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Menstrual Phase Differences in Muscle Deoxygenation, Respiration and Blood Lactate Concentration Do Not Influence Exercise Performance

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Abstract

The purpose was to evaluate muscle deoxygenation (Δ[HHb]), oxygen uptake (VO_2), ventilation (V_E), and arterialized blood lactate [La^-] throughout exhaustive incremental (RAMP) and severe-intensity exercise (SI) during follicular (FOL) and luteal (LUT) menstrual phases. During RAMP, Δ[HHb]/VO_2 was 65% lower in LUT below gas exchange threshold ($p<0.05$), without changes in VO_2, V_E and Δ[HHb] ($p>0.05$). During SI, Δ[HHb]/VO_2 was 18% lower in LUT during the kinetic phase ($p<0.05$), V_E was higher (FOL: 83 ± 19 $L \cdot min^{-1}$; LUT: 89 ± 17 $L \cdot min^{-1}$) and post-SI [La^-] were lower (FOL: 12.9 ± 2.5 $mmol \cdot L^{-1}$; LUT: 11.7 ± 1.7 $mmol \cdot L^{-1}$) ($p<0.05$), without differences in VO_2 and Δ[HHb] ($p>0.05$). No FOL/LUT differences in RAMP-performance (FOL: 218 ± 35 W; LUT: 221 ± 29 W) or SI-endurance (FOL: 99 ± 20 s; LUT: 96 ± 15 s) ($p>0.05$) were found. These data demonstrate that physiological differences between menstrual phases exist, however, performance is unaffected.

Keywords

Muscle deoxygenation, near infrared spectroscopy, breath-by-breath pulmonary O_2 uptake, gas exchange, ramp incremental exercise, heavy intensity exercise, menstrual cycle

Co-Authorship Statement

This study was designed by Dr. G. R. Belfry and J. L. Smith with input from the advisory committee (Dr. M. Mottola). All data was collected and analyzed by J. L. Smith with the assistance of M. J. Hurtubise. J. L. Smith wrote the original manuscript for the study.

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I dedicate my thesis to my grandmothers, who always encouraged and supported me to follow my dreams and smile through the process.

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Δ[HHb] responses were then normalized from 0% to 100%, such that 0% represented the start of the tests and 100% represented test cessation. * LUT phase is significantly different from FOL ($p<0.05$). 37

List of Abbreviations

ATP – Adenosine triphosphate

A.u. – Arbitrary units

BBT – Basal body temperature

B_F – Breathing frequency

Breaths/min – Breaths per minute

CO_2 – Carbon Dioxide

FOL – Early follicular phase

GET – Gas exchange threshold

H^+ – Hydrogen Ion

[HHb] – Deoxygenated hemoglobin concentration

Δ[HHb] – Change in deoxygenated hemoglobin concentration

$\Delta HHb/\Delta VO_{2p}$ – Adjustment of the normalized [HHb]-to-VO_{2p} ratio

$[La^-]$ – Arterialized capillary blood lactate concentration

$L \cdot min^{-1}$ – Litres per minute

LUT – Mid-luteal phase

m – Meter

MC – Menstrual Cycle

min – Minute

mmHg – Millimeters of mercury

$mmol \cdot L^{-1}$ – Millimole per litre

ms – Millisecond

N_2 – Nitrogen

NIRS – Near-infrared spectroscopy

O_2 – Oxygen

O_2Sat – Oxygen saturation

PAP – Peak aerobic power

$P_{ET}CO_2$ – End-tidal partial pressure of carbon dioxide

$P_{ET}O_2$ – End-tidal partial pressure of oxygen

PO – Power output

PO_{SI} – Power output during severe-intensity step exercise

RAMP – Ramp incremental exercise test

RER – Respiratory exchange ratio

RPM – Revolutions per minute

s – Second

SD – Standard deviation

SI – Severe-intensity step exercise

[THb] – Total hemoglobin concentration

TTF – Time-to-fatigue

V_E – Minute ventilation

$VO_{2\Delta 50}$ – Oxygen uptake at 50% of the difference between VO_{2GET} and VO_{2peak}

VO_{2GET} – Oxygen uptake at estimated gas exchange threshold

VO_{2max} – Maximal oxygen uptake

VO_{2p} – Pulmonary oxygen uptake

VO_{2peak} – Peak pulmonary oxygen uptake

W – Watts

WR – Work rate

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Chapter 1

1 « Review of Literature »

Eumenorrheic females experience cyclic fluctuations of ovarian hormones estrogen and progesterone every 20 - 45 days through the naturally occurring menstrual cycle (Lebrun, Joyce, & Constantini, 2013). Amongst reproductive functions, these hormonal fluctuations have been shown to effect both cardiorespiratory and metabolic responses to exercise which might influence overall performance. As such, this day to day variability of female hormones has led to an under-representation of female participants compared to males in sport and exercise studies. For example, in a review by Bruinvels et al. (2017) of 1382 exercise studies 2011 to 2013, it was found that female participants were involved in only 39% of studies (Bruinvels et al., 2017). Further, studies that did include female participants often only tested women in the early-follicular phase of the menstrual cycle when ovarian hormones estrogen and progesterone are known to be in lowest concentration, in an attempt to minimize any possible hormonal effects. Although this research is beneficial in understanding sex differences within exercise response, it leaves many unanswered questions regarding the influence of female hormones on the physiological and performance response to exercise throughout the female menstrual cycle. Finally, menstrual cycle (MC) exercise research that exists in the literature lacks methodological consistency pertaining to menstrual phase verification and the timing of testing throughout the MC, and as such, presents equivocal results (Bruinvels et al., 2017). The present study aims to determine the physiological and performance effects of ovarian hormones during exhaustive ramp incremental and subsequent severe-intensity

step exercise in the early-follicular (FOL) and mid-luteal (LUT) menstrual phases, when hormones are known to be at the lowest and highest concentrations, respectively.

This chapter will review the background literature of menstrual phase exercise research pertaining to ramp incremental exercise and severe-intensity constant load exercise. Specifically, the physiology of the menstrual cycle, aerobic capacity and performance, cardiovascular function, and substrate metabolism will be discussed to underpin the rationale for the study. Thereafter, an overview of equipment and cardiorespiratory and metabolic data collection processes, as well as the relevance to this research study, will be presented.

1.1 « The Female Menstrual Cycle »

Through the years of adolescence and puberty, across the reproductive years to post-menopause, a woman's physiology changes substantially. During reproductive years, ovarian hormones estrogen (Est) and progesterone (Pro) fluctuate 2-10-fold throughout the ~ 28-day MC to support reproductive function of eumenorrheic females (Lebrun et al., 2013). The physiological differences pertaining to oxygen uptake, blood lactate, and ventilation associated with menstrual phase hormonal variability suggests that studying females adds a complexity female research that is not observed with research on males. Exercise performance is a multifaceted entity – there is an intricate interaction of cardiovascular, respiratory and metabolic variables that enable the body to exercise and perform successfully, that is further complicated by endocrinological factors. This section will review the physiology of the menstrual cycle, followed by the known effects of the menstrual phase on aerobic capacity and performance, cardiorespiratory function, and substrate metabolism.

1.1.1 « Physiology of the Menstrual Cycle »

The MC lasts ~ 28 days (range 21 – 45 days) in eumenorrheic women and is divided into three main components, known as the follicular, ovulatory, and luteal phases, each with a unique hormonal profile based on physiological changes that occur throughout the cycle, as shown in *Figure 1*.

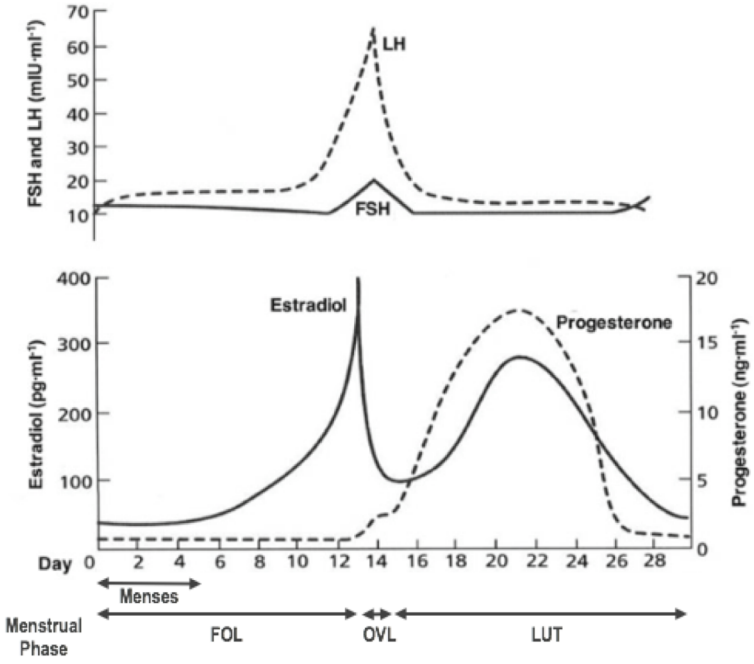


Figure 1. Ovarian and pituitary hormone concentrations across the female menstrual cycle. (FSH) Follicle stimulating hormone; (LH) Luteinizing hormone; (FOL) Follicular; (OVL) Ovulatory; (LUT) Luteal; (mIU•mL^{-1}) Milli-international units per millilitre; (pg•mL^{-1}) Pictograms per millilitre; (ng•mL^{-1}) Nanograms per millilitre. Figure modified from (Lebrun et al., 2013).

Briefly, the follicular phase begins on cycle Day 1, marked by the onset of menses, and is accompanied by low concentrations of Est and Pro. In the late follicular

phase, [Est] increases, stimulating a surge in luteinizing hormone (LH), which releases the follicle (egg) from the ovaries on ~ Day 14, known as ovulation. After ovulation occurs, the released follicle is known as the corpus luteum, which secretes Est and Pro in a parabolic function throughout the luteal phase (Day 14 – 28), reaching peak concentrations on ~ Day 21 (Lebrun et al., 2013). The present study tests participants in the early-follicular (FOL; ~ Day 2 - 3) and the mid-luteal (LUT; ~ Day 20 - 22) phases.

1.1.2 « Aerobic Capacity, Performance and the Menstrual Cycle »

Apart from results of one study (Lebrun, McKenzie, Prior, & Taunton, 1995), aerobic capacity as measured by maximal oxygen uptake (VO_{2max}) is not different across the MC (Bonen et al., 1983; De Souza, Maguire, Rubin, & Maresh, 1990; Dombovy, Bonekat, Williams, & Staats, 1987; Jurkowski, Jones, Walker, Younglai, & Sutton, 1978; Redman, Scroop, & Norman, 2003; Schoene, Robertson, Pierson, & Peterson, 1981). Lebrun et al. (1995) found that trained, eumenorrheic women had lower absolute VO_{2max} in the LUT phase compared to FOL, without any differences in maximal endurance time-to-fatigue (TTF). In contrast, there are studies that have shown menstrual phase performance differences in endurance TTF despite no changes in VO_{2max} (Jurkowski, Jones, Toews, & Sutton, 1981; Schoene et al., 1981), however, results are equivocal. Jurkowski et al. (1981) found that women exercising in the LUT phase at 90% of VO_{2max} had a greater ventilation (V_E), lower lactate ($[La^-]$) production, and greater TTF compared to the FOL phase, where Schoene et al. (1981) found shorter TTF in the LUT phase compared to FOL, despite the same changes in V_E and $[La^-]$ as presented by Jurkowski et al. (1981).

Moreover, oxygen uptake during submaximal exercise has presented differences between FOL and LUT phases. Redman et al. (2003) studied 14 sedentary females at

25% and 75% of VO_{2peak} and found that [La^-], carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were lower in the LUT phase, while VO_2 was higher. Hackney et al. (1994) also found that females exercising at 35% and 60% of VO_{2max} had lower RER in the LUT phase compared to FOL phase without changes in VO_2, VCO_2, or [La^-] (Hackney, McCracken, & Ainsworth, 1994). Hackney et al. (2000) studied women with high and low exogenous ovarian hormone concentrations (similar to the LUT and FOL phase of the MC, respectively) during prolonged exercise at 65% of VO_{2max}, and observed mean VO_2 during submaximal exercise that was trending towards being higher in the LUT phase compared to FOL ($p=0.07$; Cohen's $d = 0.5$) (Hackney, Muoio, & Meyer, 2000). These results directly oppose findings from Lebrun et al. (1995), who found that oxygen uptake was lower in the LUT phase compared to FOL. Others have reported no differences in oxygen uptake during sub-maximal exercise (Gurd, Scheid, Paterson, & Kowalchuk, 2007). As evidenced by these ambivalent results, the literature available regarding the effect of menstrual phase on oxygen uptake and endurance performance are equivocal.

