Effects of an acute priming exercise and high-intensity interval training on oxygen uptake and muscle deoxygenation kinetics in older individuals with type 2 diabetes.

Thesis submitted for the degree of Doctor of Philosophy in Physiology at the University of Dublin, Trinity College

Aaron Nevin

Submitted July 2021

Department of Physiology, School of Medicine

Trinity College, Dublin

I. DECLARATION

I declare that this thesis is entirely of my own work with the exception of the testing of 5 participants at week 0 of the intervention and 14 older participants with type 2 diabetes which was completed by Dr Norita Gildea, Dr Joel Rocha and Dr Adam McDermott, the completion of the majority of the exercise stress tests with the study participants by Dr Norita Gildea, Adam McDermott and Dr Mehmoona, the collection of blood samples by Dr Joel Rocha and Dr Noreen Boyle, the recruitment of 21 of the participants who completed the intervention by Dr Norita Gildea with the remainder recruited by Dr Adam McDermott, and the recruitment of older non-diabetic participants by Adam McDermott. This thesis has not been previously submitted for a degree in this or any other university. Trinity College Dublin may lend or copy this thesis on request.

Signature: _____ Date: _____

II. ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisor Dr Mikel Egaña for giving me the opportunity to undertake this PhD. I am very grateful for his guidance, support and patience with me throughout the last 4 years, and especially during the final year. He helped open many doors for me whether it was through funding for academic conferences or learning many skills with which I wasn't acquainted with before my time working with him. I know working with him will stand to me with whatever I do in the future.

I gratefully acknowledge the Health Research Board for the funding provided for the work described within this thesis and Trinity College Dublin for the funding that they provided me in order to undertake this PhD.

I would like to thank Dr Adam McDermott for all his help in the lab for the past few years. There's no way I would have got through the PhD without Adams help so for that I am eternally grateful to him. The chats about rugby also helped the time to fly with the exception of talking about how much better Leinster are than Munster these past 4 years.

I would like to thank Dr Norita Gildea who finished just when I had started in Trinity. While we didn't get to work together in the lab much, she was always there (along with Adam) to help out with the never-ending amounts of data analysis with this study.

I would like to thank Dr Domenico Crognale who worked as a postdoc during my first 3 months. He helped make it very easy to settle in during the first few months. I would also like to thank Dr Joel Rocha who I've never met but whose excel spreadsheets made life very easy with regards to data input.

I would like to thank all the staff at St Colmcille's and St Vincent's hospital, especially Prof Donal O'Shea. I would also like to thank the staff at Loughlinstown leisure centre for allowing us to use their facilities and help support this study.

I would like to thank all the study participants. Each and every one of them showed unbelievable commitment to the study and were all a pleasure to work with.

I would like to thank all the staff and students in the Physiology department in Trinity College Dublin that I worked with and who helped out with this project throughout the past

4 years. Special thanks goes Noreen, Aidan, Quentin, Bernard, Christine, Dave, Alice, Deirdre, Wilby, Aine, Alice (Witney) and Carol.

I would like to thank all my friends from Nenagh who put up with me over the past 4 years. Without their support and many nights out I don't think I would have got through this.

I would like to thank my landlady, Anne Seymour. Without Anne, I would have never been able to move up to Dublin in the first place and none of this would have been possible. I am forever grateful to her and the rest of her family in Nenagh.

I would like to thank my girlfriend Dervla for her support throughout the final part of the thesis and for being a light during the dark times of the Covid lockdowns, especially in recent months.

I would like to thank my family for all their support throughout the last 4 years. I will be indebted to all of them forever for supporting me throughout the past 4 years and especially the last few months. I would like to thank my grandfather and my two grandmothers, one of which I lost while doing this PhD. And finally I would like to thank my parents for helping me with everything these past 4 years. I can't put into words how grateful I am for everything you have done so thank you.

III. ABSTRACT

Peak oxygen uptake ($\dot{V}O_{2peak}$) is consistently reduced in individuals with type 2 diabetes (T2D) of all ages compared with non-diabetic (ND) controls. In middle-aged (between 45 to 60 y) individuals with T2D these reductions in $\dot{V}O_{2peak}$ are influenced by a reduced rate of microvascular blood flow adaptations, but it is unknown if these vascular impairments are apparent in older individuals with this disease. In addition, the dynamic response of $\dot{V}O_2$ at the onset of moderate-intensity exercise ($\dot{V}O_2$ kinetics) is blunted (i.e. slowed) in middle-aged, but not older (between 60 to 70 y) people with T2D compared with age-matched corresponding ND controls. This is likely due to the powerful deleterious effect of ageing *per se* mainly in untrained individuals. Understanding how to ameliorate these impairments and the confounding effects of older age is important given that these limitations may lead to cardiovascular complications and all-cause mortality.

In this regard, an acute bout of heavy-intensity priming exercise (PE) as well as short-term training interventions involving moderate-intensity continuous training (MICT) and/or low-volume high-intensity interval training (HIIT) have been shown to be effective at speeding $\dot{V}O_2$ kinetics and thus, improving exercise tolerance mainly in middle-aged adults with uncomplicated T2D. However, these effects have not been assessed in older individuals living with T2D.

To further explore these important adaptations, *Experiment 1* examined the influence of T2D on muscle deoxygenation ([HHb+Mb]) during ramp incremental cycle exercise in older adults. Fourteen older untrained adults with T2D (62.9 ± 2.8 y; 29.0 ± 3.7 $kg.m^{-2}$; 9 males/5 females) and 14 untrained older ND controls (64.8 ± 3.9 y; 27.1 ± 2.8 $kg.m^{-2}$; 10 males/4 females) completed the ramp cycle test. Normalised [HHb+Mb] profiles of the vastus lateralis muscle (%[HHb+Mb]) were plotted as a function of relative peak power output (%PO) using a bilinear model. $\dot{V}O_2$peak was significantly reduced in older individuals with T2D compared to ND controls (27.1 ± 5.7 vs. 23.3 ± 4.0 $mL.kg^{-1}.min^{-1}$), however, no difference was observed in the primary slope of the Δ%[HHb+Mb]/Δ%PO. These findings suggest that in contrast to middle-aged individuals with T2D, a greater rate of fractional O_2 extraction in the exercising musculature for a given increase in $\dot{V}O_2$, indicative of reduced O_2

delivery, is not a contributing factor to the reduction in $\dot{V}O^2$peak observed in older individuals with T2D compared to older ND controls.

Experiment 2 investigated the effects of a heavy-intensity PE on a subsequent heavy-intensity cycling exercise in older individuals with T2D (62.5 ± 3 y; 29.4 ± 4.5 kg.m^{-2}; 8 males/1 female) and older ND controls (63.3 ± 3.9 y; 28.4 ± 2.3 kg.m^{-2}; 8 males/1 female). No differences were observed between groups for $\dot{V}O_2$ [HHb+Mb] kinetics in the unprimed bout. Following PE, both groups displayed a significant acceleration for the time constant of the $\dot{V}O_2$ primary phase ($\tau\dot{V}O_{2P}$) at the onset of exercise (T2D: 40 ± 3 vs. 32 ± 3 s; ND: 43 ± 5 vs. 34 ± 6 s). A significant reduction in the slow component ($\dot{V}O_{2SC}$) amplitude was also observed in both groups (T2D: 0.3 ± 0.1 vs. 0.2 ± 0.1 L.min^{-1}; ND: 0.3 ± 0.1 vs. 0.2 ± 0.1 L.min^{-1}). This occurred in conjunction with a slower muscle [HHb+Mb] time constant of the primary phase (Δ[HHb+Mb]τ_p) in both groups, suggesting an improvement in O_2 delivery to utilisation within the exercising muscle which may enhance exercise tolerance in both groups.

When initiating high-intensity exercise from an elevated baseline (work-to-work: WtoW), middle-aged individuals with T2D display a sluggish $\dot{V}O_2$ kinetic response compared to that observed during heavy-intensity exercise initiated from an "unloaded" baseline. The objective of *Experiment 3* was to further investigate this influence of PE on $\dot{V}O_2$ and [HHb+Mb] kinetics during a subsequent WtoW exercise bout in older adults with T2D (62.2 ± 2.8 y; 30.1 ± 3.3 kg.m^{-2}; 8 males/2 females) and ND controls (64 ± 4.2 y; 27.6 ± 2.5 kg.m^{-2}; 8 males/2 females). No differences were observed between groups for any responses during the unprimed bouts. Following PE, a reduction occurred in both, the $\tau\dot{V}O_{2P}$ response (T2D: 49 ± 6 vs. 34 ± 4 s; ND: 49 ± 7 vs. 38 ± 9 s) and the amplitude of the $\dot{V}O_{2SC}$ (T2D: 0.2 ± 0.0 vs. 0.1 ± 0.0 L.min^{-1}; ND: 0.2 ± 0.1 vs. 0.1 ± 0.1 L.min^{-1}). No changes were observed for any [HHb+Mb] responses following PE. These results indicate that PE induced a speeding $\dot{V}O_2$ kinetics response due to a faster contribution from the aerobic metabolism via improvements in O_2 delivery as well as an alteration in motor unit recruitment, and that these effects were similar in both groups.

Experiment 4 investigated the effects of 12 weeks of MICT and low-volume HIIT on $\dot{V}O_2$ and [HHb+Mb] kinetics during transitions to heavy-intensity cycling exercise initiated

from an unloaded baseline. Participants with T2D were randomly assigned to a control (CON) group (53.9 ± 9.4 y; 30.5 ± 3.6 $kg.m^{-2}$; 4 males/5 females), a MICT group (54.2 ± 9.9 y; 31.3 ± 5.8 $kg.m^{-2}$; 7 males/3 females; 50 min of moderate-intensity cycling) and a HIIT group (52 ± 10.1 y; 28.8 ± 3.2 $kg.m^{-2}$; 6 males/3 females; 10 x 1 min high-intensity bouts at ~90% maximal heart rate interspersed by 1 min of unloaded cycling). Exercise groups trained 3 times per week with the intensity adjusted every 3 weeks. After 12 weeks, $\tau\dot{V}O_{2P}$ was accelerated for both MICT (35±4 vs. 24±4 s) and HIIT (33±5 vs. 26±3 s) groups, with no differences observed in the CON group (32±4 vs. 31±5 s). Additionally, a significant slowing of the $\Delta[HHb+Mb]\tau_p$ response occurred in both exercising groups but not in the control group. Taken together, these results indicate that both exercising groups induced similar benefits in $\dot{V}O_2$ kinetics due to improvements in O_2 delivery and/or distribution relative to O_2 utilisation.

In conclusion, the accumulated data for the first three experiments of this thesis indicate that no apparent differences in pathophysiological mechanisms are evident with respect to peripheral factors for the impaired exercise tolerance observed in older people with and without T2D. In addition, an acute intervention such as heavy-intensity PE may be effective at improving microvascular blood flow to the exercising muscle, thus, improving the aerobic contribution to exercise and, thereby, increasing exercise tolerance in inactive older adults with and without T2D. Finally, given that the magnitude of effects on $\dot{V}O_2$ kinetics subsequent to low-volume HIIT and MICT were similar in *Experiment 4*, it is possible that low-volume HIIT, with half the time commitment and volume than MICT, may be a more effective intervention to increase both, exercise involvement and adherence in people with uncomplicated T2D.

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V. ABBREVIATIONS AND SYMBOLS

~	approximately
β	beta
[ ]	concentration
ºC	degrees Celsius
Δ	delta; a difference or a change in value
E	extinction coefficient
≥	greater than or equal to
≤	less than or equal to
Λ	wavelength
ABI	ankle: brachial index
ACSM	American College of Sports Medicine
ADA	American Diabetes Association
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
ATPase	enzyme that catalyses the decomposition of ATP into ADP
a.u.	arbitrary units
a-vO2 diff	arteriovenous oxygen difference
BbB	breath-by-breath
beats.min^{-1}	beats per minute
BMI	body mass index
BP	blood pressure
BP	break point
Ca^{2+}	calcium
CAD	coronary artery disease
CAMKII	Ca^{2+} /calmodulin-dependent protein kinase II
CaO_2	arterial oxygen concentration
CK	creatine kinase
Cm	centimetre
CO	cardiac output

CO_2	carbon dioxide
CON	control
CVD	cardiovascular disease
CvO_2	venous oxygen concentration
CW	continuous wave
CWR	constant work rate
DBP	diastolic blood pressure
ECG	electrocardiogram
EMG	electromyogram
ET	endurance training
ETC	electron transport chain
Fig	figure
FMD	flow-mediated dilation
FPG	fasting plasma glucose
(ΔG_{ATP})	negative Gibbs free energy of ATP hydrolysis
G	change in VO_2 per unit change in external work (i.e. $\Delta VO_2/\Delta PO$)
GET	gas exchange threshold
GLP-1	Glucagon-like peptide-1
GLUT4	glucose transporter type 4
H^+	hydrogen ion
Hb	haemoglobin
HbA_{1c}	glycated haemoglobin
HbO_2	oxyhaemoglobin
$h.day^{-1}$	hours per day
HDL-C	high-density lipoprotein cholesterol
HHb	deoxyhaemoglobin
[HHb+Mb]	concentration of deoxygenated haemoglobin and myoglobin
Δ[HHb+Mb]	changes in the deoxy haemoglobin and myoglobin concentration
[HHb+Mb]-BP	deoxyhaemoglobin and myoglobin break point
[HHb+Mb]τp	time constant of deoxygenated haemoglobin and myoglobin
$\Delta[HHb+Mb]/\Delta\dot{V}O_2$	haemoglobin and myoglobin deoxygenation to oxygen uptake ratio

HIIT	high intensity interval training
HR	heart rate
HRmax	maximum heart rate
HRpeak	peak heart rate
Hz	hertz
iEMG	integrated electromyogram
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
K^+	potassium
Kg	kilogram
$Kg.m^{-2}$	kilogram per metre squared
LBF	leg blood flow
LDL-C	low-density lipoprotein cholesterol
$L.min^{-1}$	litre per minute
LOPAR	Low Level Physical Activity Recall questionnaire
LT	lactate threshold
LVC	leg vascular conductance
LVHIIT	Low volume high-intensity interval training
M	metre
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
Max	maximum
Mb	myoglobin
MET	metabolic equivalent of task
$Mg.dL^{-1}$	milligrams per decilitre per minute
MICT	Moderate-intensity continuous training
Min	minute
mL	millilitre
$mL.kg^{-1}.min^{-1}$	millilitre per kilogram per minute
mm	millimetre
mM	millimolar

mmHg	millimetre of mercury
$mmol.L^{-1}$	millimoles per litre
Mod	moderate-intensity
MRT	mean response time
ms^{-1}	millisecond
mV	milliVolt
MVC	maximum voluntary contraction
n	number of participants
Na^{+}	sodium
ND	without type 2 diabetes
NIRS	near infra-red spectroscopy
Nm	nanometre
NO	nitric oxide
N_2O	nitrous oxide
NOS	nitric oxide synthase
O_2	oxygen
P	probability (statistical)
PAD	peripheral arterial disease
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
PE	priming exercise
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator
pH	a logarithmic scale used to express the acidity or alkalinity of a solution
Phase I	cardiodynamic phase of the $\dot{V}O_2$ kinetics response
Phase II	primary phase of the $\dot{V}O_2$ kinetics response
Phase III	attainment of steady state of the $\dot{V}O_2$ kinetics response
Pi	inorganic phosphate
$Pm\dot{V}O_2$	microvascular O_2 partial pressure
PO	power output
PO_2	partial pressure of oxygen
PWV	pulse wave velocity

Qm	muscle blood flow
R	correlation coefficient
RCP	respiratory compensation point
RER	respiratory exchange ratio
RI	ramp incremental
RPE	rating of perceived exertion
RPM	revolutions per minute
RST	repeated sprint training
S	second
SBP	systolic blood pressure
SD	standard deviation
SV	stroke volume
t	time
tau, τ	time constant
τ'	effective time constant
TCD	Trinity College Dublin
TCL	total cholesterol
TD	time delay
T2D	type 2 diabetes mellitus
TTF	time to failure
$\tau\dot{V}O_{2p}$	time constant of the primary phase of the $\dot{V}O_2$ kinetics response
UKPDS	UK Prospective Diabetes Study
$\dot{V}CO_2$	carbon dioxide output
$\dot{V}E$	expired minute ventilation
$\dot{V}E/\dot{V}CO_2$	ventilatory equivalent for CO_2
$\dot{V}E/\dot{V}O_2$	ventilatory equivalent for O_2
VL	vastus lateralis
$\dot{V}O_2$	pulmonary oxygen uptake
$\dot{V}O_2max$	maximal oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
$\dot{V}O_{2SC}$	oxygen uptake 'slow-component'

Vs	versus
VT	ventilatory threshold
W	watts
$W.min^{-1}$	watts per minute
WHO	World Health Organisation
WHR	waist to hip ratio
WtoW	work-to-work
yr	year

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Chapter 1: Introduction

1.1 Overview

Due to the global prevalence of both the obesity epidemic and drastically reduced physical activity levels, type 2 diabetes (T2D) is now one of the most common chronic diseases in the world (Whiting *et al.* 2011; WHO, 2016). Individuals with T2D demonstrate impairments in physical fitness characterised by a reduced peak ($\dot{V}O_{2peak}$) and submaximal constant-load exercise tolerance, both of which correlate with cardiovascular disease and all-cause mortality.

The rate at which pulmonary oxygen uptake ($\dot{V}O_2$) adjusts at the onset of a constant-load exercise ($\dot{V}O_2$ kinetics) determines at least in part an individual's level of exercise tolerance (Jones and Poole, 2005). A sluggish increase in this response will incur a greater O_2 deficit resulting in greater utilisation of anaerobic energy stores to maintain ATP regeneration, leading to intracellular perturbations resulting in premature fatigue. Previous research has proven that the $\dot{V}O_2$ kinetic response is sluggish in middle-aged individuals with T2D when compared with non-diabetic (ND) counterparts (Green *et al.* 2015). The mechanisms responsible for this lethargic $\dot{V}O_2$ kinetic response at the onset of exercise appear to be at least in part, related to impairments in microvascular O_2 delivery and/or distribution throughout the contracting muscle.

Importantly, unlike in young middle-aged individuals with T2D, $\dot{V}O_2$ kinetic responses in older individuals with T2D are not impaired compared to age-matched ND controls during moderate-intensity cycling exercise (Wilkerson *et al.* 2012; O'Connor *et al.* 2015). Therefore, it appears that in older individuals without T2D the effect of ageing, at least in inactive otherwise healthy people, has a very powerful deconditioning effect on the cardiovascular system and skeletal muscle metabolism, which in turn leads to a slowing of the $\dot{V}O_2$ kinetic response during moderate-intensity exercise (O'Connor *et al.* 2015). Importantly, an acute heavy-intensity priming exercise (PE) bout has been shown to accelerate $\dot{V}O_2$ kinetics during a subsequent bout of moderate- to severe-intensity exercise in populations displaying an initial slowed $\dot{V}O_2$ kinetics, including older individuals (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2009) and middle-aged people with T2D (Rocha *et al.* 2019; Gildea *et al.* 2020). However, it is not known whether such an acute intervention (i.e. PE) can be

effective in speeding $\dot{V}O_2$ kinetics in subsequent constant-load exercise bouts in older individuals with T2D compared with age matched ND controls.

Furthermore, whilst exercise interventions incorporating short-term moderate-intensity continuous training (MICT) have proven effective at improving both $\dot{V}O_{2peak}$ and $\dot{V}O_2$ kinetics during low to high-intensity exercise in T2D, there is growing interest in investigating whether the time-efficient low volume high-intensity interval training (HIIT) interventions can induce comparable benefits to those observed following MICT in individuals with T2D. In the present thesis the focus will be on benefits on $\dot{V}O_2$ kinetics during heavy-intensity constant-load exercise transitions.

This introduction will discuss the characteristics and underlying mechanisms of the exercise intolerance typically observed in individuals with T2D as well as in older untrained ND individuals. It will then describe the key physiological adaptations induced by an acute PE intervention in these populations. Finally, it will briefly review the physiological and performance effects of short-term MICT and HIIT interventions on individuals with T2D with a particular focus on $\dot{V}O_2$ kinetics responses.

1.2. Type 2 Diabetes

Type 2 diabetes mellitus (T2D) is characterised by chronic elevated levels of blood glucose due to β-cell dysfunction, insulin resistance (i.e reduced insulin-dependent glucose clearance into the liver, adipose tissue or skeletal muscle) and excess hepatic glucose production (Leahy, 2005). Criteria for diagnosis of T2D includes a fasting plasma glucose level ≥7 mmol.l^{-1}; a 2-h post-prandial plasma glucose level ≥11.1 mmol.l^{-1}; and glycated haemoglobin (HbA_1c) level >6.5% (WHO, 2006; 2011).

1.2.1. Prevalence and cost

The number of adults with diabetes worldwide has almost doubled (from 4.7% to 8.7%) since 1980, with 422 million adults estimated to have the disease as of 2014 (WHO, 2016). The global prevalence of diabetes is estimated to reach 592 million by 2035 (Guariguata *et al.* 2014) with T2D accounting for 90% of the cases (Nolan *et al.* 2006). Studies from the USA indicate that the prevalence of impaired fasting glucose and glucose intolerance (prediabetes) together accounts for 30% of the adult population (≥20 years of age) (Cowie *et al.* 2009). This epidemic is occurring as a result of increased population ageing, urbanization and lifestyle changes (Chen *et al.* 2011; WHO, 2016). The fall in the age at which people develop diabetes will also be a key factor in the future for the global epidemic of T2D.

In Ireland, a 2007 estimate for doctor-diagnosed diabetes was 3.7% with it being more common among those >45 years (6.7%) compared to adults between 18 to 44 years (0.7%) (Balanda *et al.* 2013). A cross-sectional study sampling 2047 people within the age of 50 to 69 years by O'Connor *et al* (2013) found 8.5% of this population to have T2D with nearly half of this sample (41%) having undiagnosed T2D highlighting the need for more blood glucose screening, especially in people above the age of 45 years in Ireland (O'Connor *et al.* 2013). Tracey *et al* (2015) found the prevalence of T2D in Ireland to be at 8.4% in people >50 years with the highest prevalence being in those >75 years (11.9%) followed by individuals within the age of 65 to 74 years (11.1%) and in those within the age of 50 to 64 years (6.3%) (Tracey *et al.* 2015). In this study that focused on adults above the age of 50 years, the prevalence of macrovascular and microvascular complications was 15.1% and 26.0% respectively, with neuropathy and retinopathy being the most prevalent microvascular complications (Tracey *et al.* 2015). The total cost associated with diagnosed and

undiagnosed diabetes in Ireland is estimated to be €580.2 million which corresponds to 6.4% of the annual healthcare expenditure (Nolan *et al.* 2006). In patients with microvascular and macrovascular complications, such as neuropathy and angina, the healthcare cost is increased a further 1.8 and 2.9 fold compared with patients who don't display such complications (Nolan *et al.* 2006). This indicates that finding inexpensive and effective methods for preventing as well as treating diabetes and stemming the onset of further complications in individuals with diabetes is key to reducing the strain on the healthcare budget in Ireland.

1.2.2. Risk factors for T2D

The overall consensus on the development of T2D is that both genes and the environment they are exposed to are important determinants of insulin resistance and β-cell dysfunction (Mahajan *et al.* 2014). The risk of developing T2D increases by 40% in the offspring of a parent with T2D (Parikh and Groop, 2004), with insulin being the best predictor for the development of T2D indicating a strong genetic predisposition (Warram *et al.* 1990). The vast majority of loci for the prediction of T2D are related to β-cells, however, Meigs *et al* (2008) showed that using risk alleles to predict the onset of diabetes only slightly increased the prediction value more than knowledge of common risk factors alone (Meigs *et al.* 2008). Men are more likely to develop T2D than women due to developing diabetes at a lower BMI as well as having a greater predisposition to central adiposity and insulin resistance, both of which interlink in relation to liver insulin sensitivity and visceral fat (Tracey *et al.* 2016).

Since our gene pool has not changed recently, environmental factors are key for preventing, developing and treating T2D (Leahy, 2005). Key environmental factors include caloric intake, energy expenditure, nutrient composition, lifestyle, body composition (especially within the intra-abdominal cavity) and ageing (Leahy, 2005; Kahn *et al.* 2014). Therefore, interventions involving diet, exercise and improving lifestyle habits are key to slowing the progression of T2D.

1.2.3. Insulin resistance and β-cell dysfunction

Type 2 diabetes is accompanied by obesity in the vast majority of incidences, with both associated with insulin resistance (Kahn *et al.* 2006). This led to the conclusion that insulin resistance plays an important pathophysiological role in T2D by decreasing glucose uptake

into the muscle and excessive hepatic glucose production (HGP) resulting in hyperglycaemia and eventually, reduced glucose tolerance (Cerasi, 2011). This resistance results in a higher level of circulating insulin needed at the targeted tissue to activate insulin action on the cell membrane and glucose uptake into the cell (De Tata, 2008). For this to occur, a greater amount of insulin must be secreted from the pancreatic β-cells. This will place a greater strain on the pancreatic β-cells in order to compensate for insulin resistance.

However, many people who are obese and insulin-resistant do not develop T2D (Lowell & Shulman, 2005). In individuals who are insulin resistant, the mechanism for not developing reduced glucose tolerance or T2D involves simply increasing their insulin output by both increasing the mass and maintaining the number of β-cells as insulin resistance increases (Lowell & Shulman, 2005; Kahn *et al.* 2006). One conclusion stemmed from this is that in T2D, the individuals' pancreatic islet β-cells can't compensate efficiently for increased insulin resistance (Kahn *et al.* 2006). This inefficient insulin secretion by pancreatic islet β-cells is known as β-cell dysfunction. This exists in individuals who are at high risk of developing T2D even when glucose homeostasis is normal, while continued decline in β-cell function in people with T2D is associated with exacerbating the disease (Kahn, 2001). The pathogenesis of β-cell dysfunction appears to be genetic with abnormal insulin secretion from these cells displayed well before a reduced glucose tolerance in the development of T2D (Wayer *et al.* 1999; Jensen *et al.* 2002). Therefore, the progressive loss of β-cell function for insulin secretion and insulin resistance in the liver, muscle and adipose tissue are interlinked in the development of T2D (Khan, 2003; Hordern *et al.* 2012; Cerasi, 2011).

1.2.4. Ageing and T2D

Progressive skeletal muscle atrophy along with a decline in mitochondrial function is proposed to be associated with age-related reduction in exercise capacity as well as a decline in insulin sensitivity (Reusch *et al*. 2018). Muscle mass reduction occurs due to a negative balance between muscle protein synthesis and muscle protein breakdown (Koopman *et al*. 2007; Tieland *et al*. 2018). Protein breakdown rates are similar in both young and old people. However, there is a significant decline in protein synthesis in the elderly population with reduced muscle protein synthetic responses to anabolic stimuli compared to younger healthy controls (Cuthbertson et al. 2005; Wall et al. 2015; Kumar et al. 2005). Since protein breakdown is greater than synthesis, a reduction in muscle mass

results. A shift in muscle fibre type composition has been proposed especially due to inactive ageing with the majority of skeletal atrophy taking place within type II fibres (Verdijk *et al*. 2010). The loss of either type of muscle fibre with age may be attributable to a reduction in activity levels, especially high intensity activity, leading to disuse and denervation (McGregor *et al*. 2014).

There is evidence that aerobic capacity may decline at an accelerated rate after the age of 50 years (Fleg *et al*. 2005; McGregor *et al*. 2014), especially in inactive older adults (George et al. 2018). Cross-sectional evidence from healthy men and women aged 18–90 years indicates that vastus lateralis mitochondrial DNA, mRNA transcription and ATP production are inversely correlated with age (Short et al. 2005; McGregor et al. 2014; Tieland et al. 2018). Furthermore, during endurance exercise in elderly individuals, a sluggish aerobic response as represented by $\dot{V}O_2$ kinetics has been observed during the early exercise transition (Boffoli *et al*. 1994; Gurd *et al*. 2007; 2009). This may be due to a slower activation of aerobic metabolism within the contracting muscle of older adults (Boffoli *et al*. 1994; Gurd *et al*. 2007; 2009). During moderate-intensity exercise, Gurd *et al* (2007) found a slower $\tau\dot{V}O_{2P}$ in older (40 ± 17 s) compared to young (21 ± 6 s) healthy adults (Gurd *et al*. 2007). This occurred in conjunction with an attenuated activation of mitochondrial pyruvate dehydrogenase (PDH) in the older adults. This impairment in activation of a rate-limiting enzyme for oxidative phosphorylation and thus, aerobic metabolism within the contracting skeletal muscle may suggest a possible role for oxidative enzymes and provision of substrate to the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) as a source for the initial sluggish aerobic metabolism/$\dot{V}O_2$ kinetics observed at exercise onset in older inactive adults (Gurd *et al*. 2007; 2009).

Glucose tolerance declines progressively with age leading to a high incident rate of T2D in elderly populations (Chang & Halter, 2003). Impaired insulin secretory effects with ageing are magnified with other factors related to ageing such as insulin resistance, reduced energy expenditure/physical activity, obesity, sarcopenia and coexisting illness. Age-related insulin resistance is primarily correlated with obesity, sarcopenia and reduced energy expenditure/physical activity.

Blood glucose levels indicative of impaired glucose tolerance are common in people over 60 years (Chang & Halter, 2003). For instance, when taking into account body composition and

adiposity-related insulin resistance, insulin levels in response to an oral glucose tolerance test were reduced with age. Older subjects displayed dysfunctional pulsatile insulin secretion, showing reduced mass and amplitude of rapid (10 mins) insulin pulses and reduced frequency, amplitude and regularity of ultradian (50-170 mins) oscillations compared to young subjects (Chang & Halter, 2003). These results are indicative of reduced β-cell function with ageing. In addition, the second wave β-cell response to glucose is reduced with ageing along with increased relative insulin resistance which underlies the reduction in glucose tolerance with age (Chen *et al.* 1988). This second-phase reduced insulin secretion would lead to prolonged and greater post-prandial hyperglycaemia which could lead to compensatory hyperinsulinemia and eventual dysfunction of β-cell response leading to T2D. Therefore, in elderly people, interventions targeting hyperglycaemia, insulin signalling and reducing the strain on β-cell insulin secretion are necessary to prevent and treat T2D, whilst reducing the risk of developing cardiovascular complications (Kirkman *et al.* 2012).

1.2.5. Major health complications in T2D

Poor glycaemic control is proposed to play a significant role in the development of cardiovascular disease (CVD), with post-prandial hyperglycaemic spikes and hyperglycaemia throughout the day being important risk factors for the development of macrovascular and microvascular complications in T2D (Ceriello, 2005; Manders *et al.* 2009). Acute and chronic hyperglycaemia in T2D is responsible for altered homeostasis including oxidative stress leading to endothelial dysfunction and diabetic complications of the cardiovascular system (Ceriello, 2005). Peripheral arterial disease, large-artery atherosclerosis and stroke are the most common types of CVD in T2D, with poor glucose control linked with higher mortality and bad post-stroke health outcomes in stroke patients (Chen, 2016). Coronary artery disease (CAD) accounts for 80% of all deaths in T2D (Mohan *et al.* 2010). Overall, people with diabetes are nearly twice as likely to develop a range of CVD compared to non-diabetic individuals (Diabetes in the UK, 2012). Therefore, interventions that help prevent diabetic CVD complications are critical. These include reducing endothelial dysfunction, arterial stiffness, systemic vascular inflammation, and reducing basal membrane thickness of the capillaries.

1.2.6. <u>Pharmacotherapy and T2D:</u>

Pharmacotherapy helps prevent disease progression and development of other co-morbidities in T2D by managing glycaemic control. The following are the most common pharmacotherapies used in the management of T2D:

Sulfonylureas: Lower blood glucose concentration by increasing insulin secretion from the pancreas. Sulfonylureas do this by closing ATP-dependent potassium channels (Groop *et al.* 1992).

Thiazolidinediones: Act as insulin sensitizers by increasing insulin sensitivity in hepatic and adipose tissue (Yki-Järvinen, 2004). They also prevent chronic inflammation and fatty acid accumulation helping to maintain vascular health (Reusch *et al.* 2003).

Metformin: Lowers blood glucose levels in T2D. It helps improve insulin sensitivity, thus reducing insulin resistance in T2D (Bailey *et al.* 1996). Some of the mechanisms behind metformin include decreasing hepatic glucose output and enhancing glucose uptake into the muscle via increased GLUT1 and GLUT4 translocation within cells (Bailey *et al.* 1996).

Glucagon like peptide-1: Acts through its G-protein coupled receptor to enhance glucose-stimulated insulin secretion from β-cells in the pancreas (MacDonald *et al.* 2002). It also helps reduce glucagon secretion and decreases the rate of gastric emptying (Haslam, 2010; Chaudhury *et al.* 2017).

Dipeptidyl peptidase 4 (DPP-4) inhibitors: These lower high blood glucose levels by slowing the inactivation and degradation of GLP-1 (Richter *et al.* 2008).

1.3. Management of T2D through exercise and its benefits

Exercise, along with a healthy diet is key in the prevention and control of diabetes, since the effects of the two combined can lead to appropriate values of glycaemia, lipidaemia, blood pressure and lower the risk of cardiovascular events and mortality, while increasing a person's quality of life (Colberg *et al.* 2010). This is usually done in conjunction with pharmacotherapy such as insulin, insulin sensitizers, hepatic glucose producer modulators and insulin secretion promotors in order to improve insulin sensitivity in the liver, muscle and adipose tissue (Colaguiri, 2010) in people with T2D, as previously mentioned. Decreased physical activity and exercise levels are well known risk factors for developing insulin

resistance and T2D (Stanford & Goodyear, 2014). The current recommended physical activity guidelines for T2D from the American Diabetes Association (ADA) and American College of Sports Medicine (ACSM) are 150 mins.wk^{-1} of moderate or 75 mins.wk^{-1} of vigorous physical activity in conjunction with resistance exercise (≥2 times.wk^{-1}) and diet for weight management (Colberg *et al.* 2016). Indeed, increasing physical activity levels in T2D may help bring the disease into partial or full remission (i.e. normalisation of blood glucose without the need for or at least reduce pharmaceutical therapy) (Gregg *et al.* 2012).

The most well established mechanism for improving health in people with T2D through exercise is via its skeletal muscle adaptations which combat insulin resistance and enhance glucose uptake thereby reducing hyperglycaemia throughout the day (Kristin & Goodyear, 2014). Specifically, the insulin independent uptake of glucose observed with exercise may be beneficial in both insulin resistant individuals and people with T2D (Egan and Zierath, 2012). Exercise leads to increased ATP turnover and increased Ca^{2+} content due to contraction within the exercising muscle fibres. This increases the ratio of AMP:ATP resulting in activation of the 5' AMP-activated protein kinase (AMPK), P38 mitogen-activated protein kinases (p38MAPK) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) pathways (Egan and Zierath, 2012). AMPK and MAPK activation leads to downstream activation of PPARG coactivator 1 alpha (PGC-1α) which moves to the cell nucleus and binds to myocyte enhancer factor-2 (MEF2). MEF2 then binds to the MEF2/GLUT4 domain where it is in charge of hormonal GLUT4 regulation (Richter and Hargreaves, 2013). However, MEF2 on its own is not sufficient to increase GLUT4 content within the muscle. Glucose enhancing factor (GEF) and MEF2 together induce an increase (5 fold) in GLUT4 mRNA via protein synthesis resulting in increased GLUT4 content (Richter and Hargreaves, 2013). An illustration of this insulin independent pathway can be seen in Figure 1.1.

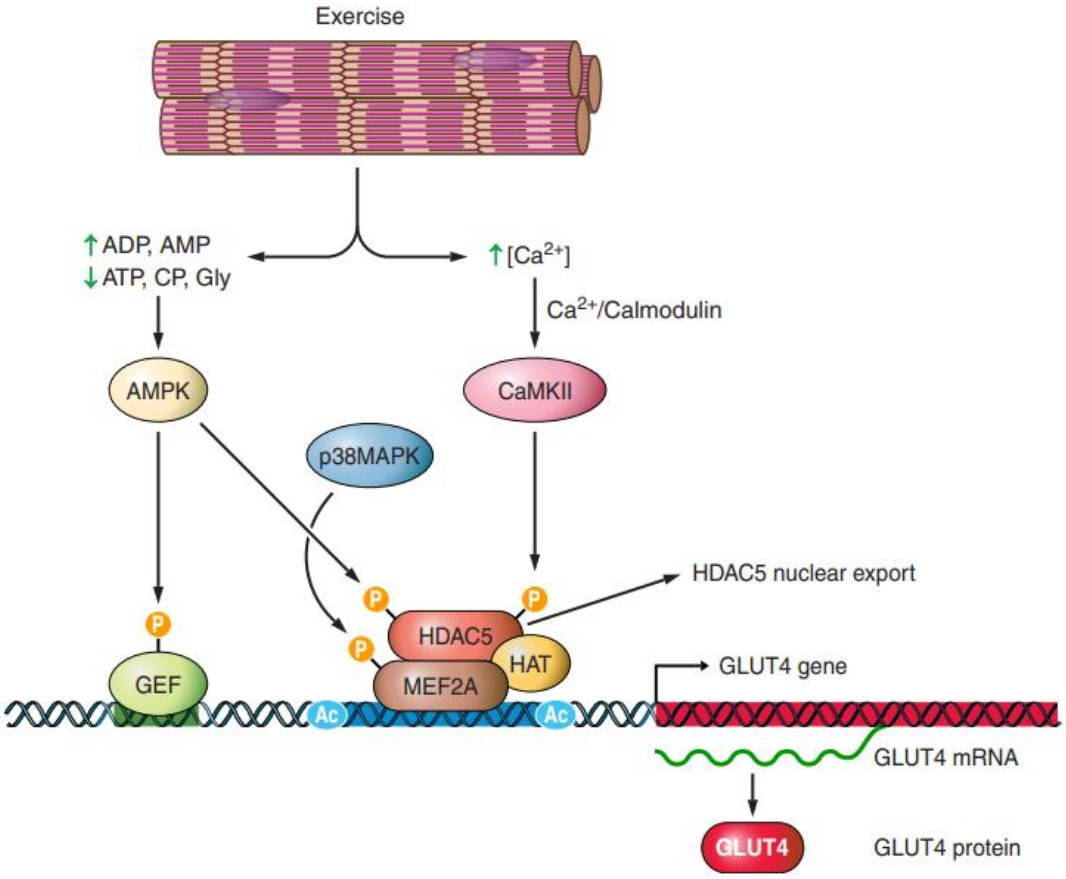

Figure 1.1 Schematic of molecular signalling involved in contraction-induced GLUT4 gene activation (taken from Richter and Hargreaves, 2013).

This occurs concomitantly with increased cardiovascular health including normalisation of blood pressure and improved endothelium-dependent vasodilation (Madsen *et al.* 2015a). Exercise is also beneficial in decreasing post-prandial hyperglycaemic excursions which in themselves are a risk factor for cardiovascular disease and mortality (Cavalot *et al.* 2006; Little *et al.* 2014).

1.4. Exercise intolerance in T2D

People with T2D appear to be more sedentary than an average healthy person (Reusch *et al.* 2013) and the development of the disease is strongly correlated with sitting time and physical inactivity throughout the day (Hamilton *et al.* 2007). One reason put forward for this is impaired exercise tolerance (Poitras *et al.* 2015). However, even when activity levels are matched between T2D and ND controls, T2D reduces cardiorespiratory fitness and thus, increases exercise intolerance (O'Connor *et al.* 2012; 2015). In addition, during moderate-

intensity exercise, women with T2D exhibited higher ratings of perceived exertion (Borg scale) compared with body mass-matched non-diabetic controls (Huebschmann *et al.* 2009) suggesting that the perception of exercise during light-to-moderate bouts of exercise in T2D is larger than in healthy counterparts. Interventions which can help increase exercise tolerance may result in people with T2D becoming more physically active, attenuating the progression of the disease and reducing associated comorbidities and mortality.

