Cardiovascular response to intermittent high intensity double- and single-legged cycling

Miss Nicole Gordon

2013
This thesis is submitted as partial fulfilment of the requirements for the degree of Bachelor of Exercise Physiology (Honours) at Murdoch University, Perth, Western Australia.

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

(Miss Nicole Gordon)
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ABSTRACT

Although the benefits of high-intensity interval training can be similar or superior to moderate- or low-intensity continuous training, it is possible that not all individuals should undertake such exercise. While high-intensity interval training is currently accepted practice within the cardiovascular rehabilitation setting, some individuals may not be suited to this type of exercise due to their decreased exercise tolerance and diminished cardiovascular function. The use of smaller muscle mass training (e.g. single-legged cycling) can allow localised high-intensity muscle training but avoid cardiac limitations associated with high blood flow demands when training using a large muscle mass. **Purpose:** To examine the differences in cardiovascular stress imposed by double- and single-legged high-intensity interval cycling in order to better understand the physiological responses of such exercise and assist in future training prescription. **Methods:** In a randomised crossover design, ten young, healthy individuals (23 ± 5 years of age, 180 ± 7 cm height, 74 ± 13 kg body weight, 51 ± 9 mL.kg\(^{-1}\).min\(^{-1}\)) performed six 1-minute double-legged 'all out' efforts interspersed with 1-minute active recovery and twelve 1-minute single-legged (six with each leg) 'all out' efforts interspersed with 1-minute active recovery in two experimental sessions. Power output, oxygen consumption and heart rate were measured throughout the interval sessions. Blood pressure, oxygen saturation, ratings of perceived exertion, pain in the quadriceps and effort were measured at baseline and immediately following each interval. All self-perceived measures were taken on a 0 - 10 scale, with 0 = no perception and 10 = maximum perception. While brain natriuretic peptide (BNP) and left ventricular function were measured pre- and post-exercise. **Results:** Significantly greater power
output (trial average: 340 ± 77 versus 301 ± 101 W, p<0.01) and workload (trial average: 916 ± 73 versus 743 ± 122 kJ, p<0.01) was observed during combined right and left single-legged cycling, when compared with double-legged cycling. Double-legged cycling resulted in greater physiological stress compared with single-legged cycling as shown by increased oxygen consumption (2.81 ± 0.69 versus 1.84 ± 0.43 L.min⁻¹, respectively; p<0.01). Additionally, greater cardiac stress was observed during and resulting from double-legged cycling when compared with single-legged cycling as shown by increased inter-interval heart rate (161 ± 7 versus 142 ± 7 bpm, respectively, p<0.01) and systolic blood pressure (180 ± 17 versus 166 ± 21 mmHg, respectively, p<0.01) as well as lower end-session left ventricular ejection fraction (pre-post change: 11.5 ± 1.8 versus 2.6 ± 1.3 %, respectively; p<0.05). BNP increased pre- to post-exercise (24 ± 8 versus 27 ± 8 pg.mL⁻¹), however, no differences were observed between conditions. Overall sessional perceived exertion was lower during single-legged, compared with double-legged cycling (7.2 ± 1.8 and 8.9 ± 0.7 units, respectively; p<0.02), even though inter-interval perceptions of exertion, pain and effort were similar between conditions. **Conclusion:** Single-legged cycling allows individuals to exercise at a greater overall power output; however, under reduced cardiovascular and physiological stress when compared with traditional double-legged cycling. Furthermore, single-legged cycling is perceived as easier, which could benefit compliance if used as a training stimulus. With increased attention placed on the use of high-intensity interval training in diseased populations, results of the present study indicate that single-legged cycling could provide an alternative approach to normal double-legged cycling giving
practitioners a method to quickly enhance metabolic function while allowing individual to exercise with less risk of experiencing an adverse cardiac event.
CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND TO STUDY

Cardiovascular disease is a major world health issue accounting for an estimated 30% of deaths globally [1]. In Australia, cardiovascular disease affects 18% of the population and is responsible for 25% ($AU372 million) of the health care costs associated with physical inactivity ($AU1.5 billion) [2, 3]. The risk of developing cardiovascular disease is drastically increased among individuals who are sedentary or undertake low levels of exercise (i.e. less than the recommended level of physical activity; <500 MET-min/week) [4]. Indeed, improving physical fitness through exercise has been shown to reduce the risk of developing numerous chronic diseases, including cardiovascular disease and diabetes [5-8].

Continuous low- to moderate-intensity exercise has traditionally been prescribed in order to improve aerobic capacity [4, 8]. The American College of Sports Medicine (ACSM) recommends the general population perform a minimum of 30 minutes of moderate-intensity exercise on most, but preferably all, days of the week. While these exercise recommendations have been shown to be effective in improving aerobic capacity and cardiorespiratory fitness [9, 10], the time required to complete such exercise is substantial. Indeed, many individuals fail to adhere to these guidelines with the primary reason being 'lack of time' [11]. Recent evidence indicates that high-intensity interval training, which involves completing high intensity efforts interspersed with periods of low intensity rest between efforts, may be as effective and a more time-efficient method of improving general health and well-being [5, 11-18]. For example, Gibala et al. [11] observed similar improvements in peak oxygen consumption (VO$_{2peak}$), exercise performance and
skeletal muscle metabolic adaptations in young, healthy men and women following six weeks of low-volume high-intensity interval training (i.e. four to six bouts of 30 seconds 'all out' efforts, with 4.5 minutes rest between, three times per week), compared to continuous moderate-intensity training (i.e. 40-60 minutes at 65% VO2peak, five times per week). It is interesting to note that within this study, the time commitment (~67%) and training volume (~90%) of high-intensity interval training was lower than continuous exercise, which is likely to improve exercise program adherence. Indeed, compared with continuous moderate-intensity exercise, high-intensity exercise has also been shown to be more motivating for participants [10] and have a higher rate of adherence [19]. As a result of improved adherence and the clear health benefits of high-intensity interval training, such training is beginning to be prescribed to high-risk individuals such as patients with cardiovascular and chronic diseases [11-15, 18].

Although the benefits of high-intensity training can be similar or superior to moderate- or low-intensity prolonged exercise [5, 11-18], it is possible that not all individuals should undertake such exercise. While high-intensity interval training is currently accepted practice within the cardiovascular rehabilitation setting [11-15, 18], some individuals may not be suited to this type of exercise due to their decreased exercise tolerance and diminished cardiovascular function [18]. Compared with low- or moderate-intensity exercise, high-intensity exercise results in increased oxygen demand, an up regulation of sympathetic neural output, increased cardiac output [20], and as a consequence greater strain on the central cardiovascular system [21]. With such high demand placed on the heart during high-intensity intervals, individuals with cardiac insufficiency may be at greater risk of
cardiac injury. For instance, a decrease in left ventricular function which persisted 30 minutes post session has been observed following a high-intensity interval session (14x1-minute efforts with two minutes recovery) in non-endurance trained individuals [17]. Such reductions in left ventricular function have been associated with the presence of the cardiac damage marker brain natriuretic peptide (BNP) [22]. Post-exercise elevations in BNP [23, 24] highlight the severe cardiac stress that can result from such unaccustomed exercise.

Although high-intensity interval training offers a viable method of exercise prescription with well documented benefits [5, 11-18, 22], the possibility to induce cardiac damage [17, 22] highlights the need for alternative methods of exercise delivery. The use of smaller muscle mass training (e.g. single-limb exercise) can allow localised high-intensity muscle training but avoid cardiac limitations associated with high blood flow demands when training using a large muscle mass [20]. Despite single-legged cycling resulting in lower cardiac output and heart rate, blood flow, muscle perfusion and oxygen extraction may be greater than normal double-legged cycling [25, 26]. Such enhanced muscle blood flow, oxygen delivery and oxygen extraction are believed to be responsible for the greater work capacity and training adaptation observed during single-legged, compared with double-legged cycling [27]. Indeed, Abbiss et al. [27] reported greater improvements in the metabolic (i.e. GLUT4 protein and AS160 protein) and mitochondrial oxidative (i.e. COX-II and IV) capacity of peripheral musculature following three weeks of single-legged, compared with double-legged cycle training. In addition to the beneficial peripheral adaptations associated with single-legged cycling [22, 25, 27], this training modality is likely to have greater adherence than high-intensity double-
legged cycling as individuals typically report lower whole body perceived exertion [26, 27], and experience similar quadriceps pain during single-legged, compared with double-legged cycling [26, 27]. To date however, there have been no studies that have investigated the effect of single-legged cycling on cardiovascular stress and function.

1.2 PURPOSE STATEMENT

Chronic cardiopulmonary diseases severely affect an individual’s quality of life and life expectancy and as a result these diseases have significant social, economic and health impacts. High-intensity interval exercise has been shown to improve cardiovascular function to a greater extent than moderate-intensity continuous exercise and is increasingly being used in cardiac rehabilitation centres. Nevertheless, high-intensity exercise with a large muscle mass places significant stress on the cardiovascular system; thus, increasing the likelihood of an adverse event. Evidence is emerging to suggest that small muscle mass high-intensity interval exercise such as single-legged cycling can provide improvements to aerobic fitness with the possibility of reduced cardiac stress. Therefore, the purpose of this study was to examine the differences in cardiovascular stress imposed by double- and single-legged high-intensity interval cycling in order to better understand the physiological responses of such exercise and assist in future training prescription.

1.3 RESEARCH QUESTIONS

The research questions that will be addressed during this study are:

1. What are the differences in oxygen consumption, blood pressure and heart rate during maximal self-paced single-legged and double-legged cycling intervals?
2. Will double-legged maximal self-paced interval cycling reduce left ventricular ejection fraction to a greater extent than single-legged cycling?

3. Will a difference in the cardiac damage marker, BNP, exist following maximal self-paced single-legged and double-legged cycling intervals?

4. What are the differences in power output and total workload during maximal self-paced single-legged and double-legged cycling intervals?

1.4 HYPOTHESES

1. During maximal self-paced intervals, oxygen consumption, blood pressure and heart rate will be higher in double-legged cycling, compared with single-legged cycling.

2. Following a bout of maximal self-paced intervals, left ventricular ejection fraction will be decreased to a greater extent in double-legged, compared with single-legged cycling.

3. The cardiac damage marker, BNP, will increase to a greater extent following maximal self-paced double-legged, compared with single-legged cycling intervals.

4. During maximal self-paced intervals, combined single-legged power and workload will be greater than during double-legged cycling.

1.5 LIMITATIONS/DELIMITATIONS

The researcher acknowledges this research has limitations as follows:

1. All subjects were asked to give maximal effort during all intervals in all testing sessions; however, this cannot be guaranteed.
2. Left ventricular ejection fraction was not measured directly, instead the area calculated at end-systole and end-diastole during echocardiography was used to indirectly measure left ventricular ejection fraction.

