EFFECTS OF CYCLING CADENCE ON RESPIRATORY AND HAEMODYNAMIC RESPONSES IN TRAINED CYCLISTS

by

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Abstract

The physiological consequences of cycling cadence selection are poorly understood.

Purpose: To determine the impact of cadence on cardiorespiratory and metabolic parameters; perceptual responses; power of breathing (P_b); electromyography of the diaphragm (EMGdi) and leg muscles; and microvascular leg blood flow.

Methods: Eleven trained cyclists (10M:1F; age=27±6yrs; $\dot{V}O_{2max}$=60.8±3.7ml·kg^{-1}·min^{-1}) completed four 6-min constant-load cycling trials at 10% below their gas exchange threshold (63±5% peak power) while pedaling at 60rpm, 90rpm, 120rpm, and a freely chosen cadence (FCC, 94.3±6.9 rpm), in randomized order, on an electromagnetically braked cycle ergometer. Ventilatory and metabolic parameters were measured using a commercially available metabolic cart. An oesophageal electrode balloon catheter was used to assess P_b and EMGdi. Surface EMG was placed on four leg muscles predominant in cycling. Blood flow index (BFI) was determined on the same muscles of the contralateral limb using near-infrared spectroscopy and indocyanine green. Perceptual responses were measured using the modified 0-10 category ratio Borg scale.

Results: With each increase in cadence there was a corresponding increase in $\dot{V}O_2$ (all pairwise comparisons p<0.05). P_b and EMG_{di} was significantly greater at 120rpm compared to all other conditions (p<0.05). There were no differences in leg EMG across cadences except for the gastrocnemius, which was significantly higher at 90rpm, FCC, and 120rpm versus 60rpm (all p<0.05). A significant main effect was observed for BFI in three out of the four muscles tested, where 120rpm was higher than 60rpm and 90rpm in the vastus medialis (9.1±3.8 vs. 7.1±1.8 and 6.8±2.5µmol·s^{-1}, respectively, both p<0.05) and semitendinosus (4.8±3.1 vs. 3.4±1.5 and 3.2±1.4µmol·s^{-1}, respectively, both p<0.05). The gastrocnemius was higher at 120rpm compared to 60rpm, 90rpm and FCC (8.1±2.0 vs. 4.1±1.5, 5.4±2.1 and 5.9±2.0 µmol·s^{-1} respectively, all

$p<0.01$). No difference in BFI was found in the vastus lateralis ($p=0.06$). There was no effect of cadence on breathing or leg discomfort ratings ($p>0.05$).

Conclusion: Relative blood flow appears to be closely linked with metabolic activity of the muscle, which was significantly elevated during the highest cadence condition. In combination with the substantial rise in respiratory variables and the P_b, cadence may represent the balance between peripheral and central stressors.

Lay Summary

Despite cycling's popularity, little is known about pedal rate selection and, in particular, what impact pedaling rate has on blood flow to the leg muscles. The purpose of this thesis was to measure blood flow of the thigh, hamstring, and calf muscles at slow, moderate, and fast pedaling rates. The results of this thesis show that, for a given cycling intensity, blood flow is the highest during fast pedaling in the majority of muscles tested. Also, heart rate, the amount of air breathed in and out, and the amount of work it takes to breathe are greater during fast pedaling. These results could be used to optimize performance in cyclists and/or to inform pulmonary rehabilitation programs on the ideal cycling cadence to reduce breathing requirements and therefore breathlessness in patients with respiratory conditions.

Preface

This thesis contains the work of the candidate, Reid Mitchell, under the supervision of Dr. Jordan A. Guenette. Experimental design and conception were the joint effort between Dr. Jordan A. Guenette and Reid Mitchell. Participant recruitment, data collection and analysis, interpretation, and document preparation are primarily the work of the candidate, Reid Mitchell.

The experiment presented in this thesis received ethical approval from the Providence Health Care Research Ethics Board (UBC-PHC REB Number: H16-00674). All data were collected at the Cardiopulmonary Exercise Physiology Laboratory within the Centre for Heart Lung Innovation at St. Paul's Hospital, in Vancouver, British Columbia, Canada.

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ΔICGmax	Change in maximum ICG concentration
BFI	Blood flow index
Deoxy[Hb+Mb]	Concentration of deoxygenated haemoglobin and myoglobin
EELV	End-expiratory lung volume
EILV	End-inspiratory lung volume
EMG	Electromyography
EMGdi	EMG of the diaphragm
EMGgm	EMG of the medial gastrocnemius
EMGst	EMG of the semitendinosus
EMGvl	EMG of the vastus lateralis
EMGvm	EMG of the vastus medialis
F_b	Breathing frequency
FCC	Freely chosen cadence
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GET	Gas exchange threshold
HR	Heart rate
IC	Inspiratory capacity
ICG	Indocyanine green
MVV	Maximum voluntary ventilation
NIRS	Near infrared spectroscopy
Oxy[Hb+Mb]	Concentration of oxygenated haemoglobin and myoglobin
P_b	Power of breathing
$\dot{Q}$	Cardiac output
RER	Respiratory exchange ratio
RMS	Root mean square
rpm	Revolutions per minute
SD	Standard deviation
Total[Hb+Mb]	Total concentration of haemoglobin and myoglobin
$\dot{V}CO_2$	Production of carbon dioxide
$\dot{V}_E$	Minute ventilation
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for oxygen
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximum oxygen consumption
V_T	Tidal volume
W	Watt
W_b	Work of breathing

Acknowledgements

This research study could not have been completed without the help and support from many individuals. First and foremost, I must thank Dr. Jordan A. Guenette and express my sincere gratitude for his continuous interest, enthusiasm and guidance throughout the past few years. The excellent training environment he has created is unparalleled and has positively impacted my development as a researcher. I cannot thank him enough for his trust in allowing me to design and execute such a highly technical and invasive physiological study as a Master's student, and I look forward to the years ahead as a doctoral student in his laboratory.

I must also thank my thesis committee members, Dr. Michael S. Koehle and Dr. William R. Henderson, as this study would not have been possible without their assistance. I am extremely grateful for the time they committed to my project as the supervising physicians. The experience of working alongside Drs. Koehle and Henderson has impacted my learning experience tremendously and the knowledge they have imparted on me is invaluable.

It has been a pleasure to work with all of the members of the Cardiopulmonary Exercise Physiology lab. I am continuously impressed by their passion for research and desire to work with patients to hopefully improve their overall quality of life. I look forward to our future collaborations, whether that is in the lab or beyond.

Of course, I must thank the participants who volunteered to take part in my study. With the invasiveness of this study, it was not the easiest to participate in. I commend them for their patience and thank them for the time they committed to my research.

Finally, I would like to thank my family and friends for the endless support through this journey. I cannot thank my parents enough for their continuous support and giving me the

opportunity to move across the country in order to complete my studies. Without them, none of this would have been possible.

Chapter 1: Background and Rationale

1.1 Introduction

Pedal rate, or cadence, is an aspect unique to cycling, which can be manipulated according to exercise intensity and environmental terrain or adapted for cycling specific training and competitive race strategy. Cadence is a highly complex, multifaceted phenomenon that has been the topic of research for the better part of a century. Benedict *et al.* (1913) were among the first scientists to investigate the effect of cadence on the net efficiency of human muscle.

> "With 70 revolutions of the pedal he found with one subject 25.1 per cent, with 80 revolutions 26.7 per cent, with 90 revolutions 28.4 per cent, while another subject with 90 revolutions he found 30.6 per cent, and with 100 revolutions 25.8 per cent, indicating approximately an optimum speed of 90 revolutions of the pedal per minute. Considering the conditions under which Amar worked and the difficulties incidental to an inadequately equipped laboratory these results are of special interest; yet, as pointed out by Lefèvre, [a] the values must be taken with considerable reserve" (p. 112).

While the authors were skeptical of this observation, the fact that cyclists self-select a cadence that is metabolically inefficient is arguably the most well established result within the cycling cadence literature (Hagberg *et al.*, 1981; Marsh & Martin, 1993; Hansen *et al.*, 2002a; Foss & Hallén, 2004; Lucia *et al.*, 2004). However, despite decades of research, the physiological basis for the freely chosen cadence (FCC) remains unknown.

Hypotheses regarding the predominant mechanisms of self-selected cadence include the reduction in oxygen consumption ($\dot{V}O_2$) (Hagberg *et al.*, 1981; Faria *et al.*, 1982; Takaishi *et al.*, 1998), minimization of peripheral stress and/or neuromuscular fatigue (Takaishi *et al.*, 1994; Marsh & Martin, 1998), and the optimization of biomechanical factors (i.e., joint kinematics)

(MacIntosh *et al.*, 2000; Marsh *et al.*, 2000; Sanderson *et al.*, 2000; Hansen *et al.*, 2002a; Hansen *et al.*, 2002b; Mornieux *et al.*, 2007). In other words, FCC is thought to represent the pedal rate that: i) requires the least amount of oxygen for a given exercise intensity (common in other endurance sports, such as running (Cavanagh & Williams, 1982)), ii) minimizes leg discomfort and muscle strain, or iii) results in the most effective force applied to the pedals. It has been suggested that FCC is a representation of the balance between strain on the peripheral muscles and stress on the central cardiorespiratory systems (Lucia *et al.*, 2004). However, few studies that incorporate variables related to all of these hypotheses have been conducted. It is therefore unclear which factors are the most important in determining cycling cadence.

The impact of cadence has been investigated in terms of $\dot{V}O_2$ (Seabury *et al.*, 1976; Lepers *et al.*, 2001; Stebbins *et al.*, 2014); muscular efficiency (Gaesser & Brooks, 1975; Chavarren & Calbet, 1999; Hansen *et al.*, 2002a); joint kinematics (Marsh *et al.*, 2000; Mornieux *et al.*, 2007; Skovereng *et al.*, 2016); muscle activation and recruitment (Neptune *et al.*, 1997; MacIntosh *et al.*, 2000; Baum & Li, 2003; Bieuzen *et al.*, 2007); as well as lower extremity oxygenation (Boone *et al.*, 2015; Zorgati *et al.*, 2015). However, due to discrepancies in study protocols, the overall physiological impact of cycling cadence is difficult to reconcile. For example, the range of cadences tested, the fitness status of participants, and the types of exercise tests used are variable within and between studies. More importantly, exercise intensity differs substantially between studies resulting in different patterns of physiological responses. Moreover, a significant portion of the knowledge gained about the role of cadence on physiological outcomes has also been derived from studies performed using research methods that differ from dynamic cycling. In particular, knee extension exercise has been used as an *in vivo* model (Andersen *et al.*, 1985) in order to isolate the relationship between contraction frequency and a plethora of physiological

outcomes within the quadriceps muscles including arterial blood flow (Andersen & Saltin, 1985; Hoelting *et al.,* 2001), muscle $\dot{V}O_2$ (Ferguson *et al.,* 2001), and power output (Sjøgaard *et al.,* 2002). While the aforementioned studies provide critical knowledge on physiological factors such as blood flow distribution within the quadriceps, the muscle recruitment patterns of knee extension exercise and dynamic two-legged cycling are significantly different (Richardson *et al.,* 1998; Koga *et al.,* 2005). Therefore, the literature lacks congruency as results from these studies may not be generalized to the impact of cadence on whole body cycling exercise.

1.2 Cadence and the Cardiorespiratory System

In contrast to endurance running, where gait (stride rate and length) is selected to minimize $\dot{V}O_2$ for a given exercise intensity (Cavanagh & Williams, 1982), cyclists FCC is associated with a higher $\dot{V}O_2$ in comparison to lower cadences (Ansley & Cangley, 2009; Vercruyssen & Brisswalter, 2010). The selection of a metabolically inefficient cadence occurs regardless of cycling experience or individual aerobic fitness (Böning *et al.,* 1984; Marsh & Martin, 1993; Nickleberry & Brooks, 1996). However, cycling experience and aerobic fitness are partially responsible for the range in FCC reported in the cadence literature. In the laboratory setting, FCC varies from 80-100 revolutions per minute (rpm), while the cadence that minimizes $\dot{V}O_2$ (often referred to as the energetically optimal cadence) has been reported to be between 50-70 rpm (Ansley & Cangley, 2009). Despite the energetically optimal cadence being consistently lower than FCC, the impact of cadence on $\dot{V}O_2$ for a given power output, remains controversial.

Most studies report an increase in $\dot{V}O_2$ with increasing cadence (Coast & Welch, 1985; Chavarren & Calbet, 1999; Zorgati *et al.*, 2013; Stebbins *et al.*, 2014). However, others report no significant differences in whole body (Lepers *et al.*, 2001; Foss & Hallén, 2004) and muscle $\dot{V}O_2$

(Skovereng *et al.*, 2016). Foss and Hallén (2004) had participants complete multiple maximal incremental cycling tests at fixed cadences of 60, 80, 100, and 120 rpm resulting in power outputs ranging from 425-525 W, and found no significant differences in $\dot{V}O_{2max}$ at the end of each trial. The absence of any significant differences in $\dot{V}O_{2max}$ could be explained by the elite calibre of their participants, which allowed them to achieve a similar $\dot{V}O_{2max}$, irrespective of cadence. In contrast, using submaximal trials of different fixed power outputs (0-350 W), the same authors found a significant effect of cadence on $\dot{V}O_2$, except for the comparison of 60 rpm and 80 rpm below 350 W. Therefore, the exercise protocol and intensity of cycling may influence the effect of cadence on $\dot{V}O_2$. Conversely, Lepers *et al.* (2001) used cycling trials of 30-minutes at an exercise intensity corresponding to 80% $\dot{V}O_{2max}$ at FCC, 20% above FCC (103 rpm), and 20% below FCC (69 rpm) and found no effect of cadence on $\dot{V}O_2$. However, in another experiment comparing 80 and 100 rpm during 90 min trials of cycling, where the intensity ranged from 50 to 80% of $\dot{V}O_{2max}$ in identical fashion, it was reported that $\dot{V}O_2$ was significantly higher at 100 rpm (Stebbins *et al.*, 2014). Therefore, the discrepancy in the cadence-$\dot{V}O_2$ relationship can be attributed, at least in part, to differences in study design, where the duration and exercise intensity appears to influence the observed response.

Studies that manipulate both cadence and power output have provided insight into the role of exercise intensity on the cadence-$\dot{V}O_2$ relationship. Chavarren and Calbet (1999) found a positive parabolic relationship in seven competitive cyclists pedaling at 60, 80, 100, and 120 rpm across a range of fixed power outputs (124-259 W). Interestingly, these authors observed the cadence-$\dot{V}O_2$ relationship become more linear as exercise intensity increased, resulting in a near linear relationship at 259 W. The increasing linearity between cadence and $\dot{V}O_2$ is in contrast with other studies, which maintain a similar parabolic shape despite changes in power output

(Coast & Welch, 1985; Foss & Hallén, 2004). The results of Chavarren and Calbet (1999) may be confounded by fatigue as participants only received five minutes of rest between trials. However, fatigue was not measured in their study. On the contrary, an increase in the energetically optimal cadence with increasing power output has become a well-established concept (Seabury *et al.*, 1976; Coast & Welch, 1985; Hansen *et al.*, 2002a; Foss & Hallén, 2004). Coast and Welch (1985) found the energetically optimal cadence to increase from 50 rpm at 100 W to approximately 80 rpm at 300 W. On two more recent occasions, the energetically optimal cadence has been shown to increase from 50 to 60 rpm at 147 and 258 W and from 50 to 80 rpm at 125 and 350 W (Hansen *et al.*, 2002a; Foss & Hallén, 2004), respectively. Thus, studies using exercise intensities above the anaerobic threshold may be unable to distinguish between the cadence that minimizes $\dot{V}O_2$ and FCC. Furthermore, the energetically optimal cadence increases with workload, suggesting metabolic demands play a role in cadence selection; however, further research is needed to determine its overall contribution.

The increase in $\dot{V}O_2$ with increasing cadence is thought to be related to an increase in the recruitment of muscle motor units with preference to motor units containing fast twitch muscle fibres (Sargeant, 1994; Barstow *et al.*, 1996; Kohler & Boutellier, 2005; Umberger *et al.*, 2006), and greater internal work, or the work to overcome an increase in inertial force of the pedal crank, with higher cadences (Neptune & Herzog, 1999; Kautz & Neptune, 2002; Ettema & Lorås, 2009). It is known that fast twitch (type II) muscle fibres have a higher maximum shortening velocity (Hill, 1913; He *et al.*, 2000), which results in their preferential recruitment at rapid contraction frequencies in comparison to slow twitch (type I) muscle fibres. Indeed, the maximum shortening velocity of slow twitch fibres is reported to be three to five times slower than fast twitch fibres (Fitts *et al.*, 1989), with peak contraction efficiency occurring at approximately one third of their

maximum velocity (Hill, 1913). In addition, since oxygen is required for the initiation of a cross-bridge between actin and myosin during aerobic metabolism, a substantial amount of oxygen is needed for rapid contraction frequencies in comparison to long duration contractions (Hill, 1922; Hogan *et al.*, 1998; He *et al.*, 2000). Fast twitch muscle fibres are also able to generate more force in comparison to slow twitch fibres for a given level of activation (Hill, 1922; He *et al.*, 2000). Therefore, it has been suggested that fast twitch fibres are also preferentially recruited at low cadences (<60 rpm), which provides an explanation for the parabolic "U-shaped" cadence-$\dot{V}O_2$ relationship observed in some studies (Takaishi *et al.*, 1998; Ansley & Cangley, 2009). Secondly, using multiple simulations and mathematical modeling (Neptune & Hull, 1999; Kautz & Neptune, 2002; Umberger *et al.*, 2006), it has been shown that negative internal work, which is the work associated with control of limb movement with respect to the body's center of mass (Ettema & Lorås, 2009), increases significantly above 90 rpm (Neptune & Herzog, 1999). The increase in negative work leads to co-activation of antagonist muscles (i.e. hamstrings) to control limb movement (Neptune & Herzog, 1999). The concept of negative work will be further described in the following section, but it is logical that recruitment of additional muscle mass will increase the oxygen demand, causing an increase in $\dot{V}O_2$. Furthermore, an increase in negative work requires a proportional increase in positive work resulting in decreased efficiency of the skeletal muscle system (Neptune & Herzog, 1999).