1.5. Maximal exercise capacity in T2D

Maximum or peak $\dot{V}O_2$ is positively correlated with quality of life while it's negatively correlated with risk of all-cause mortality and morbidity. In studies which examine maximal exercise in T2D, $\dot{V}O_{2max}$, or $\dot{V}O_{2peak}$ was found to be 10-20% lower compared with age-matched ND controls (Baldi *et al.* 2003; Regensteiner *et al.* 2009; Reusch *et al.* 2013; Green *et al.* 2015; O'Connor *et al.* 2015; Gildea *et al.* 2019). When accounting for ageing *per se*, $\dot{V}O_{2peak}$ declines at a rate of ~10% per decade in healthy older adults (Higginbotham *et al.* 1986; Beere *et al.* 1999; Gravelle *et al.* 2012; O'Connor *et al.* 2015). Among mediators of reduced peak responses and thus, exercise tolerance in people with T2D include a reduced ability of the cardiovascular system to supply O_2 to the contracting skeletal muscle during exercise (Green *et al.* 2015; Huebschmann *et al.* 2015).

1.5.1. Influence of central mechanisms

Central factors implicated in impaired maximal exercise performance in T2D include left ventricular dysfunction (Poirer *et al.* 2000; Fang *et al.* 2005; Reusch *et al.* 2013; Baldi *et al.* 2016; Wilson *et al.* 2017a) and reduced peak heart rate (Green *et al.* 2015) while insufficient cardiac output may play a role in more severe cases (Roy *et al.* 1989).

Reductions in left ventricular performance may occur due to structural and functional changes in the diabetic heart (Regensteiner *et al.* 2009; LaLande *et al.* 2010; Baldi *et al.* 2016; Wilson *et al.* 2017a; 2017b) including increased left ventricular filling pressure and a slower diastolic relaxation time (Baldi *et al.* 2016) which may combine and thus, decrease left ventricular end diastolic volume and stroke volume in T2D. Alterations in sympathetic input (Carnethon *et al.* 2003) into the heart and a reduced responsiveness of β-adrenergic receptors within the diabetic myocardium may also lead to a decrease in systolic function

and thus, contribute to the reduced cardiac reserve observed during exercise in T2D (Baldi *et al.* 2016).

With respect to impaired cardiac output in T2D, Roy *et al* (1987) reported peak cardiac output reductions in T2D during an incremental exercise test, however, it must be noted that the findings of Roy *et al* (1989) included diabetic participants with cardiac autonomic dysfunction which may have influenced the reduction in peak cardiac output compared to the healthy control group in their study. In contrast, during a graded test to exhaustion, Regensteiner *et al* (2009) found no difference in peak cardiac output in premenopausal women with uncomplicated T2D when comparing them to age, weight and physical activity level matched controls (Regensteiner *et al.* 2009).

With respect to older, inactive healthy individuals, the previously mentioned factors have also been implicated as causes for reduced $\dot{V}O_{2peak}$ (Higginbotham *et al.* 1986; Beere *et al.* 1999; McLay *et al.* 2017; George *et al.* 2018). Specifically, a decrease in left ventricular function with age and sedentary lifestyle may lead to a reduction in stroke volume at peak exercise (Seals *et al.* 1990). Peak heart rate also decreases, independently of training status by ~3-5% per decade (Hawkins and Wiswell, 2003; Christou *et al.* 2008). Taken together, these reductions in peak stroke volume and heart rate lead to a reduction in peak cardiac output, which in turn contributes to a reduced $\dot{V}O_{2peak}$ with ageing in inactive individuals (Sagiv *et al.* 2010; Christou *et al.* 2008).

1.5.2. Influence of peripheral mechanisms

Peripheral O_2 delivery and/or distribution appears to be impaired in participants with T2D (MacAnaney *et al.* 2011; Kiely *et al.* 2014, Green *et al.* 2015; Gildea *et al.* 2019). During an incremental intermittent plantar flexion exercise, Kiely *et al* (2014) measured leg haemodynamic responses using venous occlusion plethysmography (VOP) in age-matched participants with T2D and non-diabetic (ND) controls (Kiely *et al.* 2014). Both resting leg blood flow (LBF) and leg vascular conductance (LVC) were lower in T2D, while peak LBF responses were also significantly reduced in T2D with no sex-related differences reported. These results occurred in conjunction with a ~15% lower peak force (relative to each participant's maximal voluntary contraction; MVC) in participants with T2D and are consistent with other studies in T2D showing a ~15% reduction in peak power output during

graded cycling exercise to exhaustion (Regensteiner *et al.* 1995, 1998; MacAnaney *et al.* 2011; Wilkerson *et al.* 2012; Kiely *et al.* 2014, O'Connor *et al.* 2015; Green *et al.* 2015; Gildea *et al.* 2019). This suggests that impairments in the peak hyperaemic and vasodilatory responses during graded exercise in T2D (Kiely *et al.* 2014) may affect the reductions observed in $\dot{V}O_{2peak}$.

More recently, Gildea *et al* (2019) investigated muscle deoxygenation ([HHb+Mb]) responses of the vastus lateralis (VL) muscle in middle-aged participants with T2D during a ramp incremental (RI) exercise. They observed an increased primary slope of the normalised [HHb+Mb] relative to power output (Δ%[HHb+Mb]/Δ%PO) using a bilinear modelling procedure in T2D compared with ND controls, which represents an over-reliance on fractional oxygen extraction in T2D (see Figure 1.2). This occurred in conjunction with a 17% reduced $\dot{V}O_{2peak}$ in participants with T2D compared to the ND group. This supports the notion that impairments in O_2 delivery and/or distribution within the working muscle, at least in part, contribute to the reduced $\dot{V}O_{2peak}$ in T2D (Gildea *et al.* 2019).

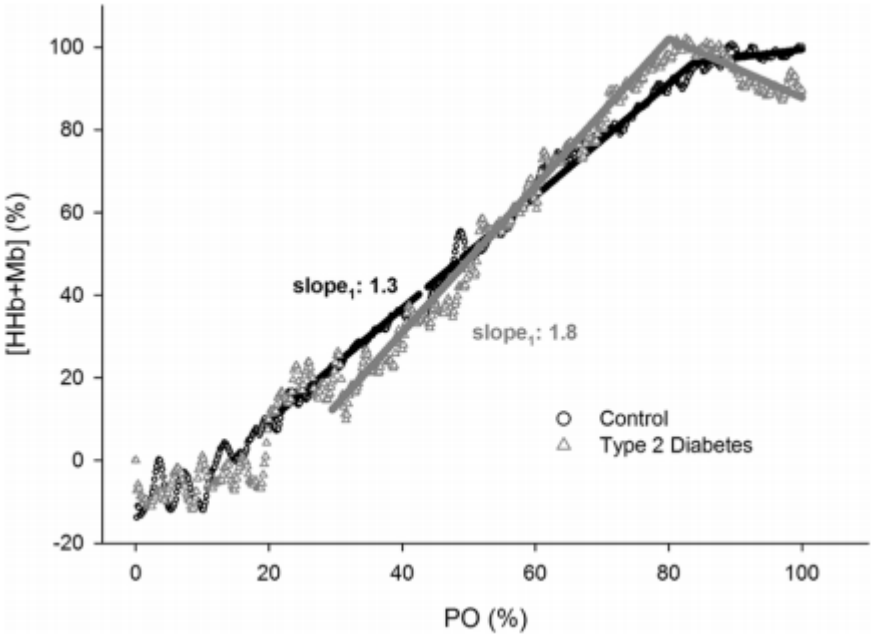

Figure 1.2. Representative profiles of the modelled muscle [HHb+Mb] response dynamics during RI exercise for a middle age individual without, and an individual with T2D when expressed as a function of relative power output (PO%). Double-linear regression models are superimposed on the data. The first Δ%[HHb+Mb]/Δ%PO slope (slope1) of the double linear regression is indicated beside each curve. Taken from Gildea *et al* (2019).

To date, there has been no studies reporting muscle [HHb+Mb] responses during RI exercise in older participants with T2D, while only one previous study reported on these responses in

older healthy adults (Gravelle *et al.* 2012). Specifically, Gravelle *et al* (2012) measured the adjustment of $\dot{V}O_2$ and Δ[HHb+Mb] within the VL muscle of 9 older (70 ± 3 y) and 9 younger adults (25 ± 5 y). $\dot{V}O_{2peak}$ and peak power output responses were lower in the older group. In addition, the slope of the normalised [HHb+Mb] relative to absolute power output (%Δ[HHb+Mb]/PO (W)) response was greater in older compared with younger adults (older: 0.027 ± 0.01 %/W; younger: 0.017 ± 0.01 %/W), suggesting impaired O_2 delivery and/or distribution leading to metabolic instability at lower absolute power outputs in the older group (Gravelle *et al.* 2012). However, when matched for relative power output (%PO), no differences were observed in the slopes. Gravelle *et al* (2012) suggested that at the same relative intensity of the RI exercise, fractional O_2 extraction responds at a similar rate regardless of age due to similar physiological mechanisms including sympathetic activation, parasympathetic withdrawal, and vasodilatory metabolic production and release. However it should be noted that the muscle Δ[HHb+Mb] responses in the study of Gravelle *et al* (2012) were modelled using a sigmoid function and the study participants were recreationally active. It is therefore necessary to expand on the modelling procedures used by the different studies while analysing the muscle [HHb+Mb] responses during RI cycling exercise.

1.5.2.1 Modelling the muscle [HHb+Mb] profile during ramp incremental exercise

Initially, the increase in muscle [HHb+Mb] responses during RI exercise were modelled using a sigmoidal fit (Ferreira *et al.* 2007) as observed in Figure 1.3. The sigmoid function characterizes the whole response, whereby, implying that the lower and upper curvatures of the muscle [HHb+Mb] response are symmetrical (Spencer *et al.* 2012). Briefly, the sigmoid model is broken into three regions. The first region shows a blunted initial muscle [HHb+Mb] response and reflects a greater increase in muscle blood flow and O_2 supply relative to O_2 utilisation in the exercising muscle at the onset of the RI exercise test. The second phase of the response reflects the increase in the rate of O_2 extraction as the power output gradually increases. The third and final region indicates a plateau of the muscle [HHb+Mb] response within the skeletal muscle microvasculature (Ferreira *et al.* 2007).

Later work by Spencer *et al* (2012) described the muscle [HHb+Mb] response as having 3 distinct phases different from those previously reported for the sigmoidal fit (Spencer *et al.* 2012). The first phase was described as the period in time where O_2 delivery matches or is in

excess of O_2 utilisation at the beginning of RI exercise when power output (W) is low. This increased O_2 supply may be attributed to muscle pump action associated with the onset of exercise. When modelling the muscle [HHb+Mb] response, this phase is excluded from analysis thereby giving us a "double-linear" or "bilinear" fit to evaluate the muscle [HHb+Mb] response. The first linear slope represents the increase in O_2 extraction of the working muscle in order to satisfy metabolic demands (see Figure 1.3). The steepness of this slope is important as it can help distinguish how reliant individuals or populations are on O_2 extraction during RI exercise when modelling the normalised muscle deoxygenation response vs relative power output (Δ%[HHb+Mb]/Δ%PO) (Gildea *et al.* 2019). After this linear increase, which takes up the majority of the muscle [HHb+Mb] response, a breakpoint is reached where a plateau-like muscle [HHb+Mb] response occurs. Whilst the precise mechanisms are yet to be fully elucidated, the breakpoint in the muscle [HHb+Mb] response is believed to reflect the upper limit of O_2 extraction in the vastus lateralis muscle towards the end of the RI cycling exercise (Murias *et al.* 2013). The model first developed by Spencer *et al* (2012) is believed to get a superior fit of the response compared to the sigmoidal modelling procedure (Spencer *et al.* 2012). While the sigmoidal model is believed to be a good fit of the response, it is limited by its assumption that the lower and upper curves of the response are symmetrical, thereby, jeopardizing one area to satisfy another (i.e. underestimating the reliance on O_2 extraction at the beginning of exercise and overestimating it at the end of exercise). Therefore, using the bilinear model to fit the Δ%[HHb+Mb]/Δ%PO response, enables us to study the O_2 extraction reliance in T2D vs. ND controls during RI exercise (Gildea *et al.* 2019), as well as whether different training interventions reduce this over-reliance on O_2 extraction in T2D (McDermott *et al.* 2018).

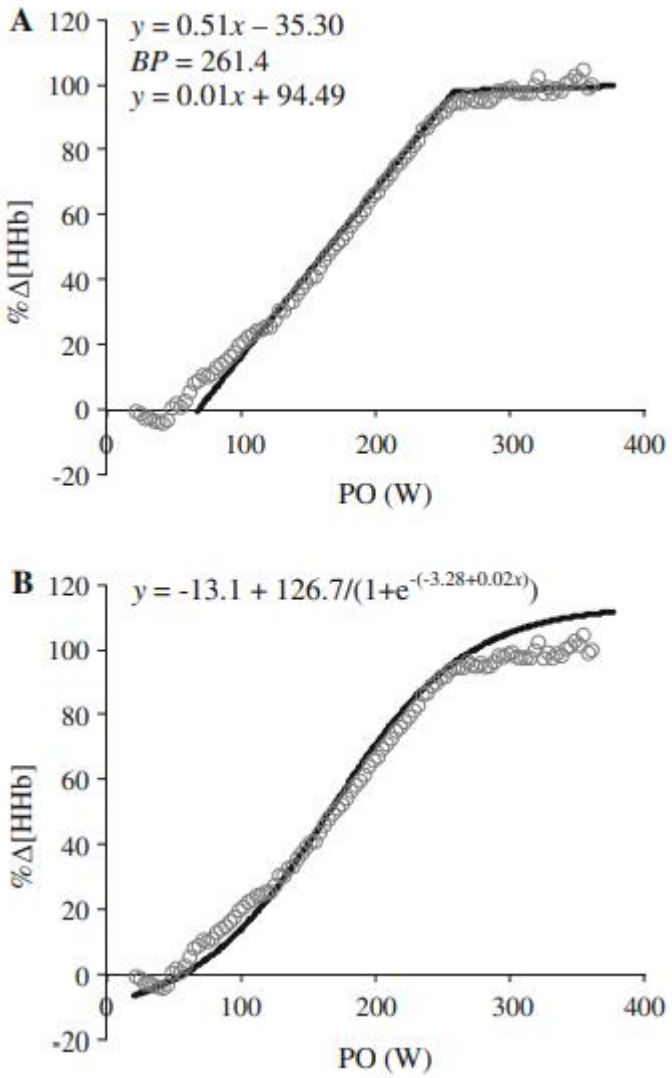

Figure 1.3. Bilinear (A) and sigmoid (B) fit superimposed onto normalised [HHb+Mb] responses as a function of power output. Taken from Spencer *et al* (2012).

1.6. $\dot{V}O_2$ kinetics during submaximal exercise onset

Non-oxidative muscle energy stores are finite therefore coordination between the pulmonary, cardiovascular and skeletal muscle systems is key to quickly increase the O_2 flux from the atmosphere to the exercising muscle and the mitochondria within, allowing ATP production via oxidative phosphorylation when transitioning from rest/elevated baseline to a higher exercise intensity (Poole & Jones, 2012). The transitory phase leading to a steady-state in $\dot{V}O_2$ is thought to give an insight into muscle energetics and metabolic control (Poole & Jones, 2012). In healthy, exercise-trained young and middle-aged participants carrying out a constant power output exercise below the anaerobic threshold, the control of $\dot{V}O_2$ on-kinetics is said to be located within the muscle mitochondria, therefore metabolic

inertia or the ability of the mitochondria to increase the rate of oxidative phosphorylation to maintain ATP homeostasis is key (Poole & Jones, 2012; Spencer *et al.* 2012). A slowing of the $\dot{V}O_2$ kinetic response is observed due to a delayed onset of oxidative phosphorylation which is hypothesized to be centred on O_2 delivery to/within the exercising muscle in both T2D (Wilkerson *et al.* 2011; O'Connor *et al.* 2015) and ageing (DeLorey *et al.* 2004; Murias *et al.* 2015) in conjunction with metabolic limitations seen in these groups. This limited $\dot{V}O_2$ kinetic response and delayed onset of oxidative phosphorylation will lead to an over-reliance on the phosphagen system and anaerobic glycolytic ATP production pathways leading to a build-up of metabolites correlated with muscle fatigue and task failure (Grassi *et al.* 2015). Therefore, interventions (i.e chronic exercise interventions or acute priming exercise bouts) which speed up/reduce the time for $\dot{V}O_2$ to achieve a steady-state and reduce the metabolites from non-oxidative energy stores may enhance exercise tolerance in T2D and ageing.

1.6.1. $\dot{V}O_2$ kinetics parameters

Oxygen uptake kinetics have been characterised using a multiple component exponential function, each with its own time constant (τ), amplitude (A) and a time delay (TD) (Poole & Jones, 2005). Phase I or the cardio-dynamic phase is indicative of an increase in pulmonary blood flow associated with an increase in tidal volume (V_T) which allows for rapid increase in $\dot{V}O_2$ at the onset of exercise in the absence of altered arterial or venous O_2 differences (Whipp *et al.* 1982; Poole & Jones, 2012). The amplitude of this phase will depend on the protocol before exercise commencement (static resting vs unloaded pedalling) (Whipp *et al.* 1982; Barstow & Mole, 1991). The exponential rise seen during constant-power output exercise is known as phase II pulmonary $\dot{V}O_2$ kinetics or the primary phase ($\dot{V}O_{2p}$). This is indicative of venous blood perfusion from all the compartments of the body, especially from the exercising muscles which have increased levels of fractional O_2 extraction (Poole & Jones, 2005). This time for arrival of deoxygenated blood will decide the TD for Phase II or the end of the cardio-dynamic phase. Phase II is said to reflect $\dot{V}O_2$ on-kinetics at the exercising muscle ($\dot{V}O_2m$) with its time constant ($\tau\dot{V}O_{2p}$) being used as a surrogate for the muscle τ (Poole & Jones, 2005). During constant power output exercise below the anaerobic threshold (AT) or ventilatory threshold (VT), $\dot{V}O_2$ plateaus to a steady state which is known as Phase III (Fig. 1.4). During exercise above the anaerobic threshold, an additional

exponential term is added to the end of Phase II known as the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) which is indicative of decreased efficiency within the exercising muscle when the amplitude is large (Fig. 1.4) (Jones *et al.* 2011).

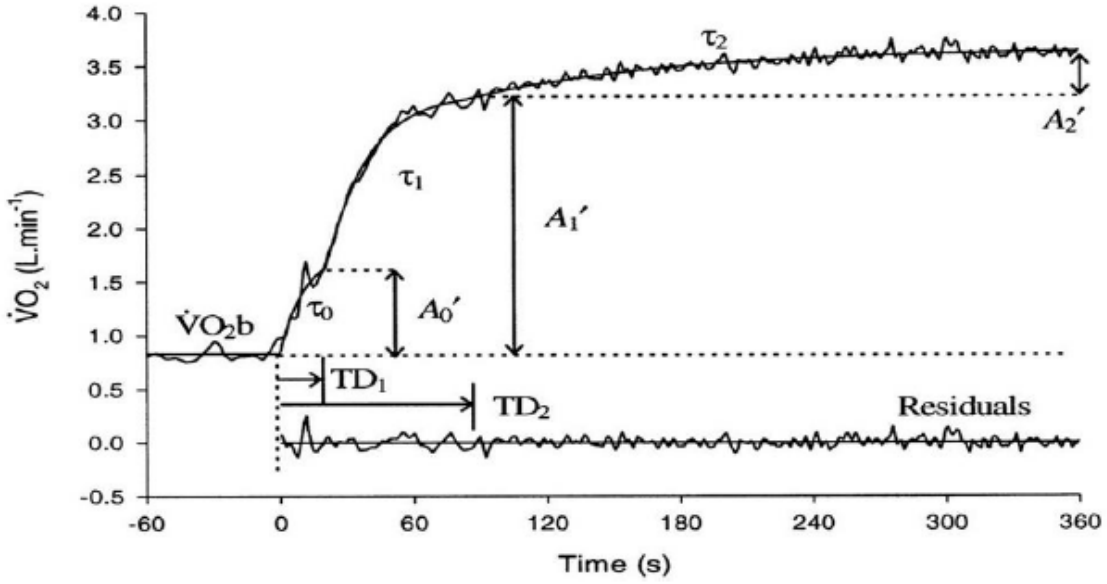

Figure 1.4. Schematic illustration of the exponential model that quantifies the $\dot{V}O_2$ kinetic response at the onset of moderate and heavy intensity exercise. The three-exponential terms, each with a τ (time constant); TD (time delay) and A (amplitude), correspond to the Phase I (Cardiodynamic phase), Phase II (Primary phase) and slow component portions of the response. The subscript numbers represent the specific phases (0 = phase I, 1 = phase II and 2 = $\dot{V}O_{2SC}$). The dotted line represents the $\dot{V}O_2$ steady state (i.e. phase III) below the level of the ventilatory threshold (VT) that is achieved within 2-3 mins during moderate intensity exercise. $\dot{V}O_2b$ is the $\dot{V}O_2$ at baseline (Taken from Burnley *et al.* 2000).

1.6.2. <u>Importance to exercise tolerance</u>

The speed at which pulmonary $\dot{V}O_2$ can reach steady-state will decide how large an O_2 deficit is incurred which is indicative of intracellular perturbations due to substrate phosphorylation, making the $\dot{V}O_2$ kinetic response of the exercise a good indicator of muscle metabolism (Xu & Rhodes, 1999; Poole & Jones, 2012; Grassi *et al.* 2015). During moderate-intensity exercise $\dot{V}O_2$ increases exponentially to a steady-state after a brief delay, with $\tau\dot{V}O_{2p}$ and the $\dot{V}O_2$ amplitude of the primary phase ($A\dot{V}O_{2p}$) used as an indicator of $\dot{V}O_2$ within the exercising muscle. Once steady-state $\dot{V}O_2$ is reached, the rate of mitochondrial respiration is said to match ATP hydrolysis meaning ATP homeostasis within the cell is maintained via oxidative phosphorylation. During heavy intensity exercise above the AT or VT and below the participants' critical power, the attainment of steady-state $\dot{V}O_2$ is delayed

due to the above-mentioned addition of a slowly developing $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) (Jones *et al.* 2011). The $\dot{V}O_{2sc}$ is one of the key parameters of $\dot{V}O_2$ kinetics due to its association to the loss of muscle efficiency and the development of fatigue (Jones *et al.* 2011; Korzeniewski *et al.* 2015; Zolads *et al.* 2016). Therefore, a long $\tau\dot{V}O_{2p}$ and enhanced $\dot{V}O_{2SC}$ amplitude will lead to greater substrate phosphorylation which will have negative implications for exercise tolerance.

1.6.3. $\dot{V}O_2$ kinetics during heavy intensity exercise

At the onset of heavy-intensity exercise above the AT or VT, both slow and fast twitch fibre types are recruited simultaneously. Because of their slower $\dot{V}O_2$ kinetics, the $\dot{V}O_2$ requirements of fast twitch fibres do not become apparent immediately due to the lower mitochondrial content of these fibres (Poole *et al.* 1994). Evidence in healthy people also suggests that a large slow component ($\dot{V}O_{2SC}$) amplitude is linked to a greater glycolytic fibre content within the vastus lateralis muscle, while greater cycling efficiency (reduced $\dot{V}O_{2SC}$ amplitude) is positively correlated with a higher slow twitch fibre type content within the VL (Coyle *et al.* 1992). However, the decreased efficiency of the exercising muscle is not dictated by the recruitment of fibre types *per se*, rather a decreased contraction efficiency of the low oxidative fibres and/or a lower level of metabolic stability play a more important role (Da Boit *et al.* 2014; Korzeniewski & Zoladz, 2015). Korzeniewski & Zoladz (2015) theorised that an intensified anaerobic glycolysis at the onset of heavy intensity exercise (possibly due to either recruitment of fast twitch fibres and impaired O_2 delivery at the microvasculature) will result in less ATP being generated from oxidative phosphorylation to match ATP demand. This would lead to exacerbated substrate phosphorylation, cytosolic acidification, decreased glycolytic flux, decreased stability of PCr and Pi and an increase in markers indicative of fatigue or decreased efficiency causing metabolic instability and increased ATP usage. The decreased glycolytic flux (accompanied by a slow decrease in ATP supply by creatine kinase) is compensated by the increased $\dot{V}O_2$ cost (Korzeniewski & Zoladz, 2015).

1.6.4. $\dot{V}O_2$ kinetics in T2D

A significantly slower $\tau\dot{V}O_{2P}$ during moderate-intensity constant-load exercise has been observed in young and middle-aged people with T2D compared with age-matched ND

controls (Regensteiner *et al.* 1998; Brandenburg *et al.* 1999; MacAnaney *et al.* 2011; O'Connor *et al.* 2015). However, in contrast, no differences in $\dot{V}O_2$ kinetics have been observed in older (60 – 70 years) people with T2D compared with healthy age-matched controls, even in the presence of a significantly reduced $\dot{V}O_{2peak}$ (Wilkerson *et al.* 2011; O'Connor *et al.* 2015). The slowed $\tau\dot{V}O_{2P}$ in middle-aged people with T2D is said to be caused by impaired O_2 delivery, poor blood flow distribution to the contracting muscle, impaired arteriolar vasodilation, metabolic inertia and/or greater recruitment of fast twitch fibres that have slower $\dot{V}O_2$ kinetics which may be due to a reduced microvascular oxygen partial pressure ($Pm\dot{V}O_2$) (Poole *et al.* 1994*;* Poole & Jones, 2005; Caron *et al.* 2017; Poole *et al.* 2008). One of the reasons why $\tau\dot{V}O_{2P}$ is not different between older people with T2D and ND older controls is because, at least in deconditioned older people, ageing *per se* has a very powerful deconditioning effect on the cardiovascular system and skeletal muscle metabolism which in turn leads to an impaired $\dot{V}O_2$ kinetic response (O'Connor *et al.* 2015).

1.6.5. $\dot{V}O_2$ kinetics in older adults (>60 years)

Oxygen uptake kinetics are slowed in older relative to younger adults at moderate- to severe-intensity domains (Barstow and Scheuermann, 2005, in Jones and Poole, 2005; Sabapathy *et al.* 2004; DeLorey *et al.* 2004; 2005; 2007; Gurd *et al.* 2007; 2008; 2009; DuManoir *et al.* 2010; Poole and Jones, 2012; McKlay *et al.* 2017). However, what remains up for debate is whether ageing *per se* or a decreased habitual physical activity or fitness levels is the main cause for the slowed $\dot{V}O_2$ kinetic responses observed with ageing (Poole and Jones, 2012; George *et al.* 2018). Whilst it is known that both central and peripheral mechanisms, such as reduced O_2 delivery to O_2 utilisation along with impaired oxidative capacity within the working muscle at the initiation of exercise may reduce exercise tolerance in older relative to younger adults (Gurd *et al.* 2008; 2009; DuManoir *et al.* 2010; Poole and Jones, 2012; George *et al.* 2018), a similar $\tau\dot{V}O_{2p}$ during moderate-intensity exercise have been reported between young and older adults when both groups were trained (Grey, 2015; McKlay *et al.* 2017; George *et al.* 2018). Moreover, George *et al* (2018) observed that $\dot{V}O_2$ kinetics were slower in young, inactive compared with older trained individuals. These findings implicate that activity levels, rather than ageing *per se*, dictate $\dot{V}O_2$ kinetics and exercise tolerance in both young and older healthy adults (George *et al.* 2018). Therefore, when comparing older aged adults across populations, it is critical to

account for underlying diseases and medications which may affect cardiorespiratory health, along with disparities in fitness and/or habitual physical activity levels (Barstow and Scheuermann, 2005, in Jones and Poole, 2005; Poole and Jones, 2012).

1.7. Priming as an acute intervention for enhancing exercise tolerance:

A prior bout of heavy-intensity exercise or "priming exercise" (PE) is believed to prime the physiological response mainly by increasing the oxidative phosphorylation contribution of a subsequent exercise bout in both healthy untrained and diseased populations (Gerbino *et al.* 1996; Burnley *et al.* 2000, 2001, 2002; Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Jones *et al.* 2006; DiMenna *et al.* 2008; Goulding *et al.* 2017; 2018 Rocha *et al.* 2019), thereby enhancing exercise tolerance.

1.7.1. Effects of PE on subsequent $\dot{V}O_2$ kinetics during moderate-intensity exercise transitions

Heavy intensity PE has been shown to accelerate the $\dot{V}O_2$ kinetic response during a subsequent bout of moderate-intensity exercise in populations displaying an initial slowed $\tau\dot{V}O_{2P}$ response such as older individuals (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2009) or those with T2D (Rocha *et al.* 2019). Among studies focusing on older individuals, Scheuermann *et al* (2002) initially reported significantly faster $\tau\dot{V}O_{2P}$ during a moderate-intensity (80% VT) cycling bout subsequent to heavy-intensity priming cycling exercise at 50% Δ (midway point of power output between VT and peak power output recorded during RI exercise), with the faster $\tau\dot{V}O_{2P}$ kinetic response being attributed to enhanced muscle perfusion (Scheuermann *et al.* 2002). These authors found the older group to have an elevated baseline HR at the beginning of the subsequent bout of moderate exercise further highlighting a likely enhanced O_2 delivery to the exercising muscle as a potential reason for the faster $\dot{V}O_2$ kinetic response. In a follow-up investigation, DeLorey *et al* (2004) also observed a faster $\tau\dot{V}O_{2P}$ following a heavy-intensity PE which occurred in conjunction with a slowing of the time constant (τ) of the [HHb+Mb] response among older adults (unprimed: 9 ± 3 s; primed: 32 ± 17 s) (DeLorey *et al.* 2004). This suggests that the faster $\tau\dot{V}O_{2P}$ during the subsequent moderate-intensity exercise bout in older individuals after PE may be related to improvements in local muscle perfusion.

It may be possible that the sluggish $\dot{V}O_2$ kinetics responses observed during the early exercise transition in older adults may also be due to a slower activation of aerobic muscle metabolism (Boffoli *et al.* 1994; Gurd *et al.* 2007; 2009). During moderate-intensity exercise, Gurd *et al* (2007) found a slower $\tau\dot{V}O_{2P}$ in older (40 ± 17 s) compared with young (21 ± 6 s) healthy adults (Gurd *et al.* 2007). This occurred in conjunction with an attenuated activation of mitochondrial pyruvate dehydrogenase (PDH) in the older adults. This impairment in the activation of a rate-limiting enzyme for oxidative phosphorylation may suggest a possible role for oxidative enzymes and provision of substrate to the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) as a source for the initial sluggish $\dot{V}O_2$ kinetics at exercise onset in older inactive adults (Gurd *et al.* 2007; 2009). Adding on from these findings the same authors (Gurd *et al.* 2009) investigated the effects of PE on PDH activity and phosphocreatine (PCr) breakdown in conjunction with $\dot{V}O_2$ and [HHb+Mb] kinetic responses in a subsequent bout of moderate-intensity cycling exercise in older participants. PE induced a faster $\tau\dot{V}O_{2P}$ in older individuals and this effect was accompanied by reduced PCr breakdown and a significantly higher PDH activity during exercise onset. This likely indicates a greater bioavailability of oxidative substrate which may have resulted in a faster activation of oxidative phosphorylation.

More recently, heavy-intensity PE has also been proven effective as an acute intervention for accelerating $\tau\dot{V}O_{2P}$ in middle-aged participants with T2D (unprimed: 42 ± 12 s; primed: 32 ± 9 s) during moderate intensity exercise (Rocha *et al.* 2019). The speeding of the $\dot{V}O_2$ primary phase kinetic response observed in participants with T2D was accompanied by a reduction in the Δ[HHb+MB]/ $\Delta\dot{V}O_{2p}$ ratio (unprimed: 1.17 ± 0.17; primed: 1.05 ± 0.14) during the moderate-intensity bout subsequent to PE. This suggests that PE induces a better matching of O_2 delivery to O_2 utilisation within the exercising muscles in conjunction with faster metabolic activation of oxidative phosphorylation through enhanced substrate availability in middle-aged individuals with T2D (Gurd *et al.* 2009; Rocha *et al.* 2019).

1.7.2. Effects of PE on subsequent $\dot{V}O_2$ kinetics during heavy-intensity exercise transitions

With respect to heavy-intensity upright cycle exercise, Gerbino *et al* (1996) identified that PE could speed the overall mean response time (MRT) of the $\dot{V}O_2$ kinetic response when assessed with a mono-exponential function. They attributed this improved $\dot{V}O_2$ kinetics response to enhanced O_2 availability via improved muscle-blood perfusion as a consequence

of improved vasodilation and a shift to the right in the HbO_2 dissociation curve due to "lactic acidosis" from the prior heavy intensity bout (Gerbino *et al.* 1996; Poole & Jones, 2005). However, whilst the main findings of the pioneering work of Gerbino and colleagues (1996) still hold true to today, the results do not distinguish whether this speeding of the kinetic response was as a result of changes in time and amplitude of the primary and/or the slow component phases.

When a bi-exponential function is employed in order to discriminate between the primary phase and the slow component (whilst excluding the cardio-dynamic phase) the speeding of the $\dot{V}O_2$ kinetics response during heavy-intensity exercise subsequent to PE is attributed to a reduction in the amplitude of the $\dot{V}O_{2SC}$ and/or an increase in the amplitude of the primary phase $\dot{V}O_2$ ($\dot{V}O_{2P}$), with no changes in $\tau\dot{V}O_{2P}$ (Burnley *et al.* 2000, 2001; Jones *et al.* 2006; Goulding *et al.* 2017). It must be stressed that these results were found in studies investigating PE during upright cycle exercise in healthy, active young participants where O_2 delivery to the exercising musculature is believed to be adequate/not a limiting factor of $\dot{V}O_2$ kinetics (Jones *et al.* 2006; Goulding *et al.* 2017; Gildea *et al.* 2020).

In contrast, when at the outset $\dot{V}O_2$ kinetic responses are "slowed" due to impaired O_2 delivery, a speeding of the $\tau\dot{V}O_{2P}$ can be observed with PE during the subsequent heavy-intensity exercise (Jones *et al.* 2011; Goulding *et al.* 2017; Gildea *et al.* 2020). For instance, supine cycle exercise has been utilised in some previous research to induce a condition where the primary phase $\dot{V}O_2$ is limited by O_2 availability (Koga *et al.* 1999; Jones *et al.* 2006; Goulding *et al.* 2017, 2018). The O_2 limitation during supine cycling is attributed to a loss of hydrostatic gradient effect of gravity resulting in a lowering of the pressure head for blood-to-myocyte O_2 diffusion in the working muscles, thus, slowing the rate of increase in $\dot{V}O_2$ at the onset of exercise (Koga *et al.* 1999; Goulding *et al.* 2017). Jones *et al* (2006) found that heavy-intensity PE allowed for a faster $\tau\dot{V}O_{2P}$ response during a subsequent bout of heavy-intensity exercise when performed in the supine but not upright position. These authors suggested that the faster $\dot{V}O_2$ kinetic response in the supine position after priming was as a result of a reduced constraint in O_2 availability to the exercising muscle during the subsequent exercise bout (Jones *et al.* 2006). This improvement in blood flow distribution is hypothesized to result from improved muscle vasodilation and a rightward shift in the HbO_2 dissociation curve. In a similar study, Goulding *et al* (2017) also found a faster $\tau\dot{V}O_{2P}$ during a

subsequent bout of heavy-intensity supine exercise following a prior PE bout. Goulding and colleagues also observed a slower τ[HHb+Mb] response post-PE in the supine condition indicative of enhanced O_2 supply compared with O_2 utilisation within the exercising muscle. In contrast, in these two studies (Jones *et al.* 2006; Goulding *et al.* 2017), PE induced an enhanced $\dot{V}O_{2P}$ amplitude and reduced $\dot{V}O_{2sc}$ amplitude during upright cycling among the same participants with no changes in $\tau\dot{V}O_{2P}$.

More recently, Gildea *et al* (2020) studied the effects of PE on $\dot{V}O_2$ and [HHb+Mb] kinetics responses during a heavy-intensity cycle exercise in untrained middle-aged people with and without uncomplicated T2D. A faster $\tau\dot{V}O_{2P}$ response was observed in both groups following PE. In addition, a 42% reduction (non-significant) in the amplitude of the $\dot{V}O_{2sc}$ was observed in the T2D group. Importantly, both groups showed a significant reduction in the MRT of the overall $\dot{V}O_2$ response. An interesting finding from this study was the fact that PE induced a faster $\tau\dot{V}O_{2P}$ response in the ND group. This may have occurred as a result of a lower fitness level of participants in the study by Gildea *et al* (2020) ($\dot{V}O_{2peak}$ = 28.3 ± 6.3 $ml.kg^{-1}.min^{-1}$) compared with healthy participants from other studies where $\tau\dot{V}O_{2P}$ was not affected by PE in the subsequent heavy-intensity exercise in the upright position (Jones *et al.* (2006): $\dot{V}O_{2max}$ = 43.5 ± 0.34 $ml.kg^{-1}.min^{-1}$; Goulding *et al.* (2017): $\dot{V}O_{2peak}$ = 51.8 ± 6.5 $ml.kg^{-1}.min^{-1}$).

1.7.3. <u>Effects of PE on subsequent $\dot{V}O_2$ kinetics during Work-to-Work exercise transitions</u>

When constant-load exercise is initiated from an elevated baseline power output of moderate-intensity (<VT) to a heavy-intensity power output (between VT and CP) or severe intensity (between CP and $\dot{V}O_{2max}$) also known as work-to-work (WtoW), a slowing in the $\tau\dot{V}O_{2P}$ is observed when compared with a condition where the transition to the same heavy/severe-intensity exercise is initiated from an unloaded/resting condition (Wilkerson *et al.* 2007; DiMenna *et al.* 2008; 2010a; 2010b; Gildea *et al.* 2019; Goulding *et al.* 2018). It is important to study these WtoW transitions in both health and disease, along with interventions to help improve $\dot{V}O_2$ kinetics during these WtoW transitions because abrupt transitions from lower to higher metabolic rates occur in our everyday lives, making WtoW a relatively ecologically valid laboratory experiment (DaBoit *et al.* 2014).

While the precise mechanisms for the altered $\dot{V}O_2$ kinetic response observed during WtoW compared with transitions initiated from unloaded/rest remain inconclusive, it appears that

the slower $\tau\dot{V}O_{2P}$ response may be intramuscular in origin with a higher type II fibre recruitment playing a role along with decreased cellular energetic state due to a prior moderate-intensity exercise bout (DiMenna *et al.* 2010b; Meyer & Foley, 1996; Goulding *et al.* 2018). Oxygen delivery may also feature in scenarios where blood flow is limited (DiMenna *et al.* 2010a).