The researcher acknowledges the following delimitations:

1. The findings are only applicable to young (age range: 19-34), healthy adults.
CHAPTER TWO: CRITICAL REVIEW OF LITERATURE

2.1 OVERVIEW
The incidence of cardiovascular disease is increasing within most developed countries and has become a major health epidemic, with significant social and economic impacts [2, 3, 5, 28]. Among other factors (e.g. diet, genetics, and smoking), physical inactivity and a subsequent lack of cardiovascular fitness is a major risk factor for cardiovascular disease. As such, it is suggested that the general population and cardiovascular disease patients perform regular aerobic exercise [10-14, 18, 29-31]. Traditionally, moderate-intensity continuous training (60 – 75% aerobic capacity) is used to improve cardiovascular fitness [4, 8], however, with 'lack of time' being the most commonly cited barrier to exercise [11, 32-34] alternative methods are being explored. High-intensity interval training is an effective method of improving cardiovascular health, requiring a much shorter time commitment, compared with traditional aerobic-based exercise programs [5, 10-16, 30, 31, 35-37]. As such, high-intensity interval training provides an alternative method of inducing favourable training adaptations.

Although the benefits of high-intensity training can be similar or superior to moderate- or low-intensity prolonged exercise [5, 11-18], it is possible that such exercise may place certain individuals at risk. Indeed, while high-intensity interval training is gaining acceptance within the cardiovascular rehabilitation setting [11-15, 18], a decreased exercise tolerance and diminished cardiovascular function may indicate some of these patients may not be suited to this type of exercise [18]. The possibility of high-intensity interval training to induce cardiac damage [17, 22] highlights the need for alternative methods of high-intensity interval delivery.
Indeed, while the benefits of high-intensity interval training may outweigh the risks [11-15, 18], it is possible that alternate strategies of exercise delivery may provide safer methods to improve physical function. The use of smaller muscle mass training (e.g. single-limb exercise) can localise high-intensity muscle training but avoid cardiac stress imposed by central limitations (e.g. cardiac output) and high blood flow demands [20]. Indeed, it has been suggested that despite lower cardiac output and heart rate, blood flow, muscle perfusion and oxygen extraction are higher in active tissue during single-legged cycling, compared with double-legged cycling [25, 26]. Such improvements in tissue oxygen delivery and utilisation are believed to be responsible for the greater work capacity and training adaptation observed during single-legged, compared with double-legged cycling [27].

Despite the increasing use of high-intensity interval training in clinical settings and the possible benefits of reduced muscle mass aerobic interval training, little is known on the cardiovascular stress induced by these exercise modalities. Reduced muscle mass aerobic exercise delivery (i.e. single-legged cycling) may allow for the significant peripheral adaptations induced by high-intensity interval training, without causing excessive stress to the cardiovascular system. The purpose of this literature review will be to examine areas of aerobic exercise delivery, highlighting moderate-intensity continuous training, high-intensity interval training and small muscle mass training (i.e. single-legged cycling) and to propose the best possible method of exercise delivery especially with regards to cardiovascular rehabilitation.

2.2 CARDIOVASCULAR DISEASE AND PHYSICAL ACTIVITY

Cardiovascular disease, presents a burden on society [2, 3, 7, 28] accounting for 30% of deaths worldwide [1]. In Australia, cardiovascular disease affects 18% of
the population and accounts for 25% of the health care costs associated with a sedentary lifestyle [2, 3]. Although a genetic component exists for the risk of developing cardiovascular disease [8], several other risk factors have been identified, and include; low cardiorespiratory fitness (i.e. \( VO_{2\text{max}} \)) [4, 5, 7], elevated body mass index [7], high blood pressure [8], elevated low-density lipoprotein [5, 8, 28] and high-sensitivity C-reactive protein levels [7, 28], as well as decreased high-density lipoprotein concentrations [5, 7, 8, 28]. The majority of these risk factors are directly influenced by diet and exercise [5, 7, 28]. Indeed, an improvement in cardiorespiratory fitness (i.e. \( VO_{2\text{max}} \)) is associated with beneficial changes in the majority of established risk factors (e.g. lipids, blood glucose, obesity) [7, 8] and thus commonly used to determine the health benefits from exercise intervention [5, 7, 10, 12]. For instance, O'Donovan et al. [5], observed a significant increase in \( VO_{2\text{max}} \) and beneficial decreases in body fat, total cholesterol, low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol in 42 previously sedentary men, following 24 weeks of aerobic cycle training (3x400-kcal per week at 60-80% \( VO_{2\text{max}} \)).

Maximal aerobic capacity is positively influenced by exercise training, regardless of age, sex, race or initial cardiovascular fitness levels [5-8, 15, 31, 38]. Indeed, Wilmore et al. [8] observed increases in \( VO_{2\text{max}} \) and associated beneficial changes in lipid and lipoprotein profile and body composition following 20 weeks of exercise training in 502 healthy previously sedentary men and women of varying age (17 to 65 years) and race [8]. In addition to reducing the risk of cardiovascular disease, exercise training may also benefit individuals already diagnosed with cardiovascular disease. For instance, a 46% increase in \( VO_{2\text{max}} \), a 40% reduction in
pro-brain natriuretic peptide (prohormone linked to cardiac damage) levels, reverse left ventricular remodelling, as well as improvements in endothelial function have been observed in 27 elderly (75±11 years) post-infarction heart failure patients following 12 weeks of high-intensity interval training (4x4 minutes walking at 90-95% HR_{peak}, interspersed with 3 minutes active rest) [17]. While improving cardiovascular fitness through exercise can drastically reduce the risk of cardiovascular disease, conjecture exists as to the most appropriate exercise regimes to increase VO_{2max} and thus overall cardiovascular disease risk [5, 9, 10, 12, 35].

2.3 MODERATE-INTENSITY CONTINUOUS EXERCISE

2.3.1 American College of Sports Medicine exercise guidelines

The American College of Sports Medicine recommends the general population perform a minimum of 30 minutes of moderate-intensity exercise on most, but preferably all, days of the week. These recommendations have shown to be effective at improving aerobic capacity and cardiorespiratory fitness with relatively low sensations of fatigue and exertion [9, 10]. Regardless, the time required to complete such exercise is substantial and therefore can be difficult, especially for those not accustomed to regular physical activity. Indeed, many individuals fail to adhere to these guidelines with the primary reason being 'lack of time' [11, 32-34].

2.3.3 Physiological adaptations to moderate-intensity exercise

The improved health and fitness associated with moderate-intensity continuous exercise is the result of both central (i.e. cardiac function and hemodynamic responses) [30, 39] and peripheral (i.e. skeletal muscle adaptations)
adaptations (as seen in Table 1) [30, 39]. For example, in female rats, Kemi et al. [30] observed a 28% improvement in $\text{VO}_{2\text{max}}$, cardiomyocyte function and capacity (as shown by improvements in fractional shortening: +23%, and $\text{Ca}^{2+}$ sensitivity: +30%) as well as endothelial function following 10 weeks of moderate-intensity running. Furthermore, in humans the use of moderate-intensity training has resulted in similar central improvements [10, 39] as well as observed increases in septal wall thickness (+7.4%) and left ventricular mass (+14%) in [39].
Table 1. Literature examining cardio-respiratory adaptations resulting from moderate- and high-intensity training in non-elite, healthy and unhealthy individuals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Age (y)</th>
<th>Mode</th>
<th>Frequency</th>
<th>Weeks</th>
<th>Duration</th>
<th>Intensity</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freyssin et al. [12]</td>
<td>26 M&amp;F</td>
<td>54±12</td>
<td>Cycling</td>
<td>C: 6d/wk</td>
<td>8</td>
<td>C: 61min</td>
<td>C: HR at VT1</td>
<td>C: ↑time at VT1, distance in 6MWT I: ↑VO_{peak}, exercise test duration, oxygen pulse, VO_2 at VT1, distance in 6MWT</td>
</tr>
<tr>
<td></td>
<td>chronic heart failure pts</td>
<td></td>
<td>I: 3d/wk</td>
<td></td>
<td></td>
<td>I: 71min</td>
<td>I: 50% then 80% max PO (12x 30sec, with 60sec PR)</td>
<td></td>
</tr>
<tr>
<td>Hottenrott et al. [35]</td>
<td>34 M&amp;F</td>
<td>43±7</td>
<td>Running</td>
<td>C: 2d/wk</td>
<td>12</td>
<td>C: 2.5h</td>
<td>C: 75-85% V_LT</td>
<td>C: ↑VO_{peak}, ↑V_LT, ↓visceral fat I: ↑VO_{peak}, ↑V_LT, ↓visceral fat</td>
</tr>
<tr>
<td></td>
<td>recreational endurance runners</td>
<td></td>
<td>I: 4d/wk</td>
<td></td>
<td></td>
<td>total</td>
<td>I: all out (10x30sec, with 90sec running at 85% V_LT)</td>
<td></td>
</tr>
<tr>
<td>Kemi et al. [30]</td>
<td>24 F</td>
<td>80-90days</td>
<td>Running</td>
<td>5d/wk</td>
<td>10</td>
<td>1h/day</td>
<td>M: 5x8min at 65-70% VO_2max, with 2min at 50-60% VO_2max H: 5x8min at 85-90% VO_2max, with 2min at 50-60% VO_2max</td>
<td>↑VO_{2max}, cardiomyocyte hypertrophy and function, Ca^{2+} sensitivity, carotid artery endothelial function</td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M: 60-73% peak treadmill HR H: 73-88% peak treadmill HR</td>
<td></td>
</tr>
<tr>
<td>King et al. [19]</td>
<td>149 M&amp; 120 F</td>
<td>56±4</td>
<td>Walking-jogging</td>
<td>M: 5d/wk</td>
<td>104</td>
<td>M: 30min</td>
<td>M: ↑HDL-C, ↓WHR ratio H: ↑VO_{2max}, test duration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sedentary</td>
<td></td>
<td>H: 3d/wk</td>
<td></td>
<td></td>
<td>H: 40min</td>
<td>H: 73-88% peak treadmill HR</td>
<td></td>
</tr>
<tr>
<td>O'Donovan et al. [5]</td>
<td>42 M</td>
<td>41±4</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>24</td>
<td>400kcal</td>
<td>M: 60% VO_2max H: 80% VO_2max</td>
<td>M: ↑VO_{2max}, ↓body fat H: as above + ↓waist girth, total cholesterol, LDL-C, non-HDL-C</td>
</tr>
<tr>
<td></td>
<td>previously sedentary healthy</td>
<td></td>
<td></td>
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<tr>
<td>Reference</td>
<td>N</td>
<td>Age (y)</td>
<td>Mode</td>
<td>Frequency</td>
<td>Weeks</td>
<td>Duration</td>
<td>Intensity</td>
<td>Results</td>
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<td>----------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Rodrigues et al. [39]</td>
<td>23M previously sedentary</td>
<td>31±3.5</td>
<td>Running</td>
<td>3d/wk</td>
<td>26</td>
<td>60min</td>
<td>HR associated with VT1 to 10% below HR associated with VT2</td>
<td>↑VO_{2peak}, septal wall thickness, LV mass and mass index, ↓HR</td>
</tr>
<tr>
<td>Skinner et al. [38]</td>
<td>633M&amp;F sedentary healthy</td>
<td>17-65</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>20</td>
<td></td>
<td>55% VO_{2max} increased to 75% VO_{2max}</td>
<td>↑VO_{2max}, fat-free mass</td>
</tr>
<tr>
<td>Wilmore et al. [8]</td>
<td>250M&amp;252F sedentary healthy</td>
<td>17-65</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>20</td>
<td></td>
<td>55% VO_{2max} increased to 75% VO_{2max}</td>
<td>↑VO_{2max}, ↓HR response at 50W, fat-free mass, ↑HDL-C</td>
</tr>
<tr>
<td>Wisloff et al. [10]</td>
<td>20M&amp;7F stable post-infarction heart failure patients</td>
<td>75±11</td>
<td>Walking</td>
<td>3d/wk</td>
<td>12</td>
<td>C: 47min</td>
<td>C: 70-75% peak HR I: 4x4min at 90-95% peak HR, 3min at 50-70% peak HR</td>
<td>C: ↑VO_{2peak} (14%), ↑relative anaerobic threshold, ↑quality of life I: ↑VO_{2peak} (46%), ↑PGC-1α, ↑SERCA, reverse LV remodelling, ↓proBNP, ↑endothelial function, ↑quality of life</td>
</tr>
</tbody>
</table>

