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Effects of a single-leg exercise training intervention on single and double leg peak power output, maximal oxygen consumption, gas exchange threshold, and the respiratory compensation point

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Effects of a single-leg exercise training intervention on single and double leg peak power output, maximal oxygen consumption, gas exchange threshold, and the respiratory compensation point.

by

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Abstract

Over the years research has attempted to understand the central vs. peripheral adaptations to training utilizing double-leg and single-leg cycling to further asses the contribution of these components respectively in the context of overall cardiovascular function and performance. Furthermore, single-leg cycling with a counterweight allows cycling with a motion similar to that of traditional double-leg cycling facilitating for more power with lower cardiovascular effort. This study examined the effects of a counterweighted single-leg cycling training program on single-leg and double-leg cycling performance during a ramp incremental test to exhaustion. Ten cardiovascular untrained otherwise healthy men were recruited. The participants underwent a series of ramp incremental tests which were comprised of traditional double-leg cycling and single-leg counterweighted cycling with each leg respectively, before and after a four-week single-leg training intervention. The results suggest that counter weighted single-leg cycling may have influenced central components leading to significant increases in double-leg maximal oxygen consumption, the gas exchange threshold and respiratory compensation point.

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List of Symbols, Abbreviations and Nomenclature

Symbols	Definition
$\dot{V}O_{2max}$	Maximal oxygen consumption
GET	Gas exchange threshold
RCP	Respiratory compensation point
MLSS	Maximal lactate steady state
$\dot{V}O_2$	Oxygen consumption
SV	Stroke volume
Q	Cardiac output
Q_{max}	Maximal cardiac output
Q_{peak}	Peak cardiac output
SL	Single-leg
VO_{2peak}	Peak oxygen uptake
DL	Double-leg
ATP	Adenosine triphosphate
$\dot{V}CO_2$	Carbon dioxide
VE	Ventilation
RI	Ramp incremental
a-vO_{2diff}	Arteriovenous oxygen difference
P_{vO2}	Partial pressure of oxygen
DMO_2	Muscular oxygen diffusive capacity
HIIT	High intensity interval training

Chapter One: Introduction

In modern sports science, athletic performance programs, rehabilitation programs, laboratory based studies, and even some recreational sport settings, often rely on the physiological evaluation of exercise performance to monitor physical fitness levels and to help prescribe exercise intensities[3]. Being able to evaluate exercise intensity boundaries enables for improved prescription of an intensity of exercise that is going to produce a stimulus that is high enough to elicit a favorable training response to the program, where progress can be monitored[4].

Cardiovascular/respiratory fitness can be measured as the maximal oxygen uptake ($\dot{V}O_{2max}$) that an individual can reach. $\dot{V}O_{2max}$ is one of the most meaningful physiological measurements when it comes to assessing aerobic fitness amongst trainers, clinicians, and researchers. $\dot{V}O_{2max}$ is defined as the highest rate at which oxygen can be taken, distributed and consumed by the body during exercise[5]. $\dot{V}O_{2max}$ is influenced by several factors such as age, body composition, heredity, mode of exercise, sex, and most importantly an individual's training status, amongst others[5]. Early studies concluded that oxygen consumption increases as the intensity of exercise increases, until it eventually reaches a maximum beyond which further increments in intensity (e.g., power output, speed, etc.) do not result in a subsequent augmentation of the $\dot{V}O_2$ response[6]. Although different views exist as to what mechanisms ultimately control the $\dot{V}O_{2max}$ response[7], interactions at different levels within the cardiovascular and respiratory system will contribute to determine one's $\dot{V}O_{2max}$[5].

In addition to measures of $\dot{V}O_{2max}$, exercise performance can be evaluated by examining submaximal responses to exercise. Although different nomenclature is used in the literature[8], two clear demarcation points can be observed during an incremental test to exhaustion that determine the boundaries between the moderate, heavy, and severe domains of exercise[9]. Commonly used

relevant threshold concepts to identify these boundaries are the gas exchange threshold (GET), which separates the moderate from the heavy exercise intensity domain, and the respiratory compensation point (RCP), which represents the metabolic rate separating the heavy from the severe exercise intensity domain[8,10]. Another widely used demarcation point evaluated through constant load exercise is the maximal lactate steady state (MLSS) determined from blood lactate concentration. The intensity of exercise at the MLSS is considered to represent the critical intensity of exercise[11], which also separates the heavy from the severe exercise intensity domains.

The administration of a controlled endurance training program is known to have a variety of positive health effects[12]. These effects can manifest in different forms of exercise training related adaptions that can range from central changes such as improvements in cardiac function, to local changes that may occur on a more peripheral or muscular level[13,14,15].

From a central adaptations perspective, it has been previously demonstrated that endurance training can lead to an increase in stroke volume (SV) and maximal cardiac output[16] when performing maximal exercise. Alterations in cardiac structure or myocardial contractility have been speculated to underlie the increases in maximal cardiac output (Q) post endurance training[17]. Different lines of research have provided information regarding this increase in maximal Q (Q_{max}) in response to exercise training. For example, some studies focused the scope on either comparing heart size amongst athletes and non-athletes or comparing heart size before and after exercise, and some have used echocardiographic imaging to illustrate that the hearts of well-trained endurance athletes are in fact larger than those of non-athletes[18,19,20]. Importantly, echocardiographic images from longitudinal training studies have provided less conclusive

evidence, as some investigations revealed increases in left ventricular size and chamber[21,22], whereas other investigators showed no such changes[16,23].

Another approach used to distinguish cardiac central changes from peripheral muscle and vascular adaptations has involved the evaluation of exercise response between a trained limb and an untrained limb. A general hypothesis five decades ago stated that changes in response to a single-leg (SL) training program in the untrained limb reflected improvement within the central component only[24]. This hypothesis was put to test in a SL cycling study where results demonstrated an increase in peak oxygen consumption ($\dot{V}O_{2peak}$) when the trained and untrained limb were exercised. Since a significant increase in Q was observed when the trained limb was exercised after the training intervention, the $\dot{V}O_{2peak}$ increase observed after exercising the untrained limb was considered to be a result of central adaptations in response to training the contralateral limb[24]. In opposition to the above-mentioned study, findings from Klausen et al.[25] suggested that the increased Q post SL training was most likely related to a decrease in peripheral resistance in the trained leg and not central adaptations.

Training a single leg enables a relatively simple discrimination of central vs. peripheral adaptations to different types of exercise training intensities (e.g., adaptations pertaining to sprint vs. endurance training)[24] and the type of impact that this training would have on double-leg (DL) and the untrained contralateral leg[24]. SL cycling was shown to increase $\dot{V}O_{2peak}$ after 4 weeks of sprint training and endurance training by 15% and 24% in the trained leg compared to baseline measurements, respectively in two different groups of participants[24]. Along with the significant improvement in the trained leg, a not significant increase in $\dot{V}O_{2peak}$ (between 3-6%) was also observed on the untrained leg [24], which made the authors speculate that SL training must have led to central circulatory improvements that could have transferred to the untrained leg.

Improvements in DL $\dot{V}O_{2max}$ after a SL training intervention are inconclusive as results from some investigations reflected an increase[24,26] in DL $\dot{V}O_{2max}$ after the exercise training intervention, whereas others did not show such improvements[27,28].

Furthermore, SL cycling was shown to be superior to DL cycling in improving single-limb aerobic capacity, vascularization, oxidative potential, and metabolic profile of the active skeletal muscles[29,25]. Skeletal muscle glucose transport was shown to increase significantly along with mitochondrial oxidative capacity in the SL cycling mode after three weeks of SL training, developing the limbs' metabolic profile faster than DL training[29]. Further, peripheral adaptations post-SL training seem to occur at a greater extent in the trained leg rather than the untrained leg or DL as demonstrated after 6 weeks of SL training where mitochondrial function significantly increased[30]. The increase in metabolic potential observed in these two previously mentioned studies could be attributed to the high power outputs achieved in one leg and the increased blood flow in the exercising limb[29,25].

Finally, there is a growing body of literature arguing that the inactive limb during SL cycling is not completely passive. Studies have shown increases in $\dot{V}O_2$, blood flow, and carbohydrate utilization above resting levels in the inactive limb[31,32,33]. Increases in muscle blood volume measured by near-infrared spectroscopy were seen in a SL graded exercise test in both the active and inactive limbs, making an argument that blood volume changes are controlled centrally rather than locally[34]. Even though both central and local adaptations to endurance training are well-accepted, the contribution of each of these components to the overall change in cardiovascular function/performance after an exercise training intervention are less clear.

Chapter Two: Literature Review

2.1 Ramp incremental testing for evaluation of maximal aerobic performance and exercise intensity thresholds

It is common that coaches and trainers prescribe exercise intensities based on several zones identified from different measures and markers. In exercise physiology laboratory settings, incremental exercise tests to exhaustion are commonly performed, from which valuable maximal and submaximal aerobic performance information can be obtained. Determination of $\dot{V}O_{2max}$ is considered the gold-standard to evaluate cardiovascular/respiratory fitness[5]. From a submaximal point of view, two clear demarcation points are commonly identified: the lactate threshold and the anaerobic threshold. As originally proposed by Beaver et al.[35], the lactate threshold separates the moderate from the heavy intensity domains, and the anaerobic threshold represents the boundary between the heavy and the severe domains of exercise. Even though these demarcation points (or thresholds) seem evident, the terminology surrounding them is often confusing due to the great variability in the denominations that are presented in the literature[36]. An important factor affecting the use of different names to refer to a similar metabolic boundary is related to the type of test used to determine the threshold. When examining pulmonary gas exchange data from an incremental test to exhaustion to identify exercise thresholds, the GET demarcates the separation between the moderate and the heavy intensity domains. Similarly, the RCP demarcates the boundary between the heavy and the severe domains of exercise, with the metabolic rate at the RCP corresponding to that observed at the MLSS[11]. When exercising at intensities in the severe domain, prolonged durations of exercise are unachievable (with time to exhaustion being exponentially shorter with progressively greater intensities) and, theoretically, $\dot{V}O_{2max}$ will be achieved.

2.1.1 Submaximal thresholds and exercise intensity domains

The GET has been used as a measure of aerobic fitness in clinical populations and endurance athletes[37,9]. It has been shown to be a useful marker in assessing the effectiveness of endurance training programs[9]. For example, elite endurance athletes with a high GET value can maintain a high fraction or percentage of their $\dot{V}O_{2max}$ for a longer period without evident signs of muscular exhaustion (unless the duration of exercise is excessively prolonged)[38]. Therefore, a change in GET is one way of gauging a change in aerobic fitness level.

In theory, GET demarcates the exercise intensity domain where aerobic adenosine triphosphate (ATP) production supports skeletal muscle metabolism[39]. Exercise intensities at or below GET do not tend towards muscular exhaustion and are maintained by the production of ATP primarily through aerobic respiration. GET usually occurs at approximately 50%-60% of $\dot{V}O_{2max}$ in untrained to moderately trained populations and may occur at around 80% of $\dot{V}O_{2max}$ in highly trained endurance athletes[39]. Typically, the GET is characterized from incremental tests to exhaustion and it has been defined as a non-linear increase in the volume CO_2 ($\dot{V}CO_2$) vs. $\dot{V}O_2$ as well as a non-linear increase in the rate of volume expired air (minute ventilation, $\dot{V}E$) vs. $\dot{V}O_2$ relationship. These physiological responses reflect increased hydrogen ions being buffered by intracellular bicarbonate thus producing CO_2 that is eliminated through the increase in ventilation[40,41].

The RCP defines a threshold where $\dot{V}E$ increases at a greater rate than the $\dot{V}CO_2$. It is characterized by a hyperventilatory response that occurs when blood bicarbonate buffering system is overwhelmed by the accumulation of intracellular hydrogen ions and CO_2[35] . At exercise intensities above GET, the ATP re-synthesis will continue to rely on aerobic respiration

as a main source of energy until the critical intensity of exercise is reached at a higher workload. RCP occurs at an intensity above the GET, at around 80-90% of $\dot{V}O_{2max}$ in an untrained to moderately trained individuals. Once this demarcation point is surpassed, exercise is performed above the critical intensity of exercise and ATP re-synthesis will rely more towards utilizing anaerobic rather than aerobic ATP production to meet the higher muscular metabolic demands, as different muscle fiber types are being recruited.

As indicated earlier, the GET demarcates the moderate exercise intensity domain from the heavy, while the RCP demarcates the heavy exercise intensity from the severe exercise intensity domain. Determining the upper limit of the aerobic system (i.e., $\dot{V}O_{2max}$) along with the GET, and the RCP provides valuable information to understand different physiological responses to exercise.

2.1.2 Physiological adaptations to exercise and factors affecting maximal aerobic performance

Testing the limit of exercise tolerance is commonly done by using a ramp incremental (RI) test to exhaustion. During this type of test, a linear increase in systemic measures of Q is typically observed in order to meet the increasing O_2 demand in response to the increased muscular work, until $\dot{V}O_{2max}$ is reached and the exercise can no longer be sustained[42]. $\dot{V}O_{2max}$ is a representation of the maximal aerobic capacity, which is widely used to asses the upper limit of cardiovascular/respiratory health and performance, as well as establishing different exercise intensities, since endurance training is often prescribed as a percent of $\dot{V}O_{2max}$[43]. $\dot{V}O_{2max}$ is achieved during severe intensity exercise, when a large portion of the muscle mass is being exercised (swimming, running, cycling), hence measuring the upper limit of the O_2

transport/utilization system. Thus, it assesses the combined functioning of the pulmonary, cardiovascular, and muscular systems to uptake, transport and utilize O_2 primarily in the contracting muscle mitochondria[7].

