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The Responses of VO2, VCO2, Substrate Utilization and Maximal Performance to Long Duration Exercise

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Abstract

Purpose: The purpose of this study was to compare and contrast the effects of a long duration exercise cycle (~3 h) by trained cyclists (RIDE) to a 3 h inactive period (SED) in recreationally active individuals (CONT) on VO_2, VCO_2, peak aerobic power, fat oxidation, anaerobic capacity (W′), arterialised-capillary lactate concentration and maximal sustainable power (CP). **Methods:** Male cyclists (n=12) and male recreationally active individuals (n=7) performed both an incremental test to volitional fatigue (RAMP) and 3 min all-out tests on a cycle ergometer, pre- and post-RIDE/SED respectively. **Results:** Increased fat oxidation rates, and reductions in VO_{2peak}, peak aerobic power anaerobic capacity (W′) and no changes in CP pre- to post-RIDE. No changes in CONT pre- to post-SED were observed. **Summary:** The decreased W′, and arterialised-capillary lactate concentrations post-RIDE, after both RAMP and 3 min all-out tests suggests diminished substrate level phosphorylation associated with the depleted glycogen stores. Critical power was unaffected by this RIDE.

Key words: long duration exercise, substrate utilization, critical power, peak aerobic power, anaerobic capacity, Tour de France

Co-Authorship Statement

This Study was designed by G. R. Belfry, M. Bitel, and M. Barnes with input from the advisory committee (J. M. Kowalchuk and C. W. J. Melling). The majority of the data was collected and analyzed by M. Bitel with the assistance of G. R. Belfry, M. Barnes and M. McCrudden. M. Bitel wrote the original manuscript for the study.

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List of Terms and Abbreviations

ATP - Adenosine Triphosphate

bpm - beats per minute

Ca^{2+} - Calcium ion

CHO - Carbohydrate

CoA - Coenzyme A

CO_2 - Carbon Dioxide

CONT - Control Group

CP - Critical Power

CPT - Carnitine Palmitoyltransferase

ETC - Electron Transport Chain

FABP - Fatty Acid Binding Protein

FAT/CD36 - Fatty Acid Translocase

FFA - Free Fatty Acid

LT – Lactate threshold/Gas Exchange Threshold

h - Hour

H^+ - Hydrogen ion

HSL - Hormone Sensitive Lipase

IMTG - Intramuscular Triglyceride

J - Joules

km - kilometre

K^+ - Potassium ion

kcal - Kilocalories

LCFA- Long Chain Fatty Acids

min - minute

MVC - Maximal Voluntary Contractions

N_2 - Nitrogen

Na^+ - Sodium ion

O_2 - Oxygen

PCr - Phosphocreatine

P_i - Inorganic Phosphate

RAMP - Incremental Ramp Test

RER - Respiratory Exchange Ratio

RIDE - Experimental Group

RPE - Rate of Perceived Exertion

RPM - Revolutions per Minute

s - Second

SD - Standard Deviation

SDH - Succinate Dehydrogenase

SE - Standard Error

SED - Sedentary

SNS - Sympathetic Nervous System

SR - Sarcoplasmic Reticulum

VCO_2 - Carbon Dioxide Production

VO_2 - Oxygen Consumption

VO_{2max} - Maximal Oxygen Consumption

VO_{2peak} - Peak Oxygen Consumption

W - Watts

W′ - Anaerobic Capacity

Chapter 1

1.0 Review of Literature

1.1 Introduction

The purpose of this study was to observe the pre- and post-responses of a long duration exercise cycle (RIDE) on VO_{2peak}, VO_2, VCO_2, peak aerobic power, fat oxidation, anaerobic capacity (W′), arterialised-capillary lactate concentration and the maximal sustainable power output (critical power (CP)) during an incremental ramp test to volitional fatigue (RAMP) and a 3 min all-out test.

The testing and intervention protocols in the present study were designed to mimic a cycling road race (stage) similar to the 21 stages performed in Le Tour de France. Typically, at the onset of each stage there is a 30-45 min period in which cyclists will attempt to break away from the main group of cyclists (peloton). This requires high intensity power outputs equivalent to CP and/or maximal oxygen consumption (VO_{2max}) (Ebert et al. 2006; Vogt et al. 2006). This initial high intensity segment of the race is typically followed by a moderate intensity cycle (below the estimated lactate threshold) that is maintained for ~3 h. With 10-20 km remaining, this previous moderate intensity segment is commonly followed by greater power outputs by the cyclists in the peloton as they endeavor to catch the cyclists that had escaped earlier. This latter phase of the race requires sustained efforts in proximity to CP, VO_{2max}, as well as supramaximal intensity efforts, culminating in a sprint to the finish line.

In the present study, the efforts of the cyclists (RIDE) during the initial phase of a stage were replicated through a RAMP and a 3 min all-out test performed on a cycle ergometer in the lab. This was followed by a moderate intensity cycle, approximately 3 h in duration, performed on the open road. Upon returning to the lab, the latter portion of the race was replicated by

performing the same RAMP and the 3 min all-out tests. These results were contrasted with a group of recreationally active individuals who performed these same tests before and after a 3 h sedentary period (CONT).

It was hypothesized that the fat oxidation profile during the RAMP test in both the RIDE and CONT group will not be dissimilar to the inverted U shape profiles observed in constant load exercise. Moreover, the rate of energy production from oxidative phosphorylation of fats up to and including CP intensities, would increase during the RAMP test post-long duration exercise. Lastly, the anaerobic capacity (W′) will be reduced post-long duration exercise as a result of glycogen depletion, with no changes in CP during the 3 min all-out test.

This chapter will review the relevant literature associated with the rationale, and the data collection equipment linked to this study.

1.2 Incremental Ramp Test

An incremental ramp test to limit of tolerance (RAMP) was utilized in the present study to simulate the intensities performed during the beginning and final phases of a race, where cyclists are working to break away from the peloton or working to catch up and/or outpace opposing cyclists respectively. The RAMP test protocol demands a progressive increase in exercise intensity until volitional exhaustion is reached. The RAMP test is used to delineate peak oxygen consumption (VO_{2peak}), estimated lactate threshold (LT) and associated power outputs (Carey et al. 2001, Martin et al. 1993, Coggan et al. 1993, Horton et al. 1998). VO_{2peak} was determined to be the VO_2 recorded over the last 15 seconds on the RAMP test, whereas estimated LT was determined to be the point at which VCO_2 begins to increase out of proportion to VO_2 (Beaver et al. 1986). In addition, progressive changes in VO_2, and VCO_2 were monitored during the RAMP test (Carey at al. 2001, Martin et al. 1993; Boone et al. 2009;

Ferreira et al. 2007). Moreover, the RAMP test can be utilized to assess performance and the associated effects of different exercise interventions, and/or utilized to observe different populations (i.e. trained versus untrained), as demonstrated in previous studies (Clark et al. 2014; Boone et al. 2009).

1.3 Critical Power (CP)

The 3 min all-out critical power test (Vanhatalo et al. 2007) was performed 15 min after the RAMP test and prior to the RIDE in the present study. The 3 min all-out test, along with the RAMP test, simulates both the initial and final efforts made by the cyclists within a racing stage. The 3 min all-out test can also be used to evaluate an individual's maximal sustainable power output (CP), anaerobic capacity and peak power (Vanhatalo et al. 2007). These are key factors in determining a cyclist's performance abilities. Critical power has been established as the boundary between heavy to very heavy exercise intensities (~80% VO_{2max}) and is the maximum power output during which a steady-state in cellular H^+, blood lactate, and inorganic phosphate concentrations, as well as VO_2 can be sustained (Jones et al. 2010, Coats et al. 2003). Moreover, the quantification of the finite capacity of the anaerobic energy systems (W′) during the initial two thirds of the 3 min all-out test, is possible, as all work performed above CP during this test constitutes W′ (Vanhatalo et al. 2007; Jones et al. 2010). Any increase in power output performed above CP, elicits an increased anaerobic contribution which results in the accumulation of H^+, lactate and inorganic phosphate. This increase in metabolites is imminently followed by fatigue. The W′ represents ATP-PCr and substrate level phosphorylation or anaerobic glycolysis (Vanhatalo et al. 2007, Jones et al. 2010).

The endurance (aerobically) trained individual, such as the cyclists (RIDE) in the present study, will manifest a greater CP compared to the untrained or to those that are sprint trained (Jones et al. 2010).

1.4 Energy Systems Associated with VO_2max and Critical Power Testing

Utilizing both the RAMP test, 3 min all-out test as well as the long duration exercise cycle to mimic a competitive cycling race requires different energy contributions at different phases of the event.

Mammalian cells generate energy through the aerobic (with O_2) and anaerobic (without O_2) energy systems. There are two systems within anaerobic metabolism, known as the alactic (no lactate production) and lactic (producing lactate) energy systems (Hill 1999; Duffield et al. 2004; Duffield et al. 2005a; Duffield et al. 2005b).

The alactic energy system, also known as the ATP-PCr system, is the major energy contributor during the most rapid demands of energy utilisation (maximal intensity exercise) and during the initial or transitioning from a lower to higher power output, as PCr breakdown facilitates ATP synthesis (Gastin 2001; Forbes 2005). Furthermore, the ATP-PCr system contributes an increasing amount of energy as exercise intensity increases from moderate to heavy to very heavy exercise (Forbes 2005). Finally, during maximal sprint exercise comparable to the 3 min all-out test, the ATP-PCr energy system contribution will predominate over the first 10 s of the exercise bout (Hill 1999; Duffield et al. 2004; Gastin 2001; Karlsson and Saltin 1970). As such this 3 min all-out test initially utilises the greatest rate of ATP demand, supporting measurement of the peak phosphorylation rates (ATP re-synthesis) originating from ATP-PCr.

The lactic anaerobic system produces energy (ATP) from glycogen and glucose stores through anaerobic glycolysis by anaerobic reactions also known as substrate level phosphorylation, resulting in lactate and hydrogen ion formation (Gastin 2001). Blood lactate can be used as an estimate of the anaerobic glycolytic contribution during and post-exercise testing (Hermansen and Stensvold 1972; Gollnick et al. 1986; Brooks 1985). Moreover, anaerobic glycolysis contribution increases when the energy supply by aerobic metabolism does not meet the demand of the energy required to perform a physical task or exercise intensity (Hill 1999; Duffield et al. 2005a; Duffield et al. 2005b; Gastin 2001; Spencer and Gastin 2001; Bangsbo et al. 1992; Brooks 1985). The same changes in energy contribution occur during the initial and final phases of a competitive cycling race when power outputs exceed LT and up to all-out efforts.

The 3 min all-out test has been utilized to quantify peak power and the capacity of this anaerobic system (ATP-PCr and anaerobic glycolysis) as well as CP (Vanhatalo et al. 2016).

The aerobic energy system predominately uses carbohydrates (CHO) and fats to phosphorylate ADP through chemical reactions in the mitochondria associated with beta-oxidation, Kreb's cycle and the electron transport chain (ETC). These processes that ultimately utilise O_2 to form H_2O in the electron transport chain are collectively known as oxidative phosphorylation, (Rakus et al. 2015; Gastin 2001; Chance and Williams 1955; Senior 1988). For the continual re-synthesis of ATP, the aerobic energy system is dependent on the availability of the reducing agents NAD^+ and FAD^+, as well as oxygen within the mitochondria for oxidative phosphorylation to proceed (Chance and Williams 1955; Senior 1988). The oxidation of CHO occurs when the end product of glycolysis, pyruvate, enters the Kreb's cycle resulting in electrons being transported to the ETC. Its maximal rate of ATP formation is much slower than anaerobic glycolysis. Moreover, fat oxidation results in the free fatty acid chains

being cleaved into fragments in beta-oxidation and used in the Kreb's cycle and ETC to produce ATP. This fat oxidation results in the slowest rate of oxidative phosphorylation compared to CHO oxidation (Senior 1988; Chance and Williams 1955). It is the result of these additional reactions within the Kreb's cycle and ETC in the mitochondria that make oxidative phosphorylation slower to reach maximal activation than both the alactic and lactic energy systems (Senior 1988; Chance and Williams 1955).