Heart rate (HR) has also been shown to increase with increasing cadence and resembles a parabolic relationship when cadences below 60 rpm are tested (Seabury *et al.*, 1976; Gottshall *et al.*, 1996; Chavarren & Calbet, 1999). In one of the few studies investigating the impact of cadence on the haemodynamic response to exercise, Gottshall *et al.* (1996) noted that with consecutive increases in cadence from 70 to 110 rpm, systemic vascular resistance decreased, while stroke

volume, cardiac output ($\dot{Q}$) and mean arterial pressure increased. Additionally, the arterial-venous oxygen difference was significantly greater at 110 rpm in comparison to 70 rpm and 90 rpm. In this study arterial venous oxygen difference was calculated as the quotient of $\dot{V}O_2$ and $\dot{Q}$ where $\dot{Q}$ is the product of stroke volume and HR. $\dot{Q}$ was measured using impedance cardiography. While the efficacy of impedance cardiography to measure $\dot{Q}$ is debated, stroke volume using this method represents whole body haemodynamics. Therefore, the arterial venous oxygen difference at the level of the microvasculature, or site of oxygen delivery, is unknown. The authors concluded that the increased arterial venous oxygen difference found in this study was the result of an effective skeletal muscle pump system. However, potential mechanical compression of blood vessels with low cadences as well as other biomechanical factors may also restrict blood flow, compromising oxygen delivery.

1.3 Biomechanical Factors

To understand the influence of biomechanics on FCC, it is critical to first define the factors that must be optimized for successful performance. Average power output is the product of the average net torque and average angular velocity of the pedal crank. In relation to cycling, torque is the perpendicular force applied to the pedal crank while average angular velocity is synonymous with cycling cadence (Abbiss *et al.*, 2009). During constant work rate cycle exercise bouts, a rise in cadence results in a proportional decrease in the force applied to the pedals in order to maintain a constant power output (Patterson & Moreno, 1990). Torque is also dependent on both the linear force produced by muscles and the distance of the moment arm relative to the joint's center of rotation. Indeed, the impact of cadence on pedal forces and joint moments has received considerable attention (Redfield & Hull, 1986; Kautz & Hull, 1993; Marsh *et al.*, 2000; Mornieux

et al., 2007; Skovereng *et al.*, 2016). For example, at a work rate of 350 W, a 15 % reduction in pedal force was observed when cadence was increased from 90 rpm to 105 rpm (Sanderson, 1991; Sanderson *et al.*, 2000). In addition, reductions in joint moments and/or the minimization of peripheral muscle stress have been considered as important factors in the selection of FCC (Redfield & Hull, 1986). However, controversy still exists regarding the role of cadence on specific joint moments of the hip, knee, and ankle.

Another concept alluded to previously is the internal work associated with increasing cadence, especially above 90 rpm (Neptune & Herzog, 1999). Briefly, internal work relates to the work involved in moving the limbs with respect to the body's center of mass and can be separated into both positive and negative components. Positive work is associated with the downward force applied to the pedal whereas negative work is the effort required to slow the limb in attempt to control inertial forces of the pedal crank (Sanderson *et al.*, 2000). Negative work results in the co-activation of antagonist muscles, which are activated at the bottom portion of the crank cycle and work in cooperation with biarticular muscles to maintain a smooth rotation of the crank (van Ingen Schenau *et al.*, 1994; Neptune & Herzog, 1999). However, inertial forces overcome the ability of co-activation to control limb movement at high cadences resulting in an inefficient muscle activation pattern and effective pedal force.

During cycling, peak power output has been shown to occur at cadences of 120-130 rpm. (Zoladz *et al.*, 2000). While this may be true, cyclists are only able to adopt this cadence for limited periods of time due to the energy requirements associated with this rapid pedaling rate (Hill, 1922; He *et al.*, 2000). Indeed, cadences above 120 rpm are commonly observed during track cycling (Craig & Norton, 2001), sprints at the end of stage races, and to rapidly accelerate away from individuals or groups (Lucia *et al.*, 2004). Combining the information regarding the

impact of cadence on the cardiorespiratory system and the role of cadence on joint moments and pedal forces, it would appear that an optimal FCC would be the cadence that minimizes metabolic cost while maximizing power output. However, other important factors such as muscle recruitment patterns or level of muscle activation and haemodynamics may also play a role in FCC.

1.4 Electromyography

Electromyography (EMG) is a recording of electrical action potential propagation from motor unit to muscle fibre. Increased EMG amplitude has been associated with the recruitment of additional muscle fibres for force generation (Olson *et al.*, 1968) and/or compensation for fatiguing motor units (Potvin, 1997). However, no consensus has been made for the impact of cycling cadence on EMG of the lower extremities. Part of the problem stems from the number of ways EMG can be analyzed. The two most prominent approaches include integrated EMG and the root mean square (RMS) EMG. Integrated EMG is calculated as the area confined to the EMG envelope and the x-y axis while RMS EMG is the square root of the mean of the squares of EMG amplitude. For example, Sarre *et al.* (2003) found no differences in RMS EMG of the knee extensors (vastus lateralis, vastus medialis, or rectus femoris) at cadences ranging from 70 % to 130 % of FCC, irrespective of changes in power out. In contrast, integrated EMG of the vastus lateralis and vastus medialis was shown to progressively increase above cadences of 60 to 75 rpm in untrained cyclists (Takaishi *et al.*, 1998). Interestingly, in the same study, Takaishi *et al.* (1998) were only able to observe a significant effect of cadence on the biceps femoris in trained cyclists. The authors proposed that untrained and trained cyclists use different muscle activation patterns based on their pedaling skill (Takaishi *et al.*, 1998).

In accordance with $\dot{V}O_2$ and HR, it has also been suggested that surface EMG can be represented by a parabolic relationship, where a minimum level of muscle activation can be obtained for a given cadence-power output combination (MacIntosh *et al.*, 2000). A parabolic relationship appears plausible given the current hypothesis of greater recruitment in fast-twitch muscle fibres causing a significant rise in $\dot{V}O_2$. However, the observation of a parabolic relationship has not been universal. MacIntosh *et al.* (2000) found a parabolic relationship in all eight participants based on the average sum of all RMS EMG values from seven lower extremity muscles tested, thought to represent overall muscle activity, but eliminates the ability to distinguish the level of activation of each individual muscle. Marsh and Martin (1995) tested a range of cadences (50, 65, 80, 95, 110 rpm, and FCC) on five different leg muscles and found a quadratic relationship to represent rectus femoris RMS EMG while the gastrocnemius and vastus lateralis displayed a positive linear rise in RMS EMG amplitude with increasing cadence. However, it should be noted that the cycling intensity (i.e., 200 W) may have been too low to elicit a response in the other muscles tested. Neptune *et al.* (1997) reported a systematic increase in RMS EMG of three thigh muscles as cadence increased from 45 to 120 rpm while cycling at 250 W. Muscle activation of the gluteus maximus and soleus depicted the parabolic relationship described above, but no difference was found in the rectus femoris or tibialis anterior muscles. Differences in activation level were attributed to differences in the function of each muscle (i.e., power producing versus transition muscles) (Marsh & Martin, 1995). Indeed, differences in activation pattern of monoarticular and biarticular muscles have been observed (van Ingen Schenau *et al.*, 1994).

Congruent with the concept of an energetically optimal cadence, an optimal neuromuscular cadence has been proposed as the cadence that minimizes neuromuscular activation/fatigue (Takaishi *et al.*, 1996; Marsh & Martin, 1997; Sarre *et al.*, 2003), and is typically defined in terms

of EMG. Takaishi *et al.* (1996) found the cadence that reduced integrated EMG of the vastus lateralis to be approximately 90 rpm during 15 min of cycling at 85 % $\dot{V}O_{2max}$. Furthermore, using a model based on experimental data to simulate muscle activation of the lower extremities at 75, 90 and 105 rpm at an intensity of 265 W, Neptune and Hull (1999) calculated the overall activation of the lower extremity (14 muscles included) to be minimized at 90 rpm. Results from these studies closely corroborate previously published values for FCC. However, when the strength capacity of an individual is taken into account by normalizing the force of a pedal stroke to a maximal voluntary contraction, it has been shown that minimal integrated EMG activity does not occur at FCC but rather increases with increasing cadence (Bieuzen *et al.*, 2007), further adding to the highly convoluted cadence-EMG literature.

In theory, an increase in EMG has been correlated with an increase in muscle force, since additional muscle fibres must be recruited in order generate maximal force (Onishi *et al.*, 2000). Accordingly, Takaishi *et al.* (2002) analyzed EMG, pedal force, and near-infrared spectroscopy (NIRS) parameters across a crank cycle and found that during periods of increased pedal force, EMG was elevated, while NIRS-derived measures of muscle oxygenation and blood volume were reduced. FCC has also been reported to be between the energetically optimal cadence and the optimal neuromuscular cadence (Bieuzen *et al.*, 2007). Thus, the FCC likely represents a compromise between central and peripheral stresses (Lucia *et al.*, 2004). It follows that other factors, such as haemodynamics, may also influence FCC.

1.5 Haemodynamic Response to Exercise

To appreciate the impact of cadence on skeletal muscle blood flow, it is essential to have an understanding of physical laws governing blood flow, as well as the proposed mechanisms

underlying the control and regulation of flow at rest, the transition from rest to exercise, and during prolonged exercise at various intensities. Poiseuille's law for fluid dynamics, when arranged in terms of flow, precisely explains the factors governing blood flow, irrespective of the region in question. The following equation represents Poiseuille's law:

$$\dot{Q} = \frac{\pi P r^4}{8nl}$$

where *P* is the pressure difference across a vascular bed, *r* is the radius of the blood vessel and is raised to the fourth power, *n* is the viscosity of blood, and *l* is the length of the vessel region. In particular, attention should be directed toward the influence of radius on flow. Since radius is raised to the fourth power, even slight changes in blood vessel diameter will have dramatic effects on blood flow. Furthermore, given that liquids flow from regions of high to low pressure, another important component of this equation is the pressure difference, or gradient, across the vasculature. The human circulatory system is designed in order to maintain this gradient, where pressure is highest close to the left ventricle of the heart and decreases progressively as it flows towards the heart's right atrium. Therefore, it has been established that blood flow to any region is dependent on the resistance to flow through the vessels (i.e., blood vessel diameter) and the pressure gradient across the vascular bed (Joyner & Casey, 2015).

The early observations of John Hunter, an 18th century Scottish surgeon, has formed the basis of our understanding of the mechanisms of blood flow regulation (Hunter *et al.*, 1794). John Hunter is credited with the concept that "blood goes where it is needed" (Rowell, 2004). Indeed, it has repeatedly been shown that blood flow is prioritized to regions of need (Saltin *et al.*, 1998; Joyner & Casey, 2015), which is perhaps best exemplified by the fact that during rest and exercise, the brain receives a relatively consistent amount of blood flow (**Figure 1**), given that it is vital for

survival and the control of other physiological systems. At rest, blood is primarily directed to all vital organs (i.e. brain, heart, liver, digestive system) responsible for maintaining resting metabolism. $\dot{Q}$ remains relatively low at approximately 5 $l \cdot min^{-1}$ in a healthy young male adult of average height and mass (Bevegard & Shepherd, 1967). Furthermore, only 15-20 % of $\dot{Q}$ is directed to the relatively inactive skeletal muscles (Joyner & Casey, 2015). However, exercise poses a significant challenge to systemic homeostasis as the skeletal muscle's demand for oxygen increases requiring an integrated response of multiple physiological systems in order to adequately maintain mean arterial pressure and organ/tissue perfusion.

The transition from rest to exercise leads to direct increases in HR, $\dot{V}_E$, blood flow, and thus $\dot{V}O_2$. In particular, in healthy untrained individuals, $\dot{Q}$ increases from 5 $l \cdot min^{-1}$ at rest to 25 $l \cdot min^{-1}$ at peak exercise (Joyner & Casey, 2015). In endurance trained individuals, $\dot{Q}$ has been shown to increase up to approximately 35-40 $l \cdot min^{-1}$ (Bevegard & Shepherd, 1967). The substantial elevation in $\dot{Q}$ is accomplished by the redistribution of blood flow from regions non-essential during exercise, such as the digestive system and spleen, and redirected toward the active skeletal muscles (**Figure 1**).

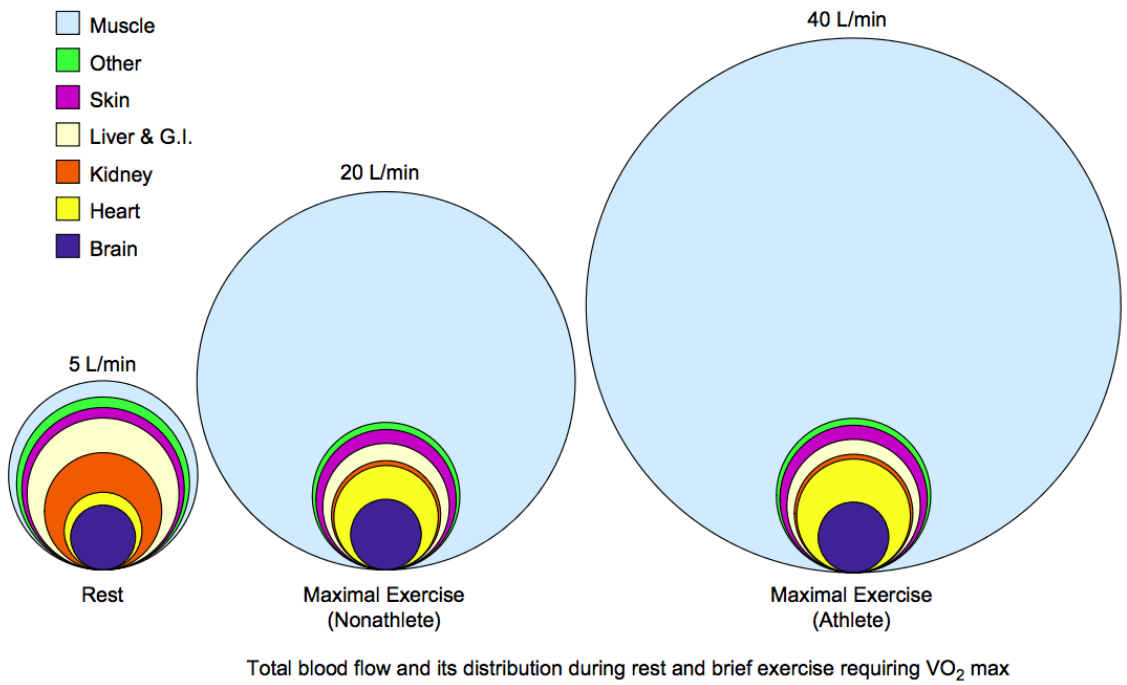

Figure 1: Blood flow distribution from rest to exercise. *Brain blood flow (purple), as a percentage of $\dot{Q}$ remains relatively consistent in trained and untrained individuals while there is a substantial rise in skeletal muscle blood flow (blue) (Joyner & Casey, 2015).*

The consistent redistribution in blood flow is the result of a number of mechanisms including sympathetic vasoconstriction and local vasodilation of muscle tissue (Saltin *et al.*, 1998; Joyner & Casey, 2015). Briefly, the sympathetic nervous system regulates blood flow by either constricting or dilating arteriolar blood vessels. Vasoconstriction results in decreased blood flow through the tissue as resistance to flow is increased. In contrast, when the diameter of a vessel is increased via vasodilation, a greater amount of blood is permissible through the given region. At the onset of exercise, the resting sympathetic vasoconstriction, or tone, of the skeletal muscles is blunted or abolished as the metabolic demands of these active tissues increases (Rosenmeier *et al.*, 2004). The localized blunting of vasoconstriction within the active skeletal muscles allows blood flow to

increase to the region of need and has been termed functional sympatholysis. Functional sympatholysis represents the balance between the metabolic demand and nervous system regulation to ensure adequate delivery of oxygen to active tissues (Sheriff *et al.*, 1993). Local vasodilation also causes the calibre of a blood vessel to increase but the mechanism in which vessel diameter increases differs. Specifically, chemical stimuli within the blood (i.e., metabolites, H^+, ATP, etc.) trigger vasodilation of the respective tissue. Thus, the sympathetic nervous system can result in a more systemic regulation to blood flow whereas local vasodilatory compounds increase blood flow to a specific region. Despite this consistent response, a debate exists regarding the mechanisms responsible for controlling blood flow, and many questions remain unanswered (Laughlin, 1987; Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996; Hamann *et al.*, 2004). The difficulty in gaining an understanding for blood flow regulation has been attributed to the body's integrative response to exercise, which creates redundant safe guards to ensure that arterial pressure remains sufficient for the delivery of oxygen to all active tissues (Saltin *et al.*, 1998).

