-738. doi:10.1016/S0002-9378(16)32480-2

40. Flannery CA, Choe GH, Cooke KM, et al. Insulin regulates glycogen synthesis in human endometrial glands through increased GYS2. *Journal of Clinical Endocrinology and Metabolism*. 2018;103(8):2843-2850. doi:10.1210/jc.2017-01759

41. Zhang H, Qi J, Wang Y, et al. Progesterone Regulates Glucose Metabolism Through Glucose Transporter 1 to Promote Endometrial Receptivity. *Front Physiol*. 2020;11:543148. doi:10.3389/fphys.2020.543148

42. Howe JC, Rumpler W V., Seale JL. Energy expenditure by indirect calorimetry in premenopausal women: variation within one menstrual cycle. *J Nutr Biochem*. 1993;4(5):268-273. doi:10.1016/0955-2863(93)90096-F

43. Costa RJS, Hoffman MD, Stellingwerff T. Considerations for ultra-endurance activities: part 1-nutrition. *Research in Sports Medicine*. 2019;27(2):166-181. doi:10.1080/15438627.2018.1502188

44. Suh S, Kim JH. Glycemic Variability: How Do We Measure It and Why Is It Important? *Diabetes Metab J*. 2015;39(4):273. doi:10.4093/DMJ.2015.39.4.273

45. Pickup JC, Freeman SC, Sutton AJ. Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data. *BMJ*. 2011;343(7815). doi:10.1136/BMJ.D3805

46. Tobias A, Ballard BD, Mohiuddin SS. Physiology, Water Balance. *StatPearls*. Published online October 7, 2020. Accessed August 6, 2021. https://www.ncbi.nlm.nih.gov/books/NBK541059/

47. Zhou Z, Sun B, Huang S, Zhu C, Bian M. Glycemic variability: adverse clinical outcomes and how to improve it? *Cardiovasc Diabetol*. 2020;19(1). doi:10.1186/S12933-020-01085-6

48. Danne T, Nimri R, Battelino T, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care*. 2017;40(12):1631-1640. doi:10.2337/dc17-1600

49. Umpierrez GE, P. Kovatchev B. Glycemic Variability: How to Measure and Its Clinical Implication for Type 2 Diabetes. *American Journal of the Medical Sciences*. 2018;356(6):518-527. doi:10.1016/J.AMJMS.2018.09.010

50. Van Enter BJ, Von Hauff E. Challenges and perspectives in continuous glucose monitoring. *Chemical Communications*. 2018;54(40):5032-5045. doi:10.1039/C8CC01678J

51. Ribet F, Stemme G, Roxhed N. Real-time intradermal continuous glucose monitoring using a minimally invasive microneedle-based system. *Biomed Microdevices*. 2018;20(4):101. doi:10.1007/s10544-018-0349-6

52. Schmelzeisen-Redeker G, Schoemaker M, Kirchsteiger H, Freckmann G, Heinemann L, Del Re L. Time Delay of CGM Sensors: Relevance, Causes, and Countermeasures. *J Diabetes Sci Technol*. 2015;9(5):1006. doi:10.1177/1932296815590154

53. Schmelzeisen-Redeker G, Staib A, Strasser M, Müller U, Schoemaker M. Overview of a novel sensor for continuous glucose monitoring. *J Diabetes Sci Technol*. 2013;7(4):808-814. doi:10.1177/193229681300700402

54. Rodbard D. Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities. *Diabetes Technol Ther*. 2016;18(S2):S23-S213. doi:10.1089/dia.2015.0417

55. Zaharieva DP, Turksoy K, McGaugh SM, et al. Lag Time Remains with Newer Real-Time Continuous Glucose Monitoring Technology during Aerobic Exercise in Adults Living with Type 1 Diabetes. *Diabetes Technol Ther*. 2019;21(6):313-321. doi:10.1089/DIA.2018.0364/ASSET/IMAGES/LARGE/FIGURE3.JPEG

56. Pleus S, Schoemaker M, Morgenstern K, et al. Rate-of-Change Dependence of the Performance of Two CGM Systems During Induced Glucose Swings. *J Diabetes Sci Technol*. 2015;9(4):801-807. doi:10.1177/1932296815578716

57. Moser O, Eckstein ML, McCarthy O, et al. Performance of the Freestyle Libre flash glucose monitoring (flash GM) system in individuals with type 1 diabetes: A secondary outcome analysis of a randomized crossover trial. *Diabetes Obes Metab*. 2019;21(11):2505-2512. doi:10.1111/dom.13835

58. Aberer F, Hajnsek M, Rumpler M, et al. Evaluation of subcutaneous glucose monitoring systems under routine environmental conditions in patients with type 1 diabetes. *Diabetes Obes Metab*. 2017;19(7):1051-1055. doi:10.1111/dom.12907

59. Freckmann G, Pleus S, Grady M, Setford S, Levy B. Measures of Accuracy for Continuous Glucose Monitoring and Blood Glucose Monitoring Devices. *J Diabetes Sci Technol*. 2019;13(3):575-583. doi:10.1177/1932296818812062

60. Biagi L, Bertachi A, Quirós C, et al. Accuracy of Continuous Glucose Monitoring before, during, and after Aerobic and Anaerobic Exercise in Patients with Type 1 Diabetes Mellitus. *Biosensors (Basel)*. 2018;8(1). doi:10.3390/BIOS8010022

61. Fokkert MJ, van Dijk PR, Edens MA, et al. Performance of continuous glucose monitoring devices during intensive exercise conditions in people with diabetes: the Mont Blanc experience. *Diabetic Medicine*. 2020;37(7):1204-1205. doi:10.1111/dme.14259

62. Iscoe KE, Campbell JE, Jamnik V, Perkins BA, Riddell MC. Efficacy of continuous real-time blood glucose monitoring during and after prolonged high-intensity cycling exercise: Spinning with a continuous glucose monitoring system. *Diabetes Technol Ther*. 2006;8(6):627-635. doi:10.1089/dia.2006.8.627

63. Adolfsson P, Nilsson S, Lindblad B. Continuous glucose monitoring system during physical exercise in adolescents with type 1 diabetes. *Acta Paediatr*. 2011;100(12):1603-1609. doi:10.1111/j.1651-2227.2011.02390.x

64. Fabra EM, Díez JL, Bondia J, Sanz AJL. A comprehensive review of continuous glucose monitoring accuracy during exercise periods. *Sensors (Switzerland)*. 2021;21(2):1-19. doi:10.3390/s21020479

65. Mian Z, Hermayer KL, Jenkins A. Continuous Glucose Monitoring: Review of an Innovation in Diabetes Management. *Am J Med Sci*. 2019;358(5):332-339. doi:10.1016/J.AMJMS.2019.07.003

66. Olczuk D, Priefer R. A history of continuous glucose monitors (CGMs) in self-monitoring of diabetes mellitus. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*. 2018;12(2):181-187. doi:10.1016/j.dsx.2017.09.005

67. Amiel SA. The consequences of hypoglycaemia. *Diabetologia*. 2021;64(5):963. doi:10.1007/S00125-020-05366-3

68. Desimone ME, Weinstock RS. Hypoglycemia. *Endotext*. Published online May 5, 2018. Accessed January 21, 2024. https://www.ncbi.nlm.nih.gov/books/NBK279137/

69. Francois ME, Cosgrove SD, Walker NM, Lucas SJ, Black KE. Physiological responses to a five-day adventure race: Continuous blood glucose, hemodynamics and metabolites the 2012 GODZone field-study. *J Exerc Sci Fit*. 2018;16(3):78-82. doi:10.1016/J.JESF.2018.07.002

70. Ishihara K, Uchiyama N, Kizaki S, Mori E, Nonaka T, Oneda H. Application of continuous glucose monitoring for assessment of individual carbohydrate requirement during ultramarathon race. *Nutrients*. 2020;12(4):1121. doi:10.3390/nu12041121

71. Costa RJS, Snipe R, Camões-Costa V, Scheer V, Murray A. The Impact of Gastrointestinal Symptoms and Dermatological Injuries on Nutritional Intake and Hydration Status During Ultramarathon Events. *Sports Med Open*. 2016;2(1). doi:10.1186/s40798-015-0041-9