With regards to PE, a small number of studies to date have investigated the effects of heavy-intensity PE on subsequent $\dot{V}O_2$ and [HHb+Mb] kinetics during WtoW transitions in healthy participants (DiMenna *et al.* 2008, 2010a). For instance, DiMenna and colleagues (2008) found no effects on $\tau\dot{V}O_{2P}$ during a WtoW transition subsequent to PE, but they observed a significant increase in the primary component amplitude (unprimed: 1.16 ± 0.25 $L.min^{-1}$; primed: 1.66 ± 0.28 $L.min^{-1}$) and a reduction in the $\dot{V}O_{2SC}$ amplitude (unprimed: 0.47 ± 0.09 $L.min^{-1}$; primed: 0.27 ± 0.13 $L.min^{-1}$) during the subsequent WtoW exercise (DiMenna *et al.* 2008). The attenuation in the amplitude of the $\dot{V}O_{2SC}$ occurred together with a reduction in the ΔiEMG between the second and final minute (i.e. 6^{th} minute) of exercise with PE. Authors hypothesized that PE induced an altered motor unit recruitment pattern and altered oxygen uptake within the recruited type 2 fibres with PE (DiMenna *et al.* 2008). In a follow-up study, the same research group investigated the effects of PE on subsequent WtoW transitions in healthy participants but in the supine cycle position, whereby muscle O_2 availability is compromised (DiMenna *et al.* 2010a). DiMenna *et al* (2010a) found that PE helped speed $\tau\dot{V}O_{2P}$ in the supine position (unprimed WtoW: 54 ± 19 s; primed WtoW: 34 ± 9 s) with no changes observed in the amplitude of the $\dot{V}O_2$ primary component or $\dot{V}O_{2SC}$. Following PE, $\tau\dot{V}O_{2P}$ during supine exercise was in fact similar to the $\tau\dot{V}O_{2P}$ obtained during upright cycling (34 vs. 38 s respectively) with the extent of the PE induced $\tau\dot{V}O_{2P}$ reduction being correlated with the difference in $\tau\dot{V}O_{2P}$ between upright and supine prior to PE (DiMenna *et al.* 2010a).

Amongst middle-aged participants with uncomplicated T2D, Gildea *et al* (2020) recently reported PE to be effective at both decreasing the amplitude of the $\dot{V}O_{2SC}$ (unprimed WtoW= 0.17 ± 0.10 $L.min^{-1}$; primed WtoW= 0.11 ± 0.05 $L.min^{-1}$) and speeding the $\tau\dot{V}O_{2P}$ (unprimed= 61 ± 17 s; primed= 39 ± 22 s) with the primed bout reaching a similar $\tau\dot{V}O_{2P}$ as the unprimed bout for the ND (42 ± 11 s). These effects resulted in a reduced $\dot{V}O_2$ MRT (unprimed WtoW= 105 ± 25 s; primed WtoW= 65 ± 22 s) (Gildea *et al.* 2020). To date, there

is no research into $\dot{V}O_2$ and [HHb+Mb] kinetics responses during WtoW transitions or the effects of PE on these responses in older people with T2D.

1.8. Low Volume High Intensity Interval Training (HIIT)

Despite exercise having clear benefits in both, health and disease, most people including those with T2D struggle to achieve the recommended daily/weekly physical activity guidelines. In recent years, HIIT, a time efficient form of interval training involving short bursts/sprints interspersed with less intense recovery periods, has governed interest within the field of exercise and clinical exercise physiology (Gibala *et al.* 2012; Little *et al.* 2011). The popularity of HIIT is due to the ability to complete the exercise session in a shorter period of time whilst getting similar physiological adaptations as following the time-oriented moderate-intensity continuous training (MICT) interventions including enhanced peak and submaximal exercise capacity, improved cardiovascular health or improved skeletal muscle metabolic capacity (glucose and lipid oxidation) (Little *et al.* 2011; Gibala *et al.* 2012; Timmons *et al.* 2012;Madsen *et al.* 2015b; Mitranun *et al.* 2014; Kindig *et al.* 2018). HIIT has also gained notoriety for its beneficial effects on glucose disposal and the prevention of T2D.

In addition, similar to MICT, HIIT is effective at improving vascular health with benefits including improved popliteal artery distensibility and endothelium-dependent vascular function as measured by flow-mediated dilation (FMD), or overall arterial structure (i.e. decreased wall thickness; increased lumen diameter and decreased wall: lumen ratio) (Rakobowchuck *et al.* 2008). Investigations into HIIT also observed increased angiogenesis in both highly oxidative and more glycolytic muscle fibres, increased capillary: fibre ratio and enhanced endothelial cell proliferation (Jensen *et al.* 2004). Finally, HIIT has proven to be as equally effective as MICT at decreasing ambulatory blood pressure (Tjonna *et al.* 2008), improving heart rate recovery, and reducing arterial stiffness in young women with high familial risk of hypertension (Guimaraes *et al.* 2010; Ciolac, 2012).

1.9. HIIT and MICT interventions for enhancing exercise tolerance in T2D

1.9.1. Peak exercise responses and vascular function

Exercise interventions involving a HIIT and/or MICT have been proven effective at enhancing $\dot{V}O_{2peak}$ of a similar magnitude of ~18% in individuals with T2D (Brandenburg *et al.* 1999; Meex *et al.* 2010; MacAnaney *et al.* 2012; Mitranun *et al.* 2014; Madsen *et al.* 2015a; 2015b;

Winding *et al.* 2018; McDermott *et al.* 2018; Green *et al.* 2020). For instance, following 12 weeks of MICT, early work by Brandenburg and colleagues (1999) reported a 27% increase in $\dot{V}O_{2peak}$ in participants with T2D (Brandenburg *et al.* 1999) while utilising concurrent exercise (MICT and resistance training), MacAnaney *et al* (2012) reported 24% increase in $\dot{V}O_{2peak}$ in individuals with T2D after 12 weeks (MacAnaney *et al.* 2012). More recently, Green *et al* (2020) reported that the benefits for $\dot{V}O_{2peak}$ following 12 weeks of MICT in T2D are not sex-specific (Green *et al.* 2020).

Following short-term HIIT interventions (2-12 weeks), Madsen *et al* (2015a; 2015b) observed an increase in $\dot{V}O_{2peak}$ of ~7% after just 8 weeks of HIIT, while among the small amount of studies that compared HIIT with MICT, Winding and colleagues (2018) reported an 20% increase in $\dot{V}O_{2peak}$ following HIIT compared to a 8% increase following MICT after an 11 week intervention in T2D (Winding *et al.* 2018). The intervention involved training 3 times per week with the HIIT protocol consisting of 10 x 60 s cycling bouts at 95% peak power output while MICT consisted of 40 mins cycling at 50% peak power output. Similarly, Mitranun *et al* (2014) reported a superior effect of 12 weeks of HIIT on $\dot{V}O_{2max}$ (12.5%) compared with MICT (11%) in T2D, although the time commitment between the two modes of exercise was similar (Mitranun *et al.* 2014). However, whilst the previous studies have shown that long training interventions involving HIIT and MICT are successful at increasing $\dot{V}O_{2peak}$ in individuals with T2D, they only reported findings from before and after training.

This led to recent work from our laboratory (McDermott *et al.* 2018) which compared the time-course effects of 12 weeks of HIIT and MICT on cardiorespiratory fitness ($\dot{V}O_{2peak}$) and fractional oxygen extraction during a ramp incremental test in individuals with T2D. After just 3 weeks of training, $\dot{V}O_{2peak}$ significantly increased ($P < 0.05$) in both exercise groups (MICT: +17%; HIIT: +8%). In concurrence with this improved aerobic capacity, the slope of the first linear component of the normalised [HHb+Mb] response relative to power output (Δ[HHb+Mb]%/Δ%PO) was significantly decreased in both groups, inferring less reliance on fractional oxygen extraction and thus, enhanced microvascular O_2 delivery and/or distribution within the exercising muscle (McDermott *et al.* 2018). These benefits, both in $\dot{V}O_{2peak}$ and in fractional O_2 extraction were maintained until the end of the 12 week training intervention in both groups.

In the previously mentioned study comparing HIIT and MICT in T2D, Mitranun and colleagues (2014) reported that flow mediated dilation (FMD) of the brachial artery improved in both groups (HIIT: +27%; MICT: +21%). However, as for $\dot{V}O_{2max}$ responses, the greatest effect was observed in the HIIT group with authors suggesting that this type of training increased blood flow and shear stress during exercise to a greater extent thus, improving NO bioavailability. It should be noted again that these results are limited by the fact that exercise modalities were matched for energy expenditure resulting in the HIIT intervention lasting the same amount of time as the MICT.

Similarly, Madsen *et al* (2015a) observed significant improvements in %FMD of the popliteal artery (~23% increase) along with improved resting haemodynamics after 8 weeks HIIT cycling exercise intervention in elderly people with T2D (Madsen *et al.* 2015a). Post-intervention, the popliteal artery diameter was also significantly increased, indicating vascular remodelling had taken place. An outward arterial remodelling of ~6% seen in that study in the T2D group is likely to lead to reduced shear rates within the artery.

1.9.2. $\dot{V}O_2$ kinetics

Oxygen uptake kinetics responses at moderate- and heavy-intensities following 12 weeks of MICT in in T2D have been shown to be enhanced (Brandenburg *et al.* 1999; MacAnaney *et al.* 2012; Green *et al.* 2020). Initially, Brandenburg *et al* (1999) reported that 12 weeks of MICT in middle-aged women with T2D was effective at speeding the overall $\dot{V}O_2$ kinetics responses at light (30 W), moderate (50 W) and heavy (80 W) absolute intensities using a mono-exponential function (i.e. without distinguishing between the $\dot{V}O_2$ primary and slow component phases).

In a similar study, MacAnaney and colleagues (2012) also reported improvements in $\dot{V}O_2$ kinetics in both moderate- and heavy-intensity exercise in participants with T2D after conducting a 12 week supervised concurrent exercise intervention consisting of MICT and resistance exercise (MacAnaney *et al.* 2012). In this study, $\dot{V}O_2$ kinetics were analysed using either a bi-exponential or tri-exponential function. Authors observed that the $\tau\dot{V}O_{2p}$ decreased during moderate- and heavy-intensity exercise whilst the amplitude of the $\dot{V}O_{2SC}$ also decreased for heavy-intensity exercise after the intervention. In addition, HR kinetics were faster at all intensities post-training and the increase in cardiac output (inert gas

rebreathing) at 30 s relative to 240 s during the moderate-intensity exercise bouts was larger post-training. Authors suggested that these benefits were associated with faster dynamic responses in HR. More recently, Green et al (2020) reported that the speeding in $\tau\dot{V}O_{2p}$ at moderate-intensity exercise following 12 weeks of MICT is not related to sex in middle-age individuals with T2D (Green *et al.* 2020). Authors also concluded that the speeding in $\tau\dot{V}O_{2p}$ was not related to changes in cardiac output. It should be noted that MacAnaney *et al* (2012) used the same absolute intensities during their pre- and post-training $\dot{V}O_2$ kinetics and cardiac output measures whereas Green *et al* (2020) used relative exercise intensities (i.e. 80%VT equivalents to pre vs. post-training incremental test).

In the previously-mentioned investigations coming out of our laboratory, the time-course effects of 12 weeks of MICT and HIIT on $\dot{V}O_2$ kinetics, both, during moderate-intensity and WtoW transitions were also explored. Regarding the findings on $\dot{V}O_2$ kinetics during moderate-intensity transitions, significant improvements in $\tau\dot{V}O_{2p}$ were reported in the exercise groups after 3 weeks of training, and the benefits were maintained until the end of the intervention (Gildea *et al.* 2018). The positive adaptations in $\dot{V}O_2$ were accompanied by significant improvements in the Δ[HHb+Mb]/ $\dot{V}O_2$ ratio for both exercise groups, again, after just 3 weeks of training with no significant improvement observed later in the intervention. These results infer improved matching of local O_2 delivery to O_2 utilisation as the mechanism behind enhanced $\dot{V}O_2$ kinetics in T2D (Gildea *et al.* 2018). Regarding the effects of HIIT and MICT on $\dot{V}O_2$ and [HHb+Mb] kinetics during WtoW transitions, after just 3 weeks the $\dot{V}O_{2SC}$ amplitude was significantly reduced by 40% and 44% in the HIIT and MICT groups respectively, with no changes observed in the control group. Significant changes were also observed in $\tau\dot{V}O_{2P}$ and $\dot{V}O_2$ MRT following 3 weeks of training in the exercise groups. These novel findings highlight the significance of HIIT as a valuable, time efficient tool for improving both fitness, exercise tolerance and quality of life in people with T2D. In most of the discussed studies, HIIT comprised of ~90 mins exercise per week vs. ~180 mins in MICT (including warm-up and cool-down periods).

1.10.Aims

It is evidently clear that $\dot{V}O_2$ kinetics are slowed in young and middle-age people with T2D. Furthermore, previous research suggests that acute interventions such as PE may help improve $\dot{V}O_2$ kinetics and thus, exercise tolerance during moderate, heavy and heavy

intensity cycle exercise initiated from an elevated baseline (WtoW) in people with T2D. However, whether the effects of PE on subsequent $\dot{V}O_2$ kinetics at different intensities are effected in older T2D is unknown. In addition, while $\dot{V}O_2$ and [HHb+Mb] kinetic responses following a 12 week exercise intervention consisting of HIIT and MICT on subsequent moderate-intensity and heavy-intensity exercise initiated from an elevated baseline have been investigated in T2D, there is no research to date on its effect on heavy-intensity exercise transitions initiated from an unloaded baseline.

Accordingly the aims of the four experiments carried out in the present thesis were the following:

- *Experiment 1*: to investigate the profile of muscle fractional oxygen extraction during ramp incremental cycle exercise in older adults with and without type 2 diabetes.
- *Experiment 2*: to assess the influence of priming exercise on $\dot{V}O_2$ and muscle [HHb+Mb] kinetics responses during a subsequent bout of heavy-intensity cycle exercise initiated from an unloaded baseline in older adults with and without type 2 diabetes.
- *Experiment 3*: to investigate the influence of priming exercise on $\dot{V}O_2$ and muscle [HHb+Mb] kinetics responses during WtoW transitions in older adults with and without type 2 diabetes.
- *Experiment 4*: to compare the effects of 12 weeks of MICT vs HIIT on $\dot{V}O_2$ and muscle [HHb+Mb] kinetics responses during heavy-intensity cycling exercise initiated from an unloaded baseline in people with type 2 diabetes.

Chapter 2: Influence of type 2 diabetes on muscle deoxygenation during ramp incremental cycle exercise in older adults

2.1 Introduction

Adult individuals with uncomplicated T2D of all ages demonstrate impaired $\dot{V}O_{2peak}$ (~20%) responses during graded exercise (Regensteiner *et al.* 1998; Baldi *et al.* 2003; MacAnaney *et al.* 2011; Kiely *et al.* 2015; Wilkerson *et al.* 2012; O'Connor *et al.* 2012; 2015; Reusch *et al.* 2013; Green *et al.* 2015; Gildea *et al.* 2019). This decrement is clinically significant given that $\dot{V}O_{2peak}$ is an established clinical predictor of cardiovascular and all-cause mortality (Regensteiner *et al.* 1995; Kodama *et al.* 2009; Reusch *et al.* 2013). When accounting for ageing, $\dot{V}O_{2peak}$ declines at a rate of ~10% per decade in healthy older adults (Higginbotham *et al.* 1986; Beere *et al.* 1999; Gravelle *et al.* 2012; O'Connor *et al.* 2015). Crucial mediators for reduced peak responses and thus exercise tolerance in people with T2D and ageing *per se* may include a reduced ability of the cardiovascular system to supply O_2 to the contracting skeletal muscle during exercise (Gravelle *et al.* 2012; Green *et al.* 2015; Huebschmann *et al.* 2015) implicating both central and/or peripheral mechanisms (Green *et al.* 2015).

Maximal oxygen uptake ($\dot{V}O_{2max}$), represents integration between the pulmonary, cardiovascular and muscular systems to uptake, transport and use O_2 respectively for ATP regeneration via oxidative processes within the mitochondria (Poole, 1997; Wagner *et al.* 1997; Levine, 2008; Gildea *et al.* 2019). Specifically, when relating to the Fick equation, $\dot{V}O_{2max}$ is governed by changes in cardiac output ($\dot{Q}$) and arteriovenous oxygen difference ($a\text{-}vO_{2diff}$), providing a volume-weighted average of fractional O_2 extraction across the whole body when measuring pulmonary $\dot{V}O_2$ (Benson *et al.* 2013; Okushima *et al.* 2016). One limitation of this is that pulmonary $\dot{V}O_2$ during a RI exercise test may not accurately represent changes in microvascular O_2 delivery and O_2 exchange within the exercising muscle (Okushima *et al.* 2015; 2016; Gildea *et al.* 2019) as the adjustment of microvascular muscle blood flow and O_2 extraction during exercise possibly being different in the working muscle as a result of redistribution of blood flow (Spencer *et al.* 2012).

In individuals with uncomplicated T2D, considerable evidence exists suggesting that peripheral O_2 delivery to the exercising muscle is impaired during steady-state exercise (Kingwell *et al.* 2003; Bauer *et al.* 2007; Lalande *et al.* 2008; MacAnaney *et al.* 2011; Kiely *et*

al. 2014; Rocha *et al.* 2019) and graded exercise to exhaustion (Kiely *et al.* 2014; Gildea *et al.* 2019). Kiely *et al* (2014) were the first to report on peak leg blood flow responses during maximal exercise in uncomplicated T2D. Using a supine single-leg plantar flexion exercise, they observed a lower peak force (relative to MVC) for the group with T2D which was accompanied by a reduced maximal blood flow and vasodilatory response. Adding on from this research, the same research group (Gildea *et al.* 2019) investigated the effects of T2D on muscle deoxygenation (Δ[HHb+Mb]) responses during RI exercise. Using near-infrared spectroscopy (NIRS), authors made non-invasive assessments of O_2 extraction in the vastus lateralis (VL) muscle by measuring the concentration changes in [HHb+Mb] during RI exercise in middle-aged individuals with T2D and age-matched non-diabetics (ND). The slope of the first linear segment of the bilinear regression function of muscle fractional O_2 extraction against relative power output (%PO), measured as Δ%[HHb+Mb]/Δ%PO was significantly steeper in T2D. These outcomes suggest that impaired O_2 delivery to the exercising muscles is an important underlying mechanism causing impaired exercise tolerance during RI exercise in middle-aged individuals with T2D (Gildea *et al.* 2019).

Whilst $\dot{V}O_{2peak}$, peak PO, time to failure, and PO at VT during the graded exercise have been reported to be significantly lower in older people with T2D compared with their ND age-matched counterparts, O'Connor *et al* (2015) found no difference for $\tau\dot{V}O_{2P}$ during moderate steady-state cycle exercise, findings that are consistent with prior research (Wilkerson *et al.* 2012). This indicates that the effects of T2D on $\dot{V}O_2$ kinetics are masked by the effects of ageing *per se* at least in sedentary men between 60 and 70 years of age (O'Connor *et al.* 2015). To date however, it is unknown whether muscle fractional O_2 extraction is different when comparing older adults with uncomplicated T2D to age-matched healthy controls.

Therefore, the aim of the present experiment was to investigate muscle deoxygenation responses during ramp incremental exercise in older adults with and without T2D.

2.2 Methodology

2.2.1 Participants

Fourteen older participants with T2D (10 males, 4 females) and 14 ND participants (10 males, 4 females) matched in age and BMI volunteered for this study (Table 2.1). All participants were non-smokers and had not smoked during the 12 months prior to the study. All participants with T2D had a history of the disease between 0.4 to 16.9 years (8.0 ± 4.1 years).

2.2.1.1 Recruitment of participants

2.2.1.1.1 T2D participant recruitment

All participants treated for T2D were recruited from St Colmcille's hospital and St Vincent's University hospital, Dublin, Ireland. Individuals with T2D eligible to partake in the study (see section *2.2.1.2.1*) were identified by chart review from study investigators and were informed about the study during a routine visit to the clinic. If interested, they were presented with a study flier which guided them to contact the principal investigators of the study and schedule a meeting. Eligible candidates were then presented with a participant information leaflet (see Appendix 2) at the first meeting with an investigator. This contained an outline to what the study involved including testing procedures. This was also an opportunity for eligible candidates to discuss the project with the investigators. Participants were then given a week to decide whether they would like to partake or not which was done by phone/email depending on the preference of the eligible candidate. Before taking part in any testing procedure, participants were requested to sign an informed consent form (see Appendix 4).

The Faculty of Health Sciences Research Ethics Committee of the University of Dublin, Trinity College Dublin and St Vincent's Healthcare Group Ethics and Medical Research Committee approved the study which was conducted in accordance with the Declaration of Helsinki.

2.2.1.1.2 Non-diabetic (ND) participant recruitment

Non-diabetic individuals were recruited from the general population using posters (see Appendix 1) distributed to multinational companies, active retirement groups and online voluntary sites aimed at people in Dublin city and suburbs.

The contact details of the principal investigators were provided for any person interested in seeking further information about the study. Individuals who sought further information received a copy of the participant information leaflet (PIL) (see Appendix 2) detailing the nature of the study, testing involved, participation requirements as well as the potential risks and benefits associated with participation. Participants were given a week to decide following provision of the study information. A private meeting was offered to each potential participant to discuss the contents of the PIL and any other elements of the study. Upon fulfilling the inclusion criteria (see section *2.2.1.2.2*), participants read and signed the informed consent form (see Appendix 4).

2.2.1.2 Inclusion/exclusion criteria for participation

2.2.1.2.1 Participants with T2D

Participants with T2D were deemed suitable for inclusion in this study if they were aged 60-70 years and diagnosed with T2D with adequately controlled HbA_{1c} levels (<10%). Individuals on exogenous insulin were deemed to be ineligible to take part. Individuals taking part also had to be untrained, defined as ≤ 2 hours of moderate intensity exercise per week for the 6 months prior to the study.

Each person then had to satisfactorily complete a 12-lead electrocardiogram (ECG) exercise stress test using the Bruce protocol, an established clinically validated diagnostic tool which aids the detection of significant coronary arterial disease (CAD), on a treadmill supervised by a medical practitioner in St. Colmcille's Hospital. The ECG complex and heart rhythm was assessed at rest, throughout exercise, and during recovery to ensure an appropriate cardiac response to exercise. Exclusion criteria from the study on the basis of an unsatisfactory exercise stress test were as follows: symptoms of chest discomfort consistent with angina, cardiac dysrhythmias more severe than occasional atrial or ventricular premature contractions, and flat/down-sloping ST segment depression ≥0.1mV. Blood pressure and heart rate were monitored continuously throughout the protocol, with a systolic blood pressure (SBP) in excess of 220 mmHg or a diastolic blood pressure (DBP) in excess of 105 mmHg during exercise being grounds for exclusion.

Additional exclusion criteria included the existence of persistent proteinuria (urine protein >200 mg/dl), high creatinine levels, SBP ≥ 170 mmHg at rest or DBP ≥ 95 mmHg at rest. The

presence of diabetic complications and/or comorbidities such as autonomic insufficiency/dysfunction, symmetrical neuropathy, abnormal cardiac function or evidence of ischaemic heart disease, angina or other cardiopulmonary symptoms which impair performance during exercise were also reasons for exclusion. The absence of such comorbidities was confirmed by medical history, physical examination and laboratory testing in the hospital by medical practitioners.

2.2.1.2.2 Participants without T2D (ND)

Non-diabetic people were deemed eligible if they were aged between 60-70 years; were untrained (≤ 2 hours of moderate intensity exercise per week for the previous 6 months) and free of cardiovascular disease (CVD) or other comorbidities impairing exercise performance. Each ND individual had a medical examination by a medical doctor at the Department of Physiology, Trinity College Dublin to ensure satisfaction of inclusion/exclusion criteria for exercise testing. This included the completion of a medical questionnaire (see Appendix 5) and physical examination.

2.2.1.2.3 Fasted venous blood sample collection

On a separate day to exercise testing and subsequent to a 12 hour overnight fast, in participants with T2D blood samples were taken from the antecubital vein for analysis in a medical room in the Department of Physiology, Trinity College Dublin. Venous blood samples were analysed in the biochemistry laboratory in St Vincent's Hospital where measurements of fasting blood glucose and HbA_{1c} were recorded from each venous blood sample.

2.2.1.2.4 Ankle: Brachial Index (ABI)

Pneumatic cuffs (Hokanson, Bellvue, WA, USA) were placed above the antecubital fossa on both arms and at the level of the malleoli on both of the participant's ankles. The flow signal was detected using Doppler Ultrasound at a frequency of 10Hz in the brachial artery, posterior tibial artery and dorsalis pedis artery with the higher of the latter 2 being used for measurement. Briefly, the Doppler ultrasound was placed at the point of interest and the pneumatic cuff was inflated until the flow signal disappeared and then slowly deflated until it reappeared again with the systolic pressure upon signal reappearance recorded. All

measures were repeated bilaterally. ABI was calculated for both sides as the highest ankle pressure (posterior tibial artery or dorsalis pedis artery) divided by the highest brachial artery pressure. Any recording within the range of 0.9 to 1.3 is indicative of an absence of peripheral arterial disease (PAD) (Aboyans *et al.* 2012). The above measures were then repeated after the participant engaged in plantar flexion exercise (30 contractions with the support of a chair to lean on if needed). This was done to see if any compromised vascular flow was apparent after exercise.

2.2.1.3 Physical activity level determination

In accordance with the exclusion criteria (see section 2.2.1.2), all participants were untrained (≤2 hours of moderate intensity activity per week) and had not completed a structured training programme in the 6 months prior. In order to determine physical activity levels for confirmation of each individual's untrained status, the RT3 Tri-axial accelerometers were used.

2.2.1.3.1 RT3 Tri-axial accelerometers

The RT3-triaxial accelerometer (Stayhealthy Inc., CAL, USA) was utilised to collect 5 day physical activity levels from each participant. The RT3 has been recognised as an effective tool for measuring physical activity in both adults and children (Rowlands *et al.* 2004). The RT3 detects movement in three planes; the mediolateral, anteroposterior and vertical to produce a vector magnitude, allowing for measurement of the frequency, intensity, duration and volume of physical activity completed by the individual equipped with the accelerometer throughout the day (Hussey *et al.* 2007). Participants wore the RT3 on their hip during all waking hours for 5 consecutive days, with exceptions made for when they were in water such as when in the shower or swimming pool. Utilising a pre-set mode, the time sampling interval is set to 1 minute and accumulated activity counts on each axis are converted to digital representation for processing. Output is then expressed as mean counts per minute for each activity which is then stored in the unit's memory chip and saved for analysis and interpretation for later (Eston *et al.* 1998). The accelerometry data from the RT3 were assessed for counts.min^{-1} for vector magnitude using previously designed cut-off values for physical activity: inactive (0-100 counts.min^{-1}), light (101-970 counts.min^{-1}), moderate (971-2333 counts.min^{-1}) and vigorous (>2334 counts.min^{-1}) (Rowlands *et al.*

2004).The accumulated physical activity counts obtained for the 5 days recorded were averaged to give a mean output for a single day, with the total time spent in each category being expressed in $hr.day^{-1}$.

2.2.2 Experimental design

2.2.2.1 Study overview

Non-diabetic participants visited the exercise physiology laboratory in the Department of Physiology in Trinity College Dublin on one occasion, whilst all T2D patients were required to visit the exercise testing facility in St Colmcille's Hospital on one occasion. In order to standardise testing conditions, participants refrained from consuming caffeine and alcohol 24 hours prior to testing. They also limited their activity throughout the day to normal activities of daily living with no strenuous exercise 24 hours prior. During this initial visit, anthropometric data was collected. After this, participants performed a RI cycling test to exhaustion.

2.2.2.2 Visit to the exercise physiology laboratory

2.2.2.2.1 Anthropometric and pulse wave velocity measures

Once wearing suitable clothing for exercise (T-shirts and exercise shorts) body mass (kg) was measured to the nearest 0.1 kg and height (m) to the nearest 0.01 m were recorded using a weighing scales and stadiometer (Seca, Hamburg, Germany). These measures were then used to calculate BMI (BMI= body mass / $height^2$). Waist circumference was measured at the halfway point between the bottom rib and the upper margin of the iliac crest, while hip circumference was measured at the point of the greater trochanter or the widest part of the hips if the greater trochanter was not palpable. Using these measures, the waist: hip ratio was then recorded (waist measurement (cm) / hip measurement (cm)).

Aortic pulse wave velocity (PWV) represents the propagation velocity of pressure waves thus, it is a direct measure of aortic stiffness (Laurent *et al.* 2001). As the majority of arterial compliance resides in the aorta, this estimate is closely related to total arterial compliance (Schram *et al.* 2004). PWV was quantified through the non-invasive method of applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia) at the femoral and carotid arteries. PWV measures the speed of the arterial pressure waves travelling along the aortic

and aortoiliac pathway. Briefly, aortic PWV was recorded by sequentially recording ECG-gated waveforms of the carotid and femoral artery of each participant. The recording of the tonometry transit distance was obtained using anthropometric measuring tape by measuring the surface of the body connecting the carotid site with the suprasternal notch and then the suprasternal notch with the femoral site. Carotid-femoral transit time allows an estimation of the average aortic distensibility thus, PWV was calculated using the mean time difference and the arterial path length between the two sites with the SphygmoCor software.

2.2.2.2.2 Ramp incremental test to exhaustion

This test was performed on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V, Groningen, Netherlands) in an upright seated position adjusted to each participant. The test involved a 2 min warm-up at 10 W followed by an incremental increase of 10-20 $W.min^{-1}$ for male participants or 10-15 $W.min^{-1}$ for females to exhaustion. Pedal frequency was kept at a constant and comfortable cadence between 60-75 revolutions per minute (RPM). The test was terminated upon dropping 3 RPM below the set cadence for more than 3 seconds and this was the time point at which the participant's peak workload was recorded. During the test breath by breath expired air was recorded on a metabolic unit (Innocor, Innovision, Odense, Denmark). Ratings of perceived exertion (RPE) was recorded every minute and heart rate was measured continuously throughout the test until failure (see section 2.2.3).

2.2.3 <u>Laboratory equipment and measurement techniques</u>

2.2.3.1 Cardio-metabolic unit

Breath by breath expired air was recorded on a cardio-metabolic unit (Innocor, Innovision, Odense, Denmark) in order to continuously determine $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_e$) and respiratory exchange ratio (RER) responses.

During each test participants wore a nose-clip in order to breathe only through the mouth using through a silicone mouth piece attached to a filter and rebreathing valve unit of the cardio-metabolic unit which allowed for expired gas collection (Fig. 2.1).

Calibration of the cardio-metabolic unit took place before each testing session according to the instructions provided by the manufacturer. This involved calibration of the oxygen sensor, by exposing the sample line to atmospheric air; the flow meter using a 3L syringe (Hans Rudolph, Kansas City, MO); and the flow-gas delay via a specific breathing technique performed by the investigator following a graphical tachymeter on the visual display unit. Calibration of CO_2 was performed every 6 months by the manufacturer.

2.2.3.2 Heart rate

Heart rate (HR) was measured continuously using a HR monitor (Polar RS800CX, Polar Electro Oy, Finland) throughout the exercise test. The heart rate monitor was secured around the participant's chest with a chest strap.

2.2.3.3 Blood pressure

Beat-to-to beat SBP and DBP were continuously measured throughout the exercise test using the volume clamp method at the level of the middle finger (Finometer, Finepress Medical Systems B.V. the Netherlands). Peak SBP and DBP were expressed as the highest mean pressures obtained in the 15 s before cessation of the exercise test. Mean arterial pressure (MAP) was then calculated from systolic and diastolic pressures (MAP: 0.33 SBP + 0.66 DBP).

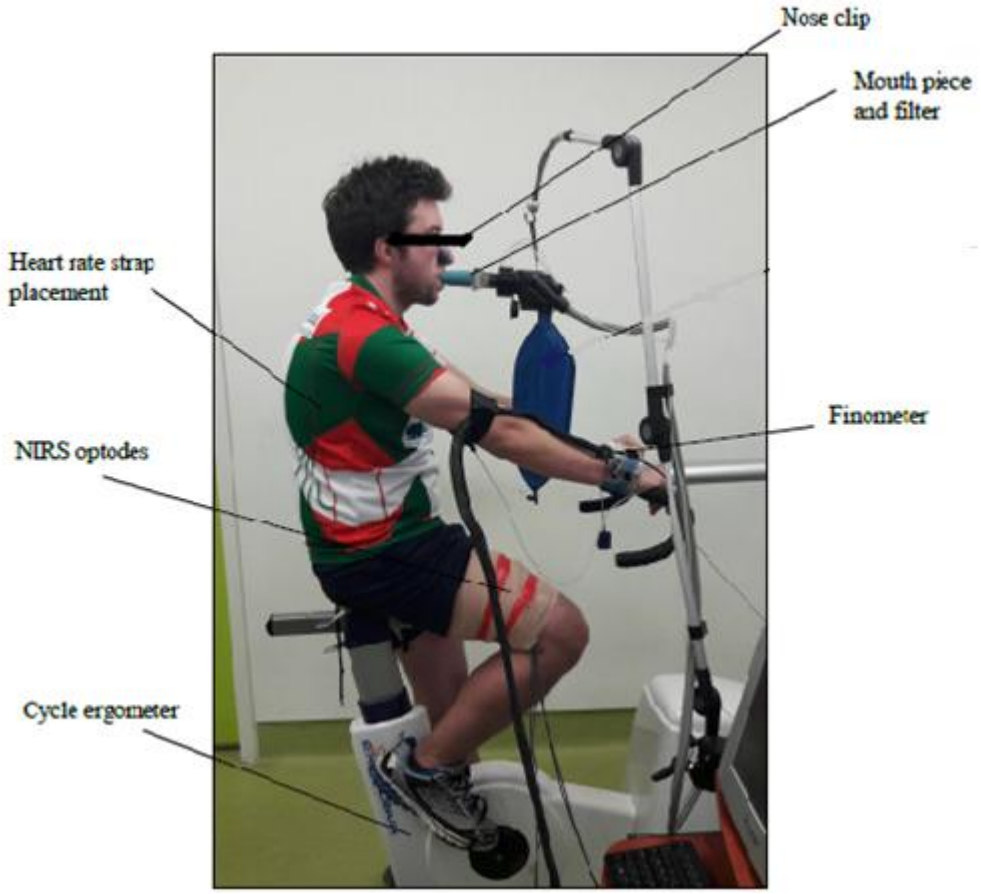

Figure 2.1. Illustration of the experimental set-up for the cycling protocol.

2.2.3.4 Ratings of perceived exertion (RPE)

Participants' RPE during exercise were obtained using a Borg scale of 6 to 20 (Borg, 1970) as a subjective measure of general exercise tolerance (see Appendix 7) at the end of each minute of the exercise test. Prior to commencing the exercise test, investigators allowed participants to become familiar with the scale and were instructed to point to the number on the scale reflective of their perceived exertion at time of prompting.

2.2.3.5 Muscle deoxygenation [HHb+Mb] and subcutaneous fat layer of the vastus lateralis

The local deoxygenation profile of the vastus lateralis (VL) muscle on the right leg was analysed non-invasively throughout all cycle exercise testing using near-infrared spectroscopy (NIRS) (Hamamatsu NIRO-200NX, Hamamatsu Photonics, Hamamatsu, Japan).

This non-invasive measure continuously records oxygenation ([O_2Hb]), deoxygenation ([HHb+Mb]), and total oxygen index (TOI) of haemoglobin in the microvasculature and myoglobin (Mb) in the skeletal muscle giving a reflection of the delivery of O_2 and consumption by the skeletal muscle during rest and exercise (DeLorey *et al.* 2003; Ferreira *et al.* 2007; Spencer *et al.* 2012). Therefore [HHb+Mb] measured via NIRS can serves as an indirect measure of fractional O_2 extraction (Elwell, 1995). Light-emitting diodes (LED λ= 735, 810, and 850 nm) were used to obtain the previously mentioned parameters with the 2 light detectors separated by 0.8 cm. The LED and light detector were 3 cm apart. The measures are grounded in the principles of diffusion approximation for high scattering semi-infinite homogenous media and Beer-Lamberts law was used to calculate parameters with the decrease in reflected light used as a measure of the source detector separation distance allowing effective light coefficient estimation (Jacobs *et al.* 2013). The spectral shape of the decay coefficient can be calculated and the tissue saturation can be estimated (Jacobs *et al.* 2013).

In the present study, the thickness of the skin and adipose tissue at the site of the optode placement was determined via 2D ultrasound operating in B-mode (Zonare Ultra Smart Cart, Software version 4.7, USA). The right-sided distal third of the VL muscle was identified via palpation from a study investigator. Hair was removed from the skin using disposable razors and the skin was sterilised using alcohol wipes. The optodes were then placed on the area of interest and stuck to the skin with the use of transparent double-sided tape. These optodes are surrounded by optically dense plastic containers which are then surrounded by black rubber holders to minimise excess light intrusion (Fig. 2.2). These were then further strapped onto the skin with surgical medical tape and then covered in cloth bandages to prevent optode movement or further light intrusion (Fig. 2.2). Before the start of the warm-up preceding every cycle test, the NIRS-derived signal was zero-set while the participant sat still on the bike allowing the NIRS data represent relative change in [HHb+Mb] from the baseline to the end of exercise.

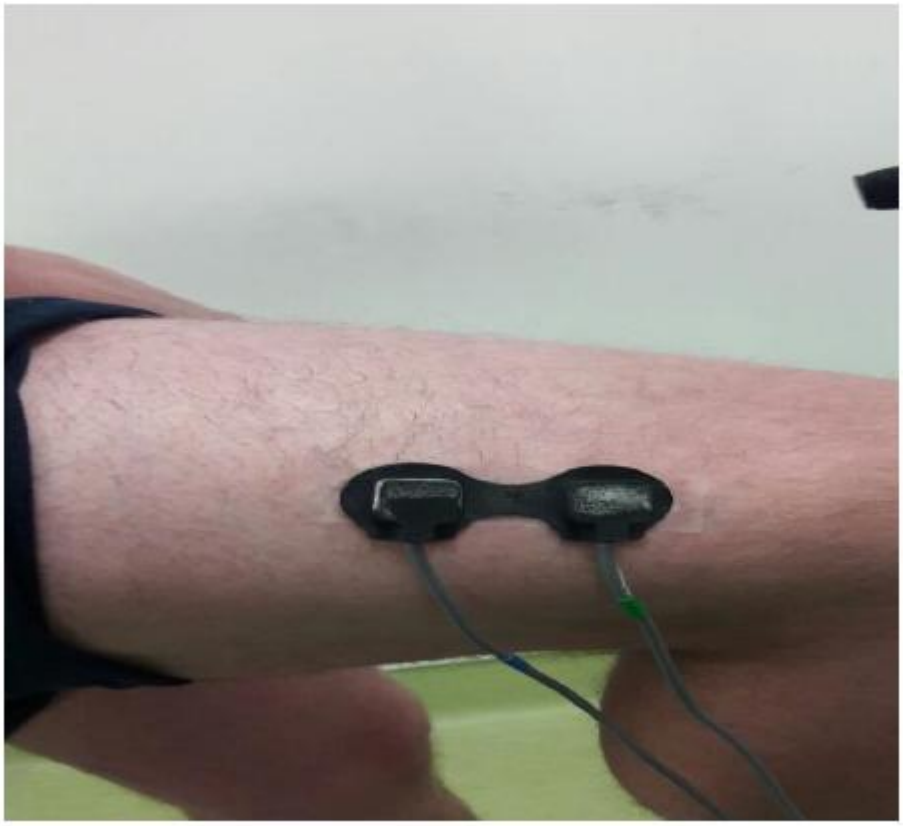

Figure 2.2. Illustration of NIRS set-up at the vastus lateralis muscle for the cycling protocol.

2.2.4 Data Analysis

2.2.4.1 Peak exercise responses

$\dot{V}O_{2peak}$ was recorded as the highest 20 s average $\dot{V}O_2$ achieved during the RI test to exhaustion and peak PO (PPO) was defined as the PO achieved at exhaustion.