Finally oxidative phosphorylation is the major energy contributor during maximal efforts of two min and longer (Hill 1999; Gastin 2001; Spencer and Gastin 2001; Jones et al. 2010; Vanhatalo et al. 2007).

1.5 Blood Lactate

During the high intensity performances during the initial and final stages of a cycling race, replicated by both the RAMP and 3 min all-out tests, cyclists will utilize heavy intensity power outputs (>65% VO_{2max}) to break away from the peloton or to catch and outpace opposing cyclists (Ebert et al. 2006; Vogt et al. 2006). This change in power output results in blood lactate accumulation that reflects anaerobic use of CHO at intensities greater than the lactate threshold (Hermansen and Stensvold 1972; Gollnick et al. 1986; Brooks 1985). The production of lactate is the result of the breakdown of glucose and glycogen molecules by glycogenolysis and/or glycolysis, and the catalytic enzyme activity of lactate dehydrogenase (LDH) to form lactate (Brooks 1985; Sjodin et al. 1982). Blood lactate accumulation during exercise occurs when lactate production exceeds the rate of lactate removal (Hermansen and Stensvold 1972; Bang 1936; Gollnick et al. 1986). This accumulation of lactate occurs during maximal efforts similar to that elicited by the RAMP and 3 min all-out tests (Hermansen and Stensvold 1972; Gollnick et al. 1986; Brooks 1985). Moreover, blood lactate concentrations can be affected by

lactate uptake and oxidation by oxidative skeletal, respiratory and cardiac muscle fibers. This oxidation of lactate is possible as monocarboxylate tranporters (MCT) transport lactate produced from the cell into the mitochondria of adjacent or neighbouring oxidative muscle fibres, where it is oxidized (McCullagh et al. 1997). This oxidizing process results in lower blood lactate levels. Moreover, during steady-state exercise the accumulation of blood lactate may reach an elevated but unchanging concentration (Hermansen and Stensvold 1972; Brooks 1985). This remains true during exercise intensities up to and including critical power (CP) (Vanhatalo et al. 2007; Jones et al. 2010; Keir et al. 2015). However, as exercise intensities increase above CP, a greater contribution from substrate level phosphorylation disrupts the steady-state condition, resulting in an increase in cellular H^+, inorganic phosphate and lactate concentrations (Vanhatalo et al. 2007; Jones et al. 2010).

1.6 Substrates for Aerobic and Anaerobic Work

The energy released in the body is obtained from carbohydrates, proteins and fats. Of these macronutrients, CHO and fats are the most readily used endogenous substrates during exercise (Ranallo and Rhodes 1998; Essen et al. 1977; Brooks and Mercier 1994; Torrens et al. 2016).

CHO are stored in the body in the form of glycogen, yielding 4 kcal per gram of CHO (glucose) derived energy (Ranallo and Rhodes 1998; Brooks and Mercier 1994; Essen et al. 1977). Muscle and liver glycogen, as well as blood glucose are the preferred energy sources during high intensity exercise (>65% VO_{2max}), where ATP is supplied either by anaerobic or oxidative (aerobic) glycolytic systems (Torrens et al. 2016; Bergman et al. 1999; Romijn et al. 1993; Brooks and Mercier 1994). During the RAMP test above LT and for the entire duration

of the 3 min all-out test, CHO are expected to be the most important substrate for ATP production.

Free fatty acids (FFA) are stored in the body as triglycerides, and are broken down through hydrolysis by a cascade of lipolytic reactions through lipases (triglyceride lipase and/or hormone sensitive lipase (HSL)) and regulatory proteins at rest and during low to moderate intensities of exercise (<65%VO_{2max}) (Aon et al. 2014). The end products are three long chain fatty acids (LCFA) and a glycerol. These end products are then released into the vasculature, as they bind with an albumin protein carrier that carries the LCFA through the circulating blood plasma (Aon et al. 2014). The LCFAs can then be utilized in beta-oxidation to produce substrate for the ETC through oxidative phosphorylation in mitochondrial respiration (Kienesberger et al. 2013). However, in order to get the LCFAs to the mitochondria to take part in beta-oxidation, the non-esterified LCFAs have to be transported through various cellular structures and membranes to reach the ETC (Aon et al. 2014; Hagberg et al. 2013).

Once LCFA reach the area of energy demand (i.e. active muscles during exercise), LCFA are transported across the endothelium and cell membrane by fatty acid translocase proteins (FAT/CD36) and fatty acid binding proteins (FABP) (Elmasri et al. 2009; Glatz et al. 2010; Hagberg et al. 2013). After the LCFAs have been transported from the blood plasma into the cell, they must be transported across the mitochondrial membrane in order to take part in beta-oxidation to produce ATP. LCFAs are esterfied to form LCFA-CoA (coenzyme A) (Ramsay and Tubbs 1975; Zammit et al. 2009), and are transported through a shuttle system of carnitine palmitoyltransferase 1 (CPT1) and 2 (CPT2) into the mitochondrial matrix (Zammit 1999a; Zammit 1999b; Zammit et al. 2009). In the mitochondrial matrix, fatty acids are cleaved into 2 carbon-acyl fragments that enter the Kreb's cycle as acetyl-CoA to eventually form ATP in the ETC (Houten et al. 2016). Greater availability of fatty acids in the mitochondrial matrix,

results in greater beta-oxidation and whole body fat oxidation (Watt et al. 2002; Watt et al. 2003). During endurance exercise (<65% VO_{2max}), whole body fat oxidation has been observed to increase its contribution to the total energy requirement as the duration of the exercise bout is lengthened (>15 min) (Bradley et al. 2012; Carey et al. 2001; Watt et al. 2003). The same response during the long duration exercise cycle in the present study is expected.

1.7 Fat Oxidation

During cycling stage racing, much of the race requires moderate to heavy intensity work. These intensities elicit an increasing contribution from fats, with a concurrent reduction in CHO use as exercise duration increases (Bradley et al. 2012; Carey et al. 2001; Watt et al. 2003; Ranallo and Rhodes 1998). The ability to transport, uptake and oxidise free fatty acids in the active muscle cells, is essential for meeting metabolic demands as CHO stores alone cannot supply the total energy required during prolonged periods of exercise (>60 min) (Ranallo and Rhodes 1998). Although fatty acids cannot provide energy as rapidly as carbohydrates (CHO), and are energetically less efficient per unit of oxygen (23 moles of O_2 per moles of fatty acid vs. 6 moles of O_2 per mole of glucose), fat provides much more energy per gram (4 kcal vs 9 kcal), making it an invaluable source of energy for low to moderate intensity (<65%VO_{2max}) exercise during prolonged exercise durations (>60 min). The ~3 h long duration exercise cycle (below LT) in the present study would utilise fat as the major substrate (Holloway et al. 2008; Romijn et al. 2000).

Moreover, with endurance training greater fat utilization at sub-maximal intensities of exercise (<LT) compared to the pre-training state is observed (Martin et al. 1993; Hurley et al. 1986; Mole et al. 1971; Henriksson 1977; Phillips et al. 1996; Coggan et al. 1993). This up-regulation of fat oxidation, post-training, allows the individual to spare their glycogen stores to

delay fatigue and reserve substrate availability for relatively greater intensities of exercise where the metabolic demand is greater and substrate level phosphorylation is necessary to meet the increased energy demand as expected in the final stages of a competitive cycling race (Phillips et al. 1996; Coggan et al. 1990, Coggan et al. 1993; Coggan et al. 1995; Holloszy and Coyle 1984; Romijin et al. 1993; Brooks and Mercier 1994).

1.8 Fat and Carbohydrate Oxidation Rates derived from VCO_2 and VO_2

During the RAMP tests, pre- and post-long duration exercise in the present study, pulmonary oxygen consumption (VO_2) and carbon dioxide output (VCO_2) were used to compare changes in fat and CHO oxidation as a consequence of the long duration exercise cycle, utilized in competitive cycling. It is from the collection of VO_2 and VCO_2 data that it is possible to derive estimations of the energy provided from CHO versus fat oxidation (Peronnet and Massicotte 1991). It assumes that other metabolic processes involved in the production and utilization of CO_2 and O_2, respectively, such as gluconeogenesis from proteins and ketone body formation, are quantitatively negligible compared to glucose and fatty acid oxidation (Peronnet and Massicotte 1991; Ferrannini 1988; Frayn 1983). Assuming that these other metabolic processes are quantitatively negligible, greater or stable VO_2 and lower VCO_2 (relatively compared to VO_2) is associated with an increased contribution in fat oxidation (Peronnet and Massicotte 1991), whereas a reduction in CHO derived substrate utilization in trained versus untrained individuals during low-moderate intensity exercise is observed (<65% VO_{2max}) (Coggan et al. 1990; Coggan et al. 1993; Hurley et al. 1986). Furthermore, lower or stable VO_2 and greater VCO_2 (relatively compared to VO_2) is linked with an increased contribution in CHO oxidation (Peronnet and Massicotte 1991).

1.9 Muscle Fibre Types

During the RAMP test, 3 min all-out test, and the long duration exercise cycle, the use of fat and CHO will depend on the recruitment of different skeletal muscle fibre types during various exercise intensities and durations. Skeletal muscle fibres come in two distinct types, Type I and Type II fibres (Schiaffino and Reggiani 1994; Thomson et al. 1979; Sant'Ana Pereira et al. 1996; Picard et al. 2011; Davie et al. 1999; Essen et al. 1975; Guegeun et al. 2005; Vanhatalo et al. 2016). Type I or slow twitch, fatigue resistant, oxidative fibres increasingly contribute with increasing exercise intensity during low to maximal O_2 consumption intensities ($\leq VO_{2max}$) (Thomson et al. 1979; Davie et al. 1999) and are expected to be the predominately active fibers during the long duration exercise cycle and up to CP intensities (~80% VO_{2max}) elicited during the RAMP and 3 min all-out test (Thomson et al. 1979; Vanhatalo et al. 2016). Type I fibers generally have two to three fold greater mitochondrial density and lower non-oxidative ATP synthesis compared to Type II fibres, relying mainly on oxidative phosphorylation of fat and glycogen for ATP supply (Picard et al. 2011; Guegeun et al. 2005; Essen et al. 1975). The high levels of oxidative phosphorylation in Type I fibres are expressed by greater succinate dehydrogenase (SDH) activity, an enzyme involved in the Kreb's cycle and ETC (oxidizing succinate to fumerate in the Kreb's cycle and reducing ubiquinone to ubiquinol in the ETC), compared to Type II fibres (Essen et al. 1975; Vanhatalo et al. 2016; Sant'Ana Pereira et al. 1996). Moreover, Type I fibres have greater oxidative metabolic machinery than Type II fibres due to larger mitochondrial size and better developed cristae (larger surface area for chemical reactions requiring oxygen), resulting in greater oxidative

phosphorylation (Vanhatalo et al. 2016; Kugelberg 1973; Schiaffino et al. 1970). Type I fibres mainly derive their energy from fat oxidation during long moderate intensity exercise (below LT) and express greater levels of sarcolemmal fatty acid transport proteins and intracellular fatty acid binding proteins compared to Type II fibres (Picard et al. 2011; Glatz et al. 2003).

Type II fibres become increasingly involved as exercise intensities increase above LT and will have a predominate contribution during intensities above CP (~80% VO_{2max}), and a considerable effect on peak power outputs during the RAMP and 3 min all-out tests in the present study (Thomson et al. 1979; Picard et al. 2011; Davies et al. 1999). These fibres display greater glycerol-3-phosphate and phosphofructokinase (PFK) activity, the regulatory enzyme of glycolysis, with only 3 ADP phosphorylated per mole of glycogen resulting in greater use of these limited glycogen stores, making them more fatigable than Type I fibres (Picard et al. 2011; Jackman and Willis 1996; Vanhatalo et al. 2016; Schiaffino and Reggiani 2011). Type II fibres have subgroups which are separated into Type IIa and Type IIx fibres (Schiaffino and Reggiani 1994; Thomson et al. 1979; Sant'Ana Pereira et al. 1996; Picard et al. 2011; Davie et al. 1999; Essen et al. 1975; Guegeun et al. 2005; Vanhatalo et al. 2016). Type IIa fibres are fast twitch, fatigue resistant, oxidative fibres and typically have a greater capacity for oxidative metabolism, but lower glycolytic metabolism and contractile force capability compared to Type IIx fibres (Thomson et al. 1979). As mentioned previously, there is greater reliance on glycolysis in Type II fibres resulting in a faster onset of fatigue than Type I fibres, but greater force producing capabilities (Vanhatalo et al. 2016; Schiaffino and Reggiani 2011).