72. Eisenmann J. Translational Gap between Laboratory and Playing Field: New Era to Solve Old Problems in Sports Science. *Translational Journal of the ACSM*. Published online 2017. doi:10.1249/TJX.0000000000000032

73. Williamson E. Nutritional implications for ultra-endurance walking and running events. *Extrem Physiol Med*. 2016;5(1). doi:10.1186/s13728-016-0054-0

74. Knechtle B. Ultramarathon runners: Nature or nurture? *Int J Sports Physiol Perform*. 2012;7(4):310-312. doi:10.1123/ijspp.7.4.310

75. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *J Acad Nutr Diet*. 2016;116(3):501-528. doi:10.1016/J.JAND.2015.12.006

76. Pramuková B, Szabadosová V, Šoltésová A. Current knowledge about sports nutrition. *Australas Med J*. 2011;4(3):107. doi:10.4066/AMJ.2011.520

77. Gonzalez JT, Fuchs CJ, Smith FE, et al. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Metab*. 2015;309(12):E1032-E1039. doi:10.1152/ajpendo.00376.2015

78. Dumke CL, Shooter L, Lind RH, Nieman DC. Indirect calorimetry during ultradistance running: a case report. *J Sports Sci Med*. 2006;5(4):692-698. Accessed July 27, 2020. http://www.ncbi.nlm.nih.gov/pubmed/24357966

79. Cuddy JS, Slivka DR, Hailes WS, Dumke CL, Ruby BC. Metabolic profile of the Ironman World Championships: A case study. *Int J Sports Physiol Perform*. 2010;5(4):570-576. doi:10.1123/ijspp.5.4.570

80. Alcock R, McCubbin A, Camões-Costa V, Costa RJS. Case Study: Providing Nutritional Support to an Ultraendurance Runner in Preparation for a Self-Sufficient Multistage Ultramarathon: Rationed Versus Full Energy Provisions. *Wilderness Environ Med*. 2018;29(4):508-520. doi:10.1016/j.wem.2018.06.004

81. Speechly DP, Taylor SR, Rogers GG. Differences in ultra-endurance exercise in performance-matched male and female runners. *Med Sci Sports Exerc*. 1996;28(3):359-365. doi:10.1097/00005768-199603000-00011

82. Hargreaves M, Spriet LL. Skeletal muscle energy metabolism during exercise. *Nature Metabolism 2020 2:9*. 2020;2(9):817-828. doi:10.1038/s42255-020-0251-4

83. Gonzalez JT, Fuchs CJ, Betts JA, Loon LJC van. Liver glycogen metabolism during and after prolonged endurance-type exercise. *https://doi.org/101152/ajpendo002322016*. 2016;311(3):E543-E553. doi:10.1152/AJPENDO.00232.2016

84. Fletcher G, Eves FF, Glover EI, et al. Dietary intake is independently associated with the maximal capacity for fat oxidation during exercise. *American Journal of Clinical Nutrition*. 2017;105(4):864-872. doi:10.3945/ajcn.116.133520

85. Horowitz JF, Mora-Rodriguez R, Byerley LO, Coyle EF. Substrate metabolism when subjects are fed carbohydrate during exercise. *Am J Physiol Endocrinol Metab*. 1999;276(5 39-5). doi:10.1152/ajpendo.1999.276.5.e828

86. Van Loon LJC, Greenhaff PL, Constantin-Teodosiu D, Saris WHM, Wagenmakers AJM. *The Effects of Increasing Exercise Intensity on Muscle Fuel Utilisation in Humans*.; 2001.

87. Achten J, Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition*. 2004;20(7-8):716-727. doi:10.1016/j.nut.2004.04.005

88. Hawley JA, Leckey JJ. Carbohydrate Dependence During Prolonged, Intense Endurance Exercise. *Sports Medicine*. 2015;45. doi:10.1007/s40279-015-0400-1

89. Cermak NM, van Loon LJC. The use of carbohydrates during exercise as an ergogenic aid. *Sports Med*. 2013;43(11):1139-1155. doi:10.1007/s40279-013-0079-0

90. Stevenson EJ, Thelwall PE, Thomas K, Smith F, Brand-Miller J, Trenell MI. Dietary glycemic index influences lipid oxidation but not muscle or liver glycogen oxidation during exercise. *Am J Physiol Endocrinol Metab*. 2009;296(5):1140-1147. doi:10.1152/ajpendo.90788.2008

91. Ronald Maughan by J, Ivy JL. Sports Nutrition, 1st Edition. Edited Chapter 8 The Regulation and Synthesis of Muscle Glycogen by Means of Nutrient Intervention. In: Wyley; 2014.

92. Jensen J, Rustad I, Kolnes AJ, et al. The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Published online 2011. doi:10.3389/fphys.2011.00112

93. Tornheim K. Glucose Metabolism and Hormonal Regulation. *Encyclopedia of Endocrine Diseases*. Published online January 1, 2018:87-94. doi:10.1016/B978-0-12-801238-3.03816-2

94. Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol (1985)*. 1986;61(1):165-172. doi:10.1152/jappl.1986.61.1.165

95. Gonzalez JT, Betts JA. Dietary sugars, exercise and hepatic carbohydrate metabolism. *Proceedings of the Nutrition Society*. 2019;78(2):246-256. doi:10.1017/S0029665118002604

96. Coggan AR, Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *J Appl Physiol*. 1987;63(6):2388-2395. doi:10.1152/jappl.1987.63.6.2388

97. Jeukendrup A, Brouns F, Wagenmakers AJM, Saris WHM. Carbohydrate-electrolyte feedings improve 1 h time trial cycling performance. *Int J Sports Med*. 1997;18(2):125-129. doi:10.1055/s-2007-972607

98. Alghannam AF, Jedrzejewski D, Tweddle MG, et al. Impact of muscle glycogen availability on the capacity for repeated exercise in man. *Med Sci Sports Exerc*. 2016;48(1):123-131. doi:10.1249/MSS.0000000000000737

99. Jeukendrup AE. Training the Gut for Athletes. *Sports Med*. 2017;47(Suppl 1):101. doi:10.1007/S40279-017-0690-6

100. Stuempfle KJ, Hoffman MD, Hew-Butler T. *Association of Gastrointestinal Distress in Ultramarathoners With Race Diet*. Vol 23.; 2013. www.IJSNEM-Journal.com

101. Jeukendrup AE, Currell K, Clarke J, Cole J, Blannin AK. Effect of beverage glucose and sodium content on fluid delivery. *Nutr Metab (Lond)*. 2009;6(1):9. doi:10.1186/1743-7075-6-9

102. Martinez IG, Mika AS, Biesiekierski JR, Costa RJS. The Effect of Gut-Training and Feeding-Challenge on Markers of Gastrointestinal Status in Response to Endurance Exercise: A Systematic Literature Review. *Sports Medicine 2023 53:6*. 2023;53(6):1175-1200. doi:10.1007/S40279-023-01841-0

103. Jeukendrup AE, Saris WHM, Wagenmakers AJM. Fat metabolism during exercise: A review. Part I: Fatty acid mobilization and muscle metabolism. *Int J Sports Med*. 1998;19(4):231-244. doi:10.1055/s-2007-971911

104. Purdom T, Kravitz L, Dokladny K, Mermier C. Understanding the factors that effect maximal fat oxidation. *Journal of the International Society of Sports Nutrition 2018 15:1*. 2018;15(1):1-10. doi:10.1186/S12970-018-0207-1

105. Volek JS, Noakes T, Phinney SD. Rethinking fat as a fuel for endurance exercise. *Eur J Sport Sci*. 2015;15(1):13-20. doi:10.1080/17461391.2014.959564

106. Spriet LL. New Insights into the Interaction of Carbohydrate and Fat Metabolism During Exercise. *Sports Med*. 2014;44(Suppl 1):87. doi:10.1007/S40279-014-0154-1

107. Burke LM, Ross ML, Garvican-Lewis LA, et al. Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J Physiol*. 2017;595(9):2785-2807. doi:10.1113/JP273230

108. Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc*. 2002;34(1):92-97. doi:10.1097/00005768-200201000-00015