2.2.4.2 Muscle deoxygenation

Similar to previous studies from this research group (Gildea *et al.* 2019), the NIRS-derived signal was normalised whereby the unloaded cycling at baseline was adjusted to zero (zero set), thus the Δ[HHb+Mb] are representative of a relative change from baseline to the end of exercise. Therefore the [HHb+Mb] data were normalised with 0% representing the final 30 seconds of unloaded cycling at baseline and 100% representing the highest mean value of the final 30 seconds of exercise to failure during the RI test. Before this analysis, [HHb+Mb] was averaged to 1 second intervals. The second-by-second [HHb+Mb] data was then averaged by applying a five-point moving average and then normalised to the peak

amplitude of the response (Δ%[HHb+Mb]). The Δ%[HHb+Mb] response dynamics were expressed in relation to relative power output (%PO) prior to analysis. The Δ%[HHb+Mb] profile for each participant was then plotted as a function of relative work rate (%POpeak) on TableCurve 2D (v5.01.01, Systat Software, CA, USA). Individual profiles were characterised by a linear function with two terms to establish the slope of increase of [HHb+Mb] (*Slope 1*), the plateau phase as maximal exercise was approached (Slope 2), and the break point (BP) located between the increasing deoxygenation and its plateau. This bilinear model was characterised using the following equation, based on the work of Spencer *et al* (2012) and previously used during RI exercise in T2D by Gildea *et al* (2019):

$$y = a + b*x - c*(x-d)*f$$

$$f=if\ (x<d,\ 0,\ 1)$$

where a and b represent the y-intercept and slope of the first linear function, d is the time delay or 'breakpoint' (namely, the x value at the break point between the two segments) and the slope of the second linear function is calculated from the estimates of b and c (namely, parameter b-c).

2.2.5 Statistical analysis

SigmaPlot version 12 (Systat Software, Point Richmond, CA) was utilised for performing statistical analysis. Normality was assessed using a Shapiro-Wilk test. Physical parameters and physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, while the Mann-Whitney U test was employed for non-parametric analyses. Statistical significance was accepted at $P \leq 0.05$. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data not normally distributed.

The key variable for this experiment is the first linear component of the bilinear model or *slope 1* (Δ%[HHb+Mb]/Δ%PO), the rate of increase in relative [HHb+Mb] (%[HHb+Mb]) normalised to peak power output (%PO) prior to break point (BP). Recent studies comparing healthy young males with female participants and exploring the effects of varying pedal rates among healthy individuals have reported a mean value for *slope 1* of 1.2 ± 0.3 (Mean ± SD) with the difference between groups or conditions being 0.4. Therefore, given that the

estimated SD of each population is 0.3 and that the minimum difference we wish to detect as significant is 0.4, the minimum sample size needed to detect with a statistical power of 80% (α=0.05) by a t-test power calculation design based on 2 groups is 12 individuals.

2.3 Results

2.3.1 Participants

2.3.1.1 Physical characteristics

Physical characteristics for the T2D and ND participants are displayed in Table 2.1. No differences were observed for any physical characteristics with the exception of ABI and HbA_{1c}, suggesting that a good matching of participants was observed between groups.

Table 2.1 ***Anthropometric, ABI, PWV, haematological parameters and resting BP data***

	T2D (n=14)	**ND (n=14)**	***P value***
Sex (M,F)	(9,5)	(10,4)	-
Age, yr	63 ± 3	65 ± 4	0.14
Height, m	*1.7 (0.1)*	*1.7 (0.2)*	0.58
Body Mass, kg	85 ± 15	78 ± 11	0.16
BMI, $kg.m^2$	29.0 ± 3.7	27.1 ± 2.8	0.13
Waist-To-Hip Ratio, a.u	1.0 ± 0.1	1.0 ± 0.1	0.97
Fat Layer VL, mm	*5.8 (3.5)*	*4.2 (1.6)*	0.08
Pre-exercise ABI, a.u	*1.1 (0.2)*	*1.0 (0.1)*	0.02
Post-exercise ABI, a.u	*1.1 (0.2)*	*1.0 (0.1)*	0.03
PWV, $m.s^{-1}$	*9.2 (5.8)*	*9.3 (1.8)*	1.00
HbA_{1c} (%)	*7.5 (2.4)*	*5.3 (0.3)**	0.005
Resting SBP, mmHg	123 ± 14	126 ± 10	0.58
Resting DBP, mmHg	74 ± 8	77 ± 11	0.40

Mean ± SD are shown for values that are normally distributed while median (interquartile range) shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample size is as follows: Waist-to-hip ratio (WHR), n = 13 (T2D); ankle-brachial index (ABI), n = 12 (ND); pulse wave velocity (PWV), n = 13 (T2D) and 5 (ND); HbA1c, n= 11 (T2D); Blood pressure (SBP and DBP), n = 13 (T2D) and n = 12 (ND).

2.3.1.2 Prescriptive medications

Table 2.2 ***Prescriptive medication for individuals with T2D and ND controls.***

	T2D	ND
Anti-hypertensives		
Angiotensin converting enzyme inhibitor	3	
Angiotensin II receptor blocker	1	
Asprin	1	
Statins	6	3
Hypoglycaemic medications		
Metformin	13	
Sulphonylureas	8	
DPP-4 inhibitors	4	

2.3.1.3 Physical activity levels

Group mean physical activity levels are shown in table 2.3. Participants with T2D engaged in more light activity throughout the day than their ND counterparts. No difference was observed for any other physical activity range between groups.

Table 2.3 ***RT3 accelerometry data for individuals with T2D and ND participants***

	T2D	ND	*P value*
Inactive, h.day^{-1}	17.03 ± 1.85	20.14 ± 3.83	0.08
Light, h.day^{-1}	5.34 ± 0.87	2.87 ± 2.25	0.02
Moderate, h.day^{-1}	1.30 ± 1.03	0.73 ± 1.12	0.14
Vigorous, h.day^{-1}	0.33 ± 0.37	0.27 ± 0.55	0.23

Mean ± SD are shown for values. n = 7 (T2D) and n = 6 (ND).

2.3.2 Performance data for Ramp incremental cycling test

2.3.2.1 Peak physiological responses

Peak physiological responses for both groups are displayed in table 2.4. When normalised to body mass, $\dot{V}O_{2peak}$ (ml.kg^{-1}.min^{-1}) was significantly lower ($P < 0.05$) in the T2D group compared with the ND group. Time to failure (TTF) during the RI test was significantly greater ($P < 0.05$) in the ND group compared with T2D. No other significant differences were observed between groups for peak parameters.

Table 2.4 ***Physiological responses at peak exercise.***

	T2D (n=14)	**ND (n=14)**	***P* value**
$\dot{V}O_{2peak}$, L.min^{-1}	2.0 ± 0.4	2.1 ± 0.6	0.45
$\dot{V}O_{2peak}$, mL.kg^{-1}.min^{-1}	23.3 ± 4.0	27.1 ± 5.7*	0.05
Peak Power, W	143 ± 35	165 ± 48	0.18
Peak HR, beats.min^{-1}	154 ± 5	151 ± 13	0.53
Peak RER, a.u	*1.1 (0.1)*	*1.1 (0.1)*	0.30
Peak RPE	16.0 ± 4.0	17.0 ± 2.0	
TTF, s	646 ± 89	732 ± 135*	0.04

Mean ± SD values are shown in normal font which were normally distributed while median (interquartile range) are shown in italic font were data showed significant skewness and were not normally distributed in one or both groups. *Significantly different than T2D.

2.3.2.2 NIRS-derived [HHb+Mb] response dynamics

Mean parameter estimates of the bilinear response model of Δ%[HHb+Mb]/Δ%PO are displayed in in Table 2.5 while Figure 2.3 displays a representative profile of the response during RI exercise for individuals with T2D and ND controls. None of the parameter estimates of Δ%[HHb+Mb]/Δ%PO was significantly different between groups. Due to technical problems with equipment data from one of the participants in the control group were excluded.

Table 2.5 ***Parameter estimates for the Δ%[HHb+Mb] profile for both individuals with T2D and ND controls plotted as a function of PO (%) during ramp incremental exercise***

	T2D (n=14)	ND (n=13)	P value
Slope1	*1.66 (0.87)*	*1.52 (0.73)*	0.48
Slope2	0.06 *(0.86)*	*-0.22 (0.91)*	0.10
BP (%)	*72.05 (4.55)*	*83.35 (3.46)*	0.18

Values are displayed median (interquartile range) due to skewness and non-normal distribution in one or both groups.

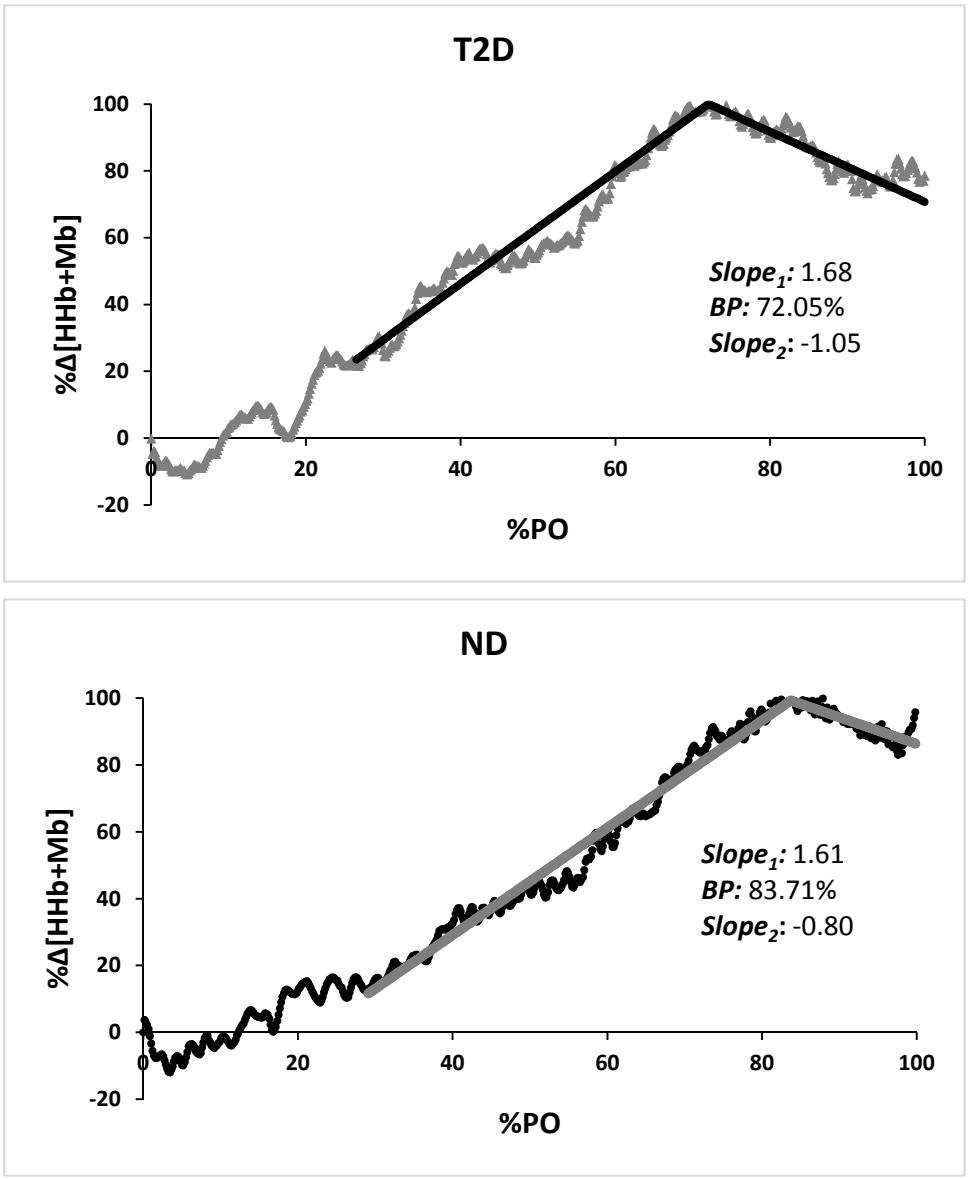

Figure 2.3. Representitive individual fits of Δ%[HHb+Mb]/Δ%PO for one participant with T2D and a ND control during ramp incremental exercise. The bilinear model is superimposed onto the %[HHb+Mb]/%PO for both individuals. Associated parameters are shown for both individuals. BP, break point; *Slope 1*, reponse slope prior to BP; *Slope 2*, response slope post-BP.

2.4 Discussion

This study examined the influence of T2D on the profile of muscle fractional oxygen extraction (as indicated by NIRS-derived [HHb+Mb] response in the vastus lateralis) during ramp incremental (RI) cycling exercise in older adults. The main finding of the current study was that the first slope (*slope 1*) of the bilinear regression function used to assess the adjustment of [HHb+Mb] between older individuals with T2D and ND controls was not different, even in the presence of a reduced $\dot{V}O_{2peak}$ (normalised to body mass) in the T2D group.

Evidence suggests that ageing (Behnke *et al.* 2005; 2006) and T2D (Behnke *et al.* 2002; Padilla *et al.* 2007) show vascular impairments which would be expected to alter microvascular function in such a way that a steeper slope of Δ%[HHb+Mb]/Δ%PO would occur during a RI cycling exercise. In the current study, the ~14% reduction in $\dot{V}O_{2peak}$ observed in the older individuals with T2D compared to ND controls is similar to the ~14% reduction reported by O'Connor *et al* (2015) in older people with T2D compared to ND controls with similar physical characteristics. Whilst the reduced $\dot{V}O_{2peak}$ in these older cohorts of individuals with T2D is numerically lower than that of middle age participants with T2D (~20%) (Regensteiner *et al.* 1998; Baldi *et al.* 2003; MacAnaney *et al.* 2011; O'Connor *et al.* 2012; 2015; Kiely *et al.* 2014; Gildea *et al.* 2019), the reduction in $\dot{V}O_{2peak}$ observed in the present study in older people with T2D cannot be attributed to an increased muscle fractional oxygen extraction which would be indicative of impairments in O_2 delivery and/or utilisation within the working muscle as reported in middle age (<55 y) participants with T2D compared to age matched ND controls (Gildea *et al.* 2019).

The slope of the first linear component (*slope 1*) of the Δ%[HHb+Mb]/Δ%PO response during the RI cycle exercise observed by Gildea *et al* (2019) in middle-aged participants with T2D (*median (interquartile range): 1.59 (1.14)*) was similar to *slope 1* reported in the current study in both older individuals with T2D and ND controls, whilst the slope was lower in the middle-aged ND control group of Gildea *et al* (2019) (*1.23 (0.51)*). This infers that in individuals with T2D and older healthy sedentary controls, O_2 availability within the microvasculature of the exercising muscle is limited (DiMenna *et al.* 2010d) which may indicate a poor matching of O_2 delivery ($\dot{Q}O_2$) both between and within the active musculature to meet the muscles $\dot{V}O_2$ demands for contraction. Furthermore, during a RI

cycling exercise in the supine posture where the exercising musculature is above the level of the heart, DiMenna and colleagues (2010d) observed a left-ward shift in the Δ%[HHb+Mb]/ Δ%PO slope compared to upright cycling in healthy young participants (DiMenna *et al.* 2010d). The supine position which may be used to mimic reductions in O_2 delivery to the active muscles (Jones *et al.* 2006; Boone *et al.* 2009; DiMenna *et al.* 2010d), creates conditions whereby gravitational assistance to the muscle blood flow response is excluded, thereby reducing muscle perfusion pressure (Jones *et al.* 2006; DiMenna *et al.* 2010d).

In animal models, during the rest-exercise transition, an undershoot in microvascular O_2 partial pressure ($Pm\dot{V}O_2$) has been reported in both older rats (Behnke *et al.* 2005; 2006) and rats with T2D (Behnke *et al.* 2002; Padilla *et al.* 2006; 2007) which may be related to a poor $\dot{Q}O_2$ response, further suggesting a mismatch of $\dot{Q}O_2$/ $\dot{V}O_2$ (Heinonen *et al.* 2016; Poole *et al.* 2020). Furthermore, in older rats that were less physically active to young rats, a reduction in blood flow to highly oxidative muscles (i.e. spinotrapezius muscle) via fewer feed arteries was reported (Behnke *et al.* 2006), highlighting a further mismatching of $\dot{Q}O_2$/ $\dot{V}O_2$.

Using rat models, Ferreira *et al* (2006) reported a higher fractional O_2 extraction at the same submaximal workloads in rats in more glycolytic fast twitch fibres compared to fibres with slow twitch oxidative fibres (Ferreira *et al.* 2006). Therefore, due to possible decreases in oxidative fibre numbers and metabolic capacity, along with an increase in the percentage of more glycolytic fibres in both individuals with T2D (Marin *et al.* 1994) and older adults (Proctor *et al.* 1995; Gurd *et al.* 2006), a further mismatch of $\dot{Q}O_2$/ $\dot{V}O_2$ and thus, lower $Pm\dot{V}O_2$ could take place. This initial rapid decline reported in rats may also indicate an attenuated rate of diffusion of O_2 across the capillary-myocyte space to the mitochondria lowering intramyocyte PO_2 and thus, impairing muscle metabolism and function (Behnke *et al.* 2002; 2005; Gravelle *et al.* 2011; Gildea *et al.* 2019).

The findings of the present study are in accordance with previous research which reported a blunted microvascular hyperaemic response during constant-load cycle exercise in both individuals with T2D (Bauer *et al.* 2007; Rocha *et al.* 2019) and older ND individuals (DeLorey *et al.* 2005). During moderate-intensity cycling, $\tau\dot{V}O_{2P}$ was found to be slower in individuals with T2D compared to ND controls (T2D: 44 ± 10 s; ND: 34 ± 8 s) (Bauer *et al.* 2007). In occurrence with this, [HHb+Mb] assessed using NIRS in the vastus lateralis muscle displayed

an overshoot at the onset of exercise in the participants with T2D. On the other hand, with respect to healthy ageing, DeLorey *et al* (2005) observed a slower $\tau\dot{V}O_{2P}$ kinetic response in older (49 ± 8 s) compared with younger (29 ± 4 s) adults during heavy-intensity cycling exercise (DeLorey *et al.* 2005). In the same study, while no difference was observed with respect to the time delay (TD) of [HHb+Mb], a significantly faster time constant (τ) was reported for the older (8 ± 2 s) compared with the young (14 ± 2 s) adults (DeLorey *et al.* 2005). These results infer a slower microvascular $\dot{Q}O_2$ during the rest-exercise transition, thereby necessitating an increased O_2 extraction to accommodate the mitochondrial $\dot{V}O_2$ requirement and thus, causing a sharp decline or undershoot in $Pm\dot{V}O_2$ as observed in the older rats (Behnke *et al.* 2005; 2006) and rats with T2D (Behnke *et al.* 2002; Padilla *et al.* 2006; 2007). This may then limit the O_2 diffusion capacity, increasing reliance on PCr breakdown and anaerobic glycogenolysis consequently increasing metabolic perturbations and premature muscle fatigue (Taylor *et al.* 1997).

In isolated muscles, using an incremental intermittent plantar flexion exercise model, Kiely *et al* (2014) measured leg haemodynamic responses using venous occlusion plethysmography (VOP) in age-matched middle-aged men and women with and without T2D (Kiely *et al.* 2014). Peak leg blood flow (LBF) responses were significantly lower in T2D with no difference reported for sex. These results occurred in conjunction with a ~15% lower peak force (relative to MVC) in participants with T2D and are consistent with studies using whole body graded protocols showing a ~15% reduction in peak power output in T2D (Regensteiner *et al.* 1995, 1998; MacAnaney *et al.* 2011; Wilkerson *et al.* 2012; Kiely *et al.* 2014, O'Connor *et al.* 2015; Green *et al.* 2015; Gildea *et al.* 2019). Thus, impairments in the peak hyperaemic and vasodilatory responses during graded exercise in T2D (Kiely *et al.* 2014) may affect the reductions observed in $\dot{V}O_{2peak}$.

The lack of difference observed in the present study for *slope 1* of the muscle [HHb+Mb] during RI exercise between older individuals with T2D and ND controls is in line with the lack of difference previously observed in $\dot{V}O_2$ kinetics in older individuals with T2D compared to age-matched ND controls during moderate intensity cycling exercise (Wilkerson *et al.* 2012; O'Connor *et al.* 2015). Therefore, it appears that in older individuals without T2D, ageing *per se*, at least in inactive otherwise healthy people, has a very powerful deconditioning effect on the cardiovascular system and skeletal muscle metabolism which in turn leads to a

slowing of aerobic metabolism and thus, the $\dot{V}O_2$ kinetic response during moderate exercise (O'Connor *et al.* 2015). This notion is further supported by George *et al* (2018) who observed that an age-related slowing of $\dot{V}O_2$ kinetics can be eliminated with endurance training while an inactive lifestyle can have negative consequences on $\dot{V}O_2$ kinetics in both young and older adults (George *et al.* 2018). Specifically, $\dot{V}O_2$ kinetics during moderate-intensity cycling were not slower in older compared with younger adults when matched for training status, while a significant difference in $\dot{V}O_2$ kinetics was observed between older endurance-trained athletes vs inactive older adults. In further support of this argument, just 12 weeks of endurance training exercise in older inactive adults was effective at significantly accelerating $\dot{V}O_2$ kinetic responses in older adults (Murias *et al.* 2010).

On the other hand, Gravelle and colleagues (2011) assessed the [HHb+Mb] response to a RI cycle test in older vs. younger recreationally active males and showed that the slope of the sigmoidal response for Δ%[HHb+Mb] relative to absolute power output (W) was greater in the older group (Gravelle *et al.* 2011). However, when Δ%[HHHb+Mb] was expressed to relative power output (Δ%PO), no difference in the sigmoidal slope was observed between groups. This may suggest that the age-related microvascular blood flow reductions are less pronounced or non-existent in active older adults. Therefore, fitness level and not exclusively ageing *per se* seems to play a critical role controlling both the [HHb+Mb] slope of increase during RI tests and the $\dot{V}O_2$ kinetic responses during constant-load exercise, in healthy adults (Gravelle *et al.* 2011; Murias *et al.* 2011; George *et al.* 2018).

Impairments in both macrovascular blood flow (Kiely *et al.* 2014; duManoir *et al.* 2016) along with impairments in endothelium-mediated function via decreased nitric oxide and increased endothelian-1 bioavailability within the microvasculature of the contracting muscle may contribute to impairments in $\dot{Q}O_2$ to match $\dot{V}O_2$ during RI cycling exercise in both T2D (Padilla *et al.* 2006; 2007; Heinonen *et al.* 2016) and ageing (Muller-Delp *et al.* 2002). Reductions in mitochondrial content and oxidative capacity in T2D (Kelley *et al.* 2002; Ritov *et al.* 2005) and ageing (Conley *et al.* 2007; Gurd *et al.* 2008), and alterations in muscle fibre type distribution towards a more glycolytic phenotype (Marin *et al.* 1994; Oberbach *et al.* 2006; Conley *et al.* 2007) may also play a role in increased fractional O_2 extraction (Ferreira *et al.* 2006).

2.4.1 Conclusion

In conclusion, $\dot{V}O_{2peak}$ was significantly reduced in the older individuals with T2D compared with ND controls, representing a 14% reduction in peak exercise capacity, however, no difference was observed in the slope of the first linear component of the Δ%[HHb+Mb]/Δ%PO response between the 2 groups. These findings suggest that in contrast to middle-aged individuals with T2D, a greater rate of fractional oxygen extraction in the exercising musculature for a given increase in $\dot{V}O_2$, indicative of reduced O_2 delivery, is not a contributing factor to the reduction in $\dot{V}O_{2peak}$ observed in older individuals with T2D compared to age-matched ND controls.

Chapter 3: Influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise in older adults with type 2 diabetes.

3.1 Introduction

In middle-aged people with uncomplicated T2D, a slowed adjustment of oxidative metabolism during a heavy-intensity constant-load cycling bout can be overcome by a prior heavy-intensity priming exercise (PE) bout with this improvement occurring as a result of an increase in the amplitude of the primary phase of the $\dot{V}O_2$ kinetics response ($A\dot{V}O_{2P}$) along with a reduction in the time constant of the $\dot{V}O_2$ primary phase ($\tau\dot{V}O_{2P}$) and of the amplitude of the $\dot{V}O_2$ slow component ($A\dot{V}O_{2SC}$) (Gildea *et al.* 2020). The resultant enhanced oxidative metabolism allows for less reliance on non-oxidative energy stores which may increase the time to fatigue and thus, exercise tolerance which in turn may reduce the risk of cardiovascular disease and all-cause mortality (Regensteiner *et al.* 1995; Kodama *et al.* 2009; Reusch *et al.* 2013).

These results observed in middle-aged people with uncomplicated T2D are in concurrence with previous studies which investigated the supine cycle exercise model as a method of exploring a condition where the primary phase of $\dot{V}O_2$ is limited by O_2 availability (Jones *et al.* 2006; Goulding *et al.* 2017; 2018). Improvements in $\tau\dot{V}O_{2P}$ observed in the supine position were attributed to enhanced O_2 delivery with PE (Jones *et al.* 2006; Goulding *et al.* 2017). The O_2 limitation during supine cycling is attributed to a loss of hydrostatic gradient effect of gravity resulting in a lowering of the pressure head for blood-to-myocyte O_2 diffusion in the working muscles, thus slowing the rate of increase in $\dot{V}O_2$ at exercise onset (Koga *et al.* 1999; Goulding *et al.* 2017). Jones *et al* (2006) observed faster $\tau\dot{V}O_{2P}$ response during a subsequent bout of heavy-intensity exercise when performed after a PE bout in the supine but not the upright position. They interpreted these results to indicate that the faster $\dot{V}O_2$ kinetic response in the supine position after priming was as a result of a reduced constraint in O_2 availability to the exercising muscle during the subsequent bout with a more appropriate blood flow distribution within the exercising muscles being key to the faster $\tau\dot{V}O_{2P}$ response (Jones *et al.* 2006). The resultant improvement in blood flow distribution is theorised to result from improved muscle vasodilation and a rightward shift in the HbO_2 dissociation curve. In a similar study, Goulding *et al* (2017) also found a faster $\tau\dot{V}O_{2P}$ during a

subsequent heavy-intensity bout of supine exercise following PE. They also observed a slower time constant of the first phase of the muscle deoxygenation ([HHb+Mb]τ_p) response with PE in the supine condition indicative of enhanced O_2 supply compared with O_2 utilisation within the exercising muscle. Both studies (Jones *et al.* 2006; Goulding *et al.* 2017) found an enhanced primary phase amplitude and reduced $\dot{V}O_{2sc}$ amplitude during upright cycling, while attributing the lack of change in the $\tau\dot{V}O_{2P}$ to limitations in adjustment of metabolic pathways due to the young age, high fitness and fast $\tau\dot{V}O_{2P}$ of the participants at the outset in both studies.

Whilst previous research has shown a slowed $\dot{V}O_2$ kinetic response in older adults when compared to young healthy participants during severe-intensity cycle exercise (Sabapathy *et al.* 2004; DeLorey *et al.* 2005), and PE has been found to be an effective intervention at enhancing exercise tolerance during moderate-intensity cycle exercise in both healthy older (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2009) and middle-aged diabetics (Rocha *et al.* 2019), to the knowledge of this author, no previous studies have investigated the effects of PE on a subsequent heavy-intensity exercise bout in both older people with and without uncomplicated T2D.

Thus, with muscle O_2 supply appearing to be impaired in both older populations with and without T2D, and heavy intensity PE being advocated as an intervention that may enhance muscle O_2 delivery in scenarios where blood flow is impaired, we tested the hypothesis that PE would increase the speed of the adjustment of the $\tau\dot{V}O_{2P}$ and/or decrease the $\dot{V}O_{2SC}$ amplitude during high-intensity cycling in both older individuals with T2D and ND controls.

3.2 Methodology

3.2.1 Participants

Nine individuals with uncomplicated T2D (8 men / 1 women), and 9 healthy individuals without T2D (ND) (8 men / 1 women) volunteered to participate in this study (Table 3.1). All of the individuals with T2D, and 7 of the ND controls also participated in *Experiment 1*. All participants were non-smokers and had not smoked during the 12 months prior to the study. Participants with T2D had a mean clinical history of diabetes of 6.3 ± 3.3 years.

3.2.1.1 Recruitment of participants

3.2.1.1.1 T2D participant recruitment

As per section 2.2.1.1.1

3.2.1.1.2 Non-diabetic (ND) participant recruitment

As per section 2.2.1.1.2

3.2.1.2 Inclusion/exclusion criteria for participation

3.2.1.2.1 Participants with T2D

As per section 2.2.1.2.1

3.2.1.2.2 Participants without T2D (ND)

As per section 2.2.1.2.2

3.2.1.2.3 Fasted venous blood sample collection

As per section 2.2.1.2.3

3.2.1.2.4 Ankle: Brachial Index (ABI)

As per section 2.2.1.2.4

3.2.1.3 Physical activity level determination

As per section 2.2.1.3

3.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

3.2.2 Experimental design

3.2.2.1 Study overview

Non-diabetic participants visited the exercise physiology laboratory in the Department of Physiology in Trinity College Dublin on two occasions, whilst all participants with T2D were required to visit the exercise testing facility in St Colmcille's Hospital on one occasion and were able to choose the exercise laboratory (between the hospital and university) for the 2nd visit. In order to standardise testing conditions, participants refrained from consuming caffeine and alcohol 24 hours prior to all testing. They also limited their activity throughout the day to normal activities of daily living with no strenuous exercise 24 hours prior. During the initial visit, anthropometric data was collected. After this, participants performed a RI cycling test to exhaustion. On the second visit, participants carried out 4 heavy intensity bouts of cycle exercise, 2 of which were carried out without prior exercise while the other 2 were carried out following prior heavy-intensity cycling exercise (see section 3.2.2.3.1).

3.2.2.2 Visit 1 overview

3.2.2.2.1 Anthropometric and pulse wave velocity measures

As per section 2.2.2.2.1

3.2.2.2.2 Ramp incremental test to exhaustion

As per section 2.2.2.2.2

3.2.2.3 Visit 2 overview

3.2.2.3.1 Heavy intensity cycle exercise and priming protocol

From the previously completed RI cycling test during visit 1, the work rates required for this protocol were calculated. A power output corresponding to 50% delta (50% Δ; the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2peak}$) was determined. Participants then performed 4 bouts of constant-load heavy-intensity cycling at this intensity. Two of these constant-load bouts were completed without prior exercise (Unprimed 50% Δ) and two bouts were completed after PE at an intensity of 50% Δ (Primed 50% Δ) so that the "unprimed" bouts were used as PE bouts (Fig 3.1). The duration of each

step transition was 6 minutes and each transition was preceded by a 3 minute baseline cycling period of 10W. Changes in WR were initiated as a step function without a warning to the individual. There was a 12 minute passive rest period between each bout of cycling, except following the first primed heavy-intensity bout where participants remained passively seated in a chair for 45 minutes. This resting period has been shown to be sufficient for physiological restoration following a bout of heavy-intensity exercise, thus having no effect on $\dot{V}O_2$ kinetics during a subsequent bout (Burnley *et al.* 2006).

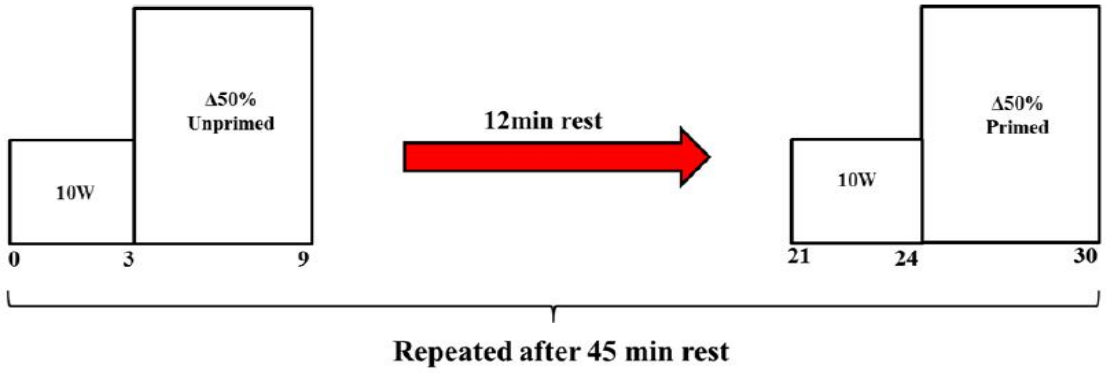

Figure 3.1. Schematic representation of the protocol for visit 2.

3.2.3 Laboratory equipment and measurement techniques

3.2.3.1 Cardio-metabolic unit

As per section 2.2.3.1

3.2.3.2 Heart rate monitor

As per section 2.2.3.2

3.2.3.3 Blood pressure

As per section 2.2.3.3

3.2.3.4 Ratings of perceived exertion (RPE)

As per section 2.2.3.4

3.2.3.5 Muscle deoxygenation [HHb+Mb] and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.5

3.2.4 Data Analysis

3.2.4.1 Peak responses

As per section 2.2.4.1

3.2.4.2 50% Δ analysis

3.2.4.2.1 $\dot{V}O_2$ kinetics

Breath by breath $\dot{V}O_2$ data for each transition were linearly interpolated providing second by second values and time aligned so that 0 represents exercise onset. Each transition was ensemble-averaged to yield a single average response and further time-aligned into 5 second bins to provide a single time-averaged response. The first 20 seconds of $\dot{V}O_2$ data were excluded to avoid fitting of Phase I or cardio-dynamic phase since it is unclear whether this phase is exponential. Therefore fitting and estimation of the model parameters were determined from 20-360 s of the step transition using a weighted least-squares nonlinear regression procedure in which the best fit was calculated by minimisation of the residual sum of squares (TableCurve 2D, Systat). Data lying outside the 95% prediction interval during the initial fitting of the model were excluded. The average and smoothed response was modelled using a bi-exponential function as follows:

Equation 1: $\dot{V}O_2(t) = \dot{V}O_2(b) + Ap(1-e^{-(tp-TDp/\tau p)})F1 + Asc(1-e^{-(tp-TDs/\tau s)})F2$

Where $\dot{V}O_2$ (t) is the absolute $\dot{V}O_2$ at a given time (*t*); $\dot{V}O_2$ (b) is the mean baseline $\dot{V}O_2$ (average of the final 60 seconds of the warm-up); Ap and Asc are the amplitudes of the increase in $\dot{V}O_2$ for Phase II or primary phase and phase III of slow component phase $\dot{V}O_{2SC}$; TDp and TDsc are the phase delays, and τp and τsc are the time constants (time duration for $\dot{V}O_2$ to increase to 63% of the amplitude of each phase) whilst F1 and F2 are conditional expressions which limit the fitting of the phase to the period at and beyond the time delay of that phase.

The absolute primary component amplitude was calculated as follows:

Absolute Ap = $\dot{V}O_2$ (b) + Ap

Because the asymptomatic value (Asc) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise,

the actual amplitude of the slow component was calculated as the absolute difference as follows:

Absolute Asc= End A – Absolute Ap

The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response:

Relative Asc= As / (Ap + As)

The functional "gain" of the primary $\dot{V}O_2$ response (Gp) was calculated as the difference between $\dot{V}O_2$ Ap and $\dot{V}O_2$ baseline normalized to the difference in power outputs between the heavy intensity exercise and unloaded cycling; and the functional gain of the entire response (i.e. end-exercise gain) was calculated in a similar manner.

The mean response time (MRT) was calculated using a mono-exponential function fitted to the entire response:

Equation 2: $\dot{V}O_2$ (*t*) = $\dot{V}O_2$ (b) + Ap $(1\text{-}e^{-(tp\text{-}TDp/\tau p)})$

Finally, the end $\dot{V}O_2$ was calculated as the average across the final 30 s of the constant load test.

3.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

Δ [HHb+Mb] kinetics of the right VL were modelled in response to exercise. As per $\dot{V}O_2$, the NIRS data was linearly interpolated for each transition to provide second by second values. Data for each bout was ensemble-averaged yielding a single average response and then time aligned into 5 second bins to give a single time-averaged response. At exercise onset, a time delay (TD) is present in the [HHb + Mb] profile before increasing exponentially to a steady-state (DeLorey *et al.* 2003), reflecting the matching of muscular local O_2 delivery and O_2 uptake (DeLorey *et al.* 2003). The TD was established via visual inspection by the study investigator as the point of exponential increase in [HHb + Mb]. The data were then fitted from the TD to end of exercise with a bi-exponential model as per $\dot{V}O_2$, to determine the time course of VL [HHb+Mb] response.

The overall change of Δ [HHb+Mb] for phase II was referred to as the effective response time: Δ[HHb+Mb]τ' = TDp + [HHb+Mb]τp

The MRT was calculated using a mono-exponential model (see *Equation 2*) and fitted from the point of increase from the onset to the end of exercise. This provided information on the overall [HHb+Mb] kinetics during heavy intensity exercise.

3.2.4.2.3 Ventilatory threshold

The ventilatory threshold (VT) which is defined as the exercise point at which $\dot{V}_E/\dot{V}O_2$ displays a systematic exponential increase without a concomitant increase in $\dot{V}_E/\dot{V}CO_2$ (Wasserman *et al*. 1973) was estimated by identifying the break-point when plotting $\dot{V}CO_2$ versus $\dot{V}O_2$ (Beaver *et al*. 1986; Amann *et al*. 2006). The first non-linear increases of $\dot{V}E$ and $\dot{V}CO_2$ due to the buffering of protons in response to the increase of blood lactate above resting values relates to the VT (Wasserman *et al*. 1973).

3.2.5 <u>Statistical analysis</u>

Data are presented as mean ± standard deviation. Statistical analysis was performed using SigmaPlot 12 (Systat Software, San Jose, Ca, USA). Normality of data was assessed using a Shapiro-Wilk test. Physical parameters and physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, while the Mann-Whitney U test was employed for non-parametric analyses. Parameter estimates for $\dot{V}O_2$ kinetics and deoxygenation [HHb+Mb] kinetics were analysed using a 2-way (group x condition) repeated measures ANOVA. Post-hoc Holm-Sidak tests were carried out in cases of significant differences being observed. The level of significance was set to $P < 0.05$.

The key variable for the current study is the rate of change in $\tau\dot{V}O_{2P}$, the time required to reach 63% of the difference between the $\dot{V}O_2$ baseline and the plateau of the primary phase. Previous studies published from our laboratory revealed $\tau\dot{V}O_{2P}$ values of 40.7 ± 8.1 s in older individuals with T2D and age-matched ND controls, during moderate-intensity exercise. With respect to previous studies investigating the effects of PE on subsequent $\tau\dot{V}O_{2P}$ (including studies comparing healthy older and younger adults; comparing supine vs. upright postures; and comparing middle-aged individuals with T2D to ND controls), a mean difference of 13.5 s was revealed when comparing $\tau\dot{V}O_{2P}$ values pre- and post-PE. Therefore, assuming that the estimated SD of each group is 8.1 s, and the mean difference we wish to detect is 13.5 s, the minimum sample size we need to detect with a statistical significance of 80% (α= 0.05) by ANOVA test for 2 groups is 8 participants.

3.3 Results

3.3.1 Participants

3.3.1.1 Physical characteristics

Physical characteristics for participants from both groups are presented in Table 3.1. Participants were well matched with no statistically significant difference for age and anthropometrical measures between groups.