Type IIx fibres are fast twitch glycolytic fibres and are predominately active during maximal to supra-maximal ($\geq VO_{2max}$) intensities of exercise (Thomson et al. 1979). These fibres rely on non-oxidative phosphorylation for ATP supply, and contain the greatest capacity for glycolytic metabolism and considerably higher resting PCr content compared to other fibre

types (Sant'Ana Pereira et al. 1996; Thomson et al. 1979; Vanhatalo et al. 2016; Gueguen et al. 2005). Since Type IIx fibres are limited to PCr and glycolytic energy supply, and have lower Krebs cycle enzyme activity compared to Type IIa fibres, fatigue occurs much quicker than Type IIa and Type I fibres (Vanhatalo et al. 2016; Sant'Ana Pereira et al. 1996; Essen et al. 1975).

1.10 Fatigue

In competitive cycling, usually the cyclists that wins the race, is the cyclists that fatigues the least (Vanhatalo et al. 2011). After cycling the prolonged light-moderate intensity part of the race, cyclists will increase their speed or power output in order to catch up or outpace the opposing cyclists. The responses of substrate utilization or other fatigue mechanisms could pose as limiting factors in maximal sustainable performance and maximal performance during the RAMP test and 3 min all-out test post-long duration exercise cycle. Fatigue has been defined as the sensations of tiredness and reductions in muscular performance and function (Abbiss and Laursen 2005; Kay et al. 2001; Green 1997; Kay and Marino 2000; Millet et al. 2000; Millet et al. 2002; Pinniger et al. 2000; St Clair Gibson et al. 2001). Numerous models have been proposed to explain the causes of fatigue during prolonged moderate intensity exercise (≤65% VO_{2max}) which include, but not limited to, substrate supply and neuromuscular mechanisms. The substrate supply model links glycogen levels with fatigue. There is a strong relationship between pre-exercise glycogen levels and cycling time to exhaustion (Abbiss and Laursen 2005; Kay and Marino 2000; Shulman and Rothman 2001; Coyle and Montain 1992; Dennis et al. 1997). The association between low glycogen levels and impaired contractile function demonstrates that the depletion of glycogen results in a reduction in the rate of ATP regeneration (Ortenblad et al. 2013). The glycogen shunt model, proposed by Shulman and

Rothman (2001), states that as glycogen concentration is reduced, muscle glycogen fails to provide the rapid burst of glucogenolysis (glycogen break down), needed to compensate for the energy demands of muscle fibres during high-intensity exercise (Chin et al. 1997; Kabbara et al. 2000; Helander et al. 2002; Duhamel et al. 2006a; Duhamel et al. 2006b; Ortenbald et al. 2011; Ortenbald et al. 2013). In addition, it is possible that the connection between glycogen and sarcoplasmic reticulum (SR) Ca^{2+} release is not related to glycogen as an energy source, but to the action of enzymes associated with the glycogen particles, which themselves might modulate the function of the SR Ca^{2+} release channels in their proximity, e.g. by phosphorylation or de-phosphorylation of proteins involved in E-C coupling during repeated contractions (Hamilton and Serysheva 2009; Sharma et al. 2012; Ortenblad et al. 2013). Following a prolonged moderate intensity exercise session (>60 min; ≤65% VO_{2max}), glycogen depletion has been observed in both Type I and II fibers (Thomson et al. 1979), and performing a similar long duration exercise cycle in the present study and competitive road race cycling could possibly result in glycogen depletion and associated fatigue.

Moreover, the neuromuscular and muscle contractile model has also been associated with fatigue. It has been suggested that fatigue can be related to the central nervous system activation failure, where it has been shown that as fatigue develops during prolonged exercise (>60 min), there is an increase in intracortical inhibition resulting in lower power outputs, reflected by decreased skeletal muscle recruitment (Abbiss and Laursen 2005; Millet et al. 2003; Paasuke et al. 1999). Fatigue has also been observed at the level of the muscle, where a decline in the action potential conduction velocity within the muscle fibre is thought to be reflective of transformations within the muscle (i.e. the accumulation of metabolic by products) resulting in decreased contractile tension of the actin-myosin cross bridges (Schillings et al. 2003). This was demonstrated in Lepers et al. (2002), where endurance trained cyclists cycled

for 5 h at 55% VO_{2max} and observed decrements in force as a result of the decreased action potential and excitability of the exercising muscle. These alterations in the muscle action potential may reflect a decrease in membrane excitability caused by imbalances in transmembrane gradients (Na^+ and K^+) resulting in reduced EMG activity during prolonged cycling exercise (Abbiss and Laursen 2005; Allman and Rice 2002; St Clair Gibson et al. 2001; Jammes et al. 2000; Nielson and Clausen 2000; Fowles et al. 2002; Hamada et al. 2003).

Muscle contractile properties have also been reported to become affected after prolonged exercise (2-5 h) (Lepers et al. 2000; Lepers et al. 2002). There may also be a reduction in Ca^{2+} release and uptake from contractile proteins to the SR, which may be responsible for an increase in the muscle relaxation time (actin-myosin cross-bridge detachment rate), resulting in lower power outputs compared to pre-exercise state (Abbiss and Laursen 2005; Hill et al. 2001; McKenna et al. 1996). This fatigue mechanism has been observed by Lepers et al. (2002), after a 5 h cycle at 55% VO_{2max} which resulted in decrements in peak twitch torque, and an increase in the contraction time of the quadriceps muscle (Lepers et al. 2000; Lepers et al. 2002; Duchateau and Hainault 1985).

1.11 Rationale

Competitive endurance sports that require physical activity for 2-4 h, such as cycling and long distance running, require long periods of sustained power outputs (Schumacher and Mueller 2002; Billat et al.2003). Improving these sustainable power outputs, allows athletes to complete a distance of a race course within a shorter period of time. These changes in sustainable power output may increase up to and including CP intensities (Joyner and Coyle 2008). In the present study, the initial period (~30 min) of a typical competitive cycling race was replicated using a RAMP test and 3 min all-out test, followed by an approximate 3 hour

light-moderate intensity cycling period (≤65% VO_{2max}). Utilizing this simulated cycling race, insight in substrate and performance changes that moderate intensity prolonged physical activity may have on the athlete can be attained. These observations could provide coaches and athletes with information on limitations to performance, and key elements in pacing strategies and tactics during a competitive racing event, especially near the end of race, where an increase in speed or work rate to outpace competing cyclists is required (Joyner and Coyle 2008). Despite there being an abundance of literature associated with the reduction in glycogen stores and increase in fat oxidation during prolonged exercise, as well as the effects glycogen depletion has on performance and fatigue (Carey et al. 2001; Bradley et al. 2012; Lepers et al. 2000; Thomson et al. 1979; Vollestad et al. 1984; Vollestad et al. 1985), little is known about what changes in maximal sustainable power outputs (CP) occur after performing at moderate intensity for a prolonged period. Knowing the limitations to maximal sustainable power outputs observed during the present study, pacing strategies and tactics could be developed by coaches and athletes to maximize the speed at which the distance of the race is completed and focusing on the goal of outpacing opposing cyclists.

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Chapter 2

2.0 The Responses of VO_2, VCO_2, Substrate Utilization and Maximal Performance to Long Duration Exercise

2.1 Introduction

The Grand Tours of cycling, such as Le Tour de France, demand some 21 days of racing (stages) by as many as 180 participants. The mean duration and distance of these stages is ~four hours and ~170 km (Le Tour, 2016). It is not unusual for these stages to begin with several high intensity efforts by the riders in the vicinity of their maximum oxygen uptake (VO_{2max}) and/or at their highest sustainable work rate (critical power (CP) (Moritani et al. 1981)), as cyclists attempt to escape from the main group of cyclists or peloton. These "escapees" may be chased down by cyclists in the peloton, and subsequently other cyclists may attempt to get away. It is not unusual for a smaller group of riders to eventually break away from the main group and gain several min advantage on the peloton. After these initial efforts the peloton may settle into two to four hours of below lactate threshold (LT) (moderate intensity) cycling, with no intention of trying to catch the cyclists in the breakaway. The peloton may now wait until the final 10-20 km to work together, taking turns breaking the wind at the front of the peloton, to catch the escapees. These efforts also require power outputs at or above CP. If the escapees are caught, the race will culminate in a mass sprint to the finish requiring maximal efforts from the cyclists.

The experimental protocol of this present study attempted to replicate the power output profile of a Grand Tour stage in cycling by performing maximal effort tests in the lab, including an incremental test to fatigue (RAMP) and a 3 min all-out effort (Vanhatalo et al. 2007), before

and after a moderate intensity, long duration solo cycle ride (~ 3 h) on the open road. The experimental group in the present study was composed of trained cyclists.

The effects of this long duration cycle ride (RIDE) on VO_2, VCO_2 (carbon dioxide production), fat oxidation, anaerobic capacity (W′), arterialised-capillary lactate concentration and CP, during the RAMP and 3 min all-out tests, before and after this RIDE, were assessed. Moreover, these results from the RIDE group were compared and contrasted with a control group consisting of recreationally active individuals (CONT) who performed the identical testing regime as the RIDE group, but substituted three hours of inactivity (SED) for the long duration cycling intervention.

Earlier work has observed increased fat oxidation rates at rest and during light, moderate and heavy intensity constant load exercise pre- and post-training, although none have observed fat oxidation rates continuously over a range of power outputs up to and including CP (Martin et al. 1993; Hurley et al. 1986; Mole et al. 1971, Carey et al. 2001; Bradley et al. 2012; Lepers et al. 2000, van Loon et al. 2001). It is suggested that increased fat oxidation, will be observed in the cyclists before the RIDE, compared to CONT during incremental exercise as observed in previous studies (Hurley et al. 1986; Carey et al. 2001), with a further increase observed after completion of this long duration ride. Since CP is performed predominately with Type 1 fibres (Vanhatalo et al. 2016), which rely on fats as substrate for oxidative phosphorylation (Essen et al. 1975), it is expected that the contribution of fats to oxidative phosphorylation will approach CP post-RIDE. Pilot data from our lab has suggested that the profile of the VO_2 and VCO_2 derived fat oxidation rates (Peronnet and Massicotte 1991) as intensity increases during RAMP exercise will not be dissimilar than those calculated from various constant load exercise bouts in the literature (van Loon et al. 2001; Achten and Jeukendrup 2004). Rowlands (2005) suggested that the VO_2 and VCO_2 derived fat oxidation

estimates will result in an overestimation of CHO oxidation rates and an underestimation of fat oxidation rates due to the excretion of non-respiratory CO_2 at work rates above estimated lactate threshold (LT). However, it was also suggested that that below LT during incremental exercise estimated substrate oxidation derived from VO_2 and VCO_2 is valid (Rowlands 2005).

Reductions in anaerobic capacity (W′) have been associated with muscle glycogen depletion (Miura et al. 2000). Moreover, glycogen depletion in Type I, Type IIa and Type IIx fibres have been reported after long duration, continuous exercise bouts at intensities equivalent to 60% VO_{2peak} (60 -120 min) in both trained and untrained individuals (Thomson et al. 1979; Gollnick et al. 1974; Vollestad et al. 1984; Vollestad et al. 1985). Importantly, it has been demonstrated that there are different glycogen depletion rates of individual fibres within the same muscle. Consequently, particular fibres within the muscle may become depleted whereas others do not (Vollestad et al. 1985). This infers that despite whole muscle glycogen being only partially reduced, glycogen depleted Type II fibres would be unable to contribute to force generation at intensities requiring substrate level phosphorylation. Accordingly, a reduction in W′ would be expected post-RIDE in the present study (Miura et al. 2000).

