The Effects of Brief Supramaximal Exercise on Maximal Aerobic Capacity

BY

Preeyaphorn Songsorn

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Faculty of Health Sciences and Sport, University of Stirling

Physiology, Exercise & Nutrition Research Group
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“It always seems impossible until it is done”

Nelson Mandela

Activist and Statesman

(1918 - 2013)
DECLARATION

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself, under the supervision of Dr Niels Vollaard and Dr Iain Gallagher. Neither the thesis, nor the original work contained therein have been submitted to this or any other institution for a higher degree.

Preeyaphorn Songsorn

Stirling, 11/01/2019

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Firstly, I would like to express gratitude to my supervisor, Dr Niels Vollaard for his continuous support, encouragement, patience and enormous knowledge. This PhD would not have been done without his guidance. I could not have imagined having a better supervisor for my PhD study.

I am particularly grateful to Thammasat University for the award of the scholarship which enabled me to undertake this PhD.

I would like to acknowledge my co-supervisor Dr Iain Gallagher for their insightful comments on this thesis.

I am very thankful to Dr José Ruffino for providing helpful guidance or advice especially at my first year of the PhD (it was difficult times!).

I would like tanks to the researchers of University of Ege, Ulster University, Swansea University, University of Worcester for their contributions to the studies in this thesis (Chapters 3 and 4)

My sincere thanks go to each of the participants who gave their valuable time and blood to the studies presented in this thesis. Without you, this thesis would have been achievable.

I am also extend my thanks to my friends: P'Tai, P'Fone, P'Ning, P'Som, P'Nui, Knot, Yui, Kade, Im and Kook for their help, encouragement and support during the hard times.

Last but not least, I would like to give a special thank you to my family: Mum, Dad, Aunt Jong, Bim and Michael for all supports and always cheering me up and giving me the belief that I could achieve this.
ABSTRACT

Non-communicable diseases (NCDs) are the leading cause of death worldwide. Physical inactivity is a risk factor of NCDs. Although exercise recommendations have been made, many people do not meet these criteria. Lack of time is one of the main perceived exercise barrier. To address this barrier, more time-efficient exercise protocols have been developed. One of these is Reduced-Exertion High-Intensity Interval Training (REHIT), which comprises two repeated 20-s ‘all-out’ sprints with in a 10-minute protocol. Health benefits from REHIT have been demonstrated; e.g. REHIT improves \( \dot{V}O_2\text{max} \) and insulin sensitivity in healthy individuals. However, no studies have examined whether the REHIT protocol can be modified to make it either shorter or easier, without affecting the beneficial changes in \( \dot{V}O_2\text{max} \). The effects of REHIT on \( \dot{V}O_2\text{max} \) in different participants still require investigation, and the mechanisms of the increase in \( \dot{V}O_2\text{max} \) remain unclear. Therefore, the aims of this thesis were to optimise the REHIT protocol to be a time-efficient and effective protocol for improved \( \dot{V}O_2\text{max} \), to investigate the mechanisms underlying the improvement in \( \dot{V}O_2\text{max} \) after REHIT, and to examine the effect of REHIT on \( \dot{V}O_2\text{max} \) in type 2 diabetes (T2D) patients. Reducing the number of sprint repetitions (Chapter 2) and sprint duration (Chapter 3) attenuated the improvements in \( \dot{V}O_2\text{max} \) with REHIT, but training fewer sessions per week (Chapter 4) did not attenuate the improvements in \( \dot{V}O_2\text{max} \). Importantly, the affective responses associated with REHIT are similar when compared with current exercise recommendations (Chapter 5). \( \dot{V}O_2\text{max} \) increases but insulin sensitivity does not change following REHIT in T2D patients (Chapter 6). Despite glycogen depletion using a protocol with single sprints, no changes in serum levels of the myokine SPARC nor \( \dot{V}O_2\text{max} \) were observed, so no information on possible mechanisms could be drawn from this thesis. Overall, it can be concluded that a manageable, effective and time-efficient exercise protocol for improving \( \dot{V}O_2\text{max} \) is REHIT, consisting of two 20-s ‘all-out’ cycle sprints within a 10-minute session performed at least two times per week.
CONTRIBUTION TO THE WORK BY OTHERS

This PhD project consists of 5 training studies and an acute exercise study. The volume of work required for such a project is too large to be performed by a single PhD student. Therefore, some of the work described in this thesis was performed by others.

Firstly, because risk assessments at the two universities at which the work was carried out prescribe that 2 researchers have to be present at all Wingate-based exercise sessions, there was a need for assistance with training supervision. Thus, undergraduate students helped with training supervision in all studies.

Two of the training studies were performed at multiple centres. Alongside the work done by myself, in Chapter 3, additional participants were recruited to the study at the University of Ege (n=18) and Ulster University (n=7). In Chapter 4, participants were also recruited to the study at Swansea University (n=10) and the University of Worcester (n=11).

The study described in Chapter 6 was funded by Diabetes UK and led by a Postdoctoral research assistant (Dr José S Ruffino). I was responsible for the study component comprising VO$_2$max testing, as well as assisting with the day-to-day running of the project overall. Dr Ruffino performed the blood analysis for this study. Malindi Haggett helped with training supervision.

The serum SPARC analysis for the study described in Chapter 2 was performed by fellow PhD student Yung-Chih Chen.
PUBLICATIONS


4. Nalcakan, G. R., Songsorn, P., Fitzpatrick, B. L., Yuzbasioglu, Y., Brick, N. E., Metcalfe, R. S. & Vollaard, N. B. J. 2018. Decreasing sprint duration from 20 to 10 s during reduced-exertion high-intensity interval training (REHIT) attenuates the increase in maximal aerobic capacity but has no effect on affective and perceptual responses. Applied Physiology, Nutrition, and Metabolism, 43(4), pp 338-344. (Chapter 3)

CONFERENCE PRESENTATIONS

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<th>Description</th>
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<tbody>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>AIT</td>
<td>Aerobic Interval Training</td>
</tr>
<tr>
<td>Akt/PKB</td>
<td>Protein Kinase B</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine Monophosphate</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP Activated Protein Kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BP</td>
<td>Blood Pressure</td>
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<tr>
<td>BRUMS</td>
<td>Brunel Mood Scale</td>
</tr>
<tr>
<td>CGM</td>
<td>Continuous Subcutaneous Glucose Monitor</td>
</tr>
<tr>
<td>CMI</td>
<td>Continuous Moderate-Intensity Exercise</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac Output</td>
</tr>
<tr>
<td>COX</td>
<td>Cytochrome Oxidase</td>
</tr>
<tr>
<td>CS</td>
<td>Citrate Synthase</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>CVI</td>
<td>Continuous vigorous-intensity exercise</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-Energy X-Ray Absorptiometry</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<tr>
<td>EPO</td>
<td>End Power Output</td>
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<tr>
<td>FAO</td>
<td>Fatty Acid B-Oxidation</td>
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</table>
FS: Feeling Scale
GLUT-4: Glucose Transporter Protein 4
HDL: High Density Lipoprotein
HEC: Hyperinsulinemic-Euglycemic Clamp
HIIT: High-Intensity Interval Training
HOMA-IR: Homeostatic Model Assessment - Insulin Resistance
HR: Heart Rate
HRR: Heart Rate Reserve
IL: Interleukin
IPAQ: International Physical Activity Questionnaire
IR: Insulin Receptor
IVGTT: Intravenous Glucose Tolerance Test
LDL: Low Density Lipoprotein
MAP: Mean Arterial Pressure
MET: Metabolic Equivalent
MICT: Moderate-intensity continuous training
MPO: Mean Power Output
mRNA: Messenger Ribonucleic Acid
NA: Negative Affect
NHS: National Health Service
NO: Nitric Oxide
O₂: Oxygen
OGTT: Oral Glucose Tolerance Test
p38 MAPK: p38 Mitogen Activated Protein Kinase
PA: Physical Activity
PACES: Physical Activity Enjoyment Scale
PANAS: Positive and Negative Affect Schedule
PAR-Q: Physical Activity Readiness Questionnaire
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PDH</td>
<td>Pyruvate Dehydrogenase</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome-proliferator Activated Receptor γ Coactivator</td>
</tr>
<tr>
<td>PPO</td>
<td>Peak Power Output</td>
</tr>
<tr>
<td>REHIT</td>
<td>Reduced-Exertion High-Intensity Interval Training</td>
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<tr>
<td>RER</td>
<td>Respiratory Exchange Ratio</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of Perceived Exertion</td>
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<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SI</td>
<td>Insulin Sensitivity</td>
</tr>
<tr>
<td>SIT</td>
<td>Sprint Interval Training</td>
</tr>
<tr>
<td>SPARC</td>
<td>Secreted Protein Acidic Rich in Cysteine</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke Volume</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 Diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>THR</td>
<td>Target Heart Rate</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>V\text{O}_2\text{max}</td>
<td>Maximal Oxygen Consumption</td>
</tr>
<tr>
<td>VT</td>
<td>Ventilatory Threshold</td>
</tr>
<tr>
<td>β-HAD</td>
<td>3-hydroxyacyl CoA dehydrogenase</td>
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CHAPTER 1

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW
1.1. General Introduction

Nowadays, many labour-saving technologies have been developed, and this has become a major factor in the high prevalence of physical inactivity (Hallal et al., 2012). Physical inactivity is a risk factor for non-communicable diseases (NCDs), which include cardiovascular diseases, cancer, diabetes, and chronic lung diseases (WHO, 2014). Importantly, NCDs were the worldwide leading cause of death in 2016 (WHO, 2018). According to the global physical activity level survey by Hallal et al. (2012), self-report questionnaires showed that 31.1% of adults worldwide were classed as ‘inactive’: performing moderate-intensity physical activity for less than 30 minutes per week, and vigorous-intensity physical activity for less than 15 minutes per week (Health and social care information center, 2014). It also indicated that women were more inactive than men at 33.9% compared to 27.9%, respectively (Hallal et al., 2012). In the United Kingdom, self-reported physical activity data revealed that 39% of UK adults were inactive (British Heart Foundation, 2017), and the data from objective measurement showed that only 6% of men and 4% of women met the recommendations (Health and Social Care Information Center, 2008).

Therefore, to decrease the risk of developing chronic diseases and the risk of death from physical inactivity, the World Health Organization announced a guideline of recommendations on physical activity (WHO, 2010). The recommendations for adults are to perform moderate-intensity aerobic physical activity at least 150 minutes per week or vigorous-intensity aerobic physical activity for 75 minutes a week, and muscle strengthening exercise should be done at least two days a week (WHO, 2010). However, there is a high proportion
there are several exercise barriers, such as lack of motivation, fear of injury, and cost, but the main exercise barrier is often cited as lack of time (Booth et al., 1997; Chao et al., 2000; Conraads et al., 2012; Dutton et al., 2005; Chinn et al., 1999; Gothe and Kendall, 2016). Therefore, time-efficient exercise protocols have been proposed as a way to address this major exercise barrier. Sprint interval training (SIT) could be one possible solution to physical inactivity. These could be used as an alternative or adjunct to current physical activity recommendations, for individuals who define lack of time as a main barrier to exercise. Importantly, SIT could be one protocol to address health problems from being inactive.

The ‘classic’ sprint interval training (SIT) protocol consists of four to six repeated 30-s supramaximal-intensity sprints, with four minutes resting periods interspersed between each exercise set (Astorino et al., 2012; Gibala and McGee, 2008; Gillen and Gibala, 2014). Several health benefits, such as improved maximal oxygen consumption (\(\dot{V}O_2\max\)), improved insulin sensitivity, and other physiological adaptations have been observed following SIT (Burgomaster et al., 2008; Rakobowchuk et al., 2008; Babraj et al., 2009; Burgomaster et al., 2007; Burgomaster et al., 2006; Burgomaster et al., 2005; Gibala et al., 2006; Granata et al., 2017). Nevertheless, there are some important limitations of the conventional SIT protocol. This protocol requires a high level of participant motivation and is associated with a high level of fatigue (Gillen and Gibala, 2014; Hawley and Gibala, 2009; Gibala and McGee, 2008). Additionally, when considering the total time required per session, it is approximately 30
minutes. The total training time per week is approximately 90 minutes, suggesting that this form of SIT requires more time than the exercise recommendations for vigorous-intensity aerobic exercise, which is 75 minutes per week (Vollaard and Metcalfe, 2017). Therefore, it might not be as time-efficient an exercise protocol as often claimed (Metcalfe et al., 2012). Importantly, based on The American College of Sports Medicine (ACSM)’s guidelines, there are two factors that need to be considered when prescribing exercise, which include increases in effectiveness and decreases in risk of injury (ACSM, 2017). Additionally, the protocol should be practical and should not be too hard to be acceptable as a regular exercise (Ekkekakis et al., 2011).

To determine whether the total training time with SIT can be reduced while retaining the health benefits, Metcalfe et al. (2012) developed the reduced-exertion high-intensity interval training (REHIT) protocol. The original REHIT protocol consists of two 20-s all-out sprints within a 10 minutes total training duration. It was shown that despite the shorter sprint time and reduced number of sprints with supramaximal-intensity exercise, this protocol was sufficient to improve aerobic fitness ($\dot{V}O_2$max) and insulin sensitivity in healthy adults (Metcalfe et al., 2012; Metcalfe et al., 2016). $\dot{V}O_2$max and insulin sensitivity are both important health parameters and are associated with chronic diseases, such as cardiovascular diseases and diabetes (Albright et al., 2000; Blair et al., 1996). Focusing on $\dot{V}O_2$max, this is a predictor of cardiovascular health and risk of death (Blair et al., 1996; Booth et al., 2012). Importantly, low $\dot{V}O_2$max is the strongest risk factor associated with all-cause mortality compared to other risk factor; for example smoking, hypertension, obesity, or high cholesterol (Blair, 2009). Nevertheless, more research is needed to better understand the effect of REHIT.
or other types of short-term supramaximal exercise protocols on \( \dot{V}O_2\text{max} \) in different groups of participants such as type 2 diabetes (T2D) patients or inactive individuals. Moreover, the mechanisms underlying these effects are still unknown.

This initial chapter will discuss the literature on the prevalence, causes, and consequences of physical inactivity, the importance of \( \dot{V}O_2\text{max} \) for health and fitness, and the potential use of SIT as an alternative exercise intervention to improve health and reduce the risk of NCDs. It will also highlight the established physiological adaptations following SIT. Moreover, the importance of affective responses and exercise adherence/adaptation is also presented in this chapter. This information will then be used to justify the overall aims of this PhD project at the end of this chapter.

1.2. Maximal Oxygen Consumption (\( \dot{V}O_2\text{max} \))

Maximal oxygen consumption (\( \dot{V}O_2\text{max} \)) is defined as the highest rate that the body can take up, deliver, and utilise oxygen during maximal exercise (Poole et al., 2008). \( \dot{V}O_2\text{max} \) can be assessed by direct and indirect methods (Grant et al., 1999). The direct methods involve a graded exercise test using either a treadmill, a cycle ergometer, or an arm-crank ergometer (McArdle et al., 2015). Measuring \( \dot{V}O_2\text{max} \) with varying exercise methods will affect the results depending on which muscle groups are used; for example, in the arm-crank test \( \dot{V}O_2\text{max} \) would be less than using a treadmill by roughly 70% because a greater amount of muscle mass is activated via the treadmill (McArdle et al., 2015). Indirect methods involve a predicted \( \dot{V}O_2\text{max} \) using a submaximal or maximal exercise test (e.g. Åstrand-Rhyming submaximal cycle ergometer (CE) test, Bruce maximal treadmill test, and Canadian Aerobic Fitness Test) (Grant et al., 1999).
\( \dot{V}O_2\text{max} \) is affected by several factors. \( \dot{V}O_2\text{max} \) could be decreased by ageing, inactivity and bed rest conditions (Betik and Hepple, 2008; Convertino, 1997; Sutton, 1992a). The rate of decline in \( \dot{V}O_2\text{max} \) by ageing is approximately 0.45 - 0.5 ml·kg\(^{-1}\)·min\(^{-1}\)·yr\(^{-1}\) (Inbar et al., 1994). The percentage change of \( \dot{V}O_2\text{max} \) is higher in active people, who performed more exercise volume (MET min/week), than inactive people (Oja, 2001). Moreover, there is a relationship between the reduction of \( \dot{V}O_2\text{max} \) and the duration of bed rest, with a decrease in \( \dot{V}O_2\text{max} \) of 0.9% of per day over 30 days of bed rest (Convertino, 1997). Genetics, gender, and body composition also have an influence on \( \dot{V}O_2\text{max} \) (McArdle et al., 2015; Wenger and Bell, 1986). For example, when comparing men and women, on average, men have a higher average absolute and relative \( \dot{V}O_2\text{max} \) than women because men are generally physically larger (bigger heart and more muscle mass) than women (McArdle et al., 2015). Furthermore, body composition has an effect on \( \dot{V}O_2\text{max} \). Basically, \( \dot{V}O_2\text{max} \) can be represented as absolute or relative \( \dot{V}O_2\text{max} \) values. Absolute \( \dot{V}O_2\text{max} \) is the actual total amount of oxygen that the body can consume and is expressed in litres of oxygen per minute (L/min). Relative \( \dot{V}O_2\text{max} \) considers body weight and is represented as millilitres of oxygen per kilogram of body weight per minute (ml/kg/min), and it can be used to compare aerobic capacity between individuals with different body sizes (McArdle et al., 2015). Relative \( \dot{V}O_2\text{max} \) is decreased in overweight individuals or people with a high percentage of body fat (Pribis et al., 2010; Shazia et al., 2015). Additionally, \( \dot{V}O_2\text{max} \) is different among people with different levels of physical activity; for example, between 18 and 22 years in men, \( \dot{V}O_2\text{max} \) in untrained, active, trained, elite, or world-class trained can range from 45, 50, 57, and 70 to greater than 80 ml/kg/min on average, respectively (Sharkey and
Gaskill, 2013). Importantly, Bouchard and Rankinen (2001) demonstrated the inter-individual difference of \( \dot{\text{VO}}_2\text{max} \) responses to regular exercise. The improvement of \( \dot{\text{VO}}_2\text{max} \) ranges between \( \sim0\% \) and \( \sim40\% \), and can be categorised into groups of high, medium, and non/low responders (Bouchard and Rankinen, 2001). Moreover, the heritability of trainability of \( \dot{\text{VO}}_2\text{max} \) is \( \sim47\% \) (Bouchard et al., 1999), so \( \sim53\% \) of the variability is not associated with genetics. Taken together, \( \dot{\text{VO}}_2\text{max} \) is affected by several factors. The researcher should consider these factors before interpreting the results of \( \dot{\text{VO}}_2\text{max} \) following training.

\( \dot{\text{VO}}_2\text{max} \) is determined by cardiac output (comprising stroke volume and heart rate) and the arteriovenous oxygen difference (a-\( \dot{\text{VO}}_2\text{diff} \)) (Plowman and Smith, 2011). \( \dot{\text{VO}}_2\text{max} \) can be limited by two main elements, which are central and peripheral factors. Central factors include heart and lung function to transfer oxygen from the lungs to muscles, while peripheral factors refer to the ability of muscles to extract oxygen from the blood (Sutton, 1992b). Cardiac output seems to be the primary limiting factor of \( \dot{\text{VO}}_2\text{max} \) (Bassett and Howley, 2000). Increases in cardiac output following exercise training are generally related to increases in stroke volume. Stroke volume is elevated by the increase in end-diastolic volume, cardiac contractility, and reduction in total peripheral resistance (Powers and Howley, 2012). Prolonged endurance training improves cardiac output, and this corresponds to an increase in \( \dot{\text{VO}}_2\text{max} \) (Ekblom et al., 1968). In addition, there is a relationship between oxygen delivery and \( \dot{\text{VO}}_2\text{max} \). It has been demonstrated that more oxygen delivery, which depends on heart pump capacity, leads to higher oxygen uptake (Saltin and Strange, 1992). Moreover, one meta-analysis showed the improvement of \( \dot{\text{VO}}_2\text{max} \) following endurance
training is associated with increasing cardiac output and oxygen extraction (Montero et al., 2015). Additionally, blood flow providing oxygen to the muscles is a key element that increases VO$_2$max (Saltin, 1985). Considering all of this evidence, it may be put forth that central factors are the limiting factors of VO$_2$max.

Additionally, the pulmonary system might be a limiting factor of VO$_2$max in some individuals such as elite athletes and patients with pulmonary diseases (Bassett and Howley, 2000). The pulmonary system is the means by which oxygen is taken from the environment into the blood via gas exchange (McArdle et al., 2015). On one hand, the pulmonary system does not limit VO$_2$max in normal people because they have a high pulmonary reserve (Sutton, 1992b). On the other hand, if an athlete trains in such a way that his or her cardiac output is enhanced, the pulmonary system may become a limiting factor because there will be a shorter transit time for gas exchange in the pulmonary capillaries, possibly resulting in arterial desaturation (Bassett and Howley, 2000). Moreover, the limitation to flow during maximal exercise in highly trained athletes caused by increasing the end-expiratory lung volume might result in shortening the respiratory muscles, leading to ineffective pulmonary functioning and affecting the gas exchange in the lungs (Sutton, 1992b). Additionally, individuals suffering from lung problems will have a less effective pulmonary system. For people who live at high altitudes, where the partial pressure of oxygen is low, there will also be an effect on VO$_2$max (Bassett and Howley, 2000). Thus, it may be concluded that the pulmonary system is a limiting factor in certain groups of people, such as patients with lung diseases and elite athletes.
In theory, another limiting factor of \( \dot{V}O_2 \text{max} \) could be peripheral factors, which predominantly involves maximal skeletal mitochondrial enzyme activity. The amount and activity of oxidative enzymes in skeletal muscle increases after both endurance exercise and SIT and is linked to improved \( \dot{V}O_2 \text{max} \) (Burgomaster et al., 2008). However, Bassett and Howley (2000) suggested that if there is a relationship between \( \dot{V}O_2 \text{max} \) and mitochondrial enzyme activity, the increase in oxygen uptake should rise proportionally to mitochondrial enzyme levels. According to Saltin et al. (1977), \( \dot{V}O_2 \text{max} \) increased 1.4-fold, while mitochondrial enzyme concentration rose 2.2-fold following training. Furthermore, there appears to be no correlation between \( \dot{V}O_2 \text{max} \) and citrate synthase (CS) activity (Holloszy and Coyle, 1984). As such, Bassett and Howley (2000) stated that mitochondrial enzyme levels after training are irrelevant in the context of elevating \( \dot{V}O_2 \text{max} \) and emphasised that \( \dot{V}O_2 \text{max} \) is limited by oxygen transportation because it measures the whole-body response, not only muscle mitochondria. Conversely, the elevation of mitochondrial enzyme capacity has been associated with an improvement in endurance performance rather than \( \dot{V}O_2 \text{max} \) (Jacobs et al., 2011). Enhanced endurance performance could be explained by examining muscle adaptations. First, an increase in mitochondrial enzymes after training is related to higher rates of fat oxidisation. Potentially, this could spare muscle glycogen and blood glucose. Second, the decrease in pH accompanying accumulation of lactate may be responsible for fatigue during exercise. A rise in mitochondrial enzyme concentrations is associated with a decrease in the production of lactate during exercise (Holloszy and Coyle, 1984). Therefore, these adaptations have led to the conclusion that improvement in endurance performance following training might be related to increased
mitochondrial enzyme activities (Bassett and Howley, 2000). Nonetheless, skeletal mitochondrial enzymes could also actually be a limiting factor of \( \dot{V}O_2 \)max, in particular in diseases related to skeletal mitochondrial enzyme deficiency (Plowman and Smith, 2011). Interestingly, Gifford et al. (2016) recently demonstrated a positive relationship between mitochondrial oxygen consumption and whole-body oxygen consumption in untrained subjects but not in trained subjects (Gifford et al., 2016). Thus, controversy remains regarding oxidative enzymes associated with \( \dot{V}O_2 \)max.

\( \dot{V}O_2 \)max is frequently used to determine cardiorespiratory fitness or aerobic power and to predict cardiovascular health and future mortality (Booth et al., 2012; Blair et al., 1996; Laukkanen et al., 2004; Myers et al., 2002). Increased cardiorespiratory fitness can reduce the risk of cardiovascular disease (CVD) (Joyner and Green, 2009; Laukkanen et al., 2004). Furthermore, healthy people with lower degrees of physical fitness also have an increased risk of death (Blair et al., 1989). Moreover, low cardiorespiratory fitness is the strongest risk factor related to all causes of deaths when compared to other risk factors such as obesity, hypertension, or smoking (Blair, 2009). Another parameter that represents aerobic power is metabolic equivalents (METs). METs refers to the metabolic cost or oxygen consumption while engaging in activities (Bouchard et al., 2012). One MET is equivalent to the resting metabolic rate (e.g. sitting at rest), which is approximately 3.5 ml O\(_2\)/kg/min (Jette et al., 1990). There is a strong relationship between exercise capacity, expressed in METs, and all-cause mortality and cardiovascular mortality. Mortality will be decreased when there is an increase in METs (Feldman et al., 2015). Furthermore, the risk of all-cause and CVD mortality are estimated to be decreased by 15% and 19%, respectively,
when cardiorespiratory fitness is increased by 1 MET (Lee et al., 2011). Overall, it can be concluded that there is a close relationship between $\dot{V}O_2{\text{max}}$ and the risk of death. Therefore, it has been proposed that increasing $\dot{V}O_2{\text{max}}$ is of equal importance to performing more physical activity (Bouchard et al., 2015).