Regular exercise, specifically endurance training, has been reported to be associated with adaptations that result in increases in $\dot{V}O_{2max}$[44]. Factors that are commonly observed and known to have an impact in improving $\dot{V}O_{2max}$ are cardiovascular, hematological and metabolic adaptations[45]. Any adaptation influencing $\dot{V}O_{2max}$ must be reflected with changes in the factors of Fick's equation: $\dot{V}O_2 = Q \times a\text{-}vO_{2diff}$ [45]. As previously mentioned, central adaptations include enhanced O_2 carrying capacity of the blood, increased blood volume, and improved heart structure/function. The above listed adaptations, may contribute to increases in arteriovenous O_2 difference ($a\text{-}vO_{2diff}$)and increases in SV affecting Q at maximal exercise, thus contributing to an increase in $\dot{V}O_{2max}$ according to the Fick equation.[45] When taking the following Q equation into consideration (Q = SV x HR), the above-mentioned increases in central adaptations are exclusively thought to be a result of enhanced SV as maximal HR is unaffected by endurance training[46,45,47]. Similarly, peripheral adaptations to training such as a decrease in vascular resistance leading to enhanced venous return may further contribute to increasing Q; while increased capillarization and more efficient blood flow may increase the $a\text{-}vO_{2diff}$, thus having an impact on $\dot{V}O_{2max}$[45]. Taken together, both central and peripheral components seem to contribute to the improvement of $\dot{V}O_{2max}$, however the extent of this contribution from each of these components mentioned in the above noted equations towards the improvement and limitation of VO_{2max} is an issue of continued debated in the field of exercise physiology.

2.1.3 Variables contributing to the determination and limitation of $\dot{V}O_{2max}$

Indeed, every step of the O_2 delivery pathway has the potential to impact the final $\dot{V}O_{2max}$ outcome, starting from ventilation, alveolar-capillary diffusion, to circulation and muscle diffusion and finally to the mitochondria, where it is utilized to produce energy. Hence it is difficult to claim one determinant of $\dot{V}O_{2max}$. Although the different components of the O_2 pathway are relatively well understood and established, there is continuous debate in terms of how each of these steps connect and work together in determining $\dot{V}O_{2max}$. It is very important to understand each of these components for the reason that they ultimately contribute to overall functions in O_2 delivery and utilization.

In an attempt to summarize the O_2 transport system (taking into consideration each of the formerly mentioned steps) Wagner et al.[2,1] portrayed a series of diagrams describing the association between $\dot{V}O_2$ to muscle venous partial pressure of O_2 $(PvO_2)^2$ as illustrated in figure 1. These diagrams show how different components of the O_2 pathway can be limiting to $\dot{V}O_{2max}$.

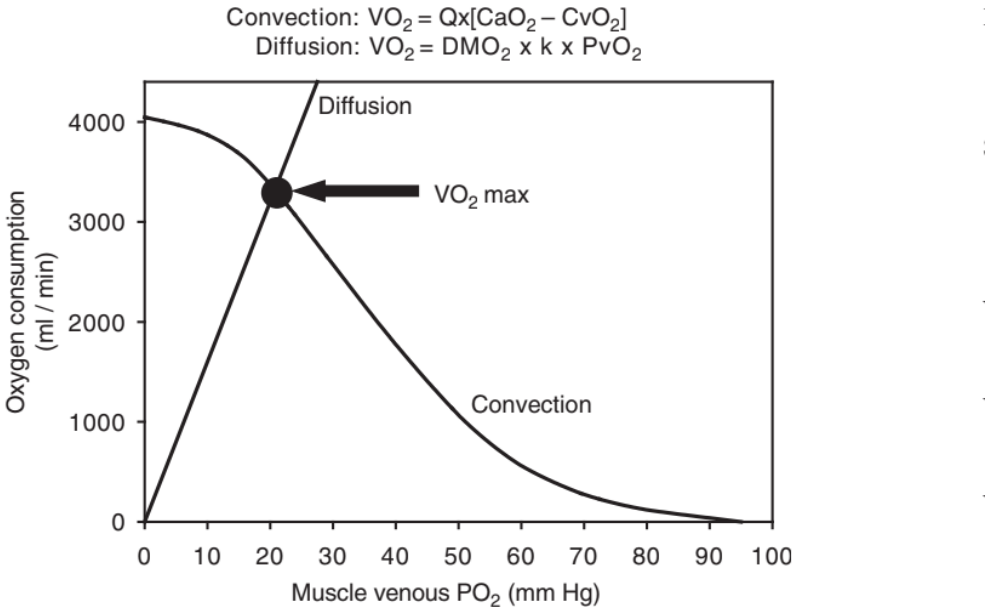

Figure 1: plot of VO_2 against muscle venous PO_2 along with the conservation of mass equations, demonstrating the convective flow of O_2 into the muscular microcirculation and the diffusive flow of O_2 from the muscular microcirculation to the mitochondria. The point of intersection represents the point where conservation of mass occurs illustrating the value of $\dot{V}O_{2max}$. Adapted from Wagner et al.[1]

The diagram was constructed based on the following two conservation of mass equations: first the Fick principle $\dot{V}O_2 = Q \times a\text{-}vO_{2diff}$ which accounts for the convection flow of O_2 into the muscular microcirculation[1]. The second equation considers the diffusive flow of O_2 from the muscular microcirculation to the mitochondria measuring the muscular O_2 diffusive capacity (DMO_2) otherwise known as Fick's law of diffusion and written as $\dot{V}O_2 = DMO_2 \times k \times P_{vO2}$[1]. The solid point in Figure 1 represents $\dot{V}O_{2max}$, as both equations yield the same $\dot{V}O_2$ and P_{vO2} requirements for mass conservation on the graph. Furthermore, Wagner et al.[2] demonstrates how individual changes in the key independent variables of these equations can impact and alter $\dot{V}O_{2max}$ as shown in Figure 2.

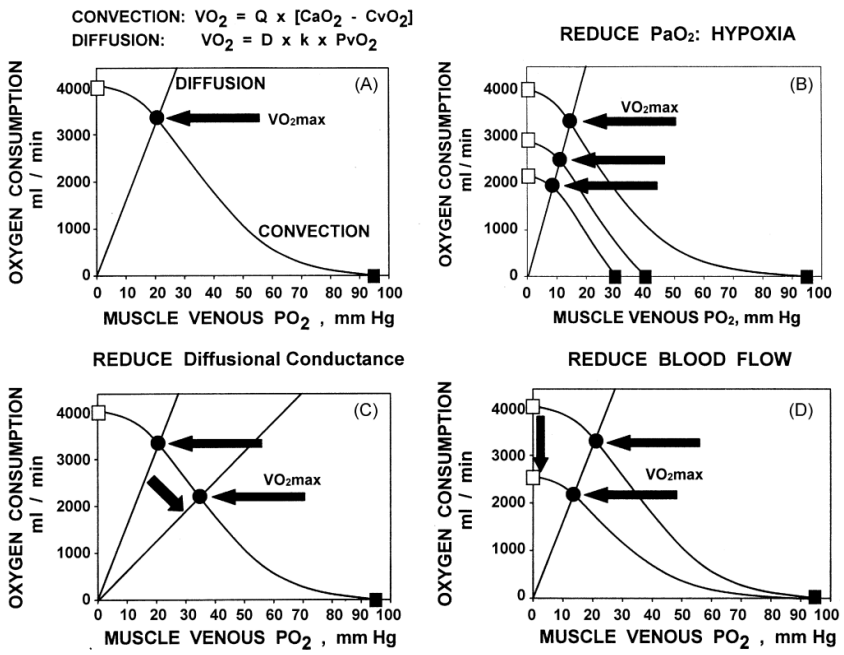

Figure 2: (Panel A) a diagram associating $\dot{V}O_2$ to P_{vO2} combining the convective circulatory and the muscular diffusive components together. (Panel B) effects of different degrees of arterial hypoxia on $\dot{V}O_{2max}$. (Panel C) the effects of reduced muscular diffusive capacity on $\dot{V}O_{2max}$. (Panel D) effects of reduced blood flow on $\dot{V}O_{2max}$. Adapted from Wagner et al.[2]

Panel A of Figure 2 represents the circulatory convective and muscular diffusive components determining $\dot{V}O_{2max}$ under regular conditions where no variable is altered. Panel A can also be used as a reference point in comparing the different outcomes observed in the other panels of Figure 2. Altering the inspired partial pressure of O_2 leads to downstream changes in ventilation and lung diffusing capacity that in turn would change the partial pressure of arterial O_2 finally shifting VO_{2max} as seen in Panel B where progressive arterial hypoxia was induced, and the circulatory convection was shifted. Panel C demonstrates a reduction muscular diffusive capacity, consequently $\dot{V}O_{2max}$ was shown to decrease while $\dot{V}O_2$ was shown to increase. On the

contrary to Panel C, Panel D reflected an increase in $\dot{V}O_{2max}$ and a decrease in $\dot{V}O_2$ due to impaired blood flow which has shifted the circulatory convective component.

Each component of the O_2 transport and utilization is crucial to the overall functionality of the O_2 uptake and consumption process. Wagner et al.[2] brought together two well established O_2 transport equations discussing both the central and peripheral components contributing to $\dot{V}O_{2max}$.Taking all variables into consideration (ventilation, a-vO_{2diff}, Q, DMO_2) it is difficult to pinpoint one factor limiting $\dot{V}O_{2max}$. The intersection point of $\dot{V}O_2$ and P_{vO2} in Figure 1 represents the point where conservation of mass occurs, indicating the value of $\dot{V}O_{2max}$ for a given Q, DMO_2, and a-vO_{2diff}. Thus altering any of these variables will result in a shift within the lines of the diagrams (Figure 2), which will eventually affect the value of $\dot{V}O_{2max}$. Therefore, each of the convective and diffusional components of O_2 transport as discussed are powerful enough to play a role in determining and limiting $\dot{V}O_{2max}$[2,1].

In general terms, two opposing arguments exist in the literature as to what factors limit $\dot{V}O_{2max}$. Whereas some advocate that cardiac output is the main limiting factor, others discuss that peripheral components are equally important in determining and limiting $\dot{V}O_{2max}$. For this reason, understanding the interplay and contributions amongst components of the central and peripheral systems is quite important.

2.2 Central vs. peripheral contributions to maximal exercise

Although some argue that cardiac output might be the main limiting factor for $\dot{V}O_{2max}$[48,49,50,51,52], others argue that peripheral components are as important[28,25,53,54]. Therefore, isolating each component of Fick's equation ($\dot{V}O_{2max}$ = Q x a-vO_{2diff}) is important as it provides further insight on training adaptations pertaining to the peripheral and central components of the

equation and their contribution to $\dot{V}O_{2max}$. It is usually accepted that improvements in $\dot{V}O_{2max}$ are mostly in response to an increase in Q_{max} post endurance training[45,55]. Studies have shown training induced increases in $\dot{V}O_{2max}$ are mainly explained by an increase in Q_{max} rather than a widening in a-vO_{2diff}. Saltin et al.[56] investigated $\dot{V}O_{2max}$ in sedentary individuals after fifty days of submaximal and maximal training, the reported difference in $\dot{V}O_{2max}$ was mainly due to improvements in Q. In another study $\dot{V}O_{2max}$ increased from 3.16 $L \cdot min^{-1}$ to 3.68 $L \cdot min^{-1}$ mainly due to the increase in Q by 8% while a-vO_{2diff} only increased by 3.6% after sixteen weeks of endurance training[16].

Alterations in blood volume seem to also play a crucial role in the increase of Q_{max}, as demonstrated after a six week training intervention[46]. Training induced changes in blood volume were shown to be the main mechanism for increasing Q_{max} by 6% after six weeks of training; after normalization of blood volume by phlebotomy, a reversal in Q_{max} to pre-training values resulted in a reduction in $\dot{V}O_{2max}$ also similar to pre-training values, indicating the significance of blood volume on Q[46]. The importance of blood volume during maximal exercise on SV and Q was also demonstrated by Krip et al.[52], who indicated that higher blood volume led to increased diastolic filling which in turn significantly increased SV, Q, and $\dot{V}O_{2max}$. Similarly, the opposite was true when blood volume was reduced by 500 ml through phlebotomy procedures, $\dot{V}O_{2max}$ decreased by 12.7% compared to baseline[52].

