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3km Track Time Trial Performance in Cross-Country Skiers After a High Intensity Training Session

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3km Track Time Trial Performance in Cross-Country Skiers
After a High Intensity Training Session

by

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A THESIS

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Abstract

Men and women have different morphology and physiology that lead to sex differences in performance and fatigability. Although women continue to increase their participation and performance in sport, the research used to guide training principles has been heavily reliant on male athletes. One reason a woman's physiology differs is because of the fluctuating hormones throughout the menstrual cycle. Estrogen and progesterone, are hormones known to influence metabolism as well as basal body temperature regulation, potentially impacting a women's training, performance and fatigue. Race-specific performance can be effectively measured by time-trials, and time trial (TT) performance is influenced by pacing strategy. Pacing strategy is a learned skill and less experienced athletes are likely to have a variable pacing strategy which may reduce performance. Therefore, the purpose of this study was to determine the influence of sex, menstrual cycle phase, age and pacing strategies on 3km track TT performance after a high intensity interval training (HIIT) session in cross-country (XC) skiers. Thirty female and nine male XC skiers completed 3 days of testing/training: a 3km track TT on Day 1 (pre-HIIT) and Day 3 (post-HIIT), and a HIIT session composed of 4-8x 800m on Day 2. An overall improvement in performance from pre- to post-HIIT TTs was observed ($p < 0.01$). Significant differences were not observed in TT performance after HIIT between sexes ($p = 0.16$) or menstrual cycle phases ($p = 0.26$). There was a trend for greater improvement in TT performance after HIIT in younger (u18) athletes compared to older (18+) athletes ($p = 0.06$). Pacing strategy of u18 athletes did not differ between those who did or did not improve their performance, although all u18 athletes improved pacing strategy in the Post-HIIT TT. Most 18+ athletes did not improve pacing strategy; however, those who did were significantly more likely to also improve their TT performance.

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Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Figures and Illustrations.....	viii
List of Symbols, Abbreviations and Nomenclature.....	ix
Statement of Contribution.....	x
Chapter 1: Introduction.....	1
1.1 Purpose.....	1
1.2 Cross-Country Skiing.....	1
1.3 High Intensity Interval Training.....	2
1.4 Rationale and Population.....	3
1.5 Objectives.....	4
1.6 Hypothesis.....	5
Chapter 2: Literature Review.....	6
2.1.1 The Menstrual Cycle.....	6
2.1.2 Exercise and Menstrual Dysfunction.....	8
2.1.3 The Influence of Hormones on Physical Performance.....	8
2.1.4 Menstrual Cycle Phase and Endurance Performance.....	12
2.2 Fatigue in Endurance Athletes.....	14
2.2.1 Exercise Induced Fatigue.....	14
2.2.2 Methods of Quantifying Fatigue.....	16
2.2.3 Sex Differences in Fatiguability.....	17
2.3.1 High Intensity Interval Training.....	18
2.3.2 Time Course in Recovery from High Intensity Interval Training.....	18
2.3.3 High Intensity Interval Training in Younger Athletes (≤ 18 years).....	19
2.3.4 Sex Differences in High Intensity Interval Training.....	20
2.3.5 Sex Differences in Adaptation to Endurance Training and High Intensity Interval Training.....	21
2.4 Using Time Trials to Measure Performance.....	23

2.5 Pacing and Performance	24
2.5.1 Pacing Strategies for Endurance Performance	24
2.5.2 Pacing Strategies for Distance Cross-Country Ski Racing.....	26
2.5.3 Pacing Strategies for Distance Running Events	26
2.5.4 Influence of Age and Performance Level on Pacing.....	27
2.5.5 Pacing Strategy and Fatigue	28
Chapter 3: Improved 3km Track Time Trial Performance after High Intensity Interval	
Training in Competitive Cross-Country Skiers (Study 1)	30
Abstract.....	31
Introduction	32
Methods	36
Results	40
Discussion.....	45
Conclusion.....	50
References	51
Chapter 4: Differences in 3km Track Time Trial Pacing and Performance by Age in	
Competitive Cross-Country Skiers: A Field Study (Study 2).....	56
Abstract.....	57
Introduction	58
Methods	61
Results	64
Discussion.....	66
Conclusion.....	69
References	70
Chapter 5: Conclusion	72
5.1 Limitations.....	73
5.2 Future Directions.....	74

Bibliography	76
Appendices	88
Appendix A: Retrospective Sample Size Calculation	88
Appendix B: Fatigue Flow Chart (Enoke & Duchateau, 2016).....	90
Appendix C: TCPS2 Core Certificate.....	91
Appendix D: Recruitment Posters	92
Appendix E: Ethics Approval	94
Appendix F: Consent Form.....	96
Appendix G: Questionnaires	100
Appendix H: Clearblue Ovulation Testing Procedures	102
Appendix I: Labrix Salivary Hormone Testing Procedures	104
Appendix J: Practice Plans for Testing Sessions	105
Appendix K: Simcoe County Track Rental Agreement & Insurance Form	108
Appendix L: Budget	112
Appendix M: Raw Data (Menstrual Cycle Phase)	113
Appendix N: Raw Data (Hormones – Labrix Results)	114
Appendix O: Hormone Descriptive Statistics	115
Appendix P: Raw Data (Pacing)	116
Appendix Q: Raw Data (Performance)	117
Appendix R: Raw Data (Rating of Fatigue Questionnaires & Feeling Scale)	118

List of Figures and Illustrations

Chapter 3: Improved 3km Track Time Trial Performance after High Intensity Interval Training in Competitive Cross-Country Skiers (Study 1)

Table 1: Self-Reported Participant Characteristics (F=30, M=9), Reported as Mean and Standard Deviation (\pm)	37
Table 2: Testing Protocol Outline.....	38
Figure 1: Performance Change in Men (n=9) and Women (n=30)	41
Figure 2 : Performance Change in Male (n=9), Female-Low Hormone (n=18), Female-High Hormone (n=8), and Female-Oral Contraceptives (n=4)	42
Table 3: Self-Reported Rating of Fatigue (ROF-Q) Grouped by Menstrual Status	43
Table 4: Self-Reported Rating of Fatigue (ROF-Q) for All Participants and Grouped by Sex.....	43
Table 5: Self-Reported Affect Score (Feeling Scale (FS)) Grouped by Menstrual Status	44
Table 6: Self-Reported Affect Score (Feeling Scale (FS)) for All Participants and Grouped by Sex	45

Chapter 4: Differences in 3km Track Time Trial Pacing and Performance by Age in Competitive Cross-Country Skiers: A Field Study (Study 2)

Table 1: Self-Reported Participant Characteristics (F=30, M=9), Reported as Mean and Standard Deviation (\pm)	61
Table 2: Testing Protocol Outline.....	62
Figure 1: Mean Performance Change (with SD bars) in u18 Participants (n=21) and 18+ Participants (n=18)	64

Figure 2: Change in Sum of Differences by Athlete Group (Performance Improvement or
Performance Decrement).....65

Table 3: Combined, u18 and 18+ Pacing Characteristics by Performance Change66

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
18+	Over 18 years of age
HH	High Hormone
HIIT	High Intensity Interval Training
HR	Heart Rate
HRV	Heart Rate Variability
LH	Low Hormone
MC	Menstrual Cycle
PD	Performance Decrement
SIT	Sprint Interval Training
TT	Time-Trial
U18	Under 18 years of age
XC	Cross-Country
$v\text{VO}_2\text{peak}$	Velocity at peak oxygen uptake
VO_2max	Maximal oxygen uptake
VO_2peak	Peak oxygen uptake, when oxygen uptake does not plateau or is not verified by a verification stage in a maximum oxygen consumption test

STATEMENT OF CONTRIBUTION

Chapter 3 (Study 1):

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Conception and design of the experiments: AW, PKDB, LP, CL. Collection of data: AW.

Analysis and interpretation of data: AW, PKDB, LP, CL. Drafting the article: AW, PKDB.

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CHAPTER 1:

INTRODUCTION

1.1 Purpose

The purpose of this thesis is to better understand how sex and menstrual cycle phase influence fatigue and performance after a high intensity interval training (HIIT) session in cross-country (XC) skiers. Additionally, considerations were made to understand how age and pacing strategy influence TT performance. The athletes were exposed to a HIIT load so as to induce acute fatigue measured by change in time-trial (TT) performance. A three-step menstrual cycle tracking protocol (calendar counting, urinary luteinizing hormone testing, and salivary estrogen and progesterone samples) was employed to ensure this method adhered to the most recent standards for menstrual cycle research in athletes (Janse De Jonge, Thompson & Han, 2019). The use of hormone verification allowed for comparison between athletes with higher and lower levels of estrogen and progesterone since low estrogen and progesterone are expected in the early and mid-follicular phase, whereas higher estrogen and progesterone are expected in the luteal phase (Janse de Jonge et al., 2019).

1.2 Cross-Country Skiing

XC skiing is a unique sport where athletes compete in several events ranging from 1.3km sprint races with knock-out heats to 10-15km individual TTs to 50km mass start races (Sandbakk & Holmberg, 2014). This wide range of performance demands shows that XC skiers are some of the fittest athletes with $VO_2\text{max}$ values reported as high as $>70\text{ml/kg/min}$ in women and $>80\text{ml/kg/min}$ in men (Ingjer, 1991). While races can often be quite long in duration (20-180mins), athletes typically select a higher intensity while climbing hill sections and use the downhill sections as recovery. This style of racing demands that skiers have an exceptional anaerobic capacity in addition to a high $VO_2\text{max}$ (Sandbakk & Holmberg, 2014). To achieve

this, elite XC skiers will train 800-900h per year with 85% of those hours spent training aerobic endurance energy systems, with 90-110 sessions per year training at intensities greater than 80% of maximal heart rate (Sandbakk & Holmberg, 2014).

Competing in a fatigued state is relevant in XC skiing since events typically consist of 2-4 races over 2-7 days with few rest days in between races. Elite XC skiers will also compete in multi-day stage races with no rest days or minimal rest between stages.

1.3 High Intensity Interval Training (HIIT)

HIIT refers to the concept of alternating between periods of highly intense work and periods of rest, where the work intervals are prescribed at an intensity between 80-100% of an individual's maximum effort (MacInnis & Gibala, 2017). HIIT is used by endurance athletes to improve performance by targeting both peripheral and cardiovascular adaptations such as increased muscle oxidative capacity and myocardium enlargement (Buchheit & Laursen, 2013). HIIT can also cause fatigue if it is not appropriately prescribed, and chronic training-induced fatigue can lead to overreaching or overtraining causing a reduction in performance (Kellmann, 2010).

Research in the field of exercise physiology has been conducted primarily with male participants and understanding of how hormones such as estrogen and progesterone may impact an athlete's response to HIIT has not been thoroughly explored (Sims & Heather, 2019; Costello, Bieuzen & Bleakley, 2014). Sex and menstrual cycle differences should be considered so that appropriate prescription of HIIT can be made without inducing overreaching or overtraining in male and female athletes.

1.4 Rationale and Population

The 3km track TT has been identified by Nordiq Canada as a field-testing protocol that athletes should perform 2-3x/year so as to track improvements in performance (Nordiq Canada, 2018). For XC ski athletes, the 3km track TT takes between 10-15 minutes to complete, indicating a combination of aerobic and anaerobic energy systems being utilized. During a 3km TT, athletes will typically sustain an intensity that is close to their anaerobic lactate threshold (Welde et al., 2003; Larsson et al., 2002), and this threshold is typically achieved at approximately 80% of VO₂max (Binder et al., 2008).

In this study the HIIT intervention was selected to be 4-8 x 800m so as to train similar metabolic pathways (combination of aerobic and anaerobic) that were being tested. Athletes were instructed to run at a pace 5% faster than recorded in their initial TT, to ensure they were training above anaerobic lactate threshold and at an intensity higher than their self-selected 3km TT pace. With this style of HIIT workout, 2-4 minutes of rest is appropriate between intervals for endurance athletes (Buchheit & Laursen, 2013b; Seiler, Joranson, Olesen & Hetlelid, 2013). A shorter rest duration causes greater metabolic strain (increase in anaerobic glycolytic pathway) and subsequently increases the overall difficulty of a session (Smilios et al., 2018). The range of 4-8 reps was employed because it would give an appropriate workout for the age and development of athletes, while also allowing for coach and athlete buy-in. As competitive XC skiers gain experience, they increase their training load systematically until they stabilize at their highest achievable training volume. Along with this increase in total training hours, athletes will also systematically increase the total volume of intensity that they perform in a given year (Solli, Tonnessen & Sandbakk, 2017). This style of HIIT training is typically performed with total work durations of approximately 15-20mins in junior XC skier racers, although the total work duration

may increase as their training capacity increases (Sandbakk, O. Sandbakk, S.B. Ettema & Welde, 2013). To objectivity add to the workout design, we set an arbitrary cut off of a 10% increase in lap time, to stop athletes from over-exerting themselves and to provide an end point to their workout. While track based HIIT is not always perfect, it can be used successfully when a reference race or TT is used to calculate lap times (Buccheit & Laursen, 2013).

Long HIIT intervals, such as described above, are used to target aerobic and anaerobic metabolic adaptations as well as neuromuscular adaptation. These HIIT sessions can lead to metabolic consequences such as glycogen depletion, accumulation of metabolites (i.e., blood lactate), and neuromuscular strain in lower body muscles (i.e., hamstrings) (Buccheit & Laursen, 2013b). Recovery time-course is highly individual; training status of the athlete and impact of the workout on different systems (ie, muscle glycogen or neuromuscular strain) will influence the amount of time needed to recover. In general, 48hrs between HIIT sessions is a commonly used guideline to ensure athletes recover adequately so that they can deliver a high level of performance in the following sessions. Excessive, repeated HIIT sessions can lead to acute and chronic (over-training) fatigue when inadequate recovery is provided (Buccheit & Laursen, 2013b). Our protocol did not adhere to the guideline of providing 48hrs between HIIT sessions and the TTs, since our objective was to increase fatigue in participants.

1.5 Objectives

The overall objective of the thesis was to understand how a HIIT session influences performance in XC skiers. The primary and secondary objectives of study 1 were to determine if sex and menstrual cycle phase influence fatigue and performance after a HIIT session in XC skiers, respectively. The primary and secondary objectives of study 2 were to investigate if age and pacing strategy influence TT performance after a HIIT session in XC skiers, respectively.

The outcomes of these objectives were directed at improving training program design and delivery in competitive and high-performance XC athletes.

1.6 Hypothesis

The primary hypothesis was that a session of HIIT would result in sex differences in performance fatiguability. This hypothesis was based on the findings of Hunter (2016) who found sex differences in fatigue with isometric strength tasks. Since the effect of menstrual cycle phase on fatigue is unclear in the literature, the secondary hypothesis was that a session of HIIT would result in fatigue and performance differences between menstrual cycle phases. The tertiary hypothesis was that differences in performance improvement after HIIT between younger (u18) and older (18+) skiers would be observed. Our final hypothesis was that an improvement in pacing strategy (a more even pace) would be associated with improved TT performance post-HIIT session. This final hypothesis was informed by Abbiss & Laursen (2008) who suggested a more even strategy (or rather, a less significant decline in speed) contributes to improved performance.

CHAPTER 2:

REVIEW OF LITERATURE

2.1 The Menstrual Cycle

The average menstrual cycle (MC) length is considered to be 28 days, but there is significant individual variation, and young healthy women can have a cycle length that ranges from 25-34 days (Bakos, Lundkvist, Wide & Bergh, 1994; Harlow, 2000). The menstrual cycle can be broken into two distinct phases, luteal and follicular, which are separated by ovulation (Oosthuysen & Bosch, 2010). The typical phase lengths for the follicular and luteal phases are 15 and 13 days, respectively, although there can be still be significant individual variation in phase lengths (Harlow, 2000; Wilcox, Dunson & Baird, 2000). A normal menstrual cycle length is not indicative of ovulation. Anovulation is present in 7% of women aged 25-34 years with average cycle lengths and more prevalent in women with shorter cycle lengths, younger girls and older women (Harlow, 2000). Amenorrhea, defined as missing three consecutive menses (Mountjoy et al., 2014), has prevalence rates as high as 37% in female distance athletes (Heikura et al., 2018).

The luteal phase can be described as the “high hormone phase” where estrogen and progesterone are highest, whereas the follicular phase can be described as the “low hormone phase” where estrogen and progesterone are lowest (Striker et al., 2006). For the purpose of understanding how the menstrual cycle impacts training and performance, our review of hormonal changes throughout the MC will be limited to estradiol (estrogen), progesterone. The start of the MC is described as the onset of menses. The follicular phase begins with the onset of menses and lasts until ovulation occurs which indicates the transition to the luteal phase (Striker et al., 2006). Both estradiol and progesterone are at their lowest values in the early follicular phase. Estradiol starts to increase in the late follicular phase with its peak immediately prior to

ovulation; following this, levels decrease slightly but remain elevated through the early, mid and late luteal phase (Striker et al., 2006). Progesterone levels remain relatively low until after ovulation, start to rise in the early luteal phase and reach a peak during the mid-luteal phase (Striker et al., 2006). Due to the mismatch in when estrogen and progesterone reach their peak, researchers may also include a third distinct phase, either named “ovulatory” or “late-follicular” which represents high estrogen and low progesterone levels (Janse De Jonge, Thompson & Han, 2019).

The early follicular, late follicular and mid-luteal phase each represent a different progesterone to estrogen ratio (Pg: E₂). In the early follicular phase the Pg: E₂ ratio is relatively well balanced; serum progesterone and estrogen levels are expected to be near 0.64 nmol/L and 150 pmol/L, respectively. In the late follicular phase the Pg: E₂ ratio is relatively low; serum progesterone and estrogen levels are expected to be near 0.64 nmol/L and 450 pmol/L, respectively. In the mid-luteal phase the Pg: E₂ ratio is relatively high; serum progesterone and estrogen levels are expected to be near 36.25 nmol/L and 496 pmol/L (Stricker et al., 2006). In addition to larger cyclical patterns across the MC, estrogen levels also fluctuate throughout the day and are often found to be the highest in the morning (Bao, Liu, van Someren, Hofman, Cao & Zhou, 2003). Aerobic exercise has also been shown to decrease salivary progesterone levels in women (Ellison & Lager, 1986). Chatterton et al. (2005) observed that women with anovulatory cycles had similar levels of salivary estrogen compared to women with ovulatory cycles, although salivary progesterone levels were significantly reduced. These findings emphasize the importance of the Pg: E₂ ratio and the use of hormone sampling to verify menstrual status.

The predominant male sex hormone, testosterone is also present in females and the rate of production of testosterone will also vary throughout the MC. Peak rate of testosterone

production occurs in the pre-ovulatory phase (within the follicular phase) and the lowest rate of production in the mid-luteal phase (Sims & Heather, 2018).

2.1.2 Exercise and Menstrual Dysfunction

High levels of physiological stress (ie, exercise) are associated with menstrual dysfunction such as amenorrhea, oligomenorrhea, and luteal phase defects (Warren & Shantha, 2000; De Souza, 2003; Goodman & Warren, 2005). Amenorrhea is the most severe menstrual disturbance experienced by athletes and is characterized by a complete follicular and luteal suppression (De Souza, 2003). Amenorrhea is often observed as the absence of menses and can present in women who have previously had a regular menstrual cycle as well as in teenagers who have delayed onset of menarche (Goodman & Warren, 2005). Oligomenorrhea is a generalized term for irregular MC, where a woman may frequently “skip” menses or have several consecutive months without menses. Luteal phase defects (ie, luteal phase shorter than 10 days) are less severe menstrual disturbances but are highly prevalent in the athletic population and experienced by up to 79% of exercising females (De Souza, 2003; De Souza et al., 1998).

2.1.3 The Influence of Hormones on Physical Performance

It is well known that both estrogen and progesterone affect female physiology and that the fluctuations of these hormones can impact the training and performance of the female athlete (Sims & Heather, 2018). Lebrun, Joyce and Constantini (2013, pp. 290) suggest many different components of physical performance that may be influenced by the MC, such as: metabolic, respiratory, cardiovascular, thermoregulatory and musculoskeletal.

Estrogen and progesterone play a role in regulating muscle glycogen storage capacity and free fatty acid availability which can alter exercising metabolism (Oosthuyse & Bosch, 2010).