### 1.3. Physical Inactivity and Chronic Diseases

According to Caspersen et al. (1985), physical activity is ‘any bodily movement produced by skeletal muscle that results in energy expenditure’ (Caspersen et al., 1985). Physical activity can for example be household tasks, occupational work, physically active transport and leisure-time activities (Hardman and Stensel, 2009). Exercise is a subset of physical activity which is defined as ‘planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness’ (Caspersen et al., 1985). The prevalence of physical inactivity has been rising in the past ten years because of the expansion of information and communication technologies, changes in transportation (Pratt et al., 2012), rapid economic growth and increasing urbanisation (Kohl et al., 2012). Development of digital and information technologies including internet access and the mobile phone have had a negative effect on physical activity levels. Additionally, using private cars or well developed public transportation are a factor that relates to physical inactivity (Pratt et al., 2012). Moreover, a decrease in occupational physical activity could be linked to urbanization (Kohl et al., 2012). Physical inactivity can be described based on the following criteria: engaging in less than five days a week of moderate-intensity physical activity for 30 min, performing less than three days a week of vigorous-intensity physical activity for 20 min, or being involved in any physical activity for less than 600 MET-min per week (Hallal et al., 2012).
Apart of being inactive, people, nowadays, become more sedentary (Clark and Sugiyama, 2015). The definition of sedentary behaviour is ‘any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture’ (Sedentary Behaviour Research Network, 2012). Sedentary behaviour is related to several behaviour domains, which include leisure (e.g. watching television, reading a book, using a computer and socializing), transport (e.g. sitting in a car) and work (e.g. office-based workers) (Clark and Sugiyama, 2015). Sedentary behaviour is associated with all-cause, cardiovascular disease and cancer mortality and also related to incidence of cardiovascular disease, cancer and type 2 diabetes (Biswas et al., 2015). Interestingly, people who meet the physical activity recommendations but still spend many hours sitting can be defined as sedentary (Tremblay et al., 2010). However, the focus of this thesis was on physical inactivity rather than sedentary behaviour.

Physical inactivity is one of the main risk factors of all-cause mortality and several chronic diseases. Lee et al. (2012) observed that 6%-10% of the causes of NCDs, including CVDs, diabetes, cancer, and chronic respiratory diseases, are the consequences of physical inactivity (Lee et al., 2012). Additionally, 71% of deaths in 2016 were caused from NCDs (WHO, 2018). Several negative health effects were found following physical inactivity, including reduced \( \dot{VO}_{2\text{max}} \), stroke volume, oxidative enzyme concentrations, muscle mass, muscle strength, insulin sensitivity, and endothelial function and increased arterial stiffness and diastolic blood pressure (Coyle et al., 1984; Drummond et al., 2012; Kortebein et al., 2007; Krogh-Madsen et al., 2010; Nosova et al., 2014; Stephens et al., 2011; Booth et al., 2012). Physical inactivity, based on estimates from the UK National Health Service, results in costs of more than one billion pounds per year,
including the effect of diseases on the health system (Allender et al., 2007). Therefore, physical inactivity is considered one of the most important health problems of the twenty-first century (Blair, 2009).

1.4. Exercise Barriers

As mentioned previously, there are many disadvantages of being inactive, but it seems that many individuals are, in fact, inactive. There are many barriers that might prevent people from performing exercise regularly. Additionally, exercise barriers can be factors that inhibit people to adopt, maintain, or restart exercise (Booth et al., 1997). Therefore, understanding exercise barriers could be a first step of finding solutions for encouraging inactive individuals to do more exercise. There are two types of perceived barriers of exercise, which including internal and external barriers (Arzu et al., 2006). According to Arzu et al. (2006) the internal barriers can be categorised as lack of energy, lack of motivation and lack of self-efficacy. The external barriers were categorised as lack of resource, lack of social support and lack of time (Arzu et al., 2006). Importantly, exercise barriers vary by age, gender and socioeconomic class (Chinn et al., 1999; Owen and Bauman, 1992). For average young adult and adult men and women, perceived lack of time is one of the main barriers to exercise (Booth et al., 1997; Arzu et al., 2006; Gómez-López et al., 2010). Additionally, not being motivated, a lack of energy, and being too tired are also well-known barriers (Chinn et al., 1999; Arzu et al., 2006; Andajani-Sutjahjo et al., 2004; Fox et al., 2012). Regarding the elderly, information from Baert's systematic review demonstrated that the main barrier is health problems (Baert et al., 2011), with other barriers including lack of time, lack of self-motivation, being too tired and feeling too old to be active (Baert et al., 2011; Buman et al., 2010). When considering the difference
between gender, in Australia, more men than women preferred to spend their free time for resting or doing relaxing activities but more women than men mentioned that their barriers are taking care for young children and having no company for exercise (Booth et al., 1997). Moreover, in Portugal, men reported lack of infrastructures in the locality more than women, and women mentioned costs more than men (Sequeira et al., 2011). Socioeconomic status (SES), which includes income, occupational and education level, is defined as a factor that related to barriers of exercise (Chinn et al., 1999) because experience and attitude of people towards the determinant of health can be influenced by SES (Marmot, 2005). In addition, a report from the European Commission (European Commission, 2014) demonstrated that people who were categorised in high SES tend to be more engaged in exercise (European Commission, 2014). According to Chinn et al. (1999), lack of money is a main barrier which is found in a lower socioeconomic class (Chinn et al., 1999). Additionally, people in lower income groups more often reported that the exercise barriers were being unable to do exercise and not wanting to do exercise (Owen and Bauman, 1992). On the other hand, more participants with higher SES reported they would do other things rather than do the exercise when they are free (Sequeira et al., 2011). Interestingly, lack of time was a barrier most cited by men and women with both high or low socioeconomic status (Sequeira et al., 2011). Overall, this could indicate that the recommended 150 minutes per week of exercise might be challenging to perform because people think that they do not have enough time for exercise; therefore, over the course of the last decade, SIT has been investigated as a more time-efficient method for achieving the health benefits of exercise (Gillen and Gibala, 2014).
1.5. Sprint Interval Training (SIT)

The concept of interval training is a repeated intense period of exercise interspersed by resting periods or low-intensity exercise (Fox et al., 1973). There are two types of interval training, which are categorised by the intensity of exercise: high-intensity interval training (HIIT) and SIT (MacInnis and Gibala, 2017). While HIIT is defined as (sub)maximal-intensity where the intensity of exercise is between ~80% and ~100% of $\dot{V}O_2$max, SIT is performed at supramaximal or all-out intensities (Vollaard and Metcalfe, 2017; Gibala et al., 2014). The protocols for SIT vary in sprint duration (10-30 seconds), number of sprints (2-15 repeated sprints), and recovery duration between sprints (50-1200 seconds) (Vollaard et al., 2017) as shown in Table 1.1. SIT training normally involves a treadmill or cycle ergometer (Kavaliauskas et al., 2018). This review focuses solely on cycle-based SIT. Wingate-based SIT appears to be the most popular protocol, and the ‘classic’ SIT protocol comprises four to seven repeats per session of 30-s all-out sprints with 0.075 kg/kg body mass of resistance, and the recovery period tends to be 4 minutes at low resistance (<30 W) (Burgomaster et al., 2005). However, limitations of Wingate-based SIT include the fact that the training requires high levels of motivation while performing the ‘all-out’ sprint and that it tends to incur much fatigue during or following training (Gillen and Gibala, 2014; Hawley and Gibala, 2009; Gibala and McGee, 2008). Further criticisms that have been made are that SIT is not a practical protocol and might not suit sedentary individuals or some groups of people (Hardcastle et al., 2014; Biddle and Batterham, 2015; Coyle, 2005).

Therefore, more practical protocols have been examined, for example, the HIIT protocol by Little et al. (2010). To match the total work completed in the classic
Wingate-based SIT protocol, this practical HIIT protocol consists of 1-minute sprints at 100% maximal aerobic power and recovery for 75 seconds at 30 W, for 8-12 cycles per training session (Little et al., 2010b). The health benefits following this protocol have been confirmed with several groups of individuals, including patients with T2D (Little et al., 2011a), sedentary adults (Hood et al., 2011), overweight or obese individuals (Gillen et al., 2013), and patients with coronary artery disease (CAD) (Currie et al., 2013).

The Wingate-based SIT protocol has also been modified in other ways. For example, various protocols have reduced the sprint duration from 30-s all-out sprints to 15-s all-out sprints (Zelt et al., 2014) or even 10-s all-out sprints (Hazell et al., 2010; Olek et al., 2018; Skleryk et al., 2013; Hellsten-Westling et al., 1993). In addition, another modified SIT protocol was developed by Metcalfe et al. (2012), namely the REHIT protocol. It reduced both the number of sprints and their duration. Based on the study by Parolin et al. (1999), glycogen depletion is known to occur predominately within the first 15 seconds of the first one or two sprint bouts (Parolin et al., 1999). Metcalfe et al. proposed that glycogen depletion could be a mechanism of SIT, which would make the REHIT protocol an effective protocol. REHIT consists of two repeated 20-s all-out sprints within a total period of 10 minutes (Metcalfe et al., 2012). It has indeed been shown that REHIT has similar health benefits (e.g. improvements in $\dot{V}O_2$max and insulin sensitivity) as the Wingate-based SIT (Metcalfe et al., 2012; Metcalfe et al., 2016).
Table 1.1 Effect of SIT on VO₂max

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Sprint duration (second)</th>
<th>Recovery duration (second)</th>
<th>Number of sprints/session</th>
<th>Number of session/week</th>
<th>Duration (week)</th>
<th>VO₂max (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katz et al. (1984)</td>
<td>8 recreational active men</td>
<td>30</td>
<td>240</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>7</td>
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<td>30</td>
<td>1200</td>
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<td>3</td>
<td>6</td>
<td>13.5</td>
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<td>Stathis et al. (1994)</td>
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<td>30</td>
<td>240</td>
<td>3-10</td>
<td>3</td>
<td>7</td>
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<td>240</td>
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<td>7</td>
<td>13.9</td>
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<td>MacDougall et al. (1998)</td>
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<td>30</td>
<td>240</td>
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<td>3</td>
<td>7</td>
<td>7.5</td>
</tr>
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<td>Harmer et al. (2000)</td>
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<td>30</td>
<td>240</td>
<td>4-10</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
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<td>3</td>
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<td>4-7</td>
<td>3</td>
<td>2</td>
<td>5.5</td>
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<td>240</td>
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<td>3</td>
<td>7</td>
<td>2.4</td>
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<tr>
<td>Study</td>
<td>Subjects</td>
<td>Sprint duration (second)</td>
<td>Recovery duration (second)</td>
<td>Number of sprints/session</td>
<td>Number of session/week</td>
<td>Duration (weeks)</td>
<td>VO₂max (%change)</td>
</tr>
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<tr>
<td>Larsen et al. (2013)</td>
<td>8 sedentary men</td>
<td>30</td>
<td>240</td>
<td>4-6</td>
<td>3</td>
<td>2</td>
<td>9.8</td>
</tr>
<tr>
<td>Shepherd et al. (2013)</td>
<td>8 sedentary men</td>
<td>30</td>
<td>270</td>
<td>4-6</td>
<td>3</td>
<td>6</td>
<td>7.7</td>
</tr>
<tr>
<td>Skleryk et al. (2013)</td>
<td>8 overweight/obese men</td>
<td>10</td>
<td>80</td>
<td>8-12</td>
<td>3</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Cochrans et al. (2013)</td>
<td>9 recreational active</td>
<td>30</td>
<td>240</td>
<td>4-7</td>
<td>3</td>
<td>6</td>
<td>6.1</td>
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<tr>
<td>Gillen et al. (2014)</td>
<td>14 overweight/obese men</td>
<td>20</td>
<td>120</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>12.1</td>
</tr>
<tr>
<td>Harris et al. (2014)</td>
<td>6 recreational active</td>
<td>30</td>
<td>270</td>
<td>4-6</td>
<td>3</td>
<td>4</td>
<td>9.1</td>
</tr>
<tr>
<td>Nalcakan (2014)</td>
<td>8 recreational active</td>
<td>30</td>
<td>270</td>
<td>4-6</td>
<td>3</td>
<td>7</td>
<td>7.7</td>
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<tr>
<td>Scalzo et al. (2014)</td>
<td>21 recreational active</td>
<td>30</td>
<td>240</td>
<td>4-8</td>
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<td>3</td>
<td>4.5</td>
</tr>
<tr>
<td>Zelt et al. (2014)</td>
<td>11 recreational active men</td>
<td>30</td>
<td>270</td>
<td>4-6</td>
<td>3</td>
<td>4</td>
<td>4.1</td>
</tr>
<tr>
<td>Zelt et al. (2014)</td>
<td>12 recreational active men</td>
<td>15</td>
<td>285</td>
<td>4-6</td>
<td>3</td>
<td>4</td>
<td>8.2</td>
</tr>
<tr>
<td>Ijichi et al. (2015)</td>
<td>10 recreational active</td>
<td>30</td>
<td>600</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>8.2</td>
</tr>
<tr>
<td>Ijichi et al. (2015)</td>
<td>10 recreational active</td>
<td>30</td>
<td>600</td>
<td>2</td>
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<td>4</td>
<td>7.1</td>
</tr>
<tr>
<td>Kiviniemi et al. (2015)</td>
<td>12 sedentary men</td>
<td>30</td>
<td>240</td>
<td>4-6</td>
<td>3</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Richardson and Gibson (2015)</td>
<td>9 recreational active</td>
<td>30</td>
<td>240</td>
<td>4-7</td>
<td>3</td>
<td>2</td>
<td>11.2</td>
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<tr>
<td>Gillen et al. (2016)</td>
<td>9 sedentary men</td>
<td>20</td>
<td>120</td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>19.2</td>
</tr>
<tr>
<td>Metcalfe et al. (2016)</td>
<td>34 sedentary subjects (17 women, 17 men)</td>
<td>20</td>
<td>200</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>9.6</td>
</tr>
<tr>
<td>Olek et al. (2018)</td>
<td>7 recreational active men</td>
<td>10</td>
<td>60</td>
<td>4-6</td>
<td>3</td>
<td>2</td>
<td>13.6</td>
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<tr>
<td>Olek et al. (2018)</td>
<td>7 recreational active men</td>
<td>10</td>
<td>240</td>
<td>4-6</td>
<td>3</td>
<td>2</td>
<td>11.9</td>
</tr>
</tbody>
</table>

*recreational active subject is a participant who performs any exercises 2 or 3 times/week but non-specifically trained

*sedentary subject is a participant who perform any exercises ≤ 1 times/week or classified as sedentary according to the criteria of the International Physical Activity Questionnaire (IPAQ)
1.6. Physiological Adaptations to SIT

It is well known that endurance exercise improves performance through several physiological adaptations (Hellsten and Nyberg, 2015; Holloszy and Coyle, 1984; Nieman and Pedersen, 1999; Petruzzello et al., 1991; Stroth et al., 2009). Many health benefits have been demonstrated following SIT (Gibala and McGee, 2008), and they are similar to those following endurance training even though the exercise volume with SIT is much lower than endurance training (Gibala et al., 2006; Burgomaster et al., 2008).

1.6.1. Cardiovascular adaptations to SIT

Many studies have shown that $\dot{V}O_2\text{max}$ improves following SIT, especially in people that are less fit (Weston et al., 2014). From the data in Table 1.1, the average percentage change of $\dot{V}O_2\text{max}$ ranges between 1.4% and 19%. The big range of changes in $\dot{V}O_2\text{max}$ might be due to the use of different protocols of SIT, specifically the sprint duration, recovery period, number of sprints per session, total exercise time, and duration of training. However, a meta-analysis by Vollaard et al. (2017) demonstrated that part of the discrepancy in improvements in $\dot{V}O_2\text{max}$ is due to the number of sprints per session. Moreover, the use of varying groups of participants, such as those that are sedentary, healthy, obese, and athletic and certain groups of patients (for example type 1 diabetes patients) may affect the results because the adaptation to training is different among those groups.

The mechanisms of how SIT affects $\dot{V}O_2\text{max}$ are still not completely understood, and as mentioned above, controversy exists on the contribution of central and peripheral adaptation mechanisms. A number of studies investigated the effect of SIT on cardiovascular adaptations, which focused on cardiac output (CO) and
stroke volume (SV), which are associated with $\dot{V}O_2$max (Macpherson et al., 2011; Smith, 2013). The results from these studies demonstrated that $\dot{V}O_2$max improves following 6 weeks of SIT, but that there was no significant change in CO or SV (Macpherson et al., 2011; Smith, 2013), suggesting that a SIT-induced increase in $\dot{V}O_2$max may depend on peripheral adaptations rather than central adaptations. This hypothesis is supported by Gibala et al. (2012) who proposed that skeletal muscle adaptations are a possible mechanism underlying the improvements in $\dot{V}O_2$max (Gibala et al., 2012). Moreover, a recent study proposed that mitochondrial oxygen demand is the limiting factor for $\dot{V}O_2$max in untrained subjects (Gifford et al., 2016). However, there is still disagreement on whether oxidative enzyme levels are associated with $\dot{V}O_2$max. The effect of SIT on peripheral adaptations will be discussed in the next section.

Other cardiovascular adaptations, including endothelial function and blood pressure, are relevant in the context of $\dot{V}O_2$max. Cardiac function is also regulated by endothelial function (Kingwell, 2002), which includes arterial distensibility and vascular function (evaluated by flow-mediated dilation (FMD)). Such indicators of cardiovascular health improve after SIT (Rakobowchuk et al., 2008). However, the effect of SIT on blood pressure was inconclusive. Systolic blood pressure (SBP) immediately decreased following 2 weeks of SIT, but diastolic blood pressure (DBP) did not change (Whyte et al., 2010). Mean arterial pressure (MAP) and DBP also decreased following 3 weeks of SIT, but a similar effect was not found in SBP (Cocks et al., 2013).

1.6.2. Skeletal muscle adaptations to SIT

Muscle oxidative enzyme capacity changes following SIT, including changes in CS, cytochrome oxidase (COX, a specific increase in COX II and COX IV protein
subunit expression), 3-hydroxyacyl CoA dehydrogenase (β-HAD), and expression of pyruvate dehydrogenase (PDH) (Burgomaster et al., 2006; Burgomaster et al., 2005; Gibala et al., 2006; Burgomaster et al., 2008; Burgomaster et al., 2007; Little et al., 2011b). First, CS is the enzyme at the first step of the Krebs cycle (Wiegand and Remington, 1986) and is considered a marker of oxidative capacity (Leek et al., 2001). Second, COX is the last enzyme of the electron transport chain within mitochondria (Li et al., 2006) and is involved in oxidative phosphorylation (Kadenbach et al., 2000). Furthermore, PDH is one of the component enzymes of the PDH complex (PDC) that is associated with pyruvate transformation into acetyl-CoA (Wieland, 1983). Additionally, 3-hydroxyacyl CoA dehydrogenase (β-HAD) is an enzyme associated with mitochondrial fatty acid b-oxidation (FAO) cycles (Yang et al., 2005). Increases in muscle oxidative capacity are associated with for example improvement of fat oxidation, glycogen sparing, and reduced insulin resistance (Gibala, 2007).

Glycogen concentration in muscle decreases on average by 20% immediately after REHIT (Metcalfe et al., 2015). Moreover, during SIT (three repeated 30-s sprints), the peak glycogenolysis rate was within the first 15 seconds of the first sprint, and the glycogenolysis rate was attenuated in the third sprint (Parolin et al., 1999). These findings confirm that glycogen is the primary source of energy during brief maximal sprints (Burgomaster et al., 2007). Moreover, glycogen depletion seems to be linked with the regulation of signalling molecules (e.g. AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK)) (Philp et al., 2012), relating to metabolic adaptations following SIT. Additionally, resting glycogen content increases, and muscle lactate accumulation decreases following a period of SIT (Burgomaster et al., 2006;
Gibala et al., 2006). Collectively, changes in utilisation of substrate after training might be connected with an elevation in mitochondrial enzyme activity (Saltin and Gollnick, 2011).

In addition, SIT affects several molecular substances in skeletal muscle, including AMPK and p38 MAPK, and it increases peroxisome proliferator-activated receptor-γ coactivator-1α activity (PGC-1α) (Metcalfe et al., 2015; Gibala et al., 2009; Burgomaster et al., 2008; Little et al., 2011b). AMPK is a signalling molecule that plays an important role in mitochondrial biogenesis and metabolic processes, including FAO (Winder and Hardie, 1996) and glucose uptake (Hayashi et al., 1998), and can be activated by endurance exercise, HIIT and SIT (Bartlett et al., 2012). In addition, p38 MAPK is a stress-stimulated protein kinase (Kramer and Goodyear, 2007) activated by SIT (Gibala et al., 2009), HIIT (Yu et al., 2003), and prolonged endurance exercise (Boppart et al., 2000) and is associated with metabolic adaptations, for example improved glucose transportation via glucose transporter type 4 (GLUT4) (Kramer and Goodyear, 2007). Furthermore, increases in AMPK and p38MAPK following exercise are also linked to increases in PGC-1α activity (Reznick and Shulman, 2006; Little et al., 2010a; Jager et al., 2007), which is a major regulator of muscle mitochondrial biogenesis (Wu et al., 1999) and is related to skeletal muscle adaptations following exercise training (Wright et al., 2007). Furthermore, PGC-1α is an important factor for improving insulin sensitivity, connected to increases in GLUT4 protein expression and enhanced FAO (Benton et al., 2008; Hawley, 2004) and exercise capacity (in mice) (Calvo et al., 2008). Acute bouts of SIT have been observed to increase PGC-1α mRNA transcription (Gibala et al., 2009; Little et al., 2011b).
The mechanisms behind skeletal muscle adaptations to SIT have been proposed as in Figure 1.1. While SIT alters the ATP/ADP/AMP ratio (Karatzafiri et al., 2001), it also increases the calcium release from the sarcoplasmic reticulum (Jeon, 2016) and increases glycogen depletion (Parolin et al., 1999), which leads to activation of AMPK. In addition, reactive oxygen species (ROS) levels following SIT, which lead to the fragmentation of ryanodine receptor type 1 (RyR1), result in AMPK activation (Place et al., 2015). Moreover, p38 MAPK is stimulated by ROS (Kang et al., 2009), and AMPK and p38 MAPK activate the signalling pathway that in turn activates PGC-1α, and the ultimate result is linked to elevated PGC-1α levels in the nucleus and the co-activation of transcription factors. Overall, these adaptations are related to mitochondrial gene transcription and lead to increased mitochondrial proteins.

**Figure 1.1** The possible mechanisms underlying skeletal adaptations to SIT. (Adapted from Gibala et al. (2012) and MacInnis and Gibala (2017))

### 1.6.3. Metabolic adaptations to SIT

The effects of SIT on metabolic function have been confirmed by several studies (Babraj et al., 2009; Whyte et al., 2010; Gillen et al., 2016; Gillen et al., 2014;
Insulin sensitivity is improved following SIT in sedentary and untrained participants (Babraj et al., 2009; Richards et al., 2010; Whyte et al., 2010; Gillen et al., 2014; Sjöros et al., 2018). The results are similar in terms of reducing the plasma insulin area under curve (AUC) and fasting plasma insulin concentration and have been seen in overweight/obese individuals at risk of developing insulin resistance (Whyte et al., 2010; Gillen et al., 2014). However, fasting plasma glucose concentrations are not changed after SIT (Babraj et al., 2009; Richards et al., 2010; Whyte et al., 2010).

The mechanism for enhancing insulin sensitivity following endurance exercise training may be based on increased oxidative enzyme capacity and GLUT4 levels (Hughes et al., 1993; Simoneau et al., 1995). Interestingly, GLUT4 level increases following SIT (Burgomaster et al., 2007; Bradley et al., 2014). Moreover, as discussed earlier, oxidative enzyme activities are enhanced after SIT; therefore, a rise in GLUT4 and oxidative enzyme activities might be responsible for the improved insulin sensitivity following SIT. However, the specific mechanism underlying the observed improvements subsequent to SIT are not clearly understood.

1.7. Psychological Responses and Exercise Adherence to REHIT

Despite the exercise recommendations that have been proposed for almost 10 years (WHO, 2010), 27.5% of the world population is still classed as inactive in 2016 (Guthold et al., 2018). Moreover, approximately 50% of people who start doing exercise then stop (James et al., 2008; Sallis et al., 1986). Barriers to exercise have been described in Section 1.4, and one of the main barriers to exercise is lack of time (Booth et al., 1997; Sequeira et al., 2011). Therefore,
more time-efficient exercise protocols have been developed for overcoming this barrier. However, many factors are associated with exercise adoption and adherence, including psychological responses. This section will focus on psychological aspects associated with exercise behaviour.