Further, Mortensen et al.[50] reported that the inability of the circulatory system in sustaining a linear increase in O_2 delivery to the working muscle limits $\dot{V}O_{2max}$[50]. The former conclusion was a result of a plateau in $\dot{V}O_2$, Q, and leg blood flow before reaching VO_{2max} during a RI test to exhaustion[50]. A decline in $\dot{V}O_2$, Q and leg blood flow once fatigue is reached during maximal exercise was also reported in a study that compared cycling under an

environmental heat stressor and normal conditions[57]. Heat stress significantly lowered $\dot{V}O_{2max}$ by causing rapid declines in Q, and mean arterial pressure which in turn led to the fall in leg blood flow, O_2 delivery and uptake compared to normal conditions[57]. The interpretation of a central limitation was made since diffusive O_2 transport across the leg muscle was not impaired, as a-vO_{2diff} and O_2 extraction increased until the end of exercise preventing any sudden drops in O_2 diffusion at the time when O_2 delivery to the leg was dropping. Therefore the authors conclude that the decline in convective O_2 transport to the leg muscle was more likely the cause of reduction in leg $\dot{V}O_2$ before exhaustion under both environmental conditions[57].

Evidence within the literature seem to favor central factors to limit $\dot{V}O_{2max}$. However peripheral factors cannot be excluded since it is known that at $\dot{V}O_{2max}$ O_2 extraction is not at 100%, meaning that a diffusion limitation of O_2 from the muscle microvasculature to the mitochondria might exist[1]. In a study that aimed to understand the roles of vascular factors limiting O_2 supply and metabolic factors limiting O_2 uptake by analyzing femoral venous blood return from the muscles during maximal exercise, the authors concluded that O_2 extraction in leg muscles is not maximal under normal conditions and that the capacity for O_2 utilization within the mitochondria contributes as a limiting step[58]. However others would argue that increasing O_2 supply to the tissue either by hyperoxia[59] or increased blood flow[60] would increase $\dot{V}O_{2max}$, an indicator that reactions within the mitochondria are not rate limiting to O_2 utilization[61]. Nevertheless, Roca et al.[61] speculated that convective O_2 delivery by circulation plays a secondary role in determining $\dot{V}O_{2max}$, and that $\dot{V}O_{2max}$ is limited by the rate of O_2 diffusion in its pathway to the mitochondria. The authors argued capillary PO_2 and the tissue diffusing capacity are two important components in determining $\dot{V}O_{2max}$.

Taken together, SV_{max} and Q are often considered the main limiting factors for $\dot{V}O_{2max}$. Although controversy exists on the issue of "what limits $\dot{V}O_{2max}$, the heart, the lungs, or the muscle?", perhaps a better question to ask is "how important are the various independent variables to $\dot{V}O_{2max}$?" as proposed by Wagner et al[2].

2.3 Evaluating the central and peripheral responses to training utilizing the single-leg cycling model.

Over the past five decades, there has been an interest in comparing traditional DL cycling training to SL cycling, mainly to investigate central adaptations vs. peripheral adaptations derived from these training conditions. An early study suggested that the main limiting factor to physical performance during DL cycling is peripheral in origin and related to O_2 utilization within the muscle[62]. It should be noted though, that this idea is supported by evidence where the exercising muscle mass is reduced (as in SL cycling not DL) and supposedly Q is no longer a key contributor to limiting performance[53]. In opposition to the idea of DL cycling being limited by the periphery as discussed by Kaijser etl al.[62]; a study by Davies and Sargeant[28] where one leg (left or right) was trained over four occasions followed by training the other leg on four separate occasions demonstrated that both the right and left leg increased $\dot{V}O_{2peak}$, while no significant impact was observed on $\dot{V}O_{2max}$ after three session of DL cycling exercise. These results support the views that many authors have, claiming that the limiting factor to achieve maximal aerobic performance during exercise where a large muscle group is engaged (e.g., DL cycling), is the ability of the cardiovascular system to transport the required volume of O_2 to the active tissues, rather than the capacity of the muscle to utilize O_2; however, the upper limit of $\dot{V}O_2$ seems to be

dependent on the aerobic capacity of the working muscle and not maximal Q during SL cycling [28,53,48,49].

Identifying exercise intensity domains is important for training adaptation and depending on the intensity of training the preponderance between peripheral and central adaptations might be shifted. In general, submaximal training programs are associated with central adaptations that may lead to an overall improvement in the total amount of physical work that can be performed, as well as increments in $\dot{V}O_{2max}$ in healthy subjects[63]. On the other hand peripheral adaptations to submaximal exercise may also play a role in increasing Q_{max}, which is thought to be brought by a decrease in systemic vascular resistance which in turn leads to an increase in venous return[25,64]. Peripheral adaptations after SL cycling at submaximal intensities were shown to increase skeletal muscle capillarization[65], mitochondrial function/content[24,29], and further enhance oxidative capacity[66]. However it is unclear how much of an impact the mentioned peripheral adaptations have on overall $\dot{V}O_{2max,}$ keeping into consideration that oxygen diffusing capacity and mitochondrial oxidative capacity exceed O_2 delivery during exercise modes involving half or more of the body's muscle mass[45,67].

Evaluating $\dot{V}O_{2max}$ is important as it allows analysis of the functional characteristics of the O_2 transport system (O_2 uptake, transport, and utilization) and its adaptations in response to exercise training[68]. Different studies have used continuous and/or interval training methods that were reported to be effective for increasing $\dot{V}O_{2max}$[53,69]. However, as previously mentioned, $\dot{V}O_{2max}$ accounts for whole body exercise at maximal performance; therefore, factors limiting $\dot{V}O_{2max}$ during DL cycling may be different compared to factors limiting $\dot{V}O_{2peak}$ during SL cycling. In general, during SL cycling the increase in $\dot{V}O_2$ is followed by a similar linear increase in Q, as also observed in DL cycling[25,70,53]. However Q and $\dot{V}O_2$ are higher during DL cycling by

difference of 2.5 $L \cdot min^{-1}$ and 0.67 $L \cdot min^{-1}$ respectively compared Q and VO_2 during submaximal SL cycling (similar differences at maximal cycling)[25]. SL increases in $\dot{V}O_{2peak}$ ranging from 16% to 25% have been reported in the trained leg after 4-6 weeks of training in all studies using a SL model[70,24,28,30]. The pronounced increase in SL $\dot{V}O_{2peak}$ is thought to be a result of an increased leg a-vO_{2diff} and an increased blood flow being directed to the active limb, the former two variables were commonly emphasized during SL cycling[25].

2.3.1 Physiological responses and adaptations to single-leg cycling.

Early investigations implemented SL cycling mainly to compare and contrast the physiological responses between SL and DL; a general conclusion has been that the periphery limits $\dot{V}O_{2peak}$ in one leg exercise unlike the double leg model, where the central mechanisms play a bigger role[28,53,27]. In a later investigation conducted by Saltin et al.[24], participants trained one leg by either sprint bouts or endurance training while the other leg remained untrained . Both groups improved $\dot{V}O_{2peak}$ from baseline values by 15% and 24% for the sprint and endurance training conditions, respectively. Importantly, the findings from the study also indicated slight improvements in the untrained leg, DL $\dot{V}O_{2max}$, and Q_{max} post SL training intervention, indicating that SL training may have some influence on the central mechanisms of adaptation. However, previous findings from Davies & Sargeant et al.[28] did not reflect any improvements in Q of contralateral and DL after SL training, thus causing a controversy within the literature.

On average, only 70 to 80% of the $\dot{V}O_{2max}$ observed during DL cycling is reached during SL cycling[25,53,27]. Similarly, Q_{max} during SL exercise has been reported to represent 87% of that reached in DL cycling at $\dot{V}O_{2max}$[27]. Nonetheless, some studies have shown that the observed increase in Q during maximal SL exercise post training was explained by an increase in SV. As

described by others[53,70], this increase in Q was only detected during exercise of the trained but not the untrained limb. Since changes in Q were not observed during exercise of the untrained limb, this information may indicate that peripheral adaptations in the trained leg such as increased leg blood flow and vascularization may play a role in contributing to an increased Q.

In a SL exercise training study by Thomas et al.[70], either the right or left leg was trained for 4 weeks, followed by 4 weeks of training the opposite leg. The results were consistent with previously mentioned findings when it comes to SL[24,25,28], as $\dot{V}O_{2peak}$ increased by 19.8%, and also a significant increase in Q was reported. An interesting finding was that Q did not further increase after training the untrained leg. In fact, a drop in SV was observed, which in turn led to a decrease in cardiac output[70]. Since no change was observed in Q when the untrained limb was tested following 4 weeks of SL training, the increase in Q and SV reported after testing the trained leg is thought to be due to peripheral adaptations in the trained leg and not to alterations in cardiac structure or function[70]. Although it seems somewhat difficult to accept that peripheral changes have an effect on Q and SV, the authors speculated that due to an increase in capillarization after SL training, total peripheral resistance in the trained leg was decreased (as demonstrated by Klausen et al.[25]) and that this would result in improvements in venous return that would allow for an enhanced diastolic filling[70]. A significant increase in DL VO_{2max} was observed even though Q and SV remained almost the same. This significant change in DL $\dot{V}O_2$ was likely the result of the non-significant increase in Q and widening of a-vO_{2diff}, as further evidence from an echocardiograph that showed no difference in the assessment of the left ventricular dimensions, mass, and performance before and after the training program[70]. Furthermore, the reported increase in DL $\dot{V}O_{2max}$ and Q was also demonstrated amongst the previously noted findings of Klausen et al.[25] after an 8-week SL training program. This increase

in DL $\dot{V}O_{2max}$ was deemed to be solely attained due to an increase in a-vO_{2diff} as maximal leg blood flow remained unchanged during that mode of cycling[25].

In support of the speculation made by Thomas et al.[70], Klausen et al.[25] reported a large improvement in $\dot{V}O_{2peak}$, Q and capillarization after exercising the trained leg. This greater $\dot{V}O_{2peak}$ was likely caused by the combined effect of increased blood flow and a-vO_{2diff} , while the increase in Q was thought to be due to the predominant increase in SV during SL cycling[25]. Additionally, the local increase in leg blood flow may play a mechanistic role in the structural remodeling of large conduit arteries such as the femoral artery. In relation to this, a SL endurance training study has been shown to induce arterial and venous expansion in the femoral artery and vein of the trained leg, which was shown to be strongly and positively correlate with changes in one-legged $\dot{V}O_{2peak}$ [65].

The impact of SL exercise training on DL maximal performance is not clear as discrepancies exist amongst reported findings. Whereas a study has reported a significant increase in DL $\dot{V}O_{2max}$ after SL training[26], and others have reported a small increase with no statistical significance[24,25], some other studies have indicated no increase in DL $\dot{V}O_{2max}$[28,53,27]. The uncertainty amongst these findings may be a result of the different durations of the exercise training protocols (4, 6, or 8 weeks), the exercise intensities (maximal or submaximal), the frequency used in each one of them, and the cycle ergometer set up across different SL exercise training studies.

2.3.2 Advantages and disadvantages of the single-leg cycling model

SL cycling is not the most comfortable mode of exercise. For example, it has been shown that this exercise modality produces an unnatural movement pattern where the hip flexor muscles

are more engaged than during normal cycling, causing undesirable fatigue and discomfort in comparison to traditional double leg cycling[71]. To overcome the biomechanical strain of SL cycling, the use of a counterweight has been proposed to facilitate a more natural coordinated pedaling movement [29]. Although not the first to utilize a counterweight all the mentioned studies in this review prior to Abbis et al.[29] have used different experimental set ups on the cycle ergometer to accommodate for SL cycling, with minimal description on the actual experimental set up. This has created a conflictive situation for interpretation and comparison of results amongst different studies. In a recent comparison between non-counterweighted SL cycling, counterweighted SL cycling and DL cycling at three different intensities (40, 80, 120W), it was evident that HR, $\dot{V}O_2$, and rate of perceived exertion were significantly higher in the non-counterweighted group as participants also reported higher discomfort during the non-counterweighted modality[72]. Counterweighted SL cycling showed similar $\dot{V}O_2$ values, HR and energy expenditure when cycling at 40 W and 80 W in comparison to DL cycling at the same POs, indicating that the counterweight facilitates a motion that is more likely to elicit physiological responses that are more comparable to that of DL cycling.

Counterweighted SL cycling has also been reported to have significantly greater femoral artery blood flow up to 45%, 90% and 68% higher blood flow in comparison to DL cycling with lower cardiac effort across different exercise intensities (40, 80, 120W)[72]. Consistent with these findings, SL exercise has been shown to have less cardiorespiratory demands during continuous and interval SL cycling (9% lower) compared to DL cycling[73].

2.4 Summary

Exercise intensity thresholds and maximal aerobic performance can be well characterized from RI test to the limit tolerance, thus providing useful information in relation to overall cardiovascular health and physical performance. Although surrounded with controversy, it is

often thought that $\dot{V}O_{2max}$ is mainly restrained by SV and Q. To settle the formerly noted controversy, exercise physiologists sought to investigate central vs. peripheral components of exercise to further understand factors limiting $\dot{V}O_{2max}$. Some of the former investigations made their arguments by comparing SL and DL cycling at maximal and submaximal intensities with different exercise programs varying in intensity, duration, and set up for the non-active limb during cycling. Most authors that investigated SL cycling conclude that: the periphery becomes the main limiting factor to performance when performing SL cycling, while central mechanisms govern $\dot{V}O_{2max}$ during DL cycling or whole-body exercise. Furthermore, some SL cycling programs demonstrated post SL cycling improvements affecting central components, while other investigators did not witness such findings. Although SL cycling is well-established, it seems that the set up used to accommodate the non-active leg was not consistent in the past; the use of a counterweight during SL cycling may provide a more accurate characterization to the training response elicited during SL cycling in a way similar to DL cycling. Therefore, there is a need to examine the exercise intensity thresholds, $\dot{V}O_{2max}$, and power output generated utilizing the counterweighted SL cycling model which was demonstrated to facilitate a smoother cycling motion almost mimicking that of DL cycling. The following chapter presents a SL training study that aimed to fill this need.