While estrogen and progesterone each play a role in regulating metabolism, their interactions can be complex. High levels of estrogen, such as in the late follicular phase, promotes glycogen uptake and storage in muscle fibres and liver at rest as well as sparing muscle glycogen stores and favouring lipid oxidation for fuel during exercise (Lebrun, Joyce & Constantini, 2013, pp. 286-287). When estrogen and progesterone are both high, such as during the luteal phase, they act to amplify storage of muscle glycogen in the liver as well as decreasing blood glucose kinetics (Lebrun, Joyce & Constantini, 2013, pp. 286-287). The actions of estrogen and progesterone may also act against each other which leads to further differences in metabolism between the late follicular phase (high estrogen, low progesterone) and the luteal phase (high estrogen, high progesterone). Estrogen decreases fat synthesis and increases lipolysis, whereas progesterone increases fat synthesis. High levels of estrogen are associated with increased mitochondrial enzyme activity (such as mitochondria carnitine acyltransferase 1) where as high levels of estrogen and progesterone together are associated with decreased mitochondrial enzyme activity (Lebrun, Joyce & Constantini, 2013, pp. 286-287). These changes in metabolism may impact physical performance by altering the availability and type of fuel for the working muscle. A reduction in glucose kinetics when estrogen and progesterone are high could be detrimental to endurance performance if glycogen stores are limited and gluconeogenesis is suppressed, although glucose ingestion during exercise may negate these effects (Oosthyse & Bosch, 2010). Additionally, increased lipid metabolism when estrogen is high could be beneficial for lower intensity endurance performance (Oosthyse & Bosch, 2010).

When performance is measured in a time-trial, increased carbohydrate metabolism in the mid-follicular phase appears to be beneficial for short duration high intensity time-trials (Campbell, Angus & Febbraio, 2001). Although, when glucose is consumed during exercise, the

influence of the MC on TT performance is reduced (Oosthuysen et al., 2005). This data suggests that increased estrogen in the mid- and late- follicular phase can be beneficial for time-trial performance, particularly when exogenous glucose is not available.

Progesterone plays a role in regulating ventilation and high progesterone levels, such as in the luteal phase, are associated with an increased rate of ventilation (Preston, Heenan & Wolfe, 2001). This may play a role in dyspnea and increased perceived exertion during exercise for non-athletes, although endurance training may negate this response (Lebrun, Joyce & Constantini, 2013, pp. 292-293). Progesterone has also been linked to the presentation of asthma; the sudden withdrawal of progesterone at the end of the luteal phase appears to aggravate asthma related respiratory symptoms and may be deleterious for performance (Lebrun, Joyce & Constantini, 2013, pp. 292-293).

Cardiovascular function is in-part regulated by both estrogen and progesterone. Estrogen increases endothelium vasodilatation which leads to increased blood supply to the heart and muscles and could potentially be beneficial for performance (Lebrun, Joyce & Constantini, 2013, pp. 285). Barba-Montero et al. (2019) recently reported that at the same submaximal intensity, absolute O₂ uptake and percentage of maximal O₂ uptake were higher in the mid-follicular phase (when estrogen starts to rise and progesterone remains low) compared to the early follicular phase (when both estrogen and progesterone are low), although these differences had a small impact on submaximal exercise capacity. Progesterone has been linked to an increase in resting heart rate (Sedlak, Shufelt, Iribarren & Merz, 2012), and a greater heart rate response to exercise has been documented in the luteal phase (Birch & Reilley, 1999). The actions of estrogen and progesterone may antagonize each other, and the impact of these changes in the cardiovascular system on physical performance is unclear (Lebrun, Joyce & Constantini, 2013, pp. 285).

Progesterone plays a role in thermoregulation (Charkoudian, Stephens, Pirkle, Kosiba & Johnson, 1999). During the luteal phase, high levels of progesterone lead to increased resting body temperature – by about 0.2C °– compared to the follicular phase (Charkoudian & Johnson, 2000; Kolka & Stephenson, 1997). While an increase in basal body temperature is reported to increase perceived exertion and decrease performance, there are conflicting reports of well-trained athletes showing no changes in performance across MC in hot and/or humid environments (Janse De Jonge, Thompson, Chuter, Silk & Thom, 2012; Lei et al., 2017).

Progesterone and estrogen have vastly different effects on protein metabolism where estrogen decreases protein oxidation and progesterone increases protein catabolism (Oothuyse & Bosch, 2010). These interactions can be extrapolated to suggest an anabolic effect of estrogen and a catabolic effect of progesterone. Testosterone is also well known for an anabolic effect on muscle; testosterone levels are highest in the follicular phase prior to ovulation and decrease during the luteal phase (Alexander, Sherwin, Bancroft & Davidson, 1990). Reis, Frick and Schmidtbleicher (1995) designed an experimental protocol that used MC-triggered training and found that improvements in strength training are increased when strength training is intensified in the follicular phase compared to the luteal phase. Researchers have suggested that periodization of training around the MC phases can maximize anabolic effects (Reis, Frick & Schmidtbleicher, 1995; Lebrun, Joyce & Constantini, 2013, pp. 298.; Sims & Yeager, 2016, pp. 8, 19-20). Graja et al. (2020) have recently demonstrated that indicators of muscle damage (creatinine kinase) are significantly higher in the pre-menstrual phase in comparison to the follicular phase and early luteal phase after intense exercise. These results suggest that athletes may be more susceptible to muscle damage in the pre-menstrual phase and that intense exercise may be better tolerated in the follicular phase (Graja et al., 2020). While some aspects of

performance have been studied relative to the MC, the influence of the MC on cardiovascular training adaptation and fatigue is less well known.

2.1.4 Menstrual Cycle Phase and Endurance Performance

Researchers continue to take interest in the influence of the MC phase on performance indicators such as VO_{2max} , endurance field tests, repeated sprint tests, and lactate thresholds (Gordon et al., 2018; Julian, Hecksteden, Fullagar & Meyer, 2017; Vaiksaar et al., 2011). Despite an increase in research on the topic, the influence of MC phase and oral contraceptives on performance shows conflicting results (Burrows & Bird, 2005; Constantini et al., 2005, Oosthuysen, Bosch & Jackson, 2005; Middleton & Wegner, 2006; Smekal et al., 2007; Rechichi, Dawson & Goodman, 2007; Oosthuysen & Bosch, 2010; Shaharudin, Ghosh & Ismail, 2011; Vaiksaar et al., 2011; Schaumberg, Jenkins, Janse De Jonge, Emmerton & Skinner, 2017; Julian et al., 2017; Gordon et al., 2018; Mattu, Iannetta, MacInnis, Doyle-Baker & Murias, 2019), possibly due to a lack of standardized methodology with assessment of the MC (Sims & Heather, 2018, Janse De Jonge et al., 2019). When conducting research on the effects of the MC on performance, the method of verification of MC phase as well as the point in the MC and the performance test(s) used will influence the findings of the study (Julian et al., 2017, Janse De Jonge et al., 2019).

Despite known physiological changes across the MC, VO_{2max} and time to exhaustion appear to be independent of MC phase (Oosthuysen & Bosch, 2010; Janse De Jonge, 2003). While a high VO_{2max} is needed to be a competitive athlete, VO_{2max} does not necessarily predict race performance amongst elite athletes as there are many other physiological measures of performance as well as technical, tactical and mental skills involved in racing. When determining

the impact of MC on performance in athletes it is important to consider aspects other than $\text{VO}_{2\text{max}}$.

Julian et al. (2017) investigated the effects of MC phase on female soccer athletes in a performance context. Throughout the study athletes maintained their typical training regime which improves transferability of these results to a high-performance domain. Significant differences were not achieved, with the small sample size, but a noticeable trend in all athletes towards a reduced maximal endurance performance in the mid-luteal phase was observed (Julian et al., 2017). These results are in line with Lebrun, McKenzie, Prior and Taunton (1995) who noticed a trend for a reduction in maximal endurance capacity in the luteal phase; again suggesting that competitive athletes' performances may be influenced by the fluctuations in estrogen and progesterone across the MC. Additionally, Shaklina et al (2016) studied competitive middle-distance track runners to investigate the changes in physical fitness and functional running performance across the MC. In this study PWC170 and a 4x400m workout were used as outcome measures and MC was broken into five separate phases (menstrual, post-menstrual, ovulatory, post-ovulatory and pre-menstrual). These researchers found that in the less competitive runners PWC170 results were significantly higher in post-ovulatory ($p<0.05$) and significantly lower in pre-menstrual ($p<0.05$) and that 4x400m lap times were significantly faster in post-menstrual and post ovulatory compared to ovulatory and pre-menstrual ($p<0.05$). While these results suggest differences in running performance across MC phases, many threats to internal validity were identified in this study such a lack of standardization in testing protocols and using indirect, submaximal tests such as the PWC170. More research is needed with 1) larger participant numbers and 2) protocols that are more robust for MC tracking to understand the true impact of the MC on training and performance of competitive endurance athletes.

2.2 Fatigue in Endurance Athletes

2.2.1 Exercise Induced Fatigue

In competitive sport, coaches and athletes strive to increase and optimize training load so as to maximize training adaptation and allow the athlete to improve the physical attributes that impact performance (Mujika et al., 2018). To stimulate an adaptation a training load must be significant enough to cause fatigue. This fatigue will result in improvements following the athlete's recovery (Borresen & Lambert, 2009). Monitoring and understanding fatigue is therefore crucial for obtaining optimal performance so that over-reaching or over-training do not occur. (Kellman, 2010).

The nature of fatigue can be classified as central, mental, or peripheral fatigue. Exercise characteristics (ie, duration, intensity) will influence the type of fatigue that occurs (Theofilidis et al., 2018). Central fatigue encompasses the nervous system which leads to a decrease in motoneuron activation and muscle recruitment for a given task (Nybo & Secher, 2004). Functionally, central fatigue can be described as a reduction in the maximal voluntary activation level for a muscular contraction (Gandevia, Mcneil, Carroll & Taylor, 2013). Central fatigue is primarily influenced by homeostatic disturbances (serotonin, ammonia, dopamine) within the central nervous system and a depletion of cerebral glycogen stores (Nybo & Secher, 2004).

Mental fatigue is a more recently researched phenomenon, which involves the brain but is independent of central fatigue. Marcora, Staiano and Manning (2009) demonstrated that mental fatigue triggered by completing cognitive tasks prior to exercising results in a decreased time to exhaustion. Mental fatigue impairs physical performance by increasing sensations of tiredness at

rest and increasing perceived exertion during exercise which in turn reduces an individual's ability to complete a physically demanding task (Marcora et al., 2015).

Peripheral fatigue refers to changes that occur at or distal to the neuromuscular junction which leads to a reduced contractile function of skeletal muscle (Wan, Qin, Wang, Sun & Liu, 2017). Common factors in peripheral fatigue include altered calcium kinetics, insufficient oxygen delivery, inadequate energy to sustain exercising metabolism and the byproducts of metabolism (Allen et al., 2008; Enoka & Duchateau, 2016; Wan, Qin, Wang, Sun & Liu, 2017). Ultimately, when attempting to understand fatigue in the context of performance, it can be difficult to separate peripheral and central fatigue to identify the "rate-limiting" cause of the reduction in performance (Enoka & Duchateau, 2016; Allen et al., 2008). The adoption of the term "fatigability" helps to discuss the different components of fatigue in relation to human performance.

Fatigability describes a decline in performance due to an inability to complete a task at the highest possible intensity (Finsterer & Mahjoub, 2014). According to Enoka and Duchateau (2016), fatigability can be broken down into two categories: 1- perceived fatigability (sensations that are interpreted by the athlete) and, 2- performance fatigability (objective measures of performance). This model suggests that perceived fatigability encompasses alterations in homeostasis (blood glucose, core temperature, hydration, etc.) as well as the psychological state of the athlete (arousal, expectations, mood, motivation, pain, etc.). Additionally, it suggests that performance fatigability encompasses contractile function (calcium kinetics, metabolism and products, blood flow, etc.) and muscle activation (voluntary activation, activation patterns, afferent feedback, etc.). A flow chart of the aspects of fatigue can be found in Appendix B taken from Enoka and Duchateau (2016).

Athletes can develop acute fatigue from a single bout of exercise or chronic fatigue can accumulate over multiple training sessions (Enoka & Duchateau, 2016). When an athlete is in a fatigued state it can be expected that they are no longer able to achieve a speed, power output or performance that they were able to achieve when they were in a recovered state (Halsen, 2014). When studying athletes, it's very challenging to identify the etiology of fatigue since many factors can influence an athlete's cognitive and physical performance at once (Enoka & Duchateau, 2016). For this reason, we will consider fatigue from a whole-body, multiple system view, without attempting to explain the specific mechanism of fatigue.

2.2.2 Methods of Quantifying Fatigue

Measurements of fatigue are task-specific, therefore when selecting a method to quantify fatigue, it is essential to understand the nature of the fatiguing exercise. There are two methods that can be used to quantify fatigue: (1) Fatigue index, (2) Performance decrement (which can be measured as decreases in time to exhaustion test or time trial (TT)). Fatigue index measures the decrease in power/force/speed throughout the duration of a prolonged fatiguing effort or contraction, or throughout a repeated series of efforts/contractions (Vandewalle, Peres & Monod, 1987; Finsterer & Mahjoub, 2014). Examples of tests include a Wingate test or repeated muscle contractions on an isokinetic dynamometer (Allen et al., 2008). Time to exhaustion tests the ability of an individual to maintain a submaximal or maximal effort/contraction until they are no longer able to sustain the required power/force/speed (Terzis, Spengos, Manta, Sarris & Geordiadis, 2008; Slawinski & Billat, 2005). Examples of tests include treadmill running tests at a constant speed or measurement of the number of repetitions completed of a submaximal load (Allen et al., 2008). TTs test the ability of an individual to execute the fastest possible completion of a pre-determined distance, such as a 5km running course or a 10km cycling

course. Performance decrement compares an individual's ability to perform before and after a fatiguing effort. Previous studies have shown that performance decrement can detect changes in performance due to muscle fatigue and mental fatigue (Marcora et al., 2008; Marcora et al., 2009)

Performance and fatigue in endurance athletes are closely related, and it can be challenging to measure one without considering the other. Endurance athletes are typically subject to competition and training schedules that demand a high level of performance despite being in a fatigued state. When considering how to measure fatigue in competitive and elite athletes, a model using performance decrement may best represent an athlete's ability to tolerate multiple aspects of fatigue and how it will impact their performance (Enoka & Duchateau, 2016).

2.2.3 Sex Differences in Fatigability

In tests of muscular strength, studies have demonstrated that men have greater performance fatigability than women in isometric contractions and lower velocity repeated dynamic tasks; however, sex differences in performance fatigability seem to be task dependant (Hunter, 2016). Additionally, males record a longer recovery time after a fatiguing exercise compared to women (Albert, Wrigley, McLean & Sleivert, 2006). Suggested mechanisms for the sex differences include, but are not limited to, contractile properties of the muscle, muscle perfusion and voluntary muscle activation (Wust, Morse, de Haan, Jones & Degens, 2008).

These studies were not conducted with consideration for MC phase in women, therefore the interpretation of sex differences may be limited. Markofski & Braun (2014) compared strength decrement and muscle soreness in an eccentric elbow extension in women in the luteal

phase and the follicular phase and found that women in the luteal phase had a greater strength decrement but noted no significant differences in muscle soreness between phases.

2.3 High Intensity Interval Training (HIIT)

HIIT can be prescribed as short duration HIIT (30s-60s effort), medium duration HIIT (60s-180s effort) or long duration HIIT (3-5mins effort), at an intensity at or above 90% of their maximum aerobic effort. In long duration HIIT, rest intervals are typically 2-5minutes, and total “working time” ranges from 8-20minutes. Duration of effort, duration of rest interval, and total “working time” (number of interval repetitions) are all factors that can be manipulated to achieve the desired physiological outcome (Buccheit & Laursen, 2013b).

HIIT is used by endurance athletes to improve performance by targeting physiological adaptations such as increased mitochondrial content, increased capillary density and maximum cardiac output (MacInnis & Gibala, 2017). HIIT has been shown to improve these metabolic and cardiovascular performance metrics after 2-8 weeks of 2-3 HIIT sessions per week resulting in performance improvements of 2-4% in well trained athletes (Daussin et al, 2007; Helgerud et al, 2007, Talanian et al., 2007; Laursen, 2010). Endurance training programs typically combine HIIT with low intensity volume training to enhance performance outcomes in sport-specific competition (Hydren & Cohen, 2015, Hebisz et al, 2019). HIIT can also cause fatigue if appropriate recovery is not prescribed and chronic training-induced fatigue can lead to overreaching or overtraining which may cause a reduction in performance (Kellmann, 2010).

2.3.2 Time-Course in Recovery from High Intensity Interval Training

The time-course of recovery from a HIIT session is still largely unknown (Buccheit & Laursen, 2013b), although there is an increased curiosity about the markers of fatigue in athletes

after they complete sport-specific workouts (Bessa et al., 2016; Binnie, Dawson, Pinnington, Landers & Pelling, 2013; Holt, Plews, Oberline-Brown, Merien & Kilding, 2019). Holt et al. (2019) demonstrated that rowers will have a larger decrease in parasympathetic heart rate variability (HRV), indicating higher fatigue, when HIIT is performed with longer intervals at anaerobic threshold. Despite the rapid HRV response after HIIT, HRV recovery accelerated after 30hrs post-HIIT, indicating a trend towards being in a more recovered state. Binnie et al. (2013) found that soccer players can have an increase in blood markers of muscle damage and inflammation at 24hrs post-HIIT, indicating that athletes had not recovered from the HIIT session after a day of recovery. Bessa et al. (2016) also found that cyclists had increased markers of skeletal muscle damage (ie, Creatine Kinase) at 3hrs, 12hrs and 24hrs post-exercise, although instead of a HIIT style workout they completed a continuous 60mins at 85% of VO₂peak.

Froyd, Millet and Noakes (2013) attempt to describe the time course of muscle fatigue in a TT. They had participants complete a quadriceps extension TT on a dynamometer (termination of TT occurred when 30,000J of work had been completed). They indicate that most of the musculoskeletal fatigue and decrease in muscular function occurs within the first 40% of the TT. Additionally, they suggest that significant musculoskeletal recovery occurs within 1-2mins of termination of the TT. While this experimental design does not accurately represent the demands of a sport-specific TT, it does give unique insight into the time-course of peripheral fatigue during a simulated TT.

2.3.3 High Intensity Interval Training and Performance in Young Athletes (≤ 18 years)

Currently HIIT in young athletes is not as well researched as in adults. A recent meta-analysis by Engel, Ackermann, Chtourou and Sperlich (2018) highlights that HIIT has a large positive effect on running speed and oxygen consumption at both aerobic and anaerobic lactate

thresholds, despite not appearing to significantly improve VO₂max in young athletes. The HIIT interventions that were included in this meta-analysis lasted from 5 days to 26 weeks and employed on average 2.5 HIIT sessions per week. Many of these studies in the meta-analysis included only male participants, indicating a possible bias of these results with an under representation of the female population.

When responses to a single HIIT session of younger males (approximately 11 years old) were compared to older males (approximately 29 years old) it was observed that younger males had a lower blood lactate and higher heart rate responses despite a lower performance level in comparison to the older males (Engel, Hartel, Strahler, Wagner & Bos, 2014). When the required recovery between HIIT sessions was analyzed by age, it appeared that prepubescent boys (9-12 years old) required less recovery time to repeat peak power, while pubescent boys (15-17 years old) and men (20-25 years old) were more similar in requiring a longer recovery time to reproduce peak power (Ratel, Bedu, Hennegrave, Dore & Duche, 2002; Zafeiridis et al., 2005). Engel et al suggested that this difference was observed because of a higher oxidative capacity in younger males, which was also supported by the observations of lower blood lactate levels (2014).