(C = moderate-intensity training; I = high-intensity training; M = male; F = female; 6MWT = 6 minute walk test; VT1 = first ventilatory threshold; V_{L} = velocity at lactate threshold; HDL-C = high-density lipoprotein cholesterol; WHR = waist to hip ratio; LDL-C = low-density lipoprotein cholesterol; LV = left ventricular; PGC-1α = peroxisome proliferative activated receptor- coactivator-1α; SERCA = sarcoplasmic reticulum Ca^{2+} ATPase; proBNP = pro-brain natriuretic peptide)
In addition to the previously mentioned central adaptations, peripheral adaptations including increased mitochondrial content [36, 40], capillary density [36, 40] and muscle respiratory capacity [36, 41] are common with moderate-intensity training (Table 1) [36]. Furthermore, inclusion of training below the first ventilatory threshold can contribute to a significantly greater skeletal muscle energy profile including increased calcium-calmodulin kinase leading to increases in peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α) which is a regulator of mitochondrial biogenesis [36]. These adaptations increase the ability to perform higher muscular power outputs for longer periods as well as improve recovery from high-intensity exercise [36].

2.3.4. Changes in cardiovascular disease risk factors associated with moderate-intensity exercise

Improvements in blood pressure [8] and lipoprotein profile [5, 8, 19] have been observed following moderate-intensity continuous training. Indeed, Wilmore et al. [8] observed significant improvements in lipid and lipoprotein profile after 26 weeks of moderate-intensity training in previously sedentary participants. Furthermore, in this study significant relationships between improvements in aerobic function (i.e. VO$_{2\text{max}}$ and power output at 60% VO$_{2\text{max}}$) and changes in percent body fat (-3.4%), fat mass (-3.4%) and fat-free mass (+0.9%) were observed [8]. In a similar cohort (sedentary individuals age: 56±4 years), greater increases in high-density lipoprotein levels were observed following two years of moderate-intensity training (five days a week; walking at 60-73% peak heart rate for 30 minutes per session), indicating frequent moderate-intensity exercise training for longer periods of time may be required to induce changes in lipoprotein profile [19].
2.4 HIGH-INTENSITY INTERVAL TRAINING

2.4.1 Immerging use of high-intensity interval training

The use of high-intensity interval training to enhance fitness and performance is common in athletic populations [35-37]. More recently, the use of high-intensity interval training has increased in non-athletic/diseased populations [11-15, 18] with evidence indicating high-intensity interval training is as effective and a more time-efficient method of improving general health and well-being when compared to moderate-intensity continuous training [5, 10-16, 30, 31, 35-37] (Table 2). High-intensity interval training is associated with greater cardiac benefits [5, 10-12, 14, 19, 29, 31, 42], as well as greater improvements in VO$_{2\text{peak/max}}$ [5, 10, 12, 14, 15, 19, 29-31, 37], exercise performance [11, 35-37] and skeletal muscle metabolic profile [10, 11, 15, 29, 35-37, 43, 44] (Table 2). The advantage of high-intensity interval training over traditional moderate-intensity exercise is that the short recovery periods allow participants to maximise the time spent at or near VO$_{2\text{max}}$ [37, 43]. Furthermore, compared with continuous moderate-intensity exercise, high-intensity exercise is more motivating for participants [10] resulting in a higher rate of adherence [19]. Regardless, due to the relatively novel use of high-intensity interval training in a non-athletic population there is a need for increased research in this area.

2.4.2 Changes in VO$_{2\text{max}}$ associated with high-intensity exercise

A plethora of research indicates that high-intensity interval training may result in greater improvements in VO$_{2\text{max}}$ compared with moderate-intensity continuous training [5, 9, 10, 12, 30, 35]. In sedentary individuals, vigorous exercise (>80% maximal aerobic power or >90% VO$_{2\text{max}}$) can dramatically increase
cardiorespiratory fitness (VO\textsubscript{2max}) [12, 15], which is associated with a decrease risk of cardiovascular disease [5, 6, 8]. For instance, Hottenrott et al. [35] compared high-intensity interval training to moderate-intensity continuous training and observed greater improvements in VO\textsubscript{2peak} (high-intensity +6.8±2.0 mL.min\textsuperscript{-1}.kg\textsuperscript{-1}; moderate-intensity +2.7±1.0 mL.min\textsuperscript{-1}.kg\textsuperscript{-1}) with high-intensity interval training [35]. While improvements have been observed in healthy individuals, high-intensity interval training has also proven safe and effective in cardiovascular disease patients with greater improvements in VO\textsubscript{2peak} (+27% versus +2%), %VO\textsubscript{2max} at the first ventilatory threshold (+22% versus +2%) and exercise capacity at the first ventilatory threshold (+111% versus +45%) observed after high-intensity interval training, compared with moderate-intensity training [12].

2.4.3 Physiological adaptations to high-intensity interval training

Similar to moderate-intensity exercise, high-intensity interval training is associated with significant central and peripheral adaptations [9, 10, 30, 43-45]. High-intensity interval training has been shown to improve cardiac function as highlighted by improving cardiomyocyte size and function [30] as well as reversing left ventricular remodelling [10]. Indeed, when compared with moderate-intensity training, high-intensity interval training in rats resulted in greater cardiomyocyte hypertrophy (+14% versus +5%), improved fractional shortening time (+45% versus +23%) and Ca\textsuperscript{2+} sensitivity (+40% versus +30%) [30]. In addition, central adaptations have also been observed in humans. For instance, in 27 stable post-infarction patients (age 75±11 years), reverse left ventricular remodelling was observed following 12 weeks of high-intensity interval training an improvement which was not observed following moderate-intensity continuous training [10]. Furthermore,
in previously sedentary individuals high-intensity interval training is associated with increased stroke volume (+15.4%), ejection fraction (+7.9%), left ventricular mass (+8.4%) and left ventricular end-diastolic volume (+7.6%) [46].
Table 2. Literature examining cardio-respiratory adaptations resulting from high-intensity interval training in non-elite, healthy and unhealthy individuals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Age (y)</th>
<th>Mode</th>
<th>Frequency</th>
<th>Weeks</th>
<th>Duration</th>
<th>Intensity</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burgomaster et al. [9]</td>
<td>20M &amp; F active untrained</td>
<td>23±1</td>
<td>Cycling</td>
<td>I: 3d/wk</td>
<td>6</td>
<td>I: 20-30min C: 40-60min</td>
<td>I: 'all out' (4-6x30sec, with 4.5min rest-50rpm, 30W) C: 65% VO₂peak</td>
<td>↑VO₂peak, PPO, mean PO, PGC-1α, CS, β-HAD, PDH, muscle glycogen, muscle PCR; ↓RER. No group differences</td>
</tr>
<tr>
<td>Cox et al. [46]</td>
<td>9M &amp; 7F previously sedentary</td>
<td>25±2</td>
<td>Cycling and running</td>
<td>6d/wk</td>
<td>7</td>
<td>Cycling: 40min Running: 50min</td>
<td>Alternated cycling (40min at 85-90% VO₂peak) and running (5x5min running at 100% VO₂max, 5min jogging), progressing weekly (~11W/wk and ~27m/wk)</td>
<td>↑septal wall thickness, stroke volume, ejection fraction, left ventricular mass, left ventricular end-diastolic volume, VO₂max ↓heart rate</td>
</tr>
<tr>
<td>Freyssin et al. [12]</td>
<td>26M &amp; F chronic heart failure patients</td>
<td>54±12</td>
<td>Cycling</td>
<td>I: 3d/wk</td>
<td>8</td>
<td>I: 71min C: 61min</td>
<td>I: 50-80% max PO (12x30sec, with 60sec PR) C: HR at VT1</td>
<td>↑VO₂peak, exercise test duration, O₂ consumption at VT1, O₂ pulse, 6MWT distance C: time at VT1, 6MWT distance</td>
</tr>
<tr>
<td>Gibala et al. [43]</td>
<td>16 M active</td>
<td>21±1</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>2</td>
<td>I: 20-30min C: 90-120min</td>
<td>I: 4-6x30sec 'all out', with 4min rest C: 65% VO₂peak</td>
<td>↑COX II and IV, muscle buffering capacity, glycogen content and exercise performance</td>
</tr>
<tr>
<td>Hottenrott et al. [35]</td>
<td>34M &amp; F recreational endurance runners</td>
<td>43±7</td>
<td>Running</td>
<td>I: 4d/wk</td>
<td>12</td>
<td>I: 30min each C: 2.5h total</td>
<td>I: all out (10x30sec, with 90sec running at 85% V̇L̇ total C: 75-85% V̇L̇</td>
<td>↑VO₂peak, ↑V̇L̇, ↓visceral fat C: ↑VO₂peak, ↑V̇L̇, ↓visceral fat</td>
</tr>
<tr>
<td>Reference</td>
<td>n</td>
<td>Age (y)</td>
<td>Mode</td>
<td>Frequency</td>
<td>Weeks</td>
<td>Duration</td>
<td>Intensity</td>
<td>Results</td>
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<tr>
<td>Kemi et al. [30]</td>
<td>24F Sprague-Dawley rats</td>
<td>80-90days</td>
<td>Running</td>
<td>5d/wk</td>
<td>10</td>
<td>60min</td>
<td>H: 5x8min at 85-90% VO$<em>{2\text{max}}$, with 2min at 50-60% VO$</em>{2\text{max}}$ M: 5x8min at 65-70% VO$<em>{2\text{max}}$, with 2min at 50-60% VO$</em>{2\text{max}}$</td>
<td>↑VO$_{2\text{max}}$, cardiomyocyte hypertrophy and function, Ca$^{2+}$ sensitivity, carotid artery endothelial function (greater improvements with high intensity)</td>
</tr>
<tr>
<td>Mandroukas et al. [16]</td>
<td>15M physical education students</td>
<td>22±3</td>
<td>Running</td>
<td>AR: 32min PR: 32min CR: til exhaustion</td>
<td>AR: 4x4min 12kph, 4min 8kph PR: 4x4min 12kph, 4min complete rest CR: 12kph til exhaustion</td>
<td>HR and VO$_2$ higher in CR during bouts 2-4. BLa higher during PR than AR. BLa during passive recovery time was higher following CR than PR or AR.</td>
<td></td>
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</tr>
<tr>
<td>Moholdt et al. [45]</td>
<td>74M &amp; 15F post-myocardial infarction patients</td>
<td>57±10</td>
<td>Treadmill</td>
<td>2d/wk</td>
<td>12</td>
<td>I: 38min C: 60min</td>
<td>I: 4x4min 85-95% HR$<em>{\text{max}}$, with 3min at 70% HR$</em>{\text{max}}$ C: aerobic exercise to music, encouraged to exert themselves</td>
<td>I: ↑VO$_{\text{2peak}}$ haemoglobin, HDL-C, endothelial function, QoL, adiponectin; ↓ferritin, resting HR C: endothelial function, QoL, adiponectin; ↓ferritin, resting HR</td>
</tr>
<tr>
<td>O'Donovan et al. [5]</td>
<td>42M previously sedentary healthy</td>
<td>41±4</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>24</td>
<td>400kcal</td>
<td>H: 80% VO$<em>{2\text{max}}$ M: 60% VO$</em>{2\text{max}}$</td>
<td>H: ↑VO$<em>{2\text{max}}$, ↓body fat, ↓waist girth, total cholesterol, LDL-C, non-HDL-C M: ↑VO$</em>{2\text{max}}$, ↓body fat</td>
</tr>
<tr>
<td>Perry et al. [44]</td>
<td>5M &amp; 3F untrained, recreationally active</td>
<td>24±1</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>6</td>
<td>60min</td>
<td>10x4min 90% VO$_{2\text{peak}}$ with 2min rest</td>
<td>↑VO$_{2\text{peak}}$ max activities of mitochondrial enzymes, content of transport proteins for fatty acids, glucose and lactate, muscle glycogen</td>
</tr>
<tr>
<td>Reference</td>
<td>n</td>
<td>Age (y)</td>
<td>Mode</td>
<td>Frequency</td>
<td>Weeks</td>
<td>Duration</td>
<td>Intensity</td>
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<tr>
<td>Trilk et al. [47]</td>
<td>28F sedentary</td>
<td>31±6</td>
<td>Cycling</td>
<td>3d/wk</td>
<td>4</td>
<td>~20-35min</td>
<td>4-7x30s sprints (5% body mass as resistance), 4min active recovery</td>
<td>↑VO$_{2\text{max}}$, stroke volume, ↓ heart rate</td>
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<tr>
<td></td>
<td>overweight/obese</td>
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<tr>
<td>Wisloff et al. [10]</td>
<td>20M&amp; 7F stable</td>
<td>75±11</td>
<td>Walking</td>
<td>3d/wk</td>
<td>12</td>
<td>I: 38min</td>
<td>I: 4x4min at 90-95% peak HR, 3min at 50-70% peak HR</td>
<td>I: ↑VO$_{2\text{peak}}$ (46%), ↑PGC-1α, ↑SERCA, reverse LV remodelling, ↓proBNP, ↑endothelial function, ↑ quality of life</td>
</tr>
<tr>
<td></td>
<td>post-infarction</td>
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<td></td>
<td>C: 47min</td>
<td>C: 70-75% peak HR</td>
<td>C: ↑VO$_{2\text{peak}}$ (14%), ↑ relative anaerobic threshold, ↑ quality of life</td>
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<td></td>
<td>heart failure</td>
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</table>