Psychological factors that are associated with exercise behaviour include self-efficacy, motivation, perceived benefits of exercise, and enjoyment (King et al., 1992; Sherwood and Jeffery, 2000). Importantly, Sallis and Hovell (1990) proposed a model, based on the natural history of exercise, for studying the determinants of exercise behaviour (Sallis and Hovell, 1990). There are four phases of the natural history of exercise; the sedentary phase, adoption phase, maintenance/drop-out phase, and resumption phase (Sallis and Hovell, 1990). The determinants of exercise behaviour focus on three transitions between four phases (Cox, 2012). The main determinant of exercise that drives people from the sedentary phase to the adoption phase is exercise self-efficacy (Cox, 2012).

Self-efficacy is the belief in one’s capability to produce actions needed to perform an exercise (Bandura, 1997). Many studies demonstrated that self-efficacy is a main factor which related to exercise adoption (the duration of engaging in exercise is less than 6 months) (Oman and King, 1998; Sallis et al., 1986; McAuley, 1992). People who have high self-efficacy adhere more to regular exercise (Sherwood and Jeffery, 2000). In addition, perceiving the importance and benefits of doing regular exercise, and exercise enjoyment are also determinants of exercise adoption (Cox, 2012).

Moreover, self-efficacy is also a determinant of the maintenance of exercise and a predictor of exercise adherence (the duration of engaging in exercise is more than 6 months) (Oman and King, 1998; McAuley and Blissmer, 2000). Several
studies confirmed that self-efficacy is associated with exercise adherence both in adults and older adults (McAuley and Jacobson, 1991; Sallis et al., 1986; McAuley et al., 2011; Garcia and King, 1991). Self-motivation, which relates to goal setting, self-monitoring and self-reinforcement, are also factors for exercise adherence (King et al., 1992). In addition, exercise enjoyment is an important determinant of exercise adherence (Jekauc, 2015; Hagberg et al., 2009). People continue to do those things that are enjoyable (Raedeke, 2007). Therefore, the exercise enjoyment is a factor that needs to be considered when designing an exercise protocol. Finally, there is a lack of research focusing on the transition from the drop-out phase to the exercise resumption phase, Thus, this area of research needs to be investigated in the future (Sallis and Hovell, 1990; Cox, 2012). Another factor that is significantly related to exercise adherence is the affective response (Rhodes and Kates, 2015). The affective response is the individual's psychological state within a given condition which is not limited by mood and emotions (Ekkekakis and Petruzzello, 2002) and it is used to evaluate the individual's experience in both positive and negative responses (Haile et al., 2015). The feeling scale is a tool for measuring affective response (Haile et al., 2015). The feeling scale comprises a range of numerical scale which represent the positive and negative feelings (Hardy and Rejeski, 1989) and it has been used to assessed the affective response before, during and after exercise (Ekkekakis, 2008; Haile et al., 2013).

There is a relationship between a positive affective response and exercise maintenance (Kwan and Bryan, 2010a; Williams et al., 2008). A positive affective response to exercise is related to greater motivation to exercise and better long term exercise adherence (Kwan and Bryan, 2010b). Moreover, sedentary
individuals who reported higher positive affective response to a single bout of exercise were more likely to participate in more minutes in exercise after 6 and 12 months (Williams et al., 2008). It might therefore be assumed that affective responses could be a predictor of future exercise participation (Williams et al., 2008). On the other hand, feeling an unpleasant or negative affective response during or following exercise may be a reason for lowering exercise adherence (Jung et al., 2014). Nevertheless, all mentioned studies focused on affective responses to moderate-intensity exercise. Interestingly, some comments suggested that a negative affective response might be found when performing SIT, which leads people to avoid this type of exercise protocol (Biddle and Batterham, 2015; Hardcastle et al., 2014). According to the dual-mode theory, performing exercise at an intensity higher than the ventilatory threshold (VT) can lead to a decrease in affective valence (Ekkekakis et al., 2008). It could be interpreted that exercising at a high intensity might make people feel unpleasant. This theory has been supported by Ekkekakis et al. (2008): the affective response did not change when exercising below or at the VT, but it decreased when the intensity was above VT (Ekkekakis et al., 2008). Similar results were found in overweight and obese individuals (Ekkekakis et al., 2010). However, these studies examined the affective responses to a continuous exercise protocol. The affective responses to other types of protocols, such as SIT or HIIT, may be different.

The psychological responses to SIT or HIIT have been investigated in a number of studies. Jung et al. (2014) demonstrated that the affective responses were significantly lower in HIIT and continuous vigorous-intensity exercise (CVI) compared to continuous moderate-intensity exercise (CMI), but there was no
significant difference between HIIT and CVI. Moreover, participants enjoyed and preferred HIIT more than CVI and CMI (Jung et al., 2014). Importantly, the affective responses gradually becomes worse when the number of sprint was increased, both in SIT (Townsend et al., 2017) and HIIT (Frazao et al., 2016), and when the sprint duration is longer (Townsend et al., 2017). Overall, it might be concluded that a negative affective response is found when performing more sprints with longer exercise duration. However, a recent study demonstrated that the affective responses were lower in HIIT and SIT compared to moderate-intensity continuous training (MICT) but not significantly different between HIIT and SIT, even when the number of sprints in SIT (which was modified by adding one more sprint to REHIT sessions) was less than HIIT (Stork et al., 2018). It would be interesting to determine whether REHIT stimulates more negative responses than MICT. Overall, affective response is a factor that is associated with exercise behaviour. People would choose to engage in the type of exercise that is most enjoyable and that is associated with the most positive affect (Miller et al., 2005). If an exercise protocol brings more negative affective responses, people might not choose that protocol as a regular exercise. Therefore, exercise specialists need to consider this factor when designing an exercise protocol.

1.8. Overall objectives of the thesis

As mentioned previously, physical inactivity is associated with increased risk of chronic diseases. However, many people are still inactive and the most cited barrier of exercise is lack of time. Therefore, time-efficient exercise protocols, e.g. REHIT, may provide an alternative for encouraging people to be more active with spending less time but still getting health benefits similar to the current exercise
recommendations. In order to confirm this, the experimental objectives of the thesis were:

- to optimise the REHIT protocol parameters and to examine the effect of modifying the REHIT protocol on changes in $\dot{V}O_2$max (Chapter 2, 3, and 4)
- to investigate the potential mechanisms for improving $\dot{V}O_2$max following SIT (Chapter 2)
- to compare psychological responses following SIT to the current exercise recommendation protocol and other HIIT protocols (Chapter 4 and 5).
- to examine the effect of REHIT on changes in $\dot{V}O_2$max and other metabolic parameters in T2D patients (Chapter 6).
CHAPTER 2

CHAPTER 2: THE EFFECT OF SINGLE-BOUT HIGH-INTENSITY TRAINING (MICROTRAINING) ON MAXIMAL OXYGEN CONSUMPTION AND SERUM LEVELS OF THE MYOKINE SPARC
2.1. Introduction

It has been confirmed that $\dot{V}O_2\text{max}$ improves following sprint interval training (SIT) (Rakobowchuk et al., 2008; Bailey et al., 2009; Astorino et al., 2012; Cochran et al., 2014; Shepherd et al., 2013; Nalcakan, 2014; Whyte et al., 2010). Therefore, SIT has been proposed as a time-efficient protocol for addressing the disease risk factor $\dot{V}O_2\text{max}$, which is related to being inactive. However, there are some limitations to the conventional SIT protocol (six repeats of 30-s ‘all-out’ sprints). High levels of fatigue and exertion were found during SIT (Gillen and Gibala, 2014; Hawley and Gibala, 2009; Gibala and McGee, 2008). Moreover, the total duration of the conventional SIT protocol is approximately 30 minutes per session, which is approximately 90 minutes per week. Thus, the time commitment in the conventional SIT is greater than the current exercise recommendation, which is 75 minutes of vigorous intensity exercise, so SIT might not be as time-efficient an exercise as often claimed (Gibala, 2007; Gibala and Little, 2010).

To address the barrier of lack of time, the conventional SIT protocol has been modified. Firstly, studies have examined the effect of reducing the sprint duration. $\dot{V}O_2\text{max}$ improved after training in the group that reduced the sprint duration to 10-s (Hazell et al., 2010) or 15-s (Zelt et al., 2014), and there was no significant difference in changes in $\dot{V}O_2\text{max}$ between reduced sprint duration protocols and conventional SIT (Hazell et al., 2010; Zelt et al., 2014). Moreover, the changes in $\dot{V}O_2\text{max}$ in both reduced sprint duration and the number of sprints were also examined. $\dot{V}O_2\text{max}$ was improved following the reduced-exertion high-intensity interval training (REHIT) (2x20-s sprints) (Metcalfe et al., 2012; Metcalfe et al., 2015; Metcalfe et al., 2016; Ruffino et al., 2017) and modified REHIT (3x20-s sprints).
sprints) (Gillen et al., 2014; Gillen et al., 2016). Moreover, the meta-analysis by Vollaard et al. (2017) confirmed that reducing the number of sprints does not attenuate the improvement of \( VO_2 \text{max} \), but may possibly even increase the improvements (Vollaard et al., 2017). It can be concluded that performing fewer and shorter sprints remains effective at improving \( VO_2 \text{max} \). However, it is unknown whether regularly performing a single sprint could be sufficient to improve \( VO_2 \text{max} \).

The mechanisms for increasing \( VO_2 \text{max} \) after SIT are still unclear. Most studies focus on peripheral adaptations such as increased mitochondrial enzymes capacity including citrate synthase (CS) (Burgomaster et al., 2005), and cytochrome c oxidase (COX) (Gibala et al., 2006). In addition, SIT is associated with an increase in the peroxisome-proliferator activated receptor \( \gamma \) coactivator (PGC)-1\( \alpha \) (Little et al., 2011b), which is a main regulator of skeletal muscle biogenesis (Wu et al., 1999). The possible mechanism underlying SIT improving \( VO_2 \text{max} \) via PGC-1\( \alpha \) is also related to the activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) (Gibala et al., 2012). Importantly, AMPK contains a glycogen binding domain and AMPK activation is related to glycogen depletion (McBride and Hardie, 2009). Parolin et al. (1999) demonstrated that glycogen depletion occurs predominantly within the first 15 s of the first sprint repetition (Parolin, 1999). It has indeed been shown that AMPK is activated following a single 30-s all-out sprint (Fuentes et al., 2012). Thus, it could be hypothesised that a single 20-s all-out sprint might be sufficient to activate skeleton muscle signalling molecules and lead to aerobic adaptation following training. Conversely, some researchers support the notion that central adaptations limit
\( \dot{V}O_2 \text{max} \) (Bassett and Howley, 2000; Sutton, 1992a). According to the HERITAGE Family Study, there are large individual differences, with low/non-responders, medium responders and high responders for \( \dot{V}O_2 \text{max} \) following 20 weeks of aerobic exercise training. The inter-individual variability of \( \dot{V}O_2 \text{max} \) in response to exercise may involve differences in gene expression (Bouchard et al., 1999). Keller et al. (2011) revealed that 86 genes can be identified as “high responder” genes, which means that these genes were up-regulated significantly more in high responders compared to low responders following 6 weeks of endurance training (Keller et al., 2011). Interestingly, many of the high responder genes are related to cardiovascular development (Keller et al., 2011). These findings might be evidence to support the fact that \( \dot{V}O_2 \text{max} \) is limited by central adaptations rather than peripheral adaptations.

Skeletal muscle is the main organ that is affected directly by supramaximal exercise. Any molecular changes which occur inside skeletal muscle during exercise may be a signal that is sent to other organs, leading to whole body adaptations. However, the mechanisms underlying adaptations are still being discussed (Catoire et al., 2014). A possible mechanism is associated with muscle-released cytokines (Pedersen and Febbraio, 2008). Several studies have found an endocrine-like function in skeletal muscles (classified as myokines). Myokines are signalling proteins or peptides that are released by skeletal muscle (Brandt and Pedersen, 2010; Pedersen et al., 2003; Bortoluzzi et al., 2006). Myokines have been described as an exercise factor which are produced from skeletal muscle during contraction and released into the bloodstream, affecting other cells or organs (Pedersen and Febbraio, 2008). Studies have shown that myokines improve glucose uptake (Busquets et al., 2006) and insulin sensitivity.
regulate fat mass and lipid metabolism (Alvarez et al., 2002) and cell proliferation signalling (Serrano et al., 2008). Interleukin (IL)-6 has been identified as the first exercise-induced myokine (Steensberg et al., 2000). It increases following exercise (Febbraio and Pedersen, 2002) and has been associated with increases in glucose uptake (Carey et al., 2006) and fat oxidation (Pedersen and Fischer, 2007). Other interleukins, including IL-8 and IL-15, were also discovered as myokines (Pedersen and Febbraio, 2008). Catoire et al. (2014) investigated novel myokines using secretome analysis. The results demonstrated that the gene expression of 69 putative myokines was up-regulated following 12 weeks of exercise training (Catoire et al., 2014). Surprisingly, 13 putative myokines are the same genes which were identified as high responder genes found by Keller et al. (2011). This finding could suggest that myokines are associated with $\dot{V}O_2^{\text{max}}$ improvement.

Secreted protein acidic and rich in cysteine (SPARC) is a ‘high responder gene’ (Keller et al., 2011), and it is also a myokine which is increased both after acute exercise and training (Aoi et al., 2013; Catoire et al., 2014; Pourteymour et al., 2017). Serum SPARC level increase following a single bout of 30 min cycling at 70% $\dot{V}O_2^{\text{max}}$ and after four weeks of cycling training at 70% $\dot{V}O_2^{\text{max}}$ for 30 minutes three times a week (Aoi et al., 2013). Moreover, SPARC gene expression was up-regulated by 12 weeks of training of cycling at 55% of maximal work load (Wmax) for 30 minutes, twice a week, combined with resistance training (Catoire et al., 2014) and it also increased after 12 weeks of cycle and whole-body strength training (Pourteymour et al., 2017). Some evidence indicates that SPARC might play a role in $\dot{V}O_2^{\text{max}}$ improvement following exercise. SPARC interacts with AMPK signalling pathways (Song et al.,
2010), it is associated with erythroid progenitors development (Luo et al., 2012) and with vascular endothelial growth factor (VEGF) signalling, which is related to cardiovascular development (Keller et al., 2011). These relevant findings could support the association between exercise-induced secretion of SPARC and the improvement of $\dot{V}O_2\text{max}$.

Thus, there were two main aims to this chapter. The first aim was to determine whether training consisting of single 20-s all-out sprints (‘microtraining’) could improve $\dot{V}O_2\text{max}$. The second aim was to determine whether microtraining increases serum SPARC levels, and whether acute changes in serum SPARC levels correlate with training-induced changes in $\dot{V}O_2\text{max}$.

2.2. Methods

2.2.1. Study 1 (the effect of microtraining on changes in $\dot{V}O_2\text{max}$)

2.2.1.1. Participants

Thirty healthy untrained men and women, aged between 18-40 years were recruited to this study. Participants were randomly allocated into a control group or a training group. Participants who had physical impairments which were an obstacle to exercise determined by a standard physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992) and who were highly physically active as classified by the International physical activity questionnaire (IPAQ) (Craig et al., 2003) were excluded from this study. Further exclusion criteria were resting heart rate $\geq$100 bpm or clinically significant hypertension (>140/90 mm Hg). All participants were informed of the protocol and potential risks from the study protocol. The experimental protocol was approved by the University of Bath REACH Ethics committee (reference EP 14/15 87).
2.2.1.2. Experimental Protocol

2.2.1.2.1. Training protocol

For the control group, participants were asked to maintain their normal activity levels for 4 weeks. Participants in the training group completed 3 exercise sessions a week for 4 weeks. A single all-out sprint was performed for 20-s without warm up or cool down on a bicycle ergometer (Ergomedic 894 E, Vansbro, Sweden) and the braking force was equivalent to 7.5% of body weight. Plasma volume change with exercise was determined at the first and last training session using finger pricks before and 0, 3, 10, and 30 minutes after exercise. Haemoglobin concentration was directly analysed (HemoCue® Hb 201+ System, Ängelholm, Sweden). Other blood samples were centrifuged at 13,000 g for 5 minutes (Haematospin 1300, Sussex, United Kingdom) and haematocrit was measured (Hawksle, Sussex, United Kingdom). Changes in plasma volume from baseline during the first and the last training session were calculated by the equations of Dill and Costill (1974). Peak power output (PPO), end power output (EPO) and mean power output (MPO) were determined by Monark Anaerobic Test software (Monark, Vansbro, Sweden) during the third and the last session of exercise. RPE was determined by the 15-point Borg scale (Borg, 1970) at the end of sessions 3, 6, 9 and 12.

2.2.1.2.2. Aerobic fitness

\( \dot{V}O_2 \text{max} \) was determined before and three days after the training protocol using an incremental cycling test to exhaustion (Lode Excalibur Sport ergometer, Groningen, the Netherlands) and a gas analysis system (Parvo Medics TrueOne 2400, Utah, USA). The test was started with a warm-up phase at 50 W for 2 minutes. Then, the resistance was increased by 1 W every 3 seconds until
volitional fatigue. The participants were asked to breathe through a mouthpiece and respired air was collected and analysed to determine \( \dot{V}O_2 \). \( \dot{VO}_2\text{max} \) was determined as the highest value of a 15-breath rolling average. The acceptance criteria of \( \dot{VO}_2\text{max} \) were: RER >1.15, maximal heart rate within 10 beats of age-predicted maximum, and volitional exhaustion. At these criteria, one participant in the control group was excluded from data analysis. Participants kept a food diary on the pre-testing day and testing day, and were asked to repeat a similar diet before the post-training \( \dot{VO}_2\text{max} \) test.

2.2.2. Study 2 (the effect of microtaining on serum SPARC levels and the relationship between changes in serum SPARC and changes in \( \dot{VO}_2\text{max} \))

2.2.2.1. Participants

Eleven healthy inactive participants, aged between 18-40 years, were recruited to this study (seven men and four women). The exclusion criteria consisted of highly physically active as classified by the International physical activity questionnaire (IPAQ) (Craig et al., 2003), physical impairments as determined by a standard physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992), resting heart rate ≥100 bpm or clinically significant hypertension (>140/90 mm Hg). The protocol and potential risks from the study were explained to all participants. The experimental protocol was approved by the University of Bath REACH Ethics Committee (reference EP 15/16 9).

2.2.2.2. Experimental Protocol

5.2.2.3.1. Training protocol

Three days after the pre-training \( \dot{VO}_2\text{max} \) test (following the same protocol as in Study 1), the acute effect of an all-out 20-s sprint was examined following an
overnight fast. Participants arrived at the laboratory in the morning. After 15 min rest, a 1.5 ml baseline blood sample was taken through a cannula inserted in a superficial forearm vein. The exercise protocol consisted of a 1-min warm up (unloaded pedalling), an all-out 20-s sprint, and a 3-min cool down (unloaded pedalling), performed on a cycle ergometer (Ergomedic 894 E, Vansbro, Sweden) and the resistance was equivalent to 7.5% of body weight. The blood samples were taken again directly after exercise, and 15 minutes and 60 minutes after exercise. Haemoglobin was analysed (HemoCue® Hb 201+ System, Ängelholm, Sweden). Haematocrit was measured (Hawksle, Sussex, United Kingdom) after blood samples were centrifuged at 13,000 g for 5 minutes (Haematospin 1300, Sussex, United Kingdom). Changes in plasma volume from before to after exercise were calculated using Dill and Costill (1974) equation. SPARC levels were analysed (Human SPARC Quantikine ELISA, RND Systems) from serum, which was collected after 30 minutes clotting at room temperature (0.5 mL clotting activator/serum micro-tubes; Sarstedt, Nümbrecht, Germany) and stored at -80°C prior to analysing. When analysing serum SPARC, 100 µL of assay diluent was added to 100 µL of sample and incubated at room temperature for 3 hours on a microplate shaker. The sample was then washed six times, 200 µL of cold Human SPARC conjugate was added and incubated for 1 hour on ice without shaking. After washing for six times again, 50 µL of stop solution was added and the optimal density was read by a microplate reader which set as 450 nm, with a wavelength correction at 570 nm.

In addition, the training effect on changes in serum SPARC levels was also examined in the present study. Participants engaged in an all-out 20-s sprint training intervention, three times a week for four weeks. The training protocol was
similar to the protocol done for the acute effect of exercise as described earlier. Peak heart rate (Polar RS400, Finland) and power output (peak, mean and end) were recorded at sessions 3 and 12. RPE was taken using the 15-point Borg scale (Borg, 1970) after 3rd, 6th, 9th and 12th session.

The second $\dot{V}O_2$max test was done three days after the 12th session. In addition, the second test for the acute effects was performed three days following the last $\dot{V}O_2$max test. The study protocol summary is shown in Figure 2.1.

![Figure 2.1 The protocol summary]

### 2.2.3. Statistics

All data are presented as mean±SD and analysed using SPSS Version 22 (Chicago, IL, USA). For Study 1, the sample size was calculated based on a coefficient of variation of the $\dot{V}O_2$max test protocol of 4%. Fourteen participants were needed in each group for detecting a 5% difference in the change in $\dot{V}O_2$max between the training group and the control group, with a power of 90% and $\alpha = 0.05$. The data followed a normal distribution, thus parametric tests were used. The differences in $\dot{V}O_2$max and maximal power output ($W_{max}$) from the exercise test between the training and the control group from pre- and post-intervention were determined using a Two-way mixed model ANOVA (group x time). The effect of a single 20-s sprint on plasma volume change were examined by Two-way repeated measures ANOVA (training session x time). The difference
in PPO, EPO and MPO between 3rd and 12th training session were analysed using paired t-tests.

For study 2, the sample size was calculated that 9 participants were needed to detect a 5% change in \( \dot{V}O_2 \text{max} \) from pre- to post-training, with a power of 90% and \( \alpha = 0.05 \). The sample size was calculated based on a coefficient of variation of the \( \dot{V}O_2 \text{max} \) test protocol of 4%. The data followed a normal distribution, thus parametric tests were used. Paired t-tests were used to determine the differences in \( \dot{V}O_2 \text{max} \) and maximal power output (Wmax) from the graded exercise testing between before and after training and the difference in PPO, EPO and MPO between 3rd and 12th training session. Serum SPARC levels and plasma volume changes were determined using a Two-way mixed model ANOVA (trial \( \times \) time) and Bonferroni Post hoc test was used to analyse the differences between time points to determine significant main effects of time. Differences in RPE between 3rd, 6th, 9th and 12th were determined using a One-way ANOVA.

2.3. Results

2.3.1. Study 1

No significant differences were observed in the baseline characteristics between the training and control groups (Table 2.1).
Table 2.1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Training</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 15, 5 men, 10 women)</td>
<td>(n = 15, 5 men, 10 women)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24 ± 6</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 ± 0.08</td>
<td>1.69 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.6 ± 15.5</td>
<td>64.4 ± 12.8</td>
</tr>
<tr>
<td>BMI (kg·m(^{-2}))</td>
<td>22.9 ± 4.5</td>
<td>22.3 ± 3.7</td>
</tr>
<tr>
<td>(\dot{V}O_2)max (ml·kg(^{-1})·min(^{-1}))</td>
<td>34 ± 8</td>
<td>32 ± 6</td>
</tr>
</tbody>
</table>

Data shown are mean±SD

There was no significant effect of time nor a significant group x time interaction effect for \(\dot{V}O_2\)max (Figure 2.2A). For maximal power output, there was a significant interaction effect for group x time \((P<0.001)\), indicating maximal power output of the training group increased significantly when comparing to the control group (Figure 2.2B).

Figure 1 Maximal oxygen consumption (A) and maximal power output (B) in the control and training group. Data are represented as mean ± SD \((n = 14\) in control group, \(n = 15\) in training group). \(\dagger\) \(p<0.001\) for the group x time interaction effect.
During the 20-s sprint, end power output (EPO) was increased significantly in the 12th training session compared to the 3rd training session (Table 2.2) but peak power output (PPO) and mean power output (MPO) did not change.

**Table 2.2** Power output during sprint (Watt)

<table>
<thead>
<tr>
<th></th>
<th>3rd training session (n = 15)</th>
<th>12th training session (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO</td>
<td>544 ± 156</td>
<td>564 ± 177</td>
</tr>
<tr>
<td>EPO</td>
<td>335 ± 123</td>
<td>365 ± 115*</td>
</tr>
<tr>
<td>MPO</td>
<td>446 ± 141</td>
<td>451 ± 149</td>
</tr>
</tbody>
</table>

*<p<0.05 for the difference between 3rd and 12th training session

After a single 20-s sprint plasma volume was significantly reduced (effect of time: \(P<0.05\)). There was no significant difference between the first and the last session (Figure 2.2). Plasma volume tended to return to baseline at 30 minutes after a single 20-s sprint.

![Figure 2.2](image)

**Figure 2.2** Changes in plasma volume; the first session and the last session training. Data are represented as mean ± SD (n = 15)
Mean RPE over 12 sessions was 15 which is equivalent to “hard” (Figure 2.3).