Finally, over the course of the RIDE a decrease in VO_{2peak} and peak aerobic power would be observed as a consequence of the decreased stroke volume and concomitant cardiac output, at any particular heart rate. This has been termed "cardiovascular drift" (Dawson et al. 2005). This reduced stroke volume, is the corollary to the reduced end diastolic volumes that result in decreased arterial pressures, lower venous return and increased HR for a given cardiac output, in previously observed long duration exercise bouts (Dawson et al. 2005; Ketelhut et al. 1994).

The purpose of this study was to assess and compare, VO_2, VCO_2, fat oxidation rates, peak power, peak aerobic power, critical power and anaerobic capacity during incremental

exercise to VO_{2peak} and a 3 min all-out test, before and after a RIDE or SED period, in endurance trained cyclists and recreationally active individuals, respectively, and observe how these responses would affect performance at the conclusion of cycling.

It was hypothesized that 1) the fat oxidation profile during the RAMP test in both the RIDE and CONT group will not be dissimilar to the inverted U shape profiles observed in constant load exercise. 2) The rate of energy production from oxidative phosphorylation of fats up to and including CP intensities, would increase during the RAMP test post-long duration exercise and, 3) W′ will be reduced post-long duration exercise as a result of glycogen depletion, with no changes in CP during the 3 min all-out test.

2.2 Methods

Participants. The control (CONT) group consisted of healthy recreationally active individuals (n=7; 26 ± 4 y, VO_{2peak} 3.81 ±0.5 L*min^{-1}, relative VO_{2peak} 48.63 ± 3.5 ml*kg^{-1}*min^{-1}). The experimental group (RIDE) consisted of healthy male trained cyclists (n = 12; 28 ± 6 y, VO_{2peak} 4.20 ± 0.5 L*min^{-1}, relative VO_{2peak} 55.63 ± 4.4 ml*kg^{-1}*min^{-1}), able to complete an 89 km ride below LT in ~3 h. All participants volunteered to participate in this study, were non-smokers with no known history of cardiovascular, respiratory, metabolic or musculoskeletal disease, and were not taking any medication that could have affected the physiological variables that were investigated. Participants and were informed all of the procedures and risks of the study prior to giving written consent to participate. The Western University Health Sciences Research Ethics Board approved this study.

Experimental Overview. Each participant was asked to arrive at the laboratory in a rested state and to avoid strenuous exercise in the 24 h proceeding the upcoming ~five hour testing and RIDE/SED protocol. The participants in both the RIDE group and CONT group (one subject

per day) performed an incremental ramp (RAMP) test and a 3 min all-out test, separated by a 15 min period of inactivity. Upon completion of the RAMP and the 3 min all-out tests, the cyclists (RIDE group) completed a long duration exercise cycle, whereas the CONT group completed a SED period. The RIDE were instructed to maintain a rating of perceived exertion (RPE) of 11 (light effort) (see Appendix C) (Borg 1982) during the RIDE along a planned route on the open road (Figure 1). This intensity is equivalent to a power output that is below the estimated lactate threshold (LT) (Ozyener et al. 2001). The RPE scale quantifies the perception of physical effort that one is exerting during exercise. Confirmation that the RIDE was performed in the moderate intensity domain was determined by maintaining their effort during the RIDE at an RPE of 11, a heart rate (HR) equal to, or below the combined mean estimated LT HR observed during the pre- and post-RAMP test, and a post-RIDE arterialised-capillary lactate of < 4 mM (Nicholson and Sleivert 2001, Pyne et al. 2001). Heart rate, distance, speed and time were recorded during the RIDE (Garmin FORERUNNER 310XT watch (Garmin, Kansa City, MO, USA)). Once completed, participants performed the identical RAMP and 3 min all-out tests, separated by 15 min of inactivity.

Food Intake. Breakfast and RIDE/SED food intake were recorded for both RIDE and CONT groups. Participants were instructed to consume their typical breakfast before a long duration exercise cycle whereas during the RIDE/SED period, participants were asked to consume their usual supplementary drinks (i.e. Gatorade) and snacks (i.e. Cliff Bar). Total grams of carbohydrates, proteins and fats were recorded for both breakfast and RIDE/SED food intake.

Ramp Incremental (RAMP) Test. All participants were measured for appropriate seat height before testing (slight bend in knee at the six o'clock position of the leg). This configuration was used for all testing protocols. All participants completed a RAMP test to volitional fatigue on an electromagnetically braked cycle ergometer (Velotron Pro, Seattle, WA, USA). This test was

performed pre- and post-RIDE/SED. This test was administered to determine peak oxygen uptake (VO_{2peak}), peak aerobic power, LT and associated VO_2 and power outputs. The test began with four min of 20 W cycling after which the work rate was increased by 30 $W^{*}min^{-1}$ to volitional fatigue. The participants were instructed to pedal at 70 RPM with the Velotron ergometer in the RPM independent of power output mode. VO_2 was measured breath-by-breath and VO_{2peak} was determined by the average VO_2 over the last 15 s of the test. The test ended when the subject was unable to maintain 60 RPM. These incremental test have been shown to give an accurate assessment of VO_{2max} when performed by young, healthy cyclists that are used to pushing themselves to exhaustion (Chidnok et al. 2013; Poole & Jones 2017). The LT was estimated by visual inspection using standard gas exchange and ventilatory variables as previously described (Beaver et al. 1986). Briefly, LT was determined to be the VO_2 at which CO_2 output (VCO_2) and ventilation (V_E) began to increase out of proportion to VO_2. This point was corroborated with the observation of an increase in end-tidal PO_2 ($PetO_2$), while the V_E-to-VCO_2 ratio and end-tidal PCO_2 ($PetCO_2$) were unchanged. Two exercise physiologists with experience in identifying LT evaluated each dataset. If a discrepancy arose between the two investigators, a mean of the identified points was utilized.

3 minute All-Out Test. Fifteen min after the RAMP test, all participants completed a 3 min all-out test on the same electromagnetically braked cycle ergometer (Velotron Pro, Seattle, WA, USA). This test was used to determine anaerobic capacity (W′) and critical power (CP) (Vanhatalo et al. 2007, Jones et al. 2010). Initially participants completed 40 s of 20 W cycling. At 35 s of this 20 W cycling period, participants were instructed to increase their RPM to over 100. At 40 s participants began the maximal effort portion of this 3 min all-out test. The participants were directed to maintain a maximal effort for the 180 s duration of this test. Participants were not informed of the elapsed time to prevent pacing and verbal encouragement

was provided throughout the test. The cycle ergometer was set using the linear mode (linear factor = power/ cadence squared) during the 3 min all-out test. The cycle ergometer was set to a resistant torque factor equal to a work rate that was 50% of the difference between LT and VO_{2peak} at 70 RPM for each subject as determined from the pre-RAMP test. This load setting was utilised for both the RIDE and CONT participants, pre- and post-RIDE/SED. The individual torque factors were calculated from Equation 1.

Equation 1

Torque factor= (Work Rate (watts) × 6.12)/ (body weight in kg × 4 meters × RPM)

Where RPM equals revolutions per minute.

Equation 2

$W' = (\sum(PO\text{-}CP)/N)$

Where PO equals power output, CP equals critical power and N equals the number of time intervals. This average power output in watts was then converted to Joules (see equation 3).

Equation 3

$J = W \times s$

Where J equals Joules, W refers to the mean wattage above W′ and s is time in seconds of the test (180).

VO_2 was measured breath-by-breath, and power output was measured every 0.1 s for the entire 3 min duration. The highest sustainable work rate (CP) was determined to be the mean power output of the last 30 s of the test. W′ was calculated as the total work completed above the calculated CP (Vanhatalo et al. 2007, Jones et al. 2010).

Long duration exercise cycle (RIDE). Participants were to cycle along a planned route that measured out to 89 km. The cyclists were instructed to familiarize themselves with the planned

route the day before testing and to maintain an RPE of 11/20 throughout the entire road cycle. The cyclists wore a Garmin FORERUNNER 310XT watch (Garmin, Kansa City, MO, USA) that continuously gathers and analyzes data from the heart rate monitor and GPS tracking system throughout the RIDE for each subject. On completion of the RIDE participants were instructed to stop the watch from recording any further information. The information was transmitted wirelessly from the watch to the computer and Garmin Connect software (Garmin.com) within 2 h of completion of the ride. At the conclusion of the RIDE participants were instructed to immediately enter the building in which the data collection lab was located and take the elevator to the appropriate floor. The post-testing protocols were initiated within 5 min of the completion of the long duration exercise cycle.

Data Collection. VO_2 was measured breath-by-breath, similar to measurements previously described (Keir et al. 2014). Briefly, the inspired and expired flow rates were analyzed by a low dead space (90mL) bidirectional turbine (Alpha Technologies VMM 110). The turbine was calibrated before each test using a 3 L syringe. Inspired and expired gases were monitored at the mouth and analyzed for concentrations of O_2, CO_2 and N_2 by a mass spectrometer (Innovision, Amis 2000, Lindvedvej, Denmark). Each subject wore a nose clip to cut off nasal airflow. The delay between volume and gas concentration was accounted for by measuring the time delay for a square wave bolus of gas to travel from the turbine transducers through a capillary line to the mass spectrometer for analysis. Flow volume data and gas concentration data were then transmitted to the lab computer. The lab computer built a profile of each breath by aligning the gas concentration information with the inspiratory and expiratory volume recordings. Breath-by-breath gas exchange was determined from the algorithms developed by Swanson (1980).

Power output from the RAMP test was recorded via the Velotron Coaching Software program (version 1.6.458 RacerMate, Inc., Seattle, WA, USA) and aligned by Powerlab software to designate a power output at each breath. Power output during the 3 min all-out test was also recorded by Velotron Wingate Software (version 1.0.2 RacerMate, Inc., Seattle, WA, USA) at 0.1 s intervals for the 180 s testing period.

During testing heart rate (HR) was collected using a Polar Wearlink Chest Strap, H1 Heart Rate Sensor and SP0180 Polar Transmitter (Polar Electro Inc., Lachine, QC, Canada) linked to a PowerLab Chart data collection system (v.7.3.1 ADInstruments Inc., Colorado, CO, USA) during both tests.

Arterialised-capillary lactate concentration was measured 3 min before both RAMP and 3 min all-out tests and 3 min after both tests. Arterialised-capillary blood was drawn using ACCU-CHEK Safe-T-Pro Plus sterile, single use lancing device and was measured by SensLab GmbH Lactate SCOUT arterialised-capillary lactate analyzer ([Lac-]; mM). Latex gloves were worn by the attending researcher. Prior to the use of the lancet, a rubbing alcohol swab was used to sterilize the finger volunteered by the participant for each test.

Data Analysis. Breath-by-breath VO_2 and VCO_2 recordings from all tests were edited by the removal of aberrant data points lying outside of 4 standard deviations of the mean (Rossiter et al. 2000; Lamarra et al. 1987). The breath-by-breath VO_2 and VCO_2 data were then interpolated to 1 s intervals, then averaged into 5 s bins for the RAMP and 3 min all-out tests, to provide a single average time response for each participant.

Heart rates at LT were recorded for each individual participant during the RAMP tests.

Carbohydrate oxidation rates were determined by the following equation (see equation 4) (Peronnet and Massicotte 1991, Bradley et al. 2012, Carey et al. 2001).

Equation 4

$$\textit{The oxidation rate of carbohydrates} = 4.585 \times VCO_2\ L*min^{-1} - 3.226 \times VO_2\ L*min^{-1}$$

Fat oxidation rates were determined by the following equation (see equation 5) (Peronnet and Massicotte 1991, Bradley et al. 2012, Carey et al. 2001).

Equation 5

$$\textit{The oxidation rate of fats} = 1.695 \times VO_2\ L*min^{-1} - 1.701 \times VCO_2\ L*min^{-1}$$

Power output data were averaged to 5 s intervals for both RAMP and 3 min all-out tests for graphic presentation and analysis.