(I: high-intensity interval training; C= moderate-intensity continuous training; M= male; F= female; PPO= peak power output; PGC-1α= peroxisome proliferative activated receptor- coactivator-1α; SERCA= sarcoplasmic reticulum Ca$^{2+}$ ATPase; proBNP= pro-brain natriuretic peptide; CS= citrate synthase; β-HAD= 3-hydroxyacyl CoA dehydrogenase; PDH= pyruvate dehydrogenase; PCR= phosphocreatinine; RER= respiratory exchange ratio; O$_2$= oxygen; 6MWT= 6 minute walk test; COX= cytochrome c oxidase; V$_{LT}$= velocity at lactate threshold; LV= left ventricular; BLa= blood lactate; HDL-C= high-density lipoprotein cholesterol; QoL= quality of life; LDL-C= low-density lipoprotein cholesterol)
Greater increases in adaptations of various peripheral metabolic indices (e.g. muscle buffering capacity, mitochondrial capacity and muscle glycogen content) have been observed following high-intensity interval training, when compared with moderate-intensity continuous training [9, 43, 44]. For instance increased maximal activity of mitochondrial enzymes (+18-29%), increased content of transport proteins for glucose, fatty acids and lactate (+14-30%) as well as increased muscle glycogen (+59%) have all been observed after high-intensity interval training in healthy adults [44]. Similarly, Gibala et al. [43] observed improvements in markers associated with mitochondrial biogenesis (i.e. COX II and IV protein content), muscle buffering capacity and glycogen content following 2 weeks of high-intensity interval training compared with moderate-intensity continuous training. Importantly, the participants of the high-intensity group had a training volume ~90% less than the moderate-intensity group, demonstrating that high-intensity interval training is a time-efficient strategy to rapidly improve skeletal muscle metabolic profile while having comparable effects with moderate-intensity continuous training [43]. It is proposed that the superior adaptations associated with high-intensity interval training are due to the increased stimulus to PGC-1α activation, which leads to an up regulation in mitochondrial biogenesis [11].

### 2.4.4 Influence of interval training on cardiovascular risk factors

#### 2.4.4.1 Body fat

Body fat (in particular, visceral fat) is an independent risk factor for cardiovascular disease [8, 35] which can be modified through high-intensity interval training [5, 8, 35]. Indeed, reductions in percent body fat and waist girth have been observed after high-intensity interval training [5]. Additionally, Hottenrott et al. [35]
measured a greater reduction in visceral fat following 12 weeks of high-intensity interval training (-16.5%) when compared with moderate-intensity continuous training (-6.5%). The authors suggested that this difference may be due to the increased workload or the greater energy expenditure resulting from a larger excess post-exercise oxygen consumption during high-intensity interval training [35].

2.4.4.2 Lipids and lipoproteins
While elevated levels of total cholesterol and low-density lipoproteins are associated with cardiovascular disease, higher levels of high-density lipoproteins can provide cardioprotective benefits [48]. Several studies indicate decreased total cholesterol and low-density lipoproteins and increased high-density lipoprotein levels post high-intensity interval training [5, 15, 45].

2.4.5 Cardiac stress associated with interval training
Despite the documented benefits of high-intensity interval training on cardiac function, skeletal muscle metabolic profile and quality of life [7, 10-12, 14, 15, 29, 31, 42] this modality can warrant caution due to the ability to induce severe cardiac stress as highlighted by decreased biventricular systolic and diastolic function immediately following high-intensity interval exercise [17, 18]. For example, in patients with non-ischemic mild heart failure an immediate decrease in end-systolic (-6%) and end-diastolic (-6%) volume were observed following four 4-minute intervals at 95% max heart rate with three minutes active rest in between efforts [18]. Nevertheless, an increase in left ventricular ejection fraction (+2.4%) was observed 30 minutes post-exercise indicating although high-intensity interval exercise results in an immediate decline in cardiac function, which could lead to cardiac injury or event, the negative effects were not long-lasting [18].
Brain natriuretic peptide (BNP) is a hormone released predominantly from the ventricles following damage or dysfunction and is increased in individuals who present with myocardial ischemia, whether it be exercise or disease-induced [23, 24]. In a study conducted by van der Zee et al. [23], NT-proBNP (BNP prohormone) was shown to increase (113-118%) following symptom-limiting exercise in patients referred for evaluation of presence or absence of inducible myocardial ischemia. Interesting to note, BNP levels increased following intense exercise in both healthy [23, 49] and unhealthy [24] individuals suggesting that ventricular wall stress, particularly that caused by intense exercise, was likely responsible for increased levels of BNP observed by van der Zee et al. [23]. Consequently, the cardiac damage associated with high-intensity interval training should be acknowledged and of concern especially in individuals with cardiovascular disease or those with multiple risk factors.

2.5 SINGLE-LEGGED CYCLE TRAINING

Although high intensity interval training offers a viable method of exercise prescription with well documented benefits [5, 11-18, 22], the possibility to induce cardiac damage [17, 22] highlights the need for alternative methods of this type of exercise delivery. The use of smaller muscle mass training (e.g. single limb exercise) can allow localised high-intensity muscle training but avoid cardiac limitations associated with high blood flow demands when training using a large muscle mass (Table 3) [20]. During normal bilateral cycling, oxygen delivery to the active muscle may be a limiting factor in the amount of work achievable [20, 25, 26, 50, 51] as documented by Knight et al. [50], who observed an increase in leg VO_{2max} and maximal oxygen delivery following incremental exercise in hyperoxic conditions
suggesting that leg VO$_{2\max}$ is limited by oxygen delivery during normoxic conditions. Single-legged cycling can offset this limitation by removing the blood flow demands of bilateral cycling and thus lowering the cardiac stress (e.g. high heart rates) associated with traditional high-intensity interval training [26, 51]. By removing the cardiac demands of a second limb it is possible that blood flow, muscle perfusion and muscle oxygen extraction will be improved in the exercising limb [20, 25, 26, 51, 52]. Such improvements are believed to be responsible for the greater work capacity and training adaptations observed during single-legged cycling [20, 25, 27, 51, 52].

2.5.1. Changes in VO$_{2\max}$ associated with single-legged cycling

Previous research has examined the influence of single-legged cycling on VO$_{2\max}$ with several reporting this modality can improve aerobic capacity [20, 27, 51, 53] likely through peripheral focused adoptions [20, 25, 27]. For instance, seven week of single-legged high-intensity interval training has been shown to improve VO$_{2\max}$ by approximately 5% [53]. Furthermore, when compared to traditional double-legged interval training, single-legged high-intensity interval training has been documented to provide similar improvements (+0.5±6.6% and -1.2±10.2%; respectively) to VO$_{2\max}$. The changes have been suggested to be due to improvements in oxidative capacity and blood flow associated with single-legged training [20, 27]. Such findings suggest that single-legged cycling may be as effective at improving VO$_{2\max}$ as double-legged cycling.

2.5.2 Physiological adaptations to single-legged cycling

Several studies have examined the peripheral benefits of single-legged cycling [20, 25, 50, 51], with improvement in mitochondrial oxidative capacity and
oxygen utilisation (as shown by increases in maximal citrate synthase activity) [25].

To date; however, no studies to the authors’ knowledge have examined the influence of single-legged cycle training on central adaptations know to improve fitness and functional capacity.