2.3.4 Sex Differences in High Intensity Interval Training

Females may recover more rapidly between short HIIT intervals (30s) in comparison to males which can affect the training stimulus from the workout (Schmitz et al., 2020). Females have also shown greater fatigue resistance than males in repeated sprints despite reporting higher perceived exertion (Laurent, Vervaecke, Kutz & Green, 2014). A possible mechanism to explain this observation is faster ATP recovery in females compared to males (Esbjornsson-Liljedahl, Bodin & Jansson, 2002) and a greater capacity to clear metabolites from working muscles due to

greater muscle perfusion (Hunter, 2014). The improved ATP recovery is a good explanation for reduced performance decrement throughout a single HIIT workout, although it is likely to be less relevant over a longer time course (ie, recovery between HIIT sessions over multiple days). After repeated sprints, females also have shown to have a smaller reduction of muscle glycogen in Type 1 muscle fibres (Esbjornsson-Liljedahl, Bodin & Jansson, 2002). This is likely attributed to sex differences in exercising metabolism, where females have a higher rate of lipid oxidation and a lower rate of glycogen breakdown (which spares muscle glycogen) in comparison to males (Devries, Hamadeh, Phillips & Tarnopolsky, 2006).

Forsyth and Burt (2019) also explored sex differences in post-exercise metabolism in response to sprint interval training (SIT), which is similar to HIIT but with shorter duration of efforts. While Forsyth and Burt (2019) report sex differences in recovery from SIT, their results contradict Laurent et al. (2014), indicating that men had significantly greater fat oxidation rates post-SIT and lower relative carbohydrate oxidation rates. Forsyth and Burt (2019) framed their paper in the context of energy expenditure and potential for weight loss rather than a competitive sport context, which explains the difference in measurement tools to understand exercise metabolism.

2.3.5 Sex Differences in Adaptation to Endurance Training and HIIT

Endurance training (ET) and HIIT are used concurrently by athletes to allow for cardiovascular and peripheral adaptation to training. Although current training strategies do not differ between men and women, it is possible the mechanisms of how males and females adapt to training may differ due to differences in body size, endocrine function, substrate use, muscle fibre type and enzyme activity (Billaut & Bishop, 2009). A meta-analysis by Diaz-Canestro and Montero (2019) demonstrated that, when the same volume of ET is performed, males will have a

significantly greater increase in absolute and relative VO₂max in comparison to females. The authors suggest that females may need to be exposed to a higher training volume than males to achieve a similar adaptation in VO₂max.

A common adaptation to ET and HIIT is increased muscle mitochondrial density (Jacobs et al., 2013), although it is not known if sex differences in mitochondrial adaptation to training exist. Montero, Madsen, Meinild-Lundby, Edin and Lundy (2018) found that when VO₂max is matched (in trained individuals), females will have increased fat and lactate oxidation during exercise and a greater mitochondrial volume density in skeletal muscle fibres compared to males. This difference disappears when adjusted for body size because females were on average smaller than males; this suggests that the greater mitochondrial volume density in females is associated with smaller body size. The authors suggest that ET may favour peripheral adaptations rather than central adaptations in females (Montero et al., 2018).

Howden et al. (2015) illustrate how sex differences in adaptation to ET and HIIT can evolve over 12 months of training. When untrained males and females follow the same periodized ET program, females experienced a greater improvement in VO₂max in comparison to males after 3 months of “base training” which prescribed higher volume of relatively low intensity exercise. Females experienced a plateau in VO₂max improvement during the remaining 9 months of training that involved an increase in the intensity of exercise prescribed. Males, in comparison, experienced improvements in VO₂max through the first 9 months of training (including both the low intensity and high intensity training), and only experienced a plateau in VO₂max in the final 3 months of training. Peripheral adaptations to training were not measured in this study, although would be interesting to see in future studies of long-term adaptation to training.

Finally, an opinion article by Cobbold (2018) discusses unpublished data that indicates that when healthy (likely untrained) males and females complete the same HIIT program for 6 weeks, males showed a significant increase in $VO_2\text{max}$ whereas females did not show a significant increase in $VO_2\text{max}$. These results suggest that males and females may have different metabolism during and post-HIIT leading to different physiological adaptations. The state of research on this topic demands that more research needs to be completed to understand the sex difference in response to HIIT from both health and performance perspectives.

2.4 Using Time Trials to Measure Performance

TTs are protocols known to be reliable and valid assessments in determining performance (Currel & Jenkendrup, 2008), and shown to be less variable and more reliable than time-to-exhaustion tests in runners completing distances of 1500m and 5000m TTs (Laursen, Francis, Abbiss, Newton & Nosaka, 2007). TTs are specific to “real-life” races since athletes are required to cover a pre-determined distance at a self-selected pace as fast as they can (Currel & Jenkendrup, 2008). Dantas, Pereira and Nakamura (2015) demonstrated that shorter TTs (5km in cycling, approximately 7-10mins in duration) correlate highly with aerobic endurance indices from incremental tests and are therefore comparable to longer (40km) TTs. Performance change can be measured by repeating a TT before and after a specific intervention, in our study the intervention will be a HIIT workout. Following an intervention a performance decrement or improvement, can indicate a negative or positive effect of an intervention, respectively. Previous studies have shown that performance decrement can detect changes in performance due to muscle fatigue and mental fatigue (Marcora et al., 2008; Marcora et al., 2009), suggesting that it may be an appropriate tool to quantify changes in performance due to fatigue.

TTs are used to assess fitness level and performance change for both younger and older competitive XC skiers. Age related differences in TT performance must be considered to effectively analyze improvements in performance over time. While older athletes may be more capable of delivering a high anaerobic power/speed output, younger athletes may have better fatigue resistance due to a greater oxidative capacity and reduced lactate production (Zafeiridis et al., 2005; Taylor, Kemp, Thompson & Radda, 1997).

TTs can be performed in both a laboratory setting and a field setting. The coefficient of variation has been reported at 1.6% for a 5km treadmill TT (Laursen et al., 2007), and at 3.4% for a 5km track TT (Denham, Feros & O'Brien, 2015). While variability of the measurement appears lower for treadmill TTs, this study selected a field testing protocol that could be easily replicated by coaches and athletes. In our study Track TTs will be used as they are more accessible for testing large groups of athletes.

2.5 Pacing and Performance

2.5.1 Pacing Strategies for Endurance Performance

In endurance events, pacing strategy can be assessed by plotting the lap time (or speed) throughout the duration of a race. Abbiss and Laursen (2008) highlight 5 different pacing strategies (even, positive, negative, “U” shape and “reverse J”) that an athlete may select during an endurance event. An even pacing strategy describes a constant speed throughout the duration of an event whereas a positive or negative pacing strategy shows a decrease or increase in speed throughout the race, respectively (Abbiss & Laursen, 2008). A negative pacing strategy can potentially increase potential for performance in endurance events because it delays significant spikes in blood lactate and muscle glycogen depletion (Abbiss & Laursen, 2005; Mattern,

Kenefick, Kertzer & Quinn, 2001). A positive pacing strategy is likely to result from starting a race too quickly and pace must be decreased in response to the homeostatic feedback caused by increased metabolites and decreased energy availability (Lander, Butterfly & Edwards, 2009; Stoggl et al., 2018; Abbiss & Laursen, 2008). Employing an even pacing strategy has been shown to be effective for successful performance in running, cycling, skiing, and other endurance events (Abbiss & Laursen, 2008). The laws of physics support the use of an even pacing strategy, where maintaining a constant speed allows for less energy to be expended during acceleration or deceleration (Swain, 1997). More complex pacing strategies can be described as “U” shape – where speed is high at the beginning, decreases mid-race, and increases again to finish at a similar speed to the start of the race – or “reverse J” shape – where speed is very high at the beginning, decreases throughout the race, but manages a small increase or “finishing sprint” at the end (Abbiss & Laursen, 2008).

Aside from shape, pacing can also be described as the variability of speed or power output from the average speed for the effort (Cangley, Passfield, Carter & Bailey, 2010). While Abbiss and Laursen (2008) suggest that less variation in speed/power from the average speed/power is beneficial for performance, this suggestion may be most relevant for events that are performed on flat terrain and constant environmental conditions. Cangley et al. (2010) compared TT performance of cyclists on variable terrain and found that athletes had improved performance when they were able to fluctuate their power output based on the terrain, rather than maintain a specific power output throughout the entire TT despite the changes in terrain. These results support using an even perceived exertion pacing strategy, rather than an even power output strategy in TTs with variable terrain (Cangley et al., 2010).

2.5.2 Pacing Strategies for Distance Cross-Country Ski Racing

A review by Stoggl et al. (2018) report that in XC ski racing, the most popular pacing strategy in 10-50km events is a positive pacing strategy. This means that skiers tend to decrease their speed of movement throughout a race, slowing down anywhere between 2-11% with each subsequent lap. It was also noted that this reduction in speed was more evident in classic events compared to skate events, suggesting that grip wax quality and ski condition may play a role in the skier's decrease in speed (Stoggl et al., 2018). Additionally, it appears that the pacing strategy of skiers is variable across different types of terrain (uphill, flat, downhill); some skiers may have a decrease in speed on hill sections on their second lap, whereas in others speed was maintained on the hill sections but decreased in the flat sections. These results agree with Cangley et al. (2010), that variable terrain demands fluctuations in speed, rather than an even speed pacing strategy. These results also suggest that skiers may employ different pacing strategies that enhance their unique physiology and morphology to best benefit their performance.

2.5.3 Pacing Strategies for Distance Running Events

In track based endurance running events, such as the 5km and 10km events (on a 400m track), the most common pacing strategy is a “U” pacing strategy where athletes start quickly, reduce speed mid-race, then finish at a speed similar to their starting speed (Foster et al., 2019). While the “U” pacing strategy is typical for elite and sub-elite runners, slower racers tended to have a positive pacing strategy, where speed continuously decreased throughout the race (Lima-Silva et al., 2010). While pacing strategy may be learned by athletes, elite athletes will not typically change their pacing strategy when they achieve new personal bests, rather they will adopt the same pacing strategy at an increased speed overall (Foster et al., 2019). De Assis

Manoel, Figueiredo and Machado (2018) also found that less experienced runners did not change their pacing strategy for a 10km track TT after 4 weeks of endurance training, similarly to elite athletes, they increased their speed overall without altering the general pacing pattern. Less experienced or slower runners likely display the positive pacing pattern by starting their race too quickly either by getting excited in a mass start race or overestimating their race speed (Esteve-Lanao, Larumbe-Zabala, Dabab, Alcocer-Gamboa & Ahumada, 2014; Renfree & St-Claire Gibson, 2013). This feedback is challenging to teach developing athletes because it is also important to understand that you can't make up for "lost time" by starting a race too slow, a slower start can be more detrimental to performance than a start that is too fast (Tucker et al., 2006; Foster et al., 1993).

Track events are unique because of the uniform racing surface and the availability of lap speed information. Running events that are more variable in terrain, such as XC running, are more similar to XC skiing events. In XC running events runners will show a significant decrease in lap speed throughout an event, although as previously observed, top finishers displayed a more even pacing pattern (less decrease in speed) than mid-pack finishers (Esteve-Lanao et al., 2014). In events with variable terrain, like XC running, there is more decision making involved to modulate speed during critical moments in the race (i.e., hills, finishing sprints), but a more even pacing strategy overall is most beneficial for performance (Esteve-Lanao et al., 2014).

2.5.4 Influence of Age and Performance Level on Pacing Strategy

While in general skiers tend to show a positive pacing strategy, the faster skiers in an event tend to have a smaller decrease in speed (sometimes even increasing speed with subsequent laps), whereas the slower skiers have a more significant decrease in speed throughout the race (Stoggl et al., 2018). It's likely that the slower skiers attempted to start the race at a

speed that was higher than was sustainable for the duration of the event, causing a more significant decrease in speed due to fatigue (Stoggl et al., 2018, Losnegard, Kjeldsen & Skattebo, 2016).

There has been interest recently in age differences in pacing strategy for marathon running and distance events (loppet) in XC skiing. It has been demonstrated that older athletes tend to have a more even pacing strategy in comparison to younger athletes in long distance events, although the athletes included in these analyses were typically 18 years of age or older (Nikolaidis, Hackney & Sharp, 2019; Nikolaidis & Knetchle, 2017; Nikolaidis et al., 2019b). Carlsson et al. (2015) studied predictors of performance in elite men for a 15km event, they found that for each additional year in age, skiers tended to have a 1% increase in lap speed, which supports older athletes employing a better pacing strategy. While there is evidence of older athletes employing better pacing strategies, there does not appear to be evidence outlining the difference in pacing strategy between young developing competitive athletes (i.e., 16-18 yrs) and older (more experienced) racers. A better understanding of the gaps in pacing strategy could help accelerate performance improvements for developing athletes.

2.5.5 Pacing Strategy and Fatigue

Pacing strategy can be regulated at both a conscious and subconscious level. An athlete selects a pacing strategy in attempt to complete the required distance in the fastest time possible while delaying onset of fatigue (Abbiss & Laursen, 2008). Throughout a race, athletes continuously make decisions about when to pick up the pace, relax a bit, or make a tactical surge to match the pace of the athletes around them (Esteve-Lanao et al., 2014). At the beginning of a race, athletes can get caught up in the excitement of starting, particularly in mass start races, and select a pace that is too fast to sustain for the duration of the event (Renfree & St Claire Gibson,

2013). Despite an athlete's best conscious intention to maintain the pace, if the starting pace is too fast, and physiological homeostasis is disturbed, subconscious regulation may overtake decision making (Noakes, 2011). Subconscious regulation likely integrates peripheral feedback such as increased metabolites, depletion of glycogen stores and remaining duration or distance in order to avoid excessive or catastrophic fatigue (Foster et al., 2004; Tucker et al., 2006).

Based on this review of the literature, two studies were completed and they are described in Chapter 3 and 4, and written in manuscript form.

CHAPTER 3:

**IMPROVED 3KM TRACK TIME TRIAL PERFORMANCE AFTER HIGH INTENSITY
INTERVAL TRAINING IN COMPETITIVE CROSS-COUNTRY SKIERS**

Improved 3km Track Time Trial Performance after High Intensity Interval Training in Competitive Cross-Country Skiers

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Abstract

Background: Research shows that a woman's metabolism, exercise capacity, and physiological responses to heat can be altered depending on which menstrual cycle phase they are in (Meendering et al, 2005; Mison et al, 2000; Oosthuysen, & Bosch, 2010) but a gap remains in understanding sex differences in fatigue and performance after high intensity interval training (HIIT). While lab-based protocols provide a controlled environment to study physiological mechanisms of fatigue, performance response in competitive athletes can be measured by time-trial (TT) to reflect competition demands. Previous studies have shown that changes in TT performance can detect changes in muscle fatigue and mental fatigue (Marcora et al., 2008; Marcora et al., 2009) The purpose of this study was to determine the influence of sex and menstrual cycle phase on 3km track TT performance after a HIIT session in XC skiers.

Methods: Competitive skiers across Canada over the age of 16 years were invited to participate. 30 female and 9 male XC skiers completed 3 days of testing/training. Participants completed a 3km track TT on Day 1 and Day 3; and a HIIT session (4-8x 800m) on Day 2. Menstrual cycle phase was verified by ovulation testing and salivary hormone samples, athletes were classified as either "Low Hormone" or "High Hormone" for analysis.

Results: An overall improvement in performance from pre- to post-HIIT TTs was observed ($p < 0.01$). No significant differences in TT performance after HIIT were observed between sexes ($p = 0.16$) or menstrual cycle phases ($p = 0.26$).

Conclusion: The results of this study indicate that competitive XC skiers will likely experience an improvement in TT performance after a bout of HIIT. Coaches and athletes should plan their workouts prior to testing accordingly to maximize TT performance. Based on these study results no special adjustments need to be considered for differences in performance after HIIT between sexes and MC phase, although further studies with a greater sample size are warranted.

Keywords: Performance, time-trial, cross-country skiing, competitive athletes, sex differences, menstrual cycle phase

INTRODUCTION

Cross-country (XC) skiing is a popular area for sport science research in Scandinavian countries, but there is a lack of studies on competitive/elite Canadian XC skiers, particularly female athletes. Also, despite significant lab-based evidence on endurance training principles, there is still a gap in field research to understand how athletes respond to training and how that may impact their performance. This study aims to elevate the quality of evidence available for competitive/elite athletes and coaches in a Canadian context.

Endurance Performance & High Intensity Interval Training

Training program periodization is an elaborate coordination of all training components (mental skills, skill acquisition, training load, diet, and recovery) to ensure an optimal environment for the competitive athlete (Mujika, Halson, Burke, Balangué & Farrow, 2018). Traditionally, training load periodization has been a primary focus for coaches and physiologists to maximize training adaptation and allow the athlete to improve the physical attributes that impact performance (Mujika et al., 2018). High Intensity Interval Training (HIIT) is used by endurance athletes to improve performance by targeting physiological adaptations such as increased mitochondrial content, increased capillary density and maximum cardiac output (Blomqvist & Saltin, 1983; Basset & Howley, 2000). HIIT has been shown to improve these metabolic and cardiovascular performance metrics after 2-8 weeks of 2-3 HIIT sessions per week resulting in performance improvements of 2-4% in well trained athletes (Daussin et al, 2008; Helgerud et al, 2007; Talanian, Galloway, Heigenhauser, Bonen & Spriet, 2007; Laursen, 2010). Endurance training programs typically combine HIIT with low intensity volume training to enhance performance outcomes in sport-specific competition (Hydren & Cohen, 2015; Hebisz, Hebisz, Borkowski & Zaton, 2019). HIIT can also cause fatigue if appropriate recovery is not

prescribed, excessive training-induced fatigue can lead to overreaching or overtraining which may cause a reduction in performance (Kellmann, 2010).

Fatigue can be characterized by both perceived fatigue and performance fatigue (Enoka & Duchateau, 2016). It can be challenging to identify the etiology of fatigue when studying it in athletes since many factors can simultaneously influence an athlete's cognitive and physical performance (Enoka & Duchateau, 2016). For this reason, we have chosen to consider fatigue from a whole-body, multiple system view, and will use decreased time-trial (TT) performance as the key indicator of fatigue in athletes.

Time Trials

TTs are a special type of performance in endurance athletes because mental strength and pacing strategies can play an integral role in performance in elite athletes. 3km track TTs are used to measure improvements in fitness and performance throughout a skier's season or career (Nordiq Canada, 2018). TTs are known to be reliable and valid assessments in determining performance and are specific to "real-life" races where athletes are required to cover a pre-determined distance at a self-selected pace as fast as they can (Currell & Jenkendrup, 2008). Changes in performance can be measured by repeating a TT before and after a specific intervention. Following an intervention a performance decrement or improvement, can indicate a negative or positive effect of an intervention, respectively. Previous studies have shown that performance decrement can detect changes in performance due to muscle fatigue and mental fatigue (Marcora, Bosio & de Morree, 2008; Marcora, Staiano & Manning, 2009).

Influence of Sex Hormones

A review by Hunter (2016) demonstrates that men have greater performance fatigability than women in isometric contractions and lower velocity in repeated dynamic tasks; however, sex differences in performance fatigability seem to be task dependant. Additionally, males record a longer recovery time after a fatiguing exercise compared to women (Albert, Wrigley, McLean & Sleivert, 2006). There is limited research describing how sex can impact the body's response to a HIIT session and if fatigue manifests differently in men vs women during and after HIIT session. While men continue to outperform women in shorter duration running events, women have demonstrated they are capable of out-performing men in ultra-distance events, suggesting the possibility that male and female energy systems are different (Brown, 2017; Chevront, Carter, DeRuisseau & Moffatt, 2005). Despite the seeming advantage women have in improved fatigue resistance, anecdotally, female athletes are often prescribed a lower intensity training load in comparison to their male counterparts (ie, fewer reps or less time at intensity). There is a gap in knowledge surrounding sex-differences in training prescription, more studies are needed to develop an evidence-based approach in this field.