109. Randell RK, Rollo I, Roberts TJ, Dalrymple KJ, Jeukendrup AE, Carter JM. Maximal Fat Oxidation Rates in an Athletic Population. *Med Sci Sports Exerc*. 2017;49(1):133-140. doi:10.1249/MSS.0000000000001084

110. Phinney SD, Bistrian BR, Evans WJ, Gervino E, Blackburn GL. The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism*. 1983;32(8):769-776. doi:10.1016/0026-0495(83)90106-3

111. Burke LM, Sharma AP, Heikura IA, et al. Crisis of confidence averted: Impairment of exercise economy and performance in elite race walkers by ketogenic low carbohydrate, high fat (LCHF) diet is reproducible. *PLoS One*. 2020;15(6). doi:10.1371/JOURNAL.PONE.0234027

112. Burke LM, Whitfield J, Heikura IA, et al. Adaptation to a low carbohydrate high fat diet is rapid but impairs endurance exercise metabolism and performance despite enhanced glycogen availability. *J Physiol*. 2021;599(3):771-790. doi:10.1113/JP280221

113. Tarnopolsky M. Protein requirements for endurance athletes. *Nutrition*. 2004;20(7-8):662-668. doi:10.1016/J.NUT.2004.04.008

114. Koopman R, Pannemans DLE, Jeukendrup AE, et al. Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. *Am J Physiol Endocrinol Metab*. 2004;287(4). doi:10.1152/AJPENDO.00543.2003

115. Smith ES, McKay AKA, Kuikman M, et al. Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. *Nutrients*. 2022;14(5). doi:10.3390/NU14050953/S1

116. Desbrow B, Tarnopolsky M, Burd NA, Moore DR, Elliott-Sale KJ. Nutrition for Special Populations: Young, Female, and Masters Athletes. *Int J Sport Nutr Exerc Metab*. 2019;29(2):220-227. doi:10.1123/IJSNEM.2018-0269

117. Devries MC. Sex-based differences in endurance exercise muscle metabolism: Impact on exercise and nutritional strategies to optimize health and performance in women. *Exp Physiol*. 2016;101(2):243-249. doi:10.1113/EP085369

118. Kim JH, Cho HT, Kim YJ. The role of estrogen in adipose tissue metabolism: Insights into glucose homeostasis regulation. *Endocr J*. 2014;61(11):1055-1067. doi:10.1507/endocrj.EJ14-0262

119. Kuikman MA, Smith ES, McKay AKA, et al. Fueling the Female Athlete: Auditing Her Representation in Studies of Acute Carbohydrate Intake for Exercise. *Med Sci Sports Exerc*. 2023;55(3):569-580. doi:10.1249/MSS.0000000000003056

120. Horton TJ, Miller EK, Glueck D, Tench K. No effect of menstrual cycle phase on glucose kinetics and fuel oxidation during moderate-intensity exercise. *Am J Physiol Endocrinol Metab*. 2002;282(4 45-4). doi:10.1152/ajpendo.00238.2001

121. Jacobs KA, Casazza GA, Suh SH, Horning MA, Brooks GA. Fatty acid reesterification but not oxidation is increased by oral contraceptive use in women. *J Appl Physiol (1985)*. 2005;98(5):1720-1731. doi:10.1152/JAPPLPHYSIOL.00685.2004

122. Oosthuyse T, Bosch AN. The effect of the menstrual cycle on exercise metabolism: Implications for exercise performance in eumenorrhoeic women. *Sports Medicine*. 2010;40(3):207-227. doi:10.2165/11317090-000000000-00000

123. Haizlip KM, Harrison BC, Leinwand LA. Sex-Based Differences in Skeletal Muscle Kinetics and Fiber-Type Composition. *Physiology*. 2015;30(1):30. doi:10.1152/PHYSIOL.00024.2014

124. Plotkin DL, Roberts MD, Haun CT, Schoenfeld BJ. Muscle Fiber Type Transitions with Exercise Training: Shifting Perspectives. *Sports*. 2021;9(9). doi:10.3390/SPORTS9090127

125. Zierath JR, Hawley JA. Skeletal Muscle Fiber Type: Influence on Contractile and Metabolic Properties. *PLoS Biol*. 2004;2(10). doi:10.1371/JOURNAL.PBIO.0020348

126. Avin KG, Naughton MR, Ford BW, et al. Sex Differences in Fatigue Resistance Are Muscle Group Dependent. *Med Sci Sports Exerc*. 2010;42(10):1943. doi:10.1249/MSS.0B013E3181D8F8FA

127. Coen PM, Goodpaster BH. Role of intramyocelluar lipids in human health. *Trends Endocrinol Metab*. 2012;23(8):391. doi:10.1016/J.TEM.2012.05.009

128. Devries MC, Lowther SA, Glover AW, Hamadeh MJ, Tarnopolsky MA. IMCL area density, but not IMCL utilization, is higher in women during moderate-intensity endurance exercise, compared with men. *Am J Physiol Regul Integr Comp Physiol*. 2007;293(6):R2336-42. doi:10.1152/ajpregu.00510.2007

129. Steffensen CH, Roepstorff C, Madsen M, Kiens B. Myocellular triacylglycerol breakdown in females but not in males during exercise. *Am J Physiol Endocrinol Metab*. 2002;282(3 45-3):634-642. doi:10.1152/AJPENDO.00078.2001/ASSET/IMAGES/LARGE/H10320751004.JPEG

130. M'Kaouar H, Péronnet F, Massicotte D, Lavoie C. Gender difference in the metabolic response to prolonged exercise with [13C]glucose ingestion. *Eur J Appl Physiol*. 2004;92(4-5):462-469. doi:10.1007/S00421-004-1122-5

131. Cano A, Ventura L, Martinez G, et al. Analysis of sex-based differences in energy substrate utilization during moderate-intensity aerobic exercise. *Eur J Appl Physiol*. 2022;122(1):29-70. doi:10.1007/S00421-021-04802-5/FIGURES/7

132. Bowler ALM, Whitfield J, Marshall L, Coffey VG, Burke LM, Cox GR. The Use of Continuous Glucose Monitors in Sport: Possible Applications and Considerations. *Int J Sport Nutr Exerc Metab*. 2023;33(2):121-132. doi:10.1123/IJSNEM.2022-0139

133. Seshadri DR, Rowbottom JR, Drummond C, Voos JE, Craker J. A review of wearable technology: Moving beyond the hype: From need through sensor implementation. In: *2016 8th Cairo*

International Biomedical Engineering Conference, CIBEC 2016. Institute of Electrical and Electronics Engineers Inc.; 2017:52-55. doi:10.1109/CIBEC.2016.7836118

134. Didyuk O, Econom N, Guardia A, Livingston K, Klueh U. Continuous Glucose Monitoring Devices: Past, Present, and Future Focus on the History and Evolution of Technological Innovation. *J Diabetes Sci Technol*. 2021;15(3):676. doi:10.1177/1932296819899394

135. Blum A. Freestyle Libre Glucose Monitoring System. *Clin Diabetes*. 2018;36(2):203. doi:10.2337/CD17-0130

136. Tang L, Chang SJ, Chen CJ, Liu JT. Non-invasive blood glucose monitoring technology: A review. *Sensors (Switzerland)*. 2020;20(23):1-32. doi:10.3390/s20236925

137. Maiorino MI, Signoriello S, Maio A, et al. Effects of continuous glucose monitoringonmetricsofglycemic control in diabetes: A Systematic Review with Meta-analysis of Randomized Controlled Trials. *Diabetes Care*. 2020;43(5):1146-1156. doi:10.2337/dc19-1459

138. Moser O, Riddell MC, Eckstein ML, et al. Glucose management for exercise using continuous glucose monitoring (CGM) and intermittently scanned CGM (isCGM) systems in type 1 diabetes: position statement of the European Association for the Study of Diabetes (EASD) and of the International Society for Pediatric and Adolescent Diabetes (ISPAD) endorsed by JDRF and supported by the American Diabetes Association (ADA). *Diabetologia 2020 63:12*. 2020;63(12):2501-2520. doi:10.1007/S00125-020-05263-9

139. Schubert-Olesen O, Kröger J, Siegmund T, Thurm U, Halle M. Continuous Glucose Monitoring and Physical Activity. *Int J Environ Res Public Health*. 2022;19(19). doi:10.3390/IJERPH191912296

140. Funtanilla VD, Candidate P, Caliendo T, Hilas O. Continuous Glucose Monitoring: A Review of Available Systems. *Pharmacy and Therapeutics*. 2019;44(9):550. Accessed August 8, 2021. /pmc/articles/PMC6705487/

141. Health Organization W. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF consultation. *World Health Organization*. Published online 2006:1-50.