1.1.3 « Cardiorespiratory Function »

The known effects of Est and Pro on basal body temperature (BBT), ventilation, vasodilation, blood flow, pulmonary diffusion capacity, and total hemoglobin concentration have been shown to affect various physiological parameters during exercise within each phase of the MC.

For example, high [Pro] at the onset of the LUT phase instigates an increase in basal body temperature (BBT) of 0.3-0.5 ^{0}C that persists until the beginning of the subsequent cycle (Lebrun et al., 1995; Marshall, 1963; Schaumberg, Jenkins, Janse de Jonge, Emmerton, & Skinner, 2017; Stephenson & Kolka, 1993), causing a rightward shift of the oxyhemoglobin dissociation curve and subsequent reduction in oxygen affinity to hemoglobin and enhanced O_2 delivery during the LUT phase (Lebrun et al., 2013). LUT phase increases in chemoreceptor sensitivity to CO_2 resulting in increases of resting and exercising V_E have also been reported (Schoene et al., 1981). This increased ventilation facilitates buffering of [H^+] removal via the carbonic anhydrase reaction and increases pH, thus eliciting a left-ward shift in the oxyhemoglobin dissociation curve, promoting oxygen affinity to hemoglobin (Dombovy et al., 1987). In terms of actual oxygen utilization, the LUT phase rise in BBT enhances O_2 delivery, while the concomitant increase in V_E promotes CO_2 elimination, most likely leading to no net effect compared to FOL phase (Lebrun et al., 2013). Moreover, oxygen carrying capacity of the blood might be affected during menstruation (the early FOL phase). Most females discharge ~ 25 – 65 mL of blood during menses, however, heavy blood loss can exceed 200 mL in some women, leading to anemia and reduced O_2 carrying capacity in the FOL phase compared to LUT (Reilly, 2010). In contrast, there have also been reports of no differences in total hemoglobin concentration across the MC (Smith, Brown, Murphy, & Harms, 2015).

Furthermore, high [Est] has been reported to enhance endothelium-dependent vasodilation in coronary and peripheral vessels of post-menopausal women (Gerhard et al., 1998; Gilligan, Quyyumi, Cannon, Johnson, & Schenke, 1994). As [Est] is high in the

LUT phase of the MC, these studies suggest that blood flow would also be enhanced in the large conductance arteries and the smaller microvascular resistance arteries, promoting O_2 delivery. Farha et al. (2007) also found a LUT phase improvement in O_2 delivery, however, this improvement was not due to changes in vasodilation, but instead a temporary LUT phase pulmonary angiogenesis that resulted in increased surface area for diffusion, eliciting an improvement in microvascular blood flow distribution (Farha et al., 2007). Further, Smith et al. (2015) have also shown LUT-phase improvements in pulmonary diffusion capacity (Smith et al., 2015), however, such changes did not impact performance.

Despite the apparent cardiovascular benefits in the LUT phase of the MC reviewed in this section, including improved oxygen delivery via increased ventilation, vasodilation, and pulmonary diffusion capacity, many studies show no performance differences between menstrual phases. The next section will review menstrual phase differences in substrate metabolism and discuss possible implications to performance across the MC.

1.1.4 « Substrate Metabolism »

There is evidence to suggest high concentrations of Est and Pro in the LUT phase of the MC shift substrate metabolism to favour lipids during sub-maximal exercise. Lower [La^-] have been reported in the LUT phase compared to FOL phase (Jurkowski et al., 1981; McCracken, Ainsworth, & Hackney, 1994), attributed to estrogen-mediated preferential lipolysis and glycogen sparing mechanisms (Bunt, 1990; Hackney et al., 1994) through the upregulation of AMPK+ activity and enhanced entry of free fatty acids into beta-oxidation (D'Eon et al., 2005). Other authours have reported a reduction in RER in the

LUT phase compared to FOL during prolonged sub-maximal exercise, indicating greater energy contribution from fat metabolism (Dombovy et al., 1987; Hackney, 1999; Hackney et al., 1994; Hackney et al., 2000; Redman et al., 2003). It is, however, important to note that if glucose is ingested prior-to or during exercise, menstrual phase differences in substrate oxidation do not persist (Campbell, Angus, & Febbraio, 2001). Exercise in a fasted state elicits menstrual phase differences in substrate metabolism, particularly an increase in LUT phase lipolysis during long duration, sub-maximal exercise bouts (Bonen et al., 1983).

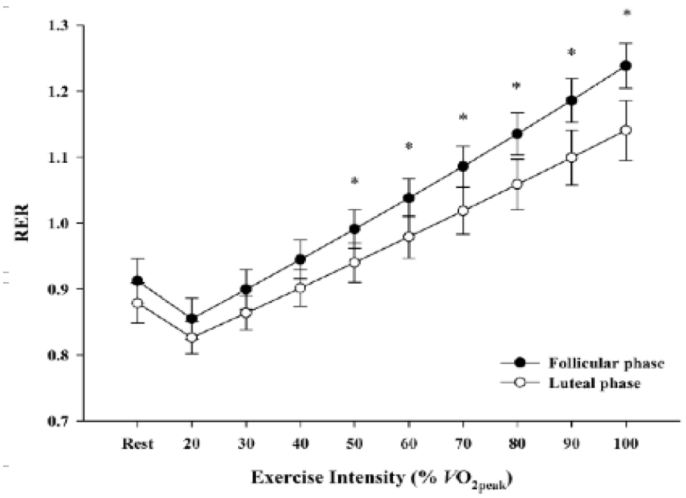


Figure 2. Effect of menstrual phase on respiratory exchange ratio (RER) during incremental exercise to exhaustion in sedentary women. (*Closed circles* Follicular phase, *open circles* LUT phase.) Values are mean (SE). *Significantly different from follicular phase, P<0.05). (Figure by Redman et. al, 2003).

1.2 « Ramp Incremental Exercise »

Maximum oxygen uptake (VO_{2max}) is achieved during exhaustive exercise using large muscle groups (running, cycling, swimming, etc.), providing an indication of the true limits of the O_2 transport/utilization systems (Poole & Jones, 2017). Ramp incremental tests begin at a light intensity baseline and increase to supra-maximal workloads via linear increase in work rate. During 20 $W \bullet min^{-1}$ ramp incremental exercise, oxygen uptake increases until VO_{2max} is attained. The gold standard for VO_{2max} achievement is a plateau in oxygen uptake despite the continual increase in work rate, however, this is not seen on every on every occasion. VO_{2max} testing is widely used in research and clinical laboratories, as well as sports and exercise facilities, as a determinant of maximal aerobic capacity.

The present study required participants to perform one ramp incremental exercise test (20 $W \bullet min^{-1}$) to exhaustion within each menstrual phase, providing an assessment of the integrated functioning of the cardiorespiratory and metabolic systems, including the uptake, transport and utilization of O_2 within the mitochondria of the contracting muscle. This section will review the different energy system contributions throughout a ramp incremental exercise test and the physiology involved in the determination of gas exchange threshold.

1.2.1 « Energy Systems »

A ramp incremental exercise test requires close integration of both glycolytic energy systems and aerobic metabolism (oxidative phosphorylation) to breakdown and utilize carbohydrates and fats and fulfil the adenosine triphosphate (ATP) requirements at the

muscle (Baker, McCormick, & Robergs, 2010). While assessing energy system contribution during incremental exercise, Damasceno et al. (2015) determined that the anaerobic/aerobic percent contribution to be ~ 94 / 6 % at the beginning and ~ 83 / 17 % at the end of the test, respectively (Damasceno, Pasqua, Lima-Silva, & Bertuzzi, 2015) (*Figure 2* created by (Bertuzzi, 2013)).

Glycolysis is the process of converting glucose (or glycogen) to pyruvate in the cytosol of the cell. Anaerobic glycolysis produces lactate from pyruvate, a process that generates a rapid, however limited, amount of ATP. Aerobic glycolysis occurs when pyruvate is transported to the mitochondria of the cell, converted to Acetyl-CoA, and enters the citric acid cycle. The citric acid cycle generates both ATP as well as electrons for passage to the electron transport chain (ETC) via high energy carriers NAD+ and FAD. An electrochemical proton gradient is established via the translocation of hydrogen ions [H^+] from the matrix to the intermembrane space within the mitochondria which drives ADP phosphorylation and ultimately substantial ATP generation via ATP synthase. This is an aerobic process, as the final reaction requires the reduction of molecules of oxygen and the formation of water (H_2O). Lipid (fatty acid) oxidation is the most plentiful source of potential energy that exists in the body and is very important during aerobic metabolism during longer duration steady state exercise (Baker et al., 2010). Following the breakdown of a triglyceride into three fatty acids and a glycerol, the fatty acids are transported into the mitochondrial matrix where they undergo ß-oxidation to generate Acetyl-CoA, which is further metabolized in the citric acid cycle and electron transport chain to generate high energy carriers and ultimately ATP (Katch, McArdle, & Katch, 2011).

During exercise, there is an increase in ATP demand and thus, an increase in O_2 utilization at the muscle, resulting in reduced O_2 partial pressure (PO_2) at the muscle (Whipp & Davis, 1979). A reduction in muscular PO_2 stimulates O_2 offloading of the myoglobin, as well as O_2 offloading from hemoglobin in the surrounding microvasculature, resulting in a higher concentration of deoxygenated hemoglobin ([HHb]) to maintain O_2 delivery (Grassi, Quaresima, Marconi, Ferrari, & Cerretelli, 1999). During ramp incremental exercise, [HHb] increases in a linear fashion until respiratory compensation threshold, where there is an evident plateau (Murias, Keir, Spencer, & Paterson, 2013). Exercising above respiratory compensation threshold results in hyperventilation and insufficient time for CO_2 removal, ultimately resulting in fatigue and exercise cessation (Baker et al., 2010).

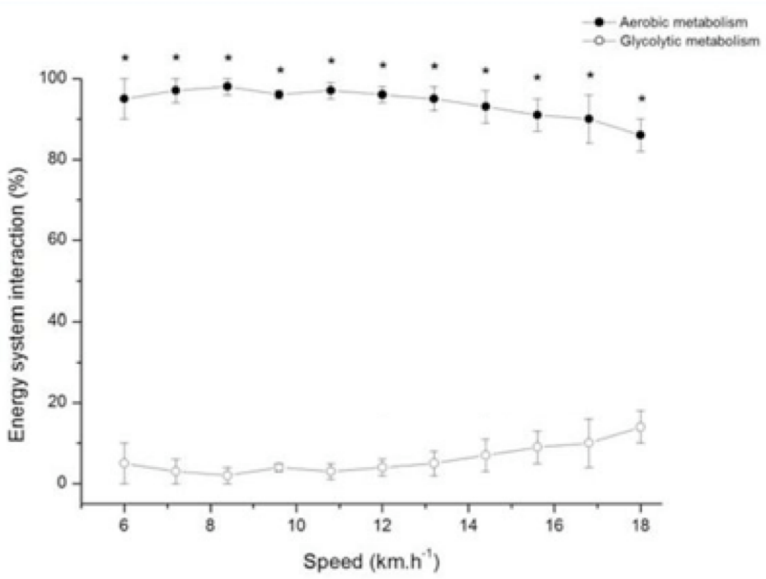
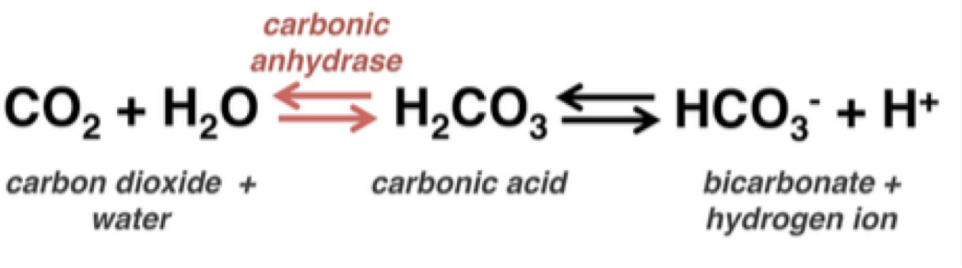


Figure 3. Energy system contributions during incremental exercise test expressed as a percentage of the total metabolic demand. Mean of n=10 (± SD). Figure modified from original by (Bertuzzi, Nascimento, Urso, Damasceno, & Lima-Silva, 2013).