Female-specific research has shown that a woman's metabolism, exercise capacity, and physiological responses to heat can be altered depending on which menstrual cycle phase they are in (Meendering, Torgrimson, Houghton, Halliwill & Minson, 2005; Minson, Halliwill, Young & Joyner, 2000; Oosthuyse, & Bosch, 2010). Changes in performance across the menstrual cycle are hypothesized because of the role of estrogen and progesterone on the cardiovascular and respiratory systems as well substrate metabolism and heat regulation (Constantini, Dubnov & Lebrun, 2005). The menstrual cycle is most frequently broken into two distinct phases, luteal and follicular, which are separated by ovulation (Oosthuyse & Bosch,

2010). The follicular phase can be described as the “low hormone phase” where estrogen and progesterone are lowest, whereas the luteal phase can be described as the “high hormone phase” where oestrogen and progesterone are highest (Striker et al., 2006). In the late luteal phase, near ovulation, estrogen rises rapidly while progesterone remains low making for a unique hormone profile which is often not considered (Constantini et al., 2005). Estrogen plays a role in regulating muscle glycogen storage capacity and free fatty acid availability that can alter exercising metabolism in comparison to when oestrogen levels are low (Oosthuyse & Bosch, 2010). When performance is measured in a time-trial, increased carbohydrate metabolism in the mid-follicular phase appears to be beneficial for short duration high intensity time-trials (Campbell, Angus & Febbraio, 2001). This suggests the possibility that an increase in estrogen can be beneficial for time-trial performance (Oosthuyse, Bosch & Jackson, 2005). Progesterone plays a role in regulating resting heart rate (Sedlak, Shufelt, Iribarren & Merz, 2012), basal body temperature and ventilation (Charkoudian et al., 1999). Exercise tolerance in hot and/or humid environments may be reduced when progesterone is high in the luteal phase, although, the more experienced the athlete, the impact of this may be reduced (Janse de Jonge, Thompson, Chuter, Silk & Thom, 2012; Tze-Huan et al., 2017).

Therefore, the objective of this current study was to determine if sex and menstrual cycle phase influence performance after a HIIT session. We hypothesized that fatigue would impact performance and differences between sexes and menstrual cycle phases would exist.

METHODS

Participants

Participants were recruited by communicating with club and provincial coaches across Canada (recruitment posters in Appendix D). All XC skiers 16 years of age and older were invited to participate in the study. The inclusion criteria for the participants were: 1) aged 16-35 years of age, 2) training year-round to optimize performance in XC skiing, and 3) competed (or intended to compete) at a national level competition or greater. No specific menstrual cycle inclusion criteria were set because of the high prevalence of menstrual cycle irregularity in endurance athletes (De Cree, 1998; Beals & Manore, 2002). Due to the novel nature of the experimental design and that it was a pilot study, sample size was unknown, although a retrospective sample size calculation was performed following the outcome of this study (Appendix A) Our recruitment targets were 50 women (ideally 25 testing in the follicular phase and 25 testing in the luteal phase) and 25 men. These targets were chosen based on expected feasibility and access to the target population. The athletes were fully informed of all experimental procedures before providing written informed consent to participate (Appendix F). The study was performed according to the Declaration of Helsinki. The research was approved by the Conjoint Health Research Ethics Board of the University of Calgary (REB19-0311) (ethics training certificate in Appendix C; Ethics approval in Appendix E).

Sixty-three competitive XC skiers from nine different teams agreed to participate. Two participants did not meet the inclusion criteria, 17 participants did not start the testing protocol because of injury/illness or schedule conflicts, and five participants dropped out of the study after Day 1 due to injury/illness. A summary of the participant characteristics are provided in Table 1.

Table 1: Self-reported participant characteristics (F=30, M=9), reported as mean and SD (\pm).

Sex	Age (yrs)	Height (m)	Weight (kg)	BMI (kg/m ²)	Running Volume (mins/wk)	Training Volume (hrs/wk)	CPL [†]
M	17.7 \pm 2.2	1.8 \pm 0.1	71.4 \pm 7.5	20.9 \pm 1.3	160.5 \pm 100.0	4.0 \pm 6.9	75.7 \pm 6.4
F	17.9 \pm 2.6	1.7 \pm 0.1	59.9 \pm 6.4	16.9 \pm 8.6	169.5 \pm 76.5	9.1 \pm 3.9	76.3 \pm 4.4

[†]CPL (Canadian Points List); Canadian XC ski racing performance indicator, a higher CPL indicates a higher level of performance.

Study Design

This study was a quasi-experimental design that investigated sex and menstrual cycle differences in TT performance after a HIIT workout. All participants completed the protocol on three consecutive days in the general preparation phase of their training program, and on a week that was deemed appropriate by their coach (typically following a rest week). Instructions were given to not do any structured workouts on the testing days as well as the day immediately prior to testing and participants completed the testing protocol with their teammates at their typical training time (varied based on team). Testing sessions took place on a local 400m track, the quality of track varied based on location ranging from competition grade tracks in larger cities (ie, Ottawa, ON) to dirt tracks in smaller towns (ie, Revelstoke, BC). Within each testing block, testing times remained consistent within the constraints of each specific location and temperature, humidity and wind speed were measured at the beginning of each testing session. Participants were asked to verbally report questionnaire scores indicating their self-reported fatigue (ie, “How fatigued are you right now”)(Rating of Fatigue Questionnaire; Micklewright, St Clair Gibson, Gladwell & Salman, 2017) and how they felt (ie, “How are you feeling right now?”)(Feeling Scale; Hardy & Rejeski, 1989) before, after and 30mins after each session (Appendix G). Maximum and average heart rate data were also collected during each session.

Detailed practice plans for the testing sessions are in Appendix J and one track facility required a rental agreement and proof of insurance (Appendix K).

Protocol sequence is presented in Table 2. On Day 2, athletes were given a lap time that was 5% faster than Day 1 and instructed to run 800m reps until they were no longer able to hold that lap time. If the participant’s pace dropped 5% below their prescribed pace, they were instructed to end their workout. The workout structure was intended to accommodate a range in training status of the participants. All participants (n = 39) completed at least 4 repetitions and 77% of participants completed all 8 repetitions.

Table 2: Testing protocol outline.

Day 1	Day 2	Day 3
Testing: 3000m Track Time Trial	Training: Critical Speed Workout (track) 4-8x800m with 2min rest (800m lap times 5% faster than Day 1)	Testing: 3000m Track Time Trial

Determination of Menstrual Cycle Phase

At least one-month prior to the athlete’s testing session, menstrual cycle was tracked by two methods: 1) counting the days between the start of two cycles to determine cycle length, and 2) ovulation testing performed by the participant, starting on day 10 of their cycle, and continued until a positive test result was obtained or until 15 test strips had been used (Clearblue ovulation testing procedures in Appendix H). If an athlete knew that they typically had a cycle length >30 days, they were given 20 test strips. Salivary estrogen and progesterone samples were collected on Day 2 of the testing protocol and were used to verify estrogen and progesterone levels (Labrix salivary hormone testing procedures in Appendix I). Participants were asked to take samples upon awakening, before lunchtime, before dinnertime and at the end of the day. Participants

typically completed the first 3 samples before training and the end of day sample after training. Hormone kits were obtained from, and analyzed by, Labrix by Doctors Data (Clackamas, OR, USA). If a positive ovulation test occurred and the participant was in the follicular phase during testing or if the participant did not have a positive ovulation test, they were classified as “Low Hormone”; if a positive ovulation test occurred and the participant was in the luteal phase during testing, they were classified as “High Hormone”. For the purpose of comparing sex differences, all female participants were analyzed together. For the purpose of analyzing the influence of menstrual cycle phase, female participants were divided into three groups: low-hormone, high-hormone, and oral contraceptives (active phase). Costs of ovulation and salivary hormone testing kits are presented in the budget (Appendix L).

Outcome Measures

The 3km track TT has been identified by Nordiq Canada (Nordiq Canada, 2018) test and track an athlete’s improvement over a season and throughout their career. Performance in the 3km track TTs on Day 1 and Day 3 was used to calculate performance change.

$$\text{Performance Change} = [((T1\text{Pre} - T2\text{Post}) / T1\text{Pre}) * 100]$$

A negative performance change is referred to as a “Performance Decrement” and a positive performance change is called “Performance Improvement”. Perceived fatigue (Rating of Fatigue Questionnaire; Micklewright et al., 2017) scores (scale of 1-10, where 10 is the highest level of fatigue) were collected at the beginning and end of each session.

Statistics

Data analysis was conducted using the statistical software SPSS v.26 (IBM Corp. Released 2019. ICM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Data are

expressed as mean \pm standard deviation (SD). A two-tailed, one sample t-test was conducted to determine the influence of HIIT on performance change. Two-tailed, unpaired t-tests were conducted to determine differences in mean response between male and female athletes. An ANOVA was used to compare performance change between male, female low-hormone, female high-hormone and female oral-contraceptives. A significance value of $p \leq 0.05$ was set. The magnitude of the mean differences between sexes and menstrual status were expressed as effect sizes (ES). The classification of the ES is follows: trivial (<0.2), small ($>0.2-0.6$), moderate ($>0.6-1.2$), large ($>1.2-2.0$), very large (>2.0), based on guidelines by Batterham and Hopkins (2006).

RESULTS

The average menstrual cycle length was 28 days, and twenty women had non-ovulatory cycles whereas ten women had ovulatory cycles. Four women were taking oral contraceptives and two had an intra-uterine devices (IUD) at the time of the data collection. One participant with an IUD ovulated and was analyzed with the “Female-High Hormone” group, one participant with an IUD did not ovulate and was analyzed with the “Female-Low Hormone” group. Salivary hormone samples reported twenty-eight women had a low progesterone: estrogen ratio, whereas only two had a balance hormone profile.

On average, performance improved in XC skiers after a HIIT session ($PC \neq 0$, $p=0.01$). 11 females and 1 male had a performance decrement whereas 19 females and 8 males had a performance improvement. Overall the group mean performance change was $1.07 \pm 2.58\%$ with the male mean higher ($2.19 \pm 3.11\%$) than the female ($0.74 \pm 2.36\%$) (see Figure 2). Despite the 1.45% greater performance improvement in men when compared to women, the overall performance change was not significantly different between sexes ($p=0.14$). The ES between

sexes is 0.058, which is regarded as a small effect size but close to a medium effect size. Raw performance change data is presented in Appendix M.

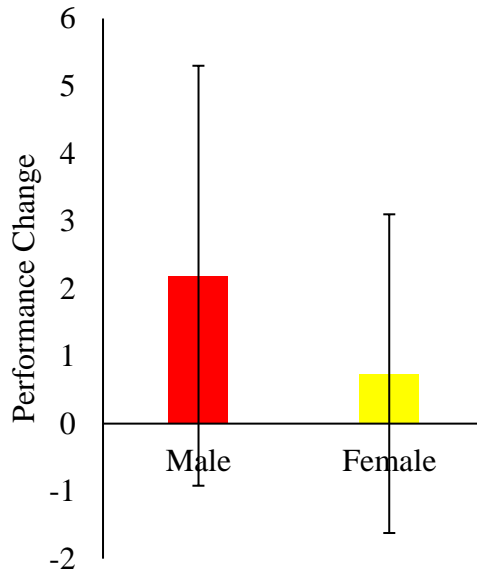


Figure 1: Mean performance change (with SD bars) in males(n=9) and females (n=30)

Performance change in female-low hormone, female-high hormone and female-oral contraceptives was $1.11 \pm 2.00\%$, $0.67 \pm 3.18\%$, and $-0.81 \pm 1.87\%$, respectively (Figure 3).

While there appears to be some differences between these groups, these results are not statistically significant ($p=0.27$). The ES between menstrual status is 0.105 which is regarded as a medium effect size. The trend shows a reduced increase in performance in the high hormone group in comparison to the low hormone group, and a performance decrement was observed on average for females taking oral contraceptives.

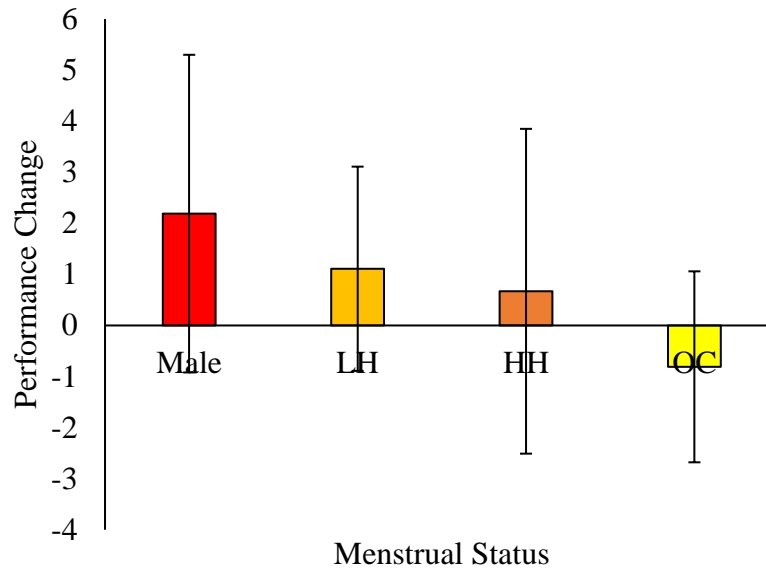


Figure 2: Mean performance change (with SD bars) in male (n=9), female-low hormone (LH) (n=18), female-high hormone (HH) (n=8), and female-oral contraceptive (OC) (n=4)

There was a significant time effect for self-reported rating of fatigue (ROF-Q) for all participants ($p < 0.0001$). ROFQ significantly increased from PreTT1 (3.44 ± 1.37) to PreTT2 (4.74 ± 1.56) ($p = 0.001$). ROFQ also significantly increased from Pre-HIIT (3.76 ± 1.56) to PostHIIT (8.90 ± 1.31) ($p < 0.0001$) and remained significantly different from PreHIIT at 30mins PostHIIT (6.25 ± 1.75) ($p < 0.001$). A significant reduction in ROFQ was reported from PostHIIT to 30mins PostHIIT ($p < 0.0001$). Further, athletes reported a significant reduction in ROF-Q from 30mins PostHIIT to PreTT2 ($p = 0.001$). Self-reported fatigue was not influenced by sex ($p = 0.295$) or menstrual status ($p = 0.285$) and there was no significant interaction between reported fatigue scores and sex ($p = 0.246$) or menstrual status ($p = 0.405$) (Table 3; Table 4).

Table 3: Self-reported rating of fatigue (ROF-Q) grouped by menstrual status.

Outcome Measure		Male [mean ± SD] N=9	F-LH [mean ± SD] N=18	F-HH [mean ± SD] N=8	F-OC [mean ± SD] N=4	Time Effect F (df) [P]	Group Effect F (df) [P]	Interaction F (df) [P]
ROF-Q	PreTT1	4.00 ± 1.12	3.00 ± 1.19	3.00 ± 1.18	3.75 ± 0.56	99.631 (4,140) [p<0.0001]	1.315 (3,35) [p=0.285]	1.052 (12,140) [p=0.405]
	PreTT2	4.78 ± 1.64	4.56 ± 1.40	5.38 ± 1.39	4.25 ± 3.00			
	PreHIIT	3.56 ± 1.13	3.72 ± 1.36	4.00 ± 1.33	3.75 ± 1.34			
	PostHIIT	9.00 ± 1.32	8.83 ± 1.14	9.50 ± 1.13	8.25 ± 1.14			
	30mins PostHIIT	7.00 ± 0.87	6.39 ± 1.48	6.39 ± 1.50	5.00 ± 0.00			

Mean ROF-Q scores for males, female low hormone (F-LH), female high-hormone (F-HH) and females on oral contraceptives (F-OC) prior to TT on Day 1 (PreTT1), prior to the TT on Day 3 (PreTT2), prior to the HIIT session on Day 2 (PreHIIT), immediately after the HIIT session on Day 2 (PostHIIT) and 30mins after the HIIT session on Day 2 (30mins PostHIIT).

Table 4: Self-reported rating of fatigue (ROF-Q) for all participants and grouped by sex.

Outcome Measure		All [mean ± SD] N=39	Male [mean ± SD] N=9	Female [mean ± SD] N=30	Time Effect F (df) [P]	Group Effect F (df) [P]	Interaction F (df) [P]
ROF-Q	PreTT1	3.44 ± 1.37	4.00 ± 1.12	3.10 ± 1.19	66.846 (4,34) [p<0.0001]	1.130 (1, 37) [p=0.295]	1.428 (4,34) [p=0.246]
	PreTT2	4.74 ± 1.56	4.78 ± 1.64	3.31 ± 1.22			
	PreHIIT	3.76 ± 1.56	3.56 ± 1.13	3.80 ± 1.35			
	PostHIIT	8.90 ± 1.31	9.00 ± 1.32	8.93 ± 1.11			
	30mins PostHIIT	6.25 ± 1.75	7.00 ± 0.87	6.27 ± 1.66			

Mean ROF-Q scores for all participants as well as means group by sex (male and female) prior to TT on Day 1 (PreTT1), prior to the TT on Day 3 (PreTT2), prior to the HIIT session on Day 2 (PreHIIT), immediately after the HIIT session on Day 2 (PostHIIT) and 30mins after the HIIT session on Day 2 (30mins PostHIIT).

There was a significant time effect for self-reported affect (Feeling Scale (FS)) for all participants ($p=0.001$). Participants reported significant reductions in FS from PreTT1 ($1.69 \pm$

1.56) to PreTT2 (0.79 ± 2.04) ($p=0.02$). While no significant difference in FS was reported from PreHIIT (1.82 ± 1.45) to PostHIIT (0.18 ± 2.44) ($p=0.07$), there was a significant increase in FS from PostHIIT to 30mins PostHIIT (1.08 ± 2.17) ($p=0.04$). FS was not influenced by sex ($p=0.997$) or menstrual status ($p=0.238$) and there was no significant interaction between reported fatigue scores and sex ($p=0.664$) or menstrual status ($p=0.291$) (Table 5, Table 6).

Table 5: Self-reported affect scores (Feeling Scale (FS)) grouped by menstrual status.

Outcome Measure		Male [mean \pm SD] N=9	F-LH [mean \pm SD] N=18	F-HH [mean \pm SD] N=8	F-OC [mean \pm SD] N=4	Time Effect F (df) [P]	Group Effect F (df) [P]	Interaction F (df) [P]
Feeling Scale	PreTT1	1.67 \pm 1.58	1.56 \pm 1.54	1.75 \pm 1.91	2.25 \pm 1.26	4.995 (4,140) [p=0.001]	1.477 (3,35) [p=0.238]	1.197 (12,140) [p=0.291]
	PreTT2	0.78 \pm 2.49	1.00 \pm 1.88	-0.13 \pm 2.23	1.75 \pm 0.96			
	PreHIIT	2.00 \pm 1.73	1.83 \pm 1.58	1.50 \pm 1.31	2.00 \pm 0.00			
	PostHIIT	-0.33 \pm 2.29	0.22 \pm 2.29	-0.63 \pm 2.93	2.75 \pm 0.50			
	30mins PostHIIT	1.44 \pm 2.30	0.83 \pm 1.95	0.13 \pm 2.42	3.25 \pm 0.96			

Mean FS scores for males, female low hormone (F-LH), female high-hormone (F-HH) and females on oral contraceptives (F-OC) prior to TT on Day 1 (PreTT1), prior to the TT on Day 3 (PreTT2), prior to the HIIT session on Day 2 (PreHIIT), immediately after the HIIT session on Day 2 (PostHIIT) and 30mins after the HIIT session on Day 2 (30mins PostHIIT).

Table 6: Self-reported affect scores (Feeling Scale (FS)) for all participants and grouped by sex.

Outcome Measure		All [mean ± SD] N=39	Male [mean ± SD] N=9	Female [mean ± SD] N=30	Time Effect F (df) [P]	Group Effect F (df) [P]	Interaction F (df) [P]
ROF-Q	PreTT1	1.69 ± 1.56	1.67 ± 1.58	1.70 ± 1.58	7.469 (4,148) [p<0.0001]	0.000 (1, 37) [p=0.997]	0.599 (4,148) [p=0.664]
	PreTT2	0.79 ± 2.04	0.78 ± 2.49	0.80 ± 1.94			
	PreHIIT	1.82 ± 1.45	2.00 ± 1.73	1.77 ± 1.38			
	PostHIIT	0.18 ± 2.44	-0.33 ± 2.29	0.33 ± 2.50			
	30mins PostHIIT	1.08 ± 2.17	1.44 ± 2.30	0.97 ± 2.16			

Mean FS scores for all participants as well as means group by sex (male and female) prior to TT on Day 1 (PreTT1), prior to the TT on Day 3 (PreTT2), prior to the HIIT session on Day 2 (PreHIIT), immediately after the HIIT session on Day 2 (PostHIIT) and 30mins after the HIIT session on Day 2 (30mins PostHIIT).

DISCUSSION

The purpose of this study was to investigate whether sex and menstrual cycle phase influence fatigue and training response after a HIIT session in competitive XC skiers. The main finding of this study was that instead of participants having a performance decrement after a HIIT session, performance, on average, significantly improved.