Active hyperaemia is the augmentation in blood flow associated with a rise in the metabolic activity of an organ or tissue. More specifically, functional or exercise hyperaemia is the rise in blood flow associated with a muscle contraction. For the purpose of this thesis, hyperaemia will refer to exercise hyperaemia. Many factors have been implicated in the hyperaemic response to a muscle contraction; however, two prominent mechanisms exist within the literature, the skeletal muscle pump effect and vasodilation (Tschakovsky *et al.*, 1996). The skeletal muscle pump effect is defined as the elevation in intramuscular pressure associated with a muscle contraction, which forces blood from the venous circulation (Laughlin, 1987). The pump effect results in a direct increase in cardiac preload, which improves the heart's ability to generate a given $\dot{Q}$. Specifically, cardiac preload is increased in two ways. First, with the expulsion of blood from the muscle,

venous pressure decreases to approximately 0 mmHg (Saltin *et al.*, 1998). Indeed, it has even been proposed that venous pressure becomes negative, as veins are pulled open during relaxation of the muscle (Laughlin, 1987). The direct increase in the arterio-venous pressure gradient causes an increase in blood flow through the vascular bed (Joyner & Casey, 2015). Secondly, preload is enhanced by the fact that 70-80 % of blood volume at rest is stored in the venous circulation, resulting in a substantial increase in blood volume toward the heart and subsequent rise in $\dot{Q}$ (Rothe, 1986). On the other hand, vasodilation is an increase in blood vessel diameter and, as mentioned before, can have a dramatic impact on blood flow according to Poiseuille's law. Multiple theories regarding vasodilation have been proposed. Of importance, each theory involves the endothelium in regulating blood vessel calibre (Delp & Laughlin, 1998). Metabolic vasodilation pertains to the increase in vessel diameter associated with the metabolic demands of the tissue. As the substrate requirements of muscle increase, blood flow will increase proportionally. In particular, during prolonged exercise, the depletion of oxygen and other substrates, as well as the accumulation of metabolic by-products stimulates the endothelium causing an increase in blood vessel diameter (Delp & Laughlin, 1998). Additionally, a myogenic vasodilation may occur due to a plethora of factors that include the physical stress on vascular walls via muscle contractions, increased flow through the vessel, or the influence of chemical stimuli within the blood that leads to an endothelium-mediated increase in blood vessel calibre (Delp & Laughlin, 1998).

Hyperaemia occurs immediately with the very first muscle contraction during exercise (Saltin *et al.*, 1998). However, considerable debate exists regarding the relative contributions from the skeletal muscle pump and vasodilation to the exercise-induced increase in blood flow. The problem in ascertaining the contribution of the pump effect and vasodilation are primarily due to

the time course in which blood flow increases. For example, while blood flow has been shown to increase after a single voluntary or passive muscular contraction, an approximate 4 to 6 s delay occurs before vasodilation can augment blood flow (Tschakovsky *et al.*, 1996). The finding from the above study suggests that the skeletal muscle pump is obligatory at the onset of exercise. Furthermore, the rhythmical pattern of intramuscular pressure generated with each muscle contraction enhances venous return, which results in the hallmark increase in stroke volume and subsequent rise in $\dot{Q}$ (Rothe, 1986). It has been proposed that the muscle pump contributes up to thirty percent of the kinetic energy required to perfuse skeletal muscle during lower limb exercise (Stegall, 1966). Indeed, when a train of tetanic contractions is delivered to deep muscles, a more efficient muscle pumping action appears to result in higher blood flows, while the same contractions do not increase blood flow to the same extent in superficial muscles where the pumping effect has less of an impact (Laughlin, 1987). If the muscle pump effect persists throughout exercise, it follows that the repetitive contraction-relaxation cycles during pedaling has the potential to impede blood flow through the active lower extremity muscles. Lutjemeier *et al.* (2005) found a reduction in mean muscle blood flow during moderate and heavy intensity knee extension exercise, suggesting that forceful contractions during rhythmic contraction-relaxation cycles can impact flow. In addition, blood flow appears to increase until approximately 60 % of maximum voluntary contraction during dynamic exercise (Wigmore *et al.*, 2004) or 83 % of $\dot{V}O_2max$ (Henderson *et al.*, 2012), where a plateau or decline in flow occurs. Using NIRS to examine changes in microvascular blood volume and oxygenation over the course of a pedal stroke, Takaishi *et al.* (2002) found that blood volume was obstructed during the contraction phase resulting in the rapid passage of a red blood cell cluster under the NIRS probe in the relaxation phase, which implies that two mechanisms may be leading to a reduction in oxygen delivery. First,

as stated above, blood flow is limited to the contraction phase resulting in a slower net flow through the muscle (Wigmore *et al.*, 2004; Osada *et al.*, 2015). Second, if red blood cells are only permitted to move through the vasculature during the relaxation phase then red blood cell transit time is increased substantially, which could affect convective oxygen supply (Walløe & Wesche, 1988; Hoelting *et al.*, 2001); however, this second mechanism is controversial. In a follow up study, Lutjemeier *et al.* (2007) used NIRS to examine the concentration of deoxygenated haemoglobin and myoglobin (deoxy[Hb+Mb]) over the course of an entire contraction and found deoxy[Hb+Mb] continues to increase during the contraction phase of knee extension exercise. Since the deoxy[Hb+Mb] signal is used as a proxy for oxygen extraction (Ferreira *et al.*, 2005), Lutjemeier *et al.* (2007) concluded that red blood cells must still be present within the microvasculature during the contraction phase and that the oxygen extraction process still occurs. A similar observation was made when using intravital microscopy in rats; red blood cell flow was still present during the contraction phases, but at a reduced rate of flow and altered distribution (Kindig *et al.*, 2002). A marked reduction in the red blood cell flux within the vessels may lead to insufficient oxygen transfer and thus premature exercise cessation.

While debate exists, many authors believe blood vessel vasodilation is the predominant mechanism responsible for hyperaemia (Lind & Williams, 1979; Tschakovsky *et al.*, 1996; Hamann *et al.*, 2004; Casey & Joyner, 2012). Using a blood pressure cuff to simulate a muscle contraction, Tschakovsky *et al.* (1996) found that blood flow increased and declined after a single compression. However, when a voluntary muscle contraction was completed, blood flow continued to increase over three consecutive cardiac cycles, suggesting that rapid vasodilation was the main contributor to the hyperaemic response. Hamann *et al.* (2003) compared exercise-induced hyperaemia to pharmacologically enhanced blood flow in the canine hindlimb. Using

ultrasound flow probes surgically inserted into the hindlimb, the dogs were first injected with adenosine, a vasodilating agent, which resulted in similar blood flow values as observed during exercise. With blood flow still elevated, the dogs were exposed to treadmill exercise in an attempt to increase blood flow with the addition of the skeletal pump effect; however, no further increase in blood flow was noted. In a separate study using the same animal model, tetanic contractions with and without infusion of potassium were completed (Hamann *et al.*, 2004). An influx of potassium into the muscle effectively clamps smooth muscle hyperpolarization and therefore removes the ability of the vessels to vasodilate. When vasodilation was absent, blood flow was reduced during the contractions, suggesting that vasodilation has the capability to at least partially compensate for mechanical compression of the vessels. Therefore, vasodilation has profound effects on microvascular blood flow, which is the site of oxygen delivery.

Skeletal muscle blood flow increases in a biphasic manner (Saltin *et al.*, 1998). While the skeletal muscle pump and vasodilation encompass much of the first phase, the second phase focuses on the close matching of blood flow to the metabolic demands of prolonged or steady state exercise. Indeed, blood flow has been linked to both the metabolic demands and contractile work of the muscle (Hamann *et al.*, 2005). While these two parameters are related, they are not interchangeable. Metabolic work corresponds to $\dot{V}O_2$, utilization of adenosine triphosphate, and clearance of metabolic by-products, whereas contractile work is associated with the duration and intensity of contractions (Hamann *et al.*, 2005). Hamann *et al.* (2005) manipulated the metabolic work of dog gastrocnemius-plantaris muscle using short and long duration contractions while overall muscle time-under tension remained equivalent. They found that consecutive short contractions led to a higher level of blood flow compared to long duration contractions. Furthermore, these short duration contractions resulted in a higher muscle $\dot{V}O_2$. Thus, blood flow

appears to be more closely related to the metabolic work of the muscle (Hamann *et al.*, 2005), a finding that is supported by work in humans involving varying intensities and types of exercise (i.e., large versus small muscle mass exercise) (Saltin *et al.*, 1998; Joyner & Casey, 2015). However, an important limitation to this study is the total number of contractions. If the muscle pump has any influence on blood flow then the cumulative effect of the muscle pump during contractions of short duration will have a greater proportional influence on blood flow than during contractions of long duration.

Finally, much of our knowledge of hyperaemia has been gained through animal research and studies incorporating research methods that differ from two-legged dynamic cycling. For instance, Hamann *et al.* (2005) explored the relationship between blood flow and the contributions of contractile work and metabolic demands in canines and Kindig *et al.* (2002) examined the heterogeneity and redistribution of red blood cells within the microvasculature of rats. Thus, caution needs to be used when generalizing these results to humans. Specifically, Andersen and Saltin (1985) found maximum blood flow to be 3-5 times greater in intact human beings compared to isolated *in vitro* muscle preparations. However, due to technological limitations, many studies in humans are restricted to knee extension exercise, which requires a different recruitment pattern in comparison to cycling (Ferreira *et al.*, 2006). While the vastus lateralis is one of the predominant muscles contributing to power output during cycling, knee extension exercise incorporates a greater level of activation from the entire quadriceps muscle group (Andersen *et al.*, 1985). Although, research using the knee extension model has provided valuable insight into the regulation of blood flow within the quadriceps, understanding the response of blood flow to dynamic whole-body exercise requires more research and technologically advanced methods.

1.6 Cadence and Skeletal Muscle Blood Flow

Successful cycling performance is dependent on maximizing oxygen delivery to regions of greatest metabolic demand (Saltin *et al.*, 1998) and blood flow is the vehicle in which metabolic demand can be satisfied. However, skeletal muscle blood flow naturally oscillates at rest and is even more pronounced during exercise (Rådegran, 1999), via the skeletal muscle pump. Recently, evidence of oscillations in arterial blood flow with the presence of anterograde and retrograde flow (Hoelting *et al.*, 2001), as well as heterogeneous flow within and between muscles (Koga *et al.*, 2014) have been shown during knee extension exercise in humans. Similar to knee extension exercise, blood flow is primarily directed to the quadriceps muscle group with preferential distribution to deep, slow-twitch muscle fibres (Okushima *et al.*, 2015) during cycling. In particular, the vastus lateralis is the main contributor in generating power output (Barstow *et al.*, 1996); however, the vastus lateralis is a superficial muscle composed predominantly of fast-twitch muscle fibres (Barstow *et al.*, 1996). Therefore, the vastus lateralis inherently receives less resting blood flow, which may influence the rate of flow during forceful muscles contractions at low cadences. Failure to deliver adequate blood flow results in a greater contribution of anaerobic metabolism leading to the generation of lactate and other metabolic by-products, which manifests in fatigue and exercise cessation.

Cadence, or contraction frequency, could impede blood flow by two separate mechanisms. First, intramuscular pressure generated by forceful muscle contractions has the ability to occlude arterial inflow and compress the vascular beds (Takaishi *et al.*, 2002), which would typically be seen in individuals who adopt a slow pedaling rate that requires greater force on the pedals per pedal stroke. Conversely, a very rapid contraction frequency may potentially impact blood flow by shortening the relaxation phase between contractions (Walløe & Wesche, 1988; Hoelting *et al.*,

2001). If this is the case and there is still enough intramuscular pressure to even partially occlude blood flow, then oxygen delivery may be affected. Work in humans (Walløe & Wesche, 1988; Hoelting *et al.*, 2001) has shown that when the relaxation phase is shortened, blood flow is significantly attenuated. A study by Hoelting *et al.* (2001) was conducted using single-leg knee extension exercise in the supine position. Contraction frequency ranged from 40 to 80 contractions per minute while the duty cycle was altered such that the contraction phase remained the same but the relaxation phase was prolonged with slower contraction frequencies. Blood flow was found to be significantly blunted with an increase in the contraction frequency suggesting that the contraction-relaxation duty cycle influences muscle blood flow. However, incorporation of a supine posture may have impacted their results since the characteristic linear increase in the blood flow-work rate relationship was not present (Andersen & Saltin, 1985). In contrast, studies using a constant duty cycle show no difference in blood flow magnitude between contraction frequencies (Ferguson *et al.*, 2001; Sjøgaard *et al.*, 2002). Since cycling cadence has a relatively constant duty cycle irrespective of the change in pedaling rate (Ferreira *et al.*, 2006), cadence may not impact muscle blood flow.

Investigations using NIRS to assess the relationship between cadence and muscle oxygenation has provided some insight into the role of cadence on muscle blood flow. As with deoxy[Hb+Mb], NIRS also provides a measure of the concentration of oxygenated haemoglobin and myoglobin (oxy[Hb+Mb]), as well as the combination the deoxygenated and oxygenated signals (total[Hb+Mb]), which has been used as an index of blood volume (Poole *et al.*, 2013). Total[Hb+Mb] accounts for both redox states of haemoglobin and myoglobin. Thus, any increase in either of these signal outputs will be the result of a hyperaemic response under the NIRS probe (Poole *et al.*, 2013). An increase in blood volume would have to be the direct result of an increase

in blood flow within the muscle. Zorgati *et al.* (2015) found total[Hb+Mb] to be significantly greater at 40 rpm compared to 100 rpm in untrained cyclists. In addition, Kounalakis and Geladas (2012) also noted a significant increase in the total[Hb+Mb] signal while comparing 40 and 80 rpm. Collectively, results of both studies suggest that blood flow may be higher at lower cadences. However, while flow may be elevated, oxygen extraction (as estimated via deoxy[Hb+Mb]) shows contradictory results. Both Zorgati *et al.* (2015) and Kounalakis and Geladas (2012) found that deoxy[Hb+Mb] was higher at 40 compared to 100 and 80 rpm, respectively. In contrast, other studies have found no difference in oxygen extraction (Ferreira *et al.*, 2006; Kounalakis & Geladas, 2012; Zorgati *et al.*, 2013) or a decrease in oxygen extraction at lower cadences (Boone *et al.*, 2015). Further research is needed to determine the impact of cadence on NIRS parameters.

1.7 Near Infrared Spectroscopy and Indocyanine Green

Near-infrared light is characterized by its short-wavelength, making it ideal for penetration of biological tissue (Quaresima *et al.*, 2004; Ferrari *et al.*, 2011). As the light travels into the tissue, near-infrared photons reflect according to the angle of the light at the transmitter-tissue interface (Jöbsis, 1977). Physical properties inherent to these photons cause them to scatter within the tissue allowing the transmitter and receiver to be placed next to each other on the surface of the same muscle (Jöbsis, 1977). Specifically, scattering causes the photons to navigate the tissue in a parabolic or "banana-shaped" curve (Boushel *et al.*, 2000). A third characteristic of near-infrared light is its ability to become absorbed by chromophores, or light absorbing molecules, in the body (Jöbsis, 1977). During NIRS assessments of skeletal muscle, haemoglobin accounts for the majority of the signal output and absorbs light at distinct wavelengths depending on its reduced or oxidized form. However, due to similarities in molecular structure, absorbance by haemoglobin

and myoglobin cannot be distinguished (Quaresima *et al.*, 2004). It has been estimated that myoglobin accounts for only 10 % of the overall NIRS signal (Kalliokoski *et al.*, 2006). Furthermore, it should be noted that cytochrome c oxidase, a component of the electron transport chain, has the ability to absorb near-infrared light at a similar wavelength but due to the relative quantity in the body compared to haemoglobin and myoglobin, has a negligible effect on signal output (Jöbsis, 1977).

Arteries and veins absorb all of the near-infrared light passing through them due to the high concentration of haemoglobin within these vessels (Boushel *et al.*, 2000). As light passes through microvascular blood vessels, only a portion of the light is absorbed allowing the remainder to be collected by a receiver. Therefore, based on the state of the tissue, differences in concentration of the combined signal of haemoglobin and myoglobin can be detected (Jöbsis, 1977; Boushel *et al.*, 2000). With respect to the modified Beer-Lambert Law, photons of near-infrared light travel through the region with the least amount of absorbance and thus NIRS provides a measure at the microvascular level (Boushel *et al.*, 2000). Since the capillary network is the site of oxygen delivery, the ability to continuously measure oxygenation at this point on the oxygen cascade has the potential to advance our understanding of oxygen delivery and utilization within target muscles.

When NIRS is used in combination with a light absorbing tracer molecule (i.e., indocyanine green (ICG)), NIRS-ICG provides an absolute or relative measure of blood flow depending on methodological design (Boushel *et al.*, 2000; Guenette *et al.*, 2008; Habazettl *et al.*, 2010; Guenette *et al.*, 2011). ICG tightly binds with albumin, a plasma protein in the blood and is broken down in the hepatic circulation (Muckle, 1976). The substance's half-life within the body is approximately 5 min, making it an ideal substance for repeated measurements (Ott *et al.*, 1994).

ICG dye is injected as a bolus into the venous circulation where it becomes mixed within the heart and pulmonary circulation (Boushel *et al.*, 2000). To obtain absolute blood flow, an arterial catheter is inserted and a photodensitometer is used to measure the arterial concentration of ICG (dye-dilution technique). A less invasive technique known as the blood flow index (BFI) method has been established, thereby eliminating the need for arterial cannulation. The BFI method measures the concentration of ICG within the target muscle over a given period of time with NIRS optodes (Kuebler *et al.*, 1998). As the concentration of ICG dye increases, it is detected by NIRS optodes and a time-ICG concentration curve is formed (**Figure 2**). The portion of the time-ICG concentration curve between 10-90 % of the rapid rise in ICG concentration is divided by the associated time it took to reach this concentration yielding a relative measure of blood flow (**Figure 2**) (Kuebler *et al.*, 1998; Habazettl *et al.*, 2010; Guenette *et al.*, 2011).