Chapter Three: Single -Leg Exercise Training Study

Effects of a single-leg training intervention on single and double-leg peak power output, maximal oxygen consumption, gas exchange threshold, and the respiratory compensation point in healthy untrained males.

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3.1 Abstract

This study examined the effects of a counterweighted single-leg (SL) cycling training program on SL and double-leg (DL) cycling performance during a ramp incremental (RI) test to exhaustion. Ten healthy untrained males (age, 28±8 yrs; height, 173±7 cm; weight, 79±3 kg) performed two DL RI tests, two counterweighted SL RI tests (one for each leg) and two to three constant-load trials to determine the power output (PO) at maximal lactate steady state (MLSS) in the leg that was randomly assigned for the counterweighted SL training intervention. Training took place 3x week for four weeks at intensities in the heavy and severe domains. The peak power output (PPO) reached during DL RI to exhaustion was greater post- (318±18 W) compared to pre-training (294±24 W). Similarly, DL maximal oxygen consumption ($\dot{V}O_{2max}$) was greater at post- (3.25±0.21 L·min^{-1}) compared to pre-training (3.07±0.22 L·min^{-1}). Both the gas exchange threshold (GET) and the respiratory compensation point (RCP) increased following exercise training (GET: post,1.93±0.11 L·min^{-1}; pre,1.65±0.13 L·min^{-1}; RCP: post,2.70±0.21 L·min^{-1}; pre, 2.53±0.16 L·min^{-1}; $p<0.05$) along with the PO associated with these thresholds (GET:post,147±16 W; pre,134±19 W; RCP:post,226±25 W; pre, 203±19 W; $p<0.05$). SL peak oxygen consumption ($\dot{V}O_{2peak}$) and PPO was greater in the trained leg after the exercise intervention ($\dot{V}O_{2peak}$: post 2.87±0.28 L·min^{-1}; pre, 2.44±0.22 L·min^{-1}; $p<0.05$). Similarly, the $\dot{V}O_2$ associated with both GET and RCP increased after exercise training in the trained leg (GET: post,1.56±0.14 L·min^{-1}; pre, 1.34±0.17 L·min^{-1}; RCP: post, 2.23±0.20 L·min^{-1}; pre, 1.84±0.17 L·min^{-1}; $p<0.05$) along with an increase in PO (GET: post, 88±18 W; pre, 73±17 W; RCP: post,128±15 W; pre,107±13 W; $p<0.05$). No post-exercise training changes were observed for the untrained leg for any of the variables evaluated in this study. In conclusion, this study

demonstrated that a SL exercise training program using a counterweighted model provided beneficial training effects to the trained leg and to the DL cycling performance.

3.2 Introduction

Testing the limit of aerobic performance (i.e., maximal oxygen uptake; $\dot{V}O_{2max}$) is one of the most widely used tools in the field of exercise physiology and human performance. Commonly, $\dot{V}O_{2max}$ is measured during incremental tests to exhaustion, which are useful not only to determine the maximal aerobic capacity of the system, but also important physiological landmarks such as the gas exchange threshold (GET) and the respiratory compensation point (RCP), which define the metabolic boundaries between the moderate and heavy, and the heavy and severe exercise intensity domains, respectively[8,10]. Thus, incremental tests to exhaustion provide valuable information to evaluate the overall maximal and submaximal performance of the cardiovascular/respiratory system[9], to classify different levels of aerobic performance[9], and to assess changes subsequent to an exercise training intervention[12,17,23].

From a maximal performance perspective, the mechanisms that control the $\dot{V}O_{2max}$ response remain a subject of continued debate[7]. Whereas some studies have proposed that the main limiting factor to $\dot{V}O_{2max}$ lies within the ability of the cardiovascular system to deliver the required volume of O_2 to the working muscles[48–51], others argue that peripheral components such as the muscle's ability to utilize O_2 also plays a critical role controlling maximal aerobic performance[1,25,28,53,54]. Despite this controversy, it is important to recognize that both central and peripheral components are part of the O_2 transport pathway, and that ignoring any single aspect of the overall system would be wrong[2].

Although different strategies can be used to try to determine the contribution of central and peripheral components to the $\dot{V}O_{2max}$ response, the single-leg (SL) cycling mode of exercise has been shown to be an effective model that allows for isolation of central (i.e., cardiac output/bulk delivery of O_2) from peripheral (i.e., within the muscle) adaptations to exercise

training[24]. SL exercise to volitional exhaustion seems to be limited by peripheral rather than central components, since Q has been shown to only reach 87% of the double leg (DL) Q_{max} during SL maximal exercise[27]. This indicates that during SL exercise, during which the heart's maximal capacity to pump blood to the active muscles has not been met and redistribution of blood flow to the working leg is increased, maximal cycling performance might be limited by the aerobic capacity of the muscle[28,48,53].

An advantage of the SL cycling model is that it enables a better characterization of peripheral adaptations to exercise, considering that one leg is being trained while the other leg remains untrained. Studies assessing the physiological responses to SL cycling have reported significant peripheral changes such as increased capillarization, femoral artery size, and mitochondrial content within the trained but not the untrained leg[25,29]. These peripheral adaptations have been speculated to decrease leg vascular resistance, thus facilitating venous return, which has been proposed as a potential cause for an enhancement in diastolic filling and increase Q and $\dot{V}O_{2peak}$ post SL training[70]. Hence, training one leg stimulates peripheral adaptations, some of which might also have a positive impact in central components of exercise.

A limitation of SL cycling is the unnatural movement pattern. For example, it has been demonstrated that SL cycling results in increased engagement of the hip flexor muscles, which causes undesirable fatigue and discomfort in comparison to DL cycling[71]. To overcome the biomechanical strain of SL cycling, a model including a counterweight attached to the crank of the non-cycling leg has been used to facilitate a more natural and coordinated pedaling movement[29]. In a recent study comparing non-counterweighted to counterweighted SL cycling (at different intensities) as well as DL cycling, greater HR, $\dot{V}O_2$, rate of perceived exertion, and discomfort were found in the non-counterweighted compared to the counterweighted group at

40, 80 and 120 Watts (W), respectively[72]. Additionally, counterweighted SL cycling was reported to have significantly greater femoral artery blood flow in comparison to DL cycling, indicating increased redistribution of blood flow to the active leg[72].

The previous findings demonstrate that utilizing counterweighted SL cycling model can contribute to characterizing physiological responses during incremental tests to exhaustion that better mimic those observed during DL cycling. Therefore, the main goal of this study was to examine the effects of a counterweighted SL endurance training program on SL and DL $\dot{V}O_{2max}$, GET, and RCP, and to examine how central (i.e., HR) responses were affected by training. We hypothesized that an increase in SL $\dot{V}O_{2peak}$, GET and RCP would lead to an increase in DL $\dot{V}O_{2max,}$ GET, and RCP, since improvements in peripheral component that are associated with SL cycling training might also impact central components.

3.3 Methods

3.3.1 Participants

Ten healthy males volunteered and completed this study (mean ± SD values: age 28 ± 8 yrs; height 173 ± 7 cm; weight 79 ± 3 kg). All participants were non-smokers, free of any musculoskeletal conditions that could limit their maximal exercise exertion and were not undergoing any medical treatment that could alter their cardiovascular responses to exercise. Participants were aware of the risks and benefits of participating in the study and signed a physical readiness questionnaire (Appendix A), and also all signed an informed consent (Appendix B) that was approved by the Conjoint Health Research Ethics Board of the University of Calgary (REB 18-0041) (Appendix C), and that was conducted in accordance with the declaration of Helsinki.

3.3.2 Experimental Design

During the pre-training testing phase, each participant visited the laboratory on 6-7 occasions to complete: *i*) two DL RI tests, *ii*) two counterweighted SL RI tests (one for each leg), and *iii*) two to three constant-load trials to determine the power output at MLSS in the leg that was later assigned for the counterweighted SL training intervention. Each test was separated by at least 48 hours and performed at a similar time of the day in an environmentally controlled laboratory (temperature: 19-20 °C; humidity 30-40%). All participants adhered to the following pre-training testing instructions: *i*) no vigorous physical activity the day prior to each test, and *ii*) no food or caffeinated beverages for at least 2 and 8 hours, respectively, prior to each test. Participants were blinded to the power output and to the elapsed time during all sessions but received visual feedback on their pedal cadence which was selected during the first testing session of each condition (i.e., DL and counterweighted SL) and maintained consistent during the following visits. The position of the handlebar and the seat was recorded during the first visit and kept consistent for the subsequent visits. Additionally, during all experimental conditions participants wore cycling shoes that attached to the pedals.

Standard anthropometric measurements (weight, height, and quadriceps skinfold and circumference) were taken during the first visit. Additionally, body fat, and lower limb fat and lean mass percentage was determined by dual energy x-ray absorptiometry (DXA) to further assess differences between the trained and untrained leg.

After the initial testing sessions, a leg was randomly assigned for SL cycling training, which was performed 3 times a week over the course of 4 weeks. The SL leg training sessions for each week were performed as follows: *i*) 30 min of constant-load SL cycling at the power output sustained during MLSS, *ii*) constant-load SL cycling at a power output 20% above MLSS

until volitional exhaustion, *iii*) 10-12 repetitions (or volitional exhaustion at any point before 12 repetitions) of HIIT SL cycling (one minute on, one minute off) at a power output corresponding to 75% of that reached during the SL RI test. The power output was re-adjusted after the second week of training by increasing the formerly mentioned percentages to keep the relative intensity of the training stimulus consistent. The re-adjustment of power output was based on participants reported RPE and measurements of [La] that were taken during every training session. The adjustment of power output was 10 W above the initial intensity. After the SL cycling training was completed, similar testing sessions as performed during pre-training were conducted to measure post-training changes in maximal and submaximal performance.

3.3.3 Cycling test measurements

Breath-by-breath gas exchange and ventilation were continuously measured using a metabolic cart (Quark CPET, COSMED, Rome, Italy), as previously described in our laboratory[74]. Calibration was performed before each test as recommended by the manufacturer.

The RI test to exhaustion was performed on an electromagnetically braked cycle ergometer (Velotron, RaceMate, Seattle, WA) were $\dot{V}O_{2max}$ ($\dot{V}O_{2peak}$ for SL exercise), peak power output (PPO), GET, and RCP were determined. The RI test consisted of a 4-min baseline cycling stage at 50 W followed by a 30 $W \cdot min^{-1}$ increment and 15 $W \cdot min^{-1}$ continuous increments in power output for DL and counterweighted SL cycling exercise, respectively. The RI test stopped when participants failed to maintain the targeted cadence by 10 rpm for more than ten consecutive seconds despite strong verbal encouragement, or when volitional exhaustion was reached.

During each counterweighted SL session, the electromagnetically braked cycle ergometer was fitted with a custom-built pedal that held a 6.84 kg counterweight. As previously

described[29,72], the use of a counterweight assists the exercising leg during the up-stroke phase, limiting the strenuous engagement of the ipsilateral hip flexor muscles. During each counterweighted SL cycling session, the non-exercising leg was kept in a resting position on a stationary platform. Two familiarization trials were performed for SL cycling before SL RI tests were conducted.

Measures of [La] were performed by drawing small blood sample through a small finger prick puncture using a lancet. A drop of blood was then immediately placed on a testing strip, which was placed into a portable lactate analyzer (Lactate Plus), which displays results within 13 seconds. The [La] measurements were taken at the beginning and at the end of every RI test as well as every 5 min during the 30-min MLSS trials. During each RI test and during the MLSS trials, participants were asked to indicate their rating of perceived exertion (RPE) using a 0-10 Borg Scale. Measures were taken at the end of the RI test, and every 5 min during the 30 min cycling MLSS protocol.

3.3.4 Data analysis

The raw gas exchange and ventilatory variables measured during the DL and SL RI tests were visually inspected by three independent experimenters for determination of GET and RCP as previously described[8]. Briefly, GET corresponded to the point at which $\dot{V}CO_2$ began to increase out of proportion in relation to $\dot{V}O_2$, coincidental with a systematic rise in the $\dot{V}E$-to-$\dot{V}O_2$ relation and end-tidal PO_2, whereas the ventilatory equivalent of $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$) and end-tidal PCO_2 were stable[8]. RCP was identified as the point corresponding to the systemic fall in end-tidal PCO_2 after a period of isocapnic buffering[8]. The RCP point was confirmed by examining the $\dot{V}_E/\dot{V}CO_2$ and $\dot{V}_E/\dot{V}O_2$ relationships as well as by identifying the second breakpoint in the $\dot{V}_E$-to-$\dot{V}O_2$ relation. The average of the three estimates from the independent

experimenters was used if the difference was within 100 ml·min^{-1}. Under the circumstances where the difference was greater than 100 ml·min^{-1}, the experimenters revaluated the profiles together until a consensus was reached. For the DL exercise, the GET and RCP were computed from the average of the two DL tests. For both DL and counterweighted SL exercise $\dot{V}O_{2max}$/$\dot{V}O_{2peak}$ corresponded to the highest VO_2 value computed from a 30 s rolling average. The highest $\dot{V}O_2$ value recorded during DL RI test corresponded to DL $\dot{V}O_{2max}$. While the highest $\dot{V}O_2$ value computed for the trained and untrained leg during SL cycling was expressed as $\dot{V}O_{2peak}$.