The study expected that the intervention would result in both performance fatigue (changes in contractile function and muscle activation) and perceived fatigue (changes in homeostasis and psychological state), therefore reducing the athlete's ability to perform the time-trial after a HIIT workout (Enoka & Duchateau, 2016). Although participants reported a significant increase in ROF-Q immediately after the HIIT workout, self-reported fatigue had significantly decreased by the following morning. Despite reporting significantly greater ROF-Q and significantly lower FS prior to the TT on Day 3 in comparison to prior to the TT on Day1, participants significantly

improved their TT performance. This finding does not align with measures of muscular fatigue where maximal power output or time exhaustion are reduced in an isolated muscle group after an acute bout of fatiguing exercise (Enoka & Duchateau, 2016). Rietjes et al. (2005) similarly found that acute fatigue in athletes does not decrease time-trial performance in the short-term. They suggest that the factors affecting peripheral fatigue are robust and may require a longer period of over-reaching to be impacted (Rietjes et al., 2005). While some aspects of fatigue are well known, there are still gaps in our knowledge on how fatigue translates to human performance and how perceived fatigue can be trained with exercise interventions (Enoka & Duchateau, 2016).

While most physiological adaptations to HIIT (such as increased capillary density and increased cardiac output) require a time frame greater than 3 days to manifest, adaptations in skeletal muscle mitochondrial content can be triggered with as little as one session of HIIT (MacInnis & Gibala, 2017; Gibala et al, 2009). Most endurance athletes will train primarily in low to moderate intensities in the general preparation phase, therefore it is possible that the HIIT session created enough stimulus to lead to improvements in performance (Mujika et al., 2018). An additional challenge in field research such as this, is that we cannot isolate physiological and psychological improvements (Noakes, 2000). An athlete's conscious effort and motivation to sustain a given exercise intensity can be significant enough to lead to an increase in performance. Due to the "against the clock" nature of TTs, the athlete's knowledge of their previous time may have motivated them to get a new personal best. Completing a vigorous intensity exercise routine (such as the HIIT workout in our protocol) can increase pain tolerance during exercise, therefore it is possible that an acute adaptation in pain tolerance could have contributed to the increase in TT performance (Jones, Booth, Taylor & Barry, 2014). It is possible that this experimental

protocol was so demanding the athletes increased their recovery methods during the testing sessions more than they would have previously. Many athletes anecdotally reported the HIIT session was the hardest workout they had done all season, and they needed an extra nap in the day or went to bed early because they were feeling tired. The recovery status of the athletes is supported by the significant reductions in self-reported fatigue once 30mins of active recovery is completed after the HIIT session, and further reductions in self-reported fatigue between after approximately 24hrs of recovery (prior to TT2). If an athlete improved their recovery methods and was able to recover adequately from the HIIT session, it may result in increased capacity to perform (Bishops, Jones & Woods, 2008).

Based on the lack of statistical significance, the impact of sex and menstrual cycle phase on performance after a HIIT session is unclear. While men appear to have a greater improvement in performance and women on oral contraceptives appear to have a greater performance decrement after HIIT, individual variation in response may play a greater role than sex or menstrual cycle phase. Previous findings on sex differences in performance fatigability suggest that women are more fatigue resistant than men in isometric, low intensity muscle contractions, but the difference between sexes is reduced during dynamic tasks (Hunter, 2016). A running time-trial is a series of dynamic, low intensity muscular contractions, leading us to believe that the mechanism responsible for sex differences in muscular fatigability may not influence training-induced fatigue after HIIT. Data-unpublished by Cobbold (2018) also suggests that men have a greater response to HIIT training, as after 6 weeks of HIIT training male participants had an increase in VO_{2max} by 11% whereas female participants did not significantly improve. Men may be more susceptible to improved performance after HIIT because of higher testosterone levels in comparison to women. Testosterone has been shown to be significantly elevated after HIIT

sessions in young male athletes (Kilian et al., 2016) and Remes, Kuppasalmi and Adlercreutz (1979) documented greater increases in VO_{2max} after training in men with higher testosterone levels. Sex differences in adaptation to training is an area of research that needs to be further developed to improve understanding of health and performance benefits of HIIT.

The lack of difference between high hormone and low hormone indicates that recovery and fatigue, as well as adaptation from a HIIT session may not be influenced by menstrual cycle phase. These findings support Hunter (2016) who remarked that MC difference in fatigability is far less significant than sex differences. Although others have hypothesized that HIIT may be better tolerated in the follicular phase because of increased carbohydrate metabolism and increased relative testosterone (Sims & Yeager, 2016, pp. 8, 19-20), the present study does not support this in the small time window that we observed these athletes. The significance of our findings on the influence of the menstrual cycle phase is limited because of the high prevalence of anovulatory cycles progesterone insufficiency reported in the salivary samples. It's likely that the majority of participants would be classified as "oligomenorrhic" with one or more missed cycles throughout the duration of tracking. All athletes with one exception were found to have an "unbalanced" estrogen to progesterone ratio, indicating some level of menstrual irregularity in nearly all participants.

The performance decrement in participants taking oral contraceptives, while not significant, can possibly be explained by Schaumberg, Jenkins, Janse de Jonge, Emmerton & Skinner (2017) who found that adaptation of VO_{2max} from sprint-interval training was dampened in women taking oral contraceptives. Our sample of women taking oral contraceptives was small (n=4), therefore no conclusions can be drawn from this.

A limitation of the study was that repeated measurements could not be completed in the luteal phase and the follicular phase (Julian et al., 2017). While repeated measurements could have added to our understanding of how the menstrual cycle impacts fatigue and training response after HIIT, it was logistically too challenging for athletes to commit to that many testing sessions. Additionally, because of the nature of individual sports training, we were not able to standardize the training and lifestyle of the participants prior to their testing sessions. While we requested that athletes do the testing following a week of lower training load to ensure fatigue levels were not too great, some athletes reported higher levels of fatigue coming into testing on Day 1. Additionally, while most competitive XC skiers should be familiar with the 3km track testing (because it is recognized as a standardized test by Nordiq Canada), some athletes indicated that they had no experience running a 3km track TT. Despite differences in athletes' training history and experience, our sample was likely representative of competitive XC skier population.

Future studies should consider including more than one sport (i.e., cycling, running, triathlon, XC skiing) to see if there is a trend that remains true amongst a variety of endurance sports. Based on our study's standard error of the sample mean, a sample of 46 male and 46 female participants would be required to be recruited to detect significant sex differences at a level of $p=0.05$. While finding a significant difference between sexes may be possible with more participants, our sample standard error indicates 168 participants are needed for High Hormone and Low Hormone groups to find a significant difference ($p=0.05$). This large sample may be very difficult to achieve and not realistic for future studies. Additionally, future research should include a longer period of exhaustive training, and consideration towards a "training camp" model may be most appropriate to reduce those extraneous factors that impact performance.

CONCLUSION

The results of this study indicate that competitive XC skiers will likely experience an improvement in time-trial performance after a bout of HIIT. Since performance, on average, improved, we are unable to comment adequately on fatigue. Performance change varies between individuals, but male athletes may have a greater response to HIIT than women. Performance change was not influenced by menstrual cycle phase in our study, although it is possible that taking oral contraceptives may play a role in reducing performance after HIIT. Coaches may want to consider an appropriate HIIT session prior to testing to maximize performance. Coaches and athletes can use a similar protocol to track performance and adaptation to HIIT throughout an athlete's season to tailor an individualized training program.

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CHAPTER 4:

**DIFFERENCES IN 3KM TRACK TIME TRIAL PACING AND PERFORMANCE BY
AGE IN COMPETITIVE CROSS-COUNTRY SKIERS: A FIELD STUDY**

Differences in 3km Track Time Trial Pacing and Performance by Age in Competitive Cross-Country Skiers: A Field Study

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Abstract

Background: Differences in fatigue and performance after HIIT in younger (u18) and older (18+) competitive cross-country (XC) skiers (vs 18+) are possible due to higher oxidative capacity and lower lactate production in younger athletes (Taylor et al., 1997; Zafeiridis et al., 2005), although this has not been studied in XC skiers. While lab-based protocols provide a controlled environment to study physiological mechanism of fatigue, performance in competitive athletes can be measured by field-based time-trials (TT) (Laursen et al., 2007; Denham et al., 2015). TT performance can be used to measure changes in fitness, although changes in pacing strategy may also impact TT performance (Abbiss & Laursen, 2008). Previous studies have demonstrated that younger and older athletes have different pacing strategies, therefore special considerations will be made to understand how pacing strategy may impact performance (Lonesgard, 2016; Stoggl et al., 2018; Nikolaidis et al., 2019). The purpose of this study was to determine the influence of sex, menstrual cycle phase, age and pacing strategies on 3km track TT performance after a high intensity training session in XC skiers.

Methods: Competitive skiers across Canada over the age of 16 years were invited to participate. 30 female and 9 male XC skiers completed 3 days of testing/training. Participants completed a 3km track TT on Day 1 and Day 3; and a HIIT session (4-8x 800m) on Day 2. Pacing strategy was calculated by sum of the differences of each individual lap from the average lap speed.

Results: A significant improvement in pacing strategy in u18 athletes was observed ($p=0.02$), although this improvement in pacing did not result in improved performance ($p=0.09$). In general, 18+ athletes did not improve their pacing strategy ($p=0.23$), although those who did improve their pacing strategy, improved their performance ($p=0.048$). A pre-trial and a lap-based HIIT session is an effective intervention to improve TT pacing strategy in u18 athletes.

Conclusion: Younger athletes showed a greater improvement in TT performance after HIIT compared to older athletes. Coaches should be aware of the age-related differences in pacing strategy and performance in younger and older skiers.

Keywords: time-trial performance, pacing, age, cross-country skiing, competitive athletes

INTRODUCTION

Physiological and/or field tests are widely used by competitive cross-country (XC) skiers to assess improvements within and between seasons, as well as to assess the success of a training intervention. Physiological parameters such as VO₂max and anaerobic lactate threshold can be measured in the lab and have been shown to correlate with performance in XC skiers (Ingier, 1991; Larsson, Olofsson, Jakobsson, Burlin & Henriksson-Laursen, 2002; Carlsson et al., 2012). Most skiers across Canada do not have access to laboratory resources, which places a greater importance on field testing, which can be performed more frequently in a familiar environment. When selecting a test, it is imperative that the test reflects the demands of the sport but also decreases the influence of confounding variables such as weather and snow conditions. Canadian XC skiers typically spend eight months of the year (April-November) doing dry-land training such as running and roller skiing, and only four months of the year training and racing on-snow. For this reason, performing dry-land field tests with athletes is an appropriate method for tracking improvements and adaptation to training interventions throughout most of the general preparation and specific preparation seasons.

Time trials (TT) are reliable and valid assessments to determining performance (Currel & Jenkendorf, 2008) and are specific to XC skiing since the “interval start” race format is common and is similar to a TT. Carlsson, M., Carlsson, T., Hammarström, Marlm & Tonkonogi (2014) found that 3km running TT performance is correlated with ski performance in competitive XC skiers, although their model was only significant for male skiers. While a 3km running TT is a short race for XC skiers (approximately 10-15mins to compete), the wide range of disciplines in XC skiing [1km sprints (2-3mins to complete) to 50km distance races (several hours to complete)], demands that XC skiers have well developed aerobic and anaerobic energy systems

(Losnegard, 2019). A 10-15min race effort requires the athlete to sustain an intensity that is close to their anaerobic lactate threshold, which is possibly a better indicator of performance than VO₂max in elite athletes (Welde, Evertsem Von Heimburg & Medbo, 2003; Larsson et al., 2002). During a 5-10km cross-country ski race, athletes will select an effort that corresponds to anaerobic lactate threshold for the majority of the race duration and will have significant accumulation of blood lactate at completion (Gonzalez-Millan et al., 2017; Welde et al., 2003). An athlete's ability to work at a high intensity is important for success in XC skiing because of the up and down nature of the courses, where uphill segments require a high energy output and downhill segments provide an opportunity for recovery (Losnegard, 2019; Gloersen, Gilgen, Dysthe, Matthe-Sorensen & Losnegard, 2019; Karlsson, Gilgen, Gloersen, Rud & Losnegard, 2018). The 3km track TT therefore as an appropriate field test to assess fitness and performance in XC skiers.

TTs are used to assess fitness level and performance for both younger and older competitive XC skiers. Age-related differences in TT performance should be considered so that coaches can effectively analyze improvements in performance over time; however, there is limited research in this area. Age may play a role in how an athlete selects their pacing strategy due to physical characteristics as well as maturity level in decision-making (Elferink-Gemser & Hettinga, 2017). While older athletes may be more capable of delivering a high anaerobic power output, younger athletes may have better fatigue resistance due to a greater oxidative capacity and reduced lactate production (Zafeiridis et al., 2005; Taylor et al., 1997). Throughout the development of an athlete, especially through the rapid changes in adolescence, an athlete will need to adapt their pacing strategy to their increasing fitness levels – a process which requires mature decision making in order to set realistic goals for their current abilities (Elferink-Gemser

& Hettinga, 2017). Pacing strategy may also be learned by young athletes, throughout a process of reflection on past experiences in order to improve future performances, suggesting that repeating the same task (or TT) can be beneficial for performance (Elferink-Gemser & Hettinga, 2017).

In endurance events, pacing strategy can be assessed by plotting the lap time (or speed) cumulatively. A positive pacing strategy shows an increase in lap time (decreased speed) near the end of the race, whereas a negative pacing strategy shows a decrease in lap time (increased speed) near the end of the race (Abbiss & Laursen, 2008). Another pacing strategy can be described as a “U” shape where speed is high at the beginning, decreases mid-race, and increases again to finish quickly (Abbiss & Laursen, 2008). Pacing can also be described by its variability when compared to the mean speed, i.e., a more even pacing strategy with lower variability can be beneficial for performance. Despite XC skiers often having a positive pacing strategy in on-snow competition, faster and older skiers will have a smaller decrease in speed compared to slower and younger skiers throughout a race (Abbiss & Laursen, 2008; Losnegard, Kjeldsen & Skattebo, 2016 2016; Stoggl, Pellegrini & Holmberg, 2018). Therefore, with this style of field testing, possible outcomes include performance improvement, no change in performance, and performance decrement alongside either improved or no-change in pacing strategy. The objective of this study was to explore if age and pacing strategy influence 3km TT performance in XC skiers during a field-testing protocol. We hypothesized that both age and pacing strategies would influence 3km track TT performance in XC skiers.

METHODS

Participants

Participants were recruited by communicating with ski club and provincial coaches across Canada. All XC skiers 16 years of age and older were invited to participate in the study. The inclusion criteria for the participants were: 1) aged 16-35 years of age, 2) training year-round to optimize performance in XC skiing, and 3) competed (or intended to compete) at a national level competition or greater. The athletes were fully informed of all experimental procedures before providing written informed consent to participate. The study was performed according to the Declaration of Helsinki. The research was approved by the Conjoint Health Research Ethics Board of the University of Calgary (REB19-0311).

Sixty-three competitive XC skiers from nine different teams agreed to participate, thirty-nine participants met the inclusion criteria and completed the testing protocol. One female participant was not included in the pacing analysis because lap times were not collected on her trial. A summary of participant characteristics is provided in Table 1.

Table 1: Self-reported participant characteristics (F=30, M=9), reported as mean and SD (\pm).

Sex	Age (yrs)	Height (m)	Weight (kg)	BMI (kg/m ²)	Running Volume (mins/wk)	Training Volume (hrs/wk)	CPL [†]
M	17.7 \pm 2.2	1.8 \pm 0.1	71.4 \pm 7.5	20.9 \pm 1.3	160.5 \pm 100.0	4.0 \pm 6.9	75.7 \pm 6.4
F	17.9 \pm 2.6	1.7 \pm 0.1	59.9 \pm 6.4	16.9 \pm 8.6	169.5 \pm 76.5	9.1 \pm 3.9	76.3 \pm 4.4

[†]CPL (Canadian Points List); Canadian XC ski racing performance indicator, a higher CPL indicates a higher level of performance.

Study Design

This quasi experimental study design investigated the influence of age and pacing strategies on 3km track TT performance. All participants completed the protocol as shown in Table 2 over three consecutive days in the general preparation phase of their training program, and on a week that was deemed appropriate by their coach (typically following a rest week). The participants were instructed not to do any structured workouts on the testing days as well as the day immediately prior to testing. On Day 2, athletes were given a target lap time that was 5% faster than Day 1 and instructed to run 800m reps until they were no longer able to reproduce that lap time. If a participant's pace dropped 5% below their prescribed pace, they were instructed to end their workout. The workout structure was intended to accommodate a range in training status of the participants.

Table 2: Testing protocol outline.

Day 1	Day 2	Day 3
Testing: 3000m Track Time Trial	Training: Critical Speed Workout (track) 4-8x800m with 2min rest (800m lap times 5% faster than Day 1)	Testing: 3000m Track Time Trial

Performance Change

The 3km track TT is a test that has been identified by Nordiq Canada (Nordiq Canada, 2018) to be used to test and track an athlete's improvement over a season and throughout their career. Performance in the 3km track TTs on Day 1 and Day 3 was used to calculate a percentage performance change.

$$\text{Performance Change} = [(T1\text{Pre} - T2\text{Post} / T1\text{Pre}) * 100]$$

A negative performance change is referred to as a “Performance Decrement” and a positive performance change is referred to as a “Performance Improvement”.

Pacing Strategies

On a 400m track, a 3km TT consists of 7.5 laps. In most cases, the first 200m lap time was not recorded, therefore the “Lap 1” time is for the first 600m, although in some cases a 200m lap time was recorded, this was recorded as “Lap 0.5” for the purpose of displaying the results. Lap times were collected using Webscorer © during the TT and lap times were converted to average speed per lap (m/s), and an average lap time for the entire TT was calculated. To describe an athlete’s pacing strategy, the sum of the differences of each individual lap from the average lap speed was calculated.

$$\text{Sum of Differences} = \sum(\bar{x} - x_i),$$

where \bar{x} = *average lap time for TT*, and x_i = *each 400m lap time*

Statistics

Data analysis was conducted using the statistical software SPSS v.26 (IBM Corp. Released 2019. ICM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Data are expressed as mean and standard deviation (SD; \pm). For the purpose of analyzing performance and pacing strategies, a two-way mixed (2 by 2) ANOVA was performed with pre- and post-sum of differences measurements as the within subject effect and performance change (performance improvement or performance decrement on Day 3) as the grouping factor between subjects. For the purpose of analyzing the influence of age, athletes were assigned to one of two groups, u18 (less than 18 years of age at the time of testing) and 18+ (18 years of age or greater at the time of

testing). A two-tailed, unpaired t-test was conducted to determine differences in mean performance change between u18 and 18+ groups. A significance value of $p \leq 0.05$ was set.

RESULTS

The primary finding of this study is that performance of u18 athletes significantly improved in the Post-HIIT TT ($p=0.005$), whereas 18+ athletes had no significant change ($p=0.671$). Although, the difference in performance change between u18 and 18+ athletes was not statistically significant, likely due to a high variability in performance, it neared statistical significance ($p=0.06$). Mean performance change in u18 and 18+ participants was $1.78 \pm 2.59\%$ and $0.24 \pm 2.38\%$, respectively (Figure 1).

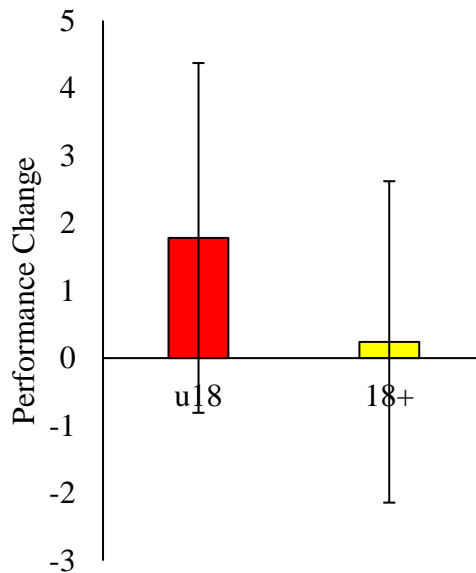


Figure 1: Performance change in u18 participants ($n=21$; 17 females and 4 males) and 18+ participants ($n=18$; 13 females and 5 males).