142. Libre Sense Glucose Sport Biosensor | Real-Time Monitoring. Accessed April 23, 2023. https://www.libresense.abbott/en/home.html

143. Mean fasting blood glucose. Accessed August 2, 2021. https://www.who.int/data/gho/indicator-metadata-registry/imr-details/2380

144. Rayas MS, Salehi M. *Non-Diabetic Hypoglycemia*. MDText.com, Inc.; 2000. Accessed January 17, 2024. https://www.ncbi.nlm.nih.gov/books/NBK355894/

145. Röder P V., Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med*. 2016;48(3):e219. doi:10.1038/EMM.2016.6

146. Mouri Mi, Badireddy M. *Hyperglycemia*. StatPearls Publishing; 2024. Accessed April 28, 2023. http://www.ncbi.nlm.nih.gov/pubmed/31242164

147. Khan A, Pessin J. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia*. 2002;45(11):1475-1483. doi:10.1007/S00125-002-0974-7

148. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. *Avicenna J Med*. 2020;10(4):174. doi:10.4103/AJM.AJM_53_20

149. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 2011;34(Suppl 1):S62. doi:10.2337/DC11-S062

150. Barata DS, Adan LF, Netto EM, Ramalho AC. The Effect of the Menstrual Cycle on Glucose Control in Women With Type 1 Diabetes Evaluated Using a Continuous Glucose Monitoring System. *Diabetes Care*. 2013;36(5):e70. doi:10.2337/DC12-2248

151. Dey S, Dasgupta D, Roy S. Blood Glucose Levels at Two Different Phases of Menstrual Cycle: A Study on a Group of Bengali-speaking Hindu Ethnic Populations of West Bengal, India. *https://doi.org/101177/0972558X19835371*. 2019;19(1):55-63. doi:10.1177/0972558X19835371

152. Good to Know: Factors Affecting Blood Glucose. *Clinical Diabetes*. 2018;36(2):202-202. doi:10.2337/cd18-0012

153. Wolever TMS, Miller JB. Sugars and blood glucose control. In: *American Journal of Clinical Nutrition*. Vol 62. Am J Clin Nutr; 1995. doi:10.1093/ajcn/62.1.212S

154. Southgate DAT. Digestion and metabolism of sugars. In: *American Journal of Clinical Nutrition*. Vol 62. Am J Clin Nutr; 1995. doi:10.1093/ajcn/62.1.203S

155. Sherwin RS, Sacca L. Effect of epinephrine on glucose metabolism in humans: Contribution of the liver. *Am J Physiol Endocrinol Metab*. 1984;10(2). doi:10.1152/ajpendo.1984.247.2.e157

156. Hwang JL, Weiss RE. Steroid-induced diabetes: a clinical and molecular approach to understanding and treatment. *Diabetes Metab Res Rev*. 2014;30(2):96. doi:10.1002/DMRR.2486

157. Emanuele N V., Swade TF, Emanuele MA. Consequences of alcohol use in diabetics. *Alcohol Research and Health*. 1998;22(3):211-219.

158. 10 Surprising Things That Can Spike Your Blood Sugar | CDC. Accessed August 25, 2021. https://www.cdc.gov/diabetes/library/spotlights/blood-sugar.html

159. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. *Arch Intern Med*. 2004;164(19):2147-2155. doi:10.1001/ARCHINTE.164.19.2147

160. Klonoff DC, Nguyen KT, Xu NY, Gutierrez A, Espinoza JC, Vidmar AP. Use of Continuous Glucose Monitors by People Without Diabetes: An Idea Whose Time Has Come?

https://doi.org/101177/19322968221110830. Published online July 20, 2022. doi:10.1177/19322968221110830

161. Kulawiec DG, Zhou T, Knopp JL, Chase JG. Continuous glucose monitoring to measure metabolic impact and recovery in sub-elite endurance athletes. *Biomed Signal Process Control*. 2021;70:103059. doi:10.1016/J.BSPC.2021.103059

162. Samant PP, Prausnitz MR. Mechanisms of sampling interstitial fluid from skin using a microneedle patch. doi:10.1073/pnas.1716772115

163. Kulcu E, Tamada JA, Reach G, Potts RO, Lesho MJ. Physiological Differences Between Interstitial Glucose and Blood Glucose Measured in Human Subjects. *Diabetes Care*. 2003;26(8):2405-2409. doi:10.2337/DIACARE.26.8.2405

164. Hoss U, Budiman ES. Factory-Calibrated Continuous Glucose Sensors: The Science Behind the Technology. *Diabetes Technol Ther*. 2017;19(S2):S44-S50. doi:10.1089/DIA.2017.0025

165. Supersapiens Application Tutorial for the Abbott Libre Sense Glucose Sport Biosensor - YouTube. Accessed April 27, 2023. https://www.youtube.com/watch?v=BoKHfVVUmLs

166. Edelman S V, Argento NB, Pettus J, Hirsch IB. Clinical Implications of Real-time and Intermittently Scanned Continuous Glucose Monitoring. *Diabetes Care*. 2018;41. doi:10.2337/dc18-1150

167. Le HT, Harris NS, Estilong AJ, Olson A, Rice MJ. Blood glucose measurement in the intensive care unit: What is the best method? *J Diabetes Sci Technol*. 2013;7(2):489-499. doi:10.1177/193229681300700226

168. Heinemann L, Schoemaker M, Schmelzeisen-Redecker G, et al. Benefits and Limitations of MARD as a Performance Parameter for Continuous Glucose Monitoring in the Interstitial Space: *https://doi.org/101177/1932296819855670*. 2019;14(1):135-150. doi:10.1177/1932296819855670

169. Gross TM, Bode BW, Einhorn D, et al. Performance Evaluation of the MiniMed® Continuous Glucose Monitoring System During Patient Home Use. *https://home-liebertpub-com.ezproxy.waikato.ac.nz/dia*. 2004;2(1):49-56. doi:10.1089/152091500316737

170. Welsh JB, Gao P, Derdzinski M, et al. Accuracy, Utilization, and Effectiveness Comparisons of Different Continuous Glucose Monitoring Systems. *Diabetes Technol Ther*. 2019;21(3):128. doi:10.1089/DIA.2018.0374

171. Alva S, Bailey T, Brazg R, et al. Accuracy of a 14-Day Factory-Calibrated Continuous Glucose Monitoring System With Advanced Algorithm in Pediatric and Adult Population With Diabetes. *J Diabetes Sci Technol*. Published online 2020. doi:10.1177/1932296820958754

172. Approval Letter for DEN170088: Dexcom G6 Integrated Continuous Glucose Monitoring System. March 27, 2018. Accessed August 9, 2021. https://www.accessdata.fda.gov/cdrh_docs/pdf17/DEN170088.pdf

173. Pemberton JS, Wilmot EG, Barnard-Kelly K, et al. CGM accuracy: Contrasting CE marking with the governmental controls of the USA (FDA) and Australia (TGA): A narrative review. *Diabetes Obes Metab*. 2023;25(4):916-939. doi:10.1111/DOM.14962

174. Davey RJ, Low C, Jones TW, Fournier PA. Contribution of an Intrinsic Lag of Continuous Glucose Monitoring Systems to Differences in Measured and Actual Glucose Concentrations Changing at Variable Rates in Vitro. *J Diabetes Sci Technol*. 2010;4(6):1393. doi:10.1177/193229681000400614

175. Nakhleh A, Shehadeh N. Hypoglycemia in diabetes: An update on pathophysiology, treatment, and prevention. *World J Diabetes*. 2021;12(12):2036. doi:10.4239/WJD.V12.I12.2036

176. Nybo L. CNS fatigue and prolonged exercise: effect of glucose supplementation. *Med Sci Sports Exerc*. 2003;35(4):589-594. doi:10.1249/01.MSS.0000058433.85789.66

177. Brun J, Dumortier M, Fedou C, Mercier J. Exercise hypoglycemia in nondiabetic subjects. *Diabetes Metab*. 2001;27:92-106.