3.3.5 Statistical Analysis

Data are presented as mean ± standard deviation (SD). For the parameters including $\dot{V}O_{2max/peak}$, $HR_{max/peak}$, PO, VO_2 and HR associated with GET and RCP a repeated measure analyzes a variance (ANOVA) was performed for 3 conditions (DL, SLT, SLU) and two time points (baseline vs. post) to detect potential differences. Where appropriate a follow up Bonferroni post hoc analysis was run to identify the differences between times and conditions. Student's t-tests were used to compare mean values for anthropometric measurements. A Shapiro-Wilk test was performed to confirm data were normally distributed. Statistical significance was set at a α-level of <0.05.

3.4 Results

3.4.1 Anthropometric measurements

Participant anthropometric measurements are displayed in table 1. Whole body DXA scans showed a significant decrease in body fat % ($p = 0.028$), overall fat mass ($p = 0.036$) the trained leg fat mass ($p = 0.05$), skinfold measurements from the VL ($p = 0.037$) from pre- to

post-training evaluations. No significant differences were found for weight, limb fat %, limb lean mass, leg fat mass, skinfold, or thigh circumference in the untrained leg.

3.4.2 Ramp Incremental test Double-leg cycling

3.4.2.1 Maximal physiological responses

Physiological responses to maximal DL ramp incremental exercise are displayed in table 2. PPO was significantly increased from pre- (294 ± 24 W) to post-training (318 ± 18 W) ($p = 0.004$). $\dot{V}O_{2max}$ also increased from pre-training (3.07 ± 0.22 $L \cdot min^{-1}$) to post-training (3.25 ± 0.21 $L \cdot min^{-1}$) ($p = 0.047$). However, HR_{max} was not statistically different at pre-training (183 ± 7 bpm) compared to post-training (180 ± 6 bpm; $p = 0.362$). Further, [La] measured at exercise exhaustion was not different at pre- (10.4 ± 2.2 $mmol \cdot L^{-1}$) compared to post-training (11.3 ± 1.9 $mmol \cdot L^{-1}$) ($p = 0.329$).

3.4.2.2 Submaximal threshold response

Table 3 summarizes the PO, $\dot{V}O_2$, and HR associated with the GET and the RCP at pre- and post-training. During the DL ramp incremental test, the PO associated with the GET was significantly increased from pre- (134 ± 19 W) to post-training (147 ± 16 W) ($p = 0.011$). Similarly, the PO associated at the RCP was significantly increased from pre- (203 ± 19 W) to post-training (226 ± 25 W) ($p = 0.009$). The $\dot{V}O_2$ at the GET significantly increased from pre- (1.65 ± 0.13 $L \cdot min^{-1}$) to post-training (1.93 ± 0.11 $L \cdot min^{-1}$) ($p = 0.008$). The $\dot{V}O_2$ associated with the RCP also increased significantly from pre- (2.53 ± 0.16 $L \cdot min^{-1}$) to post-training (2.70 ± 0.21 $L \cdot min^{-1}$) ($p = 0.010$). No differences were observed in the HR associated with the GET at pre- (131 ± 15 bpm) compared to post-training (131 ± 9 bpm) ($p = 0.928$), or in the HR associated with RCP at pre- (154 ± 12 bpm) compared to post-training (156 ± 9 bpm) ($p = 0.801$).

3.4.3 Ramp incremental test counterweighted single-leg cycling.

3.4.3.1 Maximal physiological response

Physiological responses to maximal SL ramp incremental exercise are displayed in table 2. The PPO in the trained leg was significantly increased from pre- (142 ± 10 W) to post-training (176 ± 16 W) training ($p < 0.001$). PPO in the untrained leg did not significantly increase from pre- (146 ± 14 W) to post-training (154 ± 19 W) ($p = 0.157$). $\dot{V}O_{2peak}$ of the trained leg significantly increased from pre- (2.44 ± 0.22 $L \cdot min^{-1}$) to post-training (2.87 ± 0.28 $L \cdot min^{-1}$) ($p = 0.003$). No significant differences in $\dot{V}O_{2peak}$ were found in the untrained leg between pre- (2.45 ± 0.20 $L \cdot min^{-1}$) and post-training (2.47 ± 0.26 $L \cdot min^{-1}$) ($p = 0.838$). Peak HR in the trained leg increased from pre- (165 ± 10 bpm) to post-training (175 ± 6 bpm) ($p = 0.030$). Peak HR in the untrained leg were not statistically different at pre- (163 ± 17 bpm) compared to post-training values (161 ± 11 bpm) ($p = 0.793$). Post exercise [La] were not different in the trained leg at pre- (7.1 ± 1.4 $mmol \cdot L^{-1}$) compared to post-training (8.1 ± 1.7 $mmol \cdot L^{-1}$) ($p = 0.156$). Similarly, post exercise [La] in the untrained leg was not different at pre- (6.8 ± 2 $mmol \cdot L^{-1}$) compared to post-training (6.7 ± 2 $mmol \cdot L^{-1}$) ($p = 0.849$).

3.4.3.2 Submaximal threshold response

Table 3 depicts the PO, VO_2, and HR associated with the GET and the RCP at pre- and post-training. The PO associated with GET in the trained leg was significantly increased from pre- (73 ± 7 W) to post-training (88 ± 18 W) ($p = 0.009$). The untrained leg did not exhibit significant changes in the PO from pre- (76 ± 12 W) to post-training (87 ± 16 W) ($p = 0.103$). The PO associated with the RCP in the trained leg was significantly increased from pre- (107 ± 13 W) to post-training (128 ± 15 W) ($p < 0.001$). No significant differences in the PO associated

with RCP were observed in the untrained leg from pre- (109 ± 19 W) to post-training (121 ± 16 W) ($p = 0.081$). The $\dot{V}O_2$ at the GET significantly increased from pre- (1.34 ± 0.17 $L \cdot min^{-1}$) to post-training (1.56 ± 0.14 $L \cdot min^{-1}$) ($p = 0.002$) in the trained leg. Similarly, the $\dot{V}O_2$ associated with the RCP significantly increased from pre- (1.84 ± 0.17 $L \cdot min^{-1}$) to post-training (2.23 ± 0.20 $L \cdot min^{-1}$) ($p = 0.001$). The untrained leg did not exhibit any significant changes in $\dot{V}O_2$ at the GET (pre-, 1.39 ± 0.15 $L \cdot min^{-1}$; post-training, 1.40 ± 0.10 $L \cdot min^{-1}$; $p = 0.855$) or at the RCP (pre-, 1.93 ± 0.19 $L \cdot min^{-1}$; post-training 1.95 ± 0.12 $L \cdot min^{-1}$; $p = 0.809$). The HR associated with GET (pre-, 116 ± 11 bpm; post-training 121 ± 9 bpm; $p = 0.238$) and RCP (pre-, 134 ± 12 bpm; post-training, 144 ± 8 bpm; $p = 0.071$) were not statistically different in the trained leg. The HR associated with GET (pre-, 117 ± 13 bpm; post-training 115 ± 8 bpm; $p = 0.489$) and RCP (pre-, 135 ± 17 bpm; post-training 137 ± 8 bpm; $p = 0.718$) of the untrained leg was unchanged.

3.5 Discussion

The main aim of this study was to examine the effects of a counterweighted SL endurance training program on SL $\dot{V}O_{2peak}$, GET, and RCP (trained/untrained), as well as DL $\dot{V}O_{2max}$, GET, and RCP. Additionally, the HR response was examined as an indication of central responses that were affected by training. We hypothesized that an increase in SL $\dot{V}O_{2peak}$, GET and RCP would lead to an increase in DL $\dot{V}O_{2max,}$ GET, and RCP. To our knowledge this is the first SL cycling study to investigate changes in maximal as well as submaximal responses derived from incremental tests to exhaustion following SL endurance training. Our findings demonstrated significant increases in PPO, $\dot{V}O_{2peak}$, and peak HR in the trained but not in the untrained leg during SL counterweighted RI test. Furthermore, we found that the PO and the $\dot{V}O_2$ at the GET and RCP both increased significantly in the trained but not in the untrained leg, with no significant differences observed in the HR response at these thresholds. Additionally,

these exercise training adaptations in the trained leg contributed to a significant increase in PPO and $\dot{V}O_{2max}$, as well as in the PO and $\dot{V}O_2$ associated with the GET and RCP during DL exercise. Finally, whole body DXA scan reflected a significant decrease in overall body fat percentage and fat mass, as well as a significant decrease in fat mass and skinfold measurements (VL) of the trained leg.

The increases in SL PPO and $\dot{V}O_{2peak}$ (average change 34 W and 0.43 $L \cdot min^{-1}$, respectively) after four weeks of SL endurance training in this study were somewhat greater than those presented in previous investigations[25–27,53]. A possible explanation for this large increase could be the modality of SL exercise testing and training. By using the counterweighted SL model, participants were capable of performing a cycling movement that more closely resembled that performed during DL cycling, thus allowing them to postpone the peripheral fatigue that is commonly linked to the traditional SL exercise model[29]. Although not significant, the increase in the trained leg lean mass determined by the DXA scan could have contributed to the increase in PO, since McPhee et al.[75] previously demonstrated that muscle quantity plays a role in increasing VO_{2peak} and PPO during SL cycling. Additionally, it should be noted that the exercise training protocol in this investigation was very demanding from an intensity perspective, with all sessions performed at or above the critical intensity of exercise, as determined by measuring the PO associated with the MLSS (63% of SL PPO). In relation to this, it has been shown that physiological adaptations to exercise training are intensity dependent, with higher intensities of exercise producing a greater metabolic stress and, thus, a greater stimulus for adaptations[76].

Although this study did not evaluate mechanistic aspects that might be responsible for the increase in $\dot{V}O_{2peak}$ following the exercise training intervention, different lines of speculation can be made. First, peripheral improvements have been proposed to play a major role subsequent to

SL exercise training interventions[25,28,29]. For example, improvements in mitochondrial oxidative potential have been demonstrated after SL cycling training[24,26,29]. Additionally, increases in femoral artery size and capillarization, which facilitates more efficient redistribution of an already increase blood flow towards the active tissues have been shown after high intensity exercise training[45,77]. Thus, an increased blood flow to the exercising leg, an improved redistribution of that blood flow within the capillary network, and an increased oxidative potential in the mitochondria might all contribute to the increased $\dot{V}O_{2peak}$ in the trained leg after the exercise program[25,29,70,78,79]. In support of these speculations, a study showed that after seven weeks of SL endurance training, mitochondrial oxidative capacity, O_2 extraction and blood flow were significantly greater in the trained leg compared to the untrained leg, even when measured during DL cycling[78].

Even though SL high intensity training is often related to peripheral adaptations, the effects of SL endurance training might also result in increases in SV and, subsequently Q, as reported by Thomas et al.[70]. In the current study, measures of HR can be used as a proxy to speculate about the potential changes in Q. Interestingly, it was observed that following the SL exercise training intervention, SL peak HR increased by ~10 bpm during maximal performance. Even in the presence of unchanged SV, this increased HR response would indicate that a greater Q existed after training. Additionally, this significant increase in HR must be attributed to the greater increase in VO_{2peak} that is a consequence of peripheral adaptations. In this context, an increase in Q_{peak} would result in improved convective delivery of O_2, which in turn would lead to better diffusional capacity of O_2[1,2,61].

Some investigations have shown that SL cycling is limited by the periphery and not central factors[27,28,53]. Considering that in the SL exercise model a larger percent of Q can be

distributed towards the active leg, then it might be logical to think that, unlike what the current data suggest, increases in Q during peak exercise are not necessary in this exercise modality. In this context, the abovementioned potential increases in Q_{peak} might have played a significant role in the increases observed in $\dot{V}O_{2max}$ during the DL cycling mode, as previously hypothesized[24]. For example, it has been indicated that exercise training related venous expansions and increases in capillarization contribute to a decrease in total leg peripheral resistance[25,65,79], which would result in improved venous return towards the heart, thus allowing for enhanced diastolic filling[70] and greater Q. In this scenario, local peripheral adaptations that are related to single leg exercise training might have facilitated and increased convective delivery of O_2 which, together with some of the peripheral adaptations already discussed, might explain the greater $\dot{V}O_{2max}$ observed after exercise training even during DL exercise. Interestingly, it has been shown that the available mitochondria would be able to process more O_2 if delivered[29,30,54]. Then, the increased Q_{peak} should contribute to the increase in $\dot{V}O_{2peak}$ in both the trained and untrained leg during double leg exercise. On the other hand, the lack of peripheral and central training adaptations while exercising the untrained leg resulted in an unchanged post-training $\dot{V}O_{2peak}$ response.