Participants with a performance improvement had a lower lap time sum of differences post-HIIT (0.665 ± 0.308) in comparison to pre-HIIT (0.767 ± 0.353). Participants with a

performance decrement had a greater sum of differences post-HIIT (0.887 ± 0.791) in comparison to pre-HIIT (0.718 ± 0.353) (Table 3). While the effect of time (pre vs post, $p=0.745$) and the effect of the grouping (improvement vs decrement, $p=0.192$) were not statistically significant, there was a trend suggesting that a more even pacing strategy (less deviation from the average speed) is likely to result in improved performance, whereas a more variable pacing strategy is more likely to result in a performance decrement (Figure 2). Raw pacing data is presented in Appendix N.

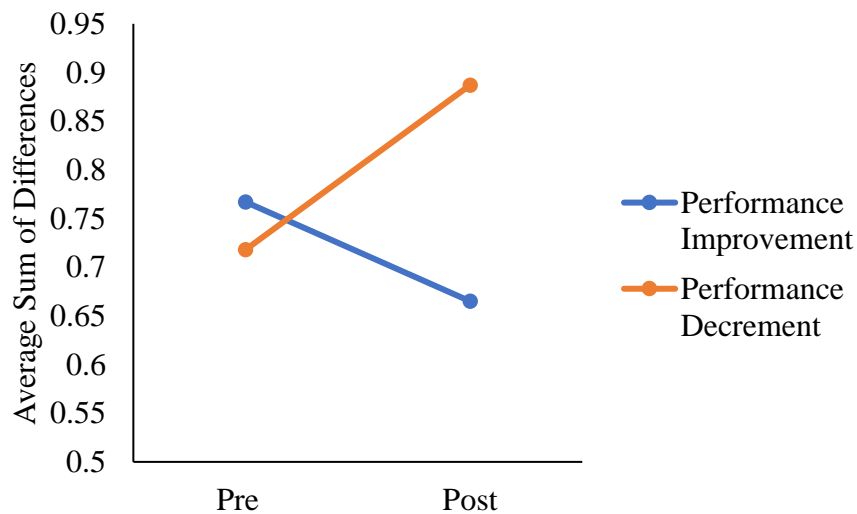


Figure 2: Change in mean sum of differences by athlete group (performance improvement or performance decrement).

When analyzed separately by age group, u18 athletes significantly improved their pacing strategy from pre to post trials ($p=0.02$). Despite observing overall improved pacing in u18 athletes, the relationship between improved performance and improved pacing is not statistically significant ($p=0.09$, Table 3). Most 18+ athletes did not show a statistically significant

improvement in their pacing strategy from pre to post trials ($p=0.23$), but those who did improve their performance also had an improvement in their pacing strategy ($p=0.048$, Table 3).

Table 3: Combined, u18 and 18+ Pacing Characteristics by Performance Change.

Outcome Measure		Performance Improvement [mean (SD)]	Performance Decrement [mean (SD)]	Time Effect F (df) [P]	Group Effect F (df) [P]	Interaction F (df) [P]
Sum of differences (All)	Pre	0.767 (0.249) n=26	0.718 (0.353) n=12	0.108(1,36) [0.745]	1.767(1,36) [0.192]	0.108(1,36) [0.397]
	Post	0.665 (0.308) n=26	0.887 (0.791) n=12			
Sum of differences (u18)	Pre	0.733 (0.212) n=15	0.944 (0.282) n=6	6.652(1,19) [0.018]	3.147(1,19) [0.092]	0.621(1,36) [0.441]
	Post	0.678 (0.346) n=15	0.646 (0.180) n=6			
Sum of differences (18+)	Pre	0.814 (0.295) n=11	0.492 (0.268) n=6	1.587(1,15) [0.227]	4.634(1,15) [0.048]	0.189(1,36) [0.670]
	Post	0.647 (0.262) n=11	0.817 (1.098) n=6			

DISCUSSION

Athlete age, as hypothesized, influenced improvement in the 3km track TT. The younger athletes (16-17yrs) were more likely to improve their 3km track TT performance after a HIIT session than older athletes' (18-28yrs). These results indicate that u18 XC skiers have the potential to significantly improve their 3km track TT performance by completing a familiarization trial and a track based HIIT workout immediately prior to testing. Improvement in performance, however, is less likely in the older athletes because of their prior training and

racing experience. The learning effect of the pre-trial and HIIT session may be more likely to influence TT performance of younger, less experienced athletes. This highlights the importance of standardizing pre-testing workouts in younger, less experienced athletes. Lack of standardization may lead to unexplained variation in testing results and an inability to make effective conclusions from testing.

Older athletes that showed an improvement in performance also had a statistically significant improvement in pacing strategy ($p=0.048$). This result suggests that it may be particularly important for older athletes to employ an even pacing strategy to improve TT performance. This finding is supported by others that also found an even pacing strategy is likely to result in better performance in endurance athletes (Abbiss & Laursen, 2008). In XC skiing specifically, the most successful athletes that have a smaller decline in velocity throughout a race are those with a more even pacing strategy and subsequent leading to a best performance (Losnegard et al., 2016).

A significant difference in pacing for the u18 group did not occur, likely due to the large degree of variability in individual athletes' performance. Elite endurance athletes have up to a 1.4% variability in performance within a season, and a greater degree of variability can be expected in sub-elite and competitive athletes (Malcata & Hopkins, 2014).

In u18 athletes, the testing protocol significantly improved their pacing strategies (a more even pacing strategy), whether they improved their performance or not. This is evidence for a pre-trial and a HIIT workout being an effective tool to teach u18 athletes pacing strategies for this style of test. The HIIT session on Day 2 was prescribed to athletes as a 400m lap time and an 800m completion time, where athletes were encouraged to run as close the lap time as possible. Running for XC ski training is mostly done in parks, on trails or on roads, not on a track. Since

track running provides a unique environment for precise pacing feedback that is not always available in other training environments, it is likely the pacing practice (through the HIIT workout) played a significant role in improving pacing strategies. Nikolaidis, Rosemann and Knetchle (2019) found that younger skiers were more variable in their pacing strategy in comparison to older skiers, indicating an improvement in pacing with age. This improvement with age could likely be accelerated if the athlete is provided with workouts designed specifically to teach pacing as a skill. These results suggest that younger athletes have a large capacity to improve their performance by learning pacing strategies and practicing them in race-specific environments.

The prescribed pace for the HIIT session was 5% faster than the athletes ran in their TT on Day 1, and despite minimal rest (2 mins between 800m reps), most athletes ran 4.8 to 6.4km at this pace. During the workout, many athletes expressed that they would have stopped running after four repeats if they had not been encouraged to keep going. It is possible that, in completing this workout, the athlete's confidence in their ability to complete the TT at a higher intensity was increased. It is possible that athletes expanded their opinion of where their limit was and were more willing to push harder to get a better result.

It is our hope that these results with additional research could be extrapolated to on-snow training and competition for XC skiers, where specific pace-based HIIT sessions can be planned prior to a race or race simulation to improve performance.

A major limitation of this study was the inability to control for other factors that may impact time-trialing and field-testing such as nutrition, recovery and mental strength (Hopkins, Hawley & Burke 1999). The testing protocol was designed to reduce athlete burden and encourage athlete participation. Standardization of all the factors of an athlete's day-to-day

routine would cause significant disruption of their lifestyle and would likely lead to lack of adherence to the protocol or an increased drop-out rate. While nutrition and recovery were not controlled outside of the training sessions, the participants were instructed to treat the 3-day testing block as they would a 3-day race weekend.

CONCLUSION

In this study the results suggests that u18 athletes are more likely to see improvements in 3km track TT performance after a HIIT session in comparison to 18+ athletes. After a session of HIIT u18 athletes are also likely to improving pacing strategy although pacing strategy is not associated with improved performance, whereas in 18+ athletes an improvement in pacing strategy is associated with improved 3km track TT performance. Finally, the results of this study also suggest that a pre-trial and a HIIT session that prescribes intensity based on lap time is an effective way to improve 3km track TT results in u18 athletes.

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CHAPTER 5: CONCLUSION

The present study sought to advance our understanding of how HITT affected performance in XC skiers. While the protocol and study design were novel, the goal was more so directed at increasing the quality of evidence in field testing within the population of Canadian competitive XC skiers. The execution of this data collection was unique because it involved travelling to locations in Montreal, QC, Barrie, ON, Ottawa, ON, Calgary, AB, and Revelstoke, BC and engaged cross-country skiers across Canada. By working directly with coaches to integrate testing sessions into the athlete's training program we were able to collect data with competitive athletes during their general preparation season. Data collection with this number of athletes would not have been possible without putting in the extra effort to communicate with and engage the community at large. The first study (Chapter 3) investigated the influence of sex and menstrual cycle phase on 3km TT performance after a session of HIIT. While we hypothesized we would observe differences across sex and MC phase, significant differences were not observed between men, women, menstrual cycle phase, or oral contraceptives. However, a somewhat surprising finding was the overall significant improvement in performance from pre- to post-HIIT TTs. This finding was not expected as the intervention was employed with the expectation to measure fatigue from the HIIT session in TT performance decrement. In Study 2 (Chapter 4), we analyzed performance change with respect to age and pacing strategy to understand how they may influence the results of the 3km TT field test. We hypothesized that differences in pacing and performance would be observed based on age, and this hypothesis proved correct. When performance improvement was analyzed separately by age group, we observed that younger (u18) athletes were likely to improve their performance, whereas older (18+) athletes were not likely to improve their performance throughout this field-testing

protocol. U18 athletes showed a more even pacing strategy in the post-HIIT TT, although the improvement in pacing strategy did not differ between athletes who improved their performance and those that did not. U18 athletes did not show a significant improvement in pacing strategy from pre- to post-HIIT TT, although the athletes who improved their TT performance had a more even pacing strategy than those who did not. Taken together, these results suggest that XC skiers should employ a pre-trial and HIIT protocol prior to doing a 3km track TT in order to optimize the outcomes of testing.

5.1 Limitations

The absence of repeated measures of TT performance of the female athletes in both the luteal phase and the follicular phase of the menstrual cycle, is a limitation of this study. Most female participants did not have a regular menstrual cycle, and verification of the luteal and follicular phases was challenging. Study groups were identified as “Low Hormone” and “High Hormone” because of the prevalence of amenorrhea and clinically low estrogen and progesterone levels. To have more success in identifying differences in MC phase, a strict exclusion criterion for amenorrhea would have been needed, and this would have limited the pool of participants from the target population.

Additionally, due to limited access to the target population, we were not able to standardize the participants’ training in the weeks leading into the testing. Athletes were tested from many different regions and clubs likely had vastly different training regimes in the weeks prior to testing. While efforts were made to plan the testing blocks after a rest week, in individual sports such as XC skiing, each athlete makes unique decisions about what a “rest week” means to them. A rest day immediately prior to the testing block was mandatory, although a single day of rest may not be sufficient to allow athletes to recover for optimal performance. Additionally, it

would have been ideal to include a familiarization trial prior to the testing block to ensure that improvements in TT performance were not due to “learning how to complete the test”. This was unfortunately not possible due to the XC ski coaches concerns about disruptions to the athletes’ typical training regime.

Finally, a limitation of the field testing is that variability in performance is high, despite best efforts to create a controlled environment. This, however, represents realistic training and testing environments for these athletes, and while significant differences may have been observed with less variability, individual variation is always present when training and testing athletes.

5.2 Future Directions

This study initially set out to understand if there were sex differences in fatigue after a HIIT session so as to improve our understanding of the effectiveness of HIIT prescription for female athletes. Since an overall performance improvement was observed, conclusions about fatigue are not possible. In order to establish conclusions about fatigue, we suggest that future studies employ a longer, more arduous training protocol to adequately fatigue athletes before testing. A study design that employs a “training camp” approach to standardize training prior to the testing block and increase athlete buy-in for a longer duration of testing could be successful.

Further studies with larger sample sizes should continue to examine the influence of sex and menstrual cycle phase on fatigue and performance after HIIT. If rigorous menstrual cycle exclusion criteria are in place, a larger target population will be required, a minimum of 46 males and 46 females would be recommended for future studies (Sample size calculation in Appendix A). This could include not only XC skiers, but also XC runners, and triathletes with the potential

for changing and adapting the protocol for other athletes such as cyclists or rowers. This would also increase the generalizability of this research.

In summary many athletes do not have access to laboratory testing and added to this, women remain significantly under-represented in exercise and sport research often because of the complexities related to the fluctuating reproductive hormones (Emmonds et al., 2019). This thesis addresses these issues by conducting research in a field setting that focuses on aspects of exercise endocrinology in an underrepresented population of Canadian athletes, namely competitive cross-country skiers.

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APPENDICES

Appendix A: Retrospective Sample Size Calculation

STATA Sample Size Calculation Output for Sex Differences:

```
. power two means 2.33 0.79, sd(2.6)
```

Performing iteration ...

Estimated sample sizes for a two-sample means test

t test assuming $sd1 = sd2 = sd$

Ho: $m2 = m1$ versus Ha: $m2 \neq m1$

Study parameters:

alpha = 0.0500

power = 0.8000

delta = -1.5400

m1 = 2.3300

m2 = 0.7900

sd = 2.6000

Estimated sample sizes

N = 92

N per group = 46

STATA Sample Size Calculation Output for Menstrual Cycle Phase Differences:

```
. power oneway 0.93 0.77 -0.78, varerror(30.82) grweights(1 1 1)
```

Performing iteration ...

Estimated sample size for one-way ANOVA

F test for group effect

Ho: $\delta = 0$ versus Ha: $\delta \neq 0$

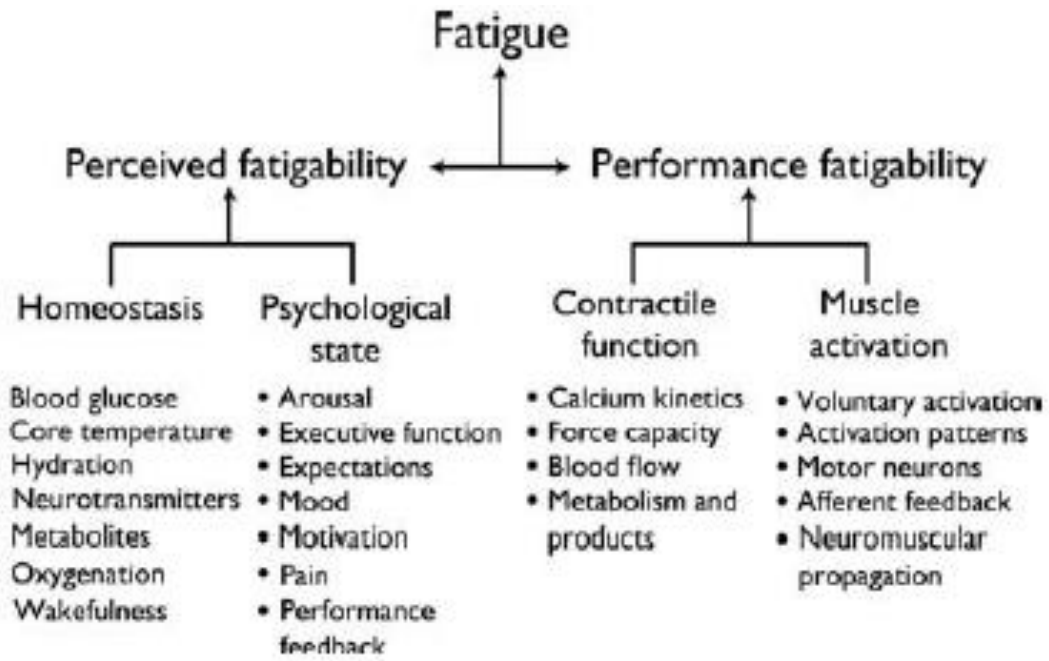
Study parameters:

```
alpha = 0.0500  
power = 0.8000  
delta = 0.1389  
N_g = 3  
m1 = 0.9300  
m2 = 0.7700  
m3 = -0.7800  
Var_m = 0.5947  
Var_e = 30.8200
```

Estimated sample sizes:

```
N = 504  
N per group = 168
```


Appendix B – Fatigue Flow Chart (Enoka & Duchateau, 2016)



Appendix C: TCPS 2: Core Certificate




Appendix D: Recruitment Posters



UNIVERSITY OF CALGARY
FACULTY OF KINESIOLOGY

The effects of the Sex & Menstrual Cycle on Fatigue and Performance Decrement in a High Intensity Training Program



**All men and women competitive cross country skiers,
Are you curious how the menstrual cycle influences your training?**

If you / your team are competing at a National or International level, are able to complete a 5-day high intensity training block as prescribed (3x 3000m Tests, 1 Track Workout, 1 rest day) and specific to women complete 2 months of menstrual cycle tracking (urinary analysis), please contact

**Anneke Winegarden in the Doyle-Baker Lab at the University of
Calgary: Anneke.winegarden1@ucalgary.ca**

Title: Effects of Sex and Menstrual Cycle Phase on Performance Decrement and Fatigue in Endurance Athletes after a High Intensity Training Session. PI. Dr. PK. Doyle-Baker. This study has been approved by the University of Calgary Conjoint Health Ethics Board (REB19-0311)

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SEX & MENSTRUAL CYCLE DIFFERENCES IN FATIGUE & PERFORMANCE DECREMENT IN XC SKIERS

Recent research has shown that a woman's metabolism, exercise capacity, and physiological responses to heat can be altered depending on which menstrual cycle (MC) phase they are in. A gap in the literature exists surrounding sex differences in fatigue development after high intensity training, and how the MC plays a role in females. It can be hypothesized that training program periodization should consider sex and MC phase to optimize training outcomes and athletic performance.

IF YOUR TEAM IS COMPETING AT A NATIONAL OR INTERNATIONAL LEVEL, ARE ABLE TO COMPLETE A 4-DAY HIGH INTENSITY TRAINING BLOCK AS PRESCRIBED (1 REST DAY, 2X 3000M TESTS, 1 TRACK WORKOUT) AND WILLING TO COMPLETE URINARY OVULATION TESTS (FEMALES ONLY), PLEASE CONSIDER PARTICIPATING!

Title: Effects of Sex and Menstrual Cycle Phase on Performance Decrement and Fatigue in Endurance Athletes after a High Intensity Training Session. PI. Dr. PK. Doyle-Baker. This study has been approved by the University of Calgary Conjoint Health Ethics Board REB19-0311.

Do males and females respond differently to a training session?

Curious, about how the menstrual cycle influences training and fatigue?

Learn about and innovative and novel methods to measure fatigue in athletes

ANNEKE WINEGARDEN
(FORMER NAKKERTOK COACH)

UNIVERSITY OF CALGARY
HIGH PERFORMANCE LAB

ANNEKE.WINEGARDEN1
@UCALGARY.CA

Appendix E: Ethics Approval

2/24/2020

<https://iriss.ucalgary.ca/IRISSPROD/sd/Doc0/L4FR731J6H84DC7EJ6T3UQ2T95/fromString.html>



Conjoint Health Research Ethics Board
Research Services Office
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chreb@ucalgary.ca

CERTIFICATION OF INSTITUTIONAL ETHICS APPROVAL

The Conjoint Health Research Ethics Board (CHREB), University of Calgary has reviewed and approved the following research protocol:

Ethics ID: REB19-0311
Principal Investigator: Patricia Katherine Doyle-Baker
Co-Investigator(s): Louis Passfield
Student Co-Investigator(s): Anneke Winegarden
Study Title: Effects of Sex and Menstrual Cycle Phase on Performance Decrement and Fatigue of Endurance Athletes after a High Intensity Training Session
Sponsor: Faculty of Kinesiology

Effective: Friday, April 26, 2019

Expires: Sunday, April 26, 2020

The following documents have been approved for use:

- Coach Flyer for Support in Recruitment, 3, April 12, 2019
- Poster, 2, March 31, 2019
- Consent Form - Clean, 4, April 25, 2019
- Questionnaire, 1, March 21, 2019
- PAR-Q, 1, March 21, 2019
- Research Proposal (Clean), 4, April 24, 2019
- App F REB 19-0311 Budget, 1, March 29, 2019

The CHREB is constituted and operates in accordance with the current version of the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* (TCPS); International Conference on Harmonization E6: Good Clinical Practice Guidelines (ICH-GCP); Part C, Division 5 of the Food and Drug regulations, Part 4 of the Natural Health Product Regulations and the Medical Device Regulations of Health Canada; Alberta's Health Information Act, RSA 2000 cH-5; and US Federal Regulations 45 CFR part 46, 21 CFR part 50 and 56.