178. Clavel P, Tiollier E, Leduc C, Fabre M, Lacome M, Buchheit M. Concurrent Validity of a Continuous Glucose-Monitoring System at Rest and During and Following a High-Intensity Interval Training Session. *Int J Sports Physiol Perform*. 2022;17(4):627-633. doi:10.1123/IJSPP.2021-0222

179. Guillot FH, Jacobs PG, Wilson LM, et al. Accuracy of the Dexcom G6 Glucose Sensor during Aerobic, Resistance, and Interval Exercise in Adults with Type 1 Diabetes. *Biosensors (Basel)*. 2020;10(10). doi:10.3390/BIOS10100138

180. Tsoukas M, Rutkowski J, El-Fathi A, et al. Accuracy of FreeStyle Libre in Adults with Type 1 Diabetes: The Effect of Sensor Age. *Diabetes Technol Ther*. 2020;22(3):203-207. doi:10.1089/dia.2019.0262

181. Thomas F, Pretty CG, Desaive T, Chase JG. Blood Glucose Levels of Subelite Athletes during 6 Days of Free Living. *J Diabetes Sci Technol*. 2016;10(6):1335-1343. doi:10.1177/1932296816648344

182. Ishihara K, Inamura N, Tani A, et al. Contribution of solid food to achieve individual nutritional requirement during a continuous 438 km mountain ultramarathon in female athlete. *Int J Environ Res Public Health*. 2021;18(10). doi:10.3390/ijerph18105153

183. Sengoku Y, Nakamura K, Ogata H, Nabekura Y, Nagasaka S, Tokuyama K. Continuous glucose monitoring during a 100-km race: A case study in an elite ultramarathon runner. *Int J Sports Physiol Perform*. 2015;10(1):124-127. doi:10.1123/ijspp.2013-0493

184. Jeukendrup AE. Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. *Curr Opin Clin Nutr Metab Care*. 2010;13(4):452-457. doi:10.1097/MCO.0B013E328339DE9F

185. Jeukendrup AE. High-carbohydrate versus high-fat diets in endurance sports. *Schweizerische Zeitschrift fur Sportmedizin und Sporttraumatologie*. 2003;51(1):17-23.

186. De Oliveira EP, Burini RC. The impact of physical exercise on the gastrointestinal tract. *Curr Opin Clin Nutr Metab Care*. 2009;12(5):533-538. doi:10.1097/MCO.0B013E32832E6776

187. de Oliveira EP, Burini RC. Carbohydrate-dependent, exercise-induced gastrointestinal distress. *Nutrients*. 2014;6(10):4191-4199. doi:10.3390/nu6104191

188. Heikura IA, Stellingwerff T, Areta JL. Low energy availability in female athletes: From the lab to the field. *Eur J Sport Sci*. 2022;22(5):709-719. doi:10.1080/17461391.2021.1915391

189. Mountjoy M, Sundgot-Borgen JK, Burke LM, et al. IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *Br J Sports Med*. 2018;52:687-697. doi:10.1136/bjsports-2018-099193

190. Smith TJ, Wilson MA, Philip Karl J, et al. Interstitial glucose concentrations and hypoglycemia during 2 days of caloric deficit and sustained exercise: a double-blind, placebo-controlled trial. *J Appl Physiol (1985)*. 2016;121(5):1208-1216. doi:10.1152/JAPPLPHYSIOL.00432.2016

191. Sun Q, Li J, Gao F. New insights into insulin: The anti-inflammatory effect and its clinical relevance. *World J Diabetes*. 2014;5(2):89. doi:10.4239/WJD.V5.I2.89

192. Hevener AL, Ribas V, Moore TM, Zhou Z. The Impact of Skeletal Muscle ERα on Mitochondrial Function and Metabolic Health. *Endocrinology (United States)*. 2020;161(2):1-16. doi:10.1210/endocr/bqz017

193. Shi H, Seeley RJ, Clegg DJ. Sexual differences in the control of energy homeostasis. *Front Neuroendocrinol*. 2009;30(3):396-404. doi:10.1016/j.yfrne.2009.03.004

194. Frank A, Brown LM, Clegg DJ. The role of hypothalamic estrogen receptors in metabolic regulation. *Front Neuroendocrinol*. 2014;35(4):550-557. doi:10.1016/j.yfrne.2014.05.002

195. Hevener AL, Zhou Z, Drew BG, Ribas V. The role of skeletal muscle estrogen receptors in metabolic homeostasis and insulin sensitivity. In: *Advances in Experimental Medicine and Biology*. Vol 1043. Adv Exp Med Biol; 2017:257-284. doi:10.1007/978-3-319-70178-3_13

196. Hevener AL, Zhou Z, Moore TM, Drew BG, Ribas V. The impact of ERα action on muscle metabolism and insulin sensitivity – Strong enough for a man, made for a woman. *Mol Metab*. 2018;15:20-34. doi:10.1016/j.molmet.2018.06.013

197. Ropero AB, Alonso-Magdalena P, Quesada I, Nadal A. The role of estrogen receptors in the control of energy and glucose homeostasis. *Steroids*. 2008;73(9-10):874-879. doi:10.1016/j.steroids.2007.12.018

198. Hevener AL, Clegg DJ, Mauvais-Jarvis F. Impaired estrogen receptor action in the pathogenesis of the metabolic syndrome. *Mol Cell Endocrinol*. 2015;418(Pt 3):306-321. doi:10.1016/j.mce.2015.05.020

199. Chen JQ, Brown TR, Russo J. Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. *Biochim Biophys Acta Mol Cell Res*. 2009;1793(7):1128-1143. doi:10.1016/j.bbamcr.2009.03.009

200. Zhang S, Osumi H, Uchizawa A, et al. Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiol Rep*. 2020;8(2). doi:10.14814/phy2.14353

201. Benton MJ, Hutchins AM, Dawes JJ. Effect of menstrual cycle on resting metabolism: A systematic review and metaanalysis. *PLoS One*. 2020;15(7 July). doi:10.1371/journal.pone.0236025

202. Chapman AB, Zamudio S, Woodmansee W, et al. Systemic and renal hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy. *Am J Physiol Renal Physiol*. 1997;273(5 42-5). doi:10.1152/ajprenal.1997.273.5.f777

203. Szmuilowicz ED, Adler GK, Williams JS, et al. Relationship between aldosterone and progesterone in the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*. 2006;91(10):3981-3987. doi:10.1210/jc.2006-1154

204. Pechère-Bertschi A, Maillard M, Stalder H, Brunner HR, Burnier M. Renal segmental tubular response to salt during the normal menstrual cycle. *Kidney Int*. 2002;61(2):425-431. doi:10.1046/j.1523-1755.2002.00158.x

205. Olson BR, Forman MR, Lanza E, et al. Relation between Sodium Balance and Menstrual Cycle Symptoms in Normal Women. *Ann Intern Med*. 1996;125(7):564-567. doi:10.7326/0003-4819-125-7-199610010-00005

206. Landau RL, Lugibihl K. The effect of progesterone on amino acid metabolism. *J Clin Endocrinol Metab*. 1961;21(11):1355-1363. doi:10.1210/jcem-21-11-1355

207. Draper CF, Duisters K, Weger B, et al. Menstrual cycle rhythmicity: metabolic patterns in healthy women. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-32647-0

208. Faustmann G, Meinitzer A, Magnes C, et al. Progesterone-associated arginine decline at luteal phase of menstrual cycle and associations with related amino acids and nuclear factor kB activation. *PLoS One*. 2018;13(7). doi:10.1371/journal.pone.0200489

209. Kriengsinyos W, Wykes LJ, Goonewardene LA, Ball RO, Pencharz PB. Phase of menstrual cycle affects lysine requirement in healthy women. *Am J Physiol Endocrinol Metab*. 2004;287(3 50-3). doi:10.1152/ajpendo.00262.2003

210. Han HS, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective. *Exp Mol Med*. 2016;48(3):e218-e218. doi:10.1038/emm.2015.122