Statistical Analysis. Statistical analyses were performed with Sigma Plot version 12.3 (Systat Software Inc., San Jose, CA). Statistical significance was accepted at an alpha level of 5%. Differences between pre- and post-RIDE/SED periods were compared to detect changes in VO_2, VCO_2 and power output over the duration of the tests. VO_2, VCO_2 and power output were analyzed using two way repeated measures ANOVA. Differences in pre- and post-arterialised-capillary lactate concentrations, VO_{2peak}, peak aerobic power, LT, max heart rate, heart rate at LT, peak power output, CP and W′, were compared using a paired t-test. Carbohydrate, fat and protein intake were also compared between CONT and RIDE groups using one way ANOVA. Table data are presented as means ± SD, and bar graphs and scatter plots are presented as means.

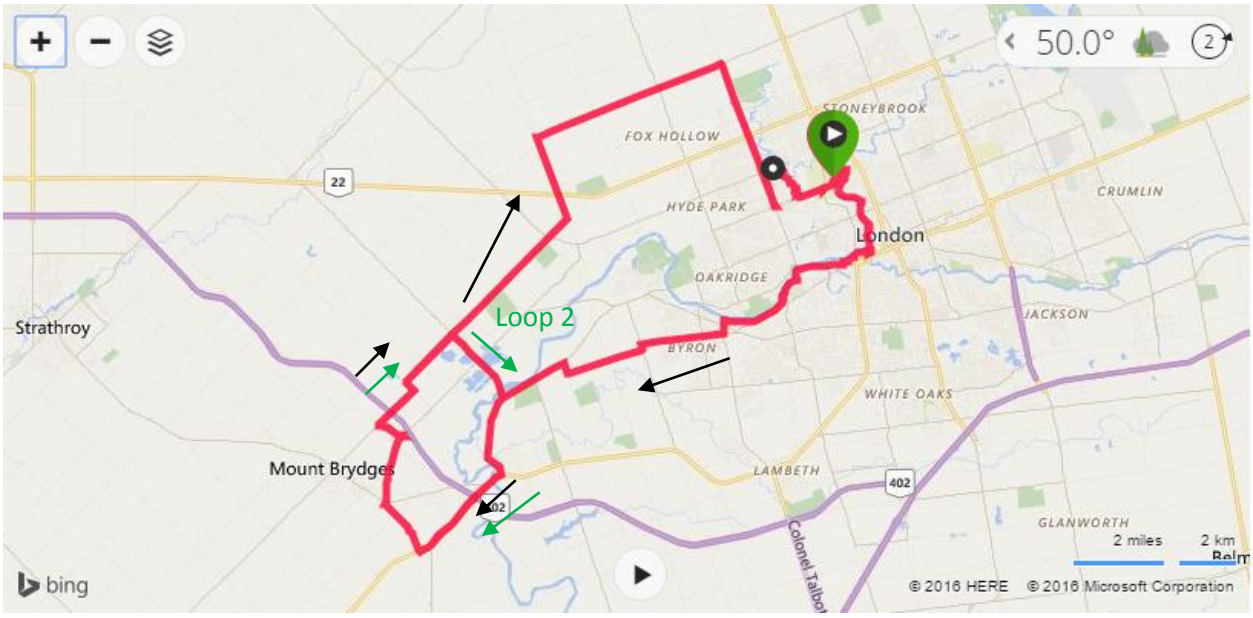

Figure 1. Long duration exercise cycle route. Black arrows indicate direction of the route and Green arrows indicate the second loop each cyclist had to complete during the long duration exercise cycle.

2.3 Results

Subject Characteristics. The mean height, weight and age of the CONT group were 180 ± 8 cm, 78 ± 7 kg, and 26 ± 4 y. Their absolute and relative VO_{2peak} was 3.81 ±0.5 $L*min^{-1}$, 48.6 ± 3.5 $ml*kg^{-1}*min^{-1}$, respectively. The mean height, weight and age of the RIDE group were 181 ± 6 cm, 76 ± 5 kg, and 28 ± 6 y respectively. Their absolute and relative VO_{2peak} was 4.20 ± 0.5 $L*min^{-1}$, and 55.63 ± 4.4 $ml*kg^{-1}*min^{-1}$, respectively. There was no difference in absolute VO_{2peak} between CONT and RIDE groups ($P>0.05$). The RIDE group was able to complete the long duration exercise cycle at an RPE of 11 on the 20 point scale. The mean HR at LT from both pre- and post-RAMP tests were not different from the mean HR during the long duration exercise cycle (140 ± 9 bpm, 140 ± 13 bpm, respectively; $P>0.05$).

Long duration Cycle (RIDE). Average distance for the intervention was 89 ± 7 kilometers which took an average of 3.25 h ± 0.36 h to complete. The average heart rate and speed were 140 ± 9 bpm and 27 ± 3 km/h respectively (Figure 1).

Incremental Ramp Test. Peak aerobic power output and VO_{2peak} decreased for the RIDE group pre- to post-RIDE ($P<0.05$), whereas no changes over the pre- to post-SED period were observed in the CONT group ($P>0.05$) (Table 1). The maximum heart rate attained during the RAMP test was unchanged pre- to post-RIDE/SED ($P>0.05$). VO_2 at LT was unchanged in both the RIDE and CONT groups post-RIDE/SED period ($P>0.05$) (Table 1). The mean HR at LT, from both pre- and post-incremental tests, was compared to the mean HR over the course of the RIDE (140 ± 9 bpm and 140 ± 13 bpm respectively; $P>0.05$). The work rate at LT was lower in pre- to post-RIDE ($P<0.05$), whereas LT in the CONT was unchanged (Table 1). HR at LT was unchanged pre- to post- RIDE/SED period ($p>0.05$) (Table 1). There were no pre- to post-differences within the CONT group's resting RER, 1.00 ± 0.12, 0.95 ± 0.11 ($P>0.05$), however the resting RER of the RIDE group was significantly lower pre- to post-long duration exercise cycle, (0.95 ± 0.1, 0.85 ± 0.1, respectively ($P<0.05$)). There were no pre-post differences within the CONT group's resting fat oxidation (0.00 ± 0.20 $g*min^{-1}$, 0.05 ± 0.27 $g*min^{-1}$ ($P>0.05$)), whereas resting fat oxidation within the RIDE group was significantly greater pre- to post-RIDE (0.08 ± 0.15 $g*min^{-1}$, 0.25 ± 0.18 $g*min^{-1}$, respectively ($P<0.05$)). Fat oxidation rates during the RAMP test was not different pre- to post-SED period in the CONT group (Figure 2a) ($P>0.05$), whereas increased fat oxidation rates were observed in the RIDE group on the post-RIDE RAMP ($P<0.05$) (Figure 2b). Carbohydrate oxidation rates during the RAMP test was not different pre- to post-SED period in the CONT group (Figure 3a) ($P>0.05$), whereas decreased carbohydrate oxidation rates were observed during the RAMP test post-RIDE (Figure 3b) ($P<0.05$). Power outputs at 0.99 RER during the RAMP were higher pre- to post-RIDE

(P<0.05), whereas no changes were observed in the CONT (P>0.05) (Table 1). Arterialised-capillary lactate concentrations were unchanged pre- to post-RAMP testing, post-SED period (P>0.05) (Figure 4a). Post-RIDE, arterialised-capillary lactate concentrations remained unchanged before both the pre- and post-RAMP tests, whereas decreases were observed after the post-RIDE RAMP (P<0.05) (Figure 4b).

3 min All-Out Test. CP remained unchanged pre- to post-RIDE/SED (Table 1). W′ was also unchanged in the CONT group pre- to post-SED (Table 1; Figure 5a) whereas W′ was lower pre- to post-RIDE during the 3 min all-out test in the RIDE group (P<0.05) (Table 1; Figure 5b). Furthermore, RER at CP was unchanged post-SED in the CONT group (Table 1), but was lower post-RIDE (P<0.05) (Table 1). Peak power was unaffected in both groups, pre- to post-RIDE/SED period (P>0.05) (Table 1). VO_2 over the duration of the test was also unchanged pre- to post- RIDE/SED period in both groups (Figure 6a & b). VCO_2 over the duration of the test was unchanged pre- to post-SED period in CONT group (Figure 7a) (P>0.05), whereas VCO_2 was lower pre- to post- RIDE (Figure 7b) (P<0.05). The maximum heart rate reached during the 3 min all-out test was also unchanged pre- to post-RIDE/SED period in both groups (P>0.05) (Table 1). Arterialised-capillary lactate concentrations were unchanged for the CONT group pre- to post-SED 3 min all-out testing (P>0.05) (Figure 4a). However, arterialised-capillary lactate concentrations were lower before and after the post-RIDE CP test (P<0.05) (Figure 4b). *Food Log.* Both groups consumed similar amounts of carbohydrates, proteins and fats for breakfast (P>0.05) (Table 2). However, protein intake was higher for the CONT group during the SED period versus the RIDE group during the long duration exercise cycle (18.6 ± 4.0 grams vs. 11.2 ± 7.5 grams (P<0.05)), with carbohydrate and fat intake remaining the same (P>0.05) (Table 2).

Table 1. Control and Experimental group, RAMP (incremental test) and 3 min all-out tests results, before and after (PRE to POST) sedentary period (SED) and long duration exercise cycle (RIDE). All values are presented as the group means with standard deviation (±).

Variable	Control PRE	Control POST	Significance	Ride PRE	Ride POST	Significance
Incremental Ramp Test						
Estimated Lactate Threshold (L/min)	2.27 ± 0.16	2.22 ± 0.16	P= 0.19	2.53 ± 0.43	2.46 ± 0.40	P= 0.48
Estimated Lactate Threshold (watts)	187 ± 20	187 ± 21	P= 0.97	211 ± 34	197 ± 29	P= 0.01*
Power Output at 0.99 RER (watts)	195 ± 40	190 ± 44	P= 0.30	266 ± 49	293 ± 26	P= 0.006*
RER at CP Work Rate	1.08 ± 0.06	1.08 ± 0.07	P= 0.83	1.08 ± 0.08	1.03 ± 0.06	P= 0.01*
Heart Rate at LT (bpm)	139 ± 7	144 ± 10	P= 0.09	138 ± 9	143 ± 9	P= 0.07
Heart Rate Max (bpm)	176 ± 9	177 ± 10	P= 0.90	179 ± 11	177 ± 11	P= 0.29
VO_{2max} (L/min)	3.81 ± 0.5	3.76 ± 0.3	P= 0.62	4.20 ± 0.5	3.84 ± 0.4	P= 0.009*
Peak Aerobic Power (watts)	356 ± 43	352 ± 44	P= 0.51	397 ± 32	366 ± 33	P=<0.001*
3 min all-out Test						
Resting RER	0.82 ± 0.1	0.81 ± 0.05	P= 0.88	0.92 ± 0.25	0.87 ± 0.31	P= 0.23
CP (watts)	243 ± 57	250 ± 51	P= 0.12	309 ± 34	306 ± 39	P= 0.77
RER at CP	1.18 ± 0.05	1.17 ± 0.05	P= 0.28	1.15 ± 0.07	1.08 ± 0.07	P= 0.01*
Peak Power (watts)	773 ± 167	777 ± 134	P= 0.92	693 ± 122	672 ± 104	P= 0.37
W′ (Joules)	15080 ± 3530	13527 ± 4537	P= 0.10	9817 ± 4135	6353 ± 2600	P= 0.004*
Heart Rate Max (bpm)	172 ± 8	174 ± 7	P= 0.06	165 ± 16	166 ± 17	P= 0.89

(*) $P<0.05$; significant within group differences before and after (PRE and POST) SED or RIDE.