You and your co-investigators are not members of the CHREB and did not participate in review or voting on this study.

Restrictions:

This Certification is subject to the following conditions:

<https://iriss.ucalgary.ca/IRISSPROD/sd/Doc0/L4FR731J6H84DC7EJ6T3UQ2T95/fromString.html>

1/2

1. Approval is granted only for the research and purposes described in the application.
2. Any modification to the approved research must be submitted to the CHREB for approval.
3. An annual application for renewal of ethics certification must be submitted and approved by the above expiry date.
4. A closure request must be sent to the CHREB when the research is complete or terminated.

Approval by the REB does not necessarily constitute authorization to initiate the conduct of this research. The Principal Investigator is responsible for ensuring required approvals from other involved organizations (e.g., Alberta Health Services, community organizations, school boards) are obtained.

Approved By:

Stacey A. Page, PhD, Chair , CHREB

Date:

Friday, April 26, 2019

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Appendix F: Consent Form



Consent Form

TITLE: Effects of Sex and Menstrual Cycle Phase on Performance Decrement and Perceived Fatigue in Endurance Athletes after a High Intensity Training Session

INVESTIGATORS: Drs. Patricia K. Doyle-Baker, Dr. PH, (CSEP-CEP); Connie Lebrun (MD); Anneke Winegarden, BScHK, CSEP-CEP (MSc. Trainee)

Anneke.winegarden1@ucalgary.ca

This consent form is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, please ask. Take the time to read this carefully and to understand any accompanying information. You will receive a copy of this form.

BACKGROUND

A decrease in performance can be used as a method to identify training stress in athletes by measuring the amount of fatigue after a workout. Performance decrease in cross-country skiers has not been studied following a high intensity training session. Males and females are known to respond to fatigue differently and there is limited knowledge on this. As well the menstrual cycle is known to (MC) influence fatigue and training adaptation in competitive endurance athletes.

This study has two parts. Part A seeks to determine the daily change in 3000m running time trial (TT) performance in cross-country skiers. Part B seeks to understand the differences between males and females and whether the MC influences fatigue development by investigating the decrease in performance in 3000m running TT before and after a high intensity training session.

Competitive cross-country skiers will be recruited across Canada to participate in our study.

WHAT IS THE PURPOSE OF THE STUDY?

This study will investigate whether sex and menstrual cycle phase (Follicular (FP) or Luteal (LP)) will influence fatigue and performance decrement from a high intensity training session in endurance athletes.

WHAT WOULD I HAVE TO DO?

You may choose to participate in the one of the three following options: 1) PART A, or 2) PART B, 3) PART A and PART B (performed at separate times). Participation in PART A and/or PART B will be determined by your availability as an athlete and your location. You can withdraw from the study by verbally telling the research assistant (RA) or thorough email (anneke.winegarden1@ucalgary.ca) to withdraw. If the final 3000m TT has been completed, the data collected will be used in the final analysis.

Equipment:

We would like you to feel as comfortable as possible and therefore ask that you use your own heart rate monitor and watch. We also ask that you export your workout files and provide this to us after each session.

1/4



Prescreening:

You must complete the following prescreening procedures prior to running the 3000m TT:

- Physical Activity Readiness Questionnaire (PAR-Q)
- Resting heart rate (HR) and resting blood pressure (BP) taken by the RA (CSEP-CEP)
- Height and weight measurements taken by the RA (CSEP-CEP)

Pre-testing:

- We ask that you complete at least 1 track intensity workout within 2 weeks prior to 3000m TT testing)
- We ask that you have at least 1 rest day prior to 3000m TT testing
- We ask that you come to the Human Performance Lab at the University of Calgary to have a height and weight measured and a (DEXA) scan which measures fat mass, fat-free mass and bone mineral density. The scan takes 5 minutes.

PART A (3hr time commitment over 3 days):

- Complete the following testing schedule:
 - Day 1 involves running the 3000m TT at race pace. We are asking for your maximum effort; run a personal best time. A standardized warm up and cool down plan will be given to you to complete. Please wear your heart rate monitor and watch so that you can provide us with your maximum heart rate and average heart rate, during the 3000m TT. Total time for 3000m TT completion will be collected and recorded by the RA. The total workout time will be approximately 1.5hrs. We ask that you consider the following hours as a partial rest day and do not do any other structured physical activity on this day.
 - Day 2: Rest day. We ask that you do not do any structured physical activity on this day.
 - Day 3: Is a repeat of day 1 3000m TT. Same instructions as above.

PART B (4.5hr time commitment over 3 days):

- Females Only: We ask that you track your menstrual cycle for 2-months prior to the first 3000m TT and email the start day and end day of your menstrual cycle to the RA.
- Females Only: We ask that you complete urinary ovulatory testing for 10 days beginning on day 10 of your cycle so that we determine the menstrual cycle phases within your last month. We will provide you with 10 kits (Clearblue Advanced Digital Ovulation tests) and instructions on how to do this and how to store them.
- Females Only: We ask that you complete a salivary hormone sample on the first day of the running tests. Instructions will be given to you later on re: the collection process. This sample will be collected by the research assistant and for estrogen and progesterone analysis to verify your menstrual cycle phase.
- We ask that you complete the following testing schedule:
 - Day 1 involves running 3000m TT at race pace. We are asking for your maximum effort; run a personal best time. A standardized warm up and cool down plan will be given to you to complete. Please wear your personal heart rate monitor and watch so that you can provide us with your maximum heart rate and average heart rate, during the 3000m TT. Total time for 3000m TT completion will be collected and recorded by the RA.
 - The RA will administer the Rating of Fatigue Questionnaire and we ask that you complete this before and after the TT. The total workout time will be approximately 1.5hrs. We ask that you consider the following hours as a partial rest day and do not do any other structured physical activity on this day.

2/4



- Day 2 involves a Track Workout. You will be asked to complete 4-6 x 800m intervals with 3 mins rest at 90% of your max aerobic speed. This will be calculated from your recent 3000m TT results. You will be provided with a goal split time and a goal heart rate for the workout. Please wear your personal heart rate monitor and watch so that you can provide us with your maximum heart rate and average heart rate, during the 3000m TT. The RA will administer the Rating of Fatigue Questionnaire and we ask that you complete this before and after the TT. Total workout time will be approximately 1.5 hrs. We ask that you do not do any structured physical activity on this day.
- Day 3: 3000m running TT. Same instructions as Day 1.

WHAT ARE THE RISKS?

The risks associated with this study may include discomfort and fatigue associated with endurance performance testing and high intensity running. There is also the risk of training related injury associated with high intensity running and radiation exposure (less than the daily exposure from the sun) from a DXA, may occur.

WILL I BENEFIT IF I TAKE PART?

If you agree to participate, upon completion of the study you will be offered a 1-1 meeting with a CSEP-Certified Exercise Physiologist to discuss your time trial results, fatigue levels and heart rate data collected during the study.

WILL WE BE PAID FOR PARTICIPATING, OR DO WE HAVE TO PAY FOR ANYTHING?

There will be no financial compensation to you. You will be responsible for covering costs of transportation and parking at training facilities as per typical training session.

WILL MY RECORDS BE KEPT PRIVATE?

All of the information collected will remain strictly confidential. Only the investigators responsible for this study, the research assistant (RA) who will be doing the baseline assessments, and the University of Calgary Conjoint Health Research Ethics Board will have access to this information. Confidentiality will be protected by using a study identification (ID) number in the database. Any results of the study reported will in no way identify you the study participant. De-identified data will be stored for possible future use by other researchers. Participants will be provided with a copy of their individual raw data.

IF I SUFFER A RESEARCH-RELATED INJURY, WILL I BE COMPENSATED?

In the event that you suffer an injury as a result of participating in this research, no compensation will be provided to you by the University of Calgary or the researchers. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages.



SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding your participation in the research project and agree to participate as a participant. In no way does this waive your legal rights nor release the investigators or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time. If you have further questions concerning matters related to this research, please contact:

Dr. PK Doyle-Baker (Principal Investigator): (403) 220-7034

If you have any questions concerning your rights as a possible participant in this research, please contact the Chair, Conjoint Health Research Ethics Board, University of Calgary at 403-220-7990.

Please check all boxes below that apply:

- I consent to participate in PART A of this study.
- I consent to participate in PART B of this study.


Participant's Name	Signature and Date
Parent/Guardian Name (If <18 years)	Signature and Date
Investigator/Delegate's Name	Signature and Date
Witness' Name	Signature and Date

The University of Calgary Conjoint Health Research Ethics Board has approved this research.

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

Appendix G: Questionnaires

Micklewright (2017) Rating of Fatigue Questionnaire: How fatigued are you right now?

10	TOTAL FATIGUE & EXHAUSTION – NOTHING LEFT	
9		
8	VERY FATIGUED	
7		
6		
5	MODERATELY FATIGUED	
4		
3	A LITTLE FATIGUED	
2		
1		
0	NOT FATIGUED AT ALL	

Feeling Scale: How do you feel right now?

Feeling Scale (FS) (Hardy & Rejeski, 1989)	
While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.	
+5	Very good
+4	
+3	Good
+2	
+1	Fairly good
0	Neutral
-1	Fairly bad
-2	
-3	Bad
-4	
-5	Very bad

Session-RPE Method for Training Load Monitoring (Foster et al., 2001): How hard was your entire workout?

Rating	Descriptor
0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	*
7	Very Hard
8	*
9	*
10	Maximal

Appendix H: Clearblue Ovulation Testing Procedures

Participants began testing 10 days after onset of menses, until a positive test occurred, onset of menses re-occurred, or 15 days passed.

Below is an excerpt from the Clearblue instructions manual, available online.

Advantages over traditional methods for determining ovulation

Detection of the LH surge using the Clearblue Digital Ovulation Test allows women to time intercourse to coincide with their most fertile time by alerting them to approaching ovulation. Thus it provides women with a prospective method of identifying their period of peak fertility and has advantages over traditional methods of identifying ovulation.

- The basal body temperature (BBT) method requires women to chart their daily temperature to detect a rise associated with ovulation. However, it is not ideal for timing intercourse because the rise in temperature cannot be detected until after ovulation has occurred.¹⁷ Furthermore, most studies examining the BBT method have concluded that it can be unreliable, due to inaccurate interpretation of temperature curves, either through patient error, or confounding factors (alcohol intake, timing of temperature reading or certain medications)^{18,19}
- Monitoring of cervical mucus can provide prospective information that ovulation has occurred, but it requires a level of training, is less accurate than monitoring LH surge, and may not be acceptable to all women²⁰
- The calendar method relies on previous cycle length to predict when ovulation is likely to occur in the current cycle. It is an unreliable method for the purpose of timing intercourse to conceive, because women's cycles are known to vary from cycle to cycle and the day of ovulation itself is therefore variable.^{17,18,21} Studies have shown that the calendar method correctly identifies women's fertile days in only a third of cycles.²²

The accuracy in predicting the LH surge to within 1 day is reported to vary between 57–70% for the basal body temperature method and 48–76% for the cervical mucus evaluation method.²³

Adapts to a woman's own LH surge threshold

The Clearblue Ovulation Test is a rapid 'sandwich' immunoassay which uses monoclonal antibodies to detect the LH molecule. An optical system, contained within the Test Holder, then measures the density of the lines formed by the binding of LH to the antibodies, and if above a defined threshold, a positive 'LH surge' result will be displayed.

As many women have low levels of LH present in their urine throughout their cycles, the Clearblue Digital Ovulation Test does not measure the LH surge to a constant uniform threshold. Instead, the Clearblue Digital Ovulation Test sets a personalised threshold level for each woman, by measuring their change in LH level from their personal baseline. This is an obvious advantage over visual tests which ignore the fact that different women have different baseline LH levels and that levels can even vary between cycles.

Easy to use

The Clearblue Digital Ovulation Test comprises of an electronic Test Holder and a supply of foil-wrapped Test Sticks. The pack also contains an instruction leaflet; an abbreviated version of these instructions is provided below.



Prior to using the Clearblue Digital Ovulation Test, the user must remove a Test Stick from its foil wrapper, take off the Test Stick cap, and insert the Test Stick into the Test Holder. The Test Stick and Test Holder are marked with pink arrows. The user must align these when inserting the Test Stick into the Test Holder.



The Test Stick clicks into place when inserted and the 'Test Ready' symbol appears on the Display.

When the 'Test Ready' symbol appears, the user simply holds the Absorbent Sampler in her urine stream for 5–7 seconds. Alternatively, she can collect a sample of urine in a clean, dry container and immerse the sampler in the collected specimen for 15 seconds. After 20 to 40 seconds, the 'Test Ready' symbol will flash to show that the test is working.

After 3 minutes, the Test Holder automatically reads and interprets the test result and delivers a ○ for a 'No LH surge' result and a ☺ for a positive 'LH surge' result.

The Clearblue Digital Ovulation Test is available in variable pack sizes to allow women to purchase a 1 or 2 month supply and to accommodate the variability in women's length of cycle.* In addition, the Test Holder is reusable the following month, should the user have Test Sticks left over from their current testing cycle.




*Not all pack sizes are available in all countries.


Appendix I: Labrix Salivary Hormone Testing Procedures

2 KIT CONTENTS


If you have questions, please call your provider before proceeding. If your kit is missing components, call (800) 325-2784 for a replacement.




Cardboard kit box




Insulated cooler with lid
(keep in kit box)




Ice pack




Plastic bag containing:
4 color-coded saliva tubes and 4 straws




Requisition form



Patient survey



(USA Only)
FedEx Clinical Pak



(USA Only)
Billable Stamp

3 PREPARATION

The testing for this profile consists of collecting 4 saliva samples for hormone analysis.

BEFORE YOU START: Please read all of the instructions carefully. Consult your physician for specific instruction and before stopping any medications. Make sure your physician has selected a test profile on the requisition form. Collect saliva on a day that is determined by you and your provider, considering the recommendations on page 4.

DAY OF COLLECTION
Do not take any supplements until after you have collected all four samples.
First saliva collection MUST be 30 minutes after awakening. Set a timer if necessary.
Do not eat or drink anything 1 hour prior to saliva collection.
Do not brush, floss, or have dental work done 30 minutes prior to saliva collection.
Do not consume food or drinks that contain caffeine.
Avoid all nuts, fruits, fruit juices, wine, rice and coffee.
Before each saliva collection, wash hands and rinse mouth with water, but wait 10 minutes to collect your saliva sample (to avoid dilution.)
Use of cosmetics, other than "anti-aging" creams is permitted.

4 RECOMMENDATIONS

WOMEN & MEN ON HORMONES (estrogen, progesterone, testosterone, DHEA)		
Typical Hormones (ovary)	Last dose prior to collection should be within a window of 12-24 hours before collecting your first sample	Do not apply topical or use sublingual hormones throughout the entire day of saliva collection. This will contaminate your sample and your test results.
Biological Hormones or Testes (discussed under tongue)	Last dose prior to collection should be within a window of 24-36 hours, followed by two full glasses of water to cleanse the tissues. Then collect your first sample.	
Oral Hormones (pills) and Transdermal Patches	Should be used as prescribed on the day of collection	
Injections, Subcutaneous Patches and Transdermal Patches	Collect at the halfway point between doses	
CONTROLLED SUBSTANCE SUPPLEMENTATION		
CONSULT WITH YOUR PROVIDER for instruction if you are taking a controlled supplement for adrenal support (or any other glucocorticoid for medical reasons). Note: certain medications, including hydrocortisone creams and asthma inhalers, contain cortisol. To evaluate your natural cortisol production, it is recommended to stop using cortisol-containing products 5 days prior to sample collection. Consult with your provider prior to stopping cortisol-containing medication(s).		
MELATONIN SUPPLEMENTATION		
Last dose should be at least 24 hours before first saliva sample collection.		
OTHER		DAY TO COLLECT
Women with menstrual cycle		Collect 1 day during days 1-6 of a 28-day cycle, counting the first day of your period as day 1
Post-menopausal women		
Women who have had a hysterectomy		
Men not on hormones		Collect any day of the month. If you are taking hormones or are on cortisol and melatonin supplementation, please read and follow the recommendations listed above.
Neurotransmitter and adrenal function profiles only (cortisol, alpha, and/or DHEA)		
Women w/ hormonal IUD (just an option)		

Call your provider with additional questions. Please do not send any medications with your kit.

5 COLLECTING SALIVA

Collect saliva on a day that is determined by you and your physician, considering the recommendations on page 4.

- 1 | Collect before breakfast in the **PINK TUBE (AM 30)**
Collect 30 minutes after awakening (set a timer)
- 2 | Collect before lunch in the **GREEN TUBE (NOON)**
- 3 | Collect before dinner in the **ORANGE TUBE (EVENING)**
- 4 | Collect right before bedtime in the **BLUE TUBE (NIGHT)**

1. 10 minutes before each saliva collection, wash hands and rinse mouth with water.
2. The first saliva collection must be 30 minutes after awakening. Timing is critical.
3. Fill all four tubes regardless of how many tests are ordered.
4. Each tube should be 3/4 full. Bubbles or foam are OK.
5. Use of the included straws to funnel saliva into each tube is optional. Dispose of straws before shipping.
6. After each saliva collection:
 - ✓ Snap saliva tube closed tightly.
 - ✓ Record the date and time of the collection on the bag and on the requisition form.
 - ✓ Place saliva in freezer immediately. When all four tubes have been collected, place all tubes into plastic collection bag with absorbent material and keep in freezer with frozen ice pack until ready to ship.

6 SHIPPING

PREPARE THE PACKAGE


1. **Ship Monday - Friday only.** You will be charged a pick-up fee if you call for a Saturday pick-up. Weekend or holiday collection: keep the specimen in the freezer until the following business day to ship.
2. **Complete all paperwork, including requisition form and patient survey.** US patients only: fill out the AEN on the back of the requisition form.
3. **Place items into the cardboard kit box in the following order:**
 - Insulated cooler
 - Plastic bag with frozen specimen tubes, zipped closed
 - Frozen ice pack
 - Requisition form, patient survey, and payment (if applicable)
4. **Replace the lid of the cooler, and close the cardboard box.** Place **inside** the Clinical Pak. Write your name and address on the Billable Stamp and tear off the Customer Receipt for your records. Place on FedEx Clinical Pak.

SCHEDULE A PICK-UP (USA ONLY)

Call FedEx toll-free at 1-800-468-3339 (1-800-GO FEDEX). At the greeting, say "Schedule a Pick-up." When prompted, say "Schedule a Pick-up using a Label or Stamp." Reply "Yes" when asked if the word "Stamp" is written on the waybill. You will then be asked for your address information. **DO NOT USE A DROP BOX.**


In some cases, Doctor's Data provides prepaid shipping from certain countries outside the United States. If that policy pertains to this collection kit, then country specific shipping materials (including an Air Waybill and Instructions) will be included in the kit contents. If there are not any shipping materials included or you choose to use a different courier or level of service than provided, you must make your own shipping arrangements at your own expense.



hormone analysis
SALIVA COLLECTION



INSTRUCTIONS

Read all instructions before collecting specimens.