Table 3.1 ***Anthropometrical data, ABI, PWV, haematological parameters and resting BP for individuals with T2D and ND controls.***

	T2D (n=9)	**ND (n=9)**	***P values***
Sex (M,F)	(8,1)	(8,1)	
Age, yr	62 ± 3	63 ± 4	0.62
Height, m	1.7 ± 0.1	1.7 ± 0.1	0.33
Body Mass, kg	89 ± 16	83 ± 8	0.30
BMI, kg.m^{-2}	29.4 ± 4.5	28.4 ± 2.3	0.56
Waist-To-Hip Ratio, a.u	*1.0 (0.1)*	*1.0 (0.1)*	*0.60*
Fat Layer VL, mm	5.2 ± 1.6	5.1 ± 1.2	0.91
Pre-exercise ABI, a.u	1.3 ± 0.3	1.0 ± 0.1	
Post-exercise ABI, a.u	1.3 ± 0.4	1.1 ± 0.2	
PWV, m.s^{-1}	*10.3 (5.1)*	*9.4 (1.1)*	*0.50*
HbA1c, %	*6.6 (1.5)*	*5.2 (0)*	0.05
Resting SBP, mmHg	124.2 ± 14.3	128.7 ± 10.5	0.27
Resting DBP, mmHg	74.4 ± 9.7	80.0 ± 9.5	0.63

Mean ± SD are shown for values that are normally distributed while median (interquartile range) shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample size is as follows: pulse wave velocity (PWV), n = 9 (T2D) and 5 (ND); glycosylated haemoglobin (HbA1c), n= 7 (T2D).

3.3.1.2 Prescriptive medications

Table 3.2 ***Prescriptive medications for participants with T2D and ND participants.***

	T2D (n=9)	ND (n=9)
Anti-hypertensives		
Angiotensin converting enzyme inhibitor	1	1
Angiotensin II receptor blocker	1	1
Asprin	1	
Statins	6	1
Hypoglycaemic medications		
Metformin	8	
Sulphonylureas	2	
DPP-4 inhibitors	3	

3.3.1.3 Physical activity levels

Group mean physical activity levels are shown in table 3.3. No difference was observed between groups for any physical activity range estimated using the RT3 accelerometry data.

Table 3.3 ***RT3 accelerometry data for individuals with T2D and ND participants***

	T2D	ND	P Value
Inactive, h.day^{-1}	17.7 ± 1.6	18.5 ± 4.3	0.71
Light, h.day^{-1}	5.2 ± 1.0	4.1 ± 2.8	0.45
Moderate, h.day^{-1}	0.9 ± 0.6	1.1 ± 1.2	0.82
Vigorous, h.day^{-1}	0.2 ± 0.2	0.4 ± 0.5	0.93

Mean ± SD are shown for values. n = 5 (T2D) and n = 6 (ND).

3.3.2 Performance data from the Ramp incremental cycling test

3.3.2.1 Physiological responses

Physiological responses at peak exercise and at VT are shown in Table 3.4. No significant differences were observed for any values at peak or VT between groups.

Table 3.4 ***Physiological responses at peak exercise and VT***

	T2D (n=9)	**ND (n=9)**	***P values***
$\dot{V}O_{2peak}$, L.min^{-1}	2.14 ± 0.4	2.20 ± 0.56	0.80
$\dot{V}O_{2peak}$, mL.kg^{-1}.min^{-1}	24.4 ± 3.4	26.6 ± 6.4	0.36
Peak PO, W	*156 (38)*	*158 (56)*	*0.79*
Peak HR, beats.min^{-1}	153 ± 13	147 ± 13	0.50
Peak RER, a.u	1.1 ± 0.1	1.2 ± 0.1	0.31
TTF, s	674 ± 75	738 ± 117	0.19
$\dot{V}O_2$ @ VT, L.min^{-1}	1.6 ± 0.3	1.5 ± 0.4	0.75
$\dot{V}O_2$ @ VT, mL.kg^{-1}.min^{-1}	18.0 ± 3.6	18.4 ± 4.4	0.83
PO @ 50% Δ, W	*125 (30)*	*127 (43)*	*0.72*

Mean ± SD are shown for values that are normally distributed while median (interquartile range) are shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups.

3.3.3 Performance data from the heavy-intensity exercise bouts

3.3.3.1 $\dot{V}O_2$ kinetics

The parameter estimates of the $\dot{V}O_2$ kinetics response during heavy intensity exercise before and after PE in individuals with T2D and ND controls are shown in table 3.5 while representative responses are shown in figure 3.2. The baseline $\dot{V}O_2$ was higher in the T2D group ($P < 0.05$). In the unprimed condition no differences for the primary component parameter estimates between groups were observed. A slow component was apparent in all individuals, with no differences observed between groups with respect to this phase in the unprimed condition. The overall MRT of the $\dot{V}O_2$ kinetic response was also similar between both groups.

Priming exercise induced an increase in $A\dot{V}O_{2p}$ ($P < 0.05$) and $\dot{V}O_2$ absolute A_p ($P < 0.001$) in both groups, while it also induced a reduction in the $\tau\dot{V}O_{2P}$ ($P < 0.001$) in both groups. The

functional gain of the primary phase also increased ($P < 0.006$) subsequent to PE. In addition, $\dot{V}O_2$ A_{SC} as well as MRT were significantly reduced following PE in both groups ($P < 0.001$ and $P < 0.05$ respectively).

Table 3.5 ***$\dot{V}O_2$ kinetic parameters during unprimed and primed heavy-intensity exercise in individuals with T2D and ND controls.***

	Unprimed		Primed	
	T2D n = 9	**ND n = 9**	**T2D n = 9**	**ND n = 9**
$\dot{V}O_2$ baseline, L.min^{-1}	1.0 ± 0.2	0.8 ± 0.1†	1.0 ± 0.2	0.9 ± 0.1†
$\dot{V}O_2$ A_p, L.min^{-1}	1.0 ± 0.3	1.1 ± 0.5	1.1 ± 0.2*	1.2 ± 0.5*
$\dot{V}O_2$ absolute A_p, L.min^{-1}	2.0 ± 0.3	1.9 ± 0.5	2.1 ± 0.2*	2.1 ± 0.5*
$\tau\dot{V}O_{2P}$, s	40.5 ± 2.9	42.8 ± 5.0	31.7 ± 3.4*	34.5 ± 5.9*
Primary $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	8.4 ± 1.7	8.8 ± 1.3	9.7 ± 1.1*	9.5 ± 1.3*
$\dot{V}O_2$ A_{sc}, L.min^{-1}	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1*	0.2 ± 0.1*
$\dot{V}O_2$ A_{sc}, %	30.6 ± 17.5	27.4 ± 8.5	14.9 ± 6.5*	20.0 ± 7.6*
$\dot{V}O_2$ TD_{sc}, s	128 ± 21	148 ± 25	133 ± 13	130 ± 10
End $\dot{V}O_2$, L.min^{-1}	2.2 ± 0.3	2.2 ± 0.6	2.3 ± 0.3	2.3 ± 0.6
$\dot{V}O_2$ MRT, s	68 ± 18	65 ± 12	52 ± 1*	53 ± 10*
End $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	11.2 ± 1.4	11.4 ± 1.4	10.8 ± 1.6	11.1 ± 1.1

Mean ± SD are shown for values of unprimed and primed heavy intensity cycle exercise $\dot{V}O_2$ kinetics parameters in participants with T2D and ND controls. A, amplitude; TD, time delay; τ, time constant; p, primary phase; sc, slow component phase; MRT, mean response time. *Significantly different from the unprimed bout within the same group ($P \leq 0.05$). †Significantly different from T2D within the same condition ($P \leq 0.05$).

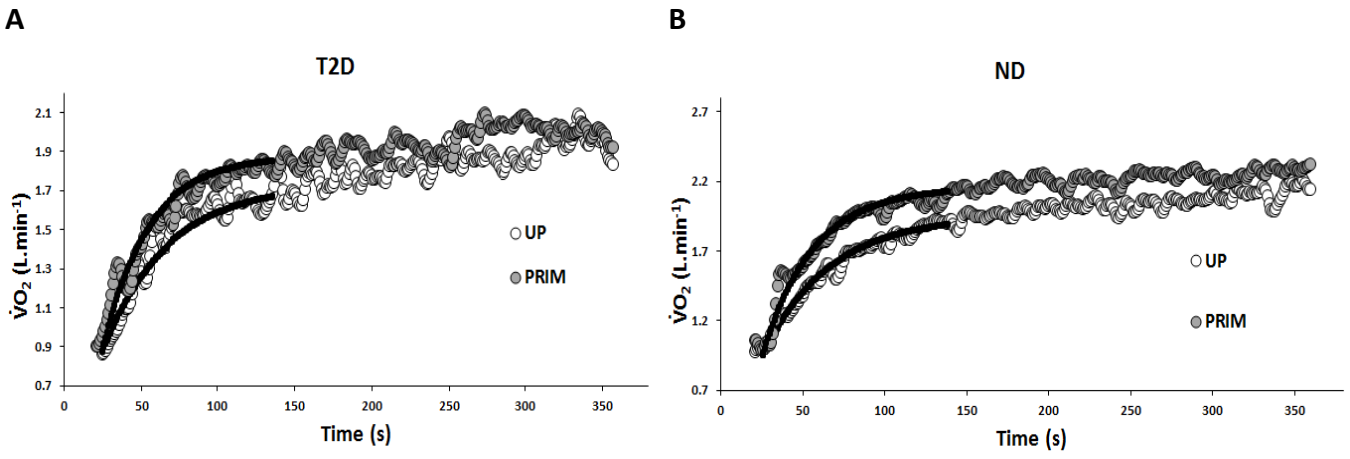

Figure 3.2 Oxygen uptake kinetic responses during heavy intensity exercise before (unprimed: ○) and after (primed: ●) PE in a representative individual with T2D (A) and a ND participant (B). The continuous lines represent the primary phase of the $\dot{V}O_2$ response. It can be observed that the $\dot{V}O_2$ A_p is increased while the $\tau\dot{V}O_{2P}$ is reduced following PE in both groups.

3.3.3.2 Muscle deoxygenation [HHb+Mb] kinetics

The parameter estimates for [HHb+Mb] kinetics during heavy-intensity exercise before and after PE in participants with T2D and ND controls are shown in table 3.6 while representative figures are shown in figure 3.3. No differences were observed between groups for any kinetic parameters.

Subsequent to PE, the TD_p occurred earlier in both groups ($P < 0.05$). In addition, following PE, the time constant of the primary phase ($\Delta[HHb+Mb]\tau_p$) as well as the $\Delta[HHb+Mb]\tau'$ became slower ($P < 0.001$) in both groups.

Table 3.6 ***[HHb+Mb] kinetic parameters during unprimed and primed heavy-intensity exercise in participants with T2D and ND controls.***

	Unprimed		Primed	
	T2D n = 9	**ND** n = 9	**T2D** n = 9	**ND** n = 9
Δ[HHb+Mb] A_p, µM*cm	162 ± 97	186 ± 78	175 ± 105	202 ± 84
Δ[HHb+Mb] TD_p, s	8.8 ± 0.8	10.0 ± 1.8	7.7 ± 1.5	8.4 ± 3.8
Δ[HHb+Mb]τ_p, s	14.7 ± 5.8	13.5 ± 6.1	23.1 ± 6.5*	19.5 ± 10.3*
Δ[HHb+Mb]τ', s	23.5 ± 5.7	23.5 ± 6.6	30.7 ± 5.3*	27.9 ± 11.9*
Δ[HHb+Mb] A_{sc}, µM*cm	16.3 ± 17.5	10.5 ± 11.0	12.0 ± 19.5	8.0 ± 13.0
Δ[HHb+Mb] TD_{sc}, s	108.8 ± 30.0	134.4 ± 24.8	131.5 ± 28.7	141.6 ± 19.9
Δ[HHb+Mb]MRT, s	19.6 ± 6.8	16.6 ± 7.2	18.2 ± 9.4	18.4 ± 7.4

Values are mean ± standard deviation. [HHb+Mb], deoxygenated haemoglobin and myoglobin concentration; A, amplitude; TD, time delay; τ, time constant; p, primary phase; sc, slow component phase; τ', effective response time (τ + TD); MRT, mean response time. *Significantly different from the unprimed bout within the same group ($P \leq 0.05$).

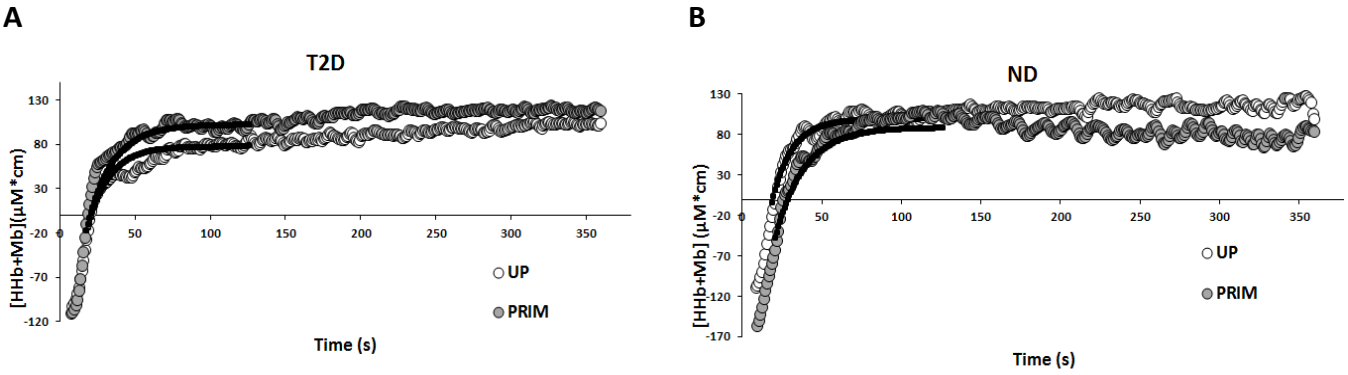

Figure 3.3 [HHb+Mb] kinetic responses during heavy intensity exercise before (unprimed: ○) and after (primed: ●) PE in a representative individual with T2D (A) and a ND participant (B). The continuous lines best represent the primary phase. It can be observed that $[HHb+Mb]\tau_p$ is larger (i.e. slower) following PE.

3.3.3.3 Heart rate responses

Heart rate responses for individuals with T2D and ND controls are represented in table 3.7. No significant differences were displayed in heart rate responses between groups. Following PE, both baseline HR and end HR responses increased ($P < 0.05$). The amplitude change in heart rate from the start to end of exercise was lower after PE in participants with T2D and ND controls ($P < 0.05$).

Table 3.7 ***Heart rate responses during heavy intensity exercise in participants with T2D and ND controls.***

	Unprimed		Primed	
	T2D n = 9	**ND n = 9**	**T2D n = 9**	**ND n = 9**
Baseline HR, beats.min^{-1}	99 ± 12	89 ± 11	109 ± 15*	102 ± 12*
End HR, beats.min^{-1}	147 ± 19	148 ± 12	153 ± 19*	154 ± 13*
Δ HR, beats.min^{-1}	48 ± 10	59 ± 12	44 ± 9*	52 ± 11*

Values are mean ± standard deviation. * Significantly different from the unprimed condition within the same group ($P \leq 0.05$).

3.4 Discussion

This study investigated the effects of a heavy intensity PE on $\dot{V}O_2$ and [HHb+Mb] kinetics during a subsequent bout of heavy intensity cycle exercise in older adults with uncomplicated T2D and age-matched ND controls. The principal findings from this study were that priming exercise significantly increased the $\dot{V}O_2$ A_p while it reduced the $\tau\dot{V}O_{2P}$ as well as the $\dot{V}O_{2SC}$ amplitude during the subsequent bout of heavy-intensity cycle exercise in both groups. Subsequently, the $\dot{V}O_2$ MRT was also significantly faster for both groups following PE. On the other hand, the Δ[HHb+Mb]τ_p and Δ[HHb+Mb]τ' were significantly larger (i.e. slower) in both groups subsequent to PE.

In accordance with Wilkerson *et al* (2011) and O'Connor *et al* (2015) who reported no difference in $\dot{V}O_2$ kinetics during moderate-intensity cycle exercise in older individuals with T2D and ND controls, in the present study, no differences were observed between older participants with T2D and ND controls with respect to $\tau\dot{V}O_{2P}$ during heavy-intensity cycle exercise in the unprimed condition. Therefore, the findings of the present study suggest that the effects of T2D on $\dot{V}O_2$ kinetics during heavy-intensity cycle exercise among older individuals are masked by ageing, at least in inactive participants between the ages of 60 and 70 years. This lack of difference was accompanied by similar [HHb+Mb] responses in both groups, suggesting converging effects of T2D (Wilkerson *et al.* 2012) and ageing (Scheuermann and Barstow, 2005; Poole *et al.* 2008) with respect to impairments in O_2 delivery and/or distribution within the exercising muscle.

The results regarding the effects of PE on a subsequent $\dot{V}O_2$ and [HHb+Mb] kinetics during a heavy-intensity exercise bout of the present study are in line with previous studies involving middle-age individuals with T2D (Gildea *et al.* 2020), as well as healthy individuals cycling in a supine posture (Jones *et al.* 2006; Goulding *et al.* 2017). Using supine heavy intensity cycle exercise to impose an O_2 delivery limitation via reduced perfusion pressure, Jones *et al* (2006) observed a faster $\tau\dot{V}O_{2P}$ with PE ($\tau\dot{V}O_{2P}$: UP = 38 ± 18 s; Prim = 24 ± 9 s) with this improvement being attributed to enhanced muscle vasodilation and a right-shift in the HbO_2 dissociation curve with PE (Jones *et al.* 2006). Using a similar protocol, Goulding *et al* (2017) more recently reported a faster $\tau\dot{V}O_{2P}$ in the supine following PE (39 s), compared with an unprimed condition (59 s). This occurred in conjunction with a slower Δ[HHb+Mb]τ_p (unprimed: 8 s; primed: 11 s) response and improved convective delivery via enhanced

heart rate kinetics with PE in the supine condition (Goulding *et al.* 2017). Authors suggested the former was indicative of enhanced O_2 supply compared with O_2 utilisation within the exercising muscle while the latter being indicative of increased bulk blood flow to the exercising limbs. On the other hand, among middle-age individuals with T2D, Gildea *et al* (2020) observed a significant reduction in $\tau\dot{V}O_{2P}$ for both middle-aged individuals with T2D (UP= 37 ± 10; primed= 31 ± 9 s) and ND controls (UP= 31 ± 5; primed= 29 ± 7 s) with no changes in $\Delta[HHb+Mb]\tau_p$, hence, indicating an improved blood flow and/or O_2 distribution within the working muscle, supporting the notion of greater O_2 delivery relative to utilisation following PE (Gildea *et al.* 2020).

The results of the current study suggest that when muscle $\dot{V}O_2$ is reduced at the outset due to limitations in O_2 delivery, PE appears to enhance muscle $\dot{V}O_2$ via improved O_2 supply, in accordance with previous research during both moderate- (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2005; 2006; 2009; Rocha *et al.* 2019) and heavy-intensity exercise (Jones *et al.* 2006; Goulding *et al.* 2017; Gildea *et al.* 2020).

The reduction in $\dot{V}O_2$ kinetic MRT observed in the present study is also consistent with previous research in T2D (Gildea *et al.* 2020), healthy younger adults (Gerbino *et al.* 1996; Burnley *et al.* 2002) and postural studies (Jones *et al.* 2006), where a reduced slow component amplitude, an increased A_p and a faster $\tau\dot{V}O_{2P}$ contribute to the overall faster $\dot{V}O_2$ kinetic response.

In the present study, PE induced an increase in HR. Although this means that bulk blood flow is increased, this may not necessarily enhance the $\tau\dot{V}O_{2P}$ response, therefore, negating the enhanced blood flow *per se* as the mechanism for improving exercise tolerance. In this regard, Fukuba *et al* (2004) reported that PE did not induce any effects in femoral artery blood flow (UP: 40.8 ± 16.9 s; Primed: 39.0 ± 17.1 s) during heavy intensity knee-extension exercise even in the presence of faster $\tau\dot{V}O_{2P}$ (UP: 68.6 ± 15.9 s; Primed: 58.0 ± 14.4 s) and elevated heart rate during a second bout. This study by Fukuba *et al* (2004) also showed that blood flow kinetics are faster than $\dot{V}O_2$ kinetics during heavy-intensity knee-extension exercise, with authors concluding that alterations in muscle metabolism help facilitate the $\dot{V}O_2$ (i.e. reduced metabolic inertia allowing enhanced O_2 extraction) in excess of the blood flow and can occur without an enhanced blood flow response during exercise (Fukuba *et al.* 2004). Thus, enhancements in vasodilation and improved distribution of blood flow

throughout the exercising muscle, in concurrence with improvements in aerobic metabolism, may allow for a superior matching of local O_2 delivery to utilisation with PE (Fukuba *et al.* 2004; DeLorey *et al.* 2004; Gurd *et al.* 2005; 2006; Murias *et al.* 2011; De Roia *et al.* 2012).

In the studies by Jones *et al* (2006) and Goulding *et al* (2017), authors found an enhanced $\dot{V}O_2$ A_p, a reduced $\dot{V}O_{2sc}$ amplitude but an unaltered $\tau\dot{V}O_{2P}$ during upright cycling, and attributed the lack of change in the $\tau\dot{V}O_{2P}$ to metabolic inertia due to the young age, high levels of fitness and the initial fast $\tau\dot{V}O_{2P}$ of the participants in both studies. The increased primary phase $\dot{V}O_2$ amplitude has been hypothesized to occur as a result of increased motor unit recruitment at the onset of the 2nd bout which in turn would mean more muscle fibres recruited to generate tension allowing for a reduction in tension per individual fibre, reducing the metabolic disturbance throughout the muscle (Burnley *et al.* 2001; 2002). Whilst the precise mechanisms for the $\dot{V}O_{2sc}$ and the decrease in its amplitude subsequent to PE are yet to be fully elucidated, altered muscle activity due to PE may explain this effects (Burnley *et al.* 2001).

Whilst the mechanisms for the PE-induced reductions in $\tau\dot{V}O_{2P}$ remain to be fully elucidated, it is possible that the enhanced O_2 availability as a consequence of improved vasodilation and a shift to the right in the HbO_2 dissociation curve due to "lactic acidosis" from the prior heavy intensity bout (Gerbino *et al.* 1996; Poole & Jones, 2005) may occur. However, PE may also enhance oxidative metabolism via up-regulation of mitochondrial enzyme activity in conjunction with enhanced O_2 delivery to utilisation as observed during moderate-intensity exercise in young and older healthy participants (Gurd *et al.* 2006; 2009). As mentioned before, it must be stated that these mechanisms of enhanced O_2 delivery to utilisation to improve the overall pulmonary $\dot{V}O_2$ kinetic response occur in scenarios where O_2 delivery is limited (Poole and Jones, 2012).

During heavy-intensity exercise, there is substantial spatial heterogeneity in muscle [HHb+Mb] which can be reduced following PE (Koga *et al.* 2007). After heavy intensity PE, this heterogeneity is reduced (Saitoh *et al.* 2009; Prieur *et al.* 2010) and this more homogenous distribution of blood flow is indicative of an improved matching of O_2 delivery to O_2 utilisation throughout the working muscle (Goulding *et al.* 2017). This may explain the faster $\tau\dot{V}O_{2P}$ and slower $[HHb+Mb]\tau_p$ observed in the present study. This improved matching

has also been observed following PE in single muscle observations (i.e. VL muscle) using NIRS in middle-age participants with T2D (Rocha *et al.* 2019) and healthy older individuals (De Roia *et al.* 2012) during moderate-intensity exercise when normalised Δ[HHb+MB]/ $\Delta\dot{V}O_{2p}$ has been used as an index of local O_2 extraction. Goulding and colleagues (2017) suggested that a slower $[HHb+Mb]\tau_p$ response observed during heavy intensity supine cycle exercise following PE contributed to the reduction in $\tau\dot{V}O_{2P}$ and increase in critical power via enhanced O_2 availability within the contracting muscle (Goulding *et al.* 2017). To date, however, it must be stressed that no relationship has been detected with regards to a reduction in [HHb+Mb] heterogeneity within the working muscle and the $\dot{V}O_{2sc}$ amplitude (Saitoh *et al.* 2009; Fukuoka *et al.* 2015; Goulding *et al.* 2017).

The present study also points to an O_2 delivery constraint in older people with T2D and healthy (untrained) older controls (indicated by an enhanced $\dot{V}O_2$ and $[HHb+Mb]\tau_p$ kinetic responses following PE) as observed in previous research investigating the effects of PE on the same parameters during moderate-intensity exercise for middle-age individuals with T2D and older inactive individuals (Gurd *et al.* 2008; 2009; De Roia *et al.* 2012; Rocha *et al.* 2019). Among older individuals, impairments in vasodilation in contracting muscles have been observed suggesting reductions in O_2 delivery (Poole *et al.* 2003; Proctor and Parker, 2006) which likely lead to the longer $\tau\dot{V}O_{2P}$ during heavy intensity exercise as observed in the present study and other previous studies (Sabapathy *et al.* 2003; DeLorey *et al.* 2005).

On the other hand, during heavy-intensity constant load plantar flexion exercise, Kiely *et al* (2014) observed impairments in the dynamic response of leg vascular conductance in both middle-age male and female participants with T2D (Kiely *et al.* 2014). The impairment in LVC kinetics were specifically characterised by a smaller initial growth phase amplitude and a large τ (time constant) of the 2nd growth phase compared with ND controls. The 2nd growth phase hyperaemic response is controlled by endothelium-(acetylcholine) mediated blood flow which may be impaired in uncomplicated T2D. This impaired hyperaemic response may affect O_2 delivery to the exercising muscle, resulting in impaired $\dot{V}O_2$ kinetics and reduced exercise tolerance in T2D. Therefore, this impaired O_2 delivery among individuals with T2D and older untrained controls may induce the initial slow $\tau\dot{V}O_{2P}$ observed, which may be overcome with PE.

*3.4.1 **Conclusion***

This study demonstrated that an acute heavy-intensity PE significantly reduced the $\tau\dot{V}O_{2P}$ and $\dot{V}O_{2sc}$ amplitude whilst increasing the primary component amplitude during a subsequent heavy intensity cycle exercise in older individuals with and without T2D. This was accompanied by a significant slowing of $[HHb+Mb]\tau_p$ in both groups suggesting PE overcame impairments in O_2 delivery to O_2 utilisation in both groups. This study supports the notion that T2D-induced impairments in $\dot{V}O_2$ kinetics are "masked" by ageing *per se* among untrained individuals. Furthermore, a prior bout of PE enhances these responses to a similar extent in older individuals with uncomplicated T2D and their age-matched ND counterparts.

Chapter 4: Influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise initiated from an elevated baseline in older adults with type 2 diabetes.

4.1 Introduction

Throughout the day, a person rarely takes part in a physical activity set at a constant intensity. Abrupt transitions from lower to higher metabolic rates occur throughout our everyday lives (DaBoit *et al.* 2014). "Work-to-Work" (WtoW) transitions are important as they allow us to investigate the effect of work rate changes involving abrupt transitions from lower to higher metabolic rates on $\dot{V}O_2$ kinetics responses which occur in both exercise training programs and everyday physical activities. When an exercise bout is initiated from an elevated baseline a slower $\tau\dot{V}O_{2P}$ is observed compared with a condition where the same exercise bout is initiated from an unloaded baseline in WtoW transitions to moderate- (Hughson & Morrisey, 1982; Brittain *et al.* 2001) and heavy-intensity domain (Wilkerson *et al.* 2007; DiMenna *et al.* 2008; 2010a; 2010b; 2010c; Goulding *et al.* 2018 Gildea *et al.* 2020).

While the precise mechanisms for the slower $\dot{V}O_2$ kinetics response observed during WtoW transitions remain to be fully elucidated, it appears that the slower $\tau\dot{V}O_{2P}$ response may be intramuscular in origin. This may include a higher proportion of type II fibre recruitment and/or a decreased cellular energetic state due to the prior moderate-intensity exercise in order to meet the new metabolic demand of the already recruited fibres (DiMenna *et al.* 2010b; Meyer & Foley, 1996; Goulding *et al.* 2018).

According to Henneman's size principle, skeletal muscle is recruited on the basis of oxidative type I fibres first, followed by larger type II glycolytic fibres (Henneman and Mendell, 1981). Based on this principle, when exercise is initiated from an elevated baseline, oxidative fibres have already been recruited along with some glycolytic fibres, resulting in the recruitment of even more glycolytic fibres which were not previously contributing to force production (Brittain *et al.* 2001; Goulding *et al.* 2018). These less efficient glycolytic fibres are thought to have slower $\dot{V}O_2$ kinetics relative to more oxidative fibres, resulting in a slower $\tau\dot{V}O_{2P}$ compared to the exercise transition initiated from an unloaded baseline where less glycolytic fibres are recruited at exercise onset (Brittain *et al.* 2001; Goulding *et al.* 2018). Non-invasive methods for assessing skeletal muscle activity have also recorded evidence

supporting increased glycolytic motor unit recruitment during WtoW exercise compared with unloaded heavy-intensity exercise, with increases in both PCr degradation (Jones *et al.* 2008) and iEMG activity (Wilkerson and Jones, 2007) occurring alongside a slower $\tau\dot{V}O_{2P}$.

A small number of studies to date have investigated the effects of PE on WtoW transitions (DiMenna *et al.* 2008; 2010a; 2010b). In active individuals cycling in the upright posture, no effect was found on $\tau\dot{V}O_{2P}$ during a WtoW transition following PE, however, a significant increase in the $\dot{V}O_2$ primary phase amplitude and a reduction in the $\dot{V}O_{2SC}$ amplitude were observed (DiMenna *et al.* 2010a). The attenuation in the $\dot{V}O_{2SC}$ amplitude occurred in coincidence with a reduction in the ΔiEMG between the second and final minute (i.e. minute 6) of exercise following PE, so authors suggested that an altered motor unit recruitment and altered intramuscular $\dot{V}O_2$ within the recruited type II fibres were likely factors influencing the outcomes (DiMenna *et al.* 2008). On the other hand, when cycling in the supine posture whereby muscle O_2 availability is compromised (DiMenna *et al.* 2010a), DiMenna *et al* (2008) found that PE helped speed the $\tau\dot{V}O_{2P}$. The authors suggested that improvements in O_2 delivery to the working muscle was a likely mechanism for the accelerated $\tau\dot{V}O_{2P}$ following PE.

Similarily, Gildea *et al* (2020) recently observed that PE induced a significant reduction in $\tau\dot{V}O_{2P}$ as well as in the amplitude of the $\dot{V}O_{2SC}$ during a WtoW transition in middle-aged individuals with T2D. This would suggest that PE is an effective acute intervention for enhancing exercise tolerance in this cohort. However, it is unknown if these adaptations are apparent in older individuals with T2D, or in non-diabetic healthy individuals. Thus, the present study aimed to investigate the influence of PE on $\dot{V}O_2$ and muscle deoxygenation ([HHb+Mb]) kinetic responses during moderate-to-heavy intensity cycling exercise transitions (WtoW) in older individuals with T2D and ND controls.

4.2 Methodology

4.2.1 Participants

Ten individuals with uncomplicated T2D (8 men / 2 women), and 10 individuals without T2D (ND) (8 men / 2 women) volunteered to participate in this study (Table 4.1). All of the individuals with T2D and 8 of the ND controls took part in the experiment described in chapter 2 while 7/10 participants from each group took part in the experiment described in chapter 3. All participants were non-smokers and had not smoked during the 12 months prior to the study.

4.2.1.1 Recruitment of participants

4.2.1.1.1 T2D participant recruitment

As per section 2.2.1.1.1

4.2.1.1.2 Non-diabetic (ND) participant recruitment

As per section 2.2.1.1.2

4.2.1.2 Inclusion/exclusion criteria for participation

4.2.1.2.1 Participants with T2D

As per section 2.2.1.2.1

4.2.1.2.2 Participants without T2D (ND)

As per section 2.2.1.2.2

4.2.1.2.3 Fasted venous blood sample collection

As per section 2.2.1.2.3

4.2.1.2.4 Ankle: Brachial Index (ABI)

As per section 2.2.1.2.4

4.2.1.3 Physical activity level determination

As per section 2.2.1.3

4.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

4.2.2 Experimental design

4.2.2.1 Study overview

Non-diabetic participants visited the exercise physiology laboratory in the Department of Physiology in Trinity College Dublin on two occasions, whilst all T2D patients were required to visit the exercise testing facility in St Colmcille's Hospital on one occasion and were able to choose between the hospital and the exercise laboratory thereafter to carry out testing. In order to standardise testing conditions, participants refrained from consuming caffeine and alcohol 24 hours prior to all testing. Participants also limited their activity throughout the day to normal activities of daily living with no strenuous exercise 24 hours prior to testing. During this initial visit, anthropometric data was collected followed by participants performing a RI cycling test to exhaustion. On the second visit, participants carried out 4 WtoW bouts of cycle exercise, 2 of which were carried out without prior exercise while the other 2 were carried out following prior high-intensity cycling exercise (see section 4.2.2.3.1).

4.2.2.2 Visit 1 overview

4.2.2.2.1 Anthropometric and pulse wave velocity measures

As per section 2.2.2.2.1

4.2.2.2.2 Ramp incremental test to exhaustion

As per section 2.2.2.2.2

4.2.2.3 Visit 2 overview

4.2.2.3.1 WtoW cycle exercise and priming protocol

From the previously completed RI cycling test during visit 1, the work rates required for this protocol were calculated. The power outputs corresponding to 80% VT and 50% Δ were determined. Participants then performed 4 bouts of constant-load, moderate-intensity exercise (80% VT) followed immediately by high-intensity cycling (50% Δ). Two of these WtoW bouts were completed without prior exercise (unprimed WtoW) and two bouts were undertaken preceded by PE at an intensity of 50% Δ (primed WtoW). The duration of each

step transition was 6 minutes, and the moderate transition was preceded by a 3 minute baseline cycling period at a workload of 10W. Changes in WR were initiated as a step function without a warning to the individual. There was a 12 minute passive rest period between each bout of cycling, except following the first primed high-intensity bout where participants rested in a chair for 45 minutes. This resting period is shown to be sufficient for physiological restoration to resting levels following a bout of heavy-intensity exercise (Burnley *et al.* 2006).

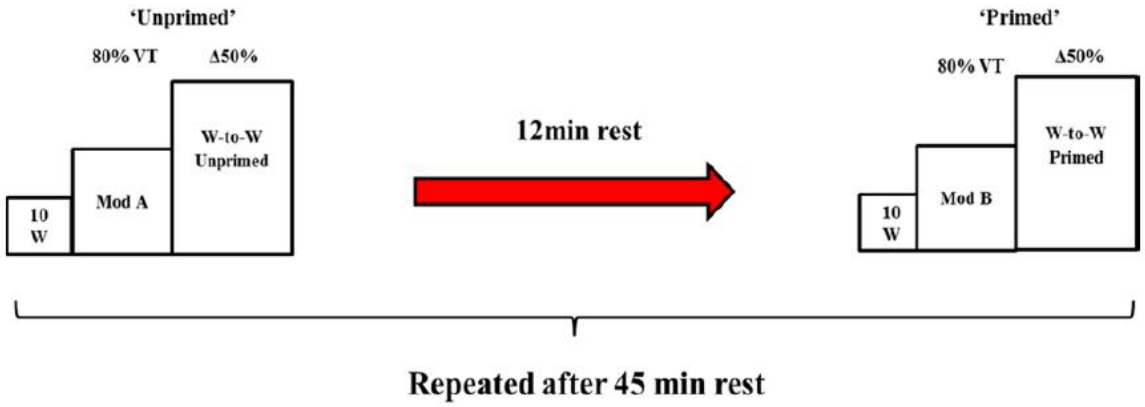

Figure 4.1. Schematic representation of the protocol for visit 2.

4.2.3 <u>Laboratory equipment and measurement techniques</u>

4.2.3.1 Cardio-metabolic unit

As per section 2.2.3.1

4.2.3.2 Heart rate monitor

As per section 2.2.3.2

4.2.3.3 Blood pressure

As per section 2.2.3.3

4.2.3.4 Ratings of perceived exertion (RPE)

As per section 2.2.3.4

4.2.3.5 Muscle deoxygenation [HHb+Mb] and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.5

4.2.4 Data Analysis

4.2.4.1 Peak responses

As per section 2.2.5.1

4.2.4.2 Elevated baseline 50% Δ analysis

4.2.4.2.1 $\dot{V}O_2$ kinetics

As per section 3.2.4.2.1

4.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

As per section 3.2.5.2.2

4.2.4.3 80%VT analysis

4.2.4.3.1 $\dot{V}O_2$ kinetics

Breath by breath $\dot{V}O_2$ data for each transition were linearly interpolated providing second by second values and time aligned so that 0 represents exercise onset. Each transition was ensemble-averaged to yield a single, average response and further time-aligned into 5 second bins to provide a single time-averaged response. The first 20 seconds of $\dot{V}O_2$ data were excluded to avoid fitting of Phase I or cardio-dynamic phase given that it's unclear whether this phase is exponential. Therefore fitting and estimation of the model parameters were determined from 20-360 s of the step transition using a weighted least-squares nonlinear regression procedure in which the best fit was calculated by minimisation of the residual sum of squares (TableCurve 2D, Systat). Data lying outside the 95% prediction interval during the initial fitting of the model were excluded. The average and smoothed response was modelled using a mono-exponential function seen in Equation 1 as follows:

Equation 1: $\dot{V}O_2(t) = \dot{V}O_2(b) + Ap(1-e^{-(tp-TDp/\tau p)})$

Where $\dot{V}O_2$ (t) is the absolute $\dot{V}O_2$ at a given time (*t*); $\dot{V}O_2$ (b) is the mean baseline $\dot{V}O_2$ (average of the final 60 seconds of the active rest); A_p is the amplitude of the increase in $\dot{V}O_2$ for Phase II or the primary phase; TD_p is the phase delay, and τ_p is the time constant (time duration for $\dot{V}O_2$ to increase to 63% of the amplitude of each phase).

In the case of a participant revealing a $\dot{V}O_{2SC}$, suggesting an overestimation of the VT, a bi-exponential function was used to model the kinetic response *as per section 3.2.4.2.1*.

4.2.4.3.2 Muscle deoxygenation [HHb+Mb] kinetics

Δ [HHb+Mb] kinetics of the right VL were modelled in response to exercise. As per $\dot{V}O_2$, the NIRS data was linearly interpolated for each transition to provide second by second values. Data for each bout was ensemble-averaged yielding a single average response and then time aligned into 5 second bins to give a single time-averaged response. At exercise onset, a time delay (TD) is present in the [HHb + Mb] profile before increasing exponentially to a steady-state (DeLorey *et al.* 2003), reflecting the matching of muscular O_2 uptake and local O_2 delivery (DeLorey *et al.* 2003). The TD was established via visual inspection by the study investigator as the point of exponential increase in [HHb + Mb]. The data were then fitted from the TD to 150 s of the response with a mono-exponential model (See *Equation 1.*).

4.2.4.2.3 Ventilatory threshold

As per section 3.2.4.2.3

4.2.5 <u>Statistical analysis</u>

Data are presented as mean ± standard deviation. Statistical analysis was performed using SigmaPlot 12 (Systat Software, San Jose, Ca, USA). Normality of data was assessed using a Shapiro-Wilk test. Physical parameters and physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, while the Mann-Whitney U test was employed for non-parametric analyses. Parameter estimates for $\dot{V}O_2$ kinetics and muscle deoxygenation [HHb+Mb] kinetics was performed using a 2-way (group x condition) repeated measures ANOVA. Post-hoc Holm-Sidak tests were carried out in the case of significant differences being observed from the ANOVA. Level of significance was set to $P < 0.05$.

The key variable for the current study is the rate of change in $\tau\dot{V}O_{2P}$. Previous studies from our laboratory revealed $\tau\dot{V}O_{2P}$ values of 45 ± 9.6 s in individuals with T2D, during high-intensity exercise initiated from an elevated-baseline. With respect PE exercise, a mean difference of 17 s was revealed when comparing $\tau\dot{V}O_{2P}$ values pre- and post-PE. Therefore, given the estimated SD of each group is 9.6 s, and the mean difference we wish to detect is 17 s, the minimum sample size needed to detect with a statistical power of 80% (α=0.05) by ANOVA test in 2 groups, is 7 participants.