From a submaximal performance perspective, this study indicated that the PO and $\dot{V}O_2$ associated with GET and RCP significantly increased in the trained leg as well as in the DL model from pre- to post-training, with no changes observed in the untrained leg. Interestingly, the HR response at the GET and RCP was not significantly different when comparing pre- to post-training values, despite the elevate work rate and metabolic rate. The lack of changes in HR in the presence of an increased metabolic demand suggests that a greater SV existed after the exercise training program as a greater Q should be expected given its linear relationship with $\dot{V}O_2$[25,28]. Similarly, these data suggest that the HR responses at the same absolute metabolic rate

should be lower, which indicates a training adaptation that is normally observed following endurance training[45]. Independently of the central adaptations indicated above, it is well accepted that when performing SL cycling, which involves a smaller muscle mass, the periphery becomes an important factor limiting maximal performance[28,48,49,53]. In fact, there is evidence that improvements in GET and RCP are normally accompanied by an increases in capillarization and improvements in mitochondrial functions[80–82]. As mentioned earlier, it is likely that these peripheral adaptations that are believed to be an important component of SL exercise training are partly responsible for an improved diastolic function and its subsequent increase in Q[24,25,53,65,70,].

Furthermore, the local reductions in fat mass and skinfold (VL) of the trained leg may indicate greater fat metabolism in the trained leg which is commonly associated with an enhanced mitochondrial content and function[83]. However, fat loss is commonly believed to occur on a whole body scale rather than a targeted location as previously demonstrated[84–87]. Evidence from a previous SL cycling study demonstrated a significant increase in net free fatty acid uptake within the muscles of the trained leg while performing submaximal DL cycling exercise and at a lower RQ compared to the untrained leg which also had a higher release of lactate[88]. These findings are indicative of a higher proportion of fat metabolism during exercise and an enhanced oxidative potential[88]. An increased mitochondrial content and function does not necessarily improve aerobic capacity, but it was previously demonstrated to be associated with the lactate threshold[82], meaning that such alterations in mitochondrial content can improve an individual's exercise capacity[73]. From this perspective, the metabolic stress induced by the constant load and HIIT SL training sessions in this study may have induced mitochondrial adaptations that led to the observed improvements in GET and RCP improving training capacity.

In conclusion, this study demonstrated that a SL exercise training program using a counterweighted model provided beneficial training effects not only to the trained leg, but also to the DL cycling performance. These improvements were observed in maximal performance as indicated by previous studies[25,26,28,29,53,65,70,78]. Importantly, a novel aspect of this study was the demonstration that the work rate and metabolic rate at GET and RCP were also increased in the trained leg during SL exercise, as well as during DL exercise. Although the present data preclude us from delineating the mechanisms controlling these maximal and submaximal changes in performance, this study suggests that the peripheral adaptations that are well-accepted to occur in response to single leg exercise training might have also contributed to some degree of central improvement.. Future studies should focus on the mechanistic bases of the adaptations to SL exercise training.

	Whole body		SLT		SLU	
	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	79.7 ± 12.5	77.9 ± 10.6	-----	-----	-----	-----
Fat (%)	16.9 ± 4.4	16.1 ± 3.7*	18.7 ± 5.9	17.9 ± 5.2	18.7 ± 6.1	18.5 ± 5.5
Fat mass (kg)	13.8 ± 5.7	12.8 ± 4.8*	2.9 ± 1.2	2.6 ± 1.1*	2.9 ± 1.3	2.7 ± 1.3
Lean mass (kg)	-----	-----	11.6 ± 1.5	12.1 ± 1.6	11.5 ± 1.5	11.6 ± 1.4
VL Skinfold (mm)	-----	-----	14.5 ± 9.8	13.3 ± 8.7*	14.7 ± 9.9	14.3 ± 9.4
VL circumference (cm)	-----	-----	54.5 ± 4	53.7 ± 3.9	54 ± 4.5	53.5 ± 4.4

Table 1. Anthropometric measurements comparing pre- and post-training values for whole body, single-leg trained, and single-leg untrained. *Significantly different from baseline ($p < 0.05$).

	DL		SLT		SLU	
	Pre	Post	Pre	Post	Pre	Post
HR (bpm)	183 ± 7	180 ± 6	165 ± 10	175 ± 16*ǂ	163 ± 14	161 ± 19
$\dot{V}O_2$ ($L \cdot min^{-1}$)	3.07 ± 0.22	3.25 ± 0.21	2.44 ± 0.22	2.87 ± 0.28*ǂ	2.45 ± 0.20	2.47 ± 0.26
PPO (W)	294 ± 24	318 ± 18*	142 ± 10	176 ± 16*ǂ	146 ± 14	154 ± 19
[La] ($mmol \cdot L^{-1}$)	10.4 ± 2.2	11.3 ± 1.9	7.0 ± 1.4	8.1 ± 1.7	6.8 ± 2	6.7 ± 2

Table 2. Pre- and post-training peak values attained during the ramp incremental test to each condition: double-leg, single-leg trained, and single-leg untrained. *Significantly different from baseline ($p < 0.05$). ǂ Significantly different from the untrained leg ($p < 0.05$).

	DL		SLT		SLU	
	Pre	Post	Pre	Post	Pre	Post
PO at GET (W)	134 ± 19	147 ± 16*	73 ± 7	88 ± 18*	76 ± 12	87 ± 16
$\dot{V}O_2$ at GET ($L \cdot min^{-1}$)	1.65 ± 0.13	1.93 ± 0.11*	1.34 ± 0.17	1.56 ± 0.14*ǂ	1.39 ± 0.15	1.40 ± 0.10
HR at GET (bpm)	131 ± 15	131 ± 9	116 ± 11	121 ± 9	117 ± 13	115 ± 8
PO (W)	203 ± 19	226 ± 25*	107 ± 13	128 ± 15*	109 ± 19	121 ± 16
$\dot{V}O_2$ at RCP ($L \cdot min^{-1}$)	2.53 ± 0.16	2.70 ± 0.21*	1.84 ± 0.17	2.23 ± 0.20*ǂ	1.93 ± 0.19	1.95 ± 0.12
HR at RCP (bpm)	154 ± 12	156 ± 9	134 ± 12	144 ± 8ǂ	135 ± 17	137 ± 8

Table 3. Pre and post-training gas exchange threshold and respiratory compensation point values for the power output and heart rate responses coinciding with those threshold values during double-leg, single-leg trained, and single-leg untrained ramp incremental test. *Significantly different from baseline ($p < 0.05$). ǂ Significantly different than untrained leg ($p < 0.05$).

Chapter Four: Overall conclusion, limitations, and future directions

4.1 Overall conclusion

The mechanisms that control the $\dot{V}O_{2max}$ response remain a subject of debate[7,2,89], with some arguing that convectional O_2 delivery to the muscle[48,49,50,51,52] is the main limiting factor determining $\dot{V}O_{2max}$, and others indicating that diffusional capacity of O_2 in the periphery also plays a crucial role in controlling maximal performance[1,28,25,53,54,60]. Despite this controversy, both factors are important in contributing to performance as a part of the O_2 delivery pathway from inspired atmospheric air into the lungs to its delivery into the mitochondria[2]. In an attempt to isolate central from peripheral adaptations to exercise training, the SL cycling exercise modality has been proven useful[28,70,78,90]. However, the mechanical constraints of this type of exercise has limited the interpretation of the results. In this sense, the addition of a counterweight in the non-cycling leg has contributed to SL cycling being able to reproduce more closely the movement of traditional DL cycling[29,73].

Thus, this thesis aimed to examine the effects of a 4-week SL exercise training intervention on physiological responses derived from a RI test in the trained and untrained leg as well as during DL cycling. The results from this investigation demonstrated significant increases in $\dot{V}O_{2peak}$, PPO, as well as the $\dot{V}O_2$ and PO associated with the GET and the RCP in the trained leg. Interestingly, similar beneficial effects were observed during DL cycling, despite the lack of peak and submaximal improvements in the untrained leg. Although it is believed that peripheral adaptation have played a role in the abovementioned adaptations to SL exercise training, as previously reported[25,70,90], HR data from this thesis suggest that SL training also contributed to central adaptations that likely mediated some of the beneficial effects seen after training.

4.2 Limitations

The main limitation of this study is the lack of more in depths mechanistic insights related to the observed exercise training adaptations. First, this study did not provide with direct measures of SV or Q. Even though the HR was used as a proxy measure for central adjustments/adaptations, not having direct information on the central component precludes us from making a stronger argument on the mechanisms that mediated the changes presented in the current investigation.

Second, no peripheral measures were analyzed to better characterize the training effect of SL exercise training on the improvements on performance. For example, analysis of non-invasive measures of blood flow or muscle tissue oxygenation would have contributed to better characterize the mechanistic aspects controlling the adaptations to the training program. Similarly, evaluations of mitochondrial function, capillarization, and vascular responsiveness were also missing in this design.

4.3 Future Directions

This thesis established that the SL cycling training program might have induced not only the expected peripheral adaptations[28,29,61,65,70,78], but also likely central adaptations that contributed to the increase in DL cycling performance. However, it has to be acknowledged that, aside from the HR data supporting the idea that central adaptations occurred, this study lacks the mechanistic evidence to go beyond speculation. Thus, further investigations into the chronic adaptations to SL counterweighted cycling should focus on the contribution of central and peripheral components to SL and DL maximal/peak and submaximal performance following an endurance-training program. In relation to this, the non-invasive measurement of tissue oxygenation using near-infrared spectroscopy can be a useful tool in detecting changes occurring at the peripheral level to further explain the profiles of muscle O_2 extraction in in response to

exercise. Additionally, measures of capillarization and mitochondrial function through muscles biopsies would contribute to further understand the mechanistic bases of the training adaptations. Similarly, to further asses central adaptations to exercise, changes in submaximal and maximal/peak Q and SV, as well as hemoglobin mass would be useful. Another aspect to consider in future investigations would be the use of instrument pedals to identify to what extent the increase in PPO observed during DL cycling was explained by changes in power distribution following training of one leg.

Finally, this study was conducted on a population of healthy individuals who did not undergo any cardiovascular training program in the past. It is plausible that potential central adaptations to training are more likely to be seen in such population in comparison to a group of endurance trained participants who already have good cardiovascular compliance and diastolic function.

Reference:

1. Wagner PD. Diffusion limitation of O2 from microvessels into muscle does contribute to the limitation of VO2max. *J Physiol*. 2015;17:3757-3758. doi:10.1113/JP270551
2. Wagner PD. Modeling O2 transport as an integrated system limiting V̇O2MAX. *Comput Methods Programs Biomed*. 2011;101(2):109-114. doi:10.1016/j.cmpb.2010.03.013
3. Caen K, Vermeire K, Bourgois J, Boone J. *Exercise Thresholds on Trial: Are They REally Equivalent ?* Vol 50.; 2018. doi:10.1249/mss.0000000000001547
4. Penedo FJ, Dahn JR. Exercise and well-being: a review of mental and physical health benefits associated with physical activity. *Curr Opin Psychiatry*. 2005;18(2):189-193. http://www.ncbi.nlm.nih.gov/pubmed/16639173.
5. Akalan C, Kravitz L, Robergs RR. V̇O2max: Essentials of the most widely used test in exercise physiology. *ACSM's Heal Fit J*. 2004;8(3):5-9. doi:10.1097/00135124-200405000-00004
6. Noakes TD. Implications of exercise testing for prediction of athletic performance: a contemporary perspective. *Med Sci Sports Exerc*. 1988;20(4):319-330. http://www.ncbi.nlm.nih.gov/pubmed/3050352.
7. Poole DC, Jones AM. Measurement of the maximum oxygen uptake V̇O2max: V̇O2peak is no longer acceptable. *J Appl Physiol*. 2017;122(4):997-1002. doi:10.1152/japplphysiol.01063.2016
8. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*. 1986;60(6):2020-2027. doi:10.1152/jappl.1986.60.6.2020

9. Pettitt RW, Clark IE, Ebner SM, Sedgeman DT, Murray SR. Gas Exchange Threshold and V[Combining Dot Above]O2max Testing for Athletes. *J Strength Cond Res*. 2013;27(2):549-555. doi:10.1519/JSC.0b013e31825770d7

10. Svedahl K, MacIntosh BR. Anaerobic Threshold: The Concept and Methods of Measurement. *Can J Appl Physiol*. 2009;28(2):299-323. doi:10.1139/h03-023

11. Keir D, Fontana FY, Robertson T, et al. Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance. *Med Sci Sport Exerc*. 2015;47(9):1932-1940. doi:10.1249/mss.0000000000000613

12. Yang H, Tsai J, Kao C, et al. The Beneficial Effect of Regular Endurance Exercise Training on Blood Pressure and Quality of Life in Patients with Hypertension. *Clin Exp Hypertens*. 2004;26(3):255-265. doi:10.1081/ceh-120030234

13. An P, Rice T, Gagnon J, et al. Familial Aggregation of Stroke Volume and Cardiac Output During Submaximal Exercise: The HERITAGE Family Study. *Int J Sports Med*. 2000;21(8):566-572. doi:10.1055/s-2000-12983

14. Thibault MC, Boulay MR, Lortie G, Bouchard C, Marcotte M, Simoneau JA. Genetic effects in human skeletal muscle fiber type distribution and enzyme activities. *Can J Physiol Pharmacol*. 2011;64(9):1245-1251. doi:10.1139/y86-210

15. Boule NG, Weisnagel SJ, Lakka TA, et al. Effects of exercise training on glucose homeostasis. *Diabetes Care*. 2005;28(1):108-114. doi:10.2337/diacare.28.1.108

16. Wallström B, Stenberg J, Astrand PO, Ekblom B, Saltin B. Effect of training on circulatory response to exercise. *J Appl Physiol*. 2017;24. doi:10.1152/jappl.1968.24.4.518

17. Clausen JP. Effect of physical training on cardiovascular adjustments to exercise in man. *Physiol Rev*. 1977;57:779-815.