### 2.5.3. Perceptual responses

Single-legged cycling [20, 27, 51, 52] as a training modality is likely to have greater adherence than high-intensity double-legged cycling as individuals typically report lower whole body perceived exertion [26, 27], while experiencing similar quadriceps pain during single-legged, compared with double-legged cycling [26, 27]. Indeed, Abbiss et al. [27] observed lower ratings of perceived exertion following six single-legged maximal self-paced 4-minute intervals, with 6 minutes active recovery (50W), when compared with three double-legged maximal self-paced 4-minute intervals, with 6 minutes active recovery (100W). The authors also noted similar ratings of perceived pain and effort between interventions [27].
Table 3. Literature examining cardio-respiratory adaptations resulting from single-legged training in non-elite, healthy and unhealthy individuals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Age (y)</th>
<th>Leg(s) trained</th>
<th>Frequency</th>
<th>Weeks</th>
<th>Protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbiss et al. [27]</td>
<td>9 trained cyclists</td>
<td>34±5</td>
<td>Single vs double leg</td>
<td>2d/wk</td>
<td>3</td>
<td>S: 6x4min 'all out', 6min AR D: 3x4min 'all out', 6min AR</td>
<td>S: ↑COX II &amp; IV, GLUT4 protein, AMPKα, VO\textsubscript{2max} (DL), time trial performance D: ↑AMPKα, VO\textsubscript{2max} (DL), time trial performance</td>
</tr>
<tr>
<td>Barker et al. [26]</td>
<td>11M recreationally trained cyclists</td>
<td>SL-assisted vs SL-unassisted vs DL</td>
<td>Acute</td>
<td>Acute</td>
<td>S: start 50W ↑25W/5min till exhaustion D: start 100W ↑50W/5min till exhaustion</td>
<td>SL-A: highest peak power SL-U: higher submax blood lactate, RPE, quad pain DL: lowest submax VO\textsubscript{2}, higher peak and submax HR Mean MAP similar between conditions</td>
<td></td>
</tr>
<tr>
<td>Bell et al. [25]</td>
<td>5M healthy</td>
<td>77±7</td>
<td>Trained vs untrained leg</td>
<td>4d/wk</td>
<td>9</td>
<td>Single-legged knee extension exercise for 40min at 75-85% VO\textsubscript{2peak}</td>
<td>Accelerated VO\textsubscript{2} kinetics, ↑maximal CS activity in trained leg</td>
</tr>
<tr>
<td>Bell et al. [53]</td>
<td>8M &amp; 1F active</td>
<td>26±3</td>
<td>Trained vs untrained leg</td>
<td>4d/wk</td>
<td>7</td>
<td>15-20x20sec 150% PO associated with single-leg VO\textsubscript{2max}, 60sec rest</td>
<td>↑VO\textsubscript{2max} (SL and DL), no significant differences between legs</td>
</tr>
<tr>
<td>Gleser et al. [51]</td>
<td>6M healthy</td>
<td>21±1</td>
<td>Trained vs untrained leg</td>
<td>2d/wk</td>
<td>4</td>
<td>Single-legged cycling (standing with partner on other pedal) at 75% VO\textsubscript{2max} till exhaustion</td>
<td>↑VO\textsubscript{2max} and cardiac output, ↓vascular resistance</td>
</tr>
<tr>
<td>Reference</td>
<td>N</td>
<td>Age (y)</td>
<td>Leg(s) trained</td>
<td>Frequency</td>
<td>Weeks</td>
<td>Protocol</td>
<td>Results</td>
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<tr>
<td>Hardman et al. [52]</td>
<td>5M &amp; 1F healthy</td>
<td>37.5±10.4</td>
<td>Trained vs untrained leg</td>
<td>3d/wk</td>
<td>6</td>
<td>Single-legged cycling starting at PO associated with 80% VO$_{2max}$ for 30min, ↑16.2W/wk</td>
<td>TL: ↑endurance time, muscle ATP and glycogen concentration, ↓muscle lactate</td>
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<td>UTL: ↑endurance time</td>
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<tr>
<td>Knight et al. [50]</td>
<td>12M healthy</td>
<td>29.4±4.5</td>
<td>100% O$_2$ vs 21% O$_2$ vs 12% O$_2$</td>
<td>Acute</td>
<td>acute</td>
<td>Incremental DL cycling at 20, 35, 50, 92 and 100% WR$_{max}$ for 3, 2, 2, 1.5 and 0.3min respectively</td>
<td>100% O$<em>2$: ↑leg VO$</em>{2max}$ by 8.1% and maximal O$<em>2$ delivery by 10.9% (leg VO$</em>{2max}$ limited by O$_2$ supply in normoxia)</td>
</tr>
<tr>
<td>Rud et al. [20]</td>
<td>6M &amp; 6F healthy</td>
<td>24±3.1</td>
<td>Trained vs untrained leg</td>
<td>4d/wk</td>
<td>7</td>
<td>SL cycling at varying intensities held constant for session (59-90% HR$_{max}$) and progressive duration (40-100min).</td>
<td>TL: ↑VO$_{2max}$, CS activity, leg O$_2$ uptake, O$_2$ extraction, leg blood flow</td>
</tr>
</tbody>
</table>

(M= male, F= female; DL= double-legged; SL= single-legged; TL= trained leg; UTL= untrained leg; SL-A= single-legged assisted; SL-U= single-legged unassisted; COX= cytochrome c oxidase; GLUT4= glucose transporter four; AMPKα= 5'-AMP-activated protein kinase α-subunit; MAP= mean arterial pressure; ATP= adenosine triphosphate; O$_2$= oxygen; WR$_{max}$= maximum work rate; CS= citrate synthase)
2.6 SUMMARY

While high-intensity interval training is currently gaining acceptance in cardiovascular rehabilitation settings, the high demands placed on the heart indicate the need to provide alternative methods of high-intensity exercise delivery. The use of single-legged cycling can induce greater peripheral adaptations while avoiding large demands on the cardiovascular system. Regardless, no studies to date have examined the central demands and cardiac stress induced by single-legged cycling. For this reason, greater research in this area is warranted with results likely providing necessary information for the use of this modality in clinical settings.
CHAPTER THREE: METHODS

3.1 STUDY DESIGN

A randomised/counterbalanced cross-over design was used for the study with subjects acting as their own controls. Participants were required to attend the Murdoch University laboratory on four separate occasions with no less than five and no greater than 10 days between testing sessions. The study consisted of a maximal exercise test, a familiarisation session and two experimental sessions. Participants were asked to maintain a similar diet (food and fluid) and avoid strenuous physical activity the day before and the day of testing. All testing was completed at a similar time of day to control for circadian rhythm.

3.2 SAMPLING METHODOLOGY

Nine males (age: 24 ± 5 years; height: 181 ± 5 cm; mass: 76 ± 11 kg; VO$_{2\text{max}}$: 52.6 ± 8.5 mL.kg$^{-1}$.min$^{-1}$) and one female (age: 19 years; height: 167 cm; mass: 55 kg; VO$_{2\text{max}}$: 38.8 mL.kg$^{-1}$.min$^{-1}$) volunteered for participation in this study. The sample size was selected based on a power analysis using unpublished heart rate data (Turner; Master’s Thesis University of Birmingham; 2010) collected during double- (146 ± 6 bpm) and single-legged (120 ± 16 bpm) cycling intervals ($\alpha$=0.05; power=0.80). All participants were be between 18 and 50 years of age and regarded as of low risk for exercise based on the Exercise & Sports Science Australia risk stratification questionnaire. All risks and benefits associated with inclusion in this study were provided to the participants in writing and informed consent was obtained prior to data collection. Ethical clearance was obtained from the Murdoch University Human Ethics Department prior to the start of this study.
3.3 PROCEDURES

During the initial testing session, participants were asked to complete a graded exercise test using a Velotron cycle ergometer (RacerMate; USA). The test started at a resistance of 70 W with 25 W-minute\(^{-1}\) increase for males, while females started at a resistance of 50 W with 20 W-minute\(^{-1}\) increase in resistance until the participant reached volitional fatigue. Maximal oxygen consumption was measured using a Parvo TrueOne metabolic cart (ParvoMedics, USA). Prior to the test, the metabolic cart was calibrated using gases of know concentration (4.0% CO\(_2\) and 16.0% O\(_2\)) and through a range of flow rates using a Hans Rudolph 3L syringe. Data collected from the graded exercise test was used for descriptive purposes (i.e. fitness level) and to calculate a power output (50% aerobic threshold) used during active recovery portions of the remaining sessions.

The second testing session was used as a familiarisation session. During this session, participants were required to complete six 1-minute cycling intervals. Prior to the interval session, participants completed a standardised 15-minute warm up cycling at 30% and 40% (5 min at 30% and 10 min at 40%) of the maximal power output measured during the graded exercise test. Participants were then instructed to cycle at their predetermined recovery power output for five minutes after which they performed two 1-minute efforts at their highest sustainable power output using normal double-legged bilateral cycling. Immediately after completing each effort, participants cycled for 1 minute at their individualised recovery intensity. Following these initial two intervals, four additional efforts were conducted in a single-legged manner. During single-legged cycling participants cycled with only one leg. A specially designed counterweight was used to compensate for the missing
limb and allow a fluid cycling motion. Participants were instructed to completed two 1-minute maximal efforts with 1 minute active recovery between efforts after which participants cycled for five minutes at half the recovery power output used during the double-legged efforts. The cycle ergometer was then adjusted to allow the participant to complete the same procedure with the opposite leg.

The remaining two experimental sessions were conducted in a randomised and counterbalanced order. During experimental sessions participants completed either six maximal 1-minute double-legged efforts with 1 minute recovery (88 ± 16 W) between efforts, or twelve (six with each leg) maximal 1-minute single-legged efforts with 1 minute recovery (44± 8 W) between each effort. Prior to exercise, participants were required to rest in a supine position for 10 minutes during which heart rate was collected beat-to-beat using a personal heart rate monitor (Polar 810i; Finland). Ultrasound measures of the left ventricle were collected during systole and diastole and a venous blood sample was obtained. Additionally, resting expired ventilation was measured via metabolic cart and measures of blood pressure and oxygen saturation (Prince-100B Fingertip Oximeter, Heal Force Bio-Meditech Holdings Limited; China) were obtained. Irrespective of interval type (i.e. double or single-legged) participants completed a 15-minute standardised (five minutes at 30% of the first ventilatory threshold, ten minutes at 40% of the first ventilatory threshold) double-legged warm-up. In the double-legged conditions, participants then cycled for an additional five minutes at 50% of the first ventilatory threshold measured during the maximal exercise test. This was immediately followed by the interval session.
Following the standardised warm-up during the single legged-condition, participants cycled for five minutes at 25% of the first ventilatory threshold, after which the interval session was started. Upon completion of the first set of single-legged intervals, the counterweight was transferred to the other pedal (~5 minutes) to allow the participant to complete the single-legged session (i.e. five minute lead in and six interval efforts) with the opposite leg. During this study the starting leg for the single-legged session was randomised and counterbalanced.