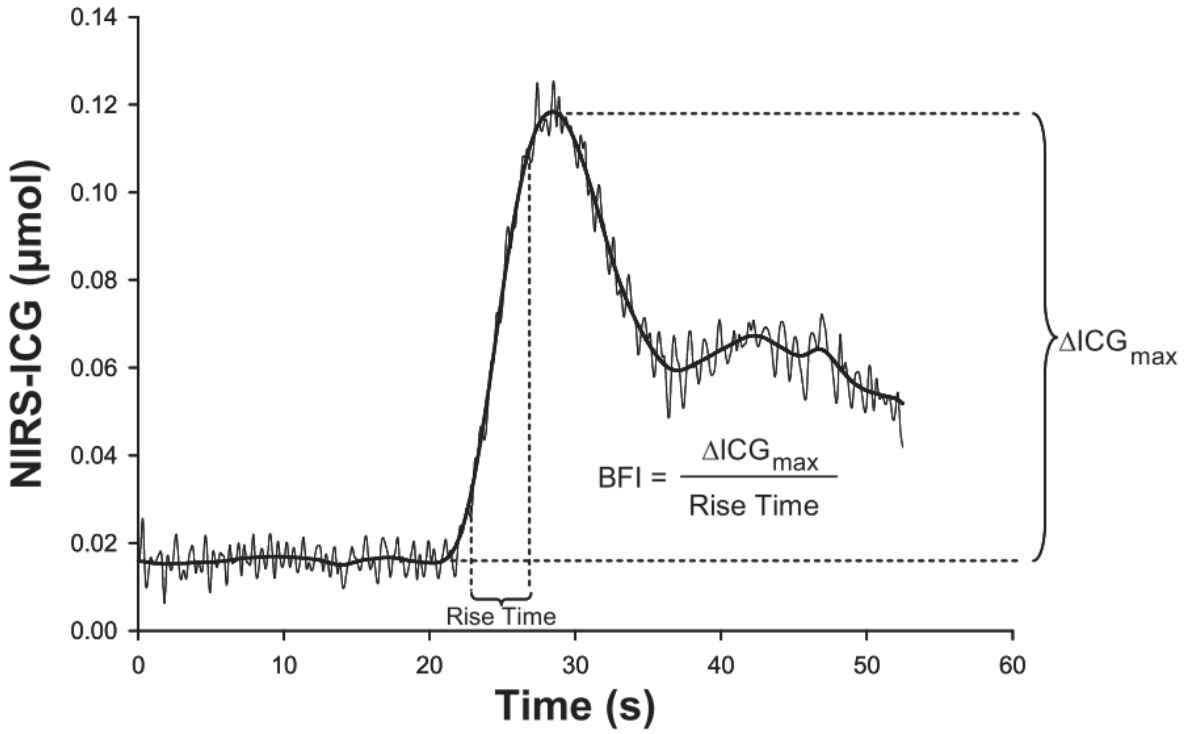

Figure 2: NIRS-ICG curve and BFI calculation. *Blood flow index is determined by dividing the change in maximal ICG concentration (ΔICGmax) and the rise time. Rise time is calculated as the time period from 10 to 90 % of ΔICGmax (Guenette et al., 2011).*

NIRS-ICG has been used to assess cerebral blood flow as well as regional muscle perfusion during exercise. Boushel *et al.* (2000) were the first to investigate absolute measures of regional muscle blood flow with NIRS-ICG; they compared blood flow values to the calf muscle and surrounding Achilles tendon region and concluded that NIRS-derived absolute blood flow correlated well with those seen using the Xe-washout technique. Kuebler *et al.* (1998) were the first to apply the BFI method to study cerebral blood flow in pigs when they compared BFI to the gold standard radioactive microsphere technique and determined BFI to be representative of cerebrovascular blood flow. While their results trended positively, the correlation between these two methods was weak and elicited a need for further research. Habazettl *et al.* (2010) validated

the BFI with NIRS-ICG derived absolute muscle blood flow. The two techniques had a strong correlation in both the 7th intercostal space and vastus lateralis with *r*-values of 0.98 and 0.96, respectively. However, these correlations should be interpreted cautiously as data from the NIRS-ICG concentration curves are included in both the BFI and absolute blood flow calculations. This results in mathematical coupling and can influence correlation analyses (Walsh & Lee, 1998). Both the dye-dilution (Guenette *et al.*, 2008) and BFI (Guenette *et al.*, 2011; Henderson *et al.*, 2012) have also been used to assess respiratory muscle blood flow in humans. Guenette and colleagues found that as $\dot{V}_E$ rose during cycling exercise, muscle blood flow values were strongly related to $\dot{Q}$, the work of breathing (W_b), and EMG of the sternocleidomastoid respiratory muscle. (Guenette *et al.*, 2008; Guenette *et al.*, 2011). In relation to the proposed study, Henderson *et al.* (2012) found BFI values of the vastus lateralis to decline between 83-100 % of maximal exercise during an incremental cycle test. They speculated that a progressive increase in intramuscular pressure with increasing workload might have restricted vascular perfusion leading to a decline in quadriceps microvascular muscle blood flow.

1.8 Aims and Hypotheses

The purpose of this thesis is to comprehensively investigate the factors influencing an individual's FCC and to gain an understanding of the impact of variables that have not yet been taken into consideration. Thus, the primary objective is to compare relative microvascular blood flow within active lower extremity muscles using NIRS-ICG at different fixed cadences. Secondary objectives include examining the effect of cadence on cardiorespiratory and metabolic parameters, P_b, EMG of the diaphragm and lower extremity muscles, and sensory responses of dyspnoea and leg discomfort.

Our central hypothesis is that microvascular blood flow to four leg muscles (i.e., vastus lateralis, vastus medialis, semitendinosus, and medial gastrocnemius) will be reduced during low cadences due to high intramuscular pressures associated with more forceful muscle contractions. Secondary hypotheses include: i) $\dot{V}O_2$, $\dot{V}_E$, and other cardiorespiratory and metabolic parameters will be elevated at high cadences; ii) EMGdi and P_b will be significantly greater at high cadences; iii) EMG of all previously mentioned leg muscles will be significantly higher during both the low and high cadence due to muscle force and contraction frequency, respectively and EMG will be minimized during FCC; iv) Dyspnoea rating will be significantly higher at high cadences while rating of leg discomfort will be greater during low cadences.

Chapter 2: Thesis Study

2.1 Methods

This study received ethical approval from the University of British Columbia – Providence Health Care Research Ethics Board (UBC REB number: H16-00674). All data was collected at the Cardiopulmonary Exercise Physiology Laboratory located at St. Paul's Hospital in Vancouver, British Columbia, Canada.

2.1.1 Subjects

A total of fourteen highly trained cyclists were enrolled in the study. Participants were recruited from various social media outlets dedicated to local cycling groups. All participants were young healthy adults that regularly train and/or compete at the regional, national, or international level as road cyclists or triathletes. Additional information regarding inclusion and exclusion criteria can be found in Table 1.

Table 1. Participant inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• 19-40 years of age (inclusive) • $FEV_1/FVC > 0.70$ • FEV_1 > 80% predicted • Body mass index between 18 and 30 $kg \cdot m^{-2}$ • Able to read and understand English • Able to ride an upright stationary bicycle • Regularly trains and or competes at a regional, national, or international level as a road cyclist or triathlete • $\dot{V}O_{2max}$ above $55ml \cdot kg^{-1} \cdot min^{-1}$	• History of or currently smoking • History of or current symptoms of cardiopulmonary disease including asthma and exercise-induced asthma • Contraindications to exercise testing including cardiovascular and/or respiratory comorbidities; a serious infection within the body; a neuromuscular or musculoskeletal disorder; or other health problem that will be made worse with exercise testing • Ulcer or tumor in the oesophagus, a nasal septum deviation, or recent nasopharyngeal surgery • Allergies to latex, local anesthetic, or iodides (i.e., shellfish)

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $\dot{V}O_{2max}$, maximal oxygen consumption.

2.1.2 Experimental Overview

Participants reported to the Cardiopulmonary Exercise Physiology laboratory for two visits. Study visits were separated by a minimum of 48-hours to ensure full recovery. During visit 1, participants provided written informed consent prior to collection of medical history, medication use, anthropometric measurements, Physical Activity Readiness questionnaire (Warburton *et al.*, 2011) and International Physical Activity questionnaire – short form (Craig *et al.*, 2003). Participants then performed routine pulmonary function tests. Participants were thoroughly familiarized with all testing procedures and symptom scales prior to the completion of a maximal incremental cycle exercise test. Visit 2 included a series of constant-work rate cycle exercise tests at a work rate corresponding to 10 % below the gas exchange threshold (GET), the point when carbon dioxide production increases in comparison to $\dot{V}O_2$, achieved during the incremental

exercise test (Caiozzo *et al.*, 1982). Both cycle exercise tests were conducted with participants breathing through a standard mouthpiece in order to monitor ventilatory and metabolic responses to exercise. During the constant work rate cycle tests, participants were also instrumented with a multi-pair oesophageal electrode balloon catheter for the assessment of oesophageal and gastric pressures, as well as electrical activity of the diaphragm. In addition, a peripheral venous catheter was inserted into the cephalic vein of the right forearm for the injection of ICG while NIRS optodes were placed on four predominant cycling muscles of the left leg to measure ICG concentration. Lastly, bipolar surface electrodes were placed contralateral to the NIRS optodes in order to measure electrical activity of the same leg muscles.

2.1.3 Exercise Protocols

Both visits were completed using the same electromagnetically braked cycle ergometer (Velotron; RacerMate Inc, Seattle, WA, USA). Each study visit began with participants seated on the ergometer for a six-minute steady state resting period for the measurement of baseline ventilatory and metabolic data. In addition, participants were asked to rate their sensation of breathing discomfort and leg discomfort using the modified 0-10 category-ratio Borg scale (Borg, 1982) and complete three resting inspiratory capacity (IC) manoeuvres (Guenette *et al.*, 2013).

During visit 1, exercise testing began with a 1-minute warm-up of unloaded pedaling followed immediately by a rapid onset in workload to 100 W, marking the beginning of the incremental exercise test, which progressed in 25 W increments every two minutes in a stepwise fashion until volitional exhaustion. During each two-minute stage, blood pressure, breathing and leg discomfort, and an IC manoeuvre were collected, in that order. Furthermore, an IC manoeuvre was conducted just prior to the completion of exercise in order to determine operating lung

volumes at peak exercise. Participants were allowed to adjust the ergometer according to their own specific measurements (i.e., seat and handlebar height, seat and handlebar position) and pedal at a self-selected cadence (> 50 rpm). In addition, cyclists used their own personal pedals and cycling shoes. The maximal work rate was determined as the highest workload sustained for at least 30 seconds. Accordingly, the GET was determined using the dual criteria approach (Caiozzo *et al.*, 1982). Briefly, the investigator and another experienced lab member independently examined graphical representations of the metabolic data from visit 1. A rise in the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) without a concomitant increase in the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), as well as the point of inflection in the relationship between carbon dioxide production ($\dot{V}CO_2$) and $\dot{V}O_2$ was defined as the GET. The GET was determined by visual inspection and empirically confirmed by calculating the second derivative of the $\dot{V}CO_2$-$\dot{V}O_2$ plot to detect the point of slope inflection.

Visit 2 started with a ten-minute warm-up at 40 % of the maximum work rate achieved from the incremental test performed during visit 1. Following this standardized warm-up, participants rested for a minimum of fifteen minutes to return HR and blood pressure back to resting values. Participants then completed four, six-minute bouts of cycle exercise at a constant work rate corresponding to 10 % below their GET while pedaling at one of four cadences: i) 60 rpm, ii) 90 rpm, iii) 120 rpm, and iv) FCC. The cadence conditions were performed in randomized counterbalanced order. Each cadence trial began with a one-minute ramp from 0 W to 10 % below GET. Participants then pedaled at each corresponding cadence for six minutes in a standard aerodynamic position using aerodynamic handlebars (Bontrager Race Aero Clip-on bars; Trek Bicycle Corporation, Waterloo, WI, USA) that were positioned consistently across cadence conditions. In addition, a commercially available cycling saddle (Power Comp; Specialized

Bicycle Components Inc., Morgan Hill, CA, USA), designed with a shortened nose, was used to reduce the potential confounding effect of cycling position on blood flow. Cadence feedback was provided via a computer monitor placed directly in front of the participant, and they were continuously encouraged by the investigators to hold each respective cadence. During the FCC trial, cadence feedback was removed and participants self-selected the cadence that was most comfortable to complete the six-minute trial. Participants were allowed to adjust the ergometer for comfort in the aerodynamic position on the first trial and the position was then held constant for subsequent cadence trials. Trials were separated by a minimum of fifteen minutes, which started with ~5 minutes of active recovery of unloaded pedaling followed by ~10 minutes of passive recovery while seated comfortably in a chair to avoid fatigue between cadence trials (shown in figure 3). Heart rate, oxygen saturation, ventilatory and metabolic parameters, as well as EMG recordings were continuously recorded throughout each trial. Furthermore, a 5mg dose of ICG was injected into the cephalic vein at the fifth minute of each trial and monitored with NIRS to obtain a relative measure of blood flow, via the BFI method.

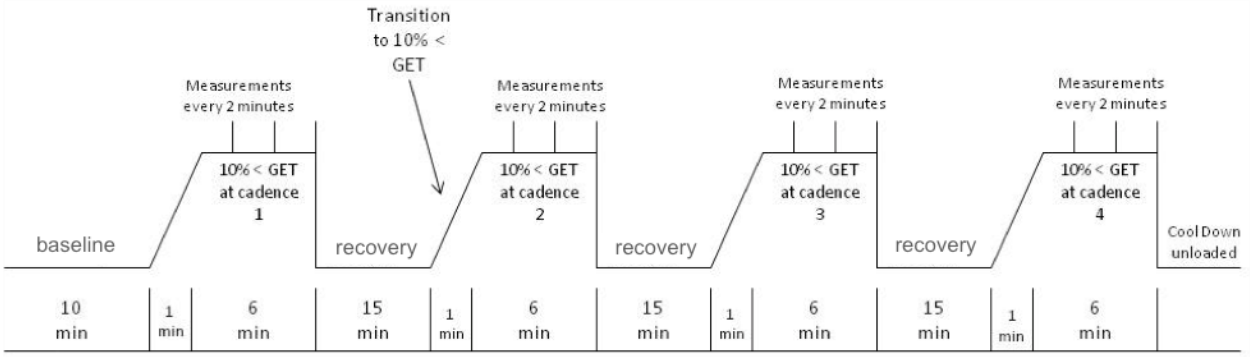

Figure 3: Schematic of the visit 2 exercise protocol. *Percentages are all based on the incremental test on the first visit. WR = work rate; GET = gas exchange threshold*

2.2 Measurements

2.2.1 Questionnaires

After providing written informed consent, a custom questionnaire regarding their medical history was completed to ensure they met study inclusion and participation in the study would not impact past medical issues. Next, participants reported their readiness to participate in physical activity using the Physical Activity Readiness questionnaire (Warburton *et al.*, 2011), and their level of physical activity using the International Physical Activity questionnaire – short form (Craig *et al.*, 2003).

2.2.2 Anthropometric Measurements

Height and mass were determined using a commercially available measuring station (Seca 769; Seca, Chino, CA, USA), which was then used to calculate body mass index.

2.2.3 Pulmonary Function

Spirometry was assessed with a commercially available cardiopulmonary testing system (Vmax 229d with Autobox 6,200 DL; SensorMedics, Yorba Linda, CA, USA) and collected according to standard recommendations (American Thoracic Society/European Respiratory, 2002). All measurements were expressed in absolute terms and as a percentage of population-specific reference values (Tan *et al.*, 2011).

2.2.4 Ventilatory and Metabolic Parameters

Both inspired and expired respiratory flows were measured continuously throughout the steady state resting period and exercise via a customized metabolic cart (TrueOne 2400; Parvo

Medics, Sandy, UT, USA) designed to provide a raw expired flow signal, while inspired flow was collected from a separate pneumotachometer (Series 3813; Hans Rudolph, Shawnee, KS, USA) and amplified (PA-1 Series 1110; Hans Rudolph, Shawnee, KS, USA). Flow was calibrated with a 3 l syringe through 150 cm long large bore tubing and connected to a two-way non-rebreathing valve (Series 2700; Hans Rudolph, Shawnee, KS, USA). Expired gases were collected in a 4 l high efficiency mixing chamber and sampled with the TrueOne 2400's Analyzer Module (oxygen/carbon dioxide analyzers). At rest and during exercise, participants completed IC manoeuvres for the determination of operating lung volumes (i.e., end-expiratory (EELV) and end-inspiratory lung volumes (EILV)), as previously described (Guenette *et al.*, 2013). Briefly, EELV was calculated as the difference between forced vital capacity and IC, while EILV was calculated as the sum of EELV and tidal volume. FVC was used instead of total lung capacity (TLC) for determination of EELV since we did not have a measure of TLC in this study. A commercially available HR monitor was used to provide a continuous measure of heart rate during exercise (Polar T34; Polar Electro Canada, Lachine, QC, Canada). Blood pressure and arterial oxygen saturation were measured using a manual sphygmomanometer and finger pulse oximeter (Radical-7 Rainbow CO-Oximetry; Masimo Corp., Irvine, CA, USA), respectively.

2.2.5 Dyspnoea and Leg Discomfort

Dyspnoea intensity (defined as "the sensation of labored or difficult breathing") and perceived leg discomfort were evaluated at rest, every two minutes during exercise, and at peak exercise using the modified 0-10 category-ratio Borg scale (Borg, 1982). Participants were familiarized with the Borg scale using a standardized script used by our laboratory where "0"

corresponds to “no breathing or leg discomfort at all” and “10” represents “the most intense breathing or leg discomfort you have ever experienced or could imagine experiencing”.

2.2.6 Diaphragmatic EMG and Respiratory Pressures

Crural EMGdi and respiratory pressures were assessed using a multi-pair oesophageal balloon catheter (Guangzhou Yinghui Medical Equipment Co. Ltd, Guangzhou, China) equipped with five pairs of EMGdi electrodes, as well as oesophageal and gastric balloons to measure EMGdi and respiratory pressures, respectively. The catheter was inserted through the nasal cavity and into the stomach by an experienced technician, as described previously (Luo *et al.*, 2008). To minimize participant discomfort, a non-aerosol lidocaine hydrochloride spray (Lidodan® Endotracheal Spray; Odan Laboratories Ltd., Montréal, QC, Canada) was applied to the nasal cavity and oropharynx, and viscous lidocaine (2% Xylocaine®; AtraZeneca Canada Inc., Mississauga, ON, Canada) was used to lubricate the catheter. Once the catheter was inserted into the naris and in position above the glottis, the participants were instructed to sip water as the technician advanced the catheter through the oesophagus and into the stomach. The catheter was then positioned to optimize the EMGdi signal. Specifically, the catheter is considered to be at the level of the diaphragm when the EMG amplitude is smallest in the middle pairing and increases progressively toward the outer pairings (Luo *et al.*, 2008). This placement also ensures that the gastric balloon remained in the stomach, to measure gastric pressure, and the oesophageal balloon was in the distal third of the oesophagus to measure oesophageal pressure (Luo *et al.*, 2008). Participants performed a Valsalva manoeuvre in order to generate a thoracic pressure to empty the balloons while they were open to atmosphere. Balloons were confirmed to be empty with the use of a glass syringe. Subsequently, 0.5 ml and 1.2 ml of air were injected into the oesophageal and

gastric balloons, respectively. Each balloon was connected to a calibrated differential pressure transducer (Model DP15-34; Validyne Engineering, Northridge, CA). The catheter was connected to a grounded bio-amplifier (Model RA-8; Yinghui Medical Technology Co. Ltd., Guangzhou, China).