Appendix J: Practice Plans for Testing Sessions

3000m TT Practice Plan		
Lead Researcher: Anneke Winegarden Extra Support: +1 Coach per every 10 athletes participating		
Time	Activity	Notes
7:00-7:15	Arrive, sign in with Anneke.	ROF-Q & Feeling Scale Data Collected upon arrival. “Start Time” will be provided to the athletes, consent forms will be signed by any withstanding athletes. Start list will also be posted inside track for athletes to refer to.
7:15-7:20	Team Meeting for final instructions	Goal: Collect time, average and maximum heart rate, go as hard as you can, race effort. Athlete Instructions: - Interval start, 15 seconds apart - 7.5 laps, which you have to count yourself - Athletes are responsible for counting their own laps in addition to data collection team, but coaches will provide a ‘bell lap’ warning when possible. - Athletes start your watch, and stop your watch as back up timing. - Give your absolute maximum effort, especially on the final lap
7:20-8:00	Warm-up 20 min – Aerobic Z1 warm-up & Dynamic exercises 1x3 min lap Z3 (Sub Threshold) 3 min off 1x1.5 min Z4/Target Race Pace 10-15 min active recovery before start	Warm up is standardized by self-lead but supervised by research assistant who is available to answer questions and provide direction when needed. Athletes start at opposite end of track on inside waterfall line. Athletes finish at standard finish line.
7:45-7:55	Data Collection Team meeting and watch synch.	Synch watches at 7:55 – first start at 5 min after. As close to 8:00 AM as possible, or 5 min after synch watch is first start

8:00 START	<p>15s interval starts, 2-5 min gaps approximately every 10 athletes to decrease congestion on track.</p> <p>Start order based on reverse CPL order</p>	<p>Starter – Victor? Lap/Finish Crew - Anneke (Webscorer) + 1 Coach (calling bib numbers, counting laps to call last lap) 3000m = 7.5 laps</p> <p>Heart Rate monitor watch must be started immediately on starting, and also stopped immediately at the finish.</p> <p>Keep everyone inside of track until all athletes have finished the 3000m effort.</p>
8:45ish	<p>Team Meeting</p> <ul style="list-style-type: none"> - HR/ROF-Q Data recording - Cool down run - ROF-Q/Feeling Scale/sRPE recording 	<p>Record – Athlete watch time, Max HR, Average HR, ROF-Q before athlete starts cool down.</p> <p>Athletes complete a 15-20min active recovery cool down (easy jogging on track), cool down is self-lead but supervised by research assistant. All athletes must report back to data collection team after cool down.</p> <p>Record ROF-Q, Feeling Scale, sRPE immediately after cool down.</p>

Lead Researcher: Anneke Winegarden
 Extra Support: +1 Coach per every 10 athletes participating

Time	Activity	Notes
7:00-7:15	Arrive, sign in with Anneke.	ROF-Q & Feeling Scale Data Collected upon arrival. Athletes will receive their "goal splits", a copy of the goal split for each athlete will be posted inside the track for athletes to reference.
7:15-7:30	Team Meeting for final instructions	Goal: Perform as many consistent 800m reps as possible. Athlete Instructions: - Aim to hit your goal split time on your first rep, if you find the goal split unachievable, use your goal heart rate to guide the pace on the 1 st and 2 nd reps, and match that pace for the remainder of the reps. - This workout should feel hard; these intervals should feel like they represent "race pace" or critical speed" intensity. - After each interval you will have 3 mins of rest. You should use your watch diligently during this workout since everyone's pace will be slightly different. - You need to press the lap button on your watch at the start and end of each interval.
7:30-8:00	Warm-up 20 min – Aerobic Z1 warm-up & Dynamic exercises 1x 5 min Z3 (Sub Threshold) 3 min active recovery 2 min rest before start	Warm up is standardized by self-lead but supervised by research assistant who is available to answer questions and provide direction when needed.
8:00 START	Workout: Race Pace / Critical Speed Int: 4-6 x 800m Rest: 3 min Format: 5s interval starts (Start order based on reverse CPL order)	When athletes finish each rep, data collection team will: <ul style="list-style-type: none"> - Record lap time from athlete watch, give athlete feedback whether or not they are on target - Record ROF-Q after each rep - Cut from workout at 20s exceeding lap time (+10%)
8:45ish	Team Meeting <ul style="list-style-type: none"> - HR Data recording - Cool down run - ROF-Q recording 	Record – Max HR & Average HR for each rep before athlete starts cool down. Athletes complete a 15-20min active recovery cool down (easy jogging on track), cool down is self-lead but supervised by research assistant. All athletes must report back to data collection team after cool down. Record ROF-Q & Session RPE immediately after cool down.

Appendix K: Simcoe County Track Rental & Insurance Form

Community
Use of Schools

Simcoe County
District School Board

Simcoe County District School Board

Address: 1170 Highway 26
Midhurst, ON L9C 1K6
Phone: (519) 228-7670
Fax: 705-728-6087

PERMIT FOR USE OF BOARD PROPERTY

Last modified: Jun 4, 2019
First booking: Jul 25, 2019 - 8:00 am

Subject to all terms, conditions, rules and regulations as stated in the Board's Policy and Procedures.
In the event of an emergency, please call the number below and follow the instructions for a building related emergency.

Please do not bring drinks containing perishable items onto the premises. The DCSB strives to provide a permit free environment.
Facilities are for emergency purposes only. Do not leave open for any other use.

Status: **Approved**

Permit number: 2019-05-21-0047 **Attendance:** 15 *May be under 18
Purpose: Track Workout
Permit type: Group A - Youth Groups

Organization: University of Calgary - High Performance Laboratory **Home phone:**
Permit holder: Anneke Winegarden **Work phone:**
Email: anneke.winegarden@gmail.com **Mobile phone:** 613-894-4850

Facilities used: Eastview SS (421 Grove Street East, Barrie, L4M 5S1)

Emergency numbers: 705-817-5154 Barrie North East, Springwater
705-794-3967 Midland, Penetanguishene Area
705-733-5888 Orillia
705-794-3609 Barrie South
705-794-3948 Barrie North West
705-817-1534 Alliston, New Tecumseth
705-715-4895 Bradford, Innisfil
705-794-4084 Barrie South West, Essa
705-794-4167 Cullingwood, Stayner

Event supervisors: Anneke, anneke.winegarden@gmail.com, 613-894-4850

Comments

For custodian

Permit is for outdoor use only. Permit will not have access inside the school. Custodial coverage is not required for field permits and custodial billing reports are not needed.

For permit holder

Permit is for outdoor use only. No access inside the school.

WFT FIFI DRAIN OUT PROCEDURE

In an effort to avoid damage to our sports fields and injury to players, the following procedures will apply:

Simcoe County District School Board fields/diamonds will be considered unplayable if the following conditions exist:

1. Ponding of water on the surface of the field/diamond.
2. Water sponging up around one's feet when walking on the field/diamond.
3. Weather conditions - lightning, thunderstorms, rain.

Permit holder: Anneke Winegarden
Organization: University of Calgary - High Performance Laboratory

Purpose: Track Workout
Permit number: 2019-05-21-0047

Page 1 of 3

If any of these conditions occur, the field/diamond will be considered "UNPLAYABLE" and the practice/game cancelled and/or rescheduled. The Board, through the direction of the principal or designate, shall have the exclusive rights to cancel any practice/game booked due to wet field conditions.

If a group arrives at a field and these conditions exist or if these conditions develop during the course of the practice/game, they are not permitted to continue playing on the field/diamond. Several factors must be considered, including the safety of the participants, the possibility of liability through accidents or injury, and the unnecessary expense of field/diamond repairs and maintenance.

If a group ignores the "UNPLAYABLE" conditions:

1. The permit holder will be required to pay for all damages to the field/diamond arising from abuse to the facility.
2. The permit holder could be held liable and responsible for accidents, or injuries incurred because of unsafe conditions.
3. The permit holder may have their permit suspended or revoked for any future use of a field/diamond.

Fields/ball diamonds shall not be used if lightning is visible. Immediately move to a safe location - away from metal structures (backstops, fences), tall structures (light, standards, trees) and out of the open field.

SCDSB is not responsible for loss or damage to vehicles or their contents.

Bookings

Status: Approved

Total hours: 6

Status	Date	Start	End	Facility and spaces
Approved	Thu, Jul 25, 2019	6:00pm	8:00pm	Eastview SS in Running Track
Approved	Fri, Jul 26, 2019	6:00pm	8:00pm	Eastview SS in Running Track
Approved	Sat, Jul 27, 2019	6:00pm	8:00pm	Eastview SS in Running Track

Cost Details

Permit costs				
	Regular	Subsidy	After subsidy	Qty
Admin Fee - Multiple:	\$25.00	-\$17.50	\$7.50	1
Field Cost - Multi-hooking:	\$50.00	-\$50.00	\$0.00	1
Booking costs				
	Regular	Subsidy	After subsidy	Qty
Rental fee:	\$0.00	-\$0.00	\$0.00	3
Total				
Subtotal:	\$75.00	-\$67.50	\$7.50	
HST:	\$9.75	-\$8.77	\$0.98	
Total:	\$84.75	-\$76.27	\$8.48	

CANADIAN UNIVERSITIES RECIPROCAL INSURANCE EXCHANGE

CERTIFICATE OF INSURANCE

INSURED

University Of Calgary
2500 University Drive NW
MS 255
Calgary, AB, T2N 1N4

Contact: Janet Stein
Title: Director, Risk Management
Tel: 403 282 2765
Email: jstein@ucalgary.ca
Reference:

CERTIFICATE HOLDER

Simcoe County District School Board

Eastview Secondary school
1170 Highway 26 West
Midhurst, Alberta, T9X 1N6

Contact:
Title:
Tel:
Fax:
Email:

Nature of Operations:
400m Outdoor Oval Running Track Rental - Faculty of Kinesiology / Human Performance Lab - Annick Wingarden - DATES:
Jul 24, 2019 - July 31, 2019

Certificate No: 79607

Issue Date: 05/29/2019

This is to confirm that insurance as described herein is in full force and effect on behalf of the Named Insured and as more fully described in said policies and any endorsements thereto and is subject to all the terms, exclusions, limits and conditions of such policies. This certificate provides proof of insurance only where a limit is shown. Where indicated the Certificate Holder has been added as an Additional Insured but only with respect to liability arising out of the operations of the Named Insured.

POLICY	EFFECTIVE	EXPIRY	LIMIT	POLICY
1907L1	01/01/2019	01/01/2020	2,000,000.00	<p>COMPREHENSIVE GENERAL LIABILITY</p> <p>Covering all premises and operations of the Named Insured including blanket contractual liability, professional and malpractice liability, cross liability, tenant's legal liability and employer's liability. The limit per occurrence is inclusive for bodily injury, personal injury and property damage.</p> <p><input checked="" type="checkbox"/> Certificate Holder as Additional Insured</p> <p>EDUCATIONAL INSTITUTIONS ERRORS AND OMISSIONS</p> <p>Covering Errors and Omissions Liability and Professional Liability of the Named Insured on a claims made basis.</p> <p>PROPERTY</p> <p>*All Risks* of direct physical loss or damage to property of the Named Insured and to property for which the Named Insured has agreed to be responsible. The limit per loss is inclusive for repair/replacement of buildings and contents, including the interests of lessors and/or mortgagees (includes Excess Property where applicable).</p> <p><input type="checkbox"/> Certificate Holder as Additional Insured/Loss Payee (ATIMA)</p>
				<p>EXCESS PROPERTY</p> <p>*All Risks* of direct physical loss or damage to property of the Named Insured and to property for which the Named Insured has agreed to be responsible. The limit per loss is in excess of \$5,000,000 and is inclusive for repair/replacement of buildings and contents, including the interests of lessors and/or mortgagees. Issued and signed on behalf of Subscribing Insurers.</p>

CURIE undertakes to provide 30 days written notice to the Certificate Holder in the event of any material change and/or cancellation of the described policies.



Authorized Representative

Appendix M: Raw Data (Menstrual Cycle Phase)

Menstrual Characteristics					MC Status (0=Male, 1= Low Hormone, 2= Luteal Phase, 3=OC)
Participant #	Phase	Ovulation Test	Day of Cycle	Phase	
1	Low Hormone	Negative			1
2	Active OC	NA			3
3	Low Hormone	Negative			1
4	Low Hormone	Positive	27	Luteal -> Menstru	2
5	Low Hormone	Negative			1
6	Active OC	NA			3
7	Low Hormone	Positive	19	Luteal	2
8	Low Hormone	Negative			1
9	Low Hormone	Negative			1
10					0
11					0
12	Low Hormone	Negative			1
13	Low Hormone	Positive	34	Luteal	2
14	Low Hormone	Negative			1
15					0
16					0
17					0
18	Low Hormone	Positive	23	ovulatory -> Lute	2
19	Low Hormone	IUD - Positive	25	Luteal	2
20	Low Hormone	Negative			1
21	Active OC	Negative			3
22	Low Hormone	Positive	8	Follicular	1
23	Low Hormone	Negative			1
24	Low Hormone	Negative			1
25					0
26					0
27					0
28	Low Hormone	Negative			1
29	Active OC	NA			3
30	Low Hormone	Negative			1
31	Low Hormone	UID - Negative			1
32					0
33	Balanced Hormones	Positive	26	Luteal	2
34	Low Hormone	Negative	8		1
35	Balanced Hormones	Positive	24	Luteal	2
36	Low Hormone	Negative	33		1
37	Low Hormone	Negative	24		1
38	Low Hormone	Positive	1	Luteal -> Menstru	2
39	Low Hormone	Negative	17		1

Appendix N: Raw Data (Hormones - Labrix Results)

Participant ID	Estradiol	Progesterone	Pg/E2 Ratio
12 813-1	0.9	62	68.9
13 813-2	1	56	56
14 813-3	1	31	31
1 86-1	1.1	32	29.1
2 86-2	1.2	64	53.3
3 86-3	1.3	22	16.9
4 86-4	2.3	58	25.2
5 86-5	1	42	42
6 86-6	1.1	80	72.7
7 86-7	2.9	126	43.4
8 86-8	0.7	46	65.7
9 86-9	1.3	60	46.2
18 93-1	1.9	74	38.9
19 93-4	1.2	70	58.3
20 93-5	1.5	166	111
21 93-6	1.3	89	68.5
22 93-7	1.5	70	46.7
23 96-1	2	50	25
24 96-2	1.1	96	87.3
28 926-1	1.5	74	49.3
29 926-2	1.3	62	47.7
30 926-3	1.5	147	98
31 926-4	2.2	104	47.3
33 725-2	1.3	280	215
34 114-1	0.7	97	139
35 114-2	0.9	180	200
36 114-3	0.7	46	65.7
37 114-4	1.3	68	52.3
38 114-5	1.4	84	60
39 114-6	1.2	61	50.8

Appendix O: Hormone Descriptive Statistics

	Estradiol (pg/mL)			Progesterone (pg/ml)			Pg/E2 ratio		
	Low	High	OC	Low	High	OC	Low	High	OC
Sample Mean	1.3	1.6	1.2	71	87	61	59.6	116.0	73.8
SD	0.4	0.7	0.1	32	75	12	38.8	78.3	13.0
Median	1.3	1.4	1.3	50	57	61	61.5	79.0	72.0
Mode	1.5	0.9	1.3	66	25	48	46.0	56.0	62.0
Reference Range	0.6-4.5			127-446			≥200		

Appendix P: Raw Data (Pacing)

Participant	Pre-Sum	Post-Sum	PD (F=Faster, S=Slower)
1	1.04	0.88	S
2	0.97	1.43	S
3	0.66	0.84	F
4	1.01	0.46	F
5	1.23	1.79	F
6	0.67	0.83	F
7	1.19	0.65	S
8	0.75	0.63	F
9	0.76	0.55	S
10	0.85	0.65	F
11	0.55	0.65	F
12	0.76	0.62	F
13	0.78	0.29	F
14	0.49	0.46	F
15	0.38	0.80	F
16	1.17	0.84	F
17	0.83	0.25	F
18	1.07	0.71	F
19	0.52	0.89	S
20	0.52	0.75	F
21	0.46	0.60	S
22	1.02	1.12	F
23	0.63	0.53	F
24	0.60	0.49	F
25	1.15	0.39	S
26	0.95	0.56	F
27	0.70	0.45	F
28	0.41	0.48	S
29	0.17	3.20	S
30	0.38	0.25	S
31	0.51	0.51	S
32	1.29	0.88	F
34	0.88	0.79	F
35	0.49	0.49	F
36	0.56	0.41	F
37	0.61	0.66	F
38	0.49	0.33	F
39	1.07	0.82	S

Appendix Q: Raw Data (Performance)

Participant #	Sex	Age (0=u18, 1=18+)	MC Status (0=Male, 1= Low Hormone, 2= Luteal Phase, 3=OC)	TT1	TT2	Performance Change
1	F	0	1	12:27	12:33	-0.8
2	F	1	3	13:18	13:41	-2.88
3	F	0	1	14:40	14:30	1.14
4	F	0	2	12:42	12:41	0.13
5	F	0	1	14:46	13:57	5.53
6	F	0	3	12:47	12:36	1.43
7	F	0	2	13:26	13:33	-0.87
8	F	0	1	12:48	12:38	1.3
9	F	0	1	11:28	11:36	-1.16
10	M	0	0	12:06	11:13	7.3
11	M	1	0	10:48	10:34	2.16
12	F	1	1	12:38	12:30	1.06
13	F	1	2	12:54	12:40	1.81
14	F	1	1	12:37	12:33	0.53
15	M	1	0	11:20	11:17	0.44
16	M	1	0	10:28	10:04	3.82
17	M	1	0	9:56	9:54	0.34
18	F	1	2	12:59	12:45	1.8
19	F	1	2	12:26	13:12	-6.17
20	F	0	1	11:39	11:34	0.72
21	F	0	3	11:56	11:57	-0.14
22	F	1	1	10:52	10:50	0.31
23	F	0	1	13:02	12:36	3.32
24	F	1	1	13:03	12:57	0.77
25	M	0	0	10:08	10:28	-3.29
26	M	0	0	11:17	10:53	3.55
27	M	0	0	11:52	11:17	4.92
28	F	1	1	12:48	12:48	0
29	F	1	3	12:07	12:19	-1.65
30	F	1	1	12:01	12:08	-0.97
31	F	1	1	11:35	11:46	-1.58
32	M	1	0	10:20	10:17	0.48
33	F	1	2	12:12	11:42	4.1
34	F	0	1	12:57	12:20	4.76
35	F	0	2	15:21	14:51	3.26
36	F	0	1	13:08	12:56	1.52
37	F	0	1	13:18	12:50	3.51
38	F	0	2	13:41	13:30	1.34
39	F	0	1	12:43	12:43	0

Appendix R: Raw Data (Rating of Fatigue Questionnaires & Feeling Scale)

Participant #	ROFQ PreT1	ROFQ PreT2	ROFQ PreHIIT	ROFQ PostHIIT	ROFQ 30min PostHIIT	FS PreT1	FS PreT2	FS PreHIIT	FS PostHIIT	FS 30min PostHIIT
1	3	3	4	9	5	1	2	3	3	3
2	3	5	4	10	5	4	2	2	3	4
3	5	4	5	9	8	4	3	3	-2	1
4	5	7	6	10	8	1	-2	3	4	-1
5	3	6	5	9	6	2	3	2	1	3
6	3	5	3	10	5	2	1	2	3	4
7	2	4	3	9	3	3	1	2	2	4
8	5	3	1	10	8	3	-1	4	-4	-3
9	2	4	3	9	5	4	3	3	4	4
10	5	3	4	10	8	2	3	3	-2	4
11	4	5	4	9	8	3	3	3	2	3
12	4	3	5	7	4	3	3	3	2	3
13	4	4	4	10	5	4	1	3	-4	-1
14	2	3	3	9	7	3	3	3	2	2
15	2	3	1	10	7	3	2	2	0	2
16	3	3	4	9	7	3	3	2	4	4
17	3	4	3	10	5	2	3	4	1	3
18	3	5	6	10	8	3	1	2	-4	-1
19	2	5	3	10	5	1	-2	1	0	1
20	3	4	3	9	5	0	0	0	0	0
21	3	2	3	8	5	1	3	2	3	3
22	2	5	2	8	7	0	0	3	-1	-1
23	2	5	4	10	8	0	0	1	-3	-2
24	3	7	5	10	7	1	-2	0	-2	-1
25	4	7	5	10	7	-1	-3	0	-1	1
26	5	6	4	8	7	0	-1	1	-2	-1
27	5	5	4	6	7	0	-2	-1	-2	-1
28	3	5	5	10	3	1	1	-2	0	0
29	6	5	5	5	5	2	1	2	2	2
30	2	6	2	8	5	-1	-2	2	-1	0
31	2	6	4	8	6	2	-2	0	-2	-1
32	5	7	3	9	7	3	-1	4	-3	-2
33	4	8	5	9	9	-2	-4	-1	-3	-4
34	2	5	2	8	8	3	3	3	0	1
35	1	5	3	9	8	1	2	1	1	2
36	3	4	3	9	7	0	1	3	3	2
37	3	4	5	8	8	2	2	1	2	2
38	3	5	2	9	7	3	2	1	-1	1
39	5	5	6	9	8	0	1	1	2	2