211. Elliott-Sale KJ, Minahan CL, de Jonge XAKJ, et al. Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. *Sports Medicine*. 2021;51(5):843-861. doi:10.1007/s40279-021-01435-8

212. Janse De Jonge XAK. Effects of the menstrual cycle on exercise performance. *Sports Medicine*. 2003;33(11):833-851. doi:10.2165/00007256-200333110-00004

213. Paul RW, Sonnier JH, Johnson EE, et al. Inequalities in the Evaluation of Male Versus Female Athletes in Sports Medicine Research: A Systematic Review. *Am J Sports Med*. 2023;51(12):3335-3342. doi:10.1177/03635465221131281

214. Deldicque L. Editorial: Women in elite sports and performance enhancement: 2021. *Front Sports Act Living*. 2022;4. doi:10.3389/fspor.2022.999969

215. Sims ST, Heather AK. Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Exp Physiol*. 2018;103(10):1309-1317. doi:10.1113/EP086797

216. Tarnopolsky LJ, MacDougall JD, Atkinson SA, Tarnopolsky MA, Sutton JR. Gender differences in substrate for endurance exercise. *J Appl Physiol*. 1990;68(1):302-308. doi:10.1152/jappl.1990.68.1.302

217. Tarnopolsky MA, Atkinson SA, Phillips SM, MacDougall JD. Carbohydrate loading and metabolism during exercise in men and women. *J Appl Physiol*. 1995;78(4):1360-1368. doi:10.1152/jappl.1995.78.4.1360

218. Horton TJ, Pagliassotti MJ, Hobbs K, Hill JO. Fuel metabolism in men and women during and after long-duration exercise. *J Appl Physiol*. 1998;85(5):1823-1832. doi:10.1152/jappl.1998.85.5.1823

219. Hackney AC. Influence of oestrogen on muscle glycogen utilization during exercise. *Acta Physiol Scand*. 1999;167(3):273-274. doi:10.1046/j.1365-201X.1999.00605.x

220. Zderic TW, Coggan AR, Ruby BC. Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *J Appl Physiol*. 2001;90(2):447-453. doi:10.1152/jappl.2001.90.2.447

221. Stannard SR, Buckley AJ, Edge JA, Thompson MW. Adaptations to skeletal muscle with endurance exercise training in the acutely fed versus overnight-fasted state. *J Sci Med Sport*. 2010;13(4):465-469. doi:10.1016/j.jsams.2010.03.002

222. Miotto PM, McGlory C, Holloway TM, Phillips SM, Holloway GP. Sex differences in mitochondrial respiratory function in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. 2018;314(6):R909-R915. doi:10.1152/AJPREGU.00025.2018

223. Fink JS. Female athletes, women's sport, and the sport media commercial complex: Have we really "come a long way, baby"? *Sport Management Review*. 2015;18(3):331-342. doi:10.1016/j.smr.2014.05.001

224. Schaumberg MA, Jenkins DG, Janse de Jonge XAK, Emmerton LM, Skinner TL. Three-step method for menstrual and oral contraceptive cycle verification. *J Sci Med Sport*. 2017;20(11):965-969. doi:10.1016/j.jsams.2016.08.013

225. Ansdell P, Thomas K, Hicks KM, Hunter SK, Howatson G, Goodall S. Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Exp Physiol*. 2020;105(12):2007-2021. doi:10.1113/EP088548

226. Oydanich M, Babici D, Zhang J, Rynecki N, Vatner DE, Vatner SF. Mechanisms of sex differences in exercise capacity. *Am J Physiol Regul Integr Comp Physiol*. 2019;316(6):R832-R838. doi:10.1152/ajpregu.00394.2018

227. Sims ST, Ware L, Capodilupo ER. Patterns of endogenous and exogenous ovarian hormone modulation on recovery metrics across the menstrual cycle. *BMJ Open Sport Exerc Med*. 2021;7(3). doi:10.1136/bmjsem-2021-001047

228. Fehring RJ, Schneider M, Raviele K. Variability in the phases of the menstrual cycle. *JOGNN - Journal of Obstetric, Gynecologic, and Neonatal Nursing*. 2006;35(3):376-384. doi:10.1111/j.1552-6909.2006.00051.x

229. Bruinvels G, Hackney AC, Pedlar CR. Menstrual Cycle: The Importance of Both the Phases and the Transitions Between Phases on Training and Performance. *Sports Medicine*. 2022;52(7):1457-1460. doi:10.1007/s40279-022-01691-2

230. Bruinvels G, Goldsmith E, Blagrove R, et al. Prevalence and frequency of menstrual cycle symptoms are associated with availability to train and compete: A study of 6812 exercising women recruited using the Strava exercise app. *Br J Sports Med*. 2021;55(8):438-443. doi:10.1136/bjsports-2020-102792

231. Hodgson TK, Braunstein GD. Physiological Effects of Androgens in Women. In: *Androgen Excess Disorders in Women*. Humana Press; 2007:49-62. doi:10.1007/978-1-59745-179-6_4

232. Farage MA, Neill S, MacLean AB. Physiological changes associated with the menstrual cycle a review. *Obstet Gynecol Surv*. 2009;64(1):58-72. doi:10.1097/OGX.0b013e3181932a37

233. de Jonge XJ, Thompson B, Ahreum HAN. Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise. *Med Sci Sports Exerc*. 2019;51(12):2610-2617. doi:10.1249/MSS.0000000000002073

234. Kendrick Z V., Ellis GS. Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. *J Appl Physiol*. 1991;71(5):1694-1699. doi:10.1152/jappl.1991.71.5.1694

235. Campbell SE, Angus DJ, Febbraio MA. Glucose kinetics and exercise performance during phases of the menstrual cycle: Effect of glucose ingestion. *Am J Physiol Endocrinol Metab*. 2001;281(4 44-4):817-825. doi:10.1152/ajpendo.2001.281.4.e817

236. Francis G, Keay N. Analysis of the variability in the timing of physiological events in the menstrual cycle. *Researchgate*. Published online March 31, 2023:1-13. doi:10.13140/RG.2.2.12049.51040

237. Casazza GA, Jacobs KA, Suh SH, Miller BF, Horning MA, Brooks GA. Menstrual cycle phase and oral contraceptive effects on triglyceride mobilization during exercise. *J Appl Physiol*. 2004;97(1):302-309. doi:10.1152/japplphysiol.00050.2004

238. Suh SH, Casazza GA, Horning MA, Miller BF, Brooks GA. Luteal and follicular glucose fluxes during rest and exercise in 3-h postabsorptive women. *J Appl Physiol (1985)*. 2002;93(1):42-50. doi:10.1152/JAPPLPHYSIOL.01080.2001

239. Boisseau N, Isacco L. Substrate metabolism during exercise: Sexual dimorphism and women's specificities. *Eur J Sport Sci*. 2022;22(5):672-683. doi:10.1080/17461391.2021.1943713

240. Wenner MM, Stachenfeld NS. Sex hormones and environmental factors affecting exercise. In: *Sex Hormones, Exercise and Women: Scientific and Clinical Aspects*. Springer International Publishing; 2016:151-170. doi:10.1007/978-3-319-44558-8_9

241. Stachenfeld NS. Sex Hormone Effects on Body Fluid Regulation. *Exerc Sport Sci Rev*. 2008;36(3):152. doi:10.1097/JES.0B013E31817BE928

242. Tollan A, Kvenild K, Strand H, Øian P, Maltau JM. Increased capillary permeability for plasma proteins in oral contraceptive users. *Contraception*. 1992;45(5):473-481. doi:10.1016/0010-7824(92)90160-U

243. Øian P, Tollan A, Fadnes HO, Noddeland H, Maltau JM. Transcapillary fluid dynamics during the menstrual cycle. *Am J Obstet Gynecol*. 1987;156(4):952-955. doi:10.1016/0002-9378(87)90364-4

244. Steinach M, Lichti J, Maggioni MA, Fähling M. A fluid shift for endurance exercise-Why hydration matters. *Acta Physiol (Oxf)*. 2019;227(1):e13347. doi:10.1111/apha.13347