2.2.7 Limb Muscle EMG

Electrical activity of the lower extremity muscles predominant in cycling was recorded using bipolar surface electrodes. In all subjects, surface electrodes were placed on the vastus lateralis (EMGvl), vastus medialis (EMGvm), semitendinosus (EMGst), and medial gastrocnemius (EMGgm) of the right leg. Skin preparation and electrode placement was performed according to SENIAM recommendations (Hermens *et al.*, 2000). The positions were as follows: EMGvl was placed 2/3 on the line from the anterior superior iliac spine to the lateral side of the patella; EMGvm was located 80 % of the line between the anterior superior iliac spine and the joint space at the anterior border of the medial knee ligament; EMGst was placed half way between the ischial tuberosity and medial epicondyle of the tibia; and EMGgm was placed on the most prominent bulge of the medial aspect of the gastrocnemius muscle while the calf region was maximally flexed (Hermens *et al.*, 2000).

2.2.8 Near-Infrared Spectroscopy and Indocyanine Green

Transmitting and receiving optodes were placed contralateral to surface electrodes with the same skin preparation. Optodes were held at a fixed position with a black plastic holder, which minimized interference from outside light. Inter-optode distance was 4 cm for all muscles, allowing near-infrared light (700-900 nm) to penetrate ~2 cm into target muscles (Boushel *et al.*,

2000). Optodes were connected to a four-channel continuous wave near-infrared spectroscopy system (Oxymon MkIII; Artinis Medical Systems, Elst, The Netherlands), that was customized by the manufacturer to detect changes in ICG (IC-Green®; Akorn, Inc., Lake Forest, IL, USA) concentration via the modified Beer-Lambert law for scattering medium. Briefly, biological tissue is not transparent causing penetrating light to scatter and allows for optodes to be placed adjacent to each other on the same muscle. ICG has a peak absorption of ~820 nm, which results in different levels of light absorption depending on the amount of ICG passing under the optodes (Jöbsis, 1977). For ICG administration, the participant was instrumented with an 18-guage peripheral venous catheter (BD Insyte™; Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT, USA) in the cephalic vein of the right forearm. The catheter was connected to a dual port intravenous set (Continu-Flo Solution Set; Baxter Healthcare Corporation, Deerfield, IL, USA) and 500 mL of intravenous fluid (0.9 % Sodium Chloride Injection USP; Baxter Corporation, Mississauga, ON, Canada). A physician rapidly delivered a 5 mg bolus of ICG through the distal port, followed immediately by a 10 mL flush of 0.9 % normal saline through the proximal inline port of the intravenous tubing.

2.3 Data Analysis

Ventilatory and metabolic parameters, respiratory pressures, and EMG data were averaged into 30s epochs, with the exception of IC derived measures. To ensure steady state conditions were reached for each cadence, the primary window of analysis was from 5:00 to 5:30 min. Assessment of breathing and leg discomfort, and an IC manoeuver followed this analysis window. The IC manoeuvre was collected as close to the end of each stage as possible and immediately

prior to test termination. In addition, a bolus of ICG was injected at the fifth minute and monitored until the end of each trial.

Ventilatory parameters, respiratory pressures, and EMG data were collected using LabChart software (7.3.7 Pro; ADInstruments Inc., Colorado Springs, CO) with a 16-channel analogue to digital acquisition system (PowerLab 16/35; ADInstruments Inc.) sampling at 2000 Hz. Raw EMGdi signals were amplified and band pass filtered between 10 Hz and 3000 Hz (Model RA-8; Yinghui Medical Technology Co. Ltd., Guangzhou, China). Post-acquisition band pass filtering between 20 Hz and 500 Hz was applied to all recordings using the acquisition software. RMS was calculated for both EMGdi and surface EMG using a moving average window of 0.1 s. During inspiration, demarcated by points of zero flow, maximum amplitude in EMGdi RMS was manually selected for each breath over the 30 s analysis window to remove cardiac artefact, as done previously (Guenette *et al.*, 2014; Schaeffer *et al.*, 2014; Faisal *et al.*, 2016). Peak RMS for EMGvl, EMGvm, EMGst, and EMGgm were selected during periods of muscle activation. All RMS EMG was averaged during the 30 s analysis window. The highest absolute EMGdi signal during an IC manoeuvre at rest or during exercise was defined as the participant's maximal EMGdi activity, which was then used to normalize EMGdi obtained during exercise and expressed as a percentage of maximal EMGdi activity (American Thoracic Society/European Respiratory, 2002).

The W_b was derived for each participant using customized software that ensemble averages flow, volume, and oesophageal pressure to make a composite average tidal oesophageal pressure-volume loop. Total W_b was defined as the area within the averaged tidal oesophageal pressure-volume loop with the addition of a triangle falling outside of this loop, representing part of the elastic W_b. P_b was then obtained from the product of W_b and breathing frequency (F_b)

Using NIRS in combination with commercially available software (Oxysoft; Artinis Medical Systems, Elst, The Netherlands), the change in ICG concentration was measured over the final minute of each cadence trial. Prior to the determination of BFI, a fourth order Butterworth filter with 50 Hz and 0.5 Hz sampling and cut-off frequencies were applied to the ICG concentration curves, respectively. BFI was defined as the maximum delta in ICG concentration (ΔICGmax) divided by the rise time, or the time to reach peak concentration. The ΔICGmax was calculated by subtracting a baseline value from the peak ICG concentration. Baseline was determined with the use of a second order derivate of the relationship between ICG concentration and time. Based on the nature of the ICG concentration curve, the initial point of inflection represents the start of the concentration curve and was defined as the baseline ICG concentration. In addition, rise time corresponded to the time interval between 10 % and 90 % of ΔICGmax (Perbeck *et al.*, 1985), which eliminated the need to determine the exact beginning and end of the ICG wash-in.

2.4 Statistical Analysis

A repeated measures study design was used to study the impact of cycling cadence on cardiorespiratory, metabolic, ventilatory variables; EMG of the diaphragm and lower extremity muscles; sensory responses; and haemodynamics of the lower limb. Data are presented as means ± standard deviation (SD) unless otherwise specified. Each variable was tested in relation to the assumption of sphericity and when this assumption was violated, variables were adjusted using the Greenhouse-Geisser correction. When significant main effects were detected, these variables were further analyzed via least significant difference post hoc pairwise comparison (i.e., paired *t*-tests). Alpha, or the level of significance, was set at 0.05 and any result below this level was considered

a statistically significant finding. All statistical analyses were performed using a commercially available statistics program (SPSS v22; International Business Machines Corporation, Armonk, NY, USA).

2.5 Sample Size

After reviewing the literature, a sample size was calculated based on the work of Boone *et al.* (2015) comparing the impact of cycling cadence on whole body $\dot{V}O_2$, since there has yet to be a study to investigate the effect of cadence on BFI using NIRS-ICG. From comparison of $\dot{V}O_2$ during cycling trials at 60 rpm and 100 rpm, a sample size of ten individuals was derived to detect significant differences in BFI. Henderson *et al.* (2012) recruited a sample of seven participants to examine respiratory and leg blood flow during cycling exercise using NIRS-ICG. Guenette *et al.* (2011) analyzed data from seven participants to determine whether BFI could be used to detect differences in respiratory muscle blood flow while another study recruited five participants to quantify regional muscle blood flow within the calf muscles (Boushel *et al.*, 2000). Finally, Habazettl *et al.* (2010) compared the BFI method with the absolute NIRS-ICG technique for blood flow measurement using a sample of ten subjects. Therefore, ten participants appear sufficient to detect potential differences in BFI across cadence conditions.

2.6 Results

2.6.1 Participant Characteristics

Participant characteristics, including demographics and anthropometrics, spirometry, and peak exercise data from visit 1, are presented in Table 2. Of the fourteen highly trained cyclists enrolled, eleven completed the study in its entirety. A total of three participants were excluded

from the study: two participants did not meet the fitness criteria (see Table 1), one participant could not complete the second visit due to intolerance to the peripheral venous catheter, and BFI data could not be obtained from one participant. Of the eleven participants, 10 were male and 1 was female with an age range of 21 to 39 years. According to Ansley and Cangley (2009), the participants in this study can be categorized as elite or category 1 cyclists based on their aerobic capacity ($\dot{V}O_{2max}$= 61 ± 4 ml·kg^{-1}·min^{-1}).

Table 2. Participant characteristics and incremental exercise data

Demographics/Anthropometrics	
Sex, M:F	10 : 1
Age, years	27 ± 6
Height, cm	179 ± 3
Mass, kg	76 ± 7
BMI, $kg \cdot m^{-2}$	24 ± 2
Spirometry	
FVC, l	5.87 ± 0.62
FVC, % predicted	105 ± 8
FEV_1, l	4.54 ± 0.60
FEV_1, % predicted	98 ± 11
FEV_1/FVC, %	78 ± 5
FEV_1/FVC, % predicted	94 ± 6
Peak incremental exercise	
Work rate, W	373 ± 26
$\dot{V}O_2$, $l \cdot min^{-1}$	4.63 ± 0.38
$\dot{V}O_2$, $ml \cdot kg^{-1} \cdot min^{-1}$	61 ± 4
$\dot{V}CO_2$, $l \cdot min^{-1}$	5.14 ± 0.36
RER	1.11 ± 0.05
$\dot{V}_E$, $l \cdot min^{-1}$	169 ± 17
V_T, l	3.06 ± 0.23
F_b, $breaths \cdot min^{-1}$	55 ± 7
$\dot{V}_E/\dot{V}O_2$	37 ± 4
$\dot{V}_E/\dot{V}CO_2$	33 ± 3
$\dot{V}_E$/MVV, %	94 ± 12
HR, $beats \cdot min^{-1}$	182 ± 11
HR, % predicted	95 ± 4
Dyspnoea, 0-10 Borg scale	6.8 ± 1.9
Leg discomfort, 0-10 Borg scale	8.0 ± 1.6

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expired volume in 1 second; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; V_T, tidal volume; F_b, breathing frequency; MVV, maximum voluntary ventilation; HR, heart rate. Values are means ± SD. MVV was estimated by multiplying FEV_1 by 40 (American Thoracic Society/European Respiratory, 2002).

2.6.2 Cardiorespiratory and metabolic responses

The impacts of cadence on cardiorespiratory, metabolic and sensory responses to submaximal exercise are described in Table 3. The average submaximal work rate was 233 ± 25 W, which corresponded to a group average of 63 ± 5 % of the maximal work rate or 74 ± 5 % of

$\dot{V}O_{2max}$ achieved during the incremental test. When expressed as a percentage of $\dot{V}O_{2max}$, the intensity of each trial, in order of increasing cadence, corresponded to 68 ± 5, 71 ± 6, 73 ± 6, and 83 ± 5 %$\dot{V}O_{2max}$. The observed cadences were 60.3 ± 0.4, 89.8 ± 0.5, 94.3 ± 6.9, and 118.4 ± 1.7 rpm and did not deviate substantially from the fixed cadences at which participants were asked to pedal (i.e., 60 rpm, 90 rpm, FCC, and 120 rpm, respectively).

Table 3. Physiological and sensory responses to submaximal exercise.

	60 RPM	90 RPM	FCC	120 RPM
Work rate, W	233 ± 25	233 ± 25	233 ± 25	233 ± 25
Cadence, rpm	60.3 ± 0.4	89.8 ± 0.5	94.3 ± 6.9	118.4 ± 1.7
$\dot{V}O_2$, l·min^{-1}	3.17 ± 0.40	3.30 ± 0.40^{a}	3.39 ± 0.40^{ab}	3.87 ± 0.44^{abc}
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	41 ± 5	43 ± 5^{a}	44 ± 5^{ab}	51 ± 5^{abc}
$\dot{V}CO_2$, l·min^{-1}	2.82 ± 0.45	2.95 ± 0.35	3.09 ± 0.40^{ab}	3.72 ± 0.50^{abc}
RER	0.89 ± 0.04	0.90 ± 0.03	0.91 ± 0.04	0.96 ± 0.02^{abc}
$\dot{V}_E$, l·min^{-1}	76 ± 13	80 ± 13	82 ± 13^{a}	110 ± 19^{abc}
V_T, l	2.35 ± 0.33	2.46 ± 0.33	2.57 ± 0.41^{ab}	2.78 ± 0.36^{abc}
F_b, breaths·min^{-1}	33 ± 5	33 ± 5	32 ± 5	40 ± 6^{abc}
$\dot{V}_E/\dot{V}O_2$	24 ± 2	24 ± 2	24 ± 2	28 ± 4^{abc}
$\dot{V}_E/\dot{V}CO_2$	27 ± 2	27 ± 2	27 ± 2	30 ± 4^{abc}
$\dot{V}_E$/MVV, %	42 ± 8	44 ± 9	46 ± 9^{a}	62 ± 13^{abc}
HR, beats·min^{-1}	143 ± 8	145 ± 9.0	146 ± 10	162 ± 9^{abc}
IC, l	3.73 ± 0.51	3.83 ± 0.45	3.80 ± 0.51	3.89 ± 0.41
EELV, %VC	37 ± 9	35 ± 8	35 ± 8	34 ± 8
EILV, %VC	77 ± 7	77 ± 7	79 ± 5	81 ± 5^{ab}
P_b, J·min^{-1}	118 ± 46	129 ± 40	137 ± 51^{a}	249 ± 112^{abc}
EMGdi, %max	28 ± 5	32 ± 8^{a}	34 ± 8^{ab}	46 ± 10^{abc}
Dyspnoea, 0-10 Borg scale	1.1 ± 1.2	1.3 ± 1.3	1.3 ± 1.3	1.8 ± 1.7
Leg discomfort, 0-10 Borg scale	2.5 ± 1.9	2.0 ± 1.6	2.5 ± 1.6	2.5 ± 1.7

Abbreviations: $\dot{V}O_2$, Oxygen consumption; $\dot{V}CO_2$, Carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; V_T, tidal volume; F_b, breathing frequency; MVV, maximum voluntary ventilation; HR, heart rate; IC, inspiratory capacity; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; VC, vital capacity; P_b, power of breathing; EMGdi, diaphragm electromyography. Values are means ± SD. ^{a}, $p<0.05$ *significantly different from 60 rpm;* ^{b}, $p<0.05$ *significantly different from 90 rpm;* ^{c}, $p<0.05$ *significantly different from freely chosen cadence.*

There was a main effect of cadence on the majority of cardiorespiratory and metabolic data (Table 3 and Figure 4). All cardiorespiratory and metabolic data are significantly higher while pedaling at 120 rpm in comparison to all other cadences tested, with the exception of inspiratory capacity, EELV, and the comparison of FCC and 120 rpm for EILV as a percentage of vital capacity, as shown in Table 3 ($p<0.05$). There was a systematic increase in $\dot{V}O_2$ with each rise in cadence (all $p<0.05$), where 120 rpm was significantly higher than all other conditions (Table 3 and Figure 4). Moreover, $\dot{V}O_2$ was significantly lower during the 90 rpm condition relative to the FCC condition ($p=0.03$) despite the small difference in cadence between the two conditions (~4 rpm). The greatest difference in $\dot{V}O_2$ was found between 60 and 120 rpm ($p<0.001$). Furthermore, $\dot{V}_E$, V_T, and F_b were significantly higher during the 120 rpm conditions compared to all other cadences (Table 3 and Figure 4, all $p<0.01$). During FCC, $\dot{V}_E$ and V_T, were significantly greater than 60 rpm (both $p<0.05$) while V_T was also higher at FCC compared to 90 rpm (p=0.044). There were no significant differences in $\dot{V}_E$, V_T, and F_b between 60 and 90 rpm (all $p>0.05$).