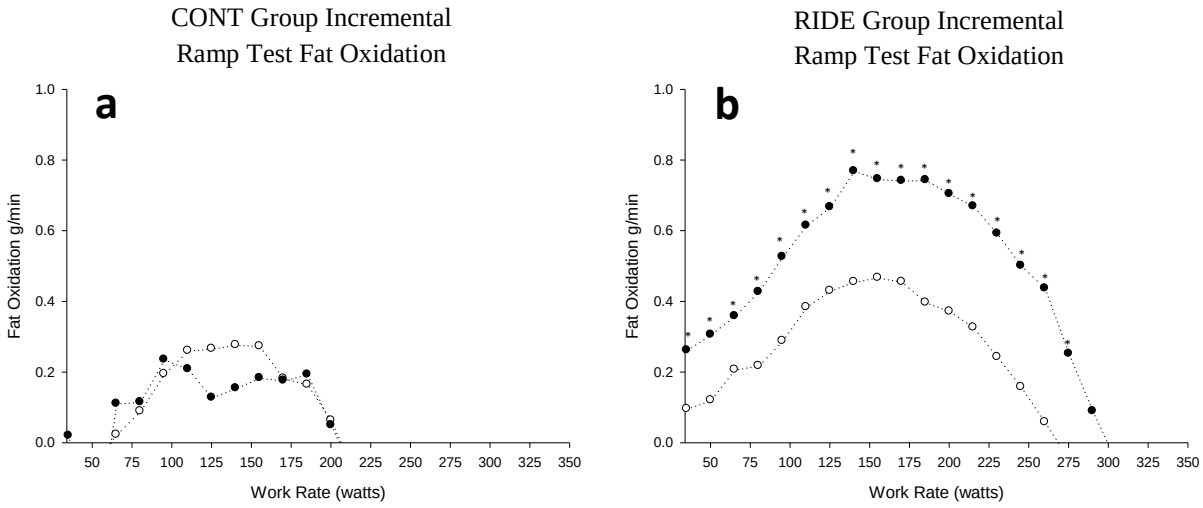

Figure 2. Mean fat oxidation (g*min^{-1}) during incremental testing (RAMP). Open circles pre-long duration exercise cycle (RIDE); closed circles post-RIDE/Control group (CONT). a. CONT; pre-post-SED (n=7); b. RIDE; pre-post-RIDE (n=11). * P<0.05 significant differences before and after (PRE and POST).

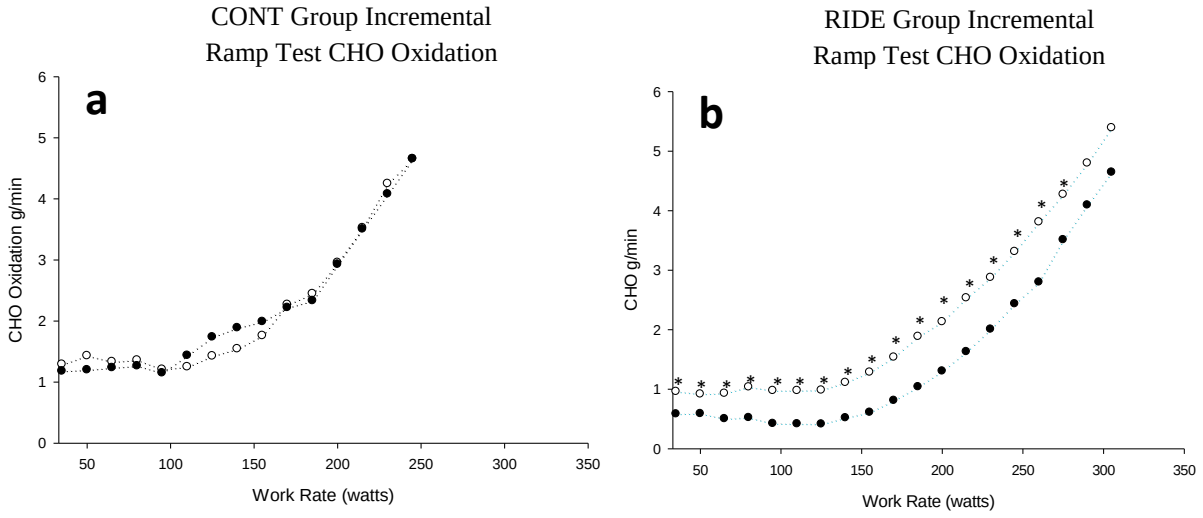

Figure 3. Mean carbohydrate (CHO) oxidation (g*min^{-1}) during incremental testing (RAMP). Open circles pre-long duration exercise cycle (RIDE); closed circles post-RIDE/Control group (CONT). **a.** CONT; pre-post-SED (n=7); **b.** RIDE; pre-post-RIDE (n=11). * P<0.05 significant differences before and after (PRE and POST).

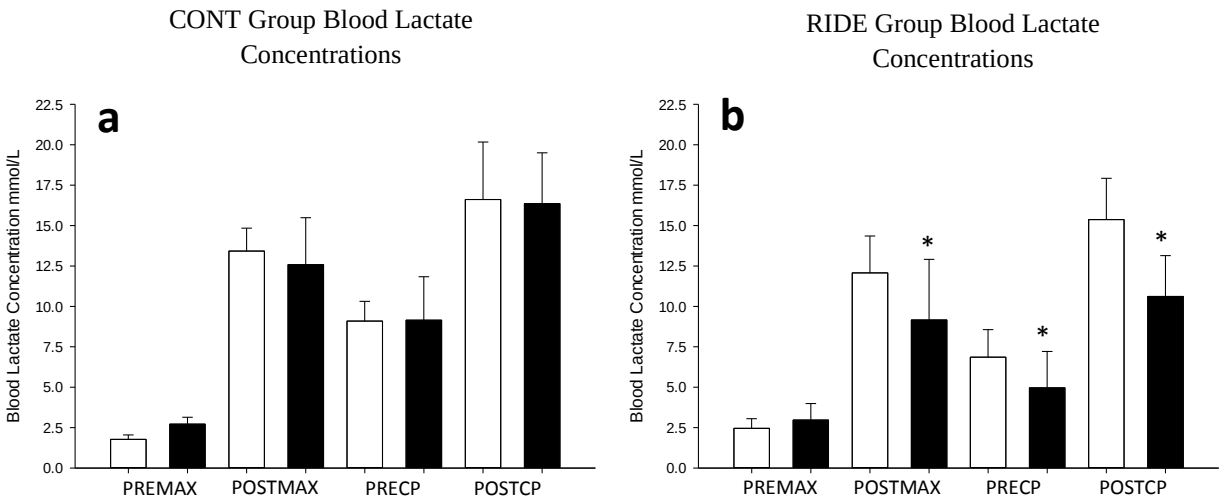

Figure 4. Mean ± SD arterialised-capillary lactate concentrations (mM). Open bars pre-long duration exercise cycle (RIDE)/ Sedentary period (CONT), and black bars post-RIDE/CONT. a. CONT; pre-post-SED (n=7); b. RIDE; pre- to post-RIDE (n=12).

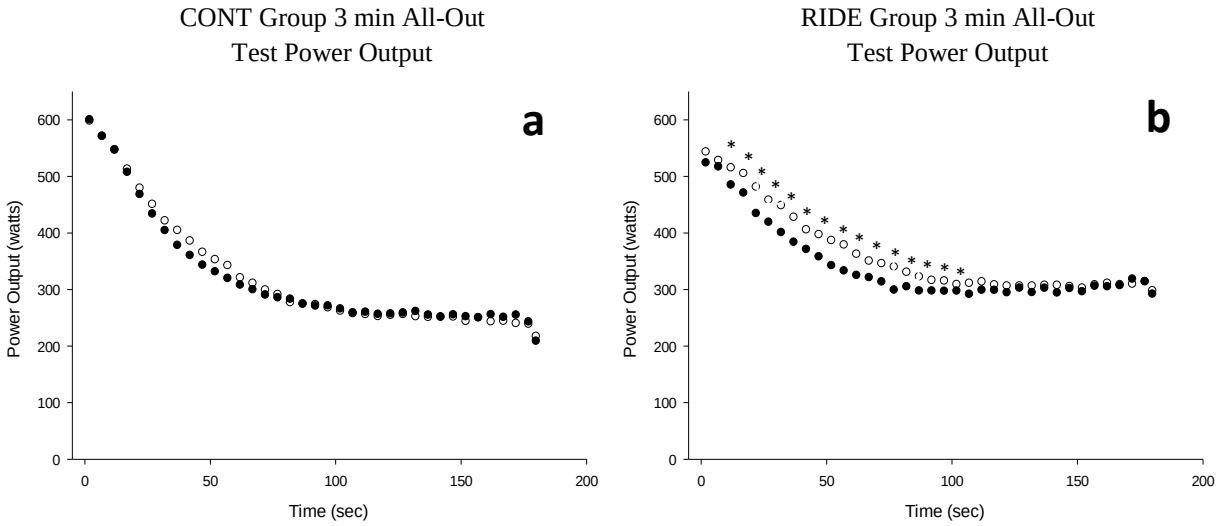

Figure 5. Mean power output during 3 min all-out testing. Open circles pre-long duration exercise cycle (RIDE); closed circles post-RIDE/Control group (CONT). a. CONT; pre-post- SED (n=7); b. RIDE; pre-post-RIDE (n=12). * $P<0.05$ significant differences before and after (PRE and POST).

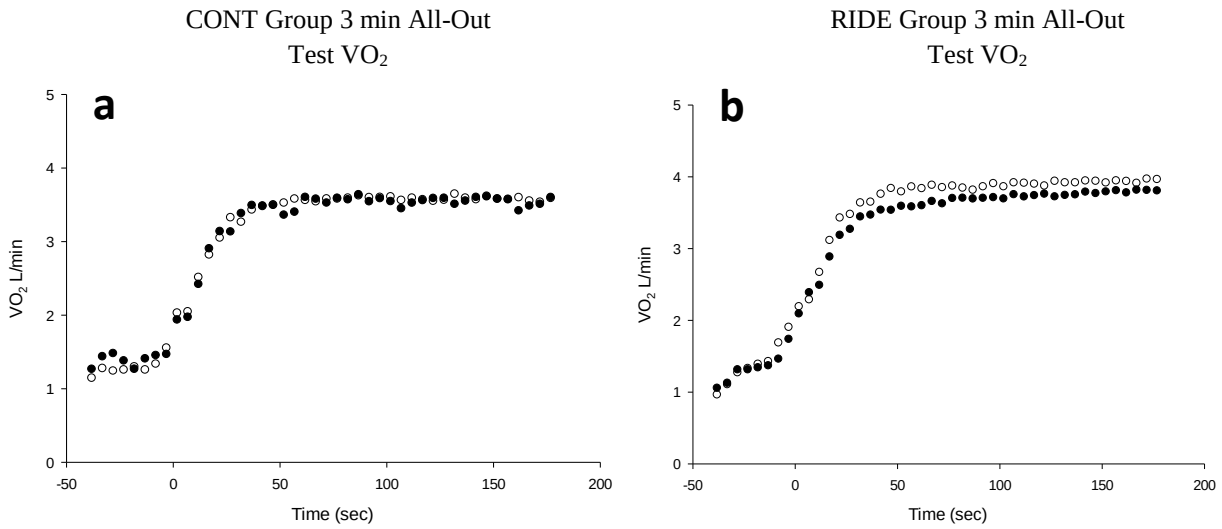

Figure 6. Mean Oxygen consumption (VO_2) $L*min^{-1}$ during 3 min all-out test. Open circles pre-long duration exercise cycle (RIDE); closed circles post-RIDE/Control group (CONT). **a.** CONT; pre-post-SED (n=6); **b.** RIDE; pre-post-RIDE (n=12). * $P<0.05$ significant differences before and after (PRE and POST).