1.2.2 « Gas Exchange Threshold »

Exercise above gas exchange threshold (GET) and respiratory compensation threshold are known as heavy- and severe-intensity exercise, respectively. During supra-GET exercise, cytosolic pyruvate production exceeds pyruvate oxidation, which accelerates anaerobic glycolysis and the lactate dehydrogenase reaction, resulting in an accumulation of lactate. To maintain electrical neutrality, lactic acid dissociates into [La^-] and hydrogen ions, which are transported into the blood (Katch et al., 2011). In the presence of bicarbonate ions (HCO_3^-) in the blood, H^+ binds to make carbonic acid, which then dissociates into carbon dioxide (CO_2) and water, catalyzed by the carbon anhydrase reaction *(Equation 1)* (Belfry, Paterson, Murias, & Thomas, 2012), and thus increases CO_2 partial pressure (PCO_2) in the blood. As peripheral chemoreceptors are highly sensitive to PCO_2 changes in arterial blood, the resulting hypercapnia stimulates increased ventilation in an attempt to remove CO_2 from the blood. If H^+ production persists beyond the capacity of ventilatory buffering, known as gas exchange threshold, H^+ will accumulate and metabolic acidosis will persist. In contrast, exercise below gas exchange threshold (GET) presents an increase in oxidative phosphorylation that is

Equation 1. Interconversion of carbon dioxide and water to bicarbonate and protons via the carbonic anhydrase reaction. Figure derived from information by (Geers & Gerolf, 2000)



matched by CO_2 production via the pyruvate to Acetyl-CoA reaction and the citric acid cycle. As such, at sub-GET workrates, VO_2 and VCO_2 stabilize in 3 and 4 minutes, respectively (Xu & Rhodes, 1999).

1.3 « Severe-Intensity Step Exercise »

Following the RAMP protocol, participants in the present study performed a 5-minute baseline cycle at 20 W, followed by a step increase to an individually determined supra-maximal workload. Participants cycled at a cadence of 70 rpm until volitional fatigue or the cycling cadence could no longer be maintained, lasting ~ 90 seconds.

As outlined by Gastin (2001), the anaerobic/aerobic percent contribution during 90 second maximal effort exercise is ~ 44 / 56 % (Gastin, 2001). At the immediate onset of the workload, energy is immediately supplied through the Adenosine Triphosphate-Phosphocreatine (ATP-PCr) system. The high energy phosphagen, in combination with stored ATP within the muscle cell, provide high power and short duration energy supply at the very beginning of heavy intensity exercise (Gastin, 2001). Along with a contribution from the ATP-PCr energy system, anaerobic glycolysis is another major energy supply. Exercising at such a high intensity elicits an accumulation of CO_2 that results in metabolic acidosis and ultimately fatigue.

1.4 « Breath by Breath Analysis of Mass Spectrometry »

In the present study, pulmonary oxygen uptake (VO_{2p}) is measured at the mouth via breath-by-breath analysis, reflecting the contribution of oxidative phosphorylation to exercising energy supply. VO_{2p}, carbon dioxide output (VCO_{2p}), and minute ventilation (V_E) values were calculated by inspired and expired flow rates from a low dead-space (90

mL) bi-directional turbine. After calibrating with fixed gas mixtures, inspired and expired gases were sampled continuously at the mouth and analyzed for concentrations of O_2 and CO_2 by mass spectrometry. Breath-by-breath gas exchange were calculated using algorithms that were developed to estimate alveolar gas exchange by accounting for changes in both functional residual capacity and alveolar gas concentrations during ventilation (Swanson & Sherrill, 1981). Total lung gas exchange was calculated and changes in gas concentration were aligned with gas volumes by measuring time delay for a square-wave bolus of gas passing through the turbine, resulting in changes in fractional gas concentrations as measured by the mass spectrometer every 20 milliseconds. Data were transferred to a computer that aligned concentrations with volumes to build a profile of each breath.

1.5 « Near Infrared Spectroscopy and Muscle Deoxygenation »

Near infrared spectroscopy (NIRS) is a method of continuous and non-invasive monitoring of muscle deoxygenation (HHb), oxygenated hemoglobin (O_2Hb), total hemoglobin (THb) and tissue hemoglobin saturation (O_2Sat) at the site of interrogation during dynamic exercise (Belfry et al., 2012; Gurd et al., 2007; McCrudden, Keir, & Belfry, 2017; K. Smith & Billaut, 2012). At the onset of exercise, PO_2 in the exercising muscle is reduced, thus increasing the PO_2 gradient and increasing O_2 delivery via enhanced O_2 offloading from hemoglobin (Hb), until the time in which O_2 delivery by blood flow increases (Laughlin & Armstrong, 1985). PO_2 during exercise can be affected by a variety of factors, including body temperature and ventilation, both of which are affected by the menstrual cycle, as discussed previously (Lebrun et al., 2013). Muscle

deoxygenation reflects the balance between oxygen delivery and utilization, and thus, provides important information regarding the adequacy of the vascular response and PO_2 gradient (Koga et al., 2011).

The theoretical basis for NIRS technology is comprehensively explained by Ferrari et. al (2004) (Ferrari, Mottola, & Quaresima, 2004). Briefly, between the visible and microwave domains of the electromagnetic spectrum is infrared light. Infrared spectroscopy exposes organic molecules hemoglobin (Hb) and myoglobin (Mb) to infrared radiation, ranging from 790 – 850 nm. Upon exposure, Hb and Mb resonate at these wavelength frequencies and are absorbed. Measurements are determined by the amount of absorption of the near-infrared light by Hb and Mb, as projected by laser diodes refracted back to the optode from muscle tissue at the area of interrogation. Deoxygenated hemoglobin (HHb) and oxygenated hemoglobin (O_2Hb) absorb different wavelengths of near-infrared (NIR) light depending on the presence of O_2-binding (690 – 760 nm and 800 – 850 nm, respectively). The difference in wavelengths emitted by the NIRS optode provides measures of [HHb], [O_2Hb] and [THb] (Koga et al., 2011).

The present study uses NIRS Oxiplex TA (model 95205, ISS, Champaign, IL) (technical reference (Franceschini, 2002). The emission probe carrying NIR light was arranged as a single channel consisting of eight laser diodes operating at two wavelengths (690 and 828 nm, 4 at each wavelength) that were pulsed in a rapid succession (frequency modulation of laser intensity was 110 MHz). Optodes were contained in an optically dense plastic holder, which were placed on the muscle belly of the vastus lateralis half-way between the lateral epicondyle and greater trochanter of the femur, and secured by a

black vinyl sheet and a tensor bandage to prevent any intrusion of extraneous light and movement of the probe.

Profiles of [HHb] during ramp incremental exercise in both men and women have been described by Murias et. al (2013). In short, both sexes show an increase in [HHb] until an evident 'plateau' at the respiratory compensation threshold, however, women demonstrate a greater overall reliance on local O_2 extraction (greater [HHb]) compared to men. Possible menstrual phase effects on local muscle deoxygenation during ramp incremental and severe intensity exercise have not been previously explored and is one of the purposes of the present study.

1.6 « Study Rationale »

Women are often under-represented in exercise physiology research, and as such, the confounding effects of the menstrual cycle on exercise response are largely unknown (Bruinvels et al., 2017). In performing a ramp incremental exercise test followed by a severe-intensity constant load bout, researchers gain a complete pulmonary and metabolic profile that presents any changes that might exist between menstrual phases.

The main purpose of this study was to compare VO_2, $[La^-]$, V_E, vastus lateralis ΔHHb, and performance (time-to-fatigue) in both the early-follicular (FOL) and mid-luteal (LUT) phases of the menstrual cycle during ramp incremental and subsequent severe-intensity constant load exercise to limit of tolerance. This information will provide valuable insight to female physiology and any potential exercise performance implications associated with such changes.

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Chapter 2

2 « Menstrual Phase Differences in Muscle Deoxygenation, Respiration, and Blood Lactate Concentration Do Not Influence Exercise Performance»

The menstrual cycle is composed of luteal (LUT) and follicular (FOL) phases wherein the ovarian hormones estrogen and progesterone are at their highest and lowest concentrations, respectively (Lebrun, Joyce, & Constantini, 2013). Notably, the vast majority of the exercise physiology literature has not included female participants in their participant pools because it is often taken for granted that the different phases of the menstrual cycle evoke increased day-to-day variability in physiological and performance variables (for review, see (Oosthuyse & Bosch, 2010). It is, however, important to recognize that a small pool of research examining the impact of the menstrual phase on the physiological responses during exercise is equivocal and is to be elucidated here.

On the one hand, Redman et. al (2003) reported increased oxygen uptake (VO_2) in the LUT phase during moderate- (25% of VO_{2peak}) and heavy-intensity (75% of VO_{2peak}) exercise compared to the FOL phase. This finding was attributed to the LUT phase eliciting increased fat and decreased carbohydrate oxidation resulting in a lower respiratory exchange ratio (RER). On the other hand, Lebrun et al. (1995) found a modest, but reliable reduction in VO_{2peak} in the LUT phase over the continuum of increasing work rates during a ramp exercise protocol; however, the authors did not offer a mechanism for this difference. Further, it is important to recognize that a number of studies have reported that LUT and FOL phases are associated with equivalent VO_2 measures for moderate-intensity exercise (Gurd, Scheid, Paterson, & Kowalchuk, 2007)

as well as exhaustive ramp exercise (Jurkowski, Jones, Walker, Younglai, & Sutton, 1978; Redman, Scroop, & Norman, 2003; Schoene, Robertson, Pierson, & Peterson, 1981).

Increases in the carbohydrate contribution to energy production associated with the imbalance between the rate of pyruvate production and oxidation that elicits increased cytoplasmic and blood lactate [La^-] and [H^+]. The increase in [H^+] undergoes the carbonic anhydrase reaction, resulting in increased VCO_2, which is a stimulus on ventilation (V_E) to increase blood pH (Green, Hughson, Orr, & Ranney, 1983).

In line with VO_2 measures, the literature has shown mixed results for exercise-based differences in [La^-] concentration across LUT and FOL phases. For example, Kendrick et al. (1991) found that estrogen supplementation in male rats resulted in decreased [La^-] and increased time-to-exhaustion via glycogen sparing mechanisms compared to a control group. In exercising human female participants, decreased [La^-] during the LUT compared to the FOL phase across moderate- to maximal-intensity exercise bouts has been reported (Jurkowski, 1981; Schoene, 1981; McCracken, 1994; Redman et al., 2003). This response was attributed to increased ventilatory buffering – a metabolically beneficial response reducing the accumulation of fatigue-inducing metabolites that is thought to positively impact endurance performance (Dombovy, Bonekat, Williams, & Staats, 1987). Jurkowski et al. (1981) observed that increased V_E associated with ventilatory buffering resulted in improved time-to-fatigue (TTF) while exercising at 90% of VO_{2max}. Others, however, have shown no menstrual phase differences in performance (Lebrun, McKenzie, Prior, & Taunton, 1995; McCracken, Ainsworth, & Hackney, 1994), despite findings of higher reported VO_{2max} in the LUT

phase (Lebrun et al., 1995) and lower LUT phase post-exercise [La^-] (McCracken, 1994; Redman et al., 2003). Furthermore, several studies have shown LUT phase increases in V_E (Jurkowski et al. 1981; Schoene et al. 1981) and decreases in [La^-] during maximal effort exercise (Jurkowski et al. 1981; Schoene et al. 1981; Redman et al., 2003). In contrast, Gurd et al. (2007) reported no exercise related changes in V_E or [La^-] changes between menstrual phases exercise suggesting consistent substrate metabolism across the menstrual cycle (Gurd et al., 2007).