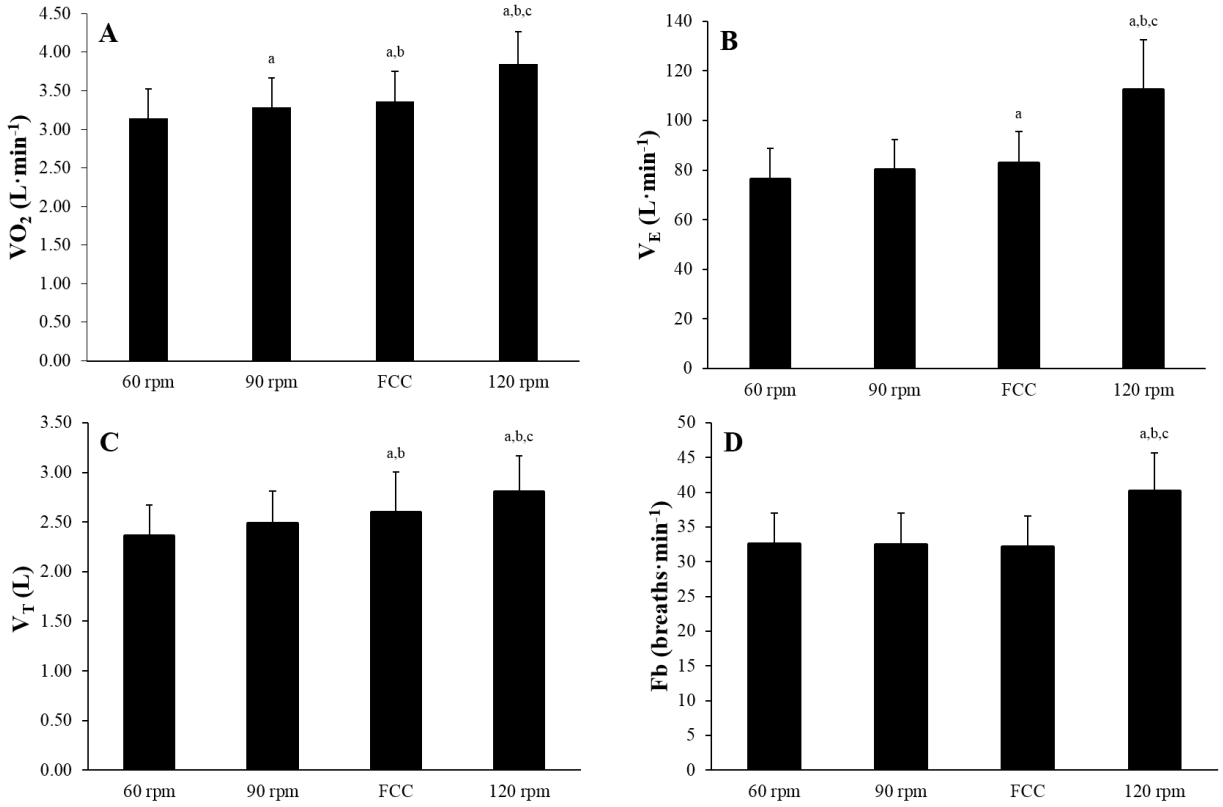

Figure 4. Impact of cycling cadence on the cardiorespiratory responses to exercise. *Panel A shows $\dot{V}O_2$ across the cadence conditions followed by minute ventilation (B), tidal volume (C), and breathing frequency (D). Values are mean ± SD. a, p<0.05 significantly different from 60 rpm; b, p<0.05 significantly different from 90 rpm; c, p<0.05 significantly different from freely chosen cadence.*

Heart rate was significantly greater at 120 rpm compared to all other cadence conditions but no significant differences were observed between 60 rpm, 90 rpm, and FCC (all p>0.05) (Table 3 and Figure 5).

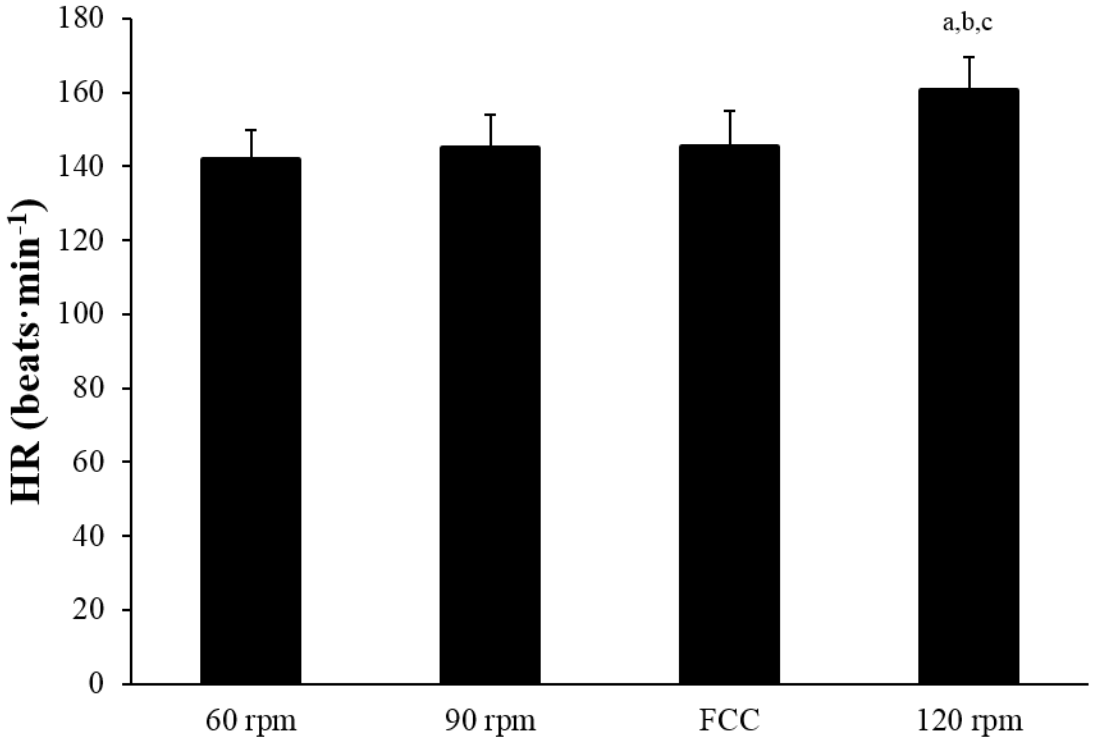

Figure 5. Impact of cycling cadence on heart rate during submaximal exercise. *Values are mean ± SD. a, p*<0.05 *significantly different from 60 rpm; b, p*<0.05 *significantly different from 90 rpm; c, p*<0.05 *significantly different from freely chosen cadence.*

2.6.3 Dyspnoea and leg discomfort

There were no significant differences in dyspnoea or leg discomfort across conditions *(p*>0.05*)* (Table 3.).

2.6.4 Diaphragm EMG and respiratory mechanics

Pedaling at 120rpm resulting in significantly greater EMGdi and P_b compared to all other cadence conditions (all $p<0.05$). In accordance with the rise in $\dot{V}_E$, there was a systemic increase in EMGdi from 60 rpm to 120 rpm (Table 3 and Figure 6). Indeed, EMGdi was also higher at FCC compared to 90 rpm ($p=0.017$) and 60 rpm ($p= 0.011$) and the 90 rpm condition was greater than 60 rpm ($p=0.033$). At 120 rpm, P_b was significantly higher than 60 rpm ($p<0.001$), 90 rpm

(p=0.001) as well as FCC (p=0.001). Moreover, P_b was higher during FCC than the 60 rpm condition (p=0.018).

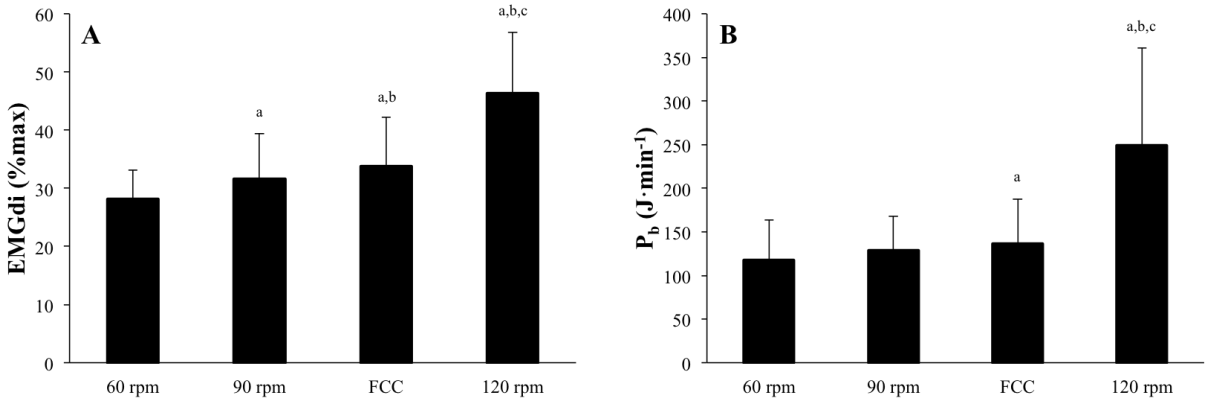

Figure 6. Impact of cycling cadence on diaphragm EMG and respiratory mechanics. *Panel A shows EMG of the diaphragm followed by the P_b (B). Values are mean ± SD. a, p<0.05 significantly different from 60 rpm; b, p<0.05 significantly different from 90 rpm; c, p<0.05 significantly different from freely chosen cadence.*

2.6.5 Surface EMG of the lower extremity

The impact of cycling cadence on EMGvl, EMGvm, EMGst, and EMGgm can be found in Table 4 and Figure 7. No main effect of cadence was detected for EMGvl, EMGvm, or EMGst (all p>0.05). However, EMGgm was significantly higher during 90 rpm, FCC and 120 rpm compared to 60 rpm (all p<0.05).

Table 4. Effect of cadence on lower extremity EMG

	60 RPM	90 RPM	FCC	120 RPM
EMGvl, μV	65.5 ± 53.8	68.4 ± 50.8	67.4 ± 43.6	78.7 ± 62.3
EMGvm, μV	76.3 ± 69.0	78.4 ± 68.3	87.8 ± 81.3	91.3 ± 79.1
EMGst, μV	48.2 ± 25.3	50.2 ± 26.1	55.2 ± 36.9	60.8 ± 26.6
EMGgm, μV	72.5 ± 46.0	99.2 ± 47.5[a]	100.2 ± 54.9[a]	113.3 ± 53.5[a]

Abbreviations: EMGvl; electromyography of the vastus lateralis; EMGvm, electromyography of the vastus medialis; EMGst, electromyography of the semitendinosus; and EMGgm, electromyography of the medial gastrocnemius. Values are mean ± SD. a, p<0.05 significantly different from 60 rpm.

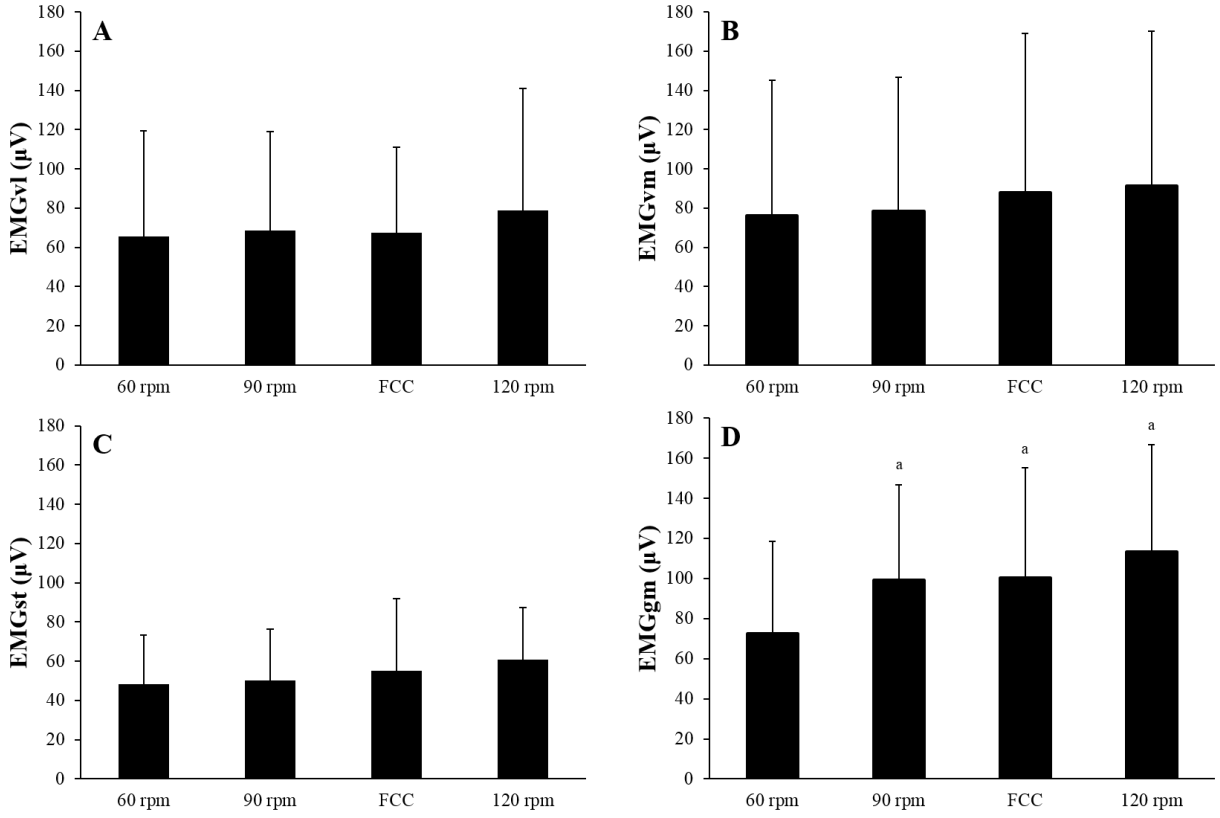

Figure 7. Cadence and lower extremity EMG. *Panels A-D corresponds to EMG of the vastus lateralis, vastus medialis, semitendinosus, and medial gastrocnemius, respectively. Values are mean ± SD. a, $p<0.05$ significantly different from 60 rpm.*

2.6.6 ICG kinetics

The influence of cadence on BFI and ICG kinetics can be found in Table 5 and Figure 8. Significant differences in BFI were observed in all muscles tested, with the exception of BFIvl, although this approached statistical significance ($p=0.064$). In particular, BFIvm and BFIst were significantly higher between 120 and 60 rpm ($p=0.038$ and $p=0.04$, respectively), as well as 120 and 90 rpm ($p=0.005$ and $p=0.029$, respectively). In the medial gastrocnemius, BFIgm was significantly greater during the 120 rpm condition compared to all other cadences (all $p<0.01$).

Table 5. Impact of cycling cadence on the kinetics of indocyanine green

	60 RPM	90 RPM	FCC	120 RPM
BFI, μmol·s^{-1}				
Vastus Lateralis	5.7 ± 1.5	5.5 ± 1.9	6.0 ± 2.1	7.0 ± 2.7
Vastus Medialis	7.1 ± 1.8	6.8 ± 2.5	7.6 ± 2.9	9.1 ± 3.8^{ab}
Semitendinosus	3.4 ± 1.5	3.2 ± 1.4	3.6 ± 1.7	4.8 ± 3.1^{ab}
Gastrocnemius	4.1 ± 1.5	5.4 ± 2.1	5.8 ± 2.0	8.1 ± 2.0^{abc}
Rise Time, s				
Vastus Lateralis	3.2 ± 0.5	3.0 ± 0.3	2.8 ± 0.5	2.5 ± 0.4^{abc}
Vastus Medialis	2.8 ± 0.4	2.7 ± 0.3	2.5 ± 0.5	2.2 ± 0.3^{abc}
Semitendinosus	3.4 ± 0.4	3.1 ± 0.6	3.0 ± 0.6	2.6 ± 0.4^{abc}
Gastrocnemius	3.3 ± 0.6	3.0 ± 0.5	2.7 ± 0.6	2.3 ± 0.2^{abc}
ΔICGmax, μmol				
Vastus Lateralis	17.4 ± 3.4	16.3 ± 4.9	16.5 ± 4.2	17.3 ± 5.9
Vastus Medialis	19.5 ± 4.5	18.0 ± 5.6	18.2 ± 4.9	19.7 ± 6.7
Semitendinosus	11.2 ± 4.7	10.0 ± 5.1	10.4 ± 4.1	11.8 ± 7.1
Gastrocnemius	13.0 ± 2.7	15.3 ± 4.1	15.1 ± 3.5	18.1 ± 3.2^{abc}

Abbreviations: ICG, indocyanine green dye; BFI, blood flow index; ΔICGmax, the change in ICG concentration. *Values are mean ± SD. a, p<0.05 significantly different from 60 rpm; b, p<0.05 significantly different from 90 rpm; c, p<0.05 significantly different from freely chosen cadence.*

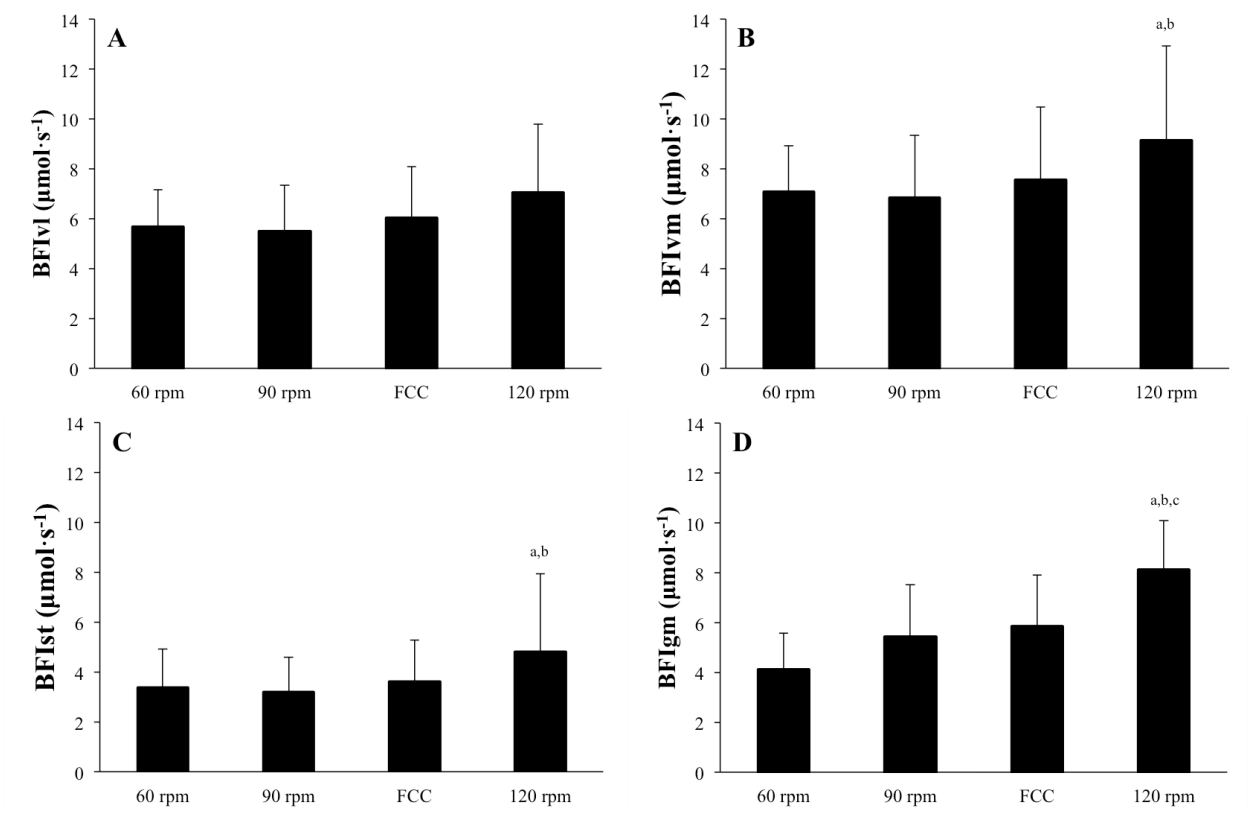

Figure 8. Impact of cadence on relative leg muscle blood flow. *Panels A-D corresponds to BFI of the vastus lateralis, vastus medialis, semitendinosus, and medial gastrocnemius, respectively. Abbreviations: BFIvl, blood flow index of the vastus lateralis; BFIvm, blood flow index of the vastus medialis; BFIst, blood flow index of the semitendinosus; BFIgm, blood flow index of the medial gastrocnemius. Values are mean ± SD. a, $p<0.05$ significantly different from 60 rpm; b, $p<0.05$ significantly different from 90 rpm; c, $p<0.05$ significantly different from freely chosen cadence.*

When deconstructing BFI into its constituents to further analyze the role of cadence on ICG kinetics, main effects can also be observed for both rise time and the total change in ICG concentration. For instance, rise time is significantly lower at 120 rpm in comparison to all other conditions in each of the muscles tested (all $p<0.05$) (Figure 9A-D). No other comparisons reveal statistical differences in rise time, irrespective of the muscle tested ($p>0.05$). Regarding ΔICGmax, there were no statistically significant differences in the vastus lateralis (Figure 10A), vastus medialis (Figure 10B), or semitendinosus muscles (Figure 10C) (all $p>0.05$), while the

change in ICG concentration was significantly greater at 120 rpm compared to 60 rpm (p=0.006), 90 rpm (p=0.02), and FCC (p=0.01) (Figure 10D).