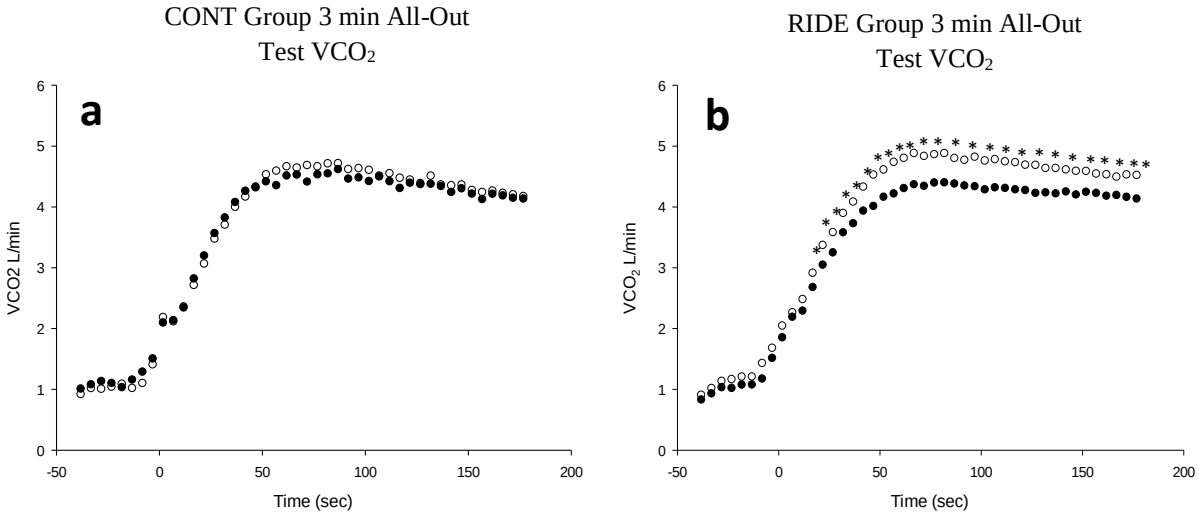

Figure 7. Mean carbon dioxide production (VCO_2) $L*min^{-1}$ during 3 min all-out testing. Open circles pre-long duration exercise cycle (RIDE); closed circles post-RIDE/Control group (CONT). **a.** CONT; pre-post-SED (n=6); **b.** RIDE; pre-post-RIDE (n=12). * $P<0.05$ significant differences before and after (PRE and POST).

Table 2. Control and Ride group dietary mean and standard deviation, breakfast and long duration exercise cycle (RIDE)/sedentary (SED) period.

Breakfast	**Control**	**Ride**	**Significance**
Carbohydrates (grams)	65.8 ± 58.7	53.7 ± 58.7	P>0.05
Proteins (grams)	19.5 ± 12.0	12.7 ± 8.8	P>0.05
Fats (grams)	15.7 ± 15.5	7.6 ± 6.1	P>0.05
Ride/Rest	**Control**	**Ride**	**Significance**
Carbohydrates (grams)	154.2 ± 28.5	131.7 ± 70.7	P>0.05
Proteins (grams)	18.6 ± 4.0	11.2 ± 7.5	P<0.05*
Fats (grams)	10.4 ± 2.2	8.8 ± 7.4	P>0.05

(*) $P<0.05$.

2.4 Discussion

In the present study, the effects of a long duration cycle (~3 h) (RIDE) and a 3 h sedentary period (SED) on, VO_2, VCO_2, substrate utilization, peak power, aerobic power, critical power (CP) and anaerobic capacity (W′) were observed in cyclists and recreationally active individuals (CONT). It was hypothesized that 1) the fat oxidation profile during the RAMP test in both the RIDE and CONT group would not be dissimilar to the inverted U shape profiles observed in constant load exercise, 2) the rate of energy production from oxidative phosphorylation of fats up to and including CP intensities, would increase during the RAMP test post-long duration exercise and, 3) W′ would be reduced post-long duration exercise as a result of glycogen depletion, with no changes in CP during the 3 min all-out test.

The major findings, resulted in both the RIDE and CONT group showing a similar inverted U shaped profile with increasing exercise intensity and showed an increase in fat utilisation up to and including CP during the ramp incremental test pre- to post-RIDE as well. Reductions in VO_{2peak}, peak aerobic power and W′ (4.20 ± 0.5 $L*min^{-1}$ vs. 3.84 ± 0.4 $L*min^{-1}$; 397 ± 32 W vs. 366 ± 33 W; ($P<0.05$) respectively), were observed post-RIDE, whereas CP remained unchanged (309 ± 34 W vs. 306 ± 39 W; ($P>0.05$)). No changes in any of these variables were observed in the CONT group pre- to post-SED.

Evidence that the RIDE in the present study was performed at or below the estimated lactate threshold (LT), was established from the unchanged mean arterialised-capillary lactate concentration of < 4 mM pre- to post-RIDE (pre-RIDE 2.45 mM and post-RIDE 2.96 mM; $P>0.05$), a sustained RPE of 11/20 (light effort) during the RIDE, and a mean heart rate (HR) during the RIDE that was similar to the mean HR at LT from both the pre- and post-RAMP (RIDE HR - 140 ± 9 bpm and RAMP LT HR 140 ± 13; $P>0.05$).

A mean arterialised-capillary lactate concentration of 4 mM within a population has been established as the power output and/or VO_2 at which the onset of blood lactate accumulation occurs (Nicholson and Sleivert 2001, Pyne et al. 2001). Moreover, an RPE of 11, on the 20 point RPE scale during exercise has been demarcated as moderate intensity exercise (Scherr et al. 2013) and moderate intensity exercise has been defined as a power output performed below LT (Ozyener et al. 2001).

As per previous research, the fat oxidation rates derived from the constant load, steady state, 20 W four min baseline exercise, increased pre- to post-RIDE (pre-0.08 ± 0.15 $g*min^{-1}$ vs. post-0.25 ± 0.18 $g*min^{-1}$;) (Bradley et al. 2012; Carey et al. 2001; Watt et al. 2003). As work rate increased during both pre- and post-RIDE/SED RAMP tests, fat oxidation rates increased in a parabolic fashion with work rate (Figure 2a & b). This inverted U shaped profile of fat oxidation rates has been observed previously by van Loon et al. (2001), during constant load exercise, 30 min in duration, at power outputs equivalent to 40%, 55% and 75% of peak aerobic power (418 W at a VO_2max of 5.4 $L*min^{-1}$) in very well trained cyclists. The calculated post-RIDE fat oxidation rates observed by van Loon et al. (2001) (0.68 $g*min^{-1}$, 0.80 $g*min^{-1}$ and 0.51 $g*min^{-1}$ respectively) at the aforementioned intensities, were observed in the present study below and above the estimated LT at intensities equivalent to 31%, 40% and 66% of VO_{2peak}. The similar fat oxidation rates at the higher power outputs observed in the van Loon et al. (2001) research, compared to the RIDE group of the present study, required a greater contribution from carbohydrates (1.44 $g*min^{-1}$; 2.04 $g*min^{-1}$; and 3.90 $g*min^{-1}$ compared to 0.49 $g*min^{-1}$; 0.42 $g*min^{-1}$; and 2.4 $g*min^{-1}$ respectively (Figure 3b)). This is not unexpected as the 30 min constant load power outputs were performed at greater relative intensities of peak aerobic power in the van Loon et al. (2001) study and would require a greater contribution from carbohydrates. This

inverted U shaped fat oxidation rate profile with increasing intensities, below and above LT has also been observed by Achten and Jeukendrup (2004).

As was hypothesised, the greater post-RIDE versus pre-RIDE fat oxidation rates continued to be observed up to and including CP (Table 1; Figure 2b). This would be predicted as the predominant recruitment of Type 1 fibres to perform CP (Vanhatalo et al. 2016), which would entail increased fat utilisation post-RIDE. The RIDE group also exhibited the anticipated greater fat oxidation rates compared to the recreationally active individuals in the CONT (Coggan et al. 1990; Coggan et al. 1993; Hurley et al. 1986) during both pre- and post-RIDE/SED on the RAMP tests.

VO_2 at LT remained the same post-RIDE, whereas power output at LT decreased (Table 1). This suggests that the reduced power output at a similar VO_2 was a function of the reduced work efficiency per litre of O_2 that is associated with oxidative phosphorylation of fats (23 mol of O_2 per fatty acid molecule) compared to that of oxidative phosphorylation of CHO (6 mol of O_2 per glucose molecule) (Sabapathy et al. 2006). The VO_2 at LT and power output pre- to post-SED in the CONT group was unchanged (Table 1).

Previous endurance training studies have suggested that the decrease in arterialised-capillary lactate accumulation post-exercise, similar to that observed after the post-RAMP and 3 min all-out tests of the RIDE group in the present study (Figure 4b), reflects a reduction in substrate level phosphorylation. This reduction in the present study may have resulted from the reduced sympathetic nervous system (SNS) stimulation linked to decreased blood epinephrine concentrations (<SNS activity) (Brooks and Mercier 1994; Gold et al. 1963; Lehmann et al. 1981) that perhaps would have transpired during the RIDE, which has been associated with increased oxidative phosphorylation of fats and reduced Type II fibre recruitment (Brooks and

Mercier 1994; Deuster et al. 1989; Bloom et al. 1976; Mora-Rodriguez and Coyle 2000; Lehmann and Keul 1986; Jubrias et al. 2003). That being said, it is more probable that glycogen depletion and the consequent reduction of substrate for substrate level phosphorylation contribution in individual Type II fibres (Vollestad et al. 1985; Shulman and Rotham 2001) during the latter stages of the RAMP and the 3 min all-out tests is the culprit.

It is suggested that the observed reduction in VO_{2peak} and peak aerobic power, post-RIDE, is a result of decreased O_2 delivery from the evolving cardiovascular drift that transpired over the RIDE. This phenomenon of cardiovascular drift has been ascribed to reduced cardiac stroke volume that decreases cardiac output at a given heart rate as a consequence of long duration exercise (Bassett and Howley 2000; Gonzalez-Alonso and Calbet 2003). The unchanged peak heart rates, and decreased VO_2 pre- to post-RIDE on the RAMP suggests CV occurred (Table 1), Using the standard O_2 cost of work ($10mls*min^{-1}*W^{-1}$) (Hansen et al. 1987; Barstow and Mole 1991) this reduction in O_2 delivery would be sufficient to explain the observed reduction in peak aerobic power post-RIDE. In comparison, no change in VO_{2peak}, peak aerobic power, or maximum heart rate were observed in the CONT pre- to post-SED period (Table 1).

W′ was lower post-RIDE (Table 1; Figure 5b). A similar reduction in W′ has been demonstrated in previous studies after performing work rates in the severe intensity domain (Simpson et al. 2012; Vanhatalo and Jones 2009). These authors proposed that the decline in W′ was a function of the reduced power output linked to Type II muscle fibre fatigue associated with glycogen depletion (Vollestad et al. 1985). A decrease in muscle glycogen has also been observed by Thomson et al. (1979) (70% reduction in Type IIa and a 30% reduction in Type IIx fibres) utilizing a comparable, although shorter, long duration moderate intensity exercise model compared to the present study (120 min at 60% VO_{2max} vs. ~195 min at 62%VO_{2peak}

respectively). An expected reduction of muscle glycogen post-RIDE in the present study would include glycogen depletion in select Type IIa/x fibres (Vollestad et al. 1985). This would result in a reduction in substrate level phosphorylation and associated power output from those glycogen depleted fibers (Vollestad et al. 1985). Moreover, the reduced arterialised-capillary lactate concentration observed after the post-RIDE RAMP and 3 min all-out tests, that originates from substrate level phosphorylation at power outputs > LT, also intimates glycogen depletion in some in Type II fibres (Genovely and Stamford 1982) (Figure 4b). Finally, the lower VCO_2 post-RIDE during the 3 min all-out test (Figure 7b from the same test, also suggests decreased substrate level phosphorylation (Tesch 1978; Karlsson et al. 1981). This reduced substrate level phosphorylation contribution would reduce substrate (H^+) for the carbonic anhydrase reaction and manifest the observed decrease in VCO_2 coupled to ventilatory buffering (Jones, 1980).

No change in W′ was observed in the CONT group post-SED period (Figure 5a). This implies that any glycogen depletion that may have occurred from the RAMP and/or the 3 min all-out tests was not great enough to elicit a drop in W′ (Vandenberghe et al. 1995; Jenkins et al. 1994). Furthermore, the unchanged arterialised-capillary lactate concentration observed post-SED period, before and after the RAMP and 3 min all-out tests (Figure 4a) also suggests that the 3 h SED period did not change the substrate level phosphorylation contribution to these performances. If there was a significant decrease in muscle glycogen after the pre-tests the return of glycogen back to pre-testing levels during the SED period may have been accelerated by the greater protein content ingested by the CONT (Table 2). A carbohydrate and protein mixture has been linked to faster glycogen repletion rates in the immediate hours post exercise than a high carbohydrate diet (Karp et al. 2006; Ivy et al. 2002).