245. Stachenfeld NS, Keefe DL. Estrogen effects on osmotic regulation of AVP and fluid balance. *Am J Physiol Endocrinol Metab*. 2002;283(4 46-4). doi:10.1152/ajpendo.00192.2002

246. McCracken M, Ainsworth B, Hackney AC. Effects of the menstrual cycle phase on the blood lactate responses to exercise. *Eur J Appl Physiol Occup Physiol*. 1994;69(2):174-175. doi:10.1007/BF00609412

247. Rodriguez-Giustiniani P, Rodriguez-Sanchez N, Galloway SDR. Fluid and electrolyte balance considerations for female athletes. *https://doi.org/101080/1746139120211939428*. 2021;22(5):697-708. doi:10.1080/17461391.2021.1939428

248. Sims ST, Rehrer NJ, Bell ML, Cotter JD. Preexercise sodium loading aids fluid balance and endurance for women exercising in the heat. *J Appl Physiol*. 2007;103(2):534-541. doi:10.1152/JAPPLPHYSIOL.01203.2006

249. Øian P, Tollan A, Fadnes HO, Noddeland H, Maltau JM. Transcapillary fluid dynamics during the menstrual cycle. *Am J Obstet Gynecol*. 1987;156(4):952-955. doi:10.1016/0002-9378(87)90364-4

250. Darwish A, Lui F. Physiology, Colloid Osmotic Pressure. *StatPearls*. Published online May 15, 2022. Accessed May 10, 2023. https://www.ncbi.nlm.nih.gov/books/NBK541067/

251. Retzlaff JA, Tauxe WN, Kiely JM, Stroebel CF. Erythrocyte Volume, Plasma Volume, and Lean Body Mass in Adult Men and Women. *Blood*. 1969;33(5):649-667. doi:10.1182/BLOOD.V33.5.649.649

252. Cable NT, Elliott KJ. The Influence of Reproductive Hormones on Muscle Strength. *http://dx.doi.org/101080/09291010412331335788*. 2010;35(3):235-244. doi:10.1080/09291010412331335788

253. McNulty KL, Elliott-Sale KJ, Dolan E, et al. The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports Med*. 2020;50(10):1813-1827. doi:10.1007/S40279-020-01319-3

254. Bull JR, Rowland SP, Scherwitzl EB, Scherwitzl R, Danielsson KG, Harper J. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ Digit Med*. 2019;2(1):83. doi:10.1038/s41746-019-0152-7

255. Dam TV, Dalgaard LB, Sevdalis V, et al. Muscle Performance during the Menstrual Cycle Correlates with Psychological Well-Being, but Not Fluctuations in Sex Hormones. *Med Sci Sports Exerc*. 2022;54(10):1678-1689. doi:10.1249/MSS.0000000000002961

256. De Souza MJ, Miller BE, Loucks AB, et al. High Frequency of Luteal Phase Deficiency and Anovulation in Recreational Women Runners: Blunted Elevation in Follicle-Stimulating Hormone Observed during Luteal-Follicular Transition. *J Clin Endocrinol Metab*. 1998;83(12):4220-4232. doi:10.1210/jcem.83.12.5334

257. Wideman L, Montgomery MM, Levine BJ, Beynnon BD, Shultz SJ. Accuracy of Calendar-Based Methods for Assigning Menstrual Cycle Phase in Women. *Sports Health*. 2013;5(2):143-149. doi:10.1177/1941738112469930

258. Stricker R, Eberhart R, Chevailler MC, Quinn FA, Bischof P, Stricker R. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT® analyzer. *Clin Chem Lab Med*. 2006;44(7):883-887. doi:10.1515/CCLM.2006.160

259. Kyei-Mensah A, Maconochie N, Zaidi J, Pittrof R, Campbell S, Seang Lin Tan. Transvaginal three-dimensional ultrasound: Reproducibility of ovarian and endometrial volume measurements. *Fertil Steril*. 1996;66(5):718-722. doi:10.1016/S0015-0282(16)58624-5

260. Su H, Yi Y, Wei T, Chang T, Cheng C. Detection of ovulation, a review of currently available methods. *Bioeng Transl Med*. 2017;2(3):238-246. doi:10.1002/btm2.10058

261. Ecochard R, Boehringer H, Rabilloud M, Marret H. Chronological aspects of ultrasonic, hormonal, and other indirect indices of ovulation. *BJOG*. 2001;108(8):822-829. doi:10.1111/j.1471-0528.2001.00194.x

262. Queenan JT, O'Brien GD, Bains LM, Simpson J, Collins WP, Campbell S. Ultrasound scanning of ovaries to detect ovulation in women. *Fertil Steril*. 1980;34(2):99-105. doi:10.1016/s0015-0282(16)44889-2

263. Sruthi Priya B, Pushpaja M, Siva kumar A V., Maruthy KN. Does the salivary fern pattern determine fertile period in reproductive female? *Clin Epidemiol Glob Health*. 2020;8(3):698-701. doi:10.1016/j.cegh.2020.01.005

264. Johnson S, Marriott L, Zinaman M. Can apps and calendar methods predict ovulation with accuracy? *Curr Med Res Opin*. 2018;34(9):1587-1594. doi:10.1080/03007995.2018.1475348

265. Barron ML, Fehring RJ. Basal body temperature assessment: Is it useful to couples seeking pregnancy? *MCN The American Journal of Maternal/Child Nursing*. 2005;30(5):290-298. doi:10.1097/00005721-200509000-00004

266. Berglund Scherwitzl E, Lindén Hirschberg A, Scherwitzl R. Identification and prediction of the fertile window using NaturalCycles. *European Journal of Contraception and Reproductive Health Care*. 2015;20(5):403-408. doi:10.3109/13625187.2014.988210

267. Killick S, Elstein M. Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. *Fertil Steril*. 1987;47(5):773-777. doi:10.1016/S0015-0282(16)59163-8

268. Blake KR, Dixson BJW, O'Dean SM, Denson TF. Standardized protocols for characterizing women's fertility: A data-driven approach. *Horm Behav*. 2016;81:74-83. doi:10.1016/j.yhbeh.2016.03.004

269. Guida M, Tommaselli GA, Palomba S, et al. Efficacy of methods for determining ovulation in a natural family planning program. *Fertil Steril*. 1999;72(5):900-904. doi:10.1016/S0015-0282(99)00365-9

270. Najmabadi S, Schliep KC, Simonsen SE, Porucznik CA, Egger MJ, Stanford JB. Cervical mucus patterns and the fertile window in women without known subfertility: a pooled analysis of three cohorts. *Human Reproduction*. 2021;36(7):1784-1795. doi:10.1093/HUMREP/DEAB049

271. Rogan MM, Black KE. Dietary energy intake across the menstrual cycle: a narrative review. *Nutr Rev*. 2023;81(7):869-886. doi:10.1093/nutrit/nuac094

272. Williams JS, Stone JC, Masood Z, Bostad W, Gibala MJ, MacDonald MJ. The impact of natural menstrual cycle and oral contraceptive pill phase on substrate oxidation during rest and acute submaximal aerobic exercise. *J Appl Physiol*. 2023;135(3):642-654. doi:10.1152/JAPPLPHYSIOL.00111.2023/ASSET/IMAGES/LARGE/JAPPLPHYSIOL.00111.2023_F003.JPEG

273. Hébert-Losier K, Finlayson SJ, Driller MW, Dubois B, Esculier JF, Beaven CM. Metabolic and performance responses of male runners wearing 3 types of footwear: Nike Vaporfly 4%, Saucony Endorphin racing flats, and their own shoes. *J Sport Health Sci*. 2022;11(3):275-284. doi:10.1016/J.JSHS.2020.11.012

274. Goodwin ML. Blood glucose regulation during prolonged, submaximal, continuous exercise: a guide for clinicians. *J Diabetes Sci Technol*. 2010;4(3):694-705. doi:10.1177/193229681000400325

275. Cengiz E, Tamborlane W V. A Tale of Two Compartments: Interstitial Versus Blood Glucose Monitoring. *Diabetes Technol Ther*. 2009;11(Suppl 1):S-11. doi:10.1089/DIA.2009.0002