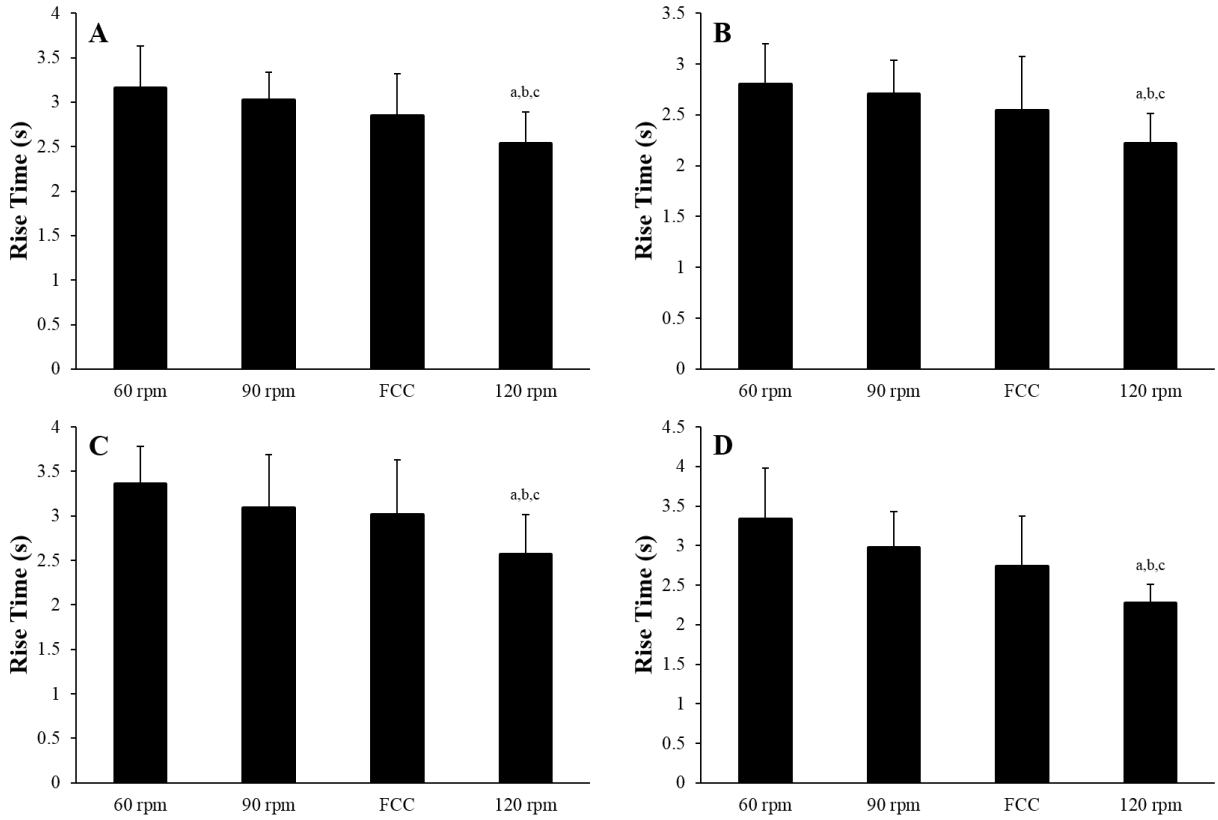

Figure 9. Rise time from 10-90 % ICG concentration at different cadences. *Panel A is the rise time in the vastus lateralis, followed by the vastus medialis (B), semitendinosus (C), and medial gastrocnemius (D). Values are mean ± SD. a, p<0.05 significantly different from 60 rpm; b, p<0.05 significantly different from 90 rpm; c, p<0.05 significantly different from freely chosen cadence.*

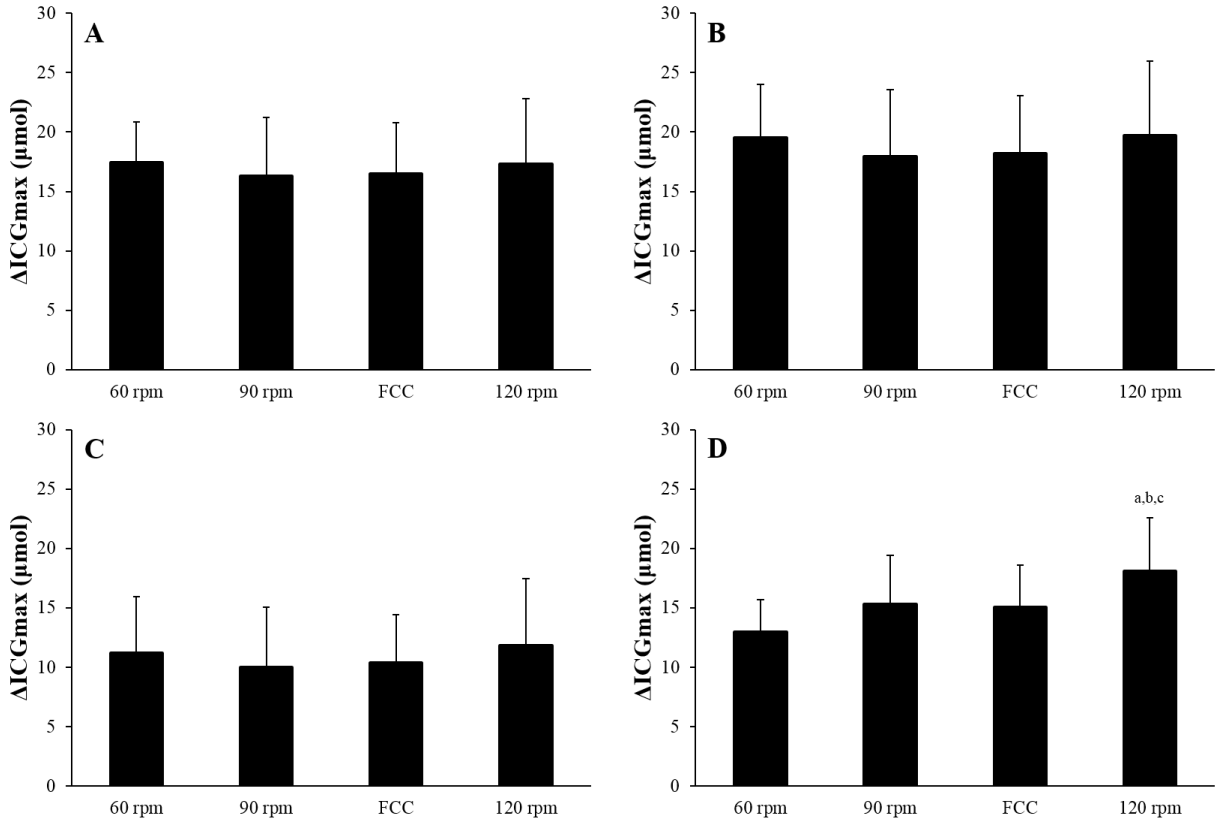

Figure 10. Effect of cadence on the change in ICG concentration in the lower extremity. *Panel A represents the change in ICG concentration in the vastus lateralis, followed by the vastus medialis (B), semitendinosus (C), and medial gastrocnemius (D). Values are mean ± SD. a, $p<0.05$ significantly different from 60 rpm; b, $p<0.05$ significantly different from 90 rpm; c, $p<0.05$ significantly different from freely chosen cadence*

2.7 Discussion:

To my knowledge, this is the first study to simultaneously investigate the impact of cycling cadence on cardiorespiratory and metabolic parameters, peripheral and respiratory muscle activation, subjective experiences of dyspnoea and leg discomfort, and the haemodynamic responses to multiple locomotor muscles during submaximal cycling exercise. The main findings are as follows: i) the $\dot{V}O_2$, $\dot{V}_E$, F_b, V_T, and HR increased as a function of cadence, and were significantly elevated at the highest pedaling rate (i.e., 120 rpm); ii) EMGdi and P_b were significantly increased at 120 rpm relative to lower cadences; iii) surface EMGgm showed significantly more activation during 90 rpm, FCC, and 120 rpm compared to 60 rpm; iv) cadence did not have an effect on the perceptual responses to submaximal exercise; and v) BFI was higher at 120 rpm than 60 rpm or 90 rpm in the vastus medialis, semitendinosus, and medial gastrocnemius, as well as 120 rpm vs. FCC for BFIgm. Collectively, the findings of the present study suggest that pedaling at a rapid cadence (>100 rpm) during submaximal exercise results in inefficiencies at both central and peripheral levels. Specifically, the elevated metabolic cost associated with rapid pedaling rates causes a substantial elevation in $\dot{V}O_2$, $\dot{V}_E$, respiratory and locomotor muscle activation, as well as BFI, which may negatively impact exercise performance in comparison to lower cadences.

2.7.1 Cardiorespiratory and Metabolic Response to Cadence

Despite reports of no differences in $\dot{V}O_2$ with increasing cadence (Lepers *et al.*, 2001; Foss & Hallén, 2004; Skovereng *et al.*, 2016), the present study provides evidence of a significant positive relationship between cadence and $\dot{V}O_2$ (Figure 4A). In accordance with our hypothesis, $\dot{V}O_2$ increased significantly with each rise in cadence from 60 to 120 rpm. Our finding of

increasing $\dot{V}O_2$ with increasing cadence is significant, particularly given that $\dot{V}O_2$ is an integral component of an individual's overall capacity for aerobic exercise (Wagner, 2000) and represents the metabolic cost of a given exercise intensity (Poole & Jones, 2012). The increase in $\dot{V}O_2$ shown in our study is in keeping with previous studies using cadences of 60 rpm or higher, where a positive relationship between $\dot{V}O_2$ and cadence was noted (Marsh & Martin, 1993; Chavarren & Calbet, 1999). According to Barstow *et al.* (1996), a possible explanation for the increase in $\dot{V}O_2$ with cadence is the progressive recruitment of fast twitch muscle fibres as cadence increases (Sargeant, 1994). Fast twitch muscle fibres become activated at increased contraction frequencies to exploit their ability to produce force during higher velocity contractions (Hill, 1922). However, this recruitment comes at the expense of an increased metabolic demand for a given exercise intensity as the initiation of a muscle contractions use more adenosine triphosphate, and by association, oxygen, in comparison to longer duration contractions (i.e., slow cadences) (He *et al.*, 2000). Moreover, the impact of cadence on $\dot{V}O_2$ in the present study is in agreement with the general trend of an increase in leg EMG activity, albeit only statistically significantly for the gastrocnemius, suggesting that muscle activation increases with increasing cadence, which presumably results in the recruitment of additional fast twitch muscles fibres.

In the present study, the energetically optimal cadence, defined as the cadence that elicits the lowest $\dot{V}O_2$ for a given exercise intensity, was 60 rpm. An energetically optimal cadence of 60 rpm is congruent with previous studies (Marsh & Martin, 1998; Lucia *et al.*, 2001; Sarre *et al.*, 2003). For example, the energetically optimal cadence observed by Chavarren and Calbet (1999) was 60 rpm at work rates ranging from 124 W to 259 W. Furthermore, our results show that 60 rpm was also the cadence to elicit the lowest $\dot{V}_E$, F_b, V_T, and HR, which is in agreement with our hypothesis that cardiorespiratory and metabolic data would be minimized at low cadences.

Although it is tempting to assume that the most energetically optimal cadence and FCC would be one and the same, as is shown in other endurance sports (i.e., running) (Cavanagh & Williams, 1982) this is may be an oversimplification due to differences in fibre type composition across different muscles in the same individual, as well as the heterogeneity in fibre composition for a given muscle between individuals. Despite oxygen being the required variable to maintain exercise at a given intensity (Saltin *et al.*, 1998), it can be presumed that the energetically optimal cadence is likely variable and specific to each individual. However, studies showing a significant increase in the energetically optimal cadence with increases in power output suggest that metabolic demand does play a role in FCC. The role of metabolic demand in FCC is evident from work assessing the impact of cadence on gross efficiency during whole body exercise (Gaesser & Brooks, 1975). Gross efficiency is calculated as the mechanical power output over the metabolic input (de Koning *et al.*, 2012), in this case $\dot{V}O_2$. In the present study, there is a 19.9 % decrease in gross efficiency during pedaling at 120 rpm in comparison to 60 rpm. Therefore, while factors such as respiratory mechanics and haemodynamics may influence cadence selection, the metabolic cost of increasing cadence cannot be disregarded with respect to FCC.

In agreement with our hypothesis and the above-mentioned impact of cadence on $\dot{V}O_2$, we observed a significant rise in $\dot{V}_E$, V_T, and f_b with increasing cadence (Figure 4B-D). In particular, $\dot{V}_E$ was substantially higher when cycling at 120 rpm and this rise in $\dot{V}_E$ appears to be driven by an increase in V_T at lower cadences (Figure 4C). A significant difference in V_T was observed between FCC and 60 rpm, as well as 90 rpm, which depict the typical rise in V_T with increasing metabolic demand below the plateau point in V_T (i.e., 68 ± 5 $\%\dot{V}O_{2max}$ at 60 rpm vs. 83 ± 5 $\%\dot{V}O_{2max}$ at 120 rpm). In addition, f_b was significantly higher while pedaling at 120 rpm in comparison to all other cadences (Figure 4D). No differences in f_b were observed between other

cadence comparisons. Again, the higher metabolic demand at 120 rpm would necessitate a rise in f_b in order to maintain a rapid cadence at the exercise intensity used in the present study. On the other hand, the central nervous system has been suggested to play a role in the ventilatory response to rapid limb movements, via group III and IV afferent fibres (Amann *et al.*, 2010). In this study, Amann *et al.* (2010) partially blocked afferent activity during rest and exercise and found a significant reduction in $\dot{V}_E$ in comparison to a placebo. Furthermore, Caterini *et al.* (2015) assessed the impact of rapid limb movements on $\dot{V}_E$ while controlling for metabolic stimulus ($\dot{V}O_2$) and found that abrupt changes to higher cadences rather than an increase in pedal load resulted in a significant increase in exercise $\dot{V}_E$. Therefore, independent of the significant rise in $\dot{V}O_2$ with each increase in cadence, it can be speculated that a significant difference in $\dot{V}_E$ would still have been observed if metabolic demand were controlled between cadence conditions.

Interestingly, this is the first study to investigate the impact of cycling cadence on EMGdi and P_b, (Figure 6). The assessment of the above parameters was accomplished with the use of a novel oesophageal catheter, as described previously (Luo *et al.*, 2008). Congruent with our hypothesis, EMGdi and P_b both showed a positive relationship with increasing cadence, where the 120 rpm condition was significantly elevated compared to all other conditions. These findings are in agreement with the rise in $\dot{V}_E$. As $\dot{V}_E$ increases as a function of cadence, EMGdi increases systematically, suggesting that a greater level of diaphragm activation is needed to satisfy the rise in metabolic demand.

Often, $\dot{V}O_2$ reported in the literature pertains to whole body $\dot{V}O_2$ because it is difficult to assess the contributions of individual muscles or muscle regions. However, in order to better understand FCC, it is important to analyze $\dot{V}O_2$, in terms of its constituents. While muscle $\dot{V}O_2$ was not specifically assessed in this study, it is inappropriate to assume that the significant effect

of cadence on $\dot{V}O_2$ was due to the increased metabolic demand of the peripheral muscles alone. Often neglected or unstated is the contribution of respiratory muscle activation to whole body $\dot{V}O_2$. Indeed, we have shown a significantly higher P_b with increasing cadence. This increase in the mechanical cost of breathing will translate directly into an increase in the metabolic or oxygen cost of breathing. Based on work of Aaron *et al.* (1992), the oxygen cost of breathing can be estimated for any given $\dot{V}_E$ using regression equations developed from voluntary hyperpnoea performed to mimic the hyperpnoea of cycling exercise. In the present study, respiratory muscle $\dot{V}O_2$ likely accounted for 3.8, 4.0, 4.2 and 6.4 % of whole body $\dot{V}O_2$ at 60 rpm, 90 rpm, FCC, and 120 rpm, respectively, based on estimates from Aaron *et al.* (1992). The sharp rise in respiratory muscle $\dot{V}O_2$ as a percentage of whole body $\dot{V}O_2$ is not surprising. Studies have shown that as $\dot{V}_E$ continues to increase, the P_b and oxygen cost of the respiratory muscles increases in a curvilinear fashion relative to $\dot{V}_E$ (Aaron *et al.*, 1992; Dominelli *et al.*, 2015). Thus, the results of this study suggest that the contributions of the respiratory muscles to both the metabolic costs of exercise and the P_b should be considered when trying to determine the factors associated with FCC.