During each interval session, expired ventilation was continuously measured, and heart rate was recorded at a frequency of 1 Hz. Power output was recorded at a frequency of 1 Hz via the Velotron cycle ergometer software. Blood pressure and oxygen saturation were measured immediately after each interval. Additionally, immediately after each interval, and at the completion of each testing session (20 minutes post completion) participants were asked to rate their perceived exertion, effort, and pain using visual analog scales (0 = none; 10 = maximal). At the completion of the final interval, participants were asked to lay supine for 10 minutes during which heart rate, blood pressure and oxygen saturation were recorded. Following this, ultrasound measures were performed and a second venous blood sample was obtained (Figure 1).
3.4 PHYSIOLOGICAL MEASURES

3.4.1 Venous Blood Sampling and analysis

Venous blood samples were obtained pre- and post-interval session using standard venipuncture techniques. All samples were obtained by a trained and certified phlebotomist from the anti-cubital vein using a 21 gauge needle and 8.0ml EDTA and SST tubes. After collection, blood samples were centrifuged and supernate was removed and stored at -80°C in individual 1.0mL samples. Plasma samples were analysed for brain natriuretic peptide (BNP) following standard commercial procedures (Western Diagnostic; Perth).

3.4.2 Ultrasound Ventricular Measures

Ultrasonic measures of left ventricular volume were measured using a hand-held portable ultrasound device (SonoSite Plus; SonoSite Inc.; USA) with a cardiac
specific ultrasound probe. A standard four-chamber apical view was used to obtain end-diastolic and end-systolic images of the left ventricle. Images were assessed for chamber volume using National Institute of Health freeware (ImageJ; NIH; USA). The difference in left ventricular area measures between end-diastole and end-systole were used to represent ejection fraction and thus ventricular function.

3.4.3 Heart Rate Analysis
Heart rate data collected during each 10 minute rest period, pre- and post-exercise, were analysed to determine the heart rate variability. Mean time domain (ms) was calculated from beat-by-beat data for the final 2 minutes of each measurement period using Polar internal software. The standard deviation in beat-by-beat measures as well as the root mean squared RMSS was calculated and used to represent sympathetic (lower standard deviation) and parasympathetic (greater standard deviation) output. Mean heart rate was calculated for each interval and session for later analysis.

3.4.4 Expired Ventilation
Expired ventilation collected during rest and intervals sessions was used to calculate the volume of oxygen consumed (VO₂) during each measurement point. Measurements obtained during the 10 minute pre-exercise rest period were used as the participant’s basal VO₂ and these values were removed from measurements recorded during exercise to provide an accurate indication of the metabolic demands associated with each effort.

3.5 DATA PROCESSING
Consistent with previous research [27], single-legged power output data was compared with double-legged data using the sum of power output from the right
and left leg. Furthermore, work-load during each interval session (only during efforts) was calculated from the mean power recorded during the double-legged and calculated from the single-legged efforts. Workload was calculated as the mean power during each effort multiplied by the time in seconds (60 seconds) corresponding to the interval. A similar methodology was used to calculate workload during recovery. Total workload was calculated as the sum of both the interval efforts and their corresponding rest periods.

In order to compare measures of oxygen consumption, oxygen saturation, ventilation, respiratory rate, heart rate, blood pressure and perceptions of exertion, pain and effort between double- and single-legged cycling, a mean value was calculated for each variable between the right and left leg. This technique has previously been used in research examining oxygen consumption during single-legged and double-legged cycling [26].

3.6 STATISTICAL ANALYSIS

Differences between conditions for mean heart rate variability, blood pressure, ventricular function, BNP, and perceived exertion obtained pre- and post-interval session were analysed using a two-way analysis of variance with repeated measures (ANOVA; condition x time). Mean VO₂, blood pressure, oxygen saturation, heart rate and perceived exertion, pain, and effort during each interval were also analysed for differences between conditions (double-legged, single-legged) using a two-way ANOVA with repeated measurements. Significant main effects or interactions were examined using a Tukey’s HSD post hoc analysis. Statistical analyses were conducted using Statistica statistical analysis software (Version 7;
StatSoft; USA) with significance set at $p \leq 0.05$. All data are presented as mean ± standard deviations unless noted otherwise.
A significant interaction ($F_{(5, 40)} = 5.7411, p<0.01$) was observed for power, with greater power measured during intervals two to six in the combined (i.e. sum of right and left leg) single-legged when compared with double-legged condition.

Furthermore, a significant interaction ($F_{(5, 40)} = 7.4060, p<0.01$) was observed for total workload, with greater total workload measured following intervals two to six in the combined single-legged when compared with the double-legged condition.

A main effect for condition ($F_{(1, 8)} = 201.39, p<0.01$) was observed for session workload, with greater external workloads measured during single-legged, compared with double-legged cycling ($743 \pm 122$ kJ versus $916 \pm 73$ kJ).
Figure 3. Mean VO₂ (top), ventilation (middle) and RR (bottom) measured pre-exercise and during each interval in double- (■) and single-legged (▲) cycling conditions. * Double-legged greater than single-legged cycling. ** Double-legged greater than single-legged condition. # Pre-exercise and interval 1 less than intervals 2-6.

A significant \( F(6, 54) = 21.373, p<0.01 \) interaction was observed for VO₂, with greater VO₂ measured during all intervals during double-legged compared with single-legged (i.e. mean of right and left leg) cycling. A significant interaction \( F(6, 54) = \)
9.7177, p<0.01) was observed for ventilation (V\textsubscript{E}), with greater V\textsubscript{E} measured during all double-legged intervals, compared with the single-legged (i.e. mean of right and left leg) condition. A main effect for condition (F\textsubscript{(1, 9)} = 6.9183, p<0.05) was observed for respiration rate (RR), with greater RR measured during double-legged when compared with the single-legged (i.e. mean of right and left leg) condition. In addition, a main effect for time (F\textsubscript{(6, 54)} = 70.976, p<0.01) was observed for RR, with a lower RR observed pre-exercise and during interval one when compared with intervals two through six.

A main effect for condition (F\textsubscript{(1, 6)} = 9.3352, p<0.05) was observed for oxygen saturation, with greater oxygen saturation measured in the single-legged (i.e. mean of right and left leg; 97% ± 1%) when compared with the double-legged (96% ± 2%) condition. In addition, a main effect for time (F\textsubscript{(7, 42)} = 5.0820, p<0.01) was observed for oxygen saturation, with greater oxygen saturation measured pre-exercise and during intervals one, two, four and five when compared with post-exercise values. Furthermore, oxygen saturation was greater in both conditions after interval one compared with interval six.
Figure 4. Heart rate measured pre-exercise and during each interval in double- (■) and single-legged (▲) cycling conditions. * Double-legged significantly greater than single-legged.

A significant interaction ($F_{(6, 54)} = 8.1881, p<0.01$) was observed for heart rate, with greater heart rate measured following all intervals in double-legged when compared with the single-legged (i.e. mean of right and left leg) condition. Furthermore, a main effect for time was observed for heart rate variability with greater standard deviation (SD1) and RMSSD measured pre- (49 ± 33 ms and 69 ± 46 ms; respectively), compared with post-trial (7 ± 4 ms and 10 ± 6 ms; respectively).
Table 4. Brain natriuertic peptide (BNP) and left ventricular ejection fraction (%) measured pre-exercise and post-exercise in the double- and single-legged cycling conditions.

<table>
<thead>
<tr>
<th></th>
<th>Double-legged</th>
<th>Single-legged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post**</td>
</tr>
<tr>
<td>BNP (pg/mL) *</td>
<td>29.9±13.3</td>
<td>32.9±12.0</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>49.4±6.8</td>
<td>37.9±5.0</td>
</tr>
</tbody>
</table>


Differences in pre- and post-exercise measurements of BNP and ejection fraction for double- and single-legged conditions are displayed in table 4. A main effect for condition was observed for BNP, with greater values measured in the double-legged when compared with single-legged condition. In addition, a significant main effect for time was observed for BNP, with greater values measured post-exercise when compared with pre-exercise. A significant interaction was observed for left ventricular ejection fraction, with a greater ejection fraction measured post-exercise in single-legged when compared with double-legged cycling.
Figure 5. Systolic (top), diastolic (middle) and mean arterial pressure (bottom) measured pre-exercise, following each interval and 10 minutes post-exercise in the double- (■) and single-legged (▲) cycling conditions. * Significant difference between double-legged and single-legged conditions. ** Pre-exercise measures less than all intervals. ^ Pre- and post-exercise measures less than all intervals. # Single-legged greater than double-legged. + Intervals 1 and 2 greater than interval 6.
A significant interaction ($F_{(8, 72)} = 6.0587, p<0.01$) was observed for systolic blood pressure, with greater systolic blood pressure measured at the end of intervals one to four in double-legged when compared with the single-legged (i.e. mean of right and left leg) condition. A main effect for condition ($F_{(1, 9)} = 7.8975, p<0.05$) was observed for diastolic blood pressure, with greater mean diastolic blood pressure measured in the single-legged (i.e. mean of right and left leg; $72 \pm 10$ mmHg) when compared with double-legged condition ($68 \pm 11$ mmHg). In addition, a main effect for time ($F_{(8, 72)} = 11.544, p<0.01$) was observed for diastolic blood pressure, with greater diastolic blood pressure observed following all intervals when compared with pre-exercise measures. A main effect for time ($F_{(8, 72)} = 89.348, p<0.01$) was observed for mean arterial pressure, with greater values observed following all intervals when compared with pre- and post-exercise values, however no significant differences were observed between pre- and post-exercise values. In addition, measures of mean arterial pressure obtained after intervals one and two were significantly greater compared with interval six.
Table 5. Ratings of perceived exertion, pain and effort measured following each interval in double- and single-legged cycling conditions.

<table>
<thead>
<tr>
<th></th>
<th>Interval 1</th>
<th>Interval 2</th>
<th>Interval 3</th>
<th>Interval 4</th>
<th>Interval 5</th>
<th>Interval 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RPE</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-leg</td>
<td>7.0±2.3</td>
<td>7.7±2.0</td>
<td>8.4±2.1</td>
<td>8.7±1.6</td>
<td>9.2±0.8</td>
<td>9.7±0.7</td>
</tr>
<tr>
<td>Single-Leg</td>
<td>6.4±2.0</td>
<td>6.8±1.9</td>
<td>7.2±2.1</td>
<td>7.7±2.2</td>
<td>7.8±2.1</td>
<td>8.0±1.8</td>
</tr>
<tr>
<td><strong>Pain</strong>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-leg</td>
<td>6.6±1.4</td>
<td>7.6±1.0</td>
<td>8.8±1.0</td>
<td>9.1±0.9</td>
<td>9.4±0.7</td>
<td>9.6±0.5</td>
</tr>
<tr>
<td>Single-Leg</td>
<td>6.7±1.7</td>
<td>8.0±1.3</td>
<td>8.7±0.9</td>
<td>9.3±0.5</td>
<td>9.6±0.5</td>
<td>9.7±0.5</td>
</tr>
<tr>
<td><strong>Effort</strong>+</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Double-leg</td>
<td>8.9±1.4</td>
<td>9.4±0.7</td>
<td>9.6±0.5</td>
<td>9.8±0.4</td>
<td>9.9±0.3</td>
<td>9.9±0.3</td>
</tr>
<tr>
<td>Single-Leg</td>
<td>9.0±0.9</td>
<td>9.2±0.8</td>
<td>9.3±0.6</td>
<td>9.5±0.5</td>
<td>9.5±0.5</td>
<td>9.7±0.5</td>
</tr>
</tbody>
</table>

* Interval 1 less than intervals 3-6, interval 2 less than intervals 5 and 6, interval 3 less than interval 6. ** Interval 1 less than intervals 2-6, interval 2 less than 4-6. + Interval 1 less than intervals 4-6.