![Figure 2.3 Rating of Perceived Exertion after the 3, 6, 9, 12 training session. Data are represented as mean ± SD (n = 15)](image)

**2.3.2. Study 2**

Eight participants completed all training session and two participants missed one training session. One participant (woman) dropped out from the study because of lack of time. The characteristics of 10 participants are shown in Table 2.3. The training characteristics are shown in Table 2.4. There was a significant difference in peak heart rate \( p<0.05 \) but no significant differences in PPO, MPO and EPO. Mean RPE score did not change over 12 training sessions (Figure 2.4).

**Table 2.3 Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th><strong>Men</strong> (n=7)</th>
<th><strong>Women</strong> (n=3)</th>
<th><strong>All</strong> (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21 ± 3</td>
<td>21 ± 3</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 ± 0.07</td>
<td>1.68 ± 0.06</td>
<td>1.74 ± 0.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.2 ± 12.8</td>
<td>58.1 ± 5.5</td>
<td>65.2 ± 11.8</td>
</tr>
<tr>
<td>BMI (kg·m(^{-2}))</td>
<td>21.8 ± 3.5</td>
<td>20.6 ± 0.8</td>
<td>21.5 ± 2.9</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{max} ) (ml·kg(^{-1})·min(^{-1}))</td>
<td>40.0 ± 6.4</td>
<td>37.8 ± 4.9</td>
<td>39.3 ± 5.7</td>
</tr>
</tbody>
</table>

Data shown are mean±SD
Table 2.4 Training characteristics

<table>
<thead>
<tr>
<th></th>
<th>3rd session</th>
<th>12th session</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (W)</td>
<td>699±154</td>
<td>642±135</td>
</tr>
<tr>
<td>MPO (W)</td>
<td>501±132</td>
<td>512±106</td>
</tr>
<tr>
<td>EPO (W)</td>
<td>354±124</td>
<td>383±95</td>
</tr>
<tr>
<td>HRpeak (bpm)</td>
<td>182±7</td>
<td>172±12*</td>
</tr>
</tbody>
</table>

Data shown are mean±SD, *p<0.05 for the difference between 3rd and 12th training session

Figure 2.4 Rating of Perceived Exertion following 3rd, 6th, 9th, 12th sessions. Data are represented as mean ± SD.

There was no statistically significant difference in VO$_2$max between pre- and post-training. However, Wmax increased significantly post-training (Table 2.5).

Table 2.5 VO$_2$max and Wmax following graded exercise test

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_2$max (L/min)</td>
<td>2.62±0.55</td>
<td>2.74±0.67</td>
</tr>
<tr>
<td>Wmax (W)</td>
<td>212±43</td>
<td>223±46*</td>
</tr>
</tbody>
</table>

Data shown are mean±SD, *p<0.05 for the difference between pre- and post-training session
There was a significant decrease in plasma volume after exercise (0 min) ($p<0.001$), which then returned to baseline at 60 min after exercise (Figure 2.5). Plasma volume did not change between pre- and post-training.

![Figure 2.5 Changes in plasma volume; pre-training (---) and post-training (-----). Data are represented as mean ± SD (n = 10), *$p<0.001$ compared to pre-exercise, †$p<0.01$ compared to post 15 min, ‡$p<0.001$ compared to post 60 min, §$p<0.05$ compared to post 60 min.](image)

Uncorrected serum SPARC levels and corrected serum SPARC levels did not differ when compared between pre- and post-training. Uncorrected serum SPARC levels (Figure 2.6A) increased significantly after exercise (0 min) ($p<0.001$) then returned to baseline at 60 min after exercise. However, this change was not observed in corrected serum SPARC levels (Figure 2.6B), suggesting that it was caused by haemoconcentration.
2.4. Discussion

This chapter examined the effect of single-bout high-intensity training (microtraining) on changes in $\dot{V}O_2\text{max}$, and acute and chronic changes in serum levels of the myokine SPARC. The results indicated that $\dot{V}O_2\text{max}$ did not improve after four weeks of 20-s single sprint training in inactive participants. In the same way, performing 20-s single sprints was insufficient to alter serum SPARC levels. Although SIT has been claimed to be a time-efficient form of exercise, there is still no clarity about the optimal number of sprint repetitions, duration of sprints and duration of training to confirm the SIT effect on cardiovascular adaptations. A study by Metcalfe et al. (2012) attempted to modify the conventional SIT protocol (4-6 repeated 30-s all-out sprints) by reducing both the number of sprints (to 2 sprints) and sprint duration (to 20 s), termed reduced-exertion high-intensity interval training (REHIT). The results demonstrated that $\dot{V}O_2\text{max}$ can be improved by regularly performing two repeated 20-s all-out sprints (Metcalfe et
al., 2012). The present study built on this by showing that $\dot{V}O_2^{\text{max}}$ does not change by regularly performing a single 20-s all-out sprint (average $\sim$4% increase in $\dot{V}O_2^{\text{max}}$, not significant). This suggests that either the total training volume is insufficient when performing single sprints, or that sprints need to be repeated to be effective. However, it should be pointed out that when considering the training duration, the training period of the present study was 4 weeks, whereas the training period of the highlighted investigation was 6 weeks (Metcalfe et al., 2012). Longer training durations of SIT (more than 6 weeks) have been seen to lead to greater improvements in $\dot{V}O_2^{\text{max}}$ (Barnett et al., 2004; Sandvei et al., 2012; Siahkouhian et al., 2013). Therefore, this could suggest that 4 weeks of training with 20-s all-out sprints might not be sufficient to see an influence on $\dot{V}O_2^{\text{max}}$. However, Ijichi et al. (2015) demonstrated that $\dot{V}O_2^{\text{max}}$ could be improved with 4 weeks of sessions involving three repeated 30-s all-out sprints. Moreover, $\dot{V}O_2^{\text{max}}$ also rises following just two weeks of classic SIT (Bailey et al., 2009; Hazell et al., 2010; Whyte et al., 2010). Nevertheless, the meta-analysis report on the effect of training duration by Weston et al. (2014) indicated that the effect was unclear, suggesting that training duration might not be the main parameter influencing $\dot{V}O_2^{\text{max}}$ improvement.

The next parameter that should be considered is the sprint duration per session. Two repeated 20-s sprints equal to 40-s per training session was observed as being adequate for increasing $\dot{V}O_2^{\text{max}}$ (Metcalfe et al., 2012), whereas single 20-s sprints did not affect $\dot{V}O_2^{\text{max}}$. This could mean that $\dot{V}O_2^{\text{max}}$ may be stimulated by repeated sprints. Otherwise, no evidence demonstrates what the optimum repeated sprint number is to have an effect on $\dot{V}O_2^{\text{max}}$. Most studies have employed protocols comprising of 4 to 10 repeated sprints per session and
revealed that \( \dot{\text{VO}}_2\text{max} \) improved by approximately 7 to 14% following training (Bayati et al., 2011; Rakobowchuk et al., 2008; Siahkouhian et al., 2013; Trilk et al., 2011; Whyte et al., 2010; Shepherd et al., 2013). These improvements in \( \dot{\text{VO}}_2\text{max} \) were lower or similar to the enhancement in \( \dot{\text{VO}}_2\text{max} \) following two repeated sprints (REHIT protocol), an improvement of approximately 12-15%, indicating that \( \dot{\text{VO}}_2\text{max} \) improvements might not depend on the number of repeated sprints. However, when combining the results from the present study and Metcalfe et al. (2012), it can be hypothesised that there is a “priming effect” during the first sprint that is essential for the following sprints to induce cardiovascular adaptations. Importantly, Vollaard et al. (2017) meta-analysed the effect of sprint number in a sprint training session on \( \dot{\text{VO}}_2\text{max} \). They demonstrated that fewer sprint repetitions did not attenuate the \( \dot{\text{VO}}_2\text{max} \) improvement and that the lowest sprint number which improves \( \dot{\text{VO}}_2\text{max} \) is two repeated sprints (Vollaard et al., 2017). It might be assumed that sprints need to be repeated in order to improve \( \dot{\text{VO}}_2\text{max} \). However, the mechanisms underlying the increases in \( \dot{\text{VO}}_2\text{max} \) following repeated sprints need to be investigated in the future.

Plasma volume from both studies dropped by an average of 8% from baseline after single 20-s sprints, tended to be normalised after 30 minutes, and were back to baseline at 60 minutes after sprints. Decreases in plasma volume were also observed in a study that utilised two repeated 20-s sprints (Metcalfe et al., 2015). According to Metcalfe et al. (2015), plasma volume decreased ~15% immediately after REHIT. Changes in plasma volume can be explained by the relationship between glycogen breakdown and extracellular fluid. Rapid glycogenolysis during sprints increases the concentration of metabolic intermediates (glucose...
and other molecules) within myocytes, causing hyperosmotic conditions. This leads to the subsequent influx of water into myocytes and results in cell swelling (Raja et al., 2006). The fluctuations in cell volume may have an influence on the function of the cell, including gene expression and cell metabolism (Häussinger, 1996). It could be put forth that glycogen depletion could be a potential reason for skeletal muscle adaptations following sprint.

Considering both the present chapter along with Metcalfe et al. (2015), there is a difference in the extent of changes in plasma volume. Although Parolin et al. (1999) demonstrated that during repeated sprints, glycogen depletion was dominant in the first bout, a prevailing rate of glycogenosis was still observed in later bouts (Parolin et al., 1999), indicating that two 20-s sprints could lead to increased glycogen breakdown and may be the cause of diminished plasma volume, more so than when performing a single 20-s sprint exclusively. It should be mentioned that in the present studies we did not measure glycogen depletion directly. Further studies need to confirm the glycogen breakdown during the first and the second 20-s sprint.

The mechanisms underlining SIT-induced improvement in \( \dot{V}O_2 \)max are poorly understood. However, a possible mechanism for improving \( \dot{V}O_2 \)max following SIT is linked to PGC-1\( \alpha \) activation and mitochondrial biogenesis. PGC-1\( \alpha \) is activated by 5'-adenosine monophosphate-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) (Gibala et al., 2012). There is a glycogen binding domain on the AMPK \( \beta \) subunit, and AMPK is activated by glycogen breakdown (McBride and Hardie, 2009). According to Parolin et al. (1999), glycogen depletion was predominantly in the first bout (Parolin et al., 1999). It could be hypothesised that AMPK would be activated by glycogen
depletion from the single 20-s all out sprint. When AMPK is activated it stimulates other molecules (e.g. PGC-1α), which could lead to increased mitochondrial content, potentially improving \( \dot{V}O_2 \text{max} \). Unfortunately, \( \dot{V}O_2 \text{max} \) did not improve following a single 20-s all-out sprint training. Therefore, these hypotheses could not be confirmed by the present study, suggesting that glycogen breakdown *per se* might not be the main stimulus for improving \( \dot{V}O_2 \text{max} \) following brief supramaximal exercise.

Another finding in the present study is that there was no change in the serum SPARC levels after acute (and four weeks of 20-s all-out sprint) training. As stated previously, information on the potential mechanism of the improvement of \( \dot{V}O_2 \text{max} \) following the exercise is limited. Myokines might be a possible mechanism which is involved in cross talk between skeletal muscle and other organs. This may lead to several physiological adaptations. Recently, many myokines have been discovered. SPARC is a myokine which has been confirmed as increasing after acute and training of endurance exercise (both in serum SPARC levels and SPARC gene expression) (Aoi et al., 2013; Catoire et al., 2014; Pourteymour et al., 2017). Importantly, SPARC is a ‘high responder gene’ which is associated to cardiovascular development (Keller et al., 2011). It might be hypothesised that serum SPARC levels would be increased following a single 20-s sprint. However, the present study showed that serum SPARC levels (corrected for changes in plasma volume) did not change following a single 20-s sprint after acute exercise both in the trained and untrained state. This finding contrasts with the findings of Aoi et al. (2013), where serum SPARC levels increased following aerobic training (30 min cycling at 70% \( \dot{V}O_2 \text{max} \)). It can be
assumed that SPARC might be stimulated by longer durations of endurance exercise rather than brief sprint exercise.

As previously described, AMPK can be activated by glycogen breakdown (McBride and Hardie, 2009) and AMPK activation increases SPARC gene expression (Song et al., 2010). Therefore, it could be hypothesised that increases in SPARC release might be associated with glycogen depletion. By combining the results from both the present studies, we clearly see increases in glycogen breakdown following single sprints (evidenced by changes in plasma volume), but the serum SPARC levels remain unchanged. This tentatively suggests that an increase in serum SPARC levels might not depend on glycogen depletion.

Further study is necessary to determine whether the first sprint has an influence on subsequent sprints, i.e. whether there is a priming effect of the first of repeated sprints that brings about cardiovascular adaptations following repeated sprint training. Additionally, other potential mechanisms responsible for increasing $\dot{V}O_2\text{max}$ after SIT should be investigated. With this recommendation in mind, there were several limitations in this study. Firstly, the design did not compare 20-s single sprints to 20-s repeated sprints or to the 30-s Wingate-based sprint. Secondly, the small number of participants led to low statistical power. However, the power was sufficient for determining a difference of 5% $\dot{V}O_2\text{max}$ between the control and training groups. There was a small change in $\dot{V}O_2\text{max}$ after the 20-s single sprint, though it was not enough to reach significance. Thirdly, the study did not use gene expression techniques to identify changes in SPARC expression. Further studies should examine SPARC levels using muscle biopsy to confirm changes in SPARC levels in the muscle.
It is possible that the assay for analysing serum SPARC levels was not accurate enough to detect changes that may have occurred in this study. However, despite the unchanged corrected serum SPARC levels, uncorrected serum SPARC levels increased significantly following sprint which was the same pattern as changes in plasma volume. Thus, it seems reasonable to assume that if single 20-s all-out sprints did have an effect on plasma SPARC levels, it would have been detected in this study. Finally, an objective measurement device such as an accelerometer was not used to confirm physical activity before and during training. However, subjective measurement (the International physical activity questionnaire (IPAQ)) was used to evaluate physical activity before training. It can be used for classifying the participants as inactive people before training. Additionally, there were not any subjective or objective measurements used for physical activity monitoring over the four weeks of training. Thus, it cannot be assumed that all participants maintained their physical activity levels over the training period, other than that they were asked to not to change their physical activity.

2.5. Conclusion

This chapter has shown that \( \dot{V}O_2 \max \) is not significantly improved by regularly performing 20-s single bouts of supramaximal-intensity exercise. Moreover, single 20-s sprints are insufficient to change serum SPARC levels but are sufficient to decrease plasma volume (which represents the occurrence of muscle glycogen depletion) after performing the sprints. It might be concluded that as neither \( \dot{V}O_2 \max \) nor SPARC levels changed, it remains unknown whether changes in SPARC levels are related to changes in \( \dot{V}O_2 \max \). Additionally, muscle glycogen depletion does not seem to be a mechanism for exercise-
induced skeletal muscle SPARC release and it cannot be the sole mechanism for improving $\dot{V}O_2$max.
CHAPTER 3

CHAPTER 3: Effect of sprint duration in reduced-exertion high-intensity interval training on changes in maximal aerobic capacity: 10-s vs 20-s sprints
3.1. Introduction

In order to provide a more time-efficient, effective alternative to SIT, reduced-exertion high-intensity interval training (REHIT) reduces the number of sprints and the sprint duration to two bouts of 20-s all-out sprints within 10 minutes of low-intensity cycling (Metcalfe et al., 2012). Despite the lower exercise volume, this protocol improves $\dot{V}O_2\text{max}$ and other health markers (e.g. insulin sensitivity) following training (Metcalfe et al., 2012; Metcalfe et al., 2015; Metcalfe et al., 2016; Ruffino et al., 2017). It is therefore suggested that some risk factors that associate with being inactive could be addressed by REHIT. Importantly, Vollaard et al. (2017) performed a meta-analysis of the effect of the number of sprints in a SIT session on the changes in $\dot{V}O_2\text{max}$ with training. The results showed that the improvement in $\dot{V}O_2\text{max}$ was not attenuated by reducing the number of sprints down to two repetitions (Vollaard et al., 2017). Conversely, we have demonstrated in Chapter 2 that $\dot{V}O_2\text{max}$ is not improved following training consisting only single sprints per session. This suggests that the lowest number of sprints that increase $\dot{V}O_2\text{max}$ is at least two sprints. However, several other factors are important in designing an effective protocol. It is affected by not only the number of sprints but also the number of sessions per week and sprint duration and recovery time, and each needs to be investigated.

Reducing the sprint duration in the conventional SIT protocol has been examined regarding whether it affects $\dot{V}O_2\text{max}$. Hazell et al. (2010) compared $\dot{V}O_2\text{max}$ following three different protocols: 10-s sprints with a 2-min rest, 10-s sprints with a 4-min rest, and 30-s sprints with a 4-min rest. $\dot{V}O_2\text{max}$ improved after training in all three groups, and there was no significant difference between the three groups (Hazell et al., 2010). Similarly, Zelt et al. (2014) demonstrated that
\( \overline{\text{VO}}_2 \text{max} \) was increased following protocols with both 15-s and 30-s sprints, and there was no significant difference between the two groups (Zelt et al., 2014). It can be concluded that improvements in \( \overline{\text{VO}}_2 \text{max} \) following SIT are not attenuated by reducing the sprint duration. However, it is unknown whether reducing the sprint duration in the REHIT protocol (which already has a lower sprint volume), affects changes in \( \overline{\text{VO}}_2 \text{max} \).

Additionally, apart from the effectiveness of the REHIT protocol, the acceptability and psychological responses of the REHIT protocol need to be considered. The affective responses and mood states are associated with exercise adherence (Kwan and Bryan, 2010b; Matsouka et al., 2005). Positive affective response and positive mood are associated with better long-term exercise adherence (Kwan and Bryan, 2010b; Matsouka et al., 2005; Kohlstedt et al., 2013; Berger and Motl, 2000). Moreover, the accumulation of short-term positive mood might be related to the long-term exercise maintenance (Kerr and Els Van den Wollenberg, 1997). On the other hand, long-term exercise adherence might be low when negative affective responses to exercise are higher (Williams et al., 2008; Garber et al., 2011). Psychological responses to exercise can be evaluated by many tools. Feeling states such as moods and emotion can be determined by self-report questionnaires; the Profile of Mood States (POMS) (Leunes and Burger, 2000) or a short version of POMS, which is the Brunel Mood Scale (BRUMS) (Lane and Lovejoy, 2001). Moreover, affective responses to exercise can be assessed by The Positive and Negative Affect Schedule (PANAS) (Kohlstedt et al., 2013) or feeling scale (Haile et al., 2013). Mood states and affective responses may be affected by exercise intensity (Kerr and Els Van den Wollenberg, 1997; Ekkekakis et al., 2008; Ekkekakis et al., 2010). Negative mood (tension and...
anxiety) increased after high intensity exercise (Steptoe and Bolton, 1988; Steptoe and Cox, 1988). Moreover, negative affective response was found following exercise above the ventilatory threshold (VT) (Ekkekakis et al., 2008; Ekkekakis et al., 2010). On the other hand, negative affect, evaluated by PANAS, did not change following high intensity exercise (Bixby et al., 2001). Interestingly, a critique from Hardcastle et al. (2014) hypothesised that SIT might increase negative affective response more than MICT and will lead to low adherence (Hardcastle et al., 2014). However, in terms of psychological responses to REHIT, there have been no studies focusing on this topic. Therefore, the objective of this chapter was to examine whether reducing sprint duration from 20 s to 10 s in the REHIT protocol affects the changes in $\dot{V}O_2$ max and the psychological responses.

3.2. Methods

3.2.1. Participants

Thirty-six healthy inactive participants, aged 18-40 years, were recruited to the present study (19 men and 17 women) from three centres (University of Stirling, University of Ege, Turkey, and Ulster University, Northern Ireland). Volunteers who had physical impairments as determined by a standard physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992) or who were classified as highly physically active using the International physical activity questionnaire (IPAQ) (Craig et al., 2003) were excluded from this study. In addition, other exclusion criteria were resting heart rate $\geq$ 100 bpm, clinically significant hypertension ($>$140/90 mm Hg), or BMI $>$ 35 kg·m$^{-2}$. All participants were randomised into two groups which were a 10 s and a 20 s sprint group. The subject characteristics of the participants are shown in Table 3.1. The study
protocol and potential risks from the study were explained to all participants and they signed an informed consent form. The experimental protocol was approved by the University of Stirling School for Sport Ethics Committee (SSREC reference 888), and the relevant ethics committees at the other two Universities.

3.2.2. Experimental Protocol

3.2.2.1. Aerobic capacity

\( \dot{V}O_2 \text{max} \) was determined before the start of training and three days after the last training session using an incremental cycling test to exhaustion (Lode Excalibur Sport ergometer, Groningen, the Netherlands). Respired gas was collected and analysed using a gas analysis system (Oxycon™ mobile device and MasterScreen™ CPX metabolic cart, New Jersey, USA and Quark C-PET, Cosmed, Rome, Italy). The testing protocol consisted of a 2-min warm-up phase at 50 W and the resistance was increased by 1 W every 3 s until volitional exhaustion or the inability to keep the pedal frequency at 60 rpm. The acceptance criteria of \( \dot{V}O_2 \text{max} \) were following those used by Metcalfe et al. (2012), which were the presence of a plateau in \( \dot{V}O_2 \) with increasing intensity, RER > 1.15, heart rate within 10 bpm of age-predicted maximal heart rate, and/or volitional exhaustion. \( \dot{V}O_2 \text{max} \) was determined as the highest value of a 15-breath rolling average of \( \dot{V}O_2 \). Participants were asked to complete a food diary on the pre-testing day and testing day of the pre-training \( \dot{V}O_2 \text{max} \) test. All participants were asked to have a similar diet before and the day of the post-training \( \dot{V}O_2 \text{max} \) test.
Table 3.1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>10-s sprint group</th>
<th>20-s sprint group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=18; M=8, F=10)</td>
<td>(n=18; M=11, F=7)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.9 ± 3.7</td>
<td>21.4 ± 1.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.08</td>
<td>1.73 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.3 ± 18.2</td>
<td>70.3 ± 11.5</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>25.3 ± 5.9</td>
<td>23.8 ± 2.4</td>
</tr>
<tr>
<td>( \dot{V}O_2 \max )</td>
<td>35.4 ± 8.7</td>
<td>39.0 ± 6.9</td>
</tr>
</tbody>
</table>

Data shown are mean±SD

3.2.2.2. Training protocol

The first training session started at least two days after the pre-training \( \dot{V}O_2 \max \) test. The training protocol was three days a week and covered six weeks. Each training session comprised two min of unloaded pedalling as a warm up, two all-out sprints with a breaking force equivalent to 7.5% of body mass separated by a recovery period after the first sprint (unloaded pedalling), and a 4-min cool-down (unloaded pedalling) after the second sprint. The total exercise time was 10 minutes. For the 10-s group, the sprint duration of the first three sessions was 5 s. Then this increased to 7.5 s in sessions 4-6 and 10 s in sessions 7-18. For the 20-s group this was 10-s sprints for the first three sessions, 15 s for sessions 4-6 and 20-s for the remaining sessions (Figure 3.1). All exercise training was performed on a cycle ergometer (Ergomedic 894 E, Vansbro, Sweden). Peak heart rate (Polar RS400, Finland) was recorded during training. RPE was taken by the 15-point Borg scale (Borg, 1970) after sessions 3, 6, 9, 12, 15 and 18. Moreover, the Brunel Mood Scale (BRUMS) (Terry and Lane, 2010) and The Positive and Negative Affect Schedule (PANAS) questionnaire (Watson et al.,
1988) were scored after finished 1st, 4th, 7th and 18th session. A questionnaire on the acceptability of HIT (Boereboom et al., 2016) was completed at the end of final training session.

Figure 3.1 The diagram of REHIT protocol; A = 10-s sprint, B = 20-s sprint

3.2.3. Statistics

Data are shown as mean±SD and analysed using SPSS Version 23 (Chicago, IL, USA). Based on a coefficient of variation of the \(\dot{V}O_2\text{max} \) test protocol of 4%, it was calculated that 14 participants were needed in each group in order to be able to detect a difference in the change in \(\dot{V}O_2\text{max} \) of 5% between 20-s group and 10-s group, with a power of 90% and \( \alpha = 0.05 \). The data have a normal distribution so parametric tests were used. Mixed model ANOVA (time × group)
was used to determine the differences between the two groups for $\dot{V}O_2\text{max}$, maximal power output ($W_{\text{max}}$), RPE. BRUMS and PANAS were analysed by mixed model ANOVA (trial x time x group). The acceptability of HIT was analysed by independent $t$-test. The significance level was set at $p < 0.05$.

### 3.3. Results

$\dot{V}O_2\text{max}$ increased significantly after training (effect of time: $p<0.001$) and the increase in $\dot{V}O_2\text{max}$ was significantly greater following 20-s than 10-s all-out sprints (effect of time x group: $p<0.05$). $\dot{V}O_2\text{max}$ was increased by on average 10% in the 20-s group and by 4% in the 10-s group (Table 3.2). Maximal power output during the maximal exercise test was increased after both training interventions (effect of time: $p<0.001$) with no significant difference between the groups (Table 3.2).