2.7.2 Peripheral Muscle EMG

The influence of cadence on electrical activation of leg muscles predominant in cycling is highly inconsistent due to differences in study design, exercise intensity, physiological differences between participants, and EMG analysis techniques. For instance, surface EMG provides insight into muscle activation of typically superficial muscles (Hug & Dorel, 2009), which differ in fibre composition amongst individuals. Cadence has been shown to increase the EMG signal of muscles predominant during cycling (Baum & Li, 2003) and the increase in EMG amplitude will differ with respect to the level of activation of fast twitch muscle fibres due to their inherent membrane

and contractile properties (Hill, 1922; Hug & Dorel, 2009). Furthermore, individuals capable of generating greater amounts of force will be working at a relatively smaller level of their strength capacity, resulting in less activation in stronger individuals (Bieuzen *et al.*, 2007; Ansley & Cangley, 2009). Both of these above-mentioned factors may play a role in the high inter-individual variability observed in surface EMG, irrespective of cycling experience (Hug *et al.*, 2008). In addition, both RMS and integrated EMG techniques have been used to assess the impact of cadence on lower extremity muscle activation. While each approach has certain advantages and limitations, it has been suggested that the difference in mean RMS EMG and integrated EMG is negligible and should not impact the interpretations of the research findings (Renshaw, 2010). Accordingly, our results are similar to that of Neptune *et al.* (1997), where a systematic increase in leg RMS EMG was observed as a function of increasing cadence (Figure 7). However, while there is a positive trend in RMS EMG with increasing cadence, no statistical differences were observed in three out of four of the muscles tested, which refutes our hypothesis. Moreover, it appears that cadences below 60 rpm are needed to determine if a parabolic relationship in RMS EMG exists, however, this was not done in the present study. Thus, we were unable to support our hypothesis that RMS EMG would be increased at the lowest cadence condition. One possible explanation for the lack of significance in EMGvl, EMGvm, and EMGst is the low statistical power to detect a main effect between cadence and EMG, as the present study was powered to detect a difference in $\dot{V}O_2$ between cadences. Conversely, we found that EMGgm was greater at 90 rpm, FCC, and 120 rpm relative to 60 rpm (Figure 7, panel D). A possible explanation for a rise in EMGgm is a difference in muscle recruitment patterns at different cadences. In this case, it appears that the gastrocnemius is recruited to a lesser extent at low cadences where cyclists may rely on a higher contribution from the quadriceps muscles to generate pedal force (Baum & Li, 2003). A linear increase in

EMGgm has also been shown in previous studies (Neptune *et al.*, 1997; Baum & Li, 2003). In addition, Neptune *et al.* (1997) found the onset of gastrocnemius muscle activity occurred later in the crank cycle at 60 rpm compared to higher pedaling rates. Therefore, the amount of time the gastrocnemius is active during the crank cycling is reduced, potentially influencing our measured level of activation. However, this shift in EMGgm onset is not a consistent finding (Baum & Li, 2003). MacIntosh *et al.* (2000) reported a similar pattern of gastrocnemius activation with increasing cadence below 400 W. At 400 W, which was the highest power output in their study, the EMG-cadence relationship was depicted by a parabolic curve where 40 rpm resulted in higher EMGgm than 60 rpm. Indeed, it has been proposed that peripheral muscle EMG increases at extremely low cadences (<60 rpm) as a function of fast-twitch muscle fibre recruitment corresponding to the increase in muscle force (Ahlquist *et al.*, 1992). However, cadences below 60 rpm were not tested in the present study.

2.7.3 Sensory Responses to Cadence

It is possible that the selection of FCC is based on the perception of peripheral muscle discomfort in response to the metabolic demands associated with a given exercise intensity or environmental terrain. Although speculative, it is possible that FCC represents the cadence that elicits the lowest (or perhaps most tolerable) degree of peripheral muscle discomfort rather than the most energetically efficient cadence. Few studies have explicitly investigated the relationship between perceived exertion and cadence (Marsh & Martin, 1998; Jameson & Ring, 2000). Marsh and Martin (1998) compared multiple power outputs and cadences in trained cyclists, runners, and recreational cyclists to determine if preferred cadence corresponded to the cadence the minimized rating of perceived exertion, as sensory feedback relating to strain of the peripheral muscles is

relayed to the central nervous system (Ekblom & Goldbarg, 1971). Marsh and Martin (1998) reported that preferred cadence was higher than the one that reduced perceived exertion during pedaling at 100, 150, and 200 W in trained cyclists. We found no effect of cadence on leg discomfort during pedaling at a group average of 233 W. A potential explanation for our finding could be the convergence of preferred cadence and the cadence that minimizes perceived exertion where at higher power outputs these two cadences become the same. The average power output used in the present study is higher than those used by Marsh and Martin (1998). Indeed, a similar phenomenon occurs with the energetically optimal cadence as work rate nears maximal exercise (Foss & Hallén, 2004).

A novel aspect to our study is the investigation of cadence on dyspnoea. Importantly, there is now a well-established link between EMGdi and exertional dyspnoea (Jolley *et al.*, 2009; Jensen *et al.*, 2011; Schaeffer *et al.*, 2014; Faisal *et al.*, 2016). Similar to the perceived strain of exercise on the peripheral muscles sending signals to the central nervous system (Ekblom & Goldbarg, 1971), sensations of breathing discomfort are also transferred to the central nervous system when the work and effort of breathing increases. EMGdi provides an indirect assessment of the inspiratory neural drive to breathe and has been strongly correlated with dyspnoea (Jolley *et al.*, 2009). However, despite the significant increase in EMGdi with each increase in cadence, as well as the high minute ventilation while pedaling at 120 rpm, there was no effect of cadence on dyspnoea in the present study. Although speculative, this may reflect the relatively low exercise intensity used in this study (i.e., 10% below GET) that resulted in low absolute Borg ratings. Indeed, absolute dyspnoea and leg discomfort ratings were consistently below 2 and 3 units, respectively across all cadence conditions on the 0-10 scale. In addition, the highest average level of activation, as a percentage of maximum, was less than 50 % during the 120 rpm conditions.

These levels may have been too low to detect physiologically meaningful and statistically significant differences between cadence conditions in healthy endurance athletes. Additional studies are needed to assess the sensory responses across different cadences at varying exercise intensities to test this hypothesis.

2.7.4 Haemodynamic Response to Cadence

Despite decades of research investigating the onset and regulation of hyperaemia, the present study is the first to examine the role of cadence on relative microvascular blood flow during dynamic cycling. To our knowledge, this is also one of the only studies to measure blood flow to multiple leg muscles simultaneously during cycling. Previous investigations into the role of cadence, or contraction frequency, on blood flow have been conducted using knee extension exercise or animal preparations, which differ quite substantially from the hyperaemic response during dynamic cycling in humans. The major finding was that BFIvm, BFIst, and BFIgm was significantly elevated at 120 rpm compared to 60 and 90 rpm (Figure 8). Furthermore, BFIgm was also significantly higher at 120 rpm compared with FCC. Two contradicting hypotheses in the literature exist regarding the impact of cadence on skeletal muscle blood flow. First, the intramuscular pressure associated with forceful muscle contractions can impede blood flow by compressing the blood vessels within the muscle's vascular bed (Takaishi *et al.*, 2002; Osada *et al.*, 2015). Indeed, it has been shown that blood flow is only permissible during the relaxation phase of a contraction cycle while blood flow is restricted during the contraction phase (Lutjemeier *et al.*, 2005). Second, the reduction in duration of the relaxation phase corresponding to rapid contraction frequencies has the potential to limit convective oxygen delivery by reducing the red blood cell transit time through the muscle capillaries (Walløe & Wesche, 1988; Hoelting *et al.*,

2001). While the results of the present study are in agreement with our hypothesis that BFI would be reduced during the lowest cadence condition, our findings do not support either of these hypotheses. For instance, with the exception of the gastrocnemius, the BFI of all other leg muscles was numerically higher during the 60 rpm condition compared to 90 rpm. While no statistically significant differences were noted, this trend suggests that blood flow restriction is not occurring during the lowest cadence condition when intramuscular pressure from forceful contractions is presumed to be the highest. This finding could be a function of the submaximal intensity used in the present study. Using an intermittent incremental cycle test protocol and NIRS-ICG to measure BFIvl, Henderson *et al.* (2012) concluded that relative microvascular blood flow in the lower extremity plateaus at approximately 60 % and subsequently declines at 83 % of peak work rate due to a potential increase in intramuscular pressure as work rate increased. Therefore, it appears that cycling at submaximal exercise intensity results in less blood flow restriction in comparison to heavy (~90 % peak power) and near maximal exercise (VO_{2peak}). Secondly, both $\dot{V}O_2$ and BFI were highest during the 120 rpm condition, suggesting that there was no limitation in convective oxygen delivery due to a reduced blood transit time. To satisfy the hypothesis that blood flow is potentially too rapid during high cadences for effective oxygen delivery to be true, a plateau in $\dot{V}O_2$ would need to be present. However, in the present study, $\dot{V}O_2$ continued to increase as a function of cadence. Again, the submaximal exercise intensity used in this study provides a possible explanation for the above finding because $\dot{V}O_2$ is not limited at the intensity used in this study. However, while whole-body or pulmonary $\dot{V}O_2$ was significantly higher during the 120 rpm condition, we did not have a measurement of muscle $\dot{V}O_2$. Therefore, further work is needed to investigate the impact of cadence on muscle $\dot{V}O_2$ in order to better understand blood flow within the muscle.

Under conditions where contractile work is held constant, Hamann *et al.* (2005) have shown that blood flow is increased during short duration (i.e., fast cadence) contractions in comparison to long duration contractions (i.e., slow cadence), suggesting that blood flow is directly related to metabolism of active tissues and not the contractile work of the muscles. The results of our study are in agreement with the aforementioned hypothesis. In particular, examination of both the rise time and the change in ICG concentration of the medial gastrocnemius provides evidence of this link between muscle activation and blood flow (Figure 9 and 10, respectively). The rise time is significantly reduced during pedaling at 120 rpm compared to all other cadences, which indicates that blood flow under the NIRS probe increases with increasing cadence. However, one of the assumptions with using NIRS-ICG is that the amount of ICG passing through muscle is the same across experimental conditions (Kuebler *et al.*, 1998). Investigation of the change in ICG concentration within the medial gastrocnemius shows a statistically significant difference in ICG concentration, where the concentration is highest at 120 rpm compared to all other conditions. Two mechanisms could explain this finding. First, compression of the blood vessels in the medial gastrocnemius could be restricting blood flow during the low cadences while this compression is lessened at higher cadences where intramuscular pressure is presumed to be lower. Second, EMGgm is distinct from the other muscles tested resulting in reduced activation at low cadences and increased recruitment as cadence increases. Analysis of EMGgm suggests that the second mechanism appears more likely as this muscle was the only one to show statistical differences in the level of activation across the cadences tested. Indeed, Guenette *et al.* (2011) examined BFI of the sternocleidomastoid muscle during voluntary hyperpnoea and found a strong correlation between both EMG (R^2=0.930) and P_b (R^2=0.863) with BFI. Thus, as it pertains to the present

study, BFIgm appears to provide evidence of a link between metabolic demands of the muscle and relative microvascular blood flow.

2.7.5 Limitations

First and foremost, the findings of the present study should be interpreted with caution. As stated throughout this thesis, exercise intensity dictates the physiological response to exercise that will be observed. For instance, exercise above anaerobic threshold will result in higher levels of metabolite production, muscle activation, and $\dot{V}O_2$ in comparison to the intensity used in the present study. Indeed, exercise above the anaerobic threshold relies on anaerobic metabolism for generation of ATP while the intensity used in the present study uses aerobic metabolism. The exercise intensity used in this study was chosen for its applicability to cycling competition and to minimize the potential confounding effect of fatigue between multiple trials performed on the same visit. For example, in professional road cycling, it has been shown that the majority of the race is completed at an intensity below approximately 70 % of $\dot{V}O_{2max}$ (Lucia *et al.*, 2001), which is similar to the intensities used in most of our cadence conditions. On the contrary, this submaximal intensity appeared to be too low to determine if any blood flow restriction occurred at low cadences. A future study incorporating a progressive increase in external power output at varying cadences may be more appropriate in determining if blood flow becomes restricted due to high intramuscular pressures near maximal exercise.

Another limitation that must be acknowledged is the use of elite cyclists. The extraordinary amount of training that these athletes have conducted manifests in specific physiological adaptations, which distinguishes them from the general population. Therefore, our results may not

generalizable to recreational athletes, older adults, or clinical populations undergoing cardiopulmonary rehabilitation.

The limitations of using surface EMG to measure electrical activity are also present in our study. These include muscle cross talk, determination of large muscle mass activity with superficial muscles fibres providing a greater contribution to the EMG signal, and motion artifacts (Hug & Dorel, 2009). Careful attention was given to skin preparation, placement of electrodes, and the use of bipolar electrodes to minimize some of these factors. Indeed, SENIAM guidelines were followed for all electrode placements to reduce cross talk (Hermens *et al.*, 2000). The same skin preparation was used in all participants and the within-subject study design was intended to minimize the between condition variability. In particular, all cadence trials were conducted during the same study visit to ensure that electrode placement was the same across all cadence trials. Furthermore, we attempted to control this factor by standardizing the relatively long recovery period between trials and randomization of the cadence trials.

Finally, there are limitations relative to the technological approach of NIRS-ICG. First, the BFI method can only provide a relative measure of blood flow across cadence conditions because there is no measure of arterial input to the muscle. Absolute blood flow would require the use of arterial cannulation, the withdrawal of blood samples and reinfusion of blood back into the body, resulting in a much more invasive procedure. In addition, an important assumption of NIRS-ICG and the BFI method is that of proportionality (Kuebler *et al.*, 1998). It is assumed that distribution and concentration of the ICG dye is representative of the flow to a region based on metabolic demand. Furthermore, it is assumed that under the same conditions, the heart and pulmonary vasculature mix the ICG dye in the same manner, such that the same concentration of dye is distributed under the NIRS optode. Despite not being able to obtain absolute measures of

blood flow, relative differences can still be compared across cadence conditions to determine the impact of cadence on microvascular leg blood flow. In addition, due to the properties of near-infrared light and scattering of this light upon entering the tissue, NIRS can only assess ICG concentration at a specific penetration depth (Jöbsis, 1977; Boushel *et al.*, 2000). In this study, the interoptode distance of 4 cm corresponds to a light penetration depth of 2 cm. Fortunately, the muscles targeted are all relatively superficial with respect to the NIRS penetration depth. Furthermore, the use of elite cyclist limits the impact of adipose tissue thickness on the NIRS signal due to their lean physique and minimal body fat composition. Overall, the within-subject design allows for the comparison of outcome variables across the cadence conditions with each individual serving as their own control, thus reducing between subject variance.

Chapter 3: Conclusion

To our knowledge, this was the first study to investigate the impact of cycling cadence on respiratory mechanics, electrical activation of both respiratory and locomotor muscles, and relative microvascular blood flow to leg muscles predominant during cycling. The present study makes novel contributions to this area of research, at least as it pertains to submaximal exercise. In particular, microvascular blood flow appears to increase with increasing cadence in most locomotor muscles studied. The use of NIRS-ICG to study the impact of cadence on BFI during dynamic two-legged cycling has improved our understanding of blood flow during exercise in the intact human being. Specifically, results from the medial gastrocnemius provide evidence of the close link between metabolic demand and skeletal muscle blood flow.

Our study is the first to examine the impact of cadence on activation of the diaphragm and the mechanical cost of breathing. Of note, with the increase in $\dot{V}_E$ as a function of cadence, EMGdi increases significantly with each rise in cadence. Furthermore, P_b was significantly elevated at the highest cadence, providing evidence that respiratory activation and mechanics may play an important role in cadence selection. The increase in respiratory muscle activation and mechanics coupled with the significant elevation in whole body $\dot{V}O_2$, $\dot{V}_E$, and HR suggests that rapid cadences represent a powerful central stimulus, which could influence cadence selection. Accordingly, the increase respiratory and cardiovascular stress likely becomes important for individuals experiencing dyspnoea or diagnosed with a chronic respiratory disease, such as chronic obstructive pulmonary disease, interstitial lung disease, or cystic fibrosis. In these cases, cycling cadence represents a method in which breathing discomfort can be reduced by pedaling at a lower cadence. In addition, these patients often experience vast peripheral

dysfunction, and thus, pedaling at a lower cadence could strengthen their peripheral muscles while allowing them to exercise for a longer duration with lower ventilatory requirements.

In conclusion, many attempts are made to control extraneous variables, such as environmental terrain and still an optimal cadence has yet to be determined. Muscle fibre type composition, aerobic fitness status, muscle strength, and the presence or absence of chronic disease all play a role in FCC, which dictates what cadence is optimal for a specific individual. Importantly, returning to the perspective of Lucia *et al.* (2004), cadence appears to represent the balance between central and peripheral stresses. Further evidence from this study shows that blood flow closely matches the metabolic demands of the peripheral muscles. Thus, haemodynamics may act as a mediator between the central and peripheral stress where a compromise is made toward the stimuli that can be tolerated for the longest duration of time.

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