To our knowledge, no research examined putative menstrual phase differences in muscle deoxygenation (HHb) during exercise. It has been suggested, however, that females, compared to males, have a greater reliance on fractional oxygen (O_2) extraction during ramp incremental exercise (Murias, Keir, Spencer, & Paterson, 2013). This sex-based difference was linked to: (1) increased muscle deoxygenation to VO_2 ratio as a consequence of a dispersal of microvascular blood flow distribution at the working muscle (Murias et al., 2013), and/or (2) a blunted vasodilatory response in females associated with limited baroreflex sensitivity to decreases in blood pressure that resulted in reduced muscle microvascular blood flow (Christou et al., 2005). Importantly, the aforementioned work did not control for menstrual phase making it uncertain whether results were menstrual phase dependent. FOL to LUT phase increases in blood flow and oxygen delivery, however, have been linked to enhanced pulmonary diffusion capacity (Smith, Brown, Murphy, & Harms, 2015) and arterial vasodilation (Gerhard et al., 1998) that may result in a LUT phase specific reduction in muscle deoxygenation.

The main purpose of this study was to compare VO_2, [La^-], V_E, vastus lateralis ΔHHb, and performance (time-to-fatigue) in both the early-follicular (FOL) and mid-

luteal (LUT) phases of the menstrual cycle during ramp incremental and subsequent severe-intensity constant load exercise to limit of tolerance. In terms of a research hypothesis, if the LUT, compared to the FOL phase is associated with lower arterialized blood lactate concentrations concomitant with increased V_E, VO_2 and enhanced local muscle blood flow distribution (shown as lower $\Delta HHb/VO_2$) then improved performance on incremental and severe-intensity constant load exercise should be observed.

2.1 « Methods »

Participants performed a ramp incremental exercise test (via cycle ergometer) followed by a constant load, severe intensity step-exercise to fatigue at 101% of their peak power attained during the ramp test. This protocol was performed during LUT and FOL phases of the menstrual cycle to assess their effects on gas exchange, vastus lateralis deoxygenation, and time to fatigue at a work rate in the proximity of peak aerobic power.

2.1.1 « Participants »

An advertisement poster on Western University campus was used to assist with volunteer participant recruitment (see Appendix E). Inclusion criteria included females who reported to be healthy and active (moderate-intensity exercise 1-3 times per week). Exclusion criteria included those who reported irregular menstrual cycles (< 21 or > 45 days), demonstrated a LUT phase deficiency, appeared to be anovulatory or ovulation could not be verified by urinary hormone testing, reported the use of hormonal contraceptives within six months of participation, taking medication that may have affected their metabolic, cardiorespiratory or hemodynamic responses to exercise, and reported a history of smoking.

2.1.2 « Ethical Approval »

Participants provided informed written consent via an approved Letter of Information form (see Appendix B) by the Health Sciences Research Ethics Board, Western University, and this work was conducted according to the ethical standards of the Declaration of Helsinki (Appendix A).

2.1.3 « Menstrual Cycle Phase Determination »

The follicular (FOL; Day 1 to ~ Day 14) and luteal (LUT; ~ Day 14 to Day 28) phases of the ~28-day menstrual cycle are separated by ovulation (~ Day 14). The onset of the FOL phase in the present study was verified by the onset of menstrual flow (Day 1) and exercise testing took place on Day 2 of this phase. The progressive rise in estrogen in the late FOL phase (~ Day 12), elicits a surge in luteinizing hormone (LH). The resulting ovulation and concomitant increase in progesterone 24-36 hours later (Schaumberg, Jenkins, Janse de Jonge, Emmerton, & Skinner, 2017) indicates the beginning of the LUT phase and was verified via urinary hormone testing (AccuMed 100-Count Ovulation (LH) Test Strips, AccuMed BioTech LLC, Houston, Texas). Progesterone reaches peak concentrations approximately six days after a positive test is detected (Reilly, 2010; Schaumberg et al., 2017) and the LUT phase exercise testing was performed six days after the verified LH surge. All participants performed the exercise testing protocol detailed below in each of the FOL and LUT phases. The order of the menstrual phase testing was counterbalanced so that 5 females began testing in the FOL phase and 5 in the LUT.

2.1.4 « Exercise Testing Protocol »

All tests were performed on an electromagnetically braked cycle ergometer (Velotron, RacerMate Inc. Seattle, WA). A ramp incremental test (RAMP), including a 4-min baseline at 20 W followed by a linear increase in power output equivalent to 20 W min^{-1} to exhaustion was performed. After a five-minute recovery period at 20 W, a constant load, severe intensity (SI) step exercise was performed to exhaustion at 101% of peak aerobic power (PAP). Exhaustion was determined when a cadence of 70 revolutions per minute (rpm) could not be maintained. Verbal encouragement was provided by the research team to facilitate maximal effort by participants.

One month prior to study onset, participants provided written consent and were informed of the exercise protocol. On the same day, participants completed a familiarization ride including both RAMP and SI protocols to acclimatize themselves with the testing procedures. All tests were performed in an overnight fasted, and hydrated state between 6:30am and 10am. Upon arrival to the lab, weight and height measurements were recorded. Participants were instructed to maintain their usual exercise and sleep regimens throughout the testing period. Nutritional food intake was also recorded 72 hours prior to their first ramp cycle ergometer test using a written food journal (Appendix C). An identical diet regimen was followed preceding the second day of testing to control for carbohydrate and protein oxidation rates that can be affected by dietary food intake during the subsequent phase tests (Bonen et al., 1983).

2.1.5 « Measurements »

During both RAMP and SI rides, local muscle deoxygenation ([HHb]) profiles from the quadriceps vastus lateralis were continuously monitored second by second via near-

infrared spectroscopy (NIRS; Oxiplex TA, model 95205, ISS, Champaign, IL). NIRS measurements were collected and recorded via well-established methods (Murias et al., 2013). In particular, an emission probe carrying near infrared (NIR) light was arranged as a single channel consisting of eight laser diodes operating at two wavelengths (690 and 828 nm, 4 at each wavelength) that were pulsed in a rapid succession (frequency modulation of laser intensity was 110 MHz). The light returning from the tissue was detected by a photodiode for eventual display of the change (Δ) in [HHb], total hemoglobin concentration ([THb]), and oxygen saturation (O_2Sat) from an exercising baseline of load less cycling (20 W) in the 30 s prior to the onset of the work rate. The optodes were contained in an optically dense plastic holder, which was placed on the muscle belly midway between the lateral epicondyle and greater trochanter of the femur. The probe was secured by a tightened elastic strap, an optically dense black vinyl sheet, and a tensor bandage to prevent movement and the intrusion of extraneous light. The NIRS probe was placed in the identical position, as demarcated by permanent marker, for each testing session.

Inspired and expired gas volumes and concentrations were sampled continuously at the mouth and analyzed every 20 ms for oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2) by mass spectrometry (AMIS 2000; Innovision, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. These data were transferred to a computer, which aligned gas concentrations with volumes to build a profile of each breath to provide measures of oxygen uptake (VO_2), carbon dioxide output (VCO_{2p}), ventilation (V_E), end-tidal oxygen pressure ($P_{ET}O_2$), end-tidal carbon dioxide pressure ($P_{ET}CO_2$), and breathing frequency (B_F). Peak oxygen uptake (VO_{2peak}) was determined

from the highest 20-breath average value. If there were no differences in VO_{2peak} between the RAMP and SI rides, this was determined to verify the participant's VO_{2max} (Rossiter, Kowalchuk, & Whipp, 2006). Oxygen uptake at estimated gas exchange threshold (VO_{2GET}), synonymous with lactate threshold, was defined as the point in which VCO_2 at which began to increase out of proportion to VO_2, coinciding with a systematic rise in V_E and $P_{ET}O_2$ (Neder & Stein, 2006).

Five arterialized-capillary blood samples were obtained and analyzed for lactate concentrations ($[La^-]$): three min pre- RAMP, at 150 watts (W) during RAMP, post-RAMP, and both immediately post- and three minutes post- SI, as outlined in *Figure 4*. Samples taken immediately post-RAMP and post-SI were taken within 5 seconds of exercise cessation to ensure consistency among all participants. $[La^-]$ were measured using a Lactate Scout analyzer (Sports Resource Group, Hawthorne, NY). A 0.2 µl blood sample was taken with AccuChek Safe-T-Pro Plus Lancets. The participant's index finger was sterilized with a rubbing alcohol swab prior to each blood draw. Latex gloves were worn by the researcher. All testing took place in the Canadian Center for Activity and Aging Cardiorespiratory Research Lab at Western University.

2.1.6 « Data Analyses »

VO_2 data were filtered by removing aberrant data points lying outside four standard deviations of the local mean and linearly interpolated to 1 s intervals which were then binned into 5 s averaged response for each participant. The second-by-second Δ[HHb] data were averaged in a similar fashion. VO_{2p} data were left-shifted by 20 s to account for the transit time lag in blood flow between the muscle and the lung to ensure that changes

in muscle VO_2 (as represented by VO_{2p}), were aligned with changes in the Δ[HHb] signal (Murias, Spencer, Kowalchuk, & Paterson, 2011). Baseline values for VO_2 and Δ[HHb] were determined as an average of the last 120 s of the four minute 20 W baseline prior to the onset of the RAMP. Since RAMP and SI bouts varied in duration for each participant, the VO_2 and Δ[HHb] responses were normalized from 0%-100% as previously described by (Murias et al., 2013). The on-transient response for VO_2 was modeled using the mono-exponential function:

$$Y_{(T)} = Y_{Bsln} + amp\left(1 - e^{-(t-TD)/\tau}\right)$$

In the above equation, $Y_{(T)}$ represents VO_{2p} at any time (t), Y_{Bsln} is the baseline VO_2 during the last 120 s of baseline 20 W cycling, τ is time-constant defined as the time for VO_2 to increase to 63% of the steady-state increase, and TD is the time delay, which was allowed to vary freely to optimize accuracy of parameter estimates (Murias et al., 2011). Data were modeled from the end of the cardio respiratory phase I to the end of the SI exercise duration. End of phase I was determined by visual inspection of the second by second data as the point in which there was a sharp decrease from baseline values in end-tidal O_2 pressure and a sharp increase in VO_2 (Whipp, Ward, Lamarra, Davis, & Wasserman, 1982). MRT in the present study is represented by the τ in the above equation.

2.1.7 « Statistical Analyses »

In most cases, data were analyzed via 2 (menstrual phase: LUT, FOL) by 20 (time: five percent increments from the onset of the RAMP and constant load exercise to volitional fatigue) fully repeated measures ANOVA. An alpha level of 0.05 was adopted and

significant main effects/interactions were decomposed via simple-effects (i.e., paired-sample t-tests). Data is presented as mean ± standard deviation.

2.2 « Results »

2.2.1 « Participants »

Participant means included age (22 years ± 1), weight (64 ± 8 kg) and height (167 ± 7 cm).

2.2.2 « Ramp Incremental Exercise »

Δ[HHb]/ΔVO_{2p} produced a menstrual phase by time interaction, $F(20,180)=1.83$ ($p=0.020$), indicating that values for the LUT phase were lower than the FOL phase at 15% ($p=0.027$), 20% ($p=0.008$), and 25% ($p=0.022$) of the RAMP protocol, whereas at 0 to 10% and 30 to 100% values did not reliably differ between phases (all $p>0.05$) *(Fig. 5C)*. In terms of VCO_2 *(Table 3),* VO_2 *(Figure 5A),* Δ[HHb] *(Figure 5B),* V_E *(Table 3),* VO_{2GET} *(Table 1),* $VO_{2\Delta 50}$ *(Table 1),* VO_{2max} *(Table 1),* oxygen saturation (O_2Sat) *(Table 4),* total hemoglobin concentration ([THb]) *(Table 4), PAP* and *TTF (Table 1)*, no reliable main effects or interactions were observed (all $p>0.05$). In contrast, $P_{ET}CO_2$ produced a main effect, $F(1,9)=19.10$, $p=0.002$), such that $P_{ET}CO_2$ values were lower in the LUT compared to the FOL phase (Cohens $d = 0.4$) *(Table 3).*

We examined arterialized-capillary [La^-] values at 3-minutes pre-RAMP, at 150 W during RAMP, and immediately post-RAMP *(Table 2)* via a 2 (menstrual phase: LUT, FOL) by 3 (time: pre-RAMP, 150 during RAMP, post-RAMP). Results did not elicit any reliable main effects or an interaction ($p>0.05$).