**Table 3.2** Effect of exercise intervention on $\dot{V}O_2\text{max}$ and $W_{\text{max}}$

<table>
<thead>
<tr>
<th></th>
<th>10 s (n = 18)</th>
<th></th>
<th>20 s (n = 17)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>$\dot{V}O_2\text{max}$ (L·min$^{-1}$)</td>
<td>2.58±0.56</td>
<td>2.67±0.61*</td>
<td>2.77±0.74</td>
<td>3.04±0.75*†</td>
</tr>
<tr>
<td>$W_{\text{max}}$ (W)</td>
<td>209±47</td>
<td>230±49*</td>
<td>228±54</td>
<td>259±59*</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, * $p<0.001$ for the effect of time, † $p<0.05$ for the effect of time x group.
Mean RPE scores of both training interventions are shown in Figure 3.2. RPE over 18 training sessions of the 10-s group corresponded to somewhat hard (13.6±0.4) and for the 20-s group corresponded to between somewhat hard and hard (14.5±0.4). There was no significant difference in RPE scores between the groups, which means that the 20-s all-out sprint intervention was not perceived to be significantly harder than the 10-s all-out sprint intervention.

**Figure 3.2** Rating of Perceived Exertion of 10-s group (—) and 20-s group (–) over 18 sessions. Data are shown as mean ± SD.
For the BRUMS, six differences subscales including tension, depression, anger, vigour, fatigue and confusion were analysed. There were significant decreases in the subscales tension \( (p<0.01) \), and depression \( (p<0.05) \) and a significant increase in the vigour subscale \( (p<0.01) \) (Table 3.3). Additionally, the PANAS questionnaire was used to measure changes in positive affect (PA) and negative affect (NA). There was a significant effect of time for PA \( (p<0.05) \) which increased from pre- to post-exercise, but no significant changes were observed for NA (Table 3.4). Finally, there was no significant difference in the acceptability of REHIT between the 10-s and 20-s groups following training (Table 3.5).
Table 3.3 The Brunel Mood Scale (BRUMS)

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-s group (n = 18)</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>18</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Tension</td>
<td>2.2±2.4</td>
<td>2.0±3.3</td>
<td>1.4±2.0</td>
<td>1.4±1.9</td>
<td>1.2±2.1</td>
<td>2.0±3.3</td>
<td>1.3±2.2</td>
<td>1.1±1.6</td>
</tr>
<tr>
<td>Depression</td>
<td>1.6±2.7</td>
<td>1.8±3.9</td>
<td>1.1±1.7</td>
<td>1.2±1.5</td>
<td>1.2±2.6</td>
<td>1.6±3.9</td>
<td>0.9±1.4</td>
<td>1.0±1.6</td>
</tr>
<tr>
<td>Anger</td>
<td>1.4±2.6</td>
<td>2.1±3.5</td>
<td>1.3±1.7</td>
<td>1.2±1.9</td>
<td>1.2±2.3</td>
<td>2.1±3.5</td>
<td>1.3±2.0</td>
<td>1.3±2.1</td>
</tr>
<tr>
<td>Vigour</td>
<td>8.2±3.2</td>
<td>7.5±3.5</td>
<td>7.6±3.3</td>
<td>7.9±3.7</td>
<td>8.4±3.7</td>
<td>8.1±3.5</td>
<td>7.9±3.2</td>
<td>9.3±2.8</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3.7±2.9</td>
<td>4.1±4.1</td>
<td>3.6±3.1</td>
<td>3.2±3.0</td>
<td>2.9±2.9</td>
<td>4.8±4.1</td>
<td>4.2±3.0</td>
<td>3.4±3.2</td>
</tr>
<tr>
<td>Confusion</td>
<td>1.1±1.6</td>
<td>1.9±2.5</td>
<td>1.1±1.5</td>
<td>0.6±1.0</td>
<td>1.1±2.1</td>
<td>1.9±2.6</td>
<td>1.1±1.6</td>
<td>0.6±1.3</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, ** p<0.01 for the effect of time

Table 3.4 PANAS

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-s group (n = 18)</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>18</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Negative affect</td>
<td>15.1±4.4</td>
<td>14.8±3.9</td>
<td>15.0±4.8</td>
<td>15.4±4.6</td>
<td>15.5±5.5</td>
<td>15.1±5.0</td>
<td>15.1±4.8</td>
<td>15.7±4.8</td>
</tr>
<tr>
<td>Positive affect</td>
<td>24.4±7.0</td>
<td>22.3±5.7</td>
<td>23.2±5.7</td>
<td>24.9±6.1</td>
<td>25.8±7.3</td>
<td>24.6±6.8</td>
<td>24.5±6.4</td>
<td>26.6±7.7</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, * p<0.05 for the effect of time
Table 3.5 Acceptability of HIT

<table>
<thead>
<tr>
<th></th>
<th>10-s group</th>
<th>20-s group</th>
</tr>
</thead>
<tbody>
<tr>
<td>I enjoyed HIT</td>
<td>2.0±0.8</td>
<td>2.1±0.6</td>
</tr>
<tr>
<td>HIT was a time burden</td>
<td>4.0±0.8</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td>I would recommend HIT to others</td>
<td>2.2±0.8</td>
<td>2.0±0.8</td>
</tr>
<tr>
<td>HIT was more demanding than expected</td>
<td>3.2±1.0</td>
<td>2.8±1.2</td>
</tr>
<tr>
<td>I would do HIT again</td>
<td>2.4±1.0</td>
<td>2.2±0.9</td>
</tr>
<tr>
<td>The travelling involved with HIT interfered with my life</td>
<td>3.8±0.9</td>
<td>3.6±1.2</td>
</tr>
<tr>
<td>The physical strain interfered with my life</td>
<td>4.1±1.1</td>
<td>3.8±1.2</td>
</tr>
<tr>
<td>I believe my fitness has improved</td>
<td>2.1±1.6</td>
<td>1.9±0.9</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, 1 = strongly agree; 5 = strongly disagree

On average the participants agreed that they enjoyed REHIT, would recommend REHIT to others, would do REHIT again and believe their fitness has improved following REHIT. Moreover, they disagreed that HIT was time consuming, or that the travelling involved with HIT and the physical strain caused by HIT interfered with their life.

3.4. Discussion

The purpose of this chapter was to examine the effect of reducing the sprint duration from 20 s to 10 s in the REHIT protocol on the changes in $\dot{V}O_2$max and mood status in inactive young adults. It was found that reducing the sprint time attenuated the improvement of $\dot{V}O_2$max. Importantly, $\dot{V}O_2$max was improved more in the 20-s group than in the 10-s group (average percentage improvement was 10% and 4% in the 20-s group and 10-s group, respectively), but there were no significant differences in the perceived exertion or mood status between the two groups. Moreover, negative affective responses were not found following
either REHIT protocol, and neither REHIT protocol deceased mood status. This suggests that two repeated 20-s all-out sprints in the REHIT protocol could be an effective and tolerable SIT protocol for improving $\dot{V}O_2\text{max}$. Overall, the findings from this chapter highlight the effect of REHIT on the key health parameter of $V_2\text{max}$. It is an important finding because $V_2\text{max}$ is a predictor of cardiovascular health and mortality (Booth et al., 2012; Blair et al., 1996; Laukkanen et al., 2004; Myers et al., 2002). Increases in $V_2\text{max}$ can reduce all causes mortality and cardiovascular mortality (Lee et al., 2011).

The present outcomes contrast with other studies, which reduced the sprint duration from the conventional SIT protocol without attenuating changes in $V_2\text{max}$ (Hazell et al., 2010; Zelt et al., 2014). $V_2\text{max}$ was improved by an average of 8% and 9% following 15-s sprints (Zelt et al., 2014) and 10-s sprints (Hazell et al., 2010), respectively, while $V_2\text{max}$ increases from 7% to 12% following the conventional SIT protocol (30-s sprints) (Rakobowchuk et al., 2008; Burgomaster et al., 2008; Bailey et al., 2009; Trilk et al., 2011; Shepherd et al., 2013). Nevertheless, the present findings showed that decreases in sprint duration in the REHIT protocol from 20 s to 10 s did attenuate the improvement of $V_2\text{max}$. Thus, combined with the findings of Chapter 2, the most effective REHIT protocol for improving $V_2\text{max}$ seems to involve two 20-s sprints.

Even though the sprint time in the 20-s group is longer than that in the 10-s group, it did not mean that the 20-s protocol was perceived as harder or having a more negative affect than the 10-s protocol. The RPE over 18 sessions corresponded to somewhat hard in the 10-s group and between somewhat hard and hard in the 20-s group, but there was no significant difference between the two groups. It might explain that the perceived exertion following the 20-s protocol was not
significantly higher than the 10-s protocol. Moreover, mood status was not significantly different between both groups. In addition, three subscales of BRUMS were changed after exercise sessions; negative mood (tension and depression) was decreased, while positive mood (vigour) was increased. The present findings agree with those of Freese et al. (2014), who demonstrated that mood status was improved following six weeks of conventional SIT (Freese et al., 2014). Moreover, the positive affect (PA) from the PANAS questionnaire was increased following the exercise sessions, but the negative affect (NA) did not change. These findings are different from those of Saanijoki et al. (2015) who demonstrated that PA was decreased and NA was increased in a SIT group compared to MICT group (Saanijoki et al., 2015). It could be explained by the difference of SIT protocol between the two studies. The SIT protocol by Saanijoki et al. (2015) comprised 4–6x 30-s all-out sprints while the protocol that was used in this study was 2x20-s or 2x10-s all-out sprints. Taken together, performing more sprints or a longer sprint duration might affect PA and NA. Interestingly, some researchers commented that a high level of motivation is needed when performing SIT, and it might not suit sedentary people (Hardcastle et al., 2014; Gillen and Gibala, 2014). The outcomes in this study suggest that REHIT is tolerable and performing the sprints did not affect mood status.

Interestingly, on average the participants in the present study reported that the REHIT protocols were enjoyable and time efficient. In addition, they would also recommend REHIT to others and believed their fitness improved following REHIT. The enjoyment and time efficiency are mentioned as the main factors for exercise adherence (Kong et al., 2016). Taken together, it could be suggested that REHIT may be a manageable exercise protocol and may be an alternative
exercise protocol (apart from the current exercise recommendations) for people who do not have enough time for doing exercise.

Limitations for this study need to be acknowledged. First, physical activity before training and during training were not confirmed by objective measurement (e.g. accelerometer). However, all participants were asked to maintain their physical activity throughout the six weeks of the training sessions. Second, the changes in mood and affect were assessed at the end of the training session, not directly after the sprints. More negative affective responses might be found immediately after finishing the sprints.

3.5. Conclusion

The study set out to determine whether the reduction of sprint duration from 20 s to 10 s in the REHIT protocol affects the improvement of $\dot{V}O_2\text{max}$ and psychological responses. The results of this investigation indicate that changes in $\dot{V}O_2\text{max}$ were attenuated when reducing the sprint duration. In addition, there was no significant difference in mood status and negative affective response between the two REHIT protocols. Therefore, it could be concluded that two 20-s all-out sprints in REHIT comprise an effective protocol for improving $\dot{V}O_2\text{max}$, and this is not associated with negative perceptual responses.
CHAPTER 4

CHAPTER 4: THE EFFECT OF THE NUMBER OF TRAINING SESSIONS PER WEEK IN REDUCED-EXERTION HIGH-INTENSITY INTERVAL TRAINING ON CHANGES IN MAXIMAL AEROBIC CAPACITY
4.1. Introduction

Along with Chapter 3, other previous studies have confirmed that $\dot{V}O_2\text{max}$ increased on average by approximately 11% following two repeated 20-s all-out sprints, three times a week for six weeks (Metcalfe et al., 2012; Metcalfe et al., 2016). However, there are various training parameters that together determine the likelihood of people doing REHIT, such as the number of sprints, sprint duration, recovery duration, or training frequency (number of training sessions per week). In Chapter 2, we were interested in whether doing fewer sprints is enough or whether doing more sprints gives greater improvement in $\dot{V}O_2\text{max}$. In Chapter 3, we were interested in whether shorter sprints are less effective and whether shorter sprints are associated with less negative responses, which may improve exercise adherence. The findings from both studies suggest that performing fewer or shorter sprints makes the protocol less effective and that the affective response did not improve when performing shorter sprints.

As stated previously, REHIT protocols can be made shorter and/or easier by reducing sprint duration, the number of sprints, or the number of training sessions. Sprint duration and the number of sprints have been studied, but there is very little information on training frequency. Most SIT/HiIT studies use three sessions per week (Gillen et al., 2016; Burgomaster et al., 2008; Gibala et al., 2006; Cochran et al., 2014; Little et al., 2011a; Little et al., 2010b). However, there is no justification provided for why three sessions of HIIT/SIT need to be performed. Adamson et al. (2014) examined the effect of two times a week of SIT (10 x 6-s ‘all-out’ sprints), and reported that $\dot{V}O_2\text{max}$ improved by 8% following eight weeks of training (Adamson et al., 2014). Additionally, Ijichi et al. (2015) demonstrated that performing SIT (3 x 30-s ‘all-out’ sprints) five times a week
increased VO\textsubscript{2}max by an average of 8% (Ijichi et al., 2015). It may be concluded that VO\textsubscript{2}max could be improved by performing SIT less or more than three sessions per week.

To date, the minimum frequency for improving VO\textsubscript{2}max and what frequency will result in the greatest improvement following REHIT are both unclear. There have been no studies that have directly compared different training frequencies in the REHIT protocol. It could be worthwhile to determine whether fewer sessions (two times per week) are equally effective and would enhance exercise adherence or whether more sessions (four times per week) would provide greater efficacy. Moreover, affective response is one of the psychological factors associated to exercise adherence (Rhodes and Kates, 2015). A positive affective response to exercise could be a predictor for better exercise adherence (Williams et al., 2008). It would be useful to examine whether more sessions would increase negative affect following REHIT or less session would increase positive affective response. It may provide evidence to support the future exercise adoption and adherence to REHIT. Therefore, the objective of this study was to determine the effect of the number of sessions, which were two, three, and four sessions a week, on the changes in VO\textsubscript{2}max and the affect responses.

4.2. Methods

4.2.1. Participants

Thirty-two healthy inactive volunteers, aged between 18-40 years were recruited to the present study from three centres (University of Stirling (n = 11), Swansea University (n = 10) and University of Worcester (n = 11)). The exclusion criteria were participants had contraindications to exercise as clarified by a physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992), classified as
highly physically active using the International physical activity questionnaire (IPAQ) (Craig et al., 2003), BMI > 35 kg·m⁻², resting heart rate ≥ 100 bpm, resting blood pressure >140/90 mm Hg, diagnosis of hypertension, cardiovascular disease, type 1 or type 2 diabetes or other chronic diseases. All participants were randomly assigned to three groups which trained two times (2X), three times (3X) or four times (4X) per week. Two participants dropped out from the study because of unrelated health issues. The participant characteristics of the remaining participants are shown in Table 4.1. The study protocol and potential risks from the study were explained to participants both verbally and in writing before providing informed consent. Ethical approval was obtained from the NHS, Invasive or Clinical Research (NICR) Committee, University of Stirling (NICR (17/18) Paper No.16) and from the ethics committees at the other two Universities.

4.2.2. Experimental Protocol

4.2.2.1. Aerobic capacity

\( \dot{V}O_2 \text{max} \) was determined before the first training session and three days after the last training session using a maximal incremental cycling test to volitional exhaustion. The protocol started with a 5-min warm-up at 50 W. Then the intensity was increased by 1 W every 3 s (Lode Excalibur Sport ergometer, Groningen, the Netherlands and VIAsprint™ 150P/200P Bicycle, Illinois, USA). Online gas analysis systems (Oxycon™ mobile device and MasterScreen™ CPX metabolic cart, New Jersey, USA; METALYZER® 3B, Leipzig, Germany; Vyntus™ CPX Metabolic Cart, Illinois, USA) were used in order to determine oxygen uptake (\( \dot{VO}_2 \)). \( \dot{VO}_2 \text{max} \) was determined as the highest value of a 15-breath rolling average of \( \dot{VO}_2 \). The acceptance criteria of \( \dot{VO}_2 \text{max} \) were to meet
two or more of the following criteria; volitional exhaustion or inability to keep the pedal frequency at 60 rpm, the presence of a plateau in \( \dot{V}O_2 \) despite increasing intensity, RER > 1.15, heart rate within 10 bpm of age-predicted maximal heart rate. The participants completed a food diary on the pre-testing day and testing day of the pre-training \( \dot{V}O_2 \)max test and they were asked to have a similar diet before and the day of the post-training \( \dot{V}O_2 \)max test. Maximal heart rate (Polar RS400, Finland) was recorded during the test.

**Table 4.1** Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>2X</th>
<th>3X</th>
<th>4X</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=11, M=8, F=3)</td>
<td>(n=10, M=5, F=5)</td>
<td>(n=9, M=7, F=2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.6 ± 6.5</td>
<td>26.3 ± 5.8</td>
<td>24.6 ± 4.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 ± 0.11</td>
<td>1.69 ± 0.07</td>
<td>1.70 ± 0.10</td>
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<tr>
<td>Weight (kg)</td>
<td>83.5 ± 24.1</td>
<td>68.5 ± 15.4</td>
<td>78.6 ± 8.2</td>
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<tr>
<td>BMI (kg·m(^{-2}))</td>
<td>26.7 ± 5.0</td>
<td>24.0 ± 5.2</td>
<td>27.1 ± 2.8</td>
</tr>
<tr>
<td>( \dot{V}O_2 )max (ml·kg(^{-1})·min(^{-1}))</td>
<td>35.1 ± 5.8</td>
<td>36.8 ± 4.1</td>
<td>35.4 ± 10.3</td>
</tr>
</tbody>
</table>

Data shown are mean±SD

**4.2.2.2. Training protocol**

At least two days after the pre-training \( \dot{V}O_2 \)max test, the first training session started. The participants performed two, three or four training sessions per week for six weeks. Training sessions involved 10 minutes on a cycle ergometer (Ergomedic 894 E and 874E, Vansbro, Sweden) which consisted of two minutes of unloaded pedalling as a warm up, two ‘all-out’ sprints against a resistance of 7.5% of body mass interspersed by unloaded pedalling recovery period after the first sprint and a four min cool-down (unloaded pedalling) after the second sprint. For the first week, the sprint duration was 10 s. Then this increased to 15 s in the
second week and 20 s in the remaining weeks. Peak heart rate (Polar RS400, Finland) was recorded during the sprint. Ratings of perceived exertion (RPE) (Borg, 1970) and the feeling scale (Hardy and Rejeski, 1989) were determined at the end of each minute during the first session, the first 20-s sprint session and the last session. Moreover, RPE and the feeling scale were also determined for the whole session in those three sessions. Additionally, the acceptability of HIT questionnaire (Boereboom et al., 2016) was completed at the end of the final session. The data were excluded from data analysis when participants missed more than three training sessions, two training sessions in the final week and last training session. In addition, there were some misunderstandings with the timings for measurement of affect and RPE at the University of Worcester. Therefore, these data were excluded from data analysis.

4.2.3. Statistics

Data are shown as mean±SD and analysed using SPSS Version 23 (Chicago, IL, USA). Fourteen participants were required in each group in order to detect a difference in the change in $\dot{V}O_2$max of 5% between the three groups, with a power of 90%, $\alpha=0.05$ and a coefficient of variation for the VO2max test protocol = 4%. The data have a normal distribution so parametric tests were used. Mixed model ANOVA (time $\times$ intervention) was used to determine the differences between the groups for the changes in $\dot{V}O_2$max and maximal power output (Wmax). Affect responses and RPE were analysed by mixed model ANOVA (time $\times$ intervention $\times$ trial). One-way ANOVA was used to examine the difference of data characteristics and the acceptability of HIT.
4.3. Results

$\dot{V}O_2\text{max}$ improved following all three training protocols (effect of time: $p<0.001$), with no significant difference among the three groups (effect of time $\times$ group: $p>0.05$) (Figure 4.1). When considering percentage change, $\dot{V}O_2\text{max}$ increased 13%, 9% and 7% in the 2X, 3X and 4X groups respectively. Maximal power output (Wmax) during the maximal exercise test was increased after training in the three groups (effect of time: $p<0.001$), and there was no time $\times$ group interaction effect ($p>0.05$) (Figure 4.2)

![Figure 4.1 VO2max following REHIT among three groups. * $p<0.001$ for effect of time.](image-url)
For the peak RPE and the average RPE at three time points, there was a main effect of time ($p<0.05$) but no effect of time x group: ($p>0.05$) (Table 4.2 and Figure 4.3). RPE was increased after both sprints and RPE at the second sprint was higher than the first sprint (the main effect of time; $p<0.05$). However, RPE returned back to baseline during the recovery period (Figure 4.3).

**Table 4.2** The peak RPE at three time points

<table>
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<tr>
<td>The first session</td>
<td>14.1±2.5</td>
<td>14.1±1.3</td>
<td>14.3±1.6</td>
</tr>
<tr>
<td>The first 20-s sprint session</td>
<td>15.4±3.5</td>
<td>16.3±1.0</td>
<td>15.8±1.6</td>
</tr>
<tr>
<td>The last session</td>
<td>15.0±2.1*</td>
<td>14.3±1.5*</td>
<td>15.2±3.3*</td>
</tr>
</tbody>
</table>

Data shown are mean±SD. * $p<0.05$ for effect of time
Figure 4.3 The average RPE from all training sessions among three groups. Data showed as mean ± SD. * $p<0.05$ for the effect of time, # $p<0.001$ for the effect of time (compared the first (2nd minute) with the second (6th minute) sprint)

For the affect responses during REHIT, there was no significant difference in the lowest affect, the end affect and the changes in affect during training between three groups (the effect of time x group; $p>0.05$) (Table 4.3). Moreover, affect was decreased after finishing both sprints (Figure 4.4).
### Table 4.3 The affect responses during REHIT

<table>
<thead>
<tr>
<th></th>
<th>2X</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>The lowest affect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- The first session</td>
<td>1.0±1.7</td>
<td>1.3±1.8</td>
<td>2.3±1.6</td>
</tr>
<tr>
<td>- The first 20-s sprint session</td>
<td>-0.7±2.6</td>
<td>-2.0±1.3</td>
<td>-0.7±2.5</td>
</tr>
<tr>
<td>- The last session</td>
<td>0.3±2.4</td>
<td>-1.0±2.4</td>
<td>1.3±3.5</td>
</tr>
<tr>
<td><strong>The end affect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- The first session</td>
<td>4.0±1.3</td>
<td>2.5±1.6</td>
<td>3.3±1.4</td>
</tr>
<tr>
<td>- The first 20-s sprint session</td>
<td>1.7±2.7</td>
<td>1.7±1.5</td>
<td>2.1±1.3</td>
</tr>
<tr>
<td>- The last session</td>
<td>1.3±2.3</td>
<td>2.7±1.9</td>
<td>2.3±2.8</td>
</tr>
<tr>
<td><strong>The change in affect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- The first session</td>
<td>-2.6±2.1</td>
<td>-1.5±2.1</td>
<td>-2.0±1.9</td>
</tr>
<tr>
<td>- The first 20-s sprint session</td>
<td>-3.6±2.1</td>
<td>-4.5±1.9</td>
<td>-4.3±2.6</td>
</tr>
<tr>
<td>- The last session</td>
<td>-2.5±1.5</td>
<td>-2.7±2.2</td>
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</tr>
</tbody>
</table>

Data shown are mean±SD.

**Figure 4.4** The average affect responses during REHIT among three groups. Data showed as mean ± SD.
Finally, there was a significant difference in only one item of acceptability of HIT which was item 7 (The physical strain interfered with my life) between 2X and 4X ($p<0.05$) and 3X and 4X ($p<0.05$). However, participants in all groups disagreed that HIT related to physical strain and travelling involved with HIT interfered with their life. Moreover, the participants agreed that they enjoyed HIT, they would recommend HIT to others and would do HIT again and they believed their fitness improved (Figure 4.5).

![Figure 4.5 Acceptability of HIT. Data presented as mean ± SD](image)

### 4.4. Discussion

The aims of this study were to examine the changes in $\dot{V}O_2\text{max}$ and the affective responses between three training frequencies of REHIT, which were two, three, and four times a week. The results demonstrated that the change in $\dot{V}O_2\text{max}$ following training was not significantly different between the three training frequencies. Moreover, the lowest, the end, and the change in affective response
were similar between the three training frequencies. Therefore, if the aim of training is to obtain the greatest improvements in $\dot{V}O_2$\text{max}, then increasing the training frequency is not a successful strategy. Moreover, decreasing the training frequency to as little as two sessions per week does not attenuate the improvements in $\dot{V}O_2$\text{max}. This is an important finding, as smaller training volumes may be more acceptable to sedentary populations. Less motivation may be needed to perform two sessions per week compared to three or more sessions. Therefore, as a public health intervention, REHIT with a reduced training frequency might be more successful.