4.3 Results

4.3.1 Participants

4.3.1.1 Physical characteristics

Physical characteristics for participants from both groups are presented in table 4.1. A larger mean body mass and HbA_{1c} was observed for individuals with T2D ($P < 0.05$).No significant difference was found for any other measure between groups.

Table 4.1 ***Anthropometrical data, ABI, PWV, haematological parameters and resting BP for individuals with T2D and ND controls.***

	T2D (n = 10)	**ND (n = 10)**	***P* values**
Sex (M,F)	(8,2)	(8,2)	
Age, yr	62 ± 3	64 ± 4	0.27
Height, m	1.7 ± 0.1	1.7 ± 0.1	0.14
Body Mass, kg	90 ± 14*	78 ± 9	0.03
BMI, $kg.m^{-2}$	30.1 ± 3.3	27.6 ± 2.5	0.07
Waist-To-Hip Ratio, a.u	1.0 ± 0.1	1.0 ± 0.1	0.25
Fat Layer VL, mm	5.4 ± 1.6	4.9 ± 1.6	0.46
Pre-exercise ABI, a.u	1.2 ± 0.4	1.0 ± 0.1	
Post-exercise ABI, a.u	1.3 ± 0.5	1.0 ± 0.1	
PWV, $m.s^{-1}$	*10.3 (5.3)*	*9.6 (8.7)*	*0.87*
HbA_{1c}, %	7.7 ± 1.3	5.2 ± 0	0.03
Resting SBP, mmHg	127 ± 14	131 ± 7	0.50
Resting DBP, mmHg	75 ± 9	83 ± 7	0.06

Mean ± SD are shown for values that are normally distributed while median (interquartile range) are shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample size is as follows: pulse wave velocity (PWV), n = 9 (T2D) and 5 (ND); glycosylated haemoglobin (HbA1c), n= 7 (T2D). *Significantly different than ND ($P < 0.05$).

4.3.1.2 Prescriptive medications

Table 4.2 ***Prescriptive medications for participants with T2D and ND participants.***

	T2D	ND
Anti-hypertensives		
Angiotensin converting enzyme inhibitor	3	1
Angiotensin II receptor blocker	1	
Asprin	1	
Statins	5	1
Hypoglycaemic medications		
Metformin	8	
Sulphonylureas	3	
DPP-4 inhibitors	3	

4.3.1.3 Physical activity levels

Group mean physical activity levels are shown in Table 4.3. No difference was observed between groups for any physical activity estimated using the RT3 accelerometry data.

Table 4.3 ***RT3 accelerometry data for individuals with T2D and ND participants.***

	T2D	ND	P value
Inactive, h.day^{-1}	*16.7 (2.2)*	*19.5 (9.2)*	*0.84*
Light, h.day^{-1}	*5.6 (1.7)*	*3.6 (6.1)*	0.84
Moderate, h.day^{-1}	1.1 ± 0.5	1.1 ± 1.3	0.94
Vigorous, h.day^{-1}	0.2 ± 0.2	0.4 ± 0.6	0.50

Mean ± SD are shown for values that are normally distributed while median (interquartile range) are shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups. n = 5 (T2D) and n = 5 (ND).

4.3.2 Performance data from the Ramp incremental cycling test

4.3.2.1 Physiological responses

Physiological responses at peak exercise and at VT are shown in Table 4.4. Relative $\dot{V}O_{2peak}$ was significantly reduced ($P < 0.05$) in individuals with T2D compared to ND controls. Time to failure was also significantly reduced ($P < 0.05$) in participants with T2D compared to the ND controls. No other significant differences were recorded for any other parameter at peak and VT between groups.

Table 4.4 ***Physiological responses at peak exercise and VT***

	T2D (n = 10)	ND (n = 10)	P-values
$\dot{V}O_{2peak}$, L.min^{-1}	2.11 ± 0.33	2.20 ± 0.54	0.31
$\dot{V}O_{2peak}$, mL.kg^{-1}.min^{-1}	23.7 ± 3.8	28.5 ± 6.1*	0.02
Peak PO, W	155 ± 28	172 ± 42	0.16
Peak HR, beats.min^{-1}	155 ± 13	151 ± 14	0.25
Peak RER, a.u	*1.1 (0.1)*	*1.2 (0.1)*	*0.09*
TTF, s	684 ± 61	766 ± 94*	0.02
$\dot{V}O_2$ @ VT, L.min^{-1}	1.50 ± 0.30	1.50 ± 0.34	0.49
$\dot{V}O_2$ @ VT, mL.kg^{-1}.min^{-1}	17.4 ± 4.1	19.8 ± 4.1	0.10
PO @ 80% VT, W	73 ± 18	82 ± 25	0.36
PO @ 50% Δ, W	124 ± 24	138 ± 36	0.32

Mean ± SD are shown for values that are normally distributed while median (interquartile range) are shown in italic font for variables which displayed skewness and were not normally distributed in one or both groups. TTF, time to failure. *Significantly different than T2D ($P < 0.05$).

4.3.3 Performance data for WtoW (50% Δ)

4.3.3.1 $\dot{V}O_2$ kinetics

The parameter estimates for $\dot{V}O_2$ kinetic responses during WtoW exercise before and after PE in individuals with T2D and ND controls are shown in Table 4.5, while representative responses are shown in Figure 4.2. No differences were observed for any of the $\dot{V}O_2$ kinetic parameters between groups.

Following PE, an increase was observed in A_p ($P < 0.05$) and absolute A_p ($P < 0.001$) for both groups. The $\tau\dot{V}O_{2P}$ also became faster following PE in both groups ($P < 0.001$). This was

accompanied by an increase in $\dot{V}O_2$ gain in the primary phase ($P < 0.05$) in both, individuals with T2D and ND controls. In addition, the $\dot{V}O_{2SC}$ amplitude was reduced following PE in both groups ($P < 0.001$) as well as the $\dot{V}O_2$ MRT ($P < 0.001$).

Table 4.5 ***$\dot{V}O_2$ kinetic parameters during unprimed and primed WtoW exercise in participants with T2D and ND controls.***

	Unprimed		Primed	
	T2D (n = 10)	**ND (n = 10)**	**T2D (n = 10)**	**ND (n = 10)**
$\dot{V}O_2$ baseline, L.min^{-1}	1.6 ± 0.3	1.6 ± 0.4	1.6 ± 0.3	1.7 ± 0.5
$\dot{V}O_2$ A_p, L.min^{-1}	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1*	0.5 ± 0.1*
$\dot{V}O_2$ absolute A_p, L.min^{-1}	2.0 ± 0.4	2.1 ± 0.5	2.1 ± 0.4*	2.2 ± 0.5*
$\tau\dot{V}O_{2P}$, s	48.9 ± 6.1	49.0 ± 7.2	34.1 ± 4.2*	38.0 ± 8.9*
Primary $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	8.7 ± 2.1	8.7 ± 1.0	9.9 ± 2.7*	9.5 ± 1.1*
$\dot{V}O_2$ A_{sc}, L.min^{-1}	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0*	0.1 ± 0.1*
$\dot{V}O_2$ A_{sc}, %	38.0 ± 9.7	35.5 ± 13.5	20.7 ± 8.8*	22.0 ± 13.5*
$\dot{V}O_2$ TDsc, s	134 ± 34	135 ± 27	124 ± 33*	117 ± 21*
End $\dot{V}O_2$, L.min^{-1}	2.2 ± 0.4	2.2 ± 0.5	2.2 ± 0.4	2.3 ± 0.6
$\dot{V}O_2$ MRT, s	66 ± 17	67 ± 14	52 ± 15*	56 ± 11*
End $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	11.9 ± 2.6	11.7 ± 1.1	12.0 ± 3.6	11.6 ± 1.5

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constant; p, primary phase; sc, slow component phase; MRT, mean response time. *Significantly different from the unprimed bout within the same group ($P \leq 0.05$).

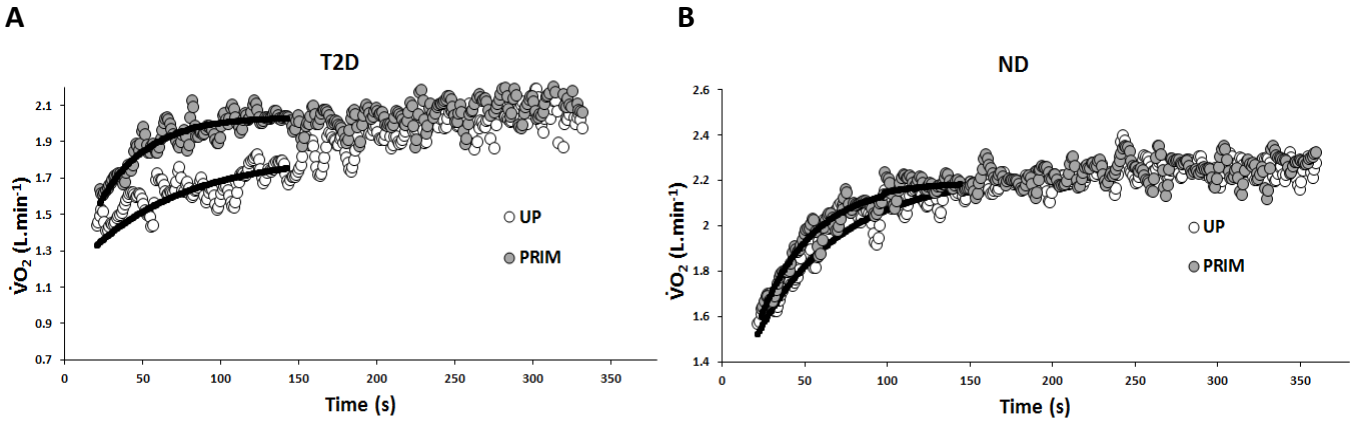

Figure 4.2 Oxygen uptake responses during high-intensity exercise without priming exercise (○) and with priming exercise (●) in a representative individual with T2D and ND participant. The continuous lines of best fit represent the primary phase of the $\dot{V}O_2$ response. Note the relatively slower $\tau\dot{V}O_{2P}$ and relatively lower A_p in the unprimed compared with primed conditions for both groups.

4.3.3.2 Muscle deoxygenation [HHb+Mb] kinetics

The parameter estimates for [HHb+Mb] kinetics during heavy intensity exercise before and after PE in participants with T2D and ND controls are shown in table 4.6 while representative figures are shown in figure 4.3. Data from one participant in the T2D group was excluded due to technical problems with equipment. No differences were observed between groups for any [HHb+Mb] kinetic parameters. No significant difference was observed following PE for either group.

Table 4.6 ***[HHb+Mb] kinetic parameters during unprimed and primed WtoW exercise***

	Unprimed		Primed	
	T2D (n = 9)	**ND (n = 10)**	**T2D (n = 9)**	**ND (n = 10)**
Δ[HHb+Mb] A_p, µM*cm	44.9 ± 45.1	73.2 ± 33.3	37.6 ± 36.6	68.0 ± 36.1
Δ[HHb+Mb] TD_p, s	6.9 ± 4.4	4.0 ± 2.1	7.4 ± 5.6	5.2 ± 4.0
Δ[HHb+Mb]τ_p, s	22.7 ± 5.0	30.0 ± 13.6	28.9 ± 5.1	26.7 ± 12.4
Δ[HHb+Mb]τ', s	29.6 ± 7.4	34.0 ± 14.6	36.4 ± 9.0	31.9 ± 13.9
Δ[HHb+Mb] A_{sc}, µM*cm	7.5 ± 7.1	5.7 ± 8.4	6.7 ± 5.9	1.3 ± 2.7
Δ[HHb+Mb] TD_{sc}, s	126 ± 68	130 ± 33	117 ± 40	130 ± 31
Δ[HHb+Mb] MRT, s	30 ± 23	39 ± 23	38 ± 37	26 ± 10

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constant; p, primary phase; sc, slow component; MRT, mean response time; τ', effective response time (τ + TD); [HHb+Mb], deoxygenated haemoglobin and myoglobin concentration.

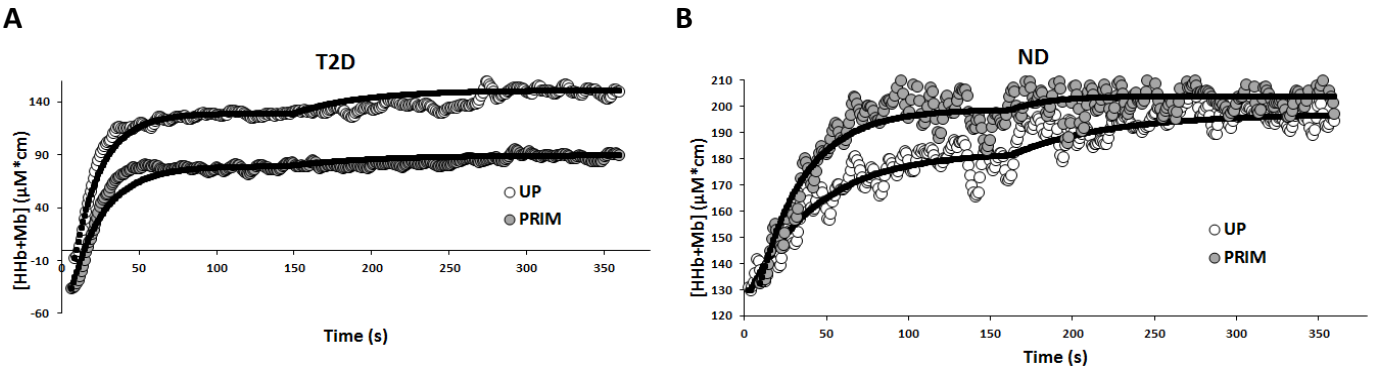

Figure 4.3 [HHb+Mb] kinetic responses during heavy intensity exercise before (unprimed: ○) and after (primed: ●) PE in a representative individual with T2D and ND participant. The continuous lines best represent the bi-exponential response of [HHb+Mb].

4.3.3.3 Heart rate responses

HR responses for individuals with T2D and ND controls are represented in table 4.7. No differences were observed between groups following PE. A significant increase was recorded for both baseline HR ($P < 0.001$) and end HR ($P < 0.05$) in both groups. In addition, the change in HR from the start to end exercise was lower after PE in both groups ($P < 0.05$).

Table 4.7 ***Heart rate responses during WtoW exercise in individuals with T2D and ND controls.***

	Unprimed		Primed	
	T2D (n = 10)	**ND (n = 10)**	**T2D (n = 10)**	**ND (n = 10)**
Baseline HR, beats.min^{-1}	122 ± 12	117 ± 10	129 ± 13*	125 ± 13*
End HR, beats.min^{-1}	149 ± 14	147 ± 11	152 ± 12*	149 ± 11*
Δ HR, beats.min^{-1}	27 ± 9	30 ± 9	23 ± 7*	24 ± 6*

Values are mean ± SD. *Significantly different from unprimed within the same group ($P < 0.05$).

4.3.4 Performance data for moderate-intensity exercise (80% VT)

4.3.4.1 $\dot{V}O_2$ kinetics

The parameter estimates of $\dot{V}O_2$ kinetic responses during moderate-intensity exercise pre and post PE in individuals with T2D and ND controls are shown in table 4.8. Following PE, the time delay of the primary phase ($\dot{V}O_2$ TD_p) increased ($P < 0.05$) for both groups, while a significant speeding of the $\tau\dot{V}O_{2P}$ ($P < 0.001$) occurred following PE in both groups.

Table 4.8 ***$\dot{V}O_2$ kinetic characteristics during unprimed and primed moderate-intensity exercise in individuals with T2D and ND controls.***

	Unprimed		Primed	
	T2D (n = 10)	ND (n = 10)	T2D (n = 10)	ND (n = 10)
$\dot{V}O_2$ baseline, L.min^{-1}	0.9 ± 0.3	0.8 ± 0.1	0.9 ± 0.3	0.8 ± 0.1
$\dot{V}O_2$ A_p, L.min^{-1}	0.6 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.3
$\dot{V}O_2$ absolute A_p, L.min^{-1}	1.6 ± 0.3	1.6 ± 0.4	1.6 ± 0.3	1.6 ± 0.4
$\dot{V}O_2$ TD_p, s	18.1 ± 8.2	19.0 ± 3.2	19.7 ± 6.2*	24.1 ± 4.8*
$\tau\dot{V}O_{2P}$, s	41.9 ± 10.7	39.4 ± 4.4	33.6 ± 6.4*	30.1 ± 3.7*
Primary $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	8.9 ± 5.6	7.8 ± 1.2	9.2 ± 5.8	8.0 ± 1.0

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constant; p, primary phase. *Significantly different from unprimed bout within the same group ($P \leq 0.05$).

4.3.4.2 Muscle deoxygenation [HHb+Mb] kinetics

The parameter estimates for [HHb+Mb] kinetics responses during moderate-intensity exercise before and after PE in individuals with T2D and ND controls are shown in Table 4.9. No differences were observed between groups for any kinetic parameters. Following PE, the primary amplitude (Δ[HHb+Mb]) increased in the ND group ($P < 0.001$) only. In addition, the time delay of the primary phase occurred earlier in both individuals with T2D and ND controls ($P < 0.05$) following PE while $\Delta[HHb+Mb]\tau_p$ and Δ[HHb+Mb]τ' were significantly prolonged ($P < 0.05$) in both groups.

Table 4.9 ***[HHb+Mb] kinetic parameters during unprimed and primed moderate exercise in individuals with T2D and ND controls.***

	Unprimed		Primed	
	T2D (n = 10)	ND (n = 10)	T2D (n = 10)	ND (n = 10)
Δ[HHb+Mb] A_p, μM*cm	104 ± 83	135 ± 70	113 ± 74	160 ± 83*
Δ[HHb+Mb] TD_p, s	11.2 ± 4.2	9.6 ± 3.2	10.2 ± 1.5*	7.7 ± 2.9
$\Delta[HHb+Mb]\tau_p$, s	14.7 ± 6.7	16.7 ± 7.3	18.6 ± 5.5*	23.1 ± 12.5*
Δ[HHb+Mb]τ', s	25.9 ± 8.0	26.3 ± 8.0	28.8 ± 5.6*	30.8 ± 10.3*

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constant; p, primary phase; τ', effective response time (τ + TD); [HHb+Mb], deoxygenated haemoglobin and myoglobin concentration. *Significantly different from unprimed within the same group ($P \leq 0.05$).

4.3.4.3 Heart rate responses

HR responses for individuals with T2D and ND controls during moderate intensity exercise are represented in table 4.10. Baseline HR was larger in participants with T2D ($P < 0.05$) compared to ND controls.

PE increased both baseline HR ($P < 0.001$) and end HR ($P < 0.001$) during the subsequent bout of moderate intensity exercise in individuals with T2D and ND controls. The change in HR from rest to end exercise (Δ HR) was lower ($P < 0.05$) following PE in both groups.

Table 4.10 ***Heart rate responses during moderate intensity exercise in individuals with T2D and ND controls.***

	Unprimed		Primed	
	T2D (n = 10)	**ND (n = 10)**	**T2D (n = 10)**	**ND (n = 10)**
Baseline HR, beats.min^{-1}	98 ± 9†	85 ± 7	108 ± 12*	99 ± 12*
End HR, beats.min^{-1}	122 ± 12	117 ± 10	129 ± 13*	125 ± 13*
Δ HR, beats.min^{-1}	24 ± 8	32 ± 9	21 ± 7*	26 ± 11*

Values are mean ± SD. *Significantly different from unprimed within the same group ($P \leq 0.05$). †Significantly different from ND ($P \leq 0.05$).

4.4 Discussion

This study investigated the effects of high-intensity PE on a subsequent bout of high-intensity cycle exercise initiated from an elevated baseline (WtoW) in older adults with uncomplicated T2D. The principal findings from this study were that PE significantly reduced $\tau\dot{V}O_{2P}$ and the amplitude of the $\dot{V}O_{2SC}$ during WtoW transitions in older individuals with T2D and ND controls.

Subsequently, the $\dot{V}O_2$ MRT was also reduced for both groups following PE while Δ[HHb+Mb] kinetic responses were unaffected. With respect to moderate-intensity exercise, a significant acceleration of $\tau\dot{V}O_{2P}$ and a significant slowing of the $\Delta[HHb+Mb]\tau_p$ occurred during moderate-intensity exercise following PE in both groups.

4.4.1 <u>High-intensity exercise (50% Δ)</u>

In the present study, a prior bout of high-intensity PE resulted in a significant reduction in $\tau\dot{V}O_{2P}$ during high-intensity cycle exercise initiated from an elevated baseline in both older individuals with T2D and ND controls. These results are in agreement with respect to findings from the same laboratory showing that PE reduced the $\tau\dot{V}O_{2P}$ during a subsequent WtoW exercise transition in middle-aged individuals with uncomplicated T2D (Gildea *et al.* 2020). However, Gildea *et al* (2020) found no reduction to $\tau\dot{V}O_{2P}$ for the age-matched (middle-age) ND control group unlike that observed in the present study. This suggests that both inactive ageing *per se* and T2D are important factors implicated in slowing the $\dot{V}O_2$ kinetics response. The improvements observed in $\tau\dot{V}O_{2P}$ with PE in T2D in the current and previous (Gildea *et al.* 2020) studies suggest that impairments in O_2 delivery and/or distribution may have contributed to the sluggish $\tau\dot{V}O_{2P}$, while PE helped improve O_2 delivery and/or distribution, thus accelerating $\tau\dot{V}O_{2P}$. This inference is postulated on previous research where blood flow was impaired deliberately when adopting the supine cycling posture. In these studies adopting the supine posture, $\tau\dot{V}O_{2P}$ was improved (i.e. reduced) during heavy-intensity exercise initiated from an unloaded baseline (Jones *et al.* 2006; Goulding *et al.* 2017) and severe-intensity exercise initiated from an elevated baseline (DiMenna *et al.* 2010).

The larger $\tau\dot{V}O_{2P}$ observed herein in the WtoW transitions compared with unloaded 50% Δ transitions observed in chapter 3 (T2D $\tau\dot{V}O_{2P}$: 50% Δ = 40.5 ± 2.9 s; WtoW= 48.9 ± 6.2 s; ND

$\tau\dot{V}O_{2P}$: 50% Δ = 42.8 ± 5.0 s; WtoW= 49.0 ± 7.1 s) suggests the larger selective recruitment during WtoW transitions of skeletal muscle fibres which have intrinsically slower $\tau\dot{V}O_{2P}$ kinetics (Poole *et al.* 2007; 2008; DiMenna *et al.* 2010). Similarily, in healthy young men cycling in the supine position, Goulding *et al* (2018) found a slower $\tau\dot{V}O_{2P}$ for severe-intensity WtoW transitions ($\tau\dot{V}O_{2P}$: 69 ± 39 s) compared to severe-intensity transitions initiated from an unloaded baseline ($\tau\dot{V}O_{2P}$: 45 ± 16 s). However, despite the slower $\tau\dot{V}O_{2P}$, $\Delta[HHb+Mb]\tau_p$ was greater when severe-intensity transitions were initiated from an elevated baseline, reflecting slower muscle deoxygenation kinetics in the VL muscle area of interest (Goulding *et al.* 2018). These authors concluded that the slower $\tau\dot{V}O_{2P}$ during the WtoW transitions occur in response to a greater recruitment of new type II fibres not previously contributing to force production, in accordance with the Henneman size principle (Henneman and Mendell, 1981), which possess inherently slower $\dot{V}O_2$ kinetics as hypothesized by DiMenna *et al* (2010b). Alternatively, or in conjunction with the previous stated hypothesis, a reduced cellular state in the already contracting fibres may play a role (Meyer and Foley, 1996; Goulding *et al.* 2018).

In the present study, significant reductions also occurred for the $\dot{V}O_{2SC}$ amplitude in both individuals with T2D (37.5%) and ND controls (35.3%) following PE. This is in agreement with previous findings observed in middle-aged individuals with T2D following the same WtoW protocol before and after PE (Gildea *et al.* 2018), as well as in healthy young individuals during upright cycling (DiMenna *et al.* 2008), and knee extension exercise (DiMenna *et al.* 2010b). In healthy young populations, the proposed mechanism for a reduced $\dot{V}O_{2SC}$ amplitude is altered motor unit recruitment and subsequent altered $\dot{V}O_2$ within the recruited type II fibres following PE (DiMenna *et al.* 2008). This hypothesis comes from an observed attenuation in the $\dot{V}O_{2SC}$ amplitude in coincidence with a reduction in the ΔiEMG between the second and final minute of cycling exercise, following PE (DiMenna *et al.* 2008). Using a knee-extension WtoW exercise protocol, DiMenna *et al* (2010b) again found a reduced $\dot{V}O_{2SC}$ amplitude following PE accompanied by a reduction in ΔiEMG between the second and final minute of exercise (i.e. minute 6), both of which were in coincidence with a significant reduction in the [PCr] slow component amplitude. Once more, the proposed mechanism appears to be an altered motor unit recruitment whereby PE induces a delayed muscle fibre activation allowing for the resultant attenuation of both [PCr] and $\dot{V}O_2$ slow

component amplitudes (Burnley *et al.* 2002; DiMenna *et al.* 2008; Bailey *et al.* 2009; DiMenna *et al.* 2010b; Gildea *et al.* 2020).

An alternative possibility is that PE induced a more homogenous muscle perfusion within the exercising muscle, attenuating the rate of fatigue development by reducing the reliance on anaerobic processes ([PCr] degradation), thus delaying the recruitment of additional motor units required to maintain force production, with a consequent reduction in the O_2 cost of exercise (Jones and Poole, 2005; DiMenna *et al.* 2008; Gildea *et al.* 2020). Therefore, enhanced O_2 delivery and/or distribution may influence both $\tau\dot{V}O_{2P}$ and $\dot{V}O_{2sc}$ by altering the kinetics of the recruited muscle fibres allowing them to reach their steady-state more rapidly (DiMenna *et al.* 2008) at least in individuals displaying a slow $\dot{V}O_2$ kinetic response such as in T2D and ageing.

The slow component observed during WtoW exercise is smaller than that observed during high-intensity exercise transitions initiated from an unloaded observed in the previous chapter (T2D $\dot{V}O_{2sc}$: 50% Δ = 0.26 ± 0.9 L.min^{-1}; WtoW= 0.16 ± 0.0 L.min^{-1}; ND $\dot{V}O_{2sc}$: 50% Δ = 0.30 ±0.1 L.min^{-1}; WtoW= 0.17 ± 0.1 L.min^{-1}). The development of the $\dot{V}O_{2SC}$ is believed to be in part due to a progressive recruitment of type II fibres, supplementary to type I fibres, resulting in an increased $\dot{V}O_2$ cost of exercise in the high-intensity domain (Krustrup *et al.* 2008; Goulding *et al.* 2018). Due to the nature of WtoW exercise, with type I fibres and some type II already recruited during moderate exercise, the increased exercise demand during the transition to heavy-intensity exercise may require a more homogenous recruitment of type II fibres which share the same metabolic properties and thus, the $\dot{V}O_{2SC}$ amplitude will not be as large as that observed during unloaded heavy-intensity exercise (Jones *et al.* 2006; Krustrup *et al.* 2009; Goulding *et al.* 2018).

Although the significant reductions in $\tau\dot{V}O_{2P}$ and the $\dot{V}O_{2SC}$ amplitude contributed to the reduced $\dot{V}O_2$ MRT following PE in the current study, there were no changes in the [HHb+Mb] kinetic responses. The NIRS-derived signal for [HHb+Mb] was accepted as a surrogate for fractional O_2 extraction (DeLorey *et al.* 2004), with the changes in [HHb+Mb] considered to be indicative of the balance between O_2 availability and utilisation in the microvasculature within the region of the vastus lateralis muscle. It should be noted among individuals with T2D that while statistical significance was not achieved, both $\Delta[HHb+Mb]\tau_p$ and Δ[HHb+Mb]MRT tended to be slower following PE. This may indicate an enhanced O_2

delivery and/or distribution to match metabolic rate which may have contributed to the faster $\tau\dot{V}O_{2P}$ and $\dot{V}O_2$ MRT.

4.4.2 Moderate intensity exercise

The findings of the present study in the unprimed condition are consistent with previous studies showing that no differences were observed for $\tau\dot{V}O_{2P}$ during moderate-intensity exercise in older individuals with T2D when compared to ND older controls (Wilkerson *et al.* 2011; O'Connor *et al.* 2015), with the effects of ageing *per se* negating the effect of T2D on older adults (O'Connor *et al.* 2015). The results regarding the effect of PE in this study are also consistent with previous research whereby heavy-intensity PE has been shown to accelerate the $\dot{V}O_2$ kinetic response during a subsequent bout of moderate-intensity exercise in elderly inactive populations (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2009), and middle-aged individuals with T2D (Rocha *et al.* 2019).

The improvements observed in the $\tau\dot{V}O_{2P}$ in these populations suggests that PE improves the adaption of muscle blood flow and O_2 delivery to O_2 utilisation within the exercising muscles in conjunction with faster metabolic activation of oxidative phosphorylation. Among healthy older adults, Scheuermann *et al* (2002) attributed this PE-induced faster $\tau\dot{V}O_{2P}$ response during a subsequent bout of moderate-intensity exercise to an enhanced muscle perfusion (Scheuermann *et al.* 2002).

DeLorey *et al* (2004) observed a faster $\tau\dot{V}O_{2P}$ during the subsequent moderate-intensity bout in the older individuals after PE accompanied with a slower $[HHb+Mb]\tau_p$ (unprimed: 9 ± 3 s; primed: 32 ± 17 s), suggesting that the enhanced $\tau\dot{V}O_{2P}$ may be related to improvements in local muscle perfusion and O_2 delivery (DeLorey *et al.* 2004). This is similar to the findings of slower $[HHb+Mb]\tau_p$ for both groups in the current investigation. Gurd *et al* (2009) reported initially that the slowed $\dot{V}O_2$ kinetics response in older adults was associated with both metabolic inertia and slower O_2 delivery to O_2 utilisation (Gurd *et al.* 2006). These researchers, in a follow-up study, investigated the effects of PE on subsequent moderate-intensity exercise oxygen uptake and metabolic markers such as pyruvate dehydrogenase (PDH) activity and phosphocreatine (PCr) breakdown. Gurd *et al* (2009) showed that in addition to a faster $\tau\dot{V}O_{2P}$ response, PCr breakdown was reduced following PE with a significantly higher PDH activity during the subsequent exercise onset, indicating greater

bioavailability of oxidative substrate. These findings, in conjunction with an elevation in NIRS-derived markers of local O_2 delivery, may have resulted in a faster activation of oxidative phosphorylation resulting in the faster primary phase $\dot{V}O_2$ kinetics, following PE (Gurd *et al.* 2009).

Recently, heavy-intensity PE has been proven effective as an intervention for accelerating $\tau\dot{V}O_{2P}$ in middle-aged participants with T2D (unprimed: 42.1 ± 12.2 s; primed: 32.2 ± 9.1 s) during moderate-intensity exercise (Rocha *et al.* 2019). The speeding of the $\dot{V}O_2$ primary phase kinetic response observed in participants with T2D was accompanied by a reduction in the Δ[HHb+Mb]/ $\Delta\dot{V}O_{2p}$ ratio (unprimed: 1.17 ± 0.17; primed: 1.05 ± 0.14) during the moderate-intensity bout subsequent to PE. These results are in line with previous studies in uncomplicated T2D showing that a blunted microvascular blow flow response at the exercise transient in this population leads to a sluggish $\tau\dot{V}O_{2P}$ (Bauer *et al.* 2007). Collectively, the improvements observed in $\tau\dot{V}O_{2P}$ in older untrained healthy individuals and individuals with T2D suggest that PE improves the adaption of muscle blood flow and O_2 delivery to O_2 utilisation within the exercising muscles in conjunction with faster metabolic activation of oxidative phosphorylation through enhanced substrate availability (Gurd *et al.* 2009; Rocha *et al.* 2019).

4.4.3 <u>Conclusion</u>

In conclusion, high-intensity PE exercise appears to be a safe and effective warm up method for generating a faster $\dot{V}O_2$ kinetic response indicative of enhanced exercise tolerance in older inactive adults with and without T2D during exercise initiated from an elevated baseline of moderate-intensity. It is important to study these WtoW transitions in both health and disease along with interventions to help improve $\dot{V}O_2$ kinetics during WtoW transitions given that abrupt transitions from lower to higher metabolic rates occur in our everyday lives (DaBoit *et al.* 2014). The results of the current study are promising given that a faster contribution from the aerobic metabolism can help increase the time to failure during activities that change to high metabolic rates (Rocha *et al.* 2019; DaBoit *et al.* 2014; McDermott *et al.* 2019).

Chapter 5: Effects of MICT vs HIIT on oxygen uptake and muscle deoxygenation kinetics during heavy intensity exercise in type 2 diabetes.

5.1 Introduction

Type 2 diabetes (T2D) results in a discernible slowing of the $\dot{V}O_2$ kinetics response during moderate- and heavy-intensity exercise in middle-aged individuals compared to healthy age-matched ND controls (Regensteiner *et al.* 1998; Brandenburg *et al.* 1999; Bauer *et al.* 2007; MacAnaney *et al.* 2012; O'Connor *et al.* 2012; O'Connor *et al.* 2015; Rocha *et al.* 2019; Gildea *et al.* 2020). This slowed response is mainly due to a slowing of the $\tau\dot{V}O_{2p}$ with the physiological significance of this being an over-reliance on the anaerobic metabolism, thereby decreasing exercise tolerance in individuals with T2D. To date, chronic exercise interventions involving moderate-intensity continuous training (MICT) have been shown to be effective at speeding the $\dot{V}O_2$ kinetics response during moderate and heavy intensity exercise in individuals with T2D (Brandenburg *et al.* 1999; MacAnaney *et al.* 2012).

Initially, Brandenburg and colleagues (1999) found a significant speeding of $\tau\dot{V}O_{2P}$ during moderate-intensity exercise after 12 weeks of endurance training in middle-aged individuals with T2D as well as a group of overweight ND controls (Brandenburg *et al.* 1999). Similarly, conducting a 12 week supervised concurrent exercise intervention consisting of 50 minutes of MICT and resistance exercise in men and women with T2D, MacAnaney *et al* (2012) observed an increase in $\dot{V}O_{2peak}$ together with an accelerated $\tau\dot{V}O_{2p}$ during moderate- and heavy-intensity cycling whilst the amplitude of the $\dot{V}O_{2sc}$ was reduced post-intervention in the same study (MacAnaney *et al.* 2012).

In addition to the well-reported benefits on $\dot{V}O_{2peak}$ and the dynamic response of $\dot{V}O_2$ in T2D, MICT has been proven to be an effective treatment to enhance endothelium-dependent vasodilation (Okada *et al.* 2010; Mitranun *et al.* 2013; Madsen *et al.* 2015a) and increase mitochondrial content and function (Meex *et al.* 2010) in this population. However, despite the clear benefits, most people with T2D struggle to achieve the recommended weekly physical activity guidelines with lack of time been reported to be an underlying factor. In recent years, low-volume high-intensity interval training (HIIT), a time efficient form of interval training involving short bursts/sprints interspersed with less intense recovery periods, has garnered much interest within both healthy and clinical populations (Gibala *et*

al. 2012; Little *et al.* 2011). The popularity of HIIT is due to the ability to exercise in a shorter period of time whilst getting similar physiological adaptations as MICT. This includes enhanced peak and submaximal exercise capacity (McDermott *et al.* 2018), improved cardiovascular health, and improved skeletal muscle metabolic capacity (glucose and lipid oxidation) (Little *et al.* 2011; Gibala *et al.* 2012; Timmons *et al.* 2012). HIIT has gained notoriety for its fast acting effects on glucose disposal and the prevention of T2D (Little *et al.* 2011; Gillen *et al.* 2012), as well as its effectiveness at improving vascular health in comparison to MICT. In this regard, benefits including improved popliteal artery distensibility, improved popliteal artery endothelium-dependent vascular function as measured by flow-mediated dilation (FMD), and overall arterial structure (decreased wall thickness; increased lumen diameter; decreased wall: lumen ratio) have been reported (Rakobowchuck *et al.* 2008; Madsen *et al.* 2015a; 2015b; 2015c). Investigations into HIIT also observed increased angiogenesis in both highly oxidative and more glycolytic muscle fibres, increased capillary: fibre ratio, and enhanced endothelial cell proliferation (Jensen *et al.* 2004).

For instance, in a short-term intervention study, Little & colleagues (2011) had 8 older individuals with T2D (62 ± 7 years) engage in 6 HIIT sessions over the course of 2 weeks. Each training session consisted of 10 x 1 min cycling intervals at 90% maximal effort interspersed by 1 min recovery (Little *et al.* 2011). After just 2 weeks, marked increases were observed in GLUT4 protein and a reduction in blood glucose concentrations measured using continuous glucose monitors. Regarding acute responses, after just one HIIT session Gillen *et al* (2012) found a significant reduction in the average post-meal peak glucose concentration as well as an attenuated average blood glucose on the 2nd hr after a meal, while the time spent in hyperglycaemia reduced by 65% in the post-HIIT condition compared to control/no exercise condition (Gillen *et al.* 2012).

Regarding $\dot{V}O_2$ kinetics, HIIT has been found to induce significant reductions in $\tau\dot{V}O_{2p}$ in untrained young healthy men (McKay *et al.* 2009; Williams *et al.* 2013), with these improvements occurring after 4 weeks. Additionally, this rapid improvement was accompanied by a significantly reduced Δ[HHb + Mb]/Δ$\dot{V}$O2 ratio (Williams *et al.* 2013). These results suggest improvements in local microvascular O_2 delivery and/or distribution at

the onset of exercise as a consequence of training (McKay *et al.* 2009; Williams *et al.* 2013), with HIIT and MICT eliciting similar improvements (McKay *et al.* 2009).

To the best of this investigators knowledge the effects of HIIT on $\dot{V}O_2$ kinetics in individuals with T2D have only been investigated in our laboratory. Comparing the effects of 12 weeks of HIIT and MICT on moderate-intensity $\dot{V}O_2$ kinetics (80% VT) in middle-age participants with T2D, Gildea *et al* (2018) reported a significant speeding in $\tau\dot{V}O_{2P}$ in the exercise groups only by the 3rd week of training (Week 3 $\tau\dot{V}O_{2p}$: HIIT= 26%; MICT= 27%; Control= 4%) with improvements being maintained thereafter until the end of the training programme. An improvement in the Δ[HHb+Mb]/ $\dot{V}O_2$ ratio for both exercise groups was also reported after 3 weeks with no significant further improvement observed thereafter. These results may infer an improved matching of local O_2 delivery to O_2 utilisation, thereby enhancing $\dot{V}O_2$ kinetics in individuals with T2D.

Moreover, the same group (McDermott *et al.* 2019) also reported the time-course effects following 12 weeks of HIIT and MICT on $\dot{V}O_2$ kinetics and [HHb+Mb] kinetics during WtoW transitions. The WtoW protocol was the same as that of chapter 4 of the current thesis. After just 3 weeks, the $\dot{V}O_{2SC}$ amplitude was reduced by 40% and 44% in the HIIT and MICT groups respectively with no changes observed in the control group (McDermott *et al.* 2019). Significant changes were also observed among the exercising groups for $\tau\dot{V}O_{2P}$ and $\dot{V}O_2$ MRT with both significantly decreasing after 3 weeks. The adaptations were maintained until the end of the 12 week training period with no additional changes. These novel findings highlight the significance of HIIT as a valuable, time efficient tool for improving exercise tolerance in people with T2D. To date however, there is no research into the effectiveness of exercise interventions involving HIIT on $\dot{V}O_2$ kinetics and [HHb+Mb] kinetics during heavy-intensity exercise initiated from an “unloaded” baseline.