276. Yousefi A, Etemad Z, Saed L, Aliakbar A, Soleimany F. Response of Growth Hormones and Cortisol to One Session of Moderate-Intensity Endurance Exercise in Patients with Type 2 Diabetes: A Quasi-experimental Study. *Med J Islam Repub Iran*. 2022;36(1). doi:10.47176/MJIRI.36.50

277. Lin G, Siddiqui R, Lin Z, et al. Blood glucose variance measured by continuous glucose monitors across the menstrual cycle. *NPJ Digit Med*. 2023;6(1). doi:10.1038/S41746-023-00884-X

278. Hummel J, Benkendorff C, Fritsche L, et al. Brain insulin action on peripheral insulin sensitivity in women depends on menstrual cycle phase. *Nature Metabolism 2023 5:9*. 2023;5(9):1475-1482. doi:10.1038/s42255-023-00869-w

279. Flockhart M, Nilsson LC, Ekblom B, Larsen FJ. A Simple Model for Diagnosis of Maladaptations to Exercise Training. *Sports Med Open*. 2022;8(1). doi:10.1186/S40798-022-00523-X

280. Coates AM, Thompson KMA, Grigore MM, et al. Altered carbohydrate oxidation during exercise in overreached endurance athletes is applicable to training monitoring with continuous glucose monitors. *Scand J Med Sci Sports*. 2024;34(1):e14551. doi:10.1111/SMS.14551

281. Maher AC, Akhtar M, Vockley J, Tarnopolsky MA. Women Have Higher Protein Content of β-Oxidation Enzymes in Skeletal Muscle than Men. *PLoS One*. 2010;5(8):e12025. doi:10.1371/JOURNAL.PONE.0012025

282. Dole A, Beaven M, Sims ST. Menstrual Cycle Tracking in Sports Research: Challenges, Progress, and Future Directions. *Physiologia 2023, Vol 3, Pages 598-610*. 2023;3(4):598-610. doi:10.3390/PHYSIOLOGIA3040044

283. JT G, CJ F, JA B, LJ van L. Glucose Plus Fructose Ingestion for Post-Exercise Recovery-Greater than the Sum of Its Parts? *Nutrients*. 2017;9(4). doi:10.3390/NU9040344

284. Qamar MI, Read AE. Effects of exercise on mesenteric blood flow in man. *Gut*. 1987;28(5):583-587. doi:10.1136/GUT.28.5.583

285. Yeung EH, Zhang C, Mumford SL, et al. Longitudinal Study of Insulin Resistance and Sex Hormones over the Menstrual Cycle: The BioCycle Study. *J Clin Endocrinol Metab*. 2010;95(12):5435. doi:10.1210/JC.2010-0702

286. Chenevière X, Borrani F, Sangsue D, Gojanovic B, Malatesta D. Gender differences in whole-body fat oxidation kinetics during exercise. *https://doi.org/101139/H10-086*. 2011;36(1):88-95. doi:10.1139/H10-086

287. Zinker BA, Britz K, Brooks GA. Effects of a 36-hour fast on human endurance and substrate utilization. *J Appl Physiol (1985)*. 1990;69(5):1849-1855. doi:10.1152/jappl.1990.69.5.1849

288. Sparks LM, Pasarica M, Sereda O, et al. Effect of adipose tissue on the sexual dimorphism in metabolic flexibility. *Metabolism*. 2009;58(11):1564-1571. doi:10.1016/J.METABOL.2009.05.008

289. Kojima C, Ishibashi A, Ebi K, Goto K. Exogenous glucose oxidation during endurance exercise under low energy availability. *PLoS One*. 2022;17(10). doi:10.1371/JOURNAL.PONE.0276002

290. Sims ST, Heather AK. Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Exp Physiol*. 2018;103(10):1309-1317. doi:10.1113/EP086797

Appendices

10.7 Ethical Approval

The University of Waikato
Private Bag 3105
Gate 1, Knighton Road
Hamilton, New Zealand

Human Research Ethics Committee
Roger Moltzen
Telephone: +64021658119
Email:humanethics@waikato.ac.nz

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

3 November 2021

Andrew Dole
School of Health
DHECS
By email: ad194@students.waikato.ac.nz

Dear Andrew

HREC(Health)2021#73 : Continuous Glucose Monitoring in Healthy Female Ultra Endurance Athletes

Thank you for your responses to the Committee feedback.

We are now pleased to provide formal approval for your project.

Please contact the Committee by email (humanethics@waikato.ac.nz) if you wish to make changes to your project as it unfolds, quoting your application number with your future correspondence. Any minor changes or additions to the approved research activities can be handled outside the monthly application cycle.

We wish you all the best with your research.

Regards,

Emeritus Professor Roger Moltzen MNZM
Chairperson
University of Waikato Human Research Ethics Committee

10.8 Co-authorship forms

THE UNIVERSITY OF WAIKATO

Co-Authorship Form

School of Graduate Research
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 5096
Email: SGR@waikato.ac.nz
Website: http://www.waikato.ac.nz/students/research-degree

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Dole A, Beaven M, Sims ST. Menstrual Cycle Tracking in Sports Research: Challenges, Progress, and Future Directions. Physiologia. 2023; 3(4):598-610. https://doi.org/10.3390/physiologia3040044

Nature of contribution by PhD candidate	Participant recruitment, study design, data collection & analysis, manuscript preparation, journal submission
Extent of contribution by PhD candidate (%)	85%

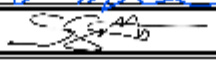
CO-AUTHORS

Name	Nature of Contribution
Beaven,M	manuscript revision
Sims S	supervision, manuscript revision

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Martyn Beaven		16/5/2024
Stacy Sims		21/05/2024

July 2015

THE UNIVERSITY OF WAIKATO

Co-Authorship Form

School of Graduate Research
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 5096
Email: SGR@waikato.ac.nz
Website: http://www.waikato.ac.nz/students/research-degree

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Dole A, Sims ST, Gan H, Beaven M. Continuous Glucose Monitor Underreports Blood Glucose During a Simulated Ultra Endurance Run in Eumenorrheic Female Runners. International Journal of Sports Physiology and Performance 2024.

Nature of contribution by PhD candidate	Research question, participant recruitment, data collection, data analysis & interpretation, manuscript preparation, manuscript submission
Extent of contribution by PhD candidate (%)	80%

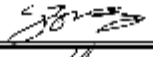
CO-AUTHORS

Name	Nature of Contribution
Sims, ST	Supervision, research question development, revision of manuscript
Gan, H	Statistical modelling and data interpretation
Beaven, M	Mansuscript revision, supervision, data analysis
Gill, N	Manuscript revision

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Stacy Sims		21/05/2024
Han Gan		24/05/2024
Martyn Beaven		18/5/2024
Nicholas Gill		25/5/24

July 2015

THE UNIVERSITY OF WAIKATO

Co-Authorship Form

School of Graduate Research
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 5096
Email: SGR@waikato.ac.nz
Website: http://www.waikato.ac.nz/students/research-degree

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Dole A, Sims ST, Beaven M. Fueling In Flux: Blood Glucose Responses and Menstrual Phase Interactions In Female Runners During a Simulated Ultra Run.Journal of Sports Sciences 2024.

Nature of contribution by PhD candidate	Research question, participant recruitment, data collection, data analysis & interpretation, manuscript preparation, manuscript submission
Extent of contribution by PhD candidate (%)	85%

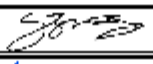
CO-AUTHORS

Name	Nature of Contribution
Sims, ST	Supervision, research question development, revision of manuscript
Beaven, M	Supervision, data analysis, revision of manuscript

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Stacy Sims		21/05/2024
Martyn Beaven		16/5/2024

July 2015

THE UNIVERSITY OF WAIKATO

Co-Authorship Form

School of Graduate Research
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 5096
Email: SGR@waikato.ac.nz
Website: http://www.waikato.ac.nz/students/research-degree

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.	
Nature of contribution by PhD candidate	Research question, participant recruitment, data collection, statistical modeling, data analysis & interpretation, manuscript preparation, manuscript submission
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

Name	Nature of Contribution
Stacy Sims	Supervision, research question development, revision of manuscript
Martyn Beaven	Supervision, data analysis, revision of manuscript

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Stacy Sims		21/05/2024
Martyn Beaven		20/5/2024

July 2015