$\dot{V}O_2$\text{max} improved by 13%, 9%, and 7% in the 2X, 3X, and 4X groups, respectively. These findings support evidence from Chapter 3 and previous studies, which demonstrated that $\dot{V}O_2$\text{max} improved by an average 11% following three times a week of REHIT (Metcalfe et al., 2012; Metcalfe et al., 2016). Moreover, the outcomes from the present study seem to be consistent with other research that found that $\dot{V}O_2$\text{max} could be increased following SIT even when training only twice a week (Yamagishi and Babraj, 2017) or more than three times a week (Ijichi et al., 2015). Although most SIT studies use a frequency of three time per week, there is no evidence to justify why performing SIT three times a week would be the most effective protocol to improve $\dot{V}O_2$\text{max}. When considering moderate-intensity continuous training (MICT), the general recommendation is to perform five or more 30-min sessions per week. Additionally, Wenger and Bell (1986) demonstrated that doing endurance exercise more often is associated with greater improvement in $\dot{V}O_2$\text{max} (Wenger and Bell, 1986). This finding contrasts with REHIT: we demonstrate that the improvement in $\dot{V}O_2$\text{max} is not increased when performing more training times.
per week. It can be suggested that the optimal REHIT protocol for improving $\dot{V}O_2\text{max}$ consists of 2x20-s all-out sprints within 10 minute of total time and with a training frequency of at least two times a week. Compared with recent exercise recommendations, which suggest that adults should engage in at least 150 minutes per week of aerobic exercise at a moderate intensity (WHO, 2010), REHIT is much shorter (20 min per week) but provides worthwhile cardiovascular adaptations. It can be proposed that REHIT could be an alternative exercise protocol for addressing one of the risk factors from being inactive.

For the psychological responses to REHIT, the peak RPE at the first session, the first 20-s session, and the last session did not differ between three groups. The average peak RPE in 2X, 3X, and 4X group were 14.9, 14.9, and 15.1, respectively. It represents ‘somewhat hard to hard’. The affect responses following REHIT were changed in the same way as the results for RPE. The lowest, the end, and the changes of affect during REHIT were not significantly different among the three groups. Importantly, those three aspects of affects (the lowest, the end, and the changes) are the most significant affective valences during exercise (Decker and Ekkekakis, 2017) and are associated with exercise adherence in the future (Williams et al., 2012). It could be concluded that doing more sessions per week did not bring more negative affect than doing less sessions per week. Despite the negative valence that has been found in all groups following the first 20-s sprint session, the affect was less negative or became more positive at the final session. This finding agrees with the results of other studies, such as that by Saanijoki et al. (2015, 2018) who demonstrated that there was a training adaptation of the affective valence during SIT (4-6x30-s ‘all-out’ sprint). The affect at the initial training sessions was low, then it was
improved over two weeks of the training duration (Saanijoki et al., 2018; Saanijoki et al., 2015). Taken together, these findings support the ‘lead-in’ period that is used in the REHIT protocol, which is the progressive increase in the sprint duration from 10 s to 20 s within the first three weeks of training. It may improve affective responses throughout the training period.

There was a significant difference in one item of the acceptability questionnaire, which showed that participants in the 4X group agreed that the physical strain interfered with their life more than the 3X and 2X groups. Nevertheless, all participants enjoyed and would do REHIT again, suggesting that performing REHIT with fewer training days per week (at little as two days a week) may lower the physical strain from the training but still be enjoyable and have an effect on health.

A number of limitations need to be considered. First, all training sessions were laboratory-based. Thus, the exercise adherence might not represent the exercise behaviour in real life. The exercise adherence in the present study over six weeks of training in the 2X, 3X, and 4X groups, was 98%, 97%, and 96%, respectively, but it remains unknown whether people will engage in REHIT for longer durations or in real-life conditions. Further studies are required to confirm and validate the long-term adherence to REHIT, preferably in non-laboratory-based settings, such as in schools, work offices, or gyms. Second, an objective measurement for the physical activity was not used for confirming the physical activity levels before and during training. However, there was no significant difference between the three groups, and all participants were asked to maintain their physical activity throughout the six weeks of training.
4.5. Conclusion

The present study examined the changes of \( \dot{V}O_2 \text{max} \) and affect responses between three different frequencies of the REHIT protocol (two, three, and four times a week). The outcomes indicated that there was no significant difference in \( \dot{V}O_2 \text{max} \) and the affect responses between the three groups, suggesting that \( \dot{V}O_2 \text{max} \) can be improved following the performance of REHIT for at least two training sessions a week. Interestingly, performing REHIT for more training days per week did not gain an improvement in \( \dot{V}O_2 \text{max} \). Moreover, training more or less than three times a week did not change the psychological responses to REHIT. Overall, to date the most time-efficient REHIT protocol that improves \( \dot{V}O_2 \text{max} \) consists of 2x20-s all-out sprints with a training frequency of at least two times a week.
CHAPTER 5: PSYCHOLOGICAL RESPONSES TO MODERATE-INTENSITY CONTINUOUS EXERCISE TRAINING (MICT), HIGH-INTENSITY INTERVAL TRAINING (HIIT) AND REDUCED-EXERTION HIGH-INTENSITY INTERVAL TRAINING (REHIT)
5.1. Introduction

Over the past decade, an increasing number of studies support that sprint interval training (SIT) improves a range of health markers, including \( \dot{V}O_{2\text{max}} \) (Rakobowchuk et al., 2008; Bailey et al., 2009; Astorino et al., 2012; Cochran et al., 2014; Shepherd et al., 2013; Nalcakan, 2014; Whyte et al., 2010), insulin sensitivity (Babraj et al., 2009; Whyte et al., 2010; Gillen et al., 2016; Gillen et al., 2014), blood pressure (Whyte et al., 2010; Cocks et al., 2013) and body composition (Whyte et al., 2010; Boer et al., 2014). However, SIT has been criticised in that it might not be adopted by sedentary people because of the high levels of motivation needed and the high level of fatigue generated (Hardcastle et al., 2014). Consequently, this might lead to increased negative affective responses and might be associated with decreased exercise adoption and adherence (Hardcastle et al., 2014; Biddle and Batterham, 2015). Conversely, an exercise protocol that is associated with positive affective responses would tend to be the one adopted as a regular exercise. Kwan and Bryan (2010a) demonstrated that positive affective responses when performing moderate intensity exercise (at 65% \( \dot{V}O_{2\text{max}} \)) were related to long-term exercise engagement. It could be suggested that the affective response is a predictor of exercise behaviour (Kwan and Bryan, 2010a). According to the dual-mode theory, negative affective responses are increased when performing high-intensity exercise (Ekkekakis et al., 2011; Ekkekakis et al., 2010; Ekkekakis et al., 2008). Therefore, sedentary individuals may feel unpleasant when performing SIT. However, the studies used to back up this theory were looking at continuous high-intensity exercise (above the ventilatory threshold), which is different in motivation, physiological and psychological responses when compared to SIT.
protocols (Biddle and Batterham, 2015). Thus, it cannot simply be assumed that SIT will have the same negative affective responses as continuous high-intensity exercise.

Jung et al. (2014) investigated the differences in affective responses between three different exercise protocols which were performed on the cycle ergometer: 1) continuous moderate-intensity exercise (CMI) (at ~40% Wpeak for 40 minutes); 2) continuous vigorous-intensity exercise (CVI) (at ~80% Wpeak for 20 minutes); 3) HIIT (one minutes cycling at ~100% Wpeak and one min interval at ~20% Wpeak for 20 minutes). The results demonstrate that the affective responses were significantly lower in HIIT and CVI compared to CMI but there was no significant difference between HIIT and CVI. Regardless of this, participants, who were inactive individuals, enjoyed and preferred HIIT more than CVI and CMI (Jung et al., 2014). When considering the affective responses following those exercise protocols, it could be concluded that the affective responses decreased significantly when the duration of exercise was longer or when the individuals were doing more sprints. This conclusion is supported by Frazao et al. (2016) who investigated the affective responses following HIIT. The affective responses declined both in active and inactive participants when performing more sprints. Interestingly, in inactive participants, affective response became negative at the 5th sprint and kept going down until the last sprint (Frazao et al., 2016). Moreover, Townsend et al. (2017) compared the affective responses between three SIT protocols: 1) 4x30-s sprints with 4 minutes rest; 2) 8x15-s sprints with 2 minutes rest; 3) 24x5-s sprints with 40 seconds rest. The results showed that more negative affective responses were found when performing more sprints in all three protocols. Moreover, the negative affective
response dropped more significantly in the 4x30-s group than the other two groups. (Townsend et al., 2017). In addition, all participants reported that the shortest sprint protocol (24x5-s sprints) was more preferable and enjoyable than the longer protocols (Townsend et al., 2017). It could be hypothesised that by doing shorter and fewer sprints, the negative affective response might be less than doing an increased number of longer sprints. Therefore, it might be assumed that the affective responses when performing shorter and fewer sprints could be similar to or favourable compared to moderate-intensity continuous exercise.

A recent study by Stork et al. (2018) compared a modified from the REHIT protocol (3x20-s sprints) to HIIT and traditional moderate-intensity continuous exercise training (MICT). The affective responses were significantly lower in HIIT and REHIT compared to MICT but there was no significant difference between HIIT and REHIT (Stork et al., 2018), suggesting that inactive participants felt more unpleasant when performing REHIT compared with MICT. However, the REHIT protocol used in this study has one more sprint added compared to the original REHIT protocol. As performing more sprints is associated with a decrease in affect, it is possible that the original REHIT protocol is associated with less negative affective responses.

Moreover, other main psychological factors affecting exercise behaviour include exercise self-efficacy and exercise enjoyment (King et al., 1992; Sherwood and Jeffery, 2000). Self-efficacy is associated with both exercise adoption and long-term adherence (McAuley, 1992; McAuley and Blissmer, 2000; Oman and King, 1998; Sallis et al., 1986). High self-efficacy is related to more exercise adherence (Sherwood and Jeffery, 2000). In addition, exercise enjoyment is a determinant of exercise adoption and adherence (King et al., 1992; Sherwood and Jeffery,
The enjoyment after exercise sessions could be a predictor of future exercise participation (Castro et al., 1999). Importantly, long-term exercise adherence could be influenced by increasing in exercise enjoyment (Hagberg et al., 2009). The effect of REHIT on psychological responses has been examined in Chapter 4. The results revealed that there was no negative affective response following REHIT. However, one of the study limitations is that the psychological responses were taken at post-exercise only. It could not detect how the affective response changed during exercise (in-task affect). According to Kwan and Bryan (2010), in-task positive affect could be a better predictor for exercise adherence than post-task positive affect (Kwan and Bryan, 2010a). Therefore, it would be useful for determining in-task affective responses to REHIT. Moreover, it is not known whether the affective responses and other psychological factors (self-efficacy, RPE and the enjoyment scale) in REHIT are similar, lower or higher when compared to other effective exercise protocols. Therefore, the affective responses and other psychological responses (self-efficacy, RPE and the enjoyment scale) should be determined and compared to the current exercise recommendation (MICT) and another type of practical high-intensity interval training (HIIT). The results will confirm whether REHIT is a feasible, time-efficient exercise which can be taken as an alternative exercise by inactive people. The objective of the present study was to compare the affective responses, RPE, self-efficacy and preference between three exercise protocols: REHIT, MICT and HIIT.
5.2. Methods

5.2.1. Participants

Twelve healthy inactive volunteers, aged between 18-40 years, were recruited to the present study (8 men and 3 women). The exclusion criteria included BMI > 35 kg·m\(^{-2}\), resting heart rate ≥ 100 bpm, hypertension (>140/90 mm Hg), chronic diseases, contraindications to exercise as classified by a physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992), on anti-depressants or other mood-altering medications. Three participants dropped out from the study. Two participants did not complete the familiarisation sessions due to feeling sick or dizziness and one participant stated that she had no time. Eight participants completed the study (seven men and one woman) and were included in the data analysis. The participant’s characteristics are shown in Table 5.1. The study protocol and potential risks of participating in the study were given to participants both verbally and in writing before informed consent was taken. Ethical approval was obtained from the NHS, Invasive or Clinical Research (NICR) Committee, University of Stirling (NICR (17/18) Paper No.17).

Table 5.1 Subject characteristics

<table>
<thead>
<tr>
<th>Subject characteristic</th>
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<tbody>
<tr>
<td>Age (years)</td>
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<td>Height (m)</td>
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<td>Weight (kg)</td>
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<td>BMI (kg·m(^{-2}))</td>
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<tr>
<td>(\dot{V}O_2) (_{\text{max}}) (ml·kg(^{-1})·min(^{-1}))</td>
<td>38.5 ± 9.7</td>
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</tbody>
</table>

Data shown are mean±SD
5.2.2. Experimental Protocol

Participants visited the laboratory eight times. The first visit was for the baseline fitness test. The next three visits were the familiarisation sessions and the last four sessions involved the testing of psychological responses to exercise. The full details of the experimental protocol are described below.

5.2.2.1. Baseline fitness test

Participants performed an incremental exercise test to exhaustion in order to determine maximal power output (Wmax) for individualising training intensities. The protocol began with a 2-min warm up at 50 W. Then the intensity increased by 1 W every 3 seconds (Lode Excalibur Sport ergometer, Groningen, the Netherlands) until volitional exhaustion. An online gas analysis system (Oxycon™ mobile device and MasterScreen™ CPX metabolic cart, New Jersey, USA) was used for determining oxygen uptake (\( \dot{V}O_2 \)). \( \dot{V}O_2\text{max} \) was determined as the highest value of a 15-breath rolling average of \( \dot{V}O_2 \). The acceptance criteria of \( \dot{V}O_2\text{max} \) were to meet two or more of the following criteria: 1) the presence of a plateau in \( \dot{V}O_2 \) despite increasing intensity, 2) volitional exhaustion, 3) inability to keep the pedal frequency more than 60 rpm, 4) heart rate within 10 bpm of age-predicted maximal heart rate, and 5) RER > 1.15. The participants were asked to refrain from any strenuous exercise, and any alcohol or caffeine-containing products 24 hours before testing. Heart rate (Polar RS400, Finland) was recorded throughout the test.

5.2.2.2. Familiarisation sessions

After the fitness test, three familiarisation sessions were performed with two days’ rest between each session. The familiarisation sessions consisted of part of each of the three exercise protocols, which were moderate-intensity continuous
exercise (MICT), high-intensity interval training (HIIT) and reduced-exertion high-intensity interval training (REHIT). The participants were asked to perform progressively longer exercise and/or increasing numbers/duration of sprints. The first familiarisation session was five min of cycling at 40% of Wmax for MICT, 1 min sprint at 100% Wmax for HIIT, two minutes of cycling at 25 W following by 10-s all-out sprint and three minutes of cycling at 25 W for REHIT. The second familiarisation session started with one minute of cycling at 25 W following by 3x1 minute sprint at 100% Wmax interspersed by one min cycling at 25 W, then performed REHIT protocol with 15-s all-out sprint and 10 minutes of MICT. The last familiarisation session was 20-s single sprint of REHIT, 10 minutes of MICT and 5x1 minute sprint of HIIT protocol, ending with two min of cycling at 25 W. The diagram of familiarisation sessions is shown in Figure 5.1.

**Figure 5.1** The diagram of familiarisation sessions. A. session 1, B. session 2 and C. session 3.

### 5.2.2.3. Exercise protocols

One week after the last familiarisation session, subjects started a randomised and counter-balanced cross-over study comparing the three protocols: MICT comprised 30 min of cycling at 40% of Wmax, HIIT involved 10x1 min cycle sprints at 100% of Wmax separated by 1 minute cycle at 25 W within a 22 minutes total time, and REHIT consisted of 2x20 s all-out sprints separated by 25 W within
a 10 minutes total time. Participants were asked to rest at least two days before starting other exercise sessions and they were asked to do all exercise sessions at the same time of the day. Heart rate (Polar RS400, Finland) was recorded throughout the exercise session and until 30 minutes after finished the exercise session.

5.2.2.4. Psychological assessments

Participants were assessed for affect responses using the one-item feeling scale (FS) (Hardy and Rejeski, 1989) and their rating of perceived exertion (RPE) using the 6-20 Borg scale (Borg, 1970) before exercise, every two min during exercise, and 10 minutes and 30 minutes after exercise. Moreover, exercise task self-efficacy questionnaire (McAuley and Mihalko, 1998) and enjoyment using Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo, 1991) were evaluated 30 minutes post-exercise. An overview of the study protocol is shown in Figure 5.2.

![Study protocol](image)

**Figure 5.2 Study protocol**

5.2.2.4.1. Affect responses

The one item feeling scale presents as an 11-point bipolar scale ranging between +5 to -5. Verbal anchors are -5 = very bad, -3 = bad, -1 = fairly bad, 0 = neutral,
+1 = fairly good, +3 = good, +5 = very good. The instructions to use the FS according to Hardy and Rejeski (1989) were given to participants before starting the sessions (Hardy and Rejeski, 1989).

5.2.2.4.2. Rating of perceived exertion (RPE)
RPE defines as the level of heaviness and strain experienced while doing physical work (Kilpatrick et al., 2015), with the scale being created by Borg (Borg, 1970). The scale ranges from 6 to 20, with 6 representing “no exertion at all” and 20 representing “maximal exertion”.

5.2.2.4.3. Exercise task self-efficacy questionnaire
The task self-efficacy questionnaire was used for assessing the confidence of participants in their ability to repeat the exercise they did (Jung et al., 2014). The questionnaire used in this study was the modified version used by Townsend et al. (2017) (Table 5.2). The scoring system was a scale from 0 (not at all) to 100 (extremely confident).

5.2.2.4.4. Enjoyment
The Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo, 1991) was used for the enjoyment of each exercise protocol. The modified PACES questionnaire used in the present study was the one described by Jung et al. (2014). Seventeen questions asked how the participants enjoyed/liked or disliked the exercise they did. The scores ranged from 1 to 7 with a maximum score of 119. Additionally, two other questions, with a scale from 1 to 9, were asked to assess the enjoyment of the exercise session that just finished and the expected enjoyment to perform the same exercise sessions three times/week for the next month.
5.2.2.4.5. Preference

Exercise preference was determined on the last visit using a “deception session” according to Townsend et al. (2017). Participants were asked to come to the laboratory and to choose the exercise protocol they preferred to do from the three protocols. This was indicated as the exercise preference. However, participants did not need to perform the exercise they have chosen for this session.

5.2.3. Statistics

Data are shown as mean±SD and analysed using SPSS Version 23 (Chicago, IL, USA). As data on the variability of the study parameters was not available, formal sample size calculations were not possible and instead a convenience sample was used for this study. The data have a normal distribution so parametric tests were used. One-way ANOVA was used to determine the differences of affect valence (lowest, end and change), RPE, self-efficacy and enjoyment scale between the three exercise protocols. The significance level was set at \( p < 0.05 \).

5.3. Results

All participants completed the familiarisation sessions and exercise sessions except one participant who completed MICT and REHIT but he did not complete his HIIT session because he felt sick after 12 minutes. The percentage of age-predicted HRmax during MICT, HIIT and REHIT was 72±5%, 96±5% and 90±5% respectively.

5.3.1. Affect responses

There were no significant differences between the three protocols in the lowest affective score (MICT: 0.7±1.4, HIIT: -1.3±2.8, REHIT: 0.6±2.4), the end affect (MICT: 0.9±1.3, HIIT: 1.7±2.1, REHIT: 2.0±2.2) (Figure 5.3) and the change in affect during exercises (MICT: -2.1±1.6 vs HIIT: -3.9±1.9 vs REHIT: -2.7±3.0).
Figure 5.3 Affect responses among three exercise protocols. Data presented as mean±SD.

5.3.2. RPE

The peak RPE was significantly lower in MICT (11.0±2.1) when compared to HIIT (16.3±2.4) and REHIT (17.0±1.9) (*p*<0.05) but there was no significant difference between HIIT and REHIT (Figure 5.4).

Figure 5.4 The RPE during three exercise protocols. Data shown are mean±SD.
5.3.3. Exercise task self-efficacy

There was no significant difference in aggregate score of self-efficacy between the three protocols. When analysing each item of self-efficacy, there was only a significant difference between MICT and REHIT in item 6 which meant participants were less confident to avoid over-exertion when performing REHIT than MICT (Table 5.2).

**Table 5.2 Exercise task self-efficacy**

<table>
<thead>
<tr>
<th></th>
<th>MICT</th>
<th>HIIT</th>
<th>REHIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidence in performing 1 bout /week for the next month</td>
<td>93.8±10.6</td>
<td>88.6±13.5</td>
<td>86.3±20.0</td>
</tr>
<tr>
<td>Confidence in performing 2 bout /week for the next month</td>
<td>85.0±19.3</td>
<td>78.6±15.7</td>
<td>71.3±21.7</td>
</tr>
<tr>
<td>Confidence in performing 3 bout /week for the next month</td>
<td>71.3±33.1</td>
<td>62.9±26.3</td>
<td>52.5±33.7</td>
</tr>
<tr>
<td>Confidence in performing 4 bout /week for the next month</td>
<td>61.3±31.4</td>
<td>51.4±38.0</td>
<td>41.3±35.2</td>
</tr>
<tr>
<td>Confidence in performing 5 bout /week for the next month</td>
<td>48.8±33.6</td>
<td>40.0±34.6</td>
<td>36.3±35.4</td>
</tr>
<tr>
<td>Avoid over-exertion?</td>
<td>93.8±9.2</td>
<td>74.3±18.1</td>
<td>68.8±23.6*</td>
</tr>
<tr>
<td>Follow the directions of the instructor/trainer?</td>
<td>93.8±11.9</td>
<td>70.4±17.9</td>
<td>93.8±10.6</td>
</tr>
</tbody>
</table>

Data shown are mean±SD, * significantly different MICT versus REHIT \(p<0.05\)

5.3.4. Enjoyment

There was no significant difference in enjoyment scale between the three protocols (Figure 5.5). The average PACES of MICT, HIIT and REHIT were 70±16, HIIT 80±19, REHIT 86±21 respectively.
5.3.5. Preference

REHIT has been chosen by seven participants out of eight (87.5%). Only one participant preferred HIIT (12.5%). Nobody chose to perform MICT.

5.4. Discussion

The purpose of the present chapter was to examine the affective responses to REHIT and compare these to MICT and HIIT in inactive young adults. The results demonstrated that the lowest, end and change in affective response were similar between REHIT, MICT and HIIT. Additionally, there was no significant difference in self-efficacy and enjoyment between these three exercise protocols. However, the majority of participants preferred REHIT. Thus, criticisms of SIT are not supported by these findings: if the number and duration of sprints are kept low, affective responses are not worse with SIT (i.e. REHIT) when compared with a popular HIIT protocol or even MICT.
Despite the variation in exercise intensities, the affect valence was not significantly different between the three protocols when comparing at the end, the lowest and the change while performing the exercises. During MICT, affect declined gradually over time. During REHIT, the affect steeply dropped at the end of supramaximal intensity bouts, but then quickly returned during the recovery period. Importantly, negative valence was not found following MICT and REHIT. During HIIT, the affect response decreased progressively over time and reached negative valence from the 7th to 10th bout of high intensity (14th to 20th minute).

These findings are consistent with Stork et al. (2018) who demonstrated that negative valence was found when performing HIIT from the 6th to 10th bout of high intensity (Stork et al., 2018). Nevertheless, the interesting outcome found in the present study (contrary to Stork et al. (2018)) is that there was no negative valence during the conventional REHIT protocol, whereas negative valence was found after the 3rd bout of the modified REHIT protocol by Stork et al. (2018). This finding may be explained by the fact that affect gets progressively more negative with each subsequent sprint, so SIT protocols should attempt to keep the number of sprint repetitions low. These findings are supported by previous studies, which demonstrated that the affect dropped to negative valence when participants did more repeated sprints during HIIT/SIT (Stork et al., 2018; Frazao et al., 2016; Jung et al., 2014; Townsend et al., 2017). Importantly, Vollaard et al. (2017) showed that performing more than two sprints does not result in greater improvements in $\dot{V}O_2$max, so the fact that more sprints result in more negative affective response means that the conventional REHIT protocol (with two sprints) is a more promising protocol than other SIT protocols.
Importantly, the present study offers evidence to argue against previous comments that SIT may be related to negative affect and could lower exercise adherence (Hardcastle et al., 2014). However, the present study examined only the acute affective responses to exercises. Future studies need to examine adherence to REHIT under ‘real-life’ conditions.

Despite the fact that the peak (both positive and negative), end and change in affect have been suggested as the most significant affective responses during exercise (Decker and Ekkekakis, 2017), the duration of exposure to low affect and high RPE also needs to be considered. During REHIT, participants experienced high RPE and low affect for very short durations. It appears that this did not make participants feel more unpleasant than 30 minutes of MICT with a bit lower RPE and a bit higher affective valence.