A main effect for time ($F_{(5, 45)}= 11.341$, p<0.01) was observed for RPE, with lower reported RPE after interval one when compared with intervals three to six. Furthermore, lower reported RPE was observed after interval two when compared with intervals five and six and lower RPE was measured after interval three when compared with interval six. A main effect for time ($F_{(5, 45)}= 5.7133$, p<0.01) was observed for perceived effort, with lower perceived effort reported after interval
one when compared with intervals four, five and six. A main effect for time ($F_{(5, 45)} = 26.101, p<0.01$) was observed for perceived pain, with lower perceived pain measured after interval one when compared with intervals two through six. Additionally, lower perceived pain was measured after interval two when compared with intervals four, five and six. No interactions observed for RPE, perceived effort or perceived pain.

Sessional RPE displayed a main effect for condition ($F_{(1, 9)} = 8.6412, p<0.02$) with greater sessional RPE measured following double-legged when compared with the single legged condition ($8.9 \pm 0.7$ versus $7.2 \pm 1.8$).
CHAPTER FIVE: DISCUSSION

The purpose of the current study was to investigate the influence of double- and single-legged cycling intervals on performance, as well as physiological and cardiac stress in young healthy adults. The main findings from the study were; 1) accumulated power output and workload were greater during combined (i.e. sum of right and left legs) single-legged when compared with double-legged cycling, 2) greater metabolic/physiological stress was observed during double-legged cycling, as measured by oxygen consumption, 3) greater cardiac stress was observed during double-legged cycling, as shown by greater inter-interval heart rate and lower end-session left ventricular function, and 4) single-legged cycling was perceived easier, as indicated by measures of sessional ratings of perceived exertion, even though inter-interval perceptions of effort and pain were similar between conditions.

In the current study we observed 12% greater power output measured during single-legged (i.e. sum of right and left leg) when compared with double-legged cycling (Figure 2). Our findings are consistent with Abbiss et al. [27] who observed similar differences (13%) when comparing the accumulated power output from single-legged with double-legged cycling during a 4-minute sustained effort. It is suggested the ability to maintain higher power outputs during single-legged cycling is due to an increased ability to deliver oxygen to the smaller active muscle mass; thus, limiting the influence of insufficient oxygen delivery which has been hypothesised to reduce double-legged cycling capacity [20, 50, 54]. This improved ability to produce greater power outputs during single-legged cycling has been associated with greater skeletal muscle metabolic adaptations [20, 25, 27, 52]. As
such, improvements in mitochondrial oxidative capacity [20, 25, 27, 52] as well as improvements in substrate storage [52] have been observed following single-legged cycle training.

Although accumulated workload was less during double-legged compared with single-legged cycling, our findings indicated double-legged cycling resulted in increased physiological stress as shown by a 34% ± 0.6% greater oxygen consumption measured during double-legged when compared with single-legged (i.e. mean of right and left leg) cycling (Figure 3). Based on the available literature, we believe this difference is due to the reduced size of active muscle mass during single-legged cycling [26] which would limit the muscles ability to consume oxygen [26, 55]. Previously, Barker et al. [26] have observed ~20% greater peak oxygen consumption during double-legged when compared with single-legged incremental cycling. We believe, although highlighting a similar phenomena, the difference between our data and Barker et al. [26] was due to the supramaximal nature of the intervals in our study which would have resulted in a large oxygen deficit and, hence, larger oxygen debt during double-legged cycling [56]. For instance, during double-legged cycling a large anaerobic contribution would be needed during the initial stages of each effort (i.e. first 15 to 20 seconds of each interval) as blood delivery is unlikely to meet the metabolic demand [57]. However, during single-legged cycling adequate oxygen delivery [20, 25, 27, 54] would have resulted in a lower anaerobic contribution to this activity. The large anaerobic contribution during the initial stages of the double-legged cycling efforts would have resulted in an increased oxygen debt [57] with elevated compensatory oxygen consumption throughout the remaining effort [56-58].
The benefits of high intensity interval training are well documented [5, 11, 13-16, 42, 43]; however, in certain populations (i.e. post myocardial infarction, chronic heart failure, diabetes mellitus and obese patients) the cardiovascular stress associated with traditional high-intensity interval training may be contraindicated [18]. Heart rate in our study was 11.8% ± 7.0% greater during double-legged compared with single-legged (i.e. mean of right and left leg) cycling (Figure 4), indicating a greater level of cardiovascular stress [10, 14, 16-18, 26, 30, 31, 59]. To further explore this, we examined change in left ventricular ejection fraction pre- and post-interval session and observed a greater decline in ejection fraction after double-legged cycling (Table 4). Indeed, an 11% decrease in left ventricular ejection fraction was observed after double-legged cycling, compared with a 2.5% decrease after single-legged cycling. Conversely, Scott et al. [17] have previously observed ~5% decrease in left ventricular ejection fraction following 14x1-minute double-legged cycling intervals at ~99% peak power output interspersed with two minutes recovery. The divergent response between our double-legged findings and those by Scott et al. [17] are possibly due to the supramaximal nature of our intervals (>99% peak power) accompanied by decreased recovery time (i.e. Scott et al. = 1:2 work to rest; current study = 1:1 work to rest) resulting in greater cardiac work during the efforts as well as increased work to compensate for oxygen deficit during the recovery [56-58]. The difference observed between double-legged and single-legged cycling in this study can be explained by the increased cardiac stress during double-legged cycling due to increase blood flow demands [20, 25, 27, 54].

Although we did not measure left ventricular ejection fraction during the interval session, a decrease in systolic blood pressure during the final two intervals
in the double-legged condition may indicate the decline in left ventricular ejection fraction began early during the exercise bout (Figure 5). As diastolic blood pressure and heart rate were consistent throughout each effort, a decline in systolic blood pressure is likely to indicate a change in stroke volume and thus a decline in left ventricular function [18, 21, 60]. This finding is of particular concern for individuals with cardiovascular disease, as this decline in stroke volume while intensity remains consistent could compromise cardiac blood flow leading to myocardial ischemia [61, 62]. Nevertheless, ultrastructural cardiomyocyte changes associated with exercise-induced myocardial ischemia are reversible once normal blood flow is returned [62].

In addition to monitoring cardiac stress through heart rate and changes in left ventricular function, brain natriuretic peptide (BNP) was measured as this cardiac metabolite is recognised as an important marker of cardiac damage in clinical populations [22-24, 49]. In the present study, both conditions resulted in increased BNP regardless of interval type (Table 4). Our findings support Krupicka et al. [49] who observed an increase in BNP levels immediately following symptom-limiting exercise. Regardless, our measures did not present with a similar level of stability as those of Krupicka et al. [49] as significant differences were recorded in baseline measures between conditions (Table 4). While BNP provides an indicator of damage, our data indicates the use of BNP to differentiate between modalities of exercise is limited by variability of the measure. Indeed, large variation in NT-proBNP measurements has previously been reported [23].

Compliance to any exercise program is influenced by participant perception of the exercise [63-65]. During both single- and double-legged efforts perceptions of
exertion, pain and effort were similar (Table 5), however, session RPE was greater following double-legged when compared with single-legged cycling. Session RPE is a subjective measure which has been shown to provide an indication of internal training load (i.e. the relative physiological stress imposed) during high-intensity, intermittent training [66]. High ventilation rates during physical activity are associated with greater perceptions of effort [67]. It is possible, the higher ventilation rates measured during double-legged cycling together with the above mentioned increase in cardiovascular stress resulted in an overall greater sense of effort and thus a higher session RPE compared with single-legged cycling. Indeed, anecdotally, subjects reported that it was ‘harder to breath’ and ‘harder on the heart’ during the double-legged cycling session. The lower session RPE observed following single-legged cycling, as well as subject reports, indicate that compliance rates may be higher for this modality if used as a training stimulus.

While findings from the current study add to the growing body of knowledge in this area, we do acknowledge limitations in data collection and interpretation which may have impacted our findings. Firstly, workload during single-legged cycling was presented as an accumulation of legs when comparing against double-legged cycling. To do this an assumption that each leg contributes equally to power production is necessary. It is possible to measure differences in individual leg power contribution using a specially designed power measuring pedal interface. Regardless, without access to such equipment we feel our methodology is valid as other researchers have utilised a similar approach within the available literature [26, 27]. Leg blood flow was not measured during this study; therefore, presenting a limitation to the interpretation of our data as we can only hypothesise differences
in blood flow between conditions. Although the measure of blood flow would have provided valuable insight into muscle oxygenation differences between conditions we feel data from previous studies investigating blood flow during smaller muscle mass training [20, 25, 54, 68] provide the necessary evidence to support our findings and discussion.

In conclusion, single-legged cycling allows individuals to exercise at a greater overall power output; however, under less cardiovascular and physiological stress, compared with traditional double-legged cycling. Importantly, double-legged cycling resulted in a greater decline in left ventricular function following this modality. These findings should be of particular interest to cardiovascular disease populations at increased risk of cardiovascular episodes. Furthermore, our findings of greater session RPE during double-legged cycling indicate single-legged interval efforts could prove to be advantageous as higher compliance rates are likely with this modality. With increased attention to the use of high-intensity interval training in diseased populations, the results of this study indicate that single-legged cycling could provide an alternative approach to normal double-legged cycling giving practitioners a method to quickly enhance metabolic function without increasing cardiac risk. Regardless, further research is needed to determine the overall effectiveness of high-intensity single-legged cycling in diseased populations.

Practical recommendations and future directions

From previously observed superior skeletal muscle metabolic adaptations [20, 25, 27, 52] and the present observations of decreased cardiovascular and physiological stress resulting from high-intensity single-legged interval cycling, we
suggest such method of exercise delivery would be safe and effective for diseased populations. Furthermore, the inclusion of high-intensity single-legged cycling into cardiovascular rehabilitation programs could prove advantageous to compliance rates as well as increase motivation. To date however, there have been no studies investigating the cardiovascular adaptations to single-legged cycle training.