2.2.3 « Severe-Intensity Step Exercise »

Δ[HHb]/ΔVO_{2p} produced a menstrual phase by time interaction, F(20,180)=1.44, p=0.020) indicating that values for the LUT phase were lower than the FOL phase at 15% (p=0.002) and 20% (p=0.005) *(Fig. 5F)* of the SI protocol. For the other time points we did not observed any reliable differences (p>0.05). In terms of VCO_2 *(Table 3),* VO_2 *(Figure 5D),* Δ[HHb] *(Figure 5E),* B_F *(Table 3),* time-to-fatigue (TTF) *(Table 1),* O_2Sat *(Table 4),* [THb] *(Table 4),* and mean response time (MRT) *(Table 3),* we did not observe any reliable main effects or interactions (all p>0.05). In contrast, mean V_E and $P_{ET}CO_2$ produced main effects, all F(1,9)=6.09 and 3.84, respectively, ps=0.036, and 0.082. V_E was higher in the LUT phase compared to the FOL phase (Cohens *d* = 0.3) *(Table 3)* and $P_{ET}CO_2$ was lower in the LUT compared to the FOL phase (Cohens *d* = 0.4) *(Table 3).* Arterialized-capillary [La^-] values were examined immediately post-SI (within 5 seconds) and 3-minutes post-SI, via a 2 (menstrual phase: LUT, FOL) by 2 (time: post-SI, 3 min post-SI) ANOVA. Results elicited an interaction, F(1,9)=2.38, p=0.070), indicating that post-SI [La^-] values were lower in LUT phase compared to FOL (p=0.037, Cohen's *d* = 0.6) , whereas 3-min post-SI LUT and FOL phase values did not differ (p>0.05).

Table 1. Group mean data ± SD for VO_{2GET}, $VO_{2\Delta 50}$, VO_{2max}, VCO_{2peak}, PAP_{RAMP}, PO_{SI}, TTF_{RAMP}, and TTF_{SI} in both FOL and LUT phases.

Variable	Unit	FOLLICULAR	LUTEAL
VO_{2max}	$L \cdot min^{-1}$	2.36 ± 0.4	2.36 ± 0.4
VCO_{2peak}	$L \cdot min^{-1}$	2.51 ± 0.5	2.48 ± 0.4
$VO_{2\Delta 50}$	$L \cdot min^{-1}$	1.77 ± 0.3	1.80 ± 0.3
VO_{2GET}	$L \cdot min^{-1}$	1.17 ± 0.2	1.23 ± 0.2
PAP_{RAMP}	W	218 ± 35	221 ± 29
PO_{SI}	W	220 ± 35	223 ± 29
TTF_{RAMP}	s	654±112	661 ± 93
TTF_{SI}	s	99.2 ± 20	95.8 ± 15

Data analyzed by paired t-tests. RAMP (ramp incremental exercise test) SI (severe-intensity step-exercise test) FOL (early-follicular phase) LUT (mid-luteal phase) VO_{2GET} (oxygen uptake at estimated gas exchange threshold during RAMP exercise) $VO_{2\Delta 50}$ (oxygen uptake at 50% of the difference between VO_{2GET} and VO_{2peak} from RAMP exercise) VO_{2max} (maximal pulmonary oxygen uptake as determined by the mean peak values from RAMP and SI. No differences between VO_{2peak} in RAMP and SI were found, $p>0.05$) VCO_{2peak} (peak carbon dioxide production) PAP (peak aerobic power output from RAMP exercise) PO_{SI} (power output during SI exercise) TTF (time-to-fatigue from SI exercise) $L \cdot min^{-1}$ (litres per minute) W (watts) s (seconds).

Table 2. Group mean data ± SD for arterialized-capillary [La^-] during RAMP and SI tests in both FOL and LUT phases.

Variable	Unit	FOLLICULAR	LUTEAL
3-min Pre-RAMP	$mmol \cdot L^{-1}$	1.5 ± 0.3	1.4 ± 0.3
150 W	$mmol \cdot L^{-1}$	4.2 ± 1.5	4.7 ± 2.0
Post-RAMP	$mmol \cdot L^{-1}$	10.2 ± 2.2	10.8 ± 2.2
Post-SI	$mmol \cdot L^{-1}$	12.9 ± 2.5	11.7 ± 1.7 *
3-min Post-SI	$mmol \cdot L^{-1}$	12.8 ± 2.5	12.1 ± 2.3

Data analyzed by paired t-tests. RAMP (ramp incremental exercise test) SI (severe-intensity step-exercise test) FOL (early-follicular phase) LUT (mid-luteal phase) [La^-] (arterialized-capillary blood lactate concentration) 3-min Pre-RAMP ([La^-] 3 minutes prior to start of RAMP) 150 W ([La^-] during RAMP when participants reach a work rate of 150 watts) Post-RAMP ([La^-] at end of RAMP test) Post-SI ([La^-] at end of SI) 3-minutes Post-SI ([La^-] 3 minutes after the end of the SI) $mmol \cdot L^{-1}$ (millimole per litre). * LUT phase is significantly different from FOL ($p<0.05$).

Table 3. Group mean data ± SD for V_E, B_F, $P_{ET}CO_2$, VCO_2, RER and MRT during RAMP and SI tests in both FOL and LUT phases.

		RAMP		SI	
Variable	**Unit**	**FOL**	**LUTEAL**	**FOL**	**LUTEAL**
V_E	$L \cdot min^{-1}$	53 ± 25	55 ± 25	83 ± 20	89 ± 17 *
B_F	Brth/min	32 ± 4	35 ± 5	45 ± 5	48 ± 8
$P_{ET}CO_2$	mmHg	37 ± 2	36 ± 3 *	27 ± 2	26 ± 3 §
VCO_2	$L \cdot min^{-1}$	1.52 ± 0.7	1.56 ± 0.7	1.90 ± 0.5	1.92 ± 0.4
RER		0.97 ± 0.14	0.97 ± 0.12	0.96 ± 0.09	0.96 ± 0.07
MRT	s	--	--	29.3 ± 6	28.6 ± 8

Data analyzed by 2 Way RM ANOVA. RAMP (ramp incremental exercise test), SI (severe-intensity step-exercise test) FOL (early-follicular phase), LUT (mid-luteal phase), V_E (ventilation), B_F (breathing frequency), $P_{ET}CO_2$ (end-tidal carbon dioxide pressure), VCO_2 (carbon dioxide production), RER (respiratory exchange ratio), MRT (mean response time), $L \cdot min^{-1}$ (litres per minute), Brth/min (breaths per minute), mmHg (millimeters of mercury) and s (seconds). LUT phase is significantly different from FOL * ($p<0.05$); § ($p=0.08$).

Table 4. Group mean data ± SD for NIRS measurements including ΔHHb, [THb] and O_2Sat during RAMP and SI in both FOL and LUT phases.

		RAMP		SI	
Variable	**Unit**	**FOL**	**LUTEAL**	**FOL**	**LUTEAL**
ΔHHb	%	55.9 ± 30	50.5 ± 33	84.8 ± 21	83.0 ± 21
[THb]	a.u.	1.87 ± 1.0	2.16 ± 1.3	2.75 ± 0.3	3.27 ± 0.2
O_2Sat	%	67.72 ± 2.3	68.45 ± 2.2	64.27 ± 1.9	64.46 ± 2.3

Data analyzed by 2 Way RM ANOVA. RAMP (ramp incremental exercise test). SI (severe-intensity step-exercise test), FOL (early-follicular phase), LUT (mid-luteal phase), ΔHHb (change in muscle deoxygenation of the vastus lateralis), [THb] (total hemoglobin concentration at the vastus lateralis), O_2Sat (oxygen saturation at the vastus lateralis), % (percent of maximum response) and a.u. (arbitrary units).

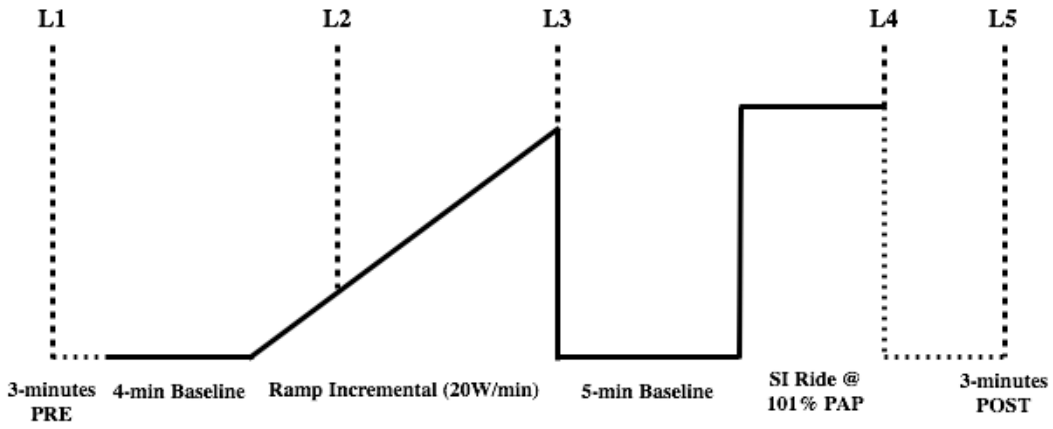

Figure 4. Schematic diagram of the RAMP and SI exercise protocols, including timing of arterialized capillary blood lactate samples (L); L1 (taken 3-minutes prior to onset of exercise), L2 (taken at 150W during ramp incremental exercise), L3 (taken immediately at ramp test cessation), L4 (taken immediately at cessation of severe-intensity step exercise at 101% of peak aerobic power), L5 (taken 3-minutes post exercise cessation) RAMP (ramp incremental exercise test), SI (severe-intensity step-exercise test), PAP (peak aerobic power).

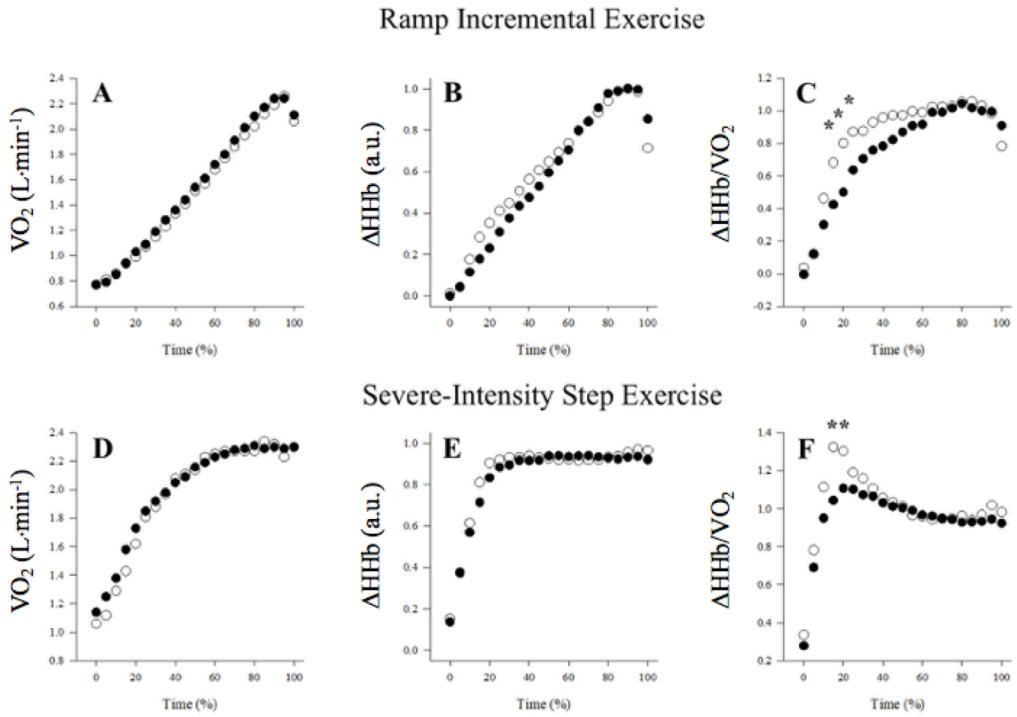

Figure 5. (A-F). Group means for A) VO_{2p} during RAMP, B) ΔHHb during RAMP, C) ΔHHb/VO2p during RAMP, D) VO2p during SI, E) ΔHHb during SI, F) ΔHHb/VO2p during SI. Open circles (○) represent the early-follicular (FOL) phase and closed circles (•) represent the mid-luteal (LUT) phase. VO_2 (pulmonary oxygen uptake) L·min^{-1} (litres per minute) ΔHHb (change in vastus lateralis muscle deoxygenation) A.u. (arbitrary units) ΔHHb/VO_2 (change in vastus lateralis muscle deoxygenation ΔHHb (a.u.) over oxygen uptake (L·min^{-1})). RAMP (ramp incremental test) and SI (severe-intensity step-exercise) varied in duration for each participant, therefore the average and time-aligned VO_2 and Δ[HHb] responses were then normalized from 0% to 100%, such that 0% represented the start of the tests and 100% represented test cessation. * LUT phase is significantly different from FOL ($p<0.05$).