Enjoyment is a factor that is related to exercise adoption (Cox, 2012) and exercise maintenance (Jekauc, 2015; Hagberg et al., 2009). The present study found similar enjoyment between the three protocols. This finding supports evidence from previous studies (Jung et al., 2014; Stork et al., 2018). However, it is contrary to other studies which have demonstrated that SIT (Astorino and Thum, 2018) or HIIT (Kong et al., 2016) were more enjoyable than MICT. Although high peak RPE was found during REHIT (~17, which corresponds to very hard) and HIIT (~16, which corresponds to between hard and very hard), the enjoyment level was not significantly different from MICT. This finding may be explained by the fact that the feeling of monotony is lower during intermittent exercise, because of the switching between “on-off” efforts (Stork et al., 2018; Jung et al., 2014). It may lead to intermittent exercise being more enjoyable and
enthralling than MICT. Additionally, REHIT is much shorter than the other two protocols. It could be a reason that seven out of eight preferred REHIT.

Affective responses during exercise have been defined as a predictor of future exercise adherence (Williams et al., 2012), but self-efficacy has also been described as a future exercise behaviour predictor (Abasi et al., 2016). Importantly, self-efficacy is a determinant of exercise adoption and adherence (Oman and King, 1998; Sallis et al., 1986; McAuley, 1992; McAuley and Blissmer, 2000). The present study found that there was no significant difference in self-efficacy between the three exercise protocols. It could be interpreted that participants felt confident that they could do REHIT, the same as that they could do MICT and HIIT. This finding is similar to the study by Jung et al. (2014), where sedentary individuals reported that there was no significant difference in the confidence to perform MICT or HIIT (Jung et al., 2014). A possible explanation is the nature of interval training: comprising of short periods of high intensity exercise interspersed by periods of recovery, making exercise more tolerable and allowing participants to gain several achievements (Jung et al., 2014). According to the self-efficacy theory, people will engage in behaviours that they feel confident they can accomplish (Bandura, 1997). Taken together, people would adopt and adhere to REHIT because they can be confident, because they can complete REHIT sessions in the same way they can complete MICT or HIIT sessions.

A few limitations to the present study need to be acknowledged. Firstly, the sample size was small. This increases the chance of assuming that there is no difference between the three protocols (type I error). Secondly, the present study
examined the acute affect responses to exercise. Long-time adherence to HIIT/REHIT still need to be investigated.

5.5. Conclusion

The present chapter was designed to compare the affect responses following REHIT, MICT and HIIT in inactive young adults. The findings indicated that there was no significant difference in affect responses, self-efficacy and enjoyment between the three exercise protocols. Importantly, negative valence was found during HIIT but was not found during REHIT (which was similar to MICT). Additionally, the majority of participants preferred REHIT over other protocols. Overall, this study strengthens the idea that SIT protocols comprising of a smaller number of sprints and shorter sprint durations, such as REHIT, may provide an effective and manageable alternative exercise intervention for improving general health.
CHAPTER 6

CHAPTER 6: THE EFFECT OF REDUCED EXERTION HIGH-INTENSITY INTERVAL TRAINING (REHIT) ON MAXIMAL OXYGEN CONSUMPTION IN TYPE 2 DIABETES (T2D) PATIENTS
6.1. Introduction

It is well established from several studies that $\dot{V}O_2\text{max}$ is improved following reduced exertion high-intensity interval training (REHIT) (Metcalfe et al., 2012; Metcalfe et al., 2016; Nalcakan et al., 2018) and may therefore be of use in disease prevention. However, it is unknown whether REHIT can also be used in the treatment of disease rather than disease prevention, for example, in type 2 diabetes (T2D) patients whose $\dot{V}O_2\text{max}$ tends to be low and which is predictive of adverse outcomes (Leite et al., 2009; Kohl et al., 1992; Wei et al., 2000).

The prevalence of diabetes is still increasing worldwide. In 2017, the estimated number of people with diabetes was 425 million adults, and this will rise to 629 million (a 48% increase) by 2045 (International Diabetes Federation, 2017). In the UK in 2017, there were almost 3.7 million adults with diabetes (Diabetes UK, 2017). Moreover, diabetes is one of the top ten leading causes of death (WHO, 2016).

T2D is the most common form of diabetes, affecting approximately 90% of people with diabetes in the UK (Diabetes UK, 2018b). Peripheral insulin resistance is the first detectable impairment in T2D (Shanik et al., 2008). Insulin resistance is also the main predictor of T2D progression (Taylor, 2012). T2D is associated with many complications, such as cardiovascular disease, kidney disease, eye disease and neuropathy (Diabetes UK, 2018a). Cardiovascular disease is one of the most significant complications (Laakso, 2001) because it is the major cause of death in diabetic patients (Morrish et al., 2001). The risk of cardiovascular disease is increased twofold in diabetic patients (Sarwar et al., 2010). In addition, mortality from cardiovascular disease is higher in diabetic patients than nondiabetic patients (Haffner et al., 1998). There is a relationship between
cardiovascular functions and $\dot{V}O_2\text{max}$ (Convertino, 1997). Low $\dot{V}O_2\text{max}$ increases the risk of death from cardiovascular and other diseases (Blair et al., 1996; Sui et al., 2007). On average, T2D patients have a 20% lower $\dot{V}O_2\text{max}$ than healthy individuals (Regensteiner et al., 1995). Importantly, increases in $\dot{V}O_2\text{max}$ are associated with reduced all-cause mortality in T2D participants (Albright et al., 2000).

It has been conclusively shown that $\dot{V}O_2\text{max}$ is improved following exercise training (Huang et al., 2016; Scribbans et al., 2016), and exercise is one of the ways of managing T2D (Colberg et al., 2010). It is well known that there are many benefits of exercise in T2D (Zanuso et al., 2010). The exercise recommendation for T2D is to perform aerobic exercise (brisk walking) at moderate intensity (40–60% of $\dot{V}O_2\text{max}$) for at least 150 minutes per week (Colberg et al., 2010). This exercise training protocol improves both insulin sensitivity (Borghouts and Keizer, 2000; Colberg et al., 2010) and $\dot{V}O_2\text{max}$ (Boule et al., 2001; Boule et al., 2003; Loimaala et al., 2003; Maiorana et al., 2002; Yeater et al., 1990). However, despite these recommendations, T2D patients do even less exercise than the general population (Morrato et al., 2007) and many patients fail to engage in exercise. Similar to the general population, lack of time is an exercise barrier in diabetes patients (Thomas, 2004).

For the last ten years, sprint interval training (SIT) has been investigated as a novel form of time-efficient exercise. It has been confirmed that the effects of SIT might be as beneficial as the current exercise recommendations (Gibala et al., 2012; Gibala and McGee, 2008). Focusing on cardiometabolic parameters, SIT improves glycaemic control in healthy sedentary individuals (Babraj et al., 2009), and improves insulin sensitivity in healthy and overweight/obese sedentary
individuals (Whyte et al., 2010; Babraj et al., 2009). Insulin-stimulated glucose uptake is increased following SIT in T2D/prediabetes participants (Sjöros et al., 2018). However, this protocol did not have an effect on glycaemic control, insulin sensitivity and insulin resistance in T2D (Shaban et al., 2014). In addition, high motivation and high levels of fatigue are the major limitations of SIT (Gillen and Gibala, 2014). Therefore, Metcalfe et al. (2012) designed the REHIT protocol. Alongside improvements in $\dot{V}O_2\text{max}$ following REHIT (Metcalfe et al., 2012; Metcalfe et al., 2016; Nalcakan et al., 2018), insulin sensitivity also improved after REHIT in healthy participants (Metcalfe et al., 2012), suggesting that REHIT might have an effect on metabolic parameters. However, what is not yet known is whether the genuinely time-efficient, lower exertion REHIT protocol has an effect on cardiometabolic parameters in patients with T2D. Thus, the objectives of this study were to examine the effect of REHIT on aerobic capacity and metabolic function, and to compare the effect of training between REHIT and the current exercise recommendation, which is moderate-intensity exercise.

6.2. Methods

6.2.1. Participants

Forty-one men with T2D aged between 40-60 years volunteered for the study. Eighteen patients did not meet the inclusion criteria. Twenty-one volunteers participated in the study. Five participants dropped out from the study: three had unrelated medical conditions, one did not have enough time for training, and one had an undisclosed personal reason. Sixteen participants completed this study. The participant characteristics of these 16 participants are shown in Table 6.1. This study compares the effect of REHIT and a walking intervention using a cross-over design study. Therefore, all participants did both exercise training
protocols, which were REHIT (eight weeks) and walking (eight weeks), separated by a wash-out period of at least eight weeks. Randomisation was applied for the first training intervention. Ten participants started the first intervention with REHIT and eleven participants started the first intervention with walking. Participants who had insulin therapy or physical impairments which are contraindications of exercise were excluded from this study. All participants were informed of the protocol and potential risks from the study protocol. The experimental protocol was approved by the NHS Research Ethics Committee (13/SW/0298).

**Table 6.1 Subject characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55±5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.7±11.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78±0.06</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>30.6±2.8</td>
</tr>
<tr>
<td>(\dot{V}\text{O}_2\text{max}) (ml·kg⁻¹·min⁻¹)</td>
<td>27.5±4.3</td>
</tr>
<tr>
<td>Duration of T2D (years since diagnosis)</td>
<td>4±4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132±12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82±7</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>99±8</td>
</tr>
</tbody>
</table>

Medication:
- None (n) 3
- Metformin (n) 9
- Sulfonylurea (n) 3
- Blood pressure lowering (n) 4
- Statins (n) 10
- Anti-depressant (n) 1

Data are shown as mean ± SD
6.2.2. Experimental Protocol

6.2.2.1. Pre-testing procedures

Participants were asked to do a familiarisation session for the maximal exercise test using a cycle ergometer (Lode Excalibur Sport ergometer, The Netherlands) and a gas analysis system (Parvo Medics TrueOne 2400, USA). The test was started with a warm up phase at 50 W for five minutes. Then, the resistance was increased by 1 W every four seconds until volitional exhaustion. The participants were asked to breathe through a mouthpiece and respired air was collected and analysed. \( \dot{V}O_2 \text{max} \) was calculated as the highest 15-breath rolling average for \( \dot{V}O_2 \) measured. Peak power output (Wmax) and maximal heart rate (HRmax) were recorded.

Then, physical activity was recorded for seven days before the testing date, using physical activity monitoring (Actiheart™, CamNtech) and a physical activity diary. The food diary was recorded three days before an oral glucose tolerance test (OGTT). There were no significant differences in total energy, carbohydrate, fat or protein content, which were calculated from nutritional scores averaged over the 3 days, before the pre- and post-intervention OGTTs (Table 6.2). Before the OGTT, participants were asked to refrain from alcohol, caffeine, any medication and heavy exercise for 24 hours and on the testing date as well. In addition, an evening meal was given to participants which was the same meal for each testing day, and participants were asked to fast for 12 hours before the testing time.
Table 6.2 Nutritional intake over the three days before the OGTTs

<table>
<thead>
<tr>
<th></th>
<th>REHIT</th>
<th>Walking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Energy (Kcal/day)</td>
<td>2276.0±495.8</td>
<td>2133.9±591.5</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>227.4±60.9</td>
<td>214.8±73.6</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>103.8±30.2</td>
<td>92.8±28.9</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>90.0±23.9</td>
<td>93.2±22.7</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>15.8±17.3</td>
<td>14.5±15.7</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD.

6.2.2.2. Pre-and post-training intervention

On the testing date, the participants arrived at the laboratory room in the morning. Body mass and height were determined, and body composition was evaluated by dual-energy X-ray absorptiometry (DEXA) scan (Discovery DXA system, Hologic, Inc. USA). A continuous subcutaneous glucose monitor (CGM) (iPro®2 Professional CGM, Medtronic, USA) was inserted into the interstitial compartment of the abdominal adipose tissue after finishing the body scan. Blood pressure was taken after resting for 30 minutes in a seated position. The value of blood pressure was averaged from three measurements which did not differ more than 10 mm Hg. The cannula was inserted at the median cubital vein and a fasting blood sample was collected. All participants were asked to drink 140 g of glucose solution (Polycal Liquid, Nutricia, UK) dissolved in 187 ml of water, equivalent to 75 g of standard glucose. Blood samples were collected at 30, 60, 90 and 120 minutes after finishing drinking. The collected blood samples were kept on ice before centrifuging at 5,000 g for 10 minutes. The plasma was separated and stored at -80°C. Plasma glucose was analysed by glucose
oxidase reaction (YSI Stat 2300, Yellow Spring, USA). Plasma insulin concentrations were determined by ELISA kits (Invitrogen, UK). Insulin sensitivity was measured using Cederholm index (Cederholm and Wibell, 1990) which was calculated following the formula:

\[
\frac{75000 + (G_0 - G_{120}) \times 1.15 \times 180 \times 0.19 \times m}{120 \times G_{\text{mean}} \times \log(I_{\text{mean}})}
\]

75000: oral glucose load in OGTT in mg

\(G_0\): plasma glucose concentration at 0 min (mmol·l\(^{-1}\))

\(G_{120}\): plasma glucose concentration at 120 min (mmol·l\(^{-1}\))

1.15: factor transferring whole venous blood glucose to plasma values

180: conversion factor to transform plasma glucose concentration from mmol·l\(^{-1}\) into mg·l\(^{-1}\)

0.19: glucose space in liter per kg of body weight

\(m\): body mass (kg)

120: OGTT duration (min)

\(G_{\text{mean}}\): mean glucose concentration during OGTT (mmol·l\(^{-1}\))

\(I_{\text{mean}}\): mean Insulin concentration during OGTT (mU·l\(^{-1}\))

Furthermore, plasma glucose area under the curve (AUC) and insulin AUC were calculated using the trapezoidal method (Purves, 1992). OGTTs were performed before and after both training interventions. \(\dot{V}O_2\)max was evaluated on the day following each OGTT. The summarising protocol is shown in Figure 6.1.

### 6.2.2.3. Training intervention protocol

**Walking**

All participants walked for 30 minutes, five times a week (three times supervised and two times non-supervised), for eight weeks at moderate intensity which was 40% of heart rate reserve (HRR) in the first 10 sessions, 50% of HRR in the
following 10 sessions and 55% of HRR in the last 20 sessions. Heart rate was monitored during walking (Polar, UK). The target heart rate (THR) was calculated using the Karvonen method (Karvonen and Vuorimaa, 1988) which is:

\[
THR = HRR \times \%\text{intensity} + HR_{rest}
\]

\[
HRR = HR_{max}(220 - age) - HR_{rest}
\]

Reduced exertion high-intensity interval training (REHIT)

Participants were asked to complete three exercise sessions per week for eight weeks, resulting in a total of 24 sessions. Each training session was 10 minutes long including a warm up and cool down, and total training time was 30 minutes per week. Each exercise session consisted of cycling at a low intensity (25 W) and one or two all-out cycling sprints (one sprint for the 1st session and two sprints for all other sessions). The sprint duration increased from 10 seconds in sessions 1-4, to 15 seconds in sessions 5-12, and 20 seconds in sessions 13-24. The schematic of the REHIT training protocol is shown in Figure 6.2. Participants sprinted against a braking force set as a constant torque equivalent to 0.65 x lean body mass. This training protocol is similar to the protocol of Metcalfe et al. (Metcalfe et al., 2012). A rating of perceived exertion (RPE) was determined using a 15-point Borg scale (Borg, 1970) at the end of each training week. Heart rate was recorded during each session (Polar, UK).
6.2.3. Statistical analysis

All data are reported as mean ± SD. All analyses were conducted using the SPSS Version 23 (Chicago, IL, USA). At least 16 participants were required to be able to detect significant differences of the change in OGTT-derived insulin sensitivity (Cederholm Index) between the two interventions with an effect size (Cohen’s d) of 0.75, β=0.80 and α=0.05. The data have a normal distribution so parametric
tests were used. The difference between the effect of the two training interventions (REHIT/walking) and time (pre/post training) were analysed using two-way repeated measures analysis of variance. The significance level was set at $p < 0.05$.

6.3. Results

6.3.1. Training characteristics

The mean adherence to the REHIT training sessions and walking sessions was 99% and 97% respectively. All training sessions, both REHIT and walking, were well tolerated by participants and no adverse effects were observed during training sessions.

The average RPE for the REHIT intervention (13.6±1.1, corresponding to “somewhat hard”) was significantly higher than for the walking intervention (11.7±1.2, corresponding to “light”) ($p<0.001$) (Figure 6.3).

![Figure 6.3](image)

**Figure 6.3** Ratings of perceived exertion during the REHIT training sessions (solid line), 10-s sprint in session 1-4, 15-s sprint in session 5-12, and 20-s sprint in 13-24. The walking sessions (dot line), the intensity is 40%HRR for session 1-10, 50%HRR for session 11-20, and 55%HRR for session 21-40. Data are shown as mean ± SD. Main effect of group: # $p<0.001$. 
The heart rate response to the REHIT intervention during sessions 1-4 (10-s sprint), sessions 5-12 (15-s sprint), and sessions 13-24 (20-s sprint) are shown in Figure 6.4. The peak heart rate during all-out sprints reached on average ~85-90% of heart rate reserve. In the walking intervention, on average participants reached their target heart rate for all intensities (Figure 6.5).

![Figure 6.4](image1.png)  
**Figure 6.4** The heart rate response to REHIT. Data are shown as mean ± SD.

![Figure 6.5](image2.png)  
**Figure 6.5** The target heart rate and the achieved heart rate in a different intensity of walking intervention. Data are shown as mean ± SD.
When comparing power output between the first and the second sprint, Peak power output (PPO), mean power output (MPO) and end power output (EPO) of the first sprint were higher than those in the second sprint (main effect of repetition; \( p<0.05 \) in PPO, \( p<0.001 \) in MPO and EPO) (Table 6.3). Additionally, MPO and EPO were decreased significantly when the sprint duration increased (main effect of time; \( p<0.001 \)).

**Table 6.3** Power output of the first and the second sprint during the REHIT intervention

<table>
<thead>
<tr>
<th></th>
<th>Sessions 1-4</th>
<th>Sessions 5-12</th>
<th>Sessions 13-24</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (1st sprint/2nd sprint; W)</td>
<td>784±129 / 759±115*</td>
<td>778±149 / 769±127*</td>
<td>765±137 / 757±151*</td>
</tr>
<tr>
<td>MPO (1st sprint/2nd sprint; W)</td>
<td>626±105 / 618±105**</td>
<td>602±105 / 589±103**</td>
<td>564±96 / 532±89***‡</td>
</tr>
<tr>
<td>EPO (1st sprint/2nd sprint; W)</td>
<td>505±100 / 500±99**</td>
<td>451±73 / 421±90**</td>
<td>393±92 / 342±78**‡</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. Main effect of time: * \( p<0.05 \), ** \( p<0.001 \). The main effect of sprint duration: ‡ \( p<0.001 \).

### 6.3.2. Training effects on cardiometabolic risk factors

There was no significant difference in baseline values between the first and the second intervention for body composition, \( \dot{V}O_2\text{max} \), blood pressure, glucose, insulin, triglycerides, plasma LDL, plasma HDL, plasma ALT and plasma fructosamine. There was no significant change in body weight, total body fat and android fat from pre- to post-training for both interventions but gynoid fat was decreased post training for both the REHIT and walking interventions (main effect of time: \( p<0.05 \)) (Table 2.3). \( \dot{V}O_2\text{max} \) was improved post REHIT intervention by an average of 7%, but only increased 1% following the walking intervention.
There was a significant interaction effect for time x intervention ($p<0.05$) (Figure 6.6).

![Figure 6.6 VO$_2$max following the REHIT and walking intervention. Data is shown as mean ± SD. Main effect of time x intervention: † $p<0.05$.]

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were improved following both training interventions (main effect of time: $p<0.05$). SBP was decreased on average 5 mm Hg and 2 mm Hg after REHIT and walking respectively. DBP was decreased on average 2 mm Hg after REHIT and 4 mm Hg after walking. MAP was decreased on average 4 mm Hg in both groups. There were no significant time x intervention interaction effects for the blood pressure parameters (Figure 6.7). No significant changes in glycaemic control and insulin sensitivity were observed from pre- to post-training for either intervention (Table 6.4). Additionally, there was no effect of training on triglycerides, plasma LDL, plasma HDL, and plasma ALT, but plasma fructosamine was decreased post training (main effect of time: $p<0.05$) (Table 6.4).
Figure 6.7 Systolic blood pressure (A), diastolic blood pressure (B) and mean arterial pressure (C) compared between pre and post training intervention (→ walking, — REHIT). Data are shown as mean ± SD. The main effect of time: * p<0.05.
### Table 6.4 Effect of the two training interventions

<table>
<thead>
<tr>
<th></th>
<th>REHIT</th>
<th>Walking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>$\dot{V}O_{2\text{max}}$ (ml·kg⁻¹·min⁻¹)</td>
<td>28.38±6.03</td>
<td>30.25±5.61</td>
</tr>
<tr>
<td>Plasma glucose AUC (mmol·min⁻¹·L⁻¹)</td>
<td>1848±543</td>
<td>1758±396</td>
</tr>
<tr>
<td>Plasma insulin AUC (mmol·min⁻¹·L⁻¹)</td>
<td>3395±679</td>
<td>3579±987</td>
</tr>
<tr>
<td>Cederholm index</td>
<td>23.3±12.1</td>
<td>24.7±14.0</td>
</tr>
<tr>
<td>(mg·L⁻²·mmol⁻¹·mU⁻¹·min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGM average glucose (mM)</td>
<td>9.5±2.9</td>
<td>9.5±3.2</td>
</tr>
<tr>
<td>Fasted plasma glucose (mmol·L⁻¹)</td>
<td>9.9±3.0</td>
<td>9.2±2.2</td>
</tr>
<tr>
<td>Fasted plasma insulin (mU·L⁻¹)</td>
<td>16.0±11.6</td>
<td>16.1±13.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>7.1±5.2</td>
<td>6.6±5.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.8±11.7</td>
<td>97.1±12.0</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>31.0±4.3</td>
<td>30.7±4.3</td>
</tr>
<tr>
<td>Android fat (%)</td>
<td>37.9±5.3</td>
<td>37.5±5.3</td>
</tr>
<tr>
<td>Gynoid fat (%)</td>
<td>28.0±5.1</td>
<td>27.3±5.1*</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>1.5±0.8</td>
<td>1.4±0.7</td>
</tr>
<tr>
<td>Plasma LDL (mM)</td>
<td>2.9±1.7</td>
<td>3.2±1.8</td>
</tr>
<tr>
<td>Plasma HDL (mM)</td>
<td>1.0±0.2</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Plasma fructosamine (µM)</td>
<td>387±65</td>
<td>376±56*</td>
</tr>
<tr>
<td>Plasma ALT (U/L)</td>
<td>36±8</td>
<td>36±9</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. AUC: area under the curve, CGM: continuous glucose monitor, HOMA-IR: homeostatic model assessment – insulin resistance, LDL: low density lipoprotein, HDL: high density lipoprotein, ALT: alanine aminotransferase. The main effect of time: * $p<0.05$. The effect of time x intervention: † $p<0.05$. 
6.4. Discussion

This is the first study investigating the effect of REHIT compared to the effect of MICT on cardiometabolic health parameters in patients with T2D. Following eight weeks of training, $\dot{V}O_2\text{max}$ improved 7% and 1% after REHIT and walking interventions, respectively. Both interventions decreased SBP and DBP. On the other hand, neither REHIT nor walking significantly improved metabolic parameters including glycaemic control, insulin sensitivity, lipid profile and body composition. When considering the total time commitment, REHIT (30 minutes per week) was 80% lower than the walking intervention (150 minutes per week). Thus, the present study confirmed the benefits of truly time-efficient exercise on health. However, REHIT might not be an option for improving insulin sensitivity in T2D individuals.

It has been confirmed that metabolic parameters improve after SIT. Classic SIT (four to seven repeats of a 30-s sprints) has an effect on insulin sensitivity in healthy sedentary participants (Babraj et al., 2009; Cocks et al., 2013; Richards et al., 2010). Additionally, previous studies have shown that insulin sensitivity can be improved following a small amount of sprint training (two to seven 20–30 second sprints per session) in both healthy and obese/overweight sedentary participants (Gillen et al., 2016; Metcalfe et al., 2012; Gillen et al., 2014; Whyte et al., 2010). The present study used a protocol of two 20-s sprints, which is the same protocol as Metcalfe et al. (2012), except for the duration of the training. Six weeks of REHIT improves glycaemic control in sedentary individuals (Metcalfe et al., 2012). Conversely, a similar effect was not seen in people with T2D following eight weeks of REHIT. This finding is consistent with data from Revdal et al. (2016), who demonstrated that 12 weeks of running-based REHIT
involving two 20-s sprints at all-out intensity improved neither glycaemic control nor insulin sensitivity in T2D patients (Revdal et al., 2016). However, another intervention presented by Revdal et al. (2016) using ten repeats of 60-s sprints at 90% HRmax, which was adapted from Little et al. (2011a), did not show the same effect on glycaemic control as the previous studies in healthy individuals either (Little et al., 2011a; Gillen et al., 2012). By combining the results of SIT or HIIT studies in T2D participants, controversy remains over the effect of SIT or HIIT on glycaemic control.