Future research involving long-term (e.g. months to years) interventions in a variety of clinical populations (e.g. individuals with cardiovascular disease, diabetes and obesity) is needed to better understand the peripheral (skeletal muscle) and central (cardiovascular) adaptations associated with single-legged cycling. Furthermore, future research should examine varying intensities and durations to assist in optimal exercise prescription.
REFERENCES


Monday, 24 September 2012

Dr Jeremiah Peiffer
School of Chiropractic and Sports Science
Murdoch University

Dear Jeremiah,

Project No. 2012/157
Project Title Examining cardiovascular differences between double- and single-legged cycling

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Approval is granted on the understanding that research will be conducted according to the standards of the National Statement on Ethical Conduct in Human Research (2007), the Australian Code for the Responsible Conduct of Research (2007) and Murdoch University policies at all times. You must also abide by the Human Research Ethics Committee’s standard conditions of approval (see attached). All reporting forms are available on the Research Ethics web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Erich von Dietze
Manager of Research Ethics

cc: Dr Chris Abbiess
    Nikky Gordon
Human Research Ethics Committee: Standard Conditions of Approval

a) The project must be conducted in accordance with the approved application, including any conditions and amendments that have been approved. You must comply with all of the conditions imposed by the HREC, and any subsequent conditions that the HREC may require.

b) You must report immediately anything which might affect ethical acceptance of your project, including:
   • Adverse effects on participants
   • Significant unforeseen events
   • Other matters that might affect continued ethical acceptability of the project.

c) Where approval has been given pending copies of documents such as letters of support / consent from other organisations or approvals from third parties, these must be provided to the Research Ethics Office before the research may commence at each relevant location.

d) Proposed changes or amendments to the research must be applied for, using an Amendment Application form, and approved by the HREC before these may be implemented.

e) An annual Report must be provided by the due date specified each year (usually the anniversary of approval) for the project to have continuing approval.

f) A closure report must be provided at the conclusion of the project.

g) If, for any reason, the project does not proceed or is discontinued, you must advise the committee in writing, using a Closure Report form.

h) If an extension is required beyond the approved end date of the project, an extension application should be made allowing sufficient time for its consideration by the committee. Extensions cannot be granted retrospectively.

i) You must advise the HREC immediately, in writing, if any complaint is made about the conduct of the project.

j) Any equipment used must meet current safety standards. Purpose built equipment must be tested and certified by independent experts for compliance with safety standards.

k) Higher degree students must have both Candidacy and Program of Study approved prior to commencing data collection.

l) You must notify the Research Ethics Office of any changes in contact details including address, phone number and email address.

m) The HREC may conduct random audits and / or require additional reports concerning the research project.

Failure to comply with the National Statement on Ethical Conduct in Human Research (2007) and with the conditions of approval may result in the suspension or withdrawal of approval for the project.

The HREC seeks to support researchers in achieving strong results and positive outcomes.
The HREC promotes a research culture in which ethics is considered and discussed at all stages of the research.
If you have any issues you wish to raise, please contact the Research Ethics Office in the first instance.
APPENDIX TWO

Project Title: Examining cardiovascular differences between double- and single-legged cycling

Investigator(s)  Dr. Jeremiah Peiffer
               Dr. Chris Abbiss
               Miss. Nikky Gordon

Contact Person  Dr. Jeremiah Peiffer
Address         Murdoch University
               90 South Street
               Murdoch, WA, 6150
Telephone No.   (61 08) 9360 7603

You are invited to participate in this study as you;
1. Are between 18 and 50 years old
2. Are a low risk candidate for exercise
3. Are able to visit the Murdoch University Exercise Physiology laboratory on four separate occasions for a total of 4 hours

Background
In recent years, a significant amount of attention has been given to the use of exercise in the treatment of many chronic disease states. For instance, exercise is prescribed routinely to individuals recovering from heart surgery or for those at risk of heart attack. Unfortunately, motivation or time issues decrease consistent participation in most programs. For this reason, the use of high-intensity interval training has gained momentum as this technique allows individuals to complete a high volume of exercise in a relatively short period of time. This technique, however, places large demands on the heart and may prove too extreme in some circumstances. The use of single limb exercise provides another alternative to normal high-intensity interval training; yet, is likely to not place such stress on the heart.

Aim of the Study
The purpose of the current study is to examine the differences in physiological responses to high-intensity cycling based intervals using the normal double-legged approach compared with a novel single-legged design.

What Does Your Participation Involve?
As a possible participant in this study, you would be asked to visit the Murdoch University Exercise Physiology laboratory on four separate occasions with no less than five and no greater than ten days between visits. Each visit would require approximately one hour to complete with your total participation estimated at four hours.

During your initial visit, you will complete a maximal exercise test on a Velotron cycle ergometer. This test will start at a light resistance and increase in resistance each minute until you are no longer able to continue (usually 8 – 20 minutes). Throughout the test, you will have your expired breath and heart rate collected. This data will be used to determine your aerobic fitness level and maximal power output.

The second visit will be used to equate you with each type of activity used in this study and with the equipment you will be using. During this session you will complete six 1-minute cycling efforts with one minute recovery between each effort. After a brief warm-up, you will complete two 1-minute efforts using a normal double-legged approach. You will then be asked to complete two 1-minute efforts using only your right leg and then two 1-minute efforts with your left leg. All efforts will be self-paced with the intent of producing the best effort possible during each 1-minute interval.
During your third and fourth visits, you will be asked to complete a double-legged and single-legged cycling session in a random order. Immediately prior to each session, you will rest for 10 minutes during which your heart rate, resting metabolic rate, muscle oxygenation levels and blood pressure will be recorded. Additionally, a non-invasive ultrasound of your heart will be obtained and an 8ml sample of venous blood will be taken from your arm by a trained and certified phlebotomist using standard techniques (similar to giving a blood sample to your doctor). You will then complete a warm-up and the exercise session. The double-legged efforts will consist of 10, 1-minute efforts with one minute recovery between each effort while the single-legged efforts will consist of 20, 1-minute efforts (10 efforts with each leg) with one minute recovery between each effort. During each effort, your expired breath, heart rate, blood pressure and muscle oxygenation levels will be recorded. At the end of the exercise session, you will rest for ten minutes during which time similar pre-exercise measures will be obtained. Before you leave the facility, you will be asked about how you feel to ensure that no untreated ill effects are being felt (such as feeling light-headed or nauseous).

Blood Collection
Throughout this study you will be asked to provide two venous blood samples during each of the two experimental exercise sessions (does not include the maximal exercise test or the first familiarisation session) for a total of four samples. After each blood draw, you will be provided the opportunity, if needed, to rest up to 30 minutes before starting any exercise. These samples are an extremely important part of this study; thus it will be necessary that you are prepared to provide all four blood samples.

Voluntary Participation
It is important that you understand that your involvement in this study is voluntary. While we would be pleased to have you participate, we respect your right to decline. There will be no consequences to you if you decide not to participate, and this will not affect your treatment/service. If you decide to discontinue participation at any time, you may do so without providing an explanation. If you withdraw, all information you have provided will be destroyed. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. All of the research will be kept on a password protected computer not accessible by non-research staff.

Students enrolled in units supervised by researchers
If you are currently a student enrolled in any unit under supervision by the researchers named on this form, it should be noted that we appreciate your willingness to participate in this study; however, you are under no obligation to do so. Your participation is completely voluntary and your willingness to participate, or not to participate, will have no influence on your current or future academic progress. As a participant, you may choose to terminate your participation in this study at any time without fear or academic repercussion.

Possible Benefits
Participants who complete this study will be provided with information such as maximal aerobic power and oxygen consumption, both of which can be used to help in the development of training programs.

Possible Risks
All exercise sessions will require a high level of effort from the participants. In rare circumstances it is possible that individuals could become light-headed and/or nauseous after completing such efforts. It is important to note that if you do become light headed and/or nauseous, you will not be allowed to leave the facility until the researcher is satisfied that you are fit to leave, or an emergency contact has been called to collect you. Nevertheless, we do not expect this to be an issue as all efforts are participant dependent; thus, no efforts will be in excess of a participant’s own ability.

No individual will be permitted to leave the research facility if complaining of any symptoms of fatigue that could impair their travel.
It is possible that during the collection of venous blood samples, participants could experience discomfort and potential bruising at the site of collection, the discomfort would not be greater than venous blood samples a participant may have provided for past medical reasons (i.e. standard medical blood test). Nevertheless, if a participant does experience pain from this procedure the researcher will immediately attend to the area with compression and ice.

**Distribution of Results**
All data will be stored and analysed in a de-identifiable manner as such, no personal results will be provided to participants with the exception of aerobic fitness and maximal power. The results of this study will be made publicly available through scientific manuscripts and through a brief description posted on the School of Chiropractic and Sports Science website within three months of full data collection (website address below). In both instances, only average data will be used and no individual data will be presented.


**Alternative Uses of Data**
All data collected during this study (including blood samples) will be stored for the duration of five years at which time data will be destroyed or electronically erased as necessary. During this period, data collected from this study may be used for scientific purposes other than those outlined above. At no time will private or identifiable participant information be used. Any data provided for alternative use will be coded so as to eliminate the ability to identify the participant.

**Questions**
If you would like to discuss any aspect of this study please feel free to contact Dr. Jeremiah Peiffer at j.peiffer@murdoch.edu.au or by phone at 9360 7603. We would be happy to discuss any aspect of the research with you. You are welcome to contact us at that time to discuss any issue relating to the research study.

We would like to thank you in advance for your assistance with this research project. We look forward to hearing from you soon.

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This study has been approved by the Murdoch University Human Research Ethics Committee (2012/157). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University’s Research Ethics Office (Tel. 08 9360 6677 (for overseas studies, +61 8 9360 6677) or e-mail ethics@murdoch.edu.au). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
APPENDIX THREE

Informed Consent

Examining cardiovascular differences between double- and single legged cycling

1. I agree voluntarily to take part in this study.

2. I have read the Information Sheet provided and been given a full explanation of the purpose of this study, of the procedures involved and of what is expected of me. The researcher has answered all my questions and has explained the possible problems that may arise as a result of my participation in this study.

   Particularly, I acknowledge that I understand the following procedures will occur during this study:
   1. I will complete one maximal exercise test
   2. I will complete three exercise sessions consisting of high intensity exercise interspersed with low intensity recovery. I understand that this type of exercise can cause fatigue; yet, it will be completed under my own self-selected pace.
   3. I will provide two venous blood samples, obtained by a trained and certified phlebotomist, from my arm (similar to giving blood at the doctor’s office) during two of the three exercise sessions (no blood during the familiarisation session). This will result in a total of four blood samples during this study.

3. I understand I am free to withdraw from the study at any time without needing to give any reason.

4. I understand I will not be identified in any publication arising out of this study.

5. I understand that my name and identity will be stored separately from the data, and these are accessible only to the investigators. All data provided by me will be analysed anonymously using code numbers.

6. I understand that all personal information provided by me is treated as confidential and will not be released by the researcher to a third party unless required to do so by law.

7. I understand that all data obtained during this study will be stored for five years during which time my data may be used in retrospective research. The use of this data will be done so in an unidentifiable manner.

Signature of Participant: __________________________ Date: ……/……/……

(Name)

Signature of Investigator: __________________________ Date: ……/……/……

(Name)