As hypothesised, the maintenance of CP during the last 30 s of the 3 min all-out test during both CONT and RIDE groups was observed (Simpson et al. 2012; Sargeant 1994; Vanhatalo et al. 2016). CP has been attributed to the exclusive recruitment of fatigue resistant Type I fibres (Vanhatalo et al. 2016). It is plausible that any reduction in oxidative phosphorylation from glycogen depletion in the Type I fibres may have been replaced by observed increased oxidative phosphorylation from fats at this power output (Metcalfe et al. 2015; Conley et al. 2001; Torrens et al. 2016; Vanhatalo et al. 2016).

Lastly, peak power outputs (0-10 s) during the 3 min all-out test remained unchanged post-RIDE (Table 1). This suggests that the ATP-PCr system was unaffected by the long duration exercise cycle (Gastin 2001; Vanhatalo and Jones 2009). Previous research focusing on the consequential effects of prolonged cycling exercise bout (2-5 h), observed reductions in a related measure of peak force production, maximal voluntary contractions (MVC) (Lepers et al. 2000; Lepers et al. 2002). It was suggested that the reductions were associated with increased muscle compound action potential durations (M-wave) and reduced isometric twitch force suggested to be accompanied by increases in H^+, P_i and/or a decrease in the sarcoplasmic reticulum release and uptake of Ca^{2+} (Metzger and Moss 1990; Lepers et al. 2000; Lepers et al. 2002). The maintenance of the peak power output observed in the present study, despite a substantially longer duration exercise, could be attributed to the lower intensity, and subsequently reduced accumulation of fatigue related metabolites (Urhausen et al. 1995), as exhibited by the lower heart rate and RPE recorded (141 bpm and RPE 11 vs 161 bpm and RPE 19 (Lepers et al. 2002)) during the RIDE, compared to this earlier research. The performance of this lower intensity in the present study would blunt the increases in H^+, P_i and/or decrease the sarcoplasmic reticulum release and uptake of Ca^{2+} that has been observed to induce reductions in

peak power output post-long duration exercise (Lepers et al. 2000, Lepers et al. 2002). Alternatively, pedaling motions on a bike resemble isotonic contractions, suggesting that the results in the present study are similar to the speeded rates of recovery of isotonic power (within 5 min) compared to isometric MVC after fatiguing contractions that have been observed elsewhere (Cheng and Rice 2005).

In summary, 1) an inverted U shaped profile of fat oxidation was observed in both the CONT and RIDE groups during incremental exercise, 2) critical power remained unchanged pre- to post- long duration cycle whereas fat oxidation rates increased at power outputs ≤ critical power during incremental exercise, and 3) the decreased W′, and lower arterialised-capillary lactate concentrations post- long duration cycle , after both incremental and 3 min all-out tests, suggests diminished substrate level phosphorylation associated with depleted glycogen stores.

This study has demonstrated that over a simulated stage race, substantial changes in substrate oxidation and performance will evolve over the duration of the stage. The novel finding that CP was unchanged post-long duration exercise suggests that CP can be used as a key parameter to devise pacing strategies and tactics to outpace opposing cyclists during the final stages of the race. This advocates that a cyclist with a high CP and low W′ relative to other cyclists, should attempt to distance themselves from the peloton with 5-10 k remaining to capitalise on their unchanged CP and the reduced W′ of the sprinters , whereas a cyclist with a low CP and a high W′ relative to other cyclists, would attempt to keep the pace of the race at a lower speed and shutting down any attempts from cyclists with high critical (read time trialling abilities) and use their relatively greater W′ near the finish line to outpace the opposing cyclists (Jones et al. 2010, Vanhatalo et al. 2011).

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Chapter 3

3.0 Limitations and Future Directions

In the present study, the changes to CP performance were only observed for the last 30 s of the 3 min all-out test, which does not provide evidence of the extended sustainability of CP performance at the end of a race. To fully understand the effects of a prolonged exercise bout on CP performance, a protocol designed to observe CP at an extended duration would need to be elicited. Moreover, leading up to the testing day, no measure of nutritional intake was collected for each individual. Therefore, individuals utilizing a high CHO diet prior to the study could

have resulted in greater stored muscle glycogen, affecting performance capacity and substrate utilization contributions (Lambert et al. 2001; Rauch et al. 1995), post-long duration exercise cycle/SED period data. Furthermore, diet was not standardized on the day of testing, as subjects were instructed to consume breakfast meals that would be their regular food intake prior to a competitive race. This allowed cyclists to consume a variety of food choices, resulting in different macronutrient and micronutrient intake for each cyclist. Diet during the long duration exercise cycle was also not standardized, as cyclists were instructed to consume their normal snack intakes during a long cycle. This also allowed the cyclists freedom to choose a variety of differing food sources, which also resulted in different nutritional intakes for each individual. Both the breakfast meals and snack intakes during the long duration cycle ride could affect performance and substrate utilization (Lambert et al. 2001; Rauch et al. 1995).

For future studies, muscle biopsies could be utilized before and after the long duration exercise cycle to measure glycogen content of the vastus lateralis, providing a better measure of glycogen depletion associated with the decrements in the performance observed post-long duration exercise cycle. In addition, observing changes in proteins and enzymes that regulate fat metabolism such as vascular endothelial growth factor B (VEGF-B) and fatty acid binding proteins (FABP) obtained from tissue samples through muscle biopsies of the vastus lateralis could provide further insight in substrate utilization changes observed post-long duration exercise cycle (Bradley et al. 2012; Watt et al. 2003; Hagberg et al. 2010; Hagberg et al. 2013; Liang and Ward 2006; Holloway et al. 2008). Moreover, blood work measures of epinephrine (Gold et al. 1963; Lehmann et al. 1981) and plasma fatty acid concentrations (Coggan et al. 1993; Romijn et al. 1993) could provide further information in the association between sympathetic nervous system activity and fat oxidation. Lastly, by performing an identical

experiment on female participants gender related differences in substrate utilization, muscle deoxygenation and performance post-long duration exercise cycle could be investigated (Murias et al. 2013; Horton et al. 1998).

3.1 References

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Appendices

Appendix A: Ethics Approval Notice

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Annual Continuing Ethics Approval Notice

Date: February 05, 2016
Principal Investigator: Dr. Glen Belfry
Department & Institution: Health Sciences\Kinesiology,Western University

Review Type: Full Board
HSREB File Number: 103432
Study Title: The necessity of long duration training sessions for maximal aerobic adaptations: The acute responses of energy system contributions, lactate threshold, and regional blood flow distribution to maximal performance before and after a 5 hour training session.

HSREB Renewal Due Date & HSREB Expiry Date:
Renewal Due -2017/01/31
Expiry Date -2017/02/05

The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

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

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

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information: Erika Basile ✓ Nicole Kaniki ___ Grace Kelly ___ Mina Mekhail ___ Vikki Tran ___

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Appendix B: Letter of Information and Consent Forms

LETTER OF INFORMATION:

Study Title: The necessity of long duration training sessions for maximal aerobic adaptations: The acute responses of energy system contributions, lactate threshold and regional blood flow distribution to maximal performance before and after a 3 hour training session.

Principal Investigator: Glen Belfry PhD Co-investigators – Michael Bitel

Introduction and Background

In such sports as swimming, rowing and road race cycling it is believed, by coaches, that long training sessions (2-6hrs) of moderate intensity work must be performed for 20-30 hours a week, for a number of months, for a number of years to be successful. The physiology of these long training sessions is not well understood. This study will attempt to discover the benefits to these long duration exercise sessions.

Purpose of Study:

You are being invited to participate in a study that examines the effects of a 3 hour exercise session on several physiological variables because you are a well trained member of the London Cycling Community that has been riding for at least 3 years. Participation in this study involves 2 visits to the laboratory of the Canadian Centre for Activity and Aging on the same day requiring less than 45 minutes. Canadian Centre for Activity and Aging Laboratory is located in the

Arthur and Sonia Labatt Health Sciences Building, at The University of Western Ontario in London, Ontario, Canada. A total of 10 healthy males will be invited to participate in this study. In order to participate you must be between 18-41 years of age. You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular, metabolic, neurological or musculoskeletal disease; or you are currently on medication; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise or exercise training protocol.

What you will be required to do if you decide to participate in this study: During the first visit to the laboratory you will complete a two cycling tests. The first test will begin with the exercise intensity being very light and easy (very little resistance). After a few minutes the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop. Following this the work rate will be reduced for 10 minutes to mild exercise and then a maximal three minute effort will be performed. This visit will last approximately 45 min. The second visit will be after you have completed the 3 hour moderate intensity exercise session as part of your regular exercise regimen. You will then come complete the identical two tests that you have performed before your ride. During both testing periods information will be collected based on your breathing and the amount of oxygen in your thigh muscle. After each test we will take your blood lactate concentration via standard finger prick apparatus. We will analyze your blood lactate levels by a portable lactate analyzer (Lactate Scout from Lactate.com). Your blood lactate concentration will enable us to determine the anaerobic energy involvement after the two cycling tests.

Breathing apparatus: During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). These will be washed and sterilized between users. This will enable us to measure the the volume of air that you breathe in and out, and measure the gas concentration

in that air. You may experience some initial discomfort from wearing the nose-clip and mouthpiece.

Thigh measurements: During the 3 minute and incremental cycling tests the relative oxygenation of the outside of your thigh muscles will be measured using near-infrared spectroscopy. This technique involves projecting light into your thigh and measures the amount of light coming out at another location. Two plastic probes will be attached to your leg approximately midway between your hip and knee. The probes will be secured with tape, covered to prevent other light from entering or leaving the area, and bound with elastic bandage to minimize movement of the probes. You may experience a bit of discomfort by having the probes secured to your leg during the exercise period.

Finger prick: After both of the incremental and 3 minute tests a pin prick will be administered to your left middle finger and a drop of blood will be used to observe the muscle by-products (lactic acid) of high intensity exercise.

Possible Risks and Discomforts: Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk, as stated by American College of Sports Medicine, is 6 in 10,000 for adverse outcome in people at higher risk – these risks would be much lower in healthy young adult athletes, who have no signs or symptoms, that would cause one to avoid exercise.

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

Potential Benefits of Participation: This is a basic physiology study and, as such, there will be no direct benefits received as a consequence of participating in the study. However, due to the nature of the exercise training there may be some

beneficial cardiovascular adaptations (increased fitness); however these may be only temporary and disappear within a few weeks of the completion of the study. If you are interested, the rationale for conducting the research and theory and significance of each of the tests will be explained, as will your individual results from each of the tests. You will also have the opportunity to learn about and better understand your physiological response to these exercise situations.

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

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

Voluntary Participation: Participation in the study is voluntary. You may refuse to participate, refuse to answer any questions and withdraw from the study at any time with no effect on your academic or employment status. You will be given a copy of this letter of information and signed consent form. You do not waive any legal rights by signing the consent form. If you have any questions regarding this study please contact Glen Belfry or Thinisia Thiruchelvam, The University of Western Ontario, London. If you have any questions about the conduct of this study or your rights as a research participant you may contact the Office of Research Ethics, The University of Western Ontario.

Initials of participant_____ Page 4 of 4

Consent Form

Study Title: The necessity of long duration training sessions for maximal aerobic adaptations: The acute responses of energy system contributions, lactate threshold, and regional blood flow distribution to maximal performance before and after a 3 hour training session.

Principal investigator: Dr Glen Belfry PhD Co-investigator

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

Participant Name (please print): _____

Signature _____

Date

Investigator (Person Responsible for Obtaining Informed Consent):

_____ _____

Name (please print) _____

Signature _____ Date: Initials of participant_____

Appendix C: Borg's Rate of Perceived Exertion (RPE) Scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Table 1. The Borg Rating of Perceived Exertion Scale

Curriculum Vitae

Name:	Michael Bitel
Post-secondary Education and Degrees:	University of Western Ontario London, Ontario, Canada 2010-2014 B.A. The University of Western Ontario London, Ontario, Canada 2014-2017 M.Sc.
Honours and Awards:	Academic All-Canadian 2012-2013
Related Work Experience	Teaching Assistant The University of Western Ontario 2014-2016
Publications:	