2.3 « Discussion »

The purpose of this study was to compare VO_2, $[La^-]$, V_E, vastus lateralis ΔHHb, and performance (time-to-fatigue) in both the early-follicular and mid-luteal phases of the menstrual cycle during ramp incremental and subsequent severe-intensity constant load exercise to limit of tolerance. The main findings demonstrated that: (1) the RAMP protocol elicited lower muscle deoxygenation at O_2 uptakes below gas exchange threshold during RAMP exercise in the LUT compared to the FOL phase – a result that was independent of menstrual phase differences in gas exchange variables or blood lactate, and (2) the severe-intensity exercise stimulus elicited higher mean ventilation and lower post-SI lactates in LUT compared to FOL, whereas the change in muscle deoxygenation to oxygen uptake ratio was lower during the kinetic phase in the LUT compared to the FOL; and (3) LUT and FOL phase performance during RAMP and severe intensity exercise were not associated with observable performance differences. The discussion first outlines results for the RAMP incremental test and then for the severe intensity test. In outlining these results, we note the importance of delineating both the identified similarities and differences in LUT and FOL phase performance and physiological measures within the context of the previous literature.

2.3.1 « Ramp Incremental Exercise »

The null difference FOL and LUT phase VO_{2max} values in RAMP and SI bouts adds to a growing body of evidence showing that cardiorespiratory performance does not vary with menstrual phase (Jurkowski et al., 1978; Redman et al., 2003; Schoene et al., 1981). The VO_2 response at moderate power outputs, however, remains equivocal as the more recent paper of Redman at al. (2003) observing modest increases in VO_2 from sub-lactate to

supra-lactate threshold power outputs in LUT compared to FOL are in juxtaposition to Gurd et al. (2007) and the current study in which menstrual phase VO_2 differences at sub-lactate threshold work rates were not observed. That in mind, Redman et. al (2003) tested participants in the mid-follicular phase (Days 5-7), when estrogen is in higher concentration compared to early-FOL, and might have effected oxygen uptake and gas exchange variables.

The hypothesized FOL to LUT phase increased V_E and decreased $[La^-]$ at VO_{2max} during RAMP was not observed. This is similar to the unchanged peak V_E observed by Redman et al. (2003). In contrast, $P_{ET}CO_2$ showed a >1 mmHg reduction in the LUT compared to the FOL phase – a salient finding given that this measure provides a non-invasive reflection of pulmonary capillary carbon dioxide pressure and a key stimulus on V_E (Benallal & Busso, 2000). It has been suggested that a 1 mmHg decrease in $P_{ET}CO_2$, elicits a 2.6 $L \cdot min^{-1}$ decrease in V_E during exercise (Clark & Godfrey, 1969). The unchanged V_E during RAMP, concomitant with the reduction in LUT phase $P_{ET}CO_2$ indicates that this change in gas pressure did not elicit a physiologically meaningful stimulus on V_E under RAMP conditions between menstrual phases. $P_{ET}CO_2$ data results were not presented by Redman et al. (2003) so no comparisons can be made.

It is suggested that the unchanged mean VCO_2, V_E and $[La^-]$ between menstrual phases in the present study, coupled with the reduced pulmonary capillary PCO_2, as reflected by $P_{ET}CO_2$ *(Table 3),* in the LUT phase was a function of the previously observed enhanced LUT phase pulmonary diffusion capacity (Farha et al., 2007) and pulmonary vasodilation (Gerhard et al., 1998). Both of these LUT specific responses would facilitate greater pulmonary capillary to alveolar CO_2 transfer and underpin the

observed decrease in $P_{ET}CO_2$ and unchanged [La^-] *(Table 2*), commensurate with the unchanged ventilatory buffering, as reflected by the stable VCO_{2max} between menstrual phases (*Table 1*). The previously observed reduction of VCO_2 at maximal exercise with similar peak lactates during RAMP (Redman et al., 2003) also suggests that the increased LUT phase pulmonary diffusion capacity (Farha et al., 2007) and pulmonary vasodilation (Gerhard et al., 1998) has increased CO_2 transfer across the pulmonary capillary/alveolar interface eliciting increased removal of CO_2.

Previous research examining muscle deoxygenation during ramp exercise has suggested that women, compared to men, have decreased microvascular blood flow to oxygen uptake (higher Δ[HHb]/VO_2) below the respiratory compensation threshold. This has been taken to evince that women have a greater reliance on muscle deoxygenation (Murias et al., 2013). The present study showed that women in the LUT phase, compared to FOL, exhibited reduced reliance on muscle deoxygenation as a function of improved microvascular blood flow (lower Δ[HHb]/VO_2) below GET. This LUT response has also been observed in post-menopausal women undergoing estrogen therapy (inducing LUT phase conditions), and was shown to enhance endothelium-dependent vasodilation (Gerhard et al., 1998; Gilligan, Quyyumi, Cannon, Johnson, & Schenke, 1994). This explanation provides a parsimonious account for the decreased LUT muscle deoxygenation to O_2 utilization observed in the present study. Moreover, Farha et al. (2007) found that ovariectomized mice, undergoing estrogen supplementation to mimic the LUT phase, showed increased pulmonary capillary density. This resulted in an increased surface area for gas diffusion (Farha et al., 2007). This would, in combination with the aforementioned LUT phase vasodilation (Gerhard et al., 1998; Gilligan et al.,

1994), enhance blood flow and also be reflected in the observed decrease of $\Delta[HHb]/\Delta VO_{2p}$ in the LUT phase of the present study.

These aforementioned LUT phase improvements of microvascular blood flow to oxygen uptake *(Figure 2C)* in the present study were unaffected at exercise intensities above GET. Above GET, the menstrual phase differences were not significant and become increasingly smaller as exercise intensity increases. This may be due to improved microvascular perfusion by enhanced vasodilation, which would be mediated by higher lactate production and the accompanying acidosis that occurs during heavy intensity exercise (Stocker, Von Oldershausen, Paternoster, Schulz, & Oberhoffer, 2017).

This demonstrates that under these higher intensity-conditions, the observed limitation in microvascular blood flow has been resolved and no change in RAMP performance (i.e. VO_{2max}, PAP, TTF) would be expected and none were observed *(Table 1)*.

2.3.2 « Severe-Intensity Step Exercise »

Similar to RAMP, VO_2 was unchanged between FOL and LUT phases and is in accord with previous work (Jurkowski et al., 1978; Redman et al., 2003; Schoene et al., 1981). In contrast to the RAMP response, however, the LUT phase produced a larger V_E and lower $P_{ET}CO_2$ compared to the FOL phase *(Table 3)*. It is suggested that this increased V_E has elicited the observed reduction of $P_{ET}CO_2$, as well as buffered, in part, the anaerobic glycolytically produced $[H^+]$. This would be reflected in the observed increase in V_E and the contemporaneous observed reduction in $[La^-]$ immediately post-SI (*Table 2*). $[La^-]$ 3-minutes post-SI was not different in FOL and LUT phases, and as there is no ventilation

data for the 3-minute recovery period, we cannot comment on the underlying mechanisms explaining the null changes in [La^-] 3-minutes post-SI.

Others have attributed reduced [La^-] in the LUT phase to increased fat oxidation resulting in glycogen sparing (Bunt, 1990; Hackney, McCracken, & Ainsworth, 1994; Jurkowski, Jones, Toews, & Sutton, 1981; McCracken et al., 1994). Furthermore, animal research has shown that progesterone treatment decreases Glut-4 protein content in insulin-responsive tissue, thereby reducing glucose transport into the cell during exercise and resulting in a greater reliance on fats as a fuel source (Campbell & Febbraio, 2001). Further, increased fat oxidation and lower glucose uptake in the LUT phase during a maximal effort time-trial performance in a fasted state has been observed (Campbell, Angus, & Febbraio, 2001). Females in the present study consumed identical diets in each menstrual phase for the 72 hours preceding testing, and all tests were performed in a similar fasted state to ensure that if substrate oxidation was to be menstrual phase dependent, changes in RER, a proxy for fat and carbohydrate oxidation rates, that this difference would not be precipitated by diet related changes in substrate oxidation. No differences in substrate oxidation, as reflected by RER (Péronnet & Massicotte, 1991) between FOL and LUT, during the pre- RAMP or pre-SI steady state light-intensity periods, or throughout either the RAMP or SI bouts ($p>0.05$; *Table 3*) were observed. Our data purport that menstrual phase is independent of substrate oxidation.

These aforementioned RAMP and SI differences between LUT and FOL phases observed in the present study indicate that the divergent contributions from the oxidative and non-oxidative phosphorylation energy systems coupled with different VO_2 kinetic activation demands of these exercise bouts resulted in a differential physiological

cardiorespiratory and metabolic responses. Notably, the non-oxidative phosphorylation contributions for RAMP, and the 90 s SI bout, have been estimated to be < 10% (Bertuzzi et al. 2013) and ~ 45% respectively (Gastin, 2001). Moreover, the mean response time of VO_2 of ~29 s during SI of the present study demonstrates a distinctly speeded activation of the phosphorylation systems to reach peak aerobic power (via the square application of the work rate) compared to the 14-min linear increase in power output during RAMP to attain the same work rate (*Table 1*).

The similar SI VO_2 kinetics for LUT and FOL phases reported here, and the observed LUT reduction of the $\Delta[HHb]/\Delta VO_{2p}$ during the kinetic phase of SI, demonstrated the FOL to LUT transition to enhanced microvascular blood flow. It is suggested that the unchanged VO_2 kinetics during the FOL compared to the LUT phase represents an increased reliance on muscle deoxygenation that is supported by LUT enhanced vasodilation and diffusion capacity that has been observed elsewhere (Christou et al., 2005; Gerhard et al., 1998; Gilligan et al., 1994). Others have associated the LUT increase in microvascular blood flow to enhanced blood volume and total hemoglobin concentration compared to the FOL-related reduction in blood volume and hemoglobin loss (Reilly, 2010), However, [THb] and O_2Sat in the present study were unchanged ($p>0.05$, *Table 4*) and as such, our data suggest that the changes in deoxygenation are the result of improved microvascular blood flow distribution via the aforementioned improved diffusion capacity and vasodilation. Accordingly, the SI and RAMP results reported here indicate that O_2 delivery is sustained by increased blood flow during LUT and increased deoxygenation during FOL.