18. Morganroth J, Maron BJ, Henry WL, Epstein SE. Comparative left ventricular dimensions in trained athletes. *Ann Intern Med*. 1975;82(4):521-524. doi:10.7326/0003-4819-82-4-521

19. Paulsen W., Boughner D., Friesen A, Persaud, J A. Ventricular response to isometric and isotonic exercise echocardiographic assessment. *Br Heart J*. 1979;42:521-527.

20. Zoneraich S, Zoneraich O, Rhee JJ, Jordan D, Ph D, Appel J. Endurance Athlete ' s Heart : A Non-Invasive Graphic Study. *Dep Med Div Cardiol Queens Hosp*.:223-239.

21. DeMaria AN, Neumann A, Lee G, Fowler W, Mason DT. Alterations in ventricular mass and performance induced by exercise training in man evaluated by echocardiography. *Circulation*. 1978;57(2):237-244. http://www.ncbi.nlm.nih.gov/pubmed/618610.

22. Ehsani AA, Hagberg JM, Hickson RC. Rapid changes in left ventricular dimensions and mass in response to physical conditioning and deconditioning. *Am J Cardiol*. 1978;42(1):52-56. doi:10.1016/0002-9149(78)90984-0

23. Wolfe L., Cunningham D., Rechnitzer P., Nichol P. Effects of endurance training on left ventricular dimensions in healthy men. *J Appl Physiol*. 1979;47(1):207-212.

24. Saltin B, Costill DL, Essén B, et al. The Nature of the Training Response; Peripheral and Central Adaptations to One-Legged Exercise. *Acta Physiol Scand*. 1976;96(3):289-305. doi:10.1111/j.1748-1716.1976.tb10200.x

25. Klausen K, Secher NH, Trap-Jensen J, Clausen JP, Hartling O. Central and regional circulatory adaptations to one-leg training. *J Appl Physiol*. 1981;52(4):976-983. doi:10.1152/jappl.1982.52.4.976

26. Bell G, Neary P, Wenger HA. The Influence of One-Legged Training on Cardiorespiratory Fitness. *J Orthop Sport Phys Ther*. 1988;10(1):8-11.

doi:10.2519/jospt.1988.10.1.8

27. Gleser MA. Effects of hypoxia and physical training on hemodynamic adjustments to one-legged exercise. *J Appl Physiol*. 1972;34(5):655-659. doi:10.1152/jappl.1973.34.5.655

28. Davies CT, Sargeant AJ. Effects of training on the physiological responses to one- and two-leg work. *J Appl Physiol*. 1975;38(3):377-375. doi:10.1152/jappl.1975.38.3.377

29. Abbiss CR, Martin JC, Hawley JA, et al. Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. *J Appl Physiol*. 2011;110(5):1248-1255. doi:10.1152/japplphysiol.01247.2010

30. Hardman AE, Williams C, Boobis LH. Influence of single-leg training on muscle metabolism and endurance during exercise with the trained limb and the untrained limb. *J Sports Sci*. 1987;5(2):105-116. doi:10.1080/02640418708729770

31. Freyschuss U, Strandell T. Circulatory adaptation to one- and two-leg exercise in supine position. *J Appl Physiol*. 1968;25(5):511-515. doi:10.1152/jappl.1968.25.5.511

32. Ahlborg G, Hagenfeldt L, Wahren J. Substrate utilization by the inactive leg during one-leg or arm exercise. *J Appl Physiol*. 1975;39(5):718-723. doi:10.1152/jappl.1975.39.5.718

33. Ishii K, Liang N, Oue A, et al. Central command contributes to increased blood flow in the noncontracting muscle at the start of one-legged dynamic exercise in humans. *J Appl Physiol*. 2012;112(12):1961-1974. doi:10.1152/japplphysiol.00075.2012

34. Cooper CE, Angus C. Blood Volume Changes are Controlled Centrally Not Locally - A Near-Infrared Spectroscopy Study of One Legged Aerobic Exercise. In: *ACSM's Health and Fitness Journal*. ; 2003:627-635. doi:10.1007/978-1-4615-0075-9_62

35. Beaver WL, Wasserman K, Whipp BJ. Bicarbonate buffering of lactic acid generated during exercise. *J Appl Physiol*. 1985;60(2):472-478. doi:10.1152/jappl.1986.60.2.472

36. Dekerle J, Baron B, Dupont L, Vanvelcenaher J, Pelayo P. Maximal lactate steady state, respiratory compensation threshold and critical power. *Eur J Appl Physiol*. 2003;89(3-4):281-288.

37. Thin AG, Linnane SJ, McKone EF, et al. Use of the gas exchange threshold to noninvasively determine the lactate threshold in patients with cystic fibrosis. *Chest*. 2002;121(6):1761-1770. doi:10.1378/chest.121.6.1761

38. Seiler S, Haugen O, Kuffel E. Autonomic Recovery after Exercise in Trained Athletes: Intensity and Duration Effects. :1366-1373. doi:10.1249/mss.0b013e318060f17d

39. Housh TJ, Cramer JT, Weir JP, Beck TW, Johnson GO. *Laboratory Manual for Exercise Physiology, Exercise Testing, And Physical Fitness*. Routledge, 2017; 2017.

40. Traylor DA, Camic CL, Bergstrom HC, et al. The Relationships Among Critical Power Determined From a 3-Min All-Out Test, Respiratory Compensation Point, Gas Exchange Threshold, and Ventilatory Threshold. *Res Q Exerc Sport*. 2013;84(2):232-238. doi:10.1080/02701367.2013.784723

41. McGehee JC, Tanner CJ, Houmard JA. A comparison of methods for estimating the lactate threshold. *J Strength Cond Res*. 2005;19(3):553-558. doi:10.1519/15444.1

42. Iannetta D, Qahtani A, Millet GY, Murias JM. Quadriceps muscles O2extraction and emg breakpoints during a ramp incremental test. *Front Physiol*. 2017;8(SEP):1-9. doi:10.3389/fphys.2017.00686

43. Coyle EF, Coggan AR, Hopper MK, Walters TJ. Determinants of endurance in well-trained cyclists. *J Appl Physiol*. 2017;64(6):2622-2630. doi:10.1152/jappl.1988.64.6.2622

44. Hawley J., Hargreaves M, Joyner M., Zierath J. Integrative biology of exercise. *Cell*. 2014:738-749.

45. Montero D, Diaz-Cañestro C, Lundby C. Endurance Training and VO2max: Role of Maximal Cardiac Output and Oxygen Extraction. *Med Sci Sports Exerc*. 2015;47(10):2024-2033. doi:10.1249/MSS.0000000000000640

46. Bonne T, Doucende G, Fluck D. Phlebotomy eliminates the maximal cardiac output response to six weeks in training. *Am J Physiol Integr Comp Physiol*. 2014;10:752-760.

47. Blomqvist C, Saltin B. Cardiovascular adaptations to physical training. *Annu Rev Physiol*. 1983:169-189.

48. Astrand PO, Saltin B. Maximal oxygen uptake and heart rate in various types of muscular activity. *J Appl Physiol*. 1961;16(6):977-981. doi:10.1152/jappl.1961.16.6.977

49. Bevegard BS, Shepherd JT. Regulation of circulation during exercise in man. *Physiol Rev*. 1967;47:178-213.

50. Mortensen SP, Dawson EA, Yoshiga CC, et al. Limitations to systemic and locomotor limb muscle oxygen delivery and uptake during maximal exercise in humans. *J Physiol*. 2005;566(1):273-285. doi:10.1113/jphysiol.2005.086025

51. Saltin B. Hemodynamic adaptations to exercise. *Am J Cardiol*. 1985;55(10). doi:10.1016/0002-9149(85)91054-9

52. Krip B, Gledhill N, Jamnik V, Warburton D. Effect of alterations in blood volume on cardiac function during maximal exercise. *Med Sci Sport Exerc*. 1997;29(11):1469-1479.

53. Davies CT, Sargeant AJ. Physiological responses to one- and two-leg exercise breathing air and 45% oxygen. *J Appl Physiol*. 1974;36(2):142-148. doi:10.1152/jappl.1974.36.2.142

54. Jacobs RA, Flück D, Bonne TC, et al. Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial

content and function. *J Appl Physiol*. 2013;115(6):785-793. doi:10.1152/japplphysiol.00445.2013

55. Baggish AL, Wang F, Weiner RB, et al. Training-specific changes in cardiac structure and function: a prospective and longitudinal assessment of competitive athletes. *J Appl Physiol*. 2008;104(4):1121-1128. doi:10.1152/japplphysiol.01170.2007

56. Saltin B, Blomquist B, Mitchell J, Johnson R, Wildenthal K, Chapman C. Response to submaximal and maximal exercise after bed rest and after training. *Circulation*. 1968;38:1-78.

57. González-Alonso J, Calbet JAL. Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation*. 2003;107(6):824-830. doi:10.1161/01.CIR.0000049746.29175.3F

58. Pirnay F, Lamy M, Dujardin J, Deroanne R, Petit J. Analysis of femoral venous blood during maximum muscular exercise. *J Appl Physiol*. 1972;33:289-292.

59. Welch H. Hyperoxia and human performance: a brief review. *Med Sci Sports Exerc*. 1982;14:253-262.

60. Rowell L, Saltin B, Kiens B, Christensen N. Is peak quadriceps flow in humans even higher during exercise with hypoxemia? *Am J Physiol*. 1986:H1038-H1044.

61. Roca J, Hogan MC, Story D, et al. Evidence for tissue diffusion limitation of VO2max in normal humans. *J Appl Physiol*. 1989;67(1):291-299. doi:10.1152/jappl.1989.67.1.291

62. Kaijser L. Limiting factors for aerobic muscle performance. *Acta Physiol Scand*. 1970;(346).

63. Bacon A, Carter R, Ogle E, Joyner M. VO2max trainability and high intensity interval training in humans: a meta-analysis. *PLoS One*. 2013;8(9):e73182.

64. Calbet JAL, Lundby C, Sander M, Robach P, Saltin B, Boushel R. Effects of ATP-induced leg vasodilation on V̇ o 2 peak and leg O 2 extraction during maximal exercise in humans . *Am J Physiol Integr Comp Physiol*. 2006;291(2):R447-R453. doi:10.1152/ajpregu.00746.2005

65. Yoshioka A, Yamamoto K, Miyachi M, Onodera S, Tanaka H, Takahashi K. Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. *J Appl Physiol*. 2017;90(6):2439-2444. doi:10.1152/jappl.2001.90.6.2439

66. MacInnis MJ, Zacharewicz E, Martin BJ, et al. Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. *J Physiol*. 2017;595(9):2955-2968. doi:10.1113/JP272570

67. Boushel R, Gnaiger E, Calbert J. Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion*. 2011;11(2):303-307.

68. Wenger H, Bell G. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. *Int J Sports Med*. 1986:346-356.

69. MacDougal J, Sale D. Continuous vs interval training a review for the athlete and coach. *Can J Appl Sport Sci*. 1981:93-97.

70. Thomas S., Cunningham D., Plyley M., Boughner D., Cook R. Central and peripheral adaptatioms of the gas transport system to one-leg training. *Can J Physiol Pharmacol*. 1981:1146-1154.

71. Wezenberg D, de Haan A, van der Woude LH, Houdijk H. Feasibility and Validity of a Graded One-Legged Cycle Exercise Test to Determine Peak Aerobic Capacity in Older People With a Lower-Limb Amputation. *Phys Ther*. 2011;92(2):329-338. doi:10.2522/ptj.20110125

72. Burns KJ, Pollock BS, Lascola P, McDaniel J. Cardiovascular responses to counterweighted single-leg cycling: Implications for rehabilitation. *Eur J Appl Physiol*. 2014;114(5):961-968. doi:10.1007/s00421-014-2830-0

73. Sonne MW, Keir PJ, MacInnis MJ, et al. Physiological responses to incremental, interval, and continuous counterweighted single-leg and double-leg cycling at the same relative intensities. *Eur J Appl Physiol*. 2017;117(7):1423-1435. doi:10.1007/s00421-017-3635-8

74. Mattioni Maturana F, Keir D, Mcklay K, Murias J. Critical power testing or self-selected cycling: which one is the best predictor of maximal metabolic steady-state? *J Sci Med Sport*. 2017;20:795-799.