Baseline insulin sensitivity might be a factor associated with exercise adaptations. A greater effect of exercise on glycaemic control tends to be seen in prediabetic and T2D patients than in individuals with a normal level of blood glucose after aerobic training (Jenkins and Hagberg, 2011; Malin and Kirwan, 2012). Conversely, there is a study that shows diminished exercise responses in patients with T2D after acute aerobic exercise (Sriwijitkamol et al., 2007). Moreover, exercise responses after aerobic exercise training are attenuated in patients with T2D who have a poor β-cell function (Dela et al., 2004; Solomon et al., 2013). Solomon et al. (2013) suggested that there is a limitation of the response of exercise after exercise training in patients with long-term T2D and insufficient glycaemic control (Solomon et al., 2013). However, Holloszy et al. (1986) demonstrated that glucose tolerance could be normalised following a long duration of exercise (≥ 60 min), high intensity (≥ 90% \( \dot{V}O_2\max \)), high frequency (≥ 5 session per week) and a long duration of the training program (≥ 12 months) (Holloszy et al., 1986). Therefore, the eight weeks of REHIT or walking training used in this study might not have been sufficient to improve insulin sensitivity and
glycaemic control in patients with T2D. A higher intensity or a longer duration of exercise might be needed to improve glycaemic control.

Additionally, attenuation of exercise-induced improvements in glycaemic control might be an effect of medication. Exercise-induced improvement of insulin sensitivity both in nondiabetic and T2D individuals might be reduced by metformin (Malin and Braun, 2016; Sharoff et al., 2010). Moreover, other medicine, such as statins, also attenuate exercise adaptations after training, including improvements in mitochondrial function and aerobic capacity (Mikus et al., 2013; Murlasits and Radák, 2014). Conversely, there have been some studies that reveal contrary effects (Boule et al., 2013; Meex et al., 2010). The present study did not examine the effects of metformin or statins on exercise adaptations as there was only a small number of participants (participants who were taking metformin/statin, n = 12; participants who were not taking metformin/statin, n = 4) so there was not enough power to determine these effects.

$\dot{V}O_2\text{max}$ improved 7% after REHIT. This result is consistent with the results seen in Chapter 4 and other REHIT studies, which demonstrate that $\dot{V}O_2\text{max}$ increased by approximately 10% in healthy sedentary individuals following REHIT (Metcalfe et al., 2012; Metcalfe et al., 2016; Nalcakan et al., 2018). This finding is important because an increase in $V_2\text{max}$ is associated with decreases in all-cause mortality in T2D, especially in patients who have low aerobic fitness (Albright et al., 2000). Moreover, risk of death increases in people who have a low fitness level, and the risk is higher than with other factors such as smoking, high blood pressure or blood sugar (Blair et al., 1989). Accordingly, Lee et al. (2011) suggested that all-cause and cardiovascular mortality decreases by 15%
and 19% respectively when exercise capacity increased every 1 MET (Lee et al., 2011). From the present data, the improvement in \( \dot{V}O_2 \text{max} \) calculated in METs is an average of 0.5 METs (pre-training 8.1 METs, post-training 8.6 METs). It might be assumed that all-cause mortality risk is reduced by an average of 8% and cardiovascular mortality risk reduced by an average of 10% following REHIT. In addition, this study demonstrated that SBP, DBP and MAP were decreased after REHIT intervention. Importantly, the meta-analysis by Emdin et al. (2015) showed that lower BP is associated with reduced all-cause mortality including cardiovascular diseases, coronary heart diseases and stroke in T2D (Emdin et al., 2015). Therefore, our study confirms that REHIT is a genuine time-efficient exercise able to reduce cardiovascular risk factors. However, it is too early to recommend REHIT to T2D patients, even though there have been no adverse effects following REHIT intervention and most of the participants enjoyed REHIT. The main outcome, which was insulin sensitivity or glycemic control, did not change after REHIT. Thus, further studies should validate the effect of REHIT in T2D and extend knowledge by investigating the effect of REHIT in other groups of patients.

In considering changes in body composition, the results revealed that eight weeks of REHIT or walking did not affect body composition. This finding is supported by Duncan et al. (2003), who showed that walking at moderate intensity does not change BMI or waist circumference (Duncan et al., 2003). However, a previous study demonstrated that body composition could be decreased by conventional SIT protocols (Whyte et al., 2010). Appetite-regulating hormones, such as ghrelin and peptide YY, are reduced following
REHIT (Metcalf et al., 2015) but the effect of REHIT on weight loss is unknown. Therefore, the effect of REHIT on body composition requires further investigation. The safety of using SIT in patients has been questioned. Most SIT research has been done in healthy sedentary individuals. Only one other study examined the effect of SIT in T2D patients (Sjöros et al., 2018). There were no serious adverse effects during or following SIT. The present study also observed no adverse events following REHIT in T2D individuals. It might be too early to conclude that REHIT is safe for T2D patients or the general population, but at a minimum this result is additional support for the safety of REHIT.

There is an argument from Hardcastle et al. (2014) that it is difficult to adapt or promote SIT to the public (Hardcastle et al., 2014). Based on exercise psychology studies, sedentary individuals might avoid involvement in SIT as it is an ‘extreme’ form of exercise, which requires strong motivation and comes with a high level of fatigue (Hardcastle et al., 2014; Gibala and McGee, 2008). In considering the RPE of the REHIT intervention in the present study, the average RPE corresponded to ‘somewhat hard’. This finding is similar to Metcalf et al. (2012), who studied sedentary people (Metcalf et al., 2012). Moreover, most of the participants in this study claimed that REHIT was enjoyable. This highlights the fact that REHIT is manageable. This finding is supported by the findings of Chapter 5, which revealed that REHIT is not associated with negative affective response and most of the participants chose REHIT as their preference. Together these findings suggest that REHIT is feasible, but further studies need to be carried out in order to validate whether REHIT is acceptable in several patient groups, or if REHIT could be implemented for public engagement.
Finally, a number of limitations need to be acknowledged. First, the present study was designed as a counter-balanced crossover study. The total time commitment to complete the study was approximately six to seven months. This might be a reason some participants dropped out of this study. Second, the eight week wash-out period between two interventions was quite a long time. Participants might change their activities even if they have been asked to maintain their lifestyle for the whole duration of the study. This would affect activity levels during/between training sessions. However, there was no significant difference between baseline physical activity levels between the two interventions.

6.5. Conclusion

This study demonstrated that eight weeks of REHIT improves $\dot{V}O_2\text{max}$ and reduces blood pressure in patients with T2D. This finding confirms that REHIT can be used as a genuinely time-efficient exercise intervention for improving cardiovascular health. However, REHIT did not change glycaemic control and insulin sensitivity in T2D patients, so the use of REHIT in this patient group remains unclear.
CHAPTER 7

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS
7.1. Overview

In 2018, HIIT was the highest-ranked fitness protocol in the worldwide fitness trend survey as rated by health and fitness professionals (Thompson, 2017) and it also has been featured in the top three ranks for fitness protocols in the worldwide fitness trend survey since 2014 (Thompson, 2013; Thompson, 2014; Thompson, 2015; Thompson, 2016). The main reason why HIIT was ranked the highest is related to the time commitment, which is shorter than other types of exercise (Thompson, 2017), suggesting that time-efficient exercise might be a suitable protocol for people who define “lack of time” as their exercise barrier. However, some researchers critiqued that HIIT/SIT is too hard and might not suit sedentary individuals (Hardcastle et al., 2014; Biddle and Batterham, 2015; Coyle, 2005).

The health benefits associated with HIIT have only been investigated for the last 10 years. Nonetheless, many types of HIIT, which differ in protocol (e.g. SIT or aerobic interval training (AIT)) and mode of exercise (e.g. cycling or running), have now been examined. This thesis focused on a specific SIT protocol which has the potential to be more time-efficient than HIIT or other protocols. SIT has the highest intensities, so the sprints can be fewer and shorter than with HIIT, and therefore the protocol has the potential to be shorter and/or more time-efficient. In fact, no HIIT protocols are shorter than current physical activity recommendations (75 min of vigorous exercise). Only short SIT protocols have the potential to be truly time-efficient.

SIT has been shown to be associated with various health benefits in lab-based studies (as described in Chapter 1). However, there are a number of limitations to the use of SIT as a ‘real-life’ practical intervention for improving health and
reducing the risk of non-communicable diseases, including the need for high motivation and high levels of exertion and fatigue during or following training (Gillen and Gibala, 2014; Hardcastle et al., 2014). Moreover, SIT is not generally a truly time-efficient protocol. The total training time per session, including warm-up, cool-down, 4-6x30-s sprints and four min recovery between sprints is approximately 30 min. The total training time commitment per week would be 90 min which is more than the current exercise recommendation (75 min of vigorous exercise). Therefore, SIT protocol parameters (e.g. exercise intensity, sprint duration and the number of sprints) have been modified. Reduced-exertion high-intensity interval training (REHIT) is a modified SIT protocol by Metcalfe et al. (2012) which reduces sprint numbers and sprint duration (Metcalf et al., 2012). Importantly, aerobic capacity and insulin sensitivity were improved following REHIT, which comprises 40-s all-out sprints and the total time commitment was 10 minutes per session (Metcalf et al., 2012), suggesting that a decrease in sprint duration and the number of sprints in REHIT do not attenuate the training effect when compared with conventional SIT. Based on the original findings, it would be interesting to examine whether modified REHIT (shorter/easier) still affects aerobic capacity (especially V̇O₂max), and can be made even more manageable. Therefore, the main objective of this thesis was to optimise the REHIT protocol to be an even more time-efficient exercise protocol that still has an effect on V̇O₂max, and to establish the least demanding protocol that is effective (Chapter 2, 3 and 4). It is not only about time-efficiency, but also about manageable perceived exertion and low negative affective responses. Psychological responses to REHIT have been examined (Chapters 3 and 4) and compared to other commonly studied exercise protocols (Chapter 5).
Additionally, a potential mechanism underlying REHIT was explored in Chapter 2. Finally, Chapter 6 aimed to compare the effect of REHIT with the current exercise recommendation on $\dot{V}O_2\text{max}$ and insulin sensitivity in T2D patients.

7.2. Discussion of Major Findings

7.2.1. Effects of modified REHIT on $\dot{V}O_2\text{max}$

$\dot{V}O_2\text{max}$ is the main outcome of the present thesis, based on the fact that it is the highest ranked risk factor associated with all causes of death (Blair, 2009). As mentioned previously, $\dot{V}O_2\text{max}$ improved on average by 11% following REHIT in sedentary individuals (Metcalfe et al., 2012; Metcalfe et al., 2016). The present thesis revealed that reducing the number of sprints (Chapter 2) or the sprint duration (Chapter 3) attenuates the improvements in $\dot{V}O_2\text{max}$. However, training less often (two times a week instead of three times) did not affect the changes in $\dot{V}O_2\text{max}$ (Chapter 4). It can be concluded that the most time-efficient REHIT protocol to improve $\dot{V}O_2\text{max}$ consists of two repeated 20-s all-out sprints across a 10 minute total duration with a training frequency of at least two times per week. In combination, a total of 36 inactive young adult participants have been trained with the REHIT protocol (Chapters 3 and 4). The improvement of $\dot{V}O_2\text{max}$ was approximately 10% following REHIT. This finding is important considering the study by Lee et al. (2011), which suggested that all causes of mortality and cardiovascular mortality reduced by 15% and 19% respectively when exercise capacity increased by 1 MET (3.5 ml/kg/min) (Lee et al., 2011). From the data presented in this thesis, $\dot{V}O_2\text{max}$ increased on average by 1 MET after REHIT, which means the risk of all causes of mortality would be reduced by 15% and cardiovascular mortality would be reduced by 19%. However, this assumption is
based on the data from inactive young adult participants. It might have a different result with different participants.

The improvement of $\dot{V}O_2\text{max}$ has also been seen following REHIT in T2D patients, improving by approximately 7% (Chapter 6). This outcome is significant for T2D patients because cardiorespiratory fitness is a strong predictor of all causes of mortality in T2D patients (Wei et al., 2000). Moreover, the risk of death from cardiovascular diseases is higher in T2D patients than in non-diabetic individuals (Stamler et al., 1993). It can be suggested that improvements in $\dot{V}O_2\text{max}$ following REHIT would decrease all causes of mortality and cardiovascular mortality in T2D patients. It could be suggested that one of the disease risk factors (low $\dot{V}O_2\text{max}$) that is related to being inactive could be addressed by REHIT.

### 7.2.2. Possible mechanisms following REHIT

The outcomes from this project highlight the beneficial effect of completing 40 seconds short burst sprints on health parameters, both in inactive and T2D individuals. However, the mechanisms underlying this adaptation are still unclear. The whole body adaptation might be initially stimulated from skeletal muscle because it is the main organ that is affected directly by all-out sprints. The first possible mechanism that is proposed in this thesis (Chapter 2) is associated with glycogen depletion in skeletal muscle and the second possible mechanism is related to the skeletal muscle myokine named SPARC.

According to Gibala et al. (2012), the potential mechanism underlying SIT which improves $\dot{V}O_2\text{max}$ is related to PGC-1$\alpha$ and mitochondrial biogenesis which involves signalling molecules including 5'-adenosine monophosphate-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) (Gibala
et al., 2012). Interestingly, glycogen depletion is associated with AMPK activation (McBride and Hardie, 2009). In Chapter 2, the hypothesis was that glycogen depletion might be related to the improvement in $\dot{V}O_2\text{max}$ via activation of AMPK and other cascade molecules, as described above. When glycogen breaks down, the metabolic molecules (glucose and other molecules) are increased within myocytes, causing hyperosmotic conditions, which then leads to the influx of water into myocytes (Raja et al., 2006). It causes a reduction of plasma volume. Therefore, the changes in $\dot{V}O_2\text{max}$ and plasma volume after a single 20-s all-out sprint have been investigated in order to find the relationship between glycogen depletion and $\dot{V}O_2\text{max}$. Despite the reduction of plasma volume (indicating glycogen depletion), $\dot{V}O_2\text{max}$ did not change following a training protocol involving single sprints, suggesting that glycogen depletion *per se* might not be a mechanism associated with increasing $\dot{V}O_2\text{max}$.

Additionally, serum SPARC levels were also examined in Chapter 2, based on the study by Song et al. (2010), which indicated that SPARC relates to AMPK, and both proteins complement each other (Song et al., 2010). Moreover, some relevant data supports that SPARC would be associated with the improvement of $\dot{V}O_2\text{max}$, such as the relationship between SPARC and erythroid progenitors development (Luo et al., 2012) or the relationship between SPARC and VEGF signalling (Keller et al., 2011). Unfortunately, neither serum SPARC levels nor $\dot{V}O_2\text{max}$ improved following a single all-out sprint training protocol. Taken together, glycogen depletion might not relate to the changes in $\dot{V}O_2\text{max}$ or serum SPARC levels. However, the present thesis could not draw a relationship between serum SPARC levels and the changes in $\dot{V}O_2\text{max}$. Therefore, the
mechanisms underlying REHIT training and the improvement in $\dot{V}O_2\text{max}$ are still unknown and further studies need to be focusing on this research area.

7.2.3. Psychological responses to REHIT

As mentioned previously, many people around the world still do not meet the exercise recommendation. Negative affective response during or following exercise might be a reason why people do not engage with exercise (Jung et al., 2014). Focusing on SIT, Hardcastle et al. (2014) gave the opinion that negative affective response might be found following SIT and it could lead people (especially sedentary individuals) to avoid this exercise (Hardcastle et al., 2014). The findings in Chapter 3 demonstrated that negative affective response did not occur following REHIT. Moreover, in-task affective responses have been examined in Chapters 4 and 5. The findings revealed that negative affective response do not occur during REHIT. Importantly, in Chapter 5, the affective responses were compared between REHIT, moderate-intensity continuous exercise training (MICT) and HIIT. The affective valences did not significantly differ between the three exercise protocols, and negative valence was found only during HIIT. It could be concluded that REHIT does not cause affective responses that are worse than those associated with current exercise recommendations. Although supra-maximal intensities are needed in the REHIT protocol, this is only for two short bursts, separated by recovery periods. This could make exercise more endurable (Jung et al., 2014). Additionally, when combining RPE data from 55 participants (Chapter 3, 4, 5 and 6), the average RPE is 13, which corresponds to “somewhat hard”, while the RPE from moderate-intensity exercise (Chapter 5 and 6) is 11, which corresponds to “light”. This suggests that the levels of exertion
following REHIT is higher than for current exercise recommendations. However, the time spent at the higher RPE scores is much shorter.

Enjoyability is a factor that is related to exercise adherence (Cox, 2012; Jekauc, 2015). The present thesis demonstrated that 27 participants (Chapters 3 and 4) on average agreed with the statement that ‘I enjoyed HIT’. Moreover, the enjoyment scores were not significantly different between REHIT, MICT and HIIT (Chapter 5). Interestingly, approximately 80% of participants (inactive young adult individuals and T2D patients) preferred REHIT compared to the current exercise recommendation and HIIT (Chapters 5 and 6). Taken together, the outcomes from the present thesis reveal evidence to oppose the comment that SIT may evoke negative affective response. The negative valence was not found during training if the sprint duration and the number of sprints were kept low. Despite the adverse relationship between negative affect and exercise adherence, this thesis did not investigate the exercise adherence in REHIT. It is still being questioned whether people will engage more in REHIT if there is no increase in negative affect following this protocol. Further studies are needed to explore this.

7.3. Limitations

7.3.1. Limitations of the training studies

Five out of six studies in this thesis are based on training studies where participants have to engage in training sessions across 4-8 weeks. A few limitations of the training study design need to be considered. Firstly, all training sessions have been supervised in order to gain the maximal adaptations to exercise. However, other lifestyle factors such as physical activity or diet might be changed during the training period which could affect the changes in $\dot{V}O_2$max.
Although all participants were asked to maintain their lifestyles over the training period, there is no objective data recorded to confirm this.

Secondly, the changes in $\dot{V}O_2\text{max}$ might be affected by a variation in diet (Zajac et al., 2014; Muoio et al., 1994). Therefore, all participants have been asked to fill in a food diary on the day before the pre-training $\dot{V}O_2\text{max}$ test. On the day before the post-training $\dot{V}O_2\text{max}$ test, they were asked to maintain a similar diet and fill in the food diary again. When considering the diet record on the day before performing the pre and post-training $\dot{V}O_2\text{max}$ tests, it was similar but it was not exactly 100% the same. Moreover, the nutrition data was not analysed in this thesis. Therefore, the difference of nutrition composition could not be identified.

In future studies, it would be better to separate the effects of the training intervention. Meals on the day before the $\dot{V}O_2\text{max}$ test should be provided for participants (although this might be costly). Furthermore, the participants were asked not to perform any moderate or strenuous exercise on the day before the $\dot{V}O_2\text{max}$ test. This information was verbally confirmed by each participant, but any objective data were not collected. In future studies, it would be beneficial to obtain an objective measure of physical activity levels prior to the $\dot{V}O_2\text{max}$ test.

7.3.2. Limitations of psychological responses to REHIT

Since all studies in this thesis were laboratory-based, the psychological responses may not be reflective of ‘real-life’ responses. Until the mechanisms for training adaptations are better understood, the only way to optimise training protocols is to try out different combinations of training parameters (frequency, intensity, duration, number of repetitions, recovery time or mode). However, there are too many possible combinations to study, and there will be varying individual responses as to what is the most effective protocol is. Therefore, it remains
unknown what ‘the most effective protocol’ is. Nonetheless, the studies in this thesis have made progress in identifying the effects of important protocol parameters.

7.4. Recommendations for Future Research

Despite the improvements of $\dot{V}O_2$max following REHIT in inactive young adults and middle aged T2D participants, as observed in this thesis, other research areas still need to be explored. Mechanisms underlying this physiological adaptation need to be examined. Moreover, the effects of REHIT on other health parameters in varying groups of people are unknown. Importantly, long term exercise adherence to REHIT also requires further investigation. Finally, “real-life” REHIT settings (e.g. gym or office) need to be investigated in order to explore the impact of REHIT in the public domain, not just in the laboratory.

7.4.1. Possible mechanisms underlying the improvement of $\dot{V}O_2$max following REHIT

In Chapter 2, some possible mechanisms have been examined following a single ‘all-out’ sprint protocol, based on the finding of Parolin et al. (1999). Indirect methods have been investigated for muscle glycogen depletion and serum SPARC levels have been assessed. According to the outcomes from Chapter 2, training sessions consisting of only a single sprint were insufficient to stimulate physiological adaptations, and therefore it is likely that glycogen depletion alone is not the sole stimulus for adaptations. Moreover, neither $\dot{V}O_2$max nor SPARC levels changed, so no conclusions about the role of SPARC in changes in $\dot{V}O_2$max can be made. Unfortunately, no potential mechanisms can be drawn from this thesis. As mentioned previously, the possible mechanisms underlying physiological adaptations following SIT are related to several signalling
molecules and mitochondria biogenesis (Gibala et al., 2012; MacInnis and Gibala, 2017), suggesting that training adaptations following SIT depend on peripheral adaptations (skeletal muscle) more than central adaptations (heart). Therefore, in future studies, it might be worth investigating any changes inside the skeletal muscles (e.g. SPARC gene expression, muscle glycogen, AMPK, P38MAPK, PGC1-α or mitochondrial enzyme activity) using muscle biopsy in order to find the possible mechanisms.

7.4.2. The health benefits of REHIT in various populations

The main health parameter used in this thesis was $\dot{V}O_2$max. Furthermore, insulin sensitivity and blood pressure were also examined in T2D individuals (Chapter 6). Importantly, $\dot{V}O_2$max is improved following REHIT, both in inactive young adult participants and T2D participants. However, the effects of REHIT in other groups of individuals are still unknown. It would be useful to conduct further research to determine the effectiveness of REHIT in other individual groups, e.g. obese/overweight, metabolic syndrome individuals or patients with other chronic diseases. Moreover, the effects of REHIT on other health parameters which related to the risk of death (e.g. blood pressure or blood lipid profiles) need to be confirmed.

Importantly, the studies in this thesis do not provide an indication that REHIT is unsafe for inactive young adult individuals or patients with T2D who have been screened for a number of risk factors (e.g. BP / PARQ / ECG / etc). It remains unknown whether there are any significant risks associated with REHIT in other populations, or people with specific (undiagnosed) risk factors, and this needs to be investigated in future studies.
7.4.3. Long term exercise adherence to REHIT

The psychological responses to REHIT have been investigated in this thesis (Chapters 3, 4 and 5). The findings demonstrated that the responses to REHIT were no more negative than those following MICT, which is the current exercise recommendation. It can be proposed that there is a possibility for people to engage in REHIT as an alternative to MICT. Nevertheless, the in-task affective responses (Chapters 4 and 5) and other questionnaires that are associated with psychological responses to REHIT (Chapter 3) might not provide sufficient information to predict long term adherence to REHIT. In terms of future studies, it would be interesting to determine the exercise adherence of REHIT under long-term, real-life conditions.

7.4.4. “Real-life” REHIT setting

All training sessions in this thesis were performed at the physiology laboratory and were supervised by specialist researchers. A number of benefits of REHIT have been seen during the training sessions. Firstly, very little sweating was caused by the REHIT sessions, and therefore the participants did not need to change their clothes or have a shower after finishing the training session. Secondly, special sportswear was not needed for REHIT. In this thesis, participants could wear whatever they wanted when they came to do the training sessions (jeans, skirts or boots), as long as those clothes allowed them to move their legs freely. This suggests that REHIT is a convenient protocol to perform. However, the REHIT protocol (and other SIT protocols) requires a special bike for training, and this might present a limitation if REHIT is promoted to the public. Nonetheless, a special bike for REHIT has been developed by a commercial company (CAR.O.L, London, UK). It would be a great opportunity to make REHIT
more widely available. Overall, it would be useful to examine the beneficial effects of REHIT and the exercise adherence to REHIT with the developed bike in “real-life” settings such as the gym, office, or school, for confirming the effectiveness of REHIT.

7.5. Conclusions

This thesis has attempted to optimise the REHIT protocol to be a truly time-efficient exercise protocol which improves $\dot{V}O_2\text{max}$. From the findings in Chapters 2, 3, 4 and 6, it could be concluded that the most effective and time-efficient exercise protocol to improve $\dot{V}O_2\text{max}$ consists of two repeated 20-s all-out sprints within 10 minutes total training time, and with a training frequency of at least two times per week. The increase in $\dot{V}O_2\text{max}$ following REHIT could be found in both inactive young adults and T2D participants. Moreover, the measurement of psychological responses to REHIT (Chapters 3, 4, 5 and 6) demonstrated that the REHIT protocol is tolerable (average RPE is 13), that there was no negative valence during REHIT, and that the majority of participants agreed that REHIT was enjoyable. This might aid long-term adherence to REHIT but this hypothesis needs to be confirmed. Finally, the mechanism underlying the improvement of $\dot{V}O_2\text{max}$ remain to be elucidated. However, the findings from this thesis provide evidence to confirm that an important health risk factor ($\dot{V}O_2\text{max}$) could be addressed by REHIT, and that REHIT is a manageable time-efficient exercise protocol. It can be proposed that REHIT could be an alternative exercise protocol alongside the current physical activity recommendations and it might be a protocol for encouraging people to be more active, especially people who define that their main exercise barrier is lack of time.


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