2.4 « Conclusion »

To our knowledge, this is the first study to examine menstrual phase differences in gas exchange, muscle deoxygenation and performance during RAMP and SI exercise. RAMP results indicate that LUT phase O_2 delivery is sustained by enhanced muscle blood flow distribution below gas exchange threshold, where FOL phase O_2 extraction and delivery is sustained via increased muscle deoxygenation below gas exchange threshold. LUT phase SI results indicated lower post exercise [La^-] and $P_{ET}CO_2$ as a function of increased V_E associated with ventilatory buffering. Further, the LUT phase kinetic portion of SI shows enhanced microvascular blood flow, whereas FOL phase VO_2 is sustained via increased muscle deoxygenation during the kinetic phase. In contrast to our hypothesis, although we have found changes in muscle deoxygenation, ventilation, and blood lactate concentrations between menstrual phases, there are no consequential deleterious effects during moderate to exhaustive exercise in recreationally active females. The social impact of this study includes the determination that recreationally active females do not have to be concerned that menstrual phase will impact exercise and/or performance.

2.5 Future Direction and Limitations

To our knowledge, the only limitation of the present study is the small sample size (n=10). Future research could investigate different subject populations including female athletes, sedentary females, and females who use oral contraceptives to determine the effects of exogenous ovarian hormones.

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Appendix

Appendix A: Ethics Approval Notice

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Amendment Approval Notice

Principal Investigator: Glen Belfry
Department & Institution: Health Sciences\Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107170
Study Title: The Effects of Periodic Breath-Holding During Intermittent Exercise on Energy System Contribution, Lactate Threshold, and Regional Blood Flow Distribution.
Sponsor: Natural Sciences and Engineering Research Council

HSREB Amendment Approval Date: October 19, 2017
HSREB Expiry Date: January 08, 2018

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Letter of Information & Consent		2017/10/17
Revised Western University Protocol		2017/10/04

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Western University, Research, Support Services Bldg., Rm. 5150
London, ON, Canada N6G 1G9 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/ethics

Appendix B: Letter of Information

Glen Belfry –
Jordyn Smith –
Cardiorespiratory Laboratory, CCAA

LETTER OF INFORMATION:

Study Title: The Effects of Periodic Breath-Holding During Intermittent Exercise on Energy System Contribution, Lactate Threshold, and Regional Blood Flow Distribution in Females.

Principal Investigator: Glen Belfry PhD
Co-investigator: Jordyn Smith MSc Candidate

Introduction, Background and Purpose of Study:

You are being invited to participate in a study that examines the effects of short duration breath holding during bike exercise on oxygen delivery to the working muscles and its energy production. Previous research on females has shown that how your breathing responds to exercise, and the way oxygen is transported in the blood may differ depending on where you are in the menstrual cycle. This research will enable a better understanding of how you will respond to exercise. Participation in this study involves 9 visits to the laboratory of the Canadian Centre for Activity and Aging requiring less than 30 minutes for each of the 10 sessions. There is one test in which you will cycle progressively harder until you get tired that will take approximately 15 minutes and then eight subsequent tests in which the response to breath holding and differing intensities of exercise will be observed. These will take approximately 10 minutes each. You will perform these tests in both phases of the your menstrual cycle. One series of tests will be completed in the follicular phase (the first half of your cycle, beginning shortly after your period begins), and the final series of tests will take place during the luteal phase (the second half of your cycle, starting ~14 days later).

The Canadian Centre for Activity and Aging Laboratory is located in the Arthur and Sonia Labatt Health Sciences Building, at Western University in London, Ontario, Canada. A total of 10 participants are required for this study. In order to participate you must be between 18-35 years of age. You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular, metabolic, neurological or musculoskeletal disease; or you are currently on medication or oral contraceptives; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise protocol.

What you will be required to do if you decide to participate in this study:

Exercise Tests :

Test 1. During the first visit to the laboratory, you will complete a complete a test that will determine your maximum ability to use oxygen. You will begin with the exercise intensity being very light and easy (very little resistance). After a few minutes, the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop. This test will take approximately 15 minutes to complete.

Tests 2-5 will be performed twice, once in each phase of the menstrual cycle (luteal and follicular phases) and be separated by 24 hours. You will begin testing either during your period or later in that same month. The attending researcher will tell you when you will be starting. See picture on page seven and eight.

Testing beginning in Luteal Phase

Test 2. The second visit will be two days after the Test 1 to ensure sufficient recovery. After cycling easily for 4 min to warm up, you will complete a 6 min continuous trial at a moderately hard intensity. This test will take 10 minutes to complete.

Test 3. After cycling easily for 4 min to warm up, you will complete a 6 min continuous trial at the same moderately hard intensity while performing repeated cycles of 25 seconds of normal breathing followed by 5 seconds of breath holding. This test will take 10 minutes to complete.

Test 4. After cycling easily for 4 min to warm up, you will complete a 6 min trial in which you will perform repeated cycles of a moderately hard intensity exercise for 25 seconds followed by 5 seconds performed at a harder intensity with normal breathing. This test will take 10 minutes to complete.

Test 5. After cycling easily for 4 min to warm up, you will complete a 6 min trial in which you will perform repeated cycles of a moderately hard intensity exercise for 25 seconds followed by 5 seconds performed at a harder intensity with breath holding. This test will take 10 minutes to complete.

Testing beginning in Luteal Phase

Test 6. Repeat Test 2.

Test 7. Repeat Test 3.

Test 8. Repeat Test 4.

Test 9. Repeat Test 5.

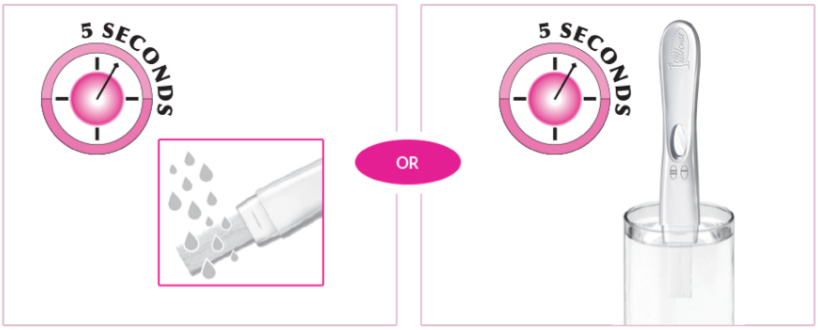
See pictures on pages 7 and 8 of this letter for an overview of your testing schedule. Throughout the study period, it is important that you maintain the same level of activity throughout the start of the study (i.e. if you were on a weight-training regimen, it must be continued throughout the full length of the testing period) to ensure changes in physical activity level will not affect the testing results.

The different phases of the menstrual cycle

The different phases of the menstrual cycle, and the appropriate testing dates associated with those phases, will be determined by: i) the start of your period/bleeding (follicular phase) and ii) verification of luteinizing hormone (LH) spike, which precedes ovulation, by urinary ovulation sticks (luteal phase).

You will be provided with the urinary testing sticks and you will be instructed by the researcher (Jordyn) to begin testing with the sticks around days 9-14 of your menstrual cycle. This will depend on your regular cycle length – Jordyn will communicate with you when you are to start testing. There are 7 sticks that are to be used, one per day, until the LH spike is detected. It is best to test your urine sample around approximately the same time each day, anywhere from 10am-8pm. Do not test your urine right in the morning (or first urination of day). Wait to test your urine with your second urination or later in the day. This is the best way to ensure the LH spike is detected.

Instructions for using the Ovulation stick are as follows: The urinary ovulation testing sticks are a small plastic stick that you are to hold in the urine stream for 5 seconds.



Hold stick by Thumb Grip, with the Absorbent Tip pointed down, and the Result Window facing away from body. Place Absorbent Tip into your urine stream for 5 seconds only.

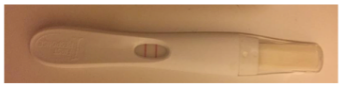
OR

Collect urine in a clean, dry cup. Dip the entire Absorbent Tip into urine for 5 seconds only.

You want the absorptive tip in the stream and the results window facing away from your body. You may also urinate in a clean, dry cup and then hold the absorptive tip in the urine for 5 seconds then remove. After 5 seconds of exposure to urine you are done taking the test. Wait 5 minutes before reading results.

In the results window, you will see 2 lines, one is a test line and one is a reference line. If the two lines are not present, or are different in colour, that is a **negative** test. You are to continue testing the following day.

If the two lines are both present and the same colour that is a **positive** test (see below).



A positive test indicates that the LH surge has occurred. Please take a photo of the

How the data will be collected for ALL participants:
During all testing periods, the volume of oxygen utilized will be monitored through a breathing apparatus, and the amount of oxygen attached to the red blood cells will be monitored by a probe fixed to your thigh muscle. The amount of lactate in your blood will be collected 3 min before and 3 min after each test via standard finger prick apparatus. We will analyze your blood lactate levels by a portable lactate analyzer (Lactate Scout from Lactate.com). Your blood lactate concentration will enable us to examine your body's energy systems after each cycling tests.

Breathing apparatus: During each of the exercise tests you will be required to wear a nose - clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). These will be washed and sterilized between before each use. This will enable us to measure the volume of air that you breathe in and out, and measure the oxygen concentration in that air. You may experience some initial discomfort from wearing the nose - clip and mouthpiece but will soon get used to it.

Thigh measurements: During each test, the relative oxygen content of the red blood cell of the thigh muscles will be measured using a machine that uses near - infrared spectroscopy. This technique involves projecting light into your thigh and measures the amount of light coming out at another location. A plastic probe will be attached to your leg approximately midway between your hip and knee. The probe will be secured with a tensor bandage tape to minimize movement, and covered with black plastic to prevent other light from entering the area. You may experience a bit of discomfort by having the probes secured to your leg during the exercise but will soon get used to it.

Finger prick: Before and after each test, a pinprick will be administered to your right middle finger and a drop of blood will be used to observe the muscle by ‐ products (lactic acid) of exercise.

Possible Risks and Discomforts: Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk, as stated by American College of Sports Medicine, is 6 in 10,000 for adverse outcomes in people at higher risk.

There may be discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise.

To monitor your safety the researcher will be present at all times during testing. The attending researcher has Cardiopulmonary Resuscitation level 3, Emergency Medical Responder (equivalent first aid as firefighters) and the Canadian Society of Exercise Physiology Certified Personal Trainer. There is also a telephone in the lab to call emergency services (911) in the advent of an adverse event.

Potential Benefits of Participation: This is a basic physiology study and, as such, there will be no direct benefits received as a consequence for participating in the study. Due to the nature of exercise, there may be some beneficial cardiovascular adaptations (increased fitness), but this will be temporary and disappear within a few weeks of the completion of the study.

If you are interested, the rationale for conducting the research and theory and significance of each of the tests will be explained, as will your individual results from each of the tests. You will also have the opportunity to learn about and better understand your physiological response to these exercise situations. You will receive information about if, and/or how the two different menstrual phases (follicular and luteal) affects your breathing as well as how your body produces and utilizes energy during exercise at various intensities.

Other Pertinent Information: You are encouraged to ask questions regarding the purpose of the study, specific measures or outcomes of your exercise tests, or overall findings and conclusions from this research study.

Privacy and Confidentiality Procedures: Data is stored for the duration of the study and then will be deleted or shredded. Your records are listed according to an identification number rather than by your name. We will ask permission to store your de ‐ identified study data in the Centre for Activity and Aging database for future research. Published reports resulting from this study will not identify you by name. Representatives of the Western University's Health Sciences Research Ethics Board may contact you or require access to your study ‐ related records to monitor the conduct of this research.

Voluntary Participation: Participation in the study is voluntary. You may refuse to participate, refuse to answer any questions and withdraw from the study at any time with no effect on your academic or employment status. You will be given a copy of this letter of information and signed consent form. You do not waive any legal rights by signing the consent form.