Therefore, the aim of the current study was to assess the effects of 12 weeks of HIIT and MICT on peak exercise responses and $\dot{V}O_2$ and [HHb+Mb] kinetics during heavy-intensity exercise at pre- and post-intervention time-points in individuals with T2D. It was hypothesized that MICT and HIIT would significantly accelerate the $\tau\dot{V}O_{2p}$ and $\dot{V}O_2$ MRT, and reduce the $\dot{V}O_{2sc}$ amplitude during heavy-intensity exercise to a similar extent in both exercising groups.

5.2 Methodology:

5.2.1 Participant recruitment

5.2.1.1 Recruitment of participants

5.2.1.1.1 T2D participant recruitment

Participants were recruited from the Diabetes Outpatient Clinics of St. Colmcille's and St. Vincent's University Hospitals (Dublin) by notice board advertisements. Eligibility was initially checked following chart review. Participants were included if they had a clinical history of diabetes <11 years, were physically inactive (≤1.5 $h.week^{-1}$ of moderate-intensity exercise in the preceding 6 months) and had HbA_{1c} levels of <10%. Participants were excluded if they were treated by exogenous insulin, were smokers, had a disease contraindicating physical training, or demonstrated evidence of liver, renal or cardiovascular disease.

Three hundred individuals with T2D were eligible for inclusion and 59 of them expressed interest in participating in the study. These individuals completed a 12-lead ECG treadmill stress test at St. Colmcille's hospital. Twelve of these 59 participants failed the treadmill exercise stress test while 12 other participants who passed the exercise stress test dropped out from the study before completing the pre-training laboratory assessments. Thus, 35 participants completed the baseline laboratory assessments and were given sealed envelopes randomly allocating them to one of the 3 intervention groups (MICT, initially *n* = 13; HIIT, initially *n* = 10 or non-exercising control, CON, initially *n* = 12). Ten participants dropped out due to personal reasons unrelated to the study (CON, *n* = 3; HIIT, *n* = 3; MICT, *n* = 4). Participants in the CON group were offered re-randomisation to one of the exercise training groups after the intervention period and 3 accepted re-allocation (HIIT, *n* = 2; MICT, *n* = 1) and completed the intervention. The final study population consisted of 25 participants undergoing the intervention of whom 3 underwent CON with either MICT or HIIT. Thus, 28 completed responses from the study intervention were included for statistical analysis (CON, *n* = 9; HIIT, *n* = 9; MICT, *n* = 10). All participants provided written informed consent prior to participation. The study was approved by the Faculty of Health Sciences' Research Ethics Committee, Trinity College Dublin, and St Vincent's Healthcare Ethics and

Medical Research Committee, and conducted in accordance with the principles outlined by the Declaration of Helsinki.

5.2.1.2 Inclusion/exclusion criteria for participation

5.2.1.2.1 Participants with T2D

As per section 2.2.1.2.1

5.2.1.2.2 Fasted venous blood sample collection

On a separate day to exercise testing and subsequent to a 12 hour overnight fast, blood samples were taken from the antecubital vein for analysis in a medical room in the Department of Physiology, Trinity College Dublin. Venous blood samples were analysed in the biochemistry laboratory in St Vincent's Hospital where measurements of HbA_{1c} were recorded from each venous blood sample.

5.2.1.2.3 Ankle: Brachial Index (ABI)

As per section 2.2.1.2.4

5.2.1.3 Physical activity level determination

As per section 2.2.1.2

5.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

5.2.2 Experimental design

5.2.2.1 Study overview

Participants carried out a pre-screening assessment in St Colmcille's hospital. During this visit, participants took part in a 12-lead ECG stress test, an ankle: brachial index test to screen for peripheral arterial disease, and pulse wave velocity to test for signs of arterial stiffness. On a separate occasion as mentioned, a fasting blood sample was drawn from the participant. Upon satisfactory completion of the pre-screening tests, the participant was allowed to partake in the study. After completing the baseline laboratory visits they were then randomised into one of 3 groups: a) non-exercising control group (CON); b) moderate-

intensity continuous training group (MICT); c) low-volume high-intensity interval training group (HIIT).

Two visits to the laboratory were conducted before randomisation and initiation of the exercise programme. Participants were instructed to avoid alcohol and caffeine intake before all exercise visits and not to take part in any strenuous activity in the 24 hour period before testing. Testing sessions were spaced at least 24 hrs apart and at the same time of day if possible. Visit 1 involved an incremental ramp test to exhaustion while visit 2 involved 2 high-intensity constant-load cycling transitions. Visits 1 and 2 were completed at pre- and post-intervention time-points while visit 1 was also completed at weeks 3, 6, and 9 to reassess peak exercise performance and adjust exercise intensity values for the training intervention.

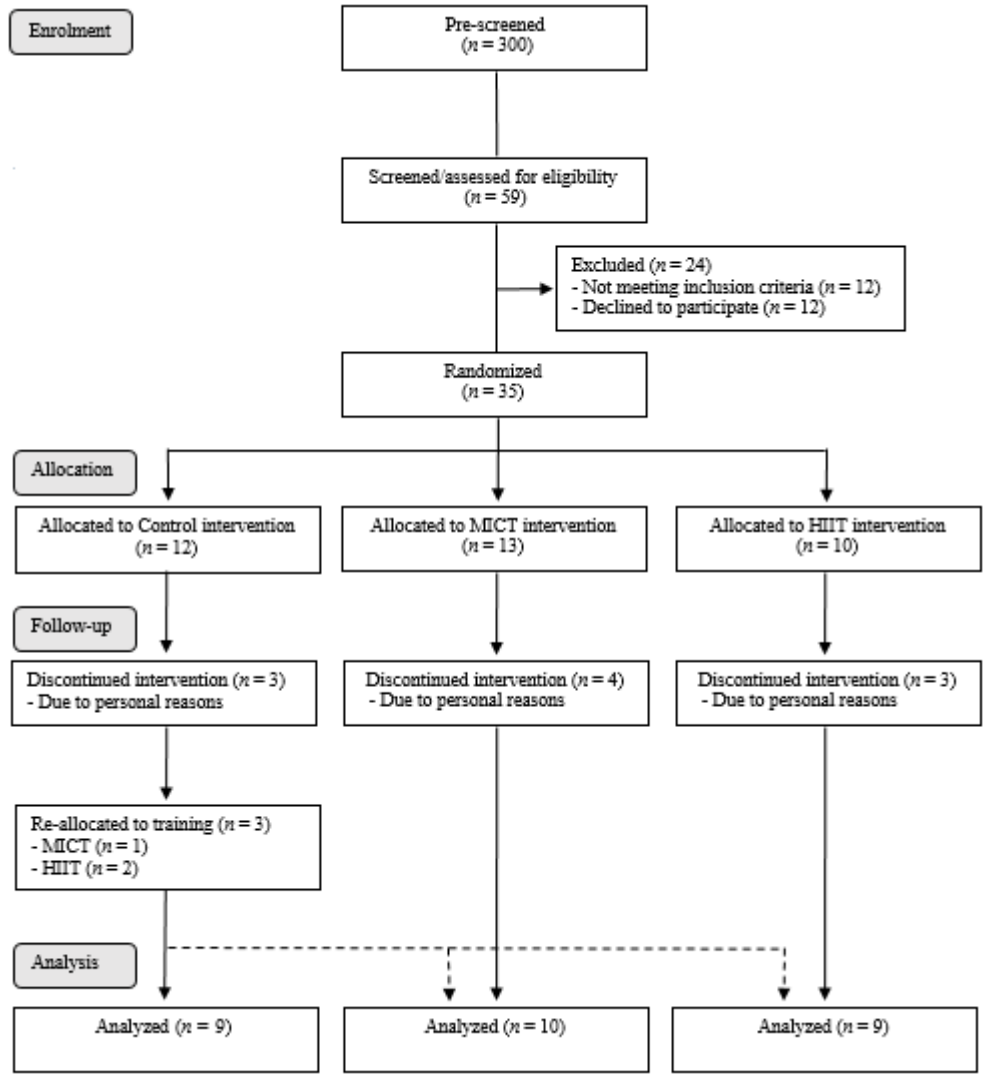

Figure 5.1. Study overview

5.2.2.2 Exercise intervention

Exercise training occurred 3 times/week over the 12 week intervention period in a community gym. Exercise was supervised by the study investigators and local gym instructors who were familiar with the exercise intervention protocols. Training sessions took place on a stationary cycle ergometer and each participant was trained on how to use the bike prior to the exercise intervention commencement. Prior to each session, it was mandatory for each participant to record both blood glucose and blood pressure in the presence of an investigator or gym instructor. Participants were prevented from training if blood glucose levels were ≤5 or ≥15 mmol/L or had elevated blood pressure ≥170/90 mmHg.

The MICT comprised of 50 minutes cycling at a power output range equivalent to ~80% of each participant's VT recorded during the RI cycling tests. Participants received their own personal heart rate monitor (CardioSport Fusion 30, Healthcare Technology Ltd, Hampshire, UK) which they used to stay within a target heart rate range associated with the specified work rate throughout the exercise session. Each MICT training session was preceded by a 5 min warm-up and 5 min cool-down using an exercise of their choice along with some basic stretching exercises taught to them by the investigator/local gym instructor.

The HIIT programme comprised of 10 x 1min bouts of cycling at an intensity equivalent to 70% Δ (70% difference between power output at VT and peak power out; designed to achieve an intensity equivalent to 90% HRmax) interspersed with 1 min active recovery (light cycling) between bouts. The same warm-up, cooldown and recording procedures used in the MICT were completed by the HIIT group.

The prescribed work rates and heart rates were adjusted every 3 weeks following reassessment of each participant's $\dot{V}O_{2peak}$ using the RI cycle test to exhaustion, ensuring training progression. The control group received no exercise training and were told to continue their normal every day routines (including physical activity and diet).

5.2.2.3 Visit 1 overview

5.2.2.3.1 Anthropometric and pulse wave velocity measures

As per section 2.2.2.2.1

5.2.2.3.2 Ramp incremental test to exhaustion

As per section 2.2.2.2.2

5.2.2.4 Visit 2 overview

5.2.2.4.1 Heavy intensity cycle exercise

From the previously completed RI cycling test during visit 1 (at pre-training), the power outputs required for this protocol were calculated. A power output corresponding to 50% delta (50% Δ; the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2peak}$) was determined. Participants then performed 2 bouts of constant-load, heavy-intensity cycling at this intensity. The duration of each step transition was 6 minutes and each transition was preceded by a 3 minute baseline cycling period at a power output of 10W. Changes in power output were initiated as a step function without a warning to the individual. There was a 45 minutes of passive rest between bouts. This resting period has been shown to be sufficient for physiological restoration following a bout of heavy-intensity exercise (Burnley *et al.* 2006). The same absolute intensity (equivalent to 50% Δ at pre-training) was used at the post-training time point.

5.2.3 <u>Laboratory equipment and measurement techniques</u>

5.2.3.1 Cardio-metabolic unit

As per section 2.2.3.1

5.2.3.2 Heart rate monitor

As per section 2.2.3.2

5.2.3.3 Blood pressure

As per section 2.2.3.3

5.2.3.4 Ratings of perceived exertion (RPE)

As per section 2.2.3.4

5.2.3.5 Muscle deoxygenation [HHb+Mb] and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.5

5.2.4 Data Analysis

5.2.4.1 Peak responses

As per section 2.2.4.1

5.2.4.2 50%Δ analysis

5.2.4.2.1 $\dot{V}O_2$ kinetics

As per section 3.2.4.2.1

5.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

As per section 3.2.4.2.2

5.2.4.2.3 Ventilatory threshold

As per section 3.2.4.2.3

5.2.5 Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was performed using SigmaPlot 12 (Systat Software, San Jose, Ca, USA). Normality of data was assessed using a Shapiro-Wilk test. Baseline data including age, anthropometric and metabolic data were compared using a one-way ANOVA. Pre and post intervention peak responses, $\dot{V}O_2$ kinetics, [HHb+Mb] kinetics and anthropometric data were analysed using a 2-way (group x time) repeated measures ANOVA. Post-hoc Holm-Sidak tests were carried out in the case of significant differences being observed from the ANOVA. The level of significance was set to $P < 0.05$.

The key variable in this study was the time constant of the $\dot{V}O_2$ primary phase ($\tau\dot{V}O_{2p}$) during heavy-intensity exercise. Published studies from our lab and others investigating 12 weeks of MICT on $\tau\dot{V}O_{2p}$ during heavy-intensity exercise in individuals with uncomplicated T2D have reported an average $\tau\dot{V}O_{2p}$ of 28 ± 3.7 s. After training, a significant shortening of $\tau\dot{V}O_{2p}$ occurred with a mean difference of ~8 s when comparing between the pre- and post-intervention values. Therefore, given that the estimated SD of each group is 3.7 s, and given

the mean difference we wish to detect is 8 s, the minimum sample size needed to detect with a statistical power of 80% (α=0.05) by ANOVA test in 3 groups, is 6 participants.

5.3 Results:

5.3.1 Participants

5.3.1.1 Exercise adherence

The mean exercise adherence was 94 ± 6% (range 31-36 sessions) in the HIIT group and 96 ± 6% (range 31-36 sessions) in the MICT group. No adverse training effects were observed.

5.3.1.2 Physical characteristics of participants

Participants' physical characteristics are presented in Table 5.1. No differences were observed for any of the measures presented, suggesting that the 3 groups were well matched.

Table 5.1. ***Baseline anthropometric and haematological parameters for the three groups.***

	CON (n = 9)	**MICT (n = 10)**	**HIIT (n = 9)**	***P* values**
Sex (M/F)	4/5	7/3	6/3	
Age, years	54 ± 9	54 ± 10	52 ± 10	0.86
Stature, m	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	0.31
Body Mass, Kg	86 ± 14	95 ± 19	87 ± 12	0.58
BMI, Kg.m^{-2}	30.5 ± 3.6	31.3 ± 5.8	28.8 ± 3.2	0.49
HbA_{1c}, %	6.8 ± 1.0	6.8 ± 0.6	7.3 ± 0.5	0.47
VL fat layer, mm	8.6 ± 3.2	6.8 ± 2.3	5.2 ± 2.2	0.22

Values are mean ± SD. BMI, body mass index; HbA_{1c}, glycosylated haemoglobin; VL, Vastus lateralis. Some variables have missing values and the sample size is as follows: HbA_{1c}, 7 (CON), 10 (MICT), 6 (HIIT); VL fat layer = 7 (CON), 10 (MICT), 8 (HIIT).

5.3.1.3 Anthropometric and HbA_{1c} data pre and post intervention for all three groups

Anthropometric and HbA_{1c} data are shown in Table 5.2. Body mass and BMI in the MICT group were reduced ($P < 0.05$) post-intervention. HbA_{1c} levels were also reduced in both the HIIT ($P < 0.001$) and MICT group ($P < 0.05$).

Table 5.2. ***Anthropometric and HbA_{1c} data pre and post intervention for all groups.***

	CON (n = 9)		MICT (n = 10)		HIIT (n = 9)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Body Mass, kg	86 ± 14	86 ± 16	95 ± 19	90 ± 18*	87 ± 12	86 ± 12
BMI, $kg.m^{-2}$	30 ± 3	31 ± 4	31 ± 6	30 ± 5*	29 ± 3	28 ± 4
Waist-to-hip Ratio, a.u	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 1.0	0.9 ± 0.1
HbA_{1c}	6.8 ± 1.0	7.0 ± 0.9	6.8 ± 0.6	6.5 ± 0.5*	7.3 ± 0.5	6.6 ± 0.8*

Values are shown for mean ± SD. *Significantly different from baseline for the same group ($P < 0.05$).

5.3.1.4 Prescriptive medication for all groups

Table 5.3 ***Prescriptive medications for the CON, MICT and HIIT groups***

	CON (n = 9)	MICT (n = 10)	HIIT (n = 9)
Anti-hypertensives			
Angiotensin converting enzyme inhibitor		1	1
Angiotensin II receptor blocker	1		
Asprin	3	1	1
Statins	5	3	3
Hypoglycaemic medications			
Metformin	7	7	6
Sulfonylureas	1	3	2
DPP-4 inhibitors			2
GLP-1 receptor agonist	1		1

5.3.1.5 Physical activity

No differences were observed between groups at baseline for physical activity ranges measured using RT3 accelerometers.

Table 5.4 ***Mean physical activity data for each group.***

	CON (n = 8)	**MICT (n = 8)**	**HIIT (n = 7)**	**P values**
Inactive, h.day^{-1}	18.2 ± 1.2	17.4 ± 1.6	17.5 ± 2.2	0.51
Light, h.day^{-1}	4.8 ± 0.9	5.2 ± 0.7	5.3 ± 1.6	0.58
Moderate, h.day^{-1}	0.8 ± 0.6	1.2 ± 1.0	1.0 ± 0.5	0.68
Vigorous, h.day^{-1}	0.2 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	0.34

Values are mean ± SD.

5.3.2 Performance data from the ramp incremental cycle test

The peak physiological responses as well as responses at VT for all 3 groups can be seen in Table 5.5. At baseline, relative $\dot{V}O_{2peak}$ and relative $\dot{V}O_2$ at VT were significantly ($P < 0.05$) higher in the HIIT group compared to the CON group. Absolute and relative $\dot{V}O_{2peak}$ as well as peak power increased significantly ($P < 0.05$) for both exercise groups after the intervention. In addition, the absolute and relative $\dot{V}O_2$ responses along with power output at VT were significantly increased following both exercise interventions. No difference was observed following the 12 week period in the CON group. Post intervention, relative $\dot{V}O_{2peak}$ as well as $\dot{V}O_2$ and power output at VT were significantly higher ($P < 0.05$) in the HIIT and MICT compared with the CON groups.

Table 5.5. ***Physiological responses at peak exercise and at VT pre and post intervention for the CON, MICT and HIIT groups.***

	CON (n = 9)		MICT (n = 10)		HIIT (n = 9)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
$\dot{V}O_{2peak}$, L.min^{-1}	1.9 ± 0.5	1.9 ± 0.5	2.2 ± 0.6	2.6 ± 0.8*	2.3 ± 0.5	2.6 ± 0.5*†
$\dot{V}O_{2peak}$, mL.kg^{-1}.min^{-1}	21.4 ± 3.5	22.0 ± 3.4	23.5 ± 3.9	28.5 ± 5.0*†	26.4 ± 4.0†	29.8 ± 3.9*†
Peak Power, W	148 ± 49	153 ± 50	176 ± 46	210 ± 64*†	187 ± 51	217 ± 55*†
Peak HR, beats.min^{-1}	164 ± 13	155 ± 18	159 ± 14	157 ± 16	162 ± 14	160 ± 14
$\dot{V}O_2$@ VT, L.min^{-1}	1.3 ± 0.3	1.4 ± 0.3	1.7 ± 0.5	2.0 ± 0.6*†	1.7 ± 0.4	2.0 ± 0.4*†
$\dot{V}O_2$ @ VT, mL.kg^{-1}.min^{-1}	15.3 ± 2.4	16.1 ± 2.2	17.7 ± 3.0	22.8 ± 4.0*†	19.7 ± 3.7†	23.0 ± 3.0*†
PO @ VT, W	82 ± 24	90 ± 27	102 ±29	140 ± 41*†	115 ± 32	148 ± 43*†
Absolute 50% Δ, W	115 ± 36	-	139 ± 37	-	151 ± 41	-

Values are mean ± SD. *Significantly different from pre-training values ($P < 0.05$). †Significantly different from the CON group ($P < 0.05$).

5.3.3 Performance data during high-intensity exercise pre and post intervention

5.3.3.1 $\dot{V}O_2$ kinetics

Mean parameter estimates for the $\dot{V}O_2$ kinetic responses during high-intensity exercise before and after the intervention in the 3 groups can be seen in Table 5.6 while individual representative responses can be viewed in Fig 5.1.

Both exercise interventions induced a significant reduction in the time constant of the $\dot{V}O_2$ primary phase ($\tau\dot{V}O_{2p}$). In addition, the $\dot{V}O_2$ MRT for both training groups was lower ($P < 0.001$) compared with the CON group post-intervention. The $\dot{V}O_2$ slow component amplitude was only reduced ($P < 0.001$) in the MICT group, only. Moreover, baseline $\dot{V}O_2$ was reduced and $\dot{V}O_2$ A_p increased following training in the HIIT group while the $\dot{V}O_2$ A_p and the primary $\dot{V}O_2$ gains were higher post-training in the MICT group.

Table 5.6. ***Dynamic response characteristics of oxygen uptake during heavy intensity exercise (50% Δ) pre and post intervention for each group.***

	CON (n = 9)		MICT (n = 10)		HIIT (n = 9)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
$\dot{V}O_2$ baseline, L.min^{-1}	0.9 ± 0.2	0.8 ± 0.1*	1.0 ± 0.2	0.9 ± 0.1*	1.0 ± 0.3	0.8 ± 0.2*
$\dot{V}O_2$ A_p, L.min^{-1}	0.8 ± 0.4	0.9 ± 0.4	0.9 ± 0.4	1.2 ± 0.5*	1.1 ± 0.4	1.3 ± 0.5 *
$\dot{V}O_2$ absolute A_p, L.min^{-1}	1.7 ± 0.5	1.6 ± 0.5	1.9 ± 0.5	2.1 ± 0.5	2.1 ± 0.5	2.1 ± 0.5
$\dot{V}O_2$ TD_p, s	19 ± 6	21 ± 3	21 ± 5	21 ± 2	20 ± 6	22 ± 6
$\tau\dot{V}O_{2p}$, s	32 ± 4	31 ± 5	35 ± 4	24 ± 4*†	33 ± 5	26 ± 3*†
Primary $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	8.0 ± 1.5	8.3 ± 1.3	7.6 ± 1.9	9.3 ± 1.4*	7.8 ± 1.5	9.2 ± 6.1
$\dot{V}O_2$ A_{sc}, L.min^{-1}	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0*	0.2 ± 0.1	0.2 ± 0.1
$\dot{V}O_2$ A_{sc}, %	24.6 ± 7.2	24.6 ± 7.4	37.1 ± 23.0	11.0 ± 3.6*†	22.0 ± 7.1	14.0 ± 7.6
$\dot{V}O_2$ TD_{sc}, s	136 ± 23	131 ± 24	143 ± 13	148 ± 17	129 ± 16	136 ± 35
End $\dot{V}O_2$, L.min^{-1}	1.9 ± 0.5	1.8 ± 0.6	2.2 ± 0.5	2.2 ± 0.6	2.3 ± 0.6	2.3 ± 0.6
$\dot{V}O_2$ MRT, s	60 ± 9	66 ± 11	75 ± 14	43 ± 5*†	58 ± 11	41 ± 6*†
End $\dot{V}O_2$ gain, ml.min^{-1}.W^{-1}	9.9 ± 1.7	10.3 ± 1.5	10.1 ± 2.0	10.3 ± 1.4	9.5 ± 1.6	10.4 ± 1.6

Values are mean ± SD. A, amplitude; TD, time delay; τ, time constants; p, primary phase; sc, slow component phase; MRT, mean response time. *Significantly different within the same group from baseline ($P < 0.05$). †Significantly different from the CON group ($P < 0.05$).

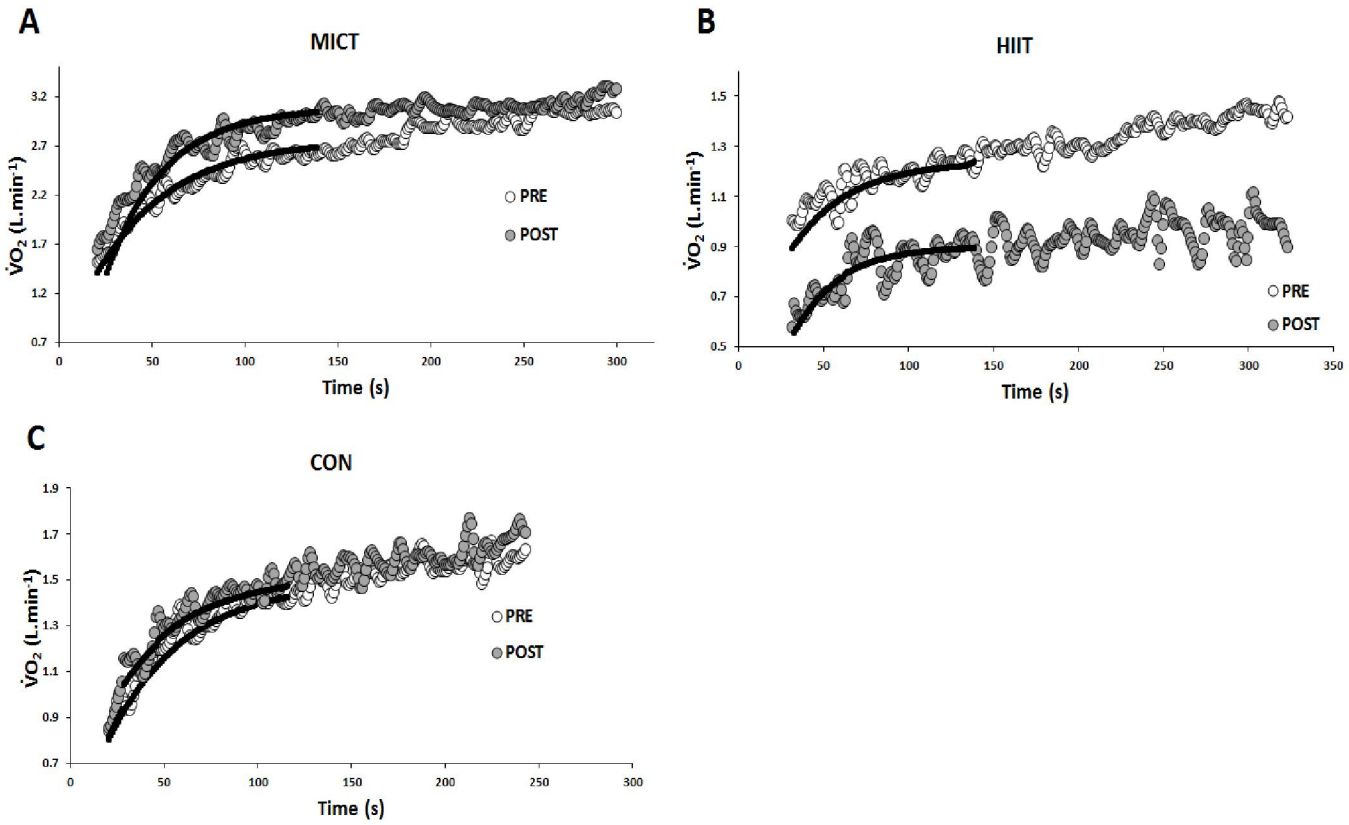

Figure 5.2. Oxygen uptake responses during high-intensity exercise for representative individuals in the CON, MICT and HIIT groups at pre (o) and post (●) exercise intervention. The continuous lines of best fit illustrate the primary phase of the $\dot{V}O_2$ kinetic response. Note the relatively slower $\tau\dot{V}O_{2p}$ in the pre-intervention time-point in the MICT and HIIT groups.

5.3.3.2 Muscle [HHb+Mb] kinetics

The parameter estimates for the [HHb+Mb] kinetics during high-intensity exercise before and after the intervention in each group are displayed in table 5.7 while individual representative responses are shown in Fig 5.2. Due to technical problems with the equipment data from 7 participants (CON: 2, MICT: 3, HIIT: 2) were excluded from the analysis. A significant slowing of the primary phase time constant ($\Delta[HHb+Mb]\tau_p$) was observed for both MICT ($P < 0.001$) and HIIT ($P < 0.05$) groups while a significant slowing ($P < 0.001$) was also observed for $\Delta[HHb+Mb]\tau'$ in the MICT group only. No other parameter estimates of muscle [HHb+Mb] were affected by the intervention.

Table 5.7. ***Kinetic parameters for Δ[HHb+Mb] during high-intensity exercise (50% Δ) pre and post intervention in the CON, MICT and HIIT groups.***

	CON (n = 7)		MICT (n = 7)		HIIT (n = 7)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Δ[HHb+Mb] Ap, μM*cm	98 ± 103	95 ± 96	125 ± 72	162 ± 48	179 ± 126	214 ± 153
Δ[HHb+Mb] TD_p, s	11.4 ± 4.7	10.9 ± 3.7	10.3 ± 3.8	9.3 ± 3.7	9.5 ± 1.9	9.3 ± 1.3
Δ[HHb+Mb]τ_p, s	14.3 ± 2.6	13.3 ± 2.2	16.3 ± 4.8	30.5 ± 7.6*†	13.9 ± 6.1	17.7 ± 4.4*†
Δ[HHb+Mb]τ', s	25.7 ± 4.9	24.1 ± 3.9	26.6 ± 6.6	39.7 ± 9.6*†	23.4 ± 7.1	27.7 ± 4.4
Δ[HHb+Mb] A_{sc}, μM*cm	20.5 ± 15.8	19.4 ± 18.6	28.1 ± 21.3	18.3 ± 12.3	14.5 ± 17.8	18.6 ± 12.3
Δ[HHb+Mb] TD_{sc}, s	121 ± 15	126 ± 23	127 ± 35	143 ± 15	122 ± 29	136 ± 18
Absolute Δ[HHb+Mb] end A, μM*cm	30.3 ± 109.6	21.1 ± 82.5	82.6 ± 92.1	109.3 ± 48.4	140.7 ± 127.5	186.5 ± 152.1
Δ[HHb+Mb]MRT, s	19.5 ± 5.5	21.6 ± 15.1	34.1 ± 34.6	24.7 ± 10.3	29.6 ± 39.6	28.2 ± 34.5

Values are mean ± SD. A, amplitude; TD, time delay, τ, time constant; p, primary phase; sc, slow component; MRT, mean response time; τ', effective response time; (τ + TD); [HHb+Mb], deoxygenated haemoglobin and myoglobin concentration. *Significantly different from baseline within the same group ($P < 0.05$). †Significantly different from the CON group ($P < 0.05$).

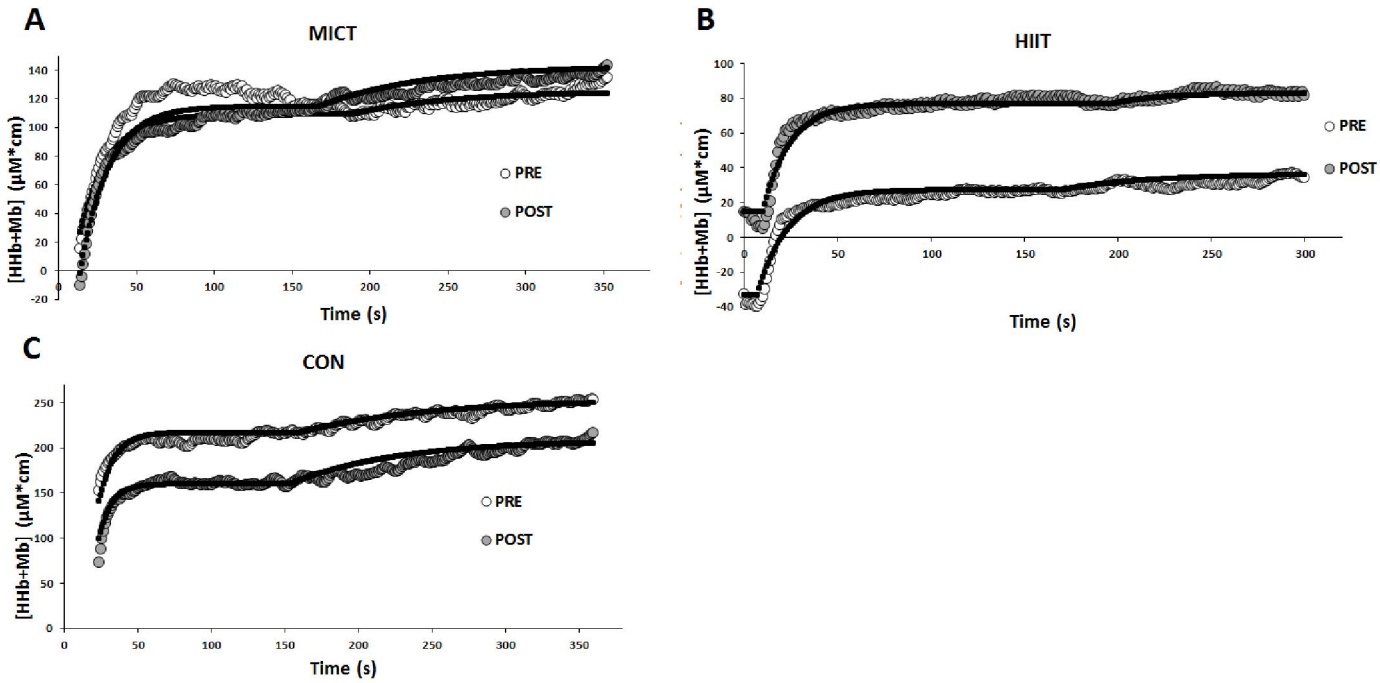

Figure 5.3. Δ[HHb+Mb] responses during high-intensity exercise for representative individuals from the CON, MICT and HIIT groups at pre (o) and post (●) exercise intervention. The continuous lines of best fit illustrate the bi-exponential [HHb+Mb] response.

5.3.3.3 Heart rate responses

Heart rate responses during the high-intensity exercise for the CON, MICT and HIIT groups' pre and post intervention are displayed in Table 5.8. Baseline HR and end-exercise HR significantly decreased ($P < 0.05$) in both exercise groups post intervention while no differences were observed for the CON group. In addition, the change in heart rate from the start to the end of the exercise bout was lower ($P < 0.05$) in the MICT group only following the intervention.

Table 5.8. ***Heart rate responses during high-intensity exercise (50% Δ) pre and post intervention for the CON, MICT and HIIT group.***

	CON (n=9)		MICT (n=10)		LVHIIT (n=9)	
	PRE	POST	PRE	POST	PRE	POST
Baseline HR, beats.min^{-1}	104 ± 10	102 ± 13	104 ± 15	96 ± 14.3*	104 ± 12	95 ± 12*
End HR, beats.min^{-1}	151 ± 16	148 ± 21	153 ± 17	133 ± 13.0*	155 ± 10	143 ± 8*
Δ HR, beats.min^{-1}	47 ± 14	46 ± 12	48 ± 13	38 ± 9.3*	51 ± 8	48 ± 9

Values are mean ± SD. *Significantly different from baseline within the same group ($P < 0.05$).

5.4 Discussion:

This study compared the effects of 12 weeks of HIIT and MICT on $\dot{V}O_2$ and [HHb+Mb] kinetics during high-intensity exercise in middle-aged adults with uncomplicated T2D. The principle findings from this study are that the time constant of the primary phase ($\tau\dot{V}O_{2P}$) and $\dot{V}O_2$ MRT significantly decreased during high-intensity exercise transitions in both the MICT and HIIT group after 12 weeks with no difference observed between exercise groups post-training. In addition, the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) amplitude decreased in the MICT group post-intervention. Simultaneously, recorded muscle oxygen extraction responses showed that the [HHb+Mb]τ_p increased (i.e. became slower) in both exercise groups post-intervention.

5.4.1 <u>Peak responses</u>

A decreased aerobic capacity is correlated with increased morbidity and mortality (Paterson *et al.* 2007). Type 2 diabetes is associated with reduced peak exercise responses compared to healthy controls (Green *et al.* 2015). In the present study, $\dot{V}O_{2peak}$ (absolute and relative) and peak power output as well as $\dot{V}O_2$ at VT (absolute and relative) and power output at VT all improved to a similar extent following 12 weeks of MICT and HIIT interventions.

Within the MICT group, relative $\dot{V}O_{2peak}$ increased by 21.1% while peak power output improved by 19.7% following the intervention. These results are similar to previous investigations into short-term MICT interventions in T2D (Brandenburg *et al.* 1999; MacAnaney *et al.* 2012; Green *et al.* 2020). For instance, MacAnaney *et al* (2012) observed a 21.5% and 26.6% improvement in relative $\dot{V}O_{2peak}$ and peak power output respectively after 12 weeks of concurrent exercise in a mixed group of men and women with uncomplicated T2D. More recently, the same laboratory showed that the improvements in $\dot{V}O_{2peak}$ following MICT are not sex-dependent in middle-age participants with uncomplicated T2D (Green *et al.* 2020).

In the HIIT group, the relative $\dot{V}O_{2peak}$ increased by 13% whilst peak power output increased by 15.8% in the current study. These findings are similar to previous findings focusing on short-term low-volume HIIT interventions in T2D (Madsen *et al.* 2015; Francois *et al.* 2017; Wilson *et al.* 2019). For instance, Madsen *et al* (2015) observed improvements in relative $\dot{V}O_{2peak}$ and peak power output of 13.2 and 10.3% respectively in individuals with T2D after

8 weeks of low-volume HIIT exercise consisting 10 x 1 minute intervals interspersed by 1 minute recovery (Madsen *et al.* 2015a; 2015b; 2015c).

5.4.2 $\dot{V}O_2$ kinetics

5.4.2.1 Time constant of the primary phase ($\tau\dot{V}O_{2P}$)

In the present study, the $\tau\dot{V}O_{2P}$ during high-intensity cycling decreased by 31.3% in the MICT group while it decreased by 20.7% in the HIIT group. The MICT group tended to have a slower $\tau\dot{V}O_{2P}$ at baseline in comparison to the HIIT group, so this may partly explain the greater decrease recorded for that group post-intervention (see Table 5.4). To this author's knowledge, only one previous study investigated the effects of MICT intervention on $\dot{V}O_2$ kinetics at heavy-intensity exercise (50% Δ) in T2D (MacAnaney *et al.* 2012). In that study, authors found a 26.1% reduction in $\tau\dot{V}O_{2P}$ in a similar 12 week intervention as previously described (MacAnaney *et al.* 2012). In addition, MacAnaney *et al* (2012) observed a 56.2% decrease in the $\dot{V}O_{2sc}$ amplitude post-intervention.

The MICT group herein trained for 50 minutes per session at 80% of their VT power output which was readjusted every 3 weeks to ensure exercise progression. While moderate intensity endurance training below the anaerobic threshold is said to recruit mainly type I or highly oxidative muscle fibres (Krustrup *et al.* 2004), the duration of the training session protocol in this study may have allowed for some recruitment of type II fibres towards the end of each individual session (Da Boit *et al.* 2014), thereby influencing the oxidative capacity of type II muscle fibres. This in turn could lead to speeding of the $\tau\dot{V}O_{2P}$ via increased oxidative activity (Meex *et al.* 2010) and increased capillarization of type II muscle fibres (Zoladz *et al.* 2016) which in turn could direct glycolytic muscle fibre characteristics towards a more oxidative phenotype. Moderate-intensity endurance training could also play a role in speeding the slow-twitch fibres maximum shortening velocity along with increasing the activity of actomyosin ATPase activity (Schluter *et al.* 2011; Da Boit *et al.* 2014). This may allow increased recruitment of oxidative fibres to contribute and satisfy power output requirements of heavy-intensity exercise which are more resistant to fatigue and also allow less contribution of glycolytic fibres, resulting in a faster $\tau\dot{V}O_{2P}$ (Da Boit *et al.* 2014) after the MICT exercise intervention.