75. Mcphee J., Williams AG, Aldred S, et al. The training stimulus experienced by the leg muscles during cycling in humans. *Exp Physiol*. 2009;94(6):684-694. doi:10.1113/expphysiol.2008.045658

76. Evans RA, Dolmage TE, Mangovski-Alzamora S, et al. One-legged cycle training for chronic obstructive pulmonary disease: A pragmatic study of implementation to pulmonary rehabilitation. *Ann Am Thorac Soc*. 2015;12(10):1490-1497. doi:10.1513/AnnalsATS.201504-231OC

77. Thijssen D, Dawson E, van den Munckhof C, Birk G, Timothy Cable N, Green D. Local and systemic effects of leg cycling training on arterial wall thickness in healthy humans. *Atherosclerosis*. 2013;229(2):282-286.

78. Rud B, Foss, Krustrup P, Secher NH, Hallén J. One-legged endurance training: Leg blood flow and oxygen extraction during cycling exercise. *Acta Physiol*. 2012;205(1):177-185. doi:10.1111/j.1748-1716.2011.02383.x

79. Klausen K, Andersen L., Pelle I. Adaptive changes in work capacity, skeletal

capillarization and enzyme levels during training and detraining. *Acta Physiol Scand*. 1981;113:9-16.

80. Nemeth BYPM, Pette D, Vrbova G. Comparison of Enzyme activiteis among single miscle fibres within defined motor units. *J Physiol*. 1981;311:489-495.

81. Mitchell EA, Martin NRW, Bailey SJ, Ferguson RA. Critical power is positively related to skeletal muscle capillarity and type I muscle fibers in endurance-trained individuals. *J Appl Physiol*. 2018;125(3):737-745. doi:10.1152/japplphysiol.01126.2017

82. Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. *J Appl Physiol*. 1980;48(3):523-527. doi:10.1152/jappl.1980.48.3.523

83. Kiens B. Effect of endurance training on fatty acid metabolism : local adaptations. 1997;29:640-645.

84. Campillo R, Andrade D, Campos-Jara C, Henriquez-Olguin C, Alvarez-Lepin C, Izquiredo M. Regional fat changes induced by localized muscle endurance resistance training. *J Strength Cond Res*. 2013;27(8):2219-2224.

85. Katch F, Clarkson P, Kroll W, Wilcox A, Mcbride T. Effects of Sit up Exercise Training on Adipose Cell Size and Adiposity. *Res Q Exerc Sport*. 1984;55(3):242-247.

86. Gwinup G, Chelvam R, Steinberg T. Thickness of subcutaneous fat and activity of underlying muscles. *Ann Intern Med*. 1971;74(3):408-411. doi:10.7326/0003-4819-74-3-408

87. Kostek M, Pescatello L, Seip R, Angelopulos T, Price T. Subcutaneous Fat Alterations Resulting from an Upper-Body Resistance Training Program. *Med Sci Sport Exerc*. 2007;39(7):1177-1185. doi:10.1249/mss.0b0138058a5cb

88. Henriksson BYJAN. Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. 1977:661-675.

89. Wagner PD. A theoretical analysis of factors determining V̇(O2)MAX at sea level and altitude. *Respir Physiol*. 1996;106(3):329-343. doi:10.1016/S0034-5687(96)00086-2

90. Saltin B. the Interplay Between Peripheral and Central Factors in the Adaptive Response To Exercise and Training. *Ann N Y Acad Sci*. 1977;301(1):224-231. doi:10.1111/j.1749-6632.1977.tb38201.x

Appendices

APPENDIX A: PAR-Q

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

› Start becoming much more physically active – start slowly and build up gradually. › Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).

› You may take part in a health and fitness appraisal.

› If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).

› If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

› You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better

› You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR

› Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?	☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/ or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?	☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm	☐ If yes, answer questions 3a-3e	☐ If no, go to question 4

	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial brillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancyrelated diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐
Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐

7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity,

please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort

exercise. **If you answered YES to one or more of the follow-up questions about your medical condition:**

› You should seek further information from a licensed health care professional before

becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information. **Delay becoming more active if:**

› You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better

› You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR

› Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

› You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.

› The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

› If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

› Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the eectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidencebased AGREE process (1) by the PARQ+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

APPENDIX B: LETTER OF INFORMED CONSENT

UNIVERSITY OF CALGARY
KINESIOLOGY

Human Performance Laboratory
Telephone: (403) 220-7955
Email: jmmurias@ucalgary.ca

Informed Consent

TITLE: Changes in MLSS and VO_2 Kinetics subsequent to a single-leg exercise training intervention.

INVESTIGATORS: Dr. Juan M. Murias

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

BACKGROUND

The administration of a controlled endurance program is known to have a variety of positive health effects. These effects can range from a systematic standpoint such as cardiorespiratory measures, to local changes that may occur on a more peripheral or muscular level. Even though both central and local adaptations to endurance training are well-accepted, the contribution of each of these components to the total change in cardiovascular function after an exercise training intervention are less clear.

Most endurance training is prescribed as a percent of maximal VO_2 (VO_{2max}). Maximal stroke volume and cardiac output are often considered the main limiting factors for VO_{2max}. Although controversy exists on this issue, this implies that O_2 supply to the active tissue is not a key limiting factor for reaching VO_{2max}. Exercising a single-leg attenuates cardiac output as a limiting factor for achieving the highest VO_2 for that exercise modality (VO_{2peak}); instead O_2 uptake would be dependent on the aerobic capacity of the working muscles. To further understand the responses to single-leg training, this study will investigate changes in VO_2 kinetics and MLSS after an endurance training intervention using a single-leg cycling model.

WHAT IS THE PURPOSE OF THE STUDY?

The goal of this study is to determine central and peripheral adaptations associated to as single-leg endurance cycling program and how this intervention affects maximal lactate steady state (MLSS) and oxygen uptake (VO_2) kinetics of the trained and untrained legs, as well as two legs cycling exercise.

WHAT WOULD I HAVE TO DO?

To be part of the study, the following admission criteria will be considered: you will have to be healthy, non-smoker, non-obese, with no peripheral vascular occlusive disease, not taking medications that are known to affect cardiovascular or hemodynamic responses to exercise (e.g., β-blockers, blood pressure medication, anti-inflammatories, anti-coagulants, etc.). You will be required to pass the Physical Activity Readiness Questionnaire (PAR-Q+) and to meet the following baseline cutoffs: resting heart rate <100 bpm, resting systolic blood pressure <144 mmHg, and resting diastolic blood pressure <95 mmHg. Moreover, you are required to be a recreationally active individual. In order to demonstrate eligibility, you will perform a VO_{2max} test in your first visit to the lab.

Once included in the study, you will be required to visit the laboratory up to 35 other separate occasions. You will be performing ramp incremental tests, 30-minute cycling at a steady workload (MLSS) and an endurance training program (3 visits a week for 4 weeks). Refer to the table at the end of this form for a detailed overview of each visit and the protocol carried out during that visit.

Measurements: All measurement techniques are used as routine measures in our laboratory. During all of the visits you will be required to wear a mask connected to a volume turbine and gas analyser so that ventilatory rates and gas concentration in the air can be measured. Masks and turbines are disinfected before each test.

During all visits the oxygenation of the thigh muscles will be continuously measured non-invasively using NIRS. NIRS projects light into a specific location of your leg muscles (the probe will be placed on your leg approximately midway between your knee and ankle during the VOT, and between your hip and your knee during the $VO_{2kinetics}$ assessment) and measures the amount of light being reflected. The amount of light detected by the NIRS probe is used to measure muscle oxygenation. The probes, once have been placed on the belly of the muscles, will be secured with tape, covered to prevent light from entering or leaving the area, and bound with elastic bandage to minimize movement.

Heart rate will be continuously monitored using a standard chest heart rate monitor. You will also be asked to rate your perceived exertion on a 10-point scale during the trials.

Measures of lactate will be performed by drawing small blood sample through a small finger prick puncture using a lancet. A drop of blood will be then immediately placed on a testing strip which is placed into a portable lactate analyzer, which displays blood lactate concentration results within 60 seconds. This is similar to blood glucose measurement conducted by diabetic patients on their own finger tips.

Furthermore a battery of cognitive tests will be performed before, during, and after each testing session. The aim of these tests is to assess your perception of fatigue, and the physical and emotional demand of each testing session.
There are no known risks associated with these procedures.

WHAT ARE THE RISKS?

Any exercise carries a slight risk or may be uncomfortable if you are unfit or not used to doing exercise. The risk of a cardiac event (heart attack, dysrhythmias, etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10000. However, the risk decreases in a previously healthy (i.e. young moderately active) population (adapted from ACSM's Guidelines for Exercise Testing and Prescription). There might be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise.

You may experience some minor discomfort from wearing the breathing mask necessary for VO_2 measurements, and by having the NIRS probes secured to your leg during the exercise period. These sensations often become less noticeable with time during the tests. You might also feel some discomfort while being occluded at the level of your upper thigh. However, this minor discomfort will only be present during the 5-min occlusion. Mild discomfort from finger prick blood samples might also be experienced. Additionally, placements of the NIRS probes might require shaving.

All testing procedures will only be conducted when a lab technician or research assistant that is certified in CPR is present. In the case of an emergency, 911 will be called using a telephone available in the testing laboratory. An automatic external defibrillator is also available within the testing building.

WILL I BENEFIT IF I TAKE PART?

This is a basic physiology/biochemistry study and, as such, there will be no direct benefits received as a consequence of participating in the study. If you are interested, the rationale for conducting the research and theory and significance of each of the tests will be explained, as well your individual results from each of the tests (i.e. VO_{2max}). You will also have the opportunity to learn about and better understand your physiological responses to an exercise situation. You are encouraged to ask questions regarding the purpose of the study, specific measures or outcomes of your exercise test, or overall findings and conclusions from this research study.

DO I HAVE TO PARTICIPATE?

Your participation in this research project is entirely voluntary. You can withdraw anytime just by sending an email to aqahtani@ucalgary.ca or by expressing this desire verbally to the investigator.

You might be withdrawn from the study for the following reasons:

- Changes in your status so that you do not fit within the admission criteria for this study.
- You cannot complete all testing sessions within the proposed period of the study.
- You are not able to comply with the instructions prior to each testing session.

If new information becomes available that might affect your willingness to participate in the study, you will be informed as soon as possible.

WHAT ELSE DOES MY PARTICIPATION INVOLVE?

Your participation in this study does not involve anything else beyond what is specified on this informed consent.

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

You will not be paid for your participation in this research project. The protocol does not require any financial or material input from the participant.

WILL MY RECORDS BE KEPT PRIVATE?

Information obtained during this research project is confidential. Nobody except the researchers will have access to your personal information. Your records are listed according to an identification number rather than by your name. Published reports resulting from this study will not identify you by name. Thus, your right to privacy will be retained. If you require it, you will be given a summary of your results and the average results for all participants in this study. Should you withdraw from the study at any time, information collected up to that point might be used for scientific purposes unless you request otherwise. In that case, the information collected will be discarded.

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

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

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding your participation in the research project and agree to participate as a participant. In no way does this waive your legal rights nor release the investigators or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time without consequences. If you have further questions concerning matters related to this research, please contact:

Dr. Juan M. Murias (403) 220-7955 or jmmurias@ucalgary.ca

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

Participant's Name	Signature and Date
Investigator/Delegate's Name	Signature and Date
Witness' Name	Signature and Date

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

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

Ethics ID: REB 18-0041Study
Title: Changes in MLSS and VO_2 Kinetics subsequent to a single-leg exercise training intervention
PI: Dr. Juan M. Murias

APPENDIX C: RECRUITMENT POSTER

UNIVERSITY OF CALGARY
KINESIOLOGY

Human Performance Laboratory
Telephone: (403) 220-7955
Email: jmmurias@ucalgary.ca

Volunteers Needed For Research Project Investigating The Effects Of a 4 Week Single-Leg Cycling Training Intervention on Central and Peripheral Adaptations

The goal of this study is to determine central and peripheral adaptations associated to as single-leg endurance cycling program and how this intervention affects maximal lactate steady state (MLSS) and oxygen uptake (VO_2) kinetics of the trained and untrained legs, as well as two legs cycling exercise.

To be part of the study, the following admission criteria will be considered: you will have to be healthy, non-smoker, non-obese, with no peripheral vascular occlusive disease, not taking medications that are known to affect cardiovascular or hemodynamic responses to exercise (e.g., β-blockers, blood pressure medication, anti-inflammatories, anti-coagulants, etc.). You will be required to pass the Physical Activity Readiness Questionnaire (PAR-Q+) and to meet the following baseline cutoffs: resting heart rate <100 bpm, resting systolic blood pressure <144 mmHg, and resting diastolic blood pressure <95 mmHg. Moreover, you are required to be a recreationally active individual. In order to demonstrate eligibility, you will perform a VO_{2max} test in your first visit to the lab.

Once included in the study, you will be required to visit the laboratory up to 35 other separate occasions. You will be performing ramp incremental tests, 30-minute cycling at a steady workload (MLSS) and an endurance training program (3 visits a week for 4 weeks). Refer to the table at the end of this form for a detailed overview of each visit and the protocol carried out during that visit.

If interested and would like to receive more information, please contact:

Ahmad Qahtani (MSc Student): (403) 399-9999 aqahtani@ucalgary.ca
Dr. Juan Murias (PhD): (403) 220-7955 jmmurias@ucalgary.ca

This study has been approved by the University of Calgary Conjoint Health Research Ethics Board (Ethics ID: REB 18-0041)

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