Version Date: 03/10/2017

Consent Form

Study Title: ***The Effects of Periodic Breath-Holding During Intermittent Exercise on Energy System Contribution, Lactate Threshold, and Regional Blood Flow Distribution in Females***

Principal investigator: Dr Glen Belfry PhD

Co-investigator: Jordyn Smith MSc Candidate

I have read the Letter of Information and have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Participant

Name (please print) _____

Signature _____ Date _____

Investigator

Name (please print) _____

Signature _____ Date _____

GROUP A FP start	Cycle Day	GROUP B LP start
Menses begins	Day 1	Menses begins
Test 1: VO_2 max test (20W/min)	**Day 2**	
	Day 3	
Test 2	**Day 4**	
Test 3	**Day 5**	
Test 4	**Day 6**	
Test 5	**Day 7**	
	Day 8	
	Day 9	
	Day 10	
Start testing with ovulation sticks	**Day 11**	Start testing with ovulation sticks
	Day 12	
	Day 13	
Postive test, LH surge detected	**Day 14**	Postive test, LH surge detected
	Day 15	
Test 6: VO_2 max test (20W/min)	**Day 16**	**Test 1**: VO_2 max test (20W/min)
	Day 17	
Test 7	**Day 18**	**Test 2**
Test 8	**Day 19**	**Test 3**
Test 9	**Day 20**	**Test 4**
Test 10	**Day 21**	**Test 5**
	Day 22	
	Day 23	
	Day 24	
	Day 25	
	Day 26	
	Day 27	
	Day 28	
Menses begins	**Day 1 (next cycle)**	Menses begins
	Day 2	**Test 6:** VO_2 max test (20W/min)
	Day 3	
	Day 4	**Test 7**
	Day 5	**Test 8**
	Day 6	**Test 9**
	Day 7	**Test 10**

Appendix C: Three Day Food Tracker

3 Day Food Tracker: Ramp Incremental Exercise Study

Instructions for Use:
This food tracker is to be used for the 3 days prior to the first wave of testing. Jordyn will tell you when you are to start using it. You are to write what your meals and snacks are for each day and count how many glasses of water (or herbal tea) you have throughout the day. Some people find it helpful to take pictures of their meals while keeping a diary to help with portion sizes. An example 'day' is outlined for you below:

Example:

Date	Breakfast	Lunch	Dinner	Snacks	Water/ Herbal Tea
Ex1	Coffee Granola – 1 cup Low fat vanilla yogurt cup Banana	Spinach salad with chicken Balsamic dressing Chocolate milk	Salmon Brown rice (~3/4 cup) Steamed broccoli and carrots.	Dark chocolate & almond granola bar Popcorn (dessert)	8

Your Meal Tracker:

Date	Breakfast	Lunch	Dinner	Snacks	Water/ Herbal Tea

Appendix D: Ovulation Testing Stick Instructions

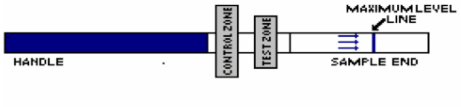
One Step Ovulation Urine Test

Cat. No. W2 –S

The Wondfo One Step Ovulation Urine Test is a qualitative test used to predict when there is a LH surge, and in turn, when you are likely to ovulate.
For *in vitro* self test use only. For external use only.

HOW DOES IT WORK?

The LH (Luteinizing Hormone) which is in the urine of normal women will increase dramatically in the middle of the menstrual cycle. The LH increase triggers ovulation which is when the egg is released periodically from Normal fertile women. WHO experts state that LH testing is a reliable way to detect ovulation. Ovulation will occur after 24-48 hours following a positive test.
When specimen is dropped into a midstream, capillary action carries the specimen to migrate along the membrane. When LH in the specimen reaches the Test Zone region of the membrane, it will form a colored line. Absence of this colored line suggests a negative result.
To serve as a procedure control, a colored line will appear at the Control Zone region, if the test has been performed properly.
The test strip detects ovulation with a high degree of certainty. It is a valuable tool in helping to achieve pregnancy since it determines the period that the egg and the sperm cells will meet in the best conditions.
Ovulation may be irregular because of circumstances, emotions and other factors in your life. You cannot presume that ovulation always occurs at the same time after menstruation. Therefore, you should test again in each menstrual cycle.



CONTENT OF THE TEST KIT

1. One pouch containing a strip and desiccant. The desiccant is for storage purposes only, and is not used in the test procedures.
2. Leaflet with instructions for use.

WHAT ELSE DO YOU NEED?

1. A clean, dry, plastic or glass container to collect the urine.
2. Timer (watch or clock)

PRECAUTIONS

1. This kit is for external use only. Do not swallow.
2. Discard after first use. The test strip cannot be used more than once.
3. Do not use test kit beyond expiration date.
4. Do not use the kit if the pouch is punctured or not well sealed.
5. Keep out of the reach of children.

STORAGE AND STABILITY

1. Store at 4° C to 30° C in the sealed pouch up to the expiration date.
2. Keep away from direct sunlight, moisture and heat.
3. DO NOT FREEZE.
4. Preferably open the pouch only shortly before the test.

SPECIMEN COLLECTION AND PREPARATION

WHEN TO COLLECT URINE FOR THE TEST?

Any urine specimen is appropriate for Ovulation Testing.

HOW TO COLLECT URINE?

Urine specimens may be collected in any clean, dry, plastic or glass container.

WHEN TO BEGIN TESTING

The length of the menstrual cycle is the duration from your first menstrual bleeding day to the day before the next bleeding begins. Determine the length of menstrual cycle before test. Please refer to the chart below to determine when you should start testing. If your cycle is shorter than 21 days or longer than 38 days, consult a physician. If you do not know your cycle length, you may begin the test 11 days after your first period since the average cycle length is 28 days. Perform 1 test each day over a 5 days period, or until the LH surge has been detected.

Cycle Chart

Cycle Length	Day of Cycle to Begin Testing
21 days	day 5
22 days	day 6
23 days	day 7
24 days	day 8
25 days	day 9
26 days	day 10
27 days	day 11
28 days	day 12
29 days	day 13
30 days	day 14
31 days	day 15
32 days	day 16
33 days	day 17
34 days	day 18
35 days	day 19
36 days	day 20
37 days	day 21
38 days	day 22
39 days	day 23
40 days	day 24

Example: If your cycle is normally twenty-six days, the Cycle Chart indicates testing should begin on day 10. The following calendar shows how to determine day 10.

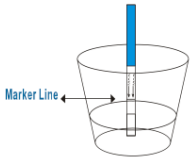
Sample calendar

1	2	3 + day1	4	5	6	7
8	9	10	11	12# day10	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28

3 + = The first day of menstrual bleeding
12# = The day to begin testing (Day 10)

HOW TO DO THE TEST

1. The test strip and urine have to be at room temperature (10°C ~30°C) for testing.
2. Remove the test strip from the sealed pouch.
3. Immerse the strip into the urine with the arrow pointing towards the urine. Take the strip out after 3 seconds and lay the strip flat on a clean, dry, non-absorbent surface (such as the mouth of the urine container).
   IMPORTANT: Do not allow the urine level to exceed the MAX (marker line), otherwise the test will not perform correctly.
4. Read results in five minutes. **Do not read results after more than 5 minutes**.



HOW TO READ THE RESULTS?

No LH Surge: Only one color band appears on the control region, or the test band appears but is lighter than the control band. This means there is no LH surge.

LH Surge: If two color bands are visible, and the test band is equal to or darker than the control band, one will probably ovulate in the next 24-48 hours. If trying to get pregnant, the best time to have intercourse is after 24 but before 48 hours.

Invalid: No visible band at all. Repeat with a new test kit. If test still fails, please contact the distributor or the store where you bought the product, with the lot number.

LIMITATIONS

1. The test works only when the test procedures are precisely followed.
2. This test may not be used as a form of birth control.
3. Consult a doctor if irregular or unusually long cycles are experienced.

QUESTIONS &ANSWERS

1. *There is much difference between the control lines of two tests. Is this a concern?* No. Variations in the color of the control band will not affect the test result. It is only effective to compare the color of the test band to that of the control band of the same device on the day the test is perform
2. *Can test results be interpreted after more than five minutes?* No. Test results must be read at 5 minutes. Though a positive result should not change for several days, a negative result may change to a false positive within minutes after the end of the testing period, which would not be an accurate reading. It is always best to read the results at the 5 minute testing period and then discard the test to avoid confusion.
3. *How long should I continue to perform the test?* At least 5 days or until the LH surge has been detected.
4. *A pink background color and vertical streaking appeared in the result area during the testing period. Is this a concern?* No. Each urine sample will vary in its chemical makeup, as will the humidity of the air in testing chamber (room). Such variations in physical conditions can cause the vertical streaking and/or the pink-rose background color but will not affect the test results. As long as the control band appears within five minutes, the test is working properly.
5. *Do alcohol or common medications affect the test*? No, but you should consult your physician if you are taking any medication.
6. *Once I see a positive result, when is the best time to have intercourse*? Ovulation is likely to occur within 24-48 hours. This is your most fertile time. Sexual intercourse is advised within this time frame.
7. *I am now using the basal body temperature method (BBT). Does this test replace BBT?* The rising in basal body temperature primarily indicates that ovulation has already occurred. This test indicates that ovulation is about to occur.
8. *I have received a positive result and had intercourse during these fertile days. I have not become pregnant. What shall I do?* Many factors can affect your ability to become pregnant. Often you may need to use the test kit for 3-4 months. You and your partner should consult your physician if pregnancy is not achieved after 3-4 months.
9. *Will the amount of liquid I drink affect the result?* We suggest that you limit your fluid intake for about two hours before you collect your urine. Because heavy intake of fluids prior to testing will dilute the hormone in your urine.

MEANING OF SYMBOLS ON PACKAGE


Keep away from sunlight


Store between 4°C and 30°C


Keep dry


Do not re-use

CE
0537

Authorized Representative :
Qarad b.v.b.a.
Volmolenheide 13
B-2400 Mol, Belgium

Appendix E: Advertisement Poster

Advertisement

WANTED:

PARTICIPANTS FOR AN EXERCISE PHYSIOLOGY EXPERIMENT

The Effects of Periodic Breath Holding During Intermittent Exercise on Energy System Contribution, Lactate Threshold, and Regional Blood Flow Distribution will be studied

Principle Investigator- Glen Belfry
Co-investigator- Jordyn Smith

We are seeking volunteers for a study that examines the energy system contribution, lactate threshold, and regional blood flow distribution to periodic breath holding during intermittent exercise.

The experiment will be conducted at the Centre for Activity and Aging using breath-by-breath oxygen uptake and near infrared spectroscopy. Participants will be required to come into the laboratory eight times a total of eight tests.

The study involves a total of 9 visits, with 1 test per visit. The first visit will be approximately 45 min long and will involve reading and signing a letter of information and an exercise test for determination of your maximal oxygen uptake test. On the second visit, another maximal oxygen uptake test will be completed with an intermittent breath holding (5 sec) and 25 sec normal breathing. Test 3 is a 6 min continuous trial at a moderately hard intensity. Test 4 is a 6 min continuous trial at moderately hard intensity with the intermittent breath holding protocol. Test 5 is a 6 min moderately hard intensity intermittent exercise with the intermittent hypoxia protocol (5 sec high power output with breath holding and 25 sec at moderately hard intensity with normal breathing). Test 6, 7, 8 will be a repeat of test 3, 4, 5, respectively.

Volunteers must be healthy, non-smoking, females between the ages of 18 and 35.

Curriculum Vitae

Name: Jordyn Lynn Smith

Post-secondary Education and Degrees:

Wilfred Laurier University
Waterloo, Ontario, Canada
2019-2020 M.B.A.

Western University
London, Ontario, Canada
2017-2019 M.Sc.

Western University
London, Ontario, Canada
2013-2017 B.Sc.

Honours and Awards:

Western Graduate Research Scholarship
2018-2019, 2017-2018

Ontario Graduate Scholarship
2017-2018

Jack Cowin Scholarship to Study Abroad
2016

Western University Deans Honours List
2013-2017

Related Work Experience

Teaching Assistant
Western University
2017-2019

Research Assistant
Western University
2018

Publications (Abstracts):

J. Smith & G. Belfry. Despite Menstrual Phase Differences in Muscle Deoxygenation, Respiration, and Blood Lactate, Exercise Performance is Preserved. *Medicine and Science in Sports and Exercise, Volume 51:5 Supplement.*

C. Brooke, J. Smith, & G. Belfry. Menstrual Phase Differences in the Physiological Resolution of Periodic Breath-Holding During Heavy Intensity Fartlek Exercise. *Medicine and Science in Sports and Exercise, Volume 51:5 Supplement.*