The low-volume HIIT protocol used in the current study was the same as that used by the group of Gibala and collegues in their landmark studies on participants with pre-diabetes and T2D (Little *et al.* 2011; Gibala *et al.* 2012). Therefore, enhanced mitochondrial capacity (Little *et al.* 2011) and vascular remodelling (Madsen *et al.* 2015a) were likely to happen in the present study due to the similarities between protocols (i.e. 10 one minute intervals at 90% Max HR interspersed with one minute recovery), and likely played a role in the underlying mechanisms for improvement in $\dot{V}O_2$ kinetics observed in the present study. There is also evidence suggesting that HIIT may be more effective than MICT at targeting type II muscle fibres with respect to enhancing oxidative enzyme capacity, mitochondrial coupling and increasing capillarization (Da Boit *et al.* 2014). HIIT training has been implicated in enhancing mitochondrial biogenesis through increased activation of upstream pathways such as the AMPK and MAPK, resulting in enhanced PGC-1α activation in both type I and II muscle fibres (Gibala *et al.* 2008; Hawley *et al.* 2012). This could enhance the metabolic capacity of type II muscle fibres allowing for increased efficiency and lessen metabolic perturbations, which may lead to faster $\tau\dot{V}O_{2P}$ (Da Boit *et al.* 2014). Conversion of highly glycolytic fibres towards a more oxidative phenotype has been reported after repeated sprint training in healthy males above $\dot{V}O_{2peak}$ (Allemeier *et al.* 1994), however, whether this occurs in submaximal HIIT training in T2D is unknown.

Overall, exercise regimes such as MICT or HIIT that employ a sufficient stimulus may increase mitochondrial content in all muscle fibres (Howard *et al.* 1985) including glycolytic fibres which, when altered due to training, may be expected to show faster $\dot{V}O_2$ kinetics (Poole *et al.* 1994). Also, due to the shift in the VT to higher exercise thresholds post-training, the necessity to recruit fast-twitch highly glycolytic fibres with slow $\dot{V}O_2$ kinetics compared to their oxidative counterparts is reduced. This allows the employment of more oxidative fibre types to facilitate ATP production, thus, speeding the overall $\dot{V}O_2$ kinetics responses and subsequently, reducing the O_2 deficit and metabolic perturbations at exercise onset and improving exercise tolerance (Poole *et al.* 1994; Korzeniewski *et al.* 2015).

5.4.2.2 Amplitude of the slow component ($\dot{V}O_{2sc}$)

The amplitude of the $\dot{V}O_{2sc}$ significantly decreased in the MICT group by 58.6% whereas a non-significant reduction of 21.7% was observed in the HIIT group. This is consistent with previous studies showing a reduction in the amplitude of the $\dot{V}O_{2sc}$ during a high-intensity

bout subsequent to a 12 week MICT intervention (MacAnaney *et al.* 2012) as well as during a WtoW transition subsequent to HIIT and MICT interventions (McDermott *et al.* 2019).

The decrease in the $\dot{V}O_{2sc}$ amplitude post-intervention observed in the current study could infer a reduction in the development of muscle inefficiency within the exercising muscle post exercise intervention which may be indicative of altered fibre type recruitment, increased blood flow perfusion to the exercising muscle(s) and/or enhanced oxidative capacity of the exercising muscle (Poole *et al.* 1994; Behnke *et al.* 2005; Poole *et al.* 2008; Meex *et al.* 2010; Korzeniewski *et al.* 2015; Heinonen *et al.* 2016).

In healthy untrained males, endurance training lasting 20 weeks resulted in a 40% increase in VEGFR2 gene (related to capillarization) within the vastus lateralis along with a tendency towards higher capillary-fibre ratio after training (Zoladz *et al.* 2016). This occurred in conjunction with a 49% decrease in the $\dot{V}O_{2sc}$ amplitude. This study by Zoladz and colleagues (2016) shows that an endurance training protocol could attenuate the magnitude of the $\dot{V}O_{2sc}$ by increasing metabolic stability due to improved O_2 delivery to the exercising muscle fibre(s). However future research is needed to elucidate if this is the case in patient populations such as T2D.

5.4.3 Muscle deoxygenation [HHb+Mb] kinetics

To our knowledge, this is the first study to report the dynamic response of [HHb+Mb] kinetics during high-intensity exercise, following a MICT or HIIT exercise intervention in individuals with T2D. Herein, a statistically significant slowing in the [HHb+Mb]τ_p was observed for both exercise groups.

During moderate-intensity cycling an overshoot in the muscle [HHb+Mb] response has been reported at the onset of exercise which was accompanied with a slower $\tau\dot{V}O_{2P}$ in middle-aged individuals with T2D (Bauer *et al.* 2007; Rocha *et al.* 2019). This indicates an increased reliance on O_2 extraction to compensate for poor microvascular blood flow delivery to the contracting muscle at exercise onset which may lead to delayed activation of oxidative phosphorylation and thus, induce a sluggish $\dot{V}O_2$ kinetic response in middle age individuals with T2D. Reasons for this impaired blood flow delivery and thus, slower $\dot{V}O_2$ kinetics in T2D may be due to impaired arteriolar vasodilation resulting from decreased nitric oxide (NO) bioavailability and increased endothelin-1 concentration (Heinonen *et al.* 2016). For

instance, Madsen *et al* (2015) observed significant improvements in %FMD of the popliteal artery (~23% increase) along with improved resting haemodynamics after 8 weeks of HIIT cycling intervention in elderly people with T2D (Madsen *et al.* 2015a). Post-intervention, the popliteal artery diameter was also significantly increased, indicating vascular remodelling had taken place. Thus, an outward arterial remodelling of ~6% seen in the T2D group could likely lead to reduced shear rates within the artery.

Moreover, a greater recruitment of fast-twitch glycolytic fibres may occur in people with T2D due to a lower oxidative to glycolytic fibre ratio (Oberbach *et al.* 2006). For instance, in animal models (rats) fast-twitch fibres have a lower microvascular O_2 partial pressure ($P_m\dot{V}O_2$) which falls faster and to a lower extent in glycolytic fibres (Behnke *et al.* 2005; Ferreira *et al.* 2006; Heinonen *et al.* 2016) leading to energetic consequences for exercise tolerance (i.e. increased Δ[ADP], Δ[PCr], Δ[Pi], and Δ[H^+]) (Poole *et al.* 2008). Therefore, it may be inferred that improvements to one of the previously mentioned variables may be responsible for the improvements in [HHb+Mb] kinetics observed with exercise.

Therefore, taken together, the faster $\tau\dot{V}O_{2P}$ and $\dot{V}O_2$ MRT as well as the reduced magnitude of $\dot{V}O_{2sc}$ and slower [HHb+Mb] kinetics suggest that local blood flow and/or distribution improved post-intervention within the exercising muscle during heavy-intensity exercise. This may occur in conjunction with an enhanced O_2 extraction capacity from the exercising musculature (Bailey *et al.* 2009).

*5.4.4 **Conclusion***

In conclusion, the present study showed that MICT and HIIT are effective interventions at improving maximal aerobic capacity ($\dot{V}O_{2peak}$) and dynamic responses ($\dot{V}O_2$ kinetics) at heavy-intensity submaximal efforts in people with T2D. Given the similar magnitude of the effects observed subsequent to HIIT and MICT, it is suggested that low-volume HIIT, with its ~50% lower training volume and time commitment than traditional MICT programmes may be a more suitable and attractive exercise modality for novice exercisers with T2D.

Chapter 6: General discussion

Individuals with T2D appear to be more sedentary than an average healthy person (Reusch *et al.* 2013) with the condition becoming exacerbated with prolonged sitting time and physical inactivity (Hamilton *et al.* 2007). Studies which examined maximal exercise capacity in adult individuals of all ages with T2D showed that $\dot{V}O_{2peak}$ is ~20% lower compared with age-matched ND controls (Baldi *et al.* 2003; Regensteiner *et al.* 2009; Reusch *et al.* 2013; Green *et al.* 2015; O'Connor *et al.* 2015; Gildea *et al.* 2019). A reduced $\dot{V}O_{2peak}$ is linked to a larger rate of O_2 extraction as a consequence of impaired blood flow in the VL muscle in middle-aged individuals with T2D compared to age-matched healthy controls (Gildea *et al.* 2019). Prior to this thesis, it was unknown whether this is apparent in older individuals with and without T2D. In addition, previous research from our laboratory and others has also demonstrated a slowed adjustment of aerobic metabolism when investigating the $\dot{V}O_2$ kinetic response as a proxy for aerobic metabolism at the onset of moderate- and heavy-intensity constant-load exercise in young and middle-aged people with T2D (Gildea *et al.* 2018; McDermott *et al.* 2019; Rocha *et al.* 2019). The speed at which pulmonary $\dot{V}O_2$ can reach steady-state will dictate how large an O_2 deficit is incurred which is indicative of intracellular perturbations due to substrate phosphorylation, making the $\dot{V}O_2$ kinetic response of exercise a good indicator of muscle metabolism (Xu & Rhodes, 1999; Poole & Jones, 2012; Grassi *et al.* 2015). Therefore, the slowed $\dot{V}O_2$ kinetic response observed in T2D will result in an increased O_2 deficit which in turn will increase the dependency on non-oxidative energy stores to match ATP demand to sustain power output during exercise onset (Green *et al.* 2015; Rocha *et al.* 2019). This resultant metabolic waste product accumulation leads to premature fatigue and increased effort perception/exercise intolerance in individuals with T2D compared to healthy young and middle-aged participants during maximal and submaximal exercise (Bauer *et al.* 2007; Huebschmann *et al.* 2009; MacAnaney *et al.* 2012; Green *et al.* 2015; Gildea *et al.* 2019; Rocha *et al.* 2019).

Whilst a significantly slower $\dot{V}O_2$ kinetic response has been observed in young and middle-aged people with T2D compared with age-matched ND controls; in contrast, no differences have been observed in older (60 – 70 years) individuals with T2D compared with healthy age-matched controls (Wilkerson *et al.* 2011; O'Connor *et al.* 2015), at least during moderate intensity exercise. This is despite elderly people with T2D showing a significantly

reduced $\dot{V}O_{2peak}$ compared to their healthy counterparts (Wilkerson *et al.* 2012; O'Connor *et al.* 2015).

With this in mind, in young and middle-aged persons with uncomplicated T2D, a prior warm-up or a heavy-intensity priming exercise (PE) has been shown to be effective at accelerating the $\dot{V}O_2$ kinetic response during cycle exercise transitions to moderate-, heavy-intensity as well as WtoW transitions (Rocha *et al.* 2016; Rocha *et al.* 2019; Gildea *et al.* 2020). However, prior to completing the experiments in the present thesis, it was unknown whether a prior PE bout would influence subsequent $\dot{V}O_2$ kinetics during high-intensity exercise transitions and/or WtoW transitions in older individuals with T2D, and whether the responses would be different when compared to older non-diabetics (ND) controls.

On the other hand, chronic exercise interventions involving short-term MICT (Brandenburg *et al.* 1999; MacAnaney *et al.* 2012; McDermott *et al.* 2018; 2019; Gildea *et al.* 2018) or low-volume HIIT (McDermott *et al.* 2018; 2019; Gildea *et al.* 2018) have also proven effective in enhancing the $\dot{V}O_2$ kinetic response at both, moderate-intensity exercise as well as WtoW high-intensity transitions. However, prior to completing the intervention period in the present thesis it was not known whether a short-term HIIT or MICT intervention could affect $\dot{V}O_2$ kinetics during transitions to high-intensity exercise (initiated from an unloaded baseline) in T2D.

6.1 Fractional O_2 extraction during a ramp incremental exercise

As previously mentioned, $\dot{V}O_{2peak}$ is positively correlated with quality of life and negatively correlated with risk of all-cause mortality and morbidity in T2D (Regensteiner *et al.* 1995). Gildea *et al* (2019) reported that the slope of the first linear segment of the Δ%[HHb+Mb]/Δ%PO relationship was significantly steeper in middle-aged individuals with T2D (aged: 35-55 y) compared to healthy age-matched controls during ramp incremental (RI) exercise to exhaustion suggesting a larger muscle fractional O_2 extraction to compensate for an impaired O_2 delivery to the exercising muscles which may likely have impaired exercise tolerance. In light of this impairment among middle-aged individuals with T2D, *Experiment 1* further investigated cardiorespiratory and microvascular responses to RI exercise in elderly individuals with and without T2D aged between 60-70 years. The primary aim of this first experiment was to investigate the influence of T2D on the profile of muscle

fractional O_2 extraction normalised to power output during RI exercise in elderly individuals. Muscle fractional O_2 was estimated using the NIRS-derived [HHb+Mb] signal derived from the vastus lateralis muscle. In contrast to the findings reported among middle-aged individuals (i.e. steeper slope of the first linear segment of the Δ%[HHb+Mb]/Δ%PO relationship in T2D) (Gildea *et al.* 2019), no differences were observed in this slope during RI exercise in older individuals with and without T2D. This implies that the influence of T2D on muscle O_2 extraction is negated with age, at least in inactive older adults.

6.2 Mechanisms for slowed $\dot{V}O_2$ kinetics

Whilst the primary locus of control for $\dot{V}O_2$ kinetics in endurance trained and otherwise healthy individuals is said to preside within the mitochondrion of skeletal muscle (Murias *et al.* 2011; Poole and Jones, 2012), it seems likely that a displacement of the locus of control upstream of the contracting muscle into the O_2 transport pathway occurs in disease, ageing and inactive participants with low fitness levels. Whilst it is acknowledged that both central and peripheral mechanisms play a role in the reduced maximal and submaximal exercise capacity in T2D and ageing, the experiments from the current thesis direct our attention towards peripheral mechanisms.

The temporal and spatial distribution of blood flow and thus O_2 delivery ($\dot{Q}O_2$) both between and within the muscle to meet their energetic requirements ($\dot{Q}O_2$/ $\dot{V}O_2$) of contraction during exercise is key to maintain mitochondrial and contractile function (Heinonen *et al.* 2016). For a balanced $\dot{Q}O_2/\dot{V}O_2$, increased cardiac output and sympathetic tone work together with local vasoactive control within the active muscles which is mediated by interactions between muscle fibre recruitment and arteriolar vasodilation ability (Heinonen *et al.* 2016). Animal models have proved useful to monitor this balance in blood flow delivery to muscle metabolic demand. These studies focused on microvascular O_2 partial pressure ($P_m\dot{V}O_2$) which represents the O_2 partial pressure driving O_2 diffusion between the blood and the myocyte making it a useful measure of $\dot{Q}O_2/\dot{V}O_2$ within the active musculature (McDonough *et al.* 2001; Behnke *et al.* 2001; 2005). In healthy and active young rats, at the initiation of contraction, mitochondrial $\dot{V}O_2$ demand increases along with an adequate or even surplus of microvascular $\dot{Q}O_2$ (Behnke *et al.* 2001). As exercise continues, after 20 s a gradual decrease in $P_m\dot{V}O_2$ is observed indicating a good matching of $\dot{Q}O_2/\dot{V}O_2$ where eventually a steady state or a plateau is reached. This decrease appears to

be exponential-like with a time constant (τ) of ~20 s. This smooth rest-contraction transition of $P_m\dot{V}O_2$ is indicative of a fast and effective contribution of oxidative phosphorylation to meet the metabolic demands of the muscle, thus, reducing the accumulation of fatigue metabolites at exercise onset. However, in rats who have T2D (Behnke *et al.* 2002; Padilla *et al.* 2006; 2007) or in older rats when compared with young rats (Behnke *et al.* 2005; 2006), after the initial time delay a rapid decline in $P_m\dot{V}O_2$ is reported before the achievement of a steady-state evidencing an impairment in $\dot{Q}O_2$ relative to mitochondrial $\dot{V}O_2$ (Behnke *et al.* 2001). Within the microvasculature, impaired arteriolar vasodilation may occur due to decreased nitric oxide (NO) bioavailability and increased endothelin-1 (Heinonen *et al.* 2016) resulting in a reduced hyperaemic response (capillary haemodynamic dysfunction/ reduced ability to vasodilate) which in turn leads to poor matching of $\dot{Q}O_2$/ $\dot{V}O_2$ in the working muscle(s) of individuals with T2D (Heinonen *et al.* 2016). The end result may be a sluggish $\dot{V}O_2$ kinetics response, an increased O_2 deficit and increased reliance on substrate phosphorylation.

In the spinotrapezius muscle capillaries of Goto-Kakizaki (GK) rats with T2D, Padilla *et al* (2006) found a decreased percentage of red blood cell (RBC) flowing capillaries (~29%) at rest compared with healthy controls, along with a 10% decrease in the capillary blood haematocrit and a significant reduction in RBC velocity and flux (Padilla *et al.* 2006). Additionally, an overall reduction of 70% was also observed in $\dot{Q}O_2$ in animals with T2D. This O_2 exchange impairment could lead to unfavourable cellular conditions within the myocyte, thus, impairing exercise tolerance. The mechanisms implicated may include a high vasomotor control with a high degree of arterial tone as a result of impaired endothelium-dependent vasodilation and increased endothelin-1 levels resulting in increased vasoconstriction. The decreased haematocrit will decrease the O_2 diffusing capacity of the muscle (DO_2m) via reduced RBC numbers available for blood-myocyte O_2 flux. DO_2m is expected to be 40% lower in spinotrapezius muscle of GK rats and, in conjunction with the 70% lower $\dot{Q}O_2$ during muscle contraction, could result in an increased reliance on O_2 extraction which could explain the lower $Pm\dot{V}O_2$ observed (Behnke *et al.* 2002; Padilla *et al.* 2007). An undershoot in $Pm\dot{V}O_2$ was indeed observed in GK rats during the transition from rest to contraction in this study (Padilla *et al.* 2007), which may be related to poor $\dot{Q}O_2$ response. This would further suggest a mismatch of $\dot{Q}O_2$/ $\dot{V}O_2$ due to the slowed RBC

haemodynamics, thus, lowering $\dot{Q}O_2$ leading to a lower $\dot{V}O_2$ achieved during steady state (Behnke *et al.* 2001; 2002; Poole *et al.* 2020).

A lower pre-contracting baseline $P_m\dot{V}O_2$ and sharp decline have also been reported in older compared with younger rats (Behnke *et al.* 2005; 2006). Oxidative metabolism was similar between old and young rats further indicating a diminished conductive O_2 delivery to the microvasculature within the working muscle and thus, an impaired blood-myocyte O_2 flux in the older rats (Behnke *et al.* 2005; 2006). During submaximal exercise the distribution pattern of $\dot{Q}O_2$ both between and within hind-limb muscles is altered in older compared with younger rats despite a similar absolute hind-limb hyperaemic response (Musch *et al.* 2004; Behnke *et al.* 2006). A reduction in $\dot{Q}O_2$ to highly oxidative muscles and an elevated $\dot{Q}O_2$ to highly glycolytic muscle fibres have been reported in older rats providing evidence of a mismatch of $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio within their oxidative musculature (Behnke *et al.* 2006). Arterial rarefaction was most evident in oxidative fibres with the authors attributing this to decreased physical activity within the older rats, subsequently reducing muscle perfusion during resting periods throughout the day and thus, providing no shear stress within the arteries. This in turn will cause less metabolic stabilization and fewer arterial networks in the oxidative fibres, at least in inactive elderly rats (Behnke *et al.* 2006). Taken together, a maldistribution of $\dot{Q}O_2$-to-$\dot{V}O_2$ may be observed within the microvasculature of older individuals with T2D and ND older controls which may cause increased metabolic perturbations, and thus, premature fatigue in both groups.

6.3 $\dot{V}O_2$ kinetics in ageing and T2D

In accordance with Wilkerson *et al* (2011) and O'Connor *et al* (2015) who reported no difference in $\dot{V}O_2$ kinetics during moderate-intensity cycle exercise in older individuals with T2D and ND controls, in the present thesis, no differences were observed between older participants with T2D and ND controls with respect to $\tau\dot{V}O_{2P}$ during heavy-intensity cycle exercise initiated from an "unloaded" or elevated baseline. These findings were accompanied by similar [HHb+Mb] responses in both groups, suggesting converging effects of T2D (Wilkerson *et al.* 2012) and ageing (Scheuermann and Barstow, 2005; Poole *et al.* 2008) with respect to impairments in O_2 delivery and/or distribution within the exercising muscle.

The slowed $\tau\dot{V}O_{2P}$ in middle-aged people with T2D is said to be caused by impaired O_2 delivery, poor blood flow distribution to the contracting muscle, impaired arteriolar vasodilation, metabolic inertia, and/or greater recruitment of fast twitch fibres which have slower $\dot{V}O_2$ kinetics which may be due to a reduced microvascular oxygen partial pressure ($Pm\dot{V}O_2$) (Poole *et al.* 1994*;* Poole & Jones, 2005; Caron *et al.* 2017; Poole *et al.* 2008). However, in elderly people with T2D, $\tau\dot{V}O_{2P}$ is not slower than ND controls even in the presence of a significantly reduced $\dot{V}O_{2peak}$ (Wilkerson *et al.* 2012; O'Connor *et al.* 2015) suggesting that the effect of T2D on $\dot{V}O_2$ kinetics is masked by age-related impairments, thus ameliorating the difference in $\tau\dot{V}O_{2P}$ due to T2D in older individuals (Bell *et al.* 1992; O'Connor *et al.* 2015).

Progressive skeletal muscle atrophy (Koopman *et al*. 2007; Tieland *et al*. 2018) along with a shift in muscle fibre type composition (Verdijk *et al*. 2010) and a decline in muscle aerobic capacity due to declines in mitochondrial number (Short *et al*. 2005; McGregor *et al*. 2014; Tieland *et al*. 2018) and function (Boffoli *et al*. 1994; Gurd *et al*. 2007; 2009) are proposed to be associated with an age-related reduction in exercise capacity in older adults. Furthermore, central mechanisms common to both T2D and ageing including left ventricular dysfunction (Baldi *et al*. 2016; Seals *et al*, 1990) and the decrease in peak heart rate commonly observed with ageing (Hawkins and Wiswell, 2003; Christou *et al*. 2008), along with peripheral factors previously mentioned, may lead to poor O_2 delivery relative to O_2 utilisation within the contracting muscle. The combined effects of these declines in central and peripheral blood flow as well as impaired skeletal muscle bioenergetics with respect to inactive ageing may have a potent effect on O_2 delivery and aerobic metabolism, thus masking the negative consequences that T2D may have on an older individual resulting in similar $\dot{V}O_2$ kinetics observed at exercise onset.

Whilst previous research has shown a slower rate of adjustment of $\tau\dot{V}O_{2P}$ in old inactive participants compared to young individuals during exercise (Bell *et al.* 2001; DeLorey *et al.* 2004; Sabapathy *et al.* 2004; duManoir *et al.* 2010; Murias *et al.* 2014), a faster $\dot{V}O_2$ kinetic response is possible with training (Murias *et al.* 2010; 2016; Keir *et al.* 2016; George *et al.* 2018) indicating that fitness level and not ageing *per se*, will determine the speed of $\tau\dot{V}O_{2P}$ in older adults. Moreover, Grey *et al* (2015) found a similar $\dot{V}O_2$ kinetic response in older endurance trained adults to that of their younger counterparts (Grey *et al.* 2015). When

comparing older and younger adults of different fitness status (inactive; recreational; endurance trained), George *et al* (2018) found no difference in $\tau\dot{V}O_{2P}$ between older and younger adults when matched for fitness status, confirming that both an age-related slowing of $\tau\dot{V}O_{2P}$ can be eliminated with training while an inactive lifestyle impacts $\tau\dot{V}O_{2P}$ to a similar extent regardless of age in both older and younger individuals (George *et al.* 2018). This negative impact of a sedentary lifestyle or at least lower fitness status on $\tau\dot{V}O_{2P}$ may also be confirmed by other studies (Murias *et al.* 2011; Murias and Paterson, 2015). In young subjects, Murias *et al* (2011) found that in individuals who were considered to have a slow or moderate $\tau\dot{V}O_{2P}$, microvascular O_2 distribution becomes a limiting factor in limiting $\tau\dot{V}O_{2P}$, therefore interventions which enhance O_2 delivery such as PE or endurance training are important for speeding $\tau\dot{V}O_{2P}$ and thus exercise tolerance (Murias *et al.* 2011). In these groups where $\tau\dot{V}O_{2P}$ was relatively slow, an "overshoot" in the Δ[HHb+Mb]/$\Delta\dot{V}O_{2P}$ occurred which was not present in the group with fast $\tau\dot{V}O_{2P}$. This infers a greater reliance on O_2 extraction to account for the mismatch in microvascular O_2 distribution to O_2 utilization. This higher Δ[HHb+Mb]/$\Delta\dot{V}O_{2P}$ was also observed when comparing older inactive and endurance trained adults indicating that in older inactive males, greater reliance on O_2 extraction to make up for the poor matching of O_2 distribution to O_2 utilization is apparent (George *et al.* 2018). Therefore, independent of what the precise mechanisms for the adjustment of $\dot{V}O_2$ kinetics are, fitness status and lifestyle plays a key role in this adjustment (George *et al.* 2018).

However, the elderly participants in the current study were inactive at the time of testing therefore producing detrimental cardiovascular adaptations which may affect O_2 transport and/or distribution to the active muscle thereby, negatively impacting their $\dot{V}O_2$ kinetic response. The slower of $\tau\dot{V}O_{2P}$ is said to be related to a slowed O_2 delivery (Hughson *et al.* 2001) which results from a slower adjustment of microvascular blood flow (duManoir *et al.* 2010), at least in older inactive individuals. However, the goal of the current thesis is not to isolate the mechanisms involved in slower $\dot{V}O_2$ kinetics therefore, intracellular mechanisms may also play an important role with in controlling aerobic metabolism with ageing (Grassi *et al.* 2001; Gurd *et al.* 2007; 2009).

6.4 PE and $\dot{V}O_2$ kinetics in older T2D and ND

Given that $\dot{V}O_2$ kinetics are normally speeded in populations with a sluggish $\tau\dot{V}O_{2P}$, in these populations, a primary mechanism for the sluggish $\tau\dot{V}O_{2P}$ may be O_2 supply. Therefore, interventions which are expected to improve O_2 delivery and/or distribution should improve this $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio and thus, accelerate the pulmonary $\dot{V}O_2$ kinetic response.

As indicated during a subsequent bout of high-intensity (*Experiment 2*) and moderate-intensity exercise transitions (*Experiment 3*), prior heavy-intensity exercise was effective at speeding the $\tau\dot{V}O_{2P}$ in both older individuals with T2D and ND controls. This occurred in conjunction with a slowing of the [HHb+Mb]τ_p in both groups following PE.

Therefore, given that peripheral blood flow is likely impaired in T2D (Behnke *et al.* 2002; Padilla *et al.* 2006; 2007; Kingwell *et al.* 2003; Kiely *et al.* 2014; Green *et al.* 2015) and ageing (Behnke *et al.* 2005; 2006; duManoir *et al.* 2010), and that PE induces the improvements in $\tau\dot{V}O_{2P}$ in middle-aged people with T2D (Rocha *et al.* 2019; Gildea *et al.* 2020) and older adults (Scheuermann *et al.* 2002; DeLorey *et al.* 2004; Gurd *et al.* 2009), it is plausible that the improvements observed in both $\dot{V}O_2$ and muscle [HHb+Mb] kinetic responses in the current thesis may be in part due to a more favourable distribution of blood flow throughout the active muscle during a subsequent bout of moderate- and heavy-intensity exercise in older individuals with T2D and ND controls.

With respect to *Experiment 3*, PE also resulted in a significant speeding of $\tau\dot{V}O_{2P}$ during heavy-intensity cycle exercise initiated from an elevated baseline in both older individuals with T2D and ND controls. These results are in agreement with findings from the same laboratory showing that a prior bout of heavy-intensity PE reduces the $\tau\dot{V}O_{2P}$ of a subsequent bout of WtoW exercise in middle-aged individuals with T2D (Gildea *et al.* 2020). However, in that study, Gildea *et al* (2020) found no reduction for $\tau\dot{V}O_{2P}$ among the middle-aged control group, thereby suggesting that inactive ageing *per se* is implicated in slowing the $\dot{V}O_2$ kinetics response and influences the PE-induced effect in on $\tau\dot{V}O_{2P}$ during the high-intensity WtoW transitions. The improvements observed in $\tau\dot{V}O_{2P}$ with PE in the current study therefore suggest impairments in O_2 delivery and/or distribution in our study participants at the outset.

In addition to the faster $\tau\dot{V}O_{2P}$ resulting from PE in *Experiments 2* and *3*, significant reductions occurred for the $\dot{V}O_{2SC}$ amplitude in both older individuals with T2D and ND controls. This is in accordance with previous research into the effects of PE in middle-aged individuals with T2D during the same transitions (Gildea *et al.* 2020), and during upright cycling (DiMenna *et al.* 2008) and knee-extension exercise (DiMenna *et al.* 2010b) in healthy populations (even if PE did not induce changes in $\tau\dot{V}O_{2P}$). The proposed mechanism in this scenario based among results obtained in healthy populations is altered motor unit recruitment and altered $\dot{V}O_{2m}$ within the recruited type II fibres subsequent to PE (DiMenna *et al.* 2008). This hypothesis comes from an observed attenuation in the $\dot{V}O_{2SC}$ amplitude in coincidence with a reduction in ΔiEMG between the second and final minute of exercise subsequent to PE (DiMenna *et al.* 2008). Using a high-intensity knee-extension exercise protocol, DiMenna *et al* (2010b) also found a reduced $\dot{V}O_{2SC}$ amplitude and ΔiEMG between the second and final minute of exercise subsequent to PE, which were accompanied with a reduction in the [PCr] slow component amplitude, resulting in a reduced [PCr] consumption. This reinforces the notion whereby PE induces a delayed muscle fibre activation allowing for the resultant attenuation of both [PCr] and $\dot{V}O_2$ slow component amplitudes (Burnley *et al.* 2002; DiMenna *et al.* 2008; Bailey *et al.* 2009; DiMenna *et al.* 2010b; Gildea *et al.* 2020).

An alternative possibility is that PE induced a more homogenous muscle perfusion within the exercising muscle, attenuating the rate of fatigue development by reducing the reliance on [PCr] degradation and glycogenolysis, thus, delaying the recruitment of additional motor units required to maintain force production with a consequent reduction in the O_2 cost of exercise (Jones and Poole, 2005; DiMenna *et al.* 2008; Gildea *et al.* 2020). Therefore, enhanced O_2 delivery and/or distribution may influence both $\tau\dot{V}O_{2P}$ and the amplitude of the $\dot{V}O_{2sc}$ by altering the kinetics of the recruited muscle fibres allowing them to reach their steady-state more rapidly (DiMenna *et al.* 2008) at least in individuals displaying a slow $\dot{V}O_2$ kinetic response as featured in the current thesis.

Collectively, an acute bout of heavy-intensity PE prior to a moderate- and heavy-intensity exercise transition as well as prior to a WtoW transition, positively effects oxidative metabolism, at least in part, by enhancing blood flow distribution within the exercising muscle. This may occur in conjunction with enhanced substrate delivery to the oxidative metabolism. These observed improvements in the current thesis with heavy-intensity PE

may increase time to fatigue and thus, exercise tolerance during heavy-intensity exercise which in turn may increase the willingness of older individuals with and without T2D to continue exercising, reducing comorbidities and increasing their quality of life.

6.5 Exercise intervention in T2D

Despite MICT interventions having clear benefits in health and disease, most people including those with T2D struggle to achieve the recommended daily/weekly physical activity guidelines. In recent years, low-volume HIIT, a time efficient form of interval training, has governed interest within the field of clinical exercise physiology (Gibala *et al.* 2012; Little *et al.* 2011). Recently, our laboratory has reported HIIT to being equally as effective as MICT at improving both peak $\dot{V}O_2$ (McDermott *et al.* 2018) and $\tau\dot{V}O_{2P}$ during submaximal exercise transitions in the moderate-intensity domain as well as WtoW transitions (Gildea *et al.* 2018; McDermott *et al.* 2019). To further explore the effects of HIIT and MICT on the $\dot{V}O_2$ kinetic response in individuals with T2D, *Experiment 4* assessed $\dot{V}O_2$ and [HHb+Mb] kinetic responses during heavy-intensity exercise transitions initiated from an unloaded baseline after 12 weeks of MICT and HIIT.

Following the intervention, the $\tau\dot{V}O_{2P}$ during heavy-intensity exercise transitions decreased in both, the MICT and HIIT groups with no differences observed between exercise groups post training. In addition, the amplitude of the $\dot{V}O_{2SC}$ decreased in the MICT group only while a trend also occurred in the HIIT group (P = 0.13). These effects induced an overall acceleration in the $\dot{V}O_2$ MRT with training, while the [HHb+Mb]τ_p became slower post-training in both groups. The significant reduction in the $\tau\dot{V}O_{2P}$ with MICT and HIIT indicates a training-induced increase in muscle blood flow and/or improved muscle perfusion to metabolic rate (Nevin *et al.* 2018; McDermott *et al.* 2019).

Endurance training has proven to be a good treatment for enhancing endothelium-dependent vasodilation in T2D (Okada *et al.* 2010; Mitranun *et al.* 2013). For instance, Mitranun and colleagues (2013) explored the effects of 12 weeks of MICT vs low volume HIIT where participants with T2D trained 3 times per week (Mitranun *et al.* 2013). Flow mediated dilation (FMD) of the brachial artery improved in both groups with the greatest effect observed in the HIIT group. The authors suggested that the HIIT increased blood flow and shear stress during exercise to a greater extent, thus, improving NO bioavailability

(Mitranun *et al.* 2013). Similarly, after 8 weeks of HIIT, similar to the protocol used in the current thesis, significant improvements in %FMD of the popliteal artery (~23% increase) along with improved resting haemodynamics were observed in individuals with T2D (Madsen *et al.* 2015a). In addition, Madsen *et al* (2015) reported an increase in the popliteal artery diameter, indicative of vascular remodelling in the T2D group following the HIIT intervention. Therefore, exercise-induced improvements in vascular function as a result of increased NO availability and increased sensitivity of smooth muscle cells to NO within the microvasculature may improve O_2 delivery and/or distribution, accelerating $\dot{V}O_2$ kinetics responses with training in middle-aged individuals with T2D (Gildea *et al.* 2018; McDermott *et al.* 2018).

The significant reduction in the amplitude of the $\dot{V}O_{2SC}$ post intervention in the present study is likely indicative of exercise-induced adaptations in the intrinsic properties of the skeletal muscle and alterations in motor unit recruitment (Meex *et al.* 2010; McDermott *et al.* 2019). However, it must be stressed that no muscle biopsies or other invasive measures were utilized within the current thesis, so conclusions regarding training-induced adaptations in intrinsic muscle properties such as increased mitochondrial number and function must be taken with caution.

6.6 Limitations

To date, NIRS remains the only available technology to inspect the microvasculature of the exercising muscle during exercise in humans. However, it must be acknowledged that it comes with limitations. First among these limitations is the inability of NIRS to record absolute concentration and changes in [HHb+Mb]. It is pertinent to note that the NIRS-derived [HHb+Mb] measure is a good estimate of fractional O_2 extraction with a time course similar to fractional O_2 extraction derived using phosphorescence quenching in rodent microvasculature (Koga *et al.* 2012).

It must also be acknowledged that at the site of interrogation, adipose tissue thickness has the potential to influence the NIRS-derived measurement via its effect on the scattering properties of the tissue (Elwell, 1995). However, no differences were observed for ultrasound-derived adipose tissue thickness among groups in the experiments conducted in the current thesis.

Large heterogeneity also exists between and within skeletal muscle with respect to fibre type and blood flow (Johnson *et a.* 1973; Kalliokoski *et al*. 2006; Ferreira *et al*. 2006; Poole et al. 2020). In the current thesis, the vastus lateralis muscle was used as the site of interrogation. In healthy individuals at least, it has been reported that this muscle contains a higher proportion of type II fibres with a low level of vascularity (Kalliokoski *et al*. 2006). This may have led to an inaccurate reflection of the [HHb+Mb] response during moderate-intensity cycle exercise where more oxidative type I fibres would be expected to be recruited. However, the majority of exercise in the current thesis was performed in the heavy-intensity domain.

Even if in the present intervention study mixed groups of men and women were included in each study arm, sex-related effects on the magnitude of responses to HIIT and MICT are likely small given that 12-weeks of MICT in individuals with T2D (Green *et al.* 2020), as well as 6 weeks of HIIT in individuals with risk factors for T2D (Phillips *et al.* 2017), showed no difference in responses between men and women.

6.7 Future research

The intramuscular [PCr] response to exercise is similar to that of pulmonary $\dot{V}O_2$ kinetics (Barstow et al. 1994; DiMenna et al. 2010). Using phosphorus magnetic resonance spectroscopy (P-MRS), previous research has shown that PE does not alter the τ for the primary phase [PCr] response, but does reduce the [PCr] slow component amplitude of the subsequent bout (Rossiter et al. 2001; DiMenna et al. 2010). To date, no research exists with respect to T2D and ageing during exercise for [PCr] kinetic responses during moderate- and heavy-intensity exercise and the effects of PE. Further research is needed in this area to elucidate the mechanisms for the altered aerobic response with respect to T2D and ageing.

Given the similar magnitude of effects following HIIT and MICT, HIIT with its shorter time commitment and exercise volume may be a more effective intervention to increase exercise participation in people living with T2D. Longer-term studies (6-12 months) are needed to elucidate this. Furthermore, due to the benefits of resistance exercise in people living with T2D (Colberg *et al*. 2016), concurrent exercise combining to 2 exercise forms should be utilised.

The current study showed no difference for $\dot{V}O_2$ kinetics and skeletal muscle [HHb+Mb] responses with respect to older adults with and without T2D indicating no difference with respect to blood flow responses during submaximal exercise. Furthermore, following 12 weeks of HIIT and MICT, due to improvements in the $\dot{V}O_2$ kinetic responses for each group, it may also inferred that an improvement in microvascular blood flow distribution to meet the metabolic demand of the muscle may be responsible for the improvements observed. However, the submaximal responses for blood flow were not assessed in the current study. Therefore, future studies should compare submaximal blood flow responses between older T2D and age-matched ND controls as well as the effects of exercise interventions employing HIIT and MICT on these responses.

6.8 Overall conclusion

Taken together, the results of the first three experiments indicate that even if older people with T2D tend to show a reduced peak exercise capacity compared to healthy older controls, the rate of O_2 extraction during a ramp incremental exercise test as well as the pulmonary $\dot{V}O_2$ response during submaximal constant-load exercise transitions are similar. In addition, in *Experiments 2* and *3*, PE induced similar enhancements in the overall $\dot{V}O_2$ kinetics response during moderate- and heavy-intensity exercise initiated from an "unloaded" and elevated baseline (WtoW) in elderly individuals with and without T2D. Specifically, the main effects of PE were an acceleration in $\tau\dot{V}O_{2P}$ during transitions to moderate- and heavy-intensity exercise, and a reduction in the amplitude of the $\dot{V}O_{2SC}$ within the heavy-intensity transition, together with a slowing of the [HHb+Mb]τ_p response. The acceleration in $\tau\dot{V}O_{2P}$ is likely moderated by an enhanced O_2 availability to the active muscles given that O_2 availability seems to be impaired at the outset in both, older inactive ND individuals as well as people with T2D. Therefore, PE may result in a more favourable distribution of blood flow within the active musculature in older people with and without T2D. The results of *Experiment 4* experiments are in line with the current body of knowledge, whereby, both, MICT and HIIT are effective interventions for improving maximal aerobic capacity ($\dot{V}O_{2peak}$) and dynamic responses of $\dot{V}O_2$ during submaximal exercise transitions specifically at heavy-intensity submaximal efforts in people with T2D. Given the similar magnitude of effects for HIIT and MICT, it is proposed that HIIT with half the time commitment and exercise volume per session compared with MICT may potentially be a

more effective intervention to increase exercise participation in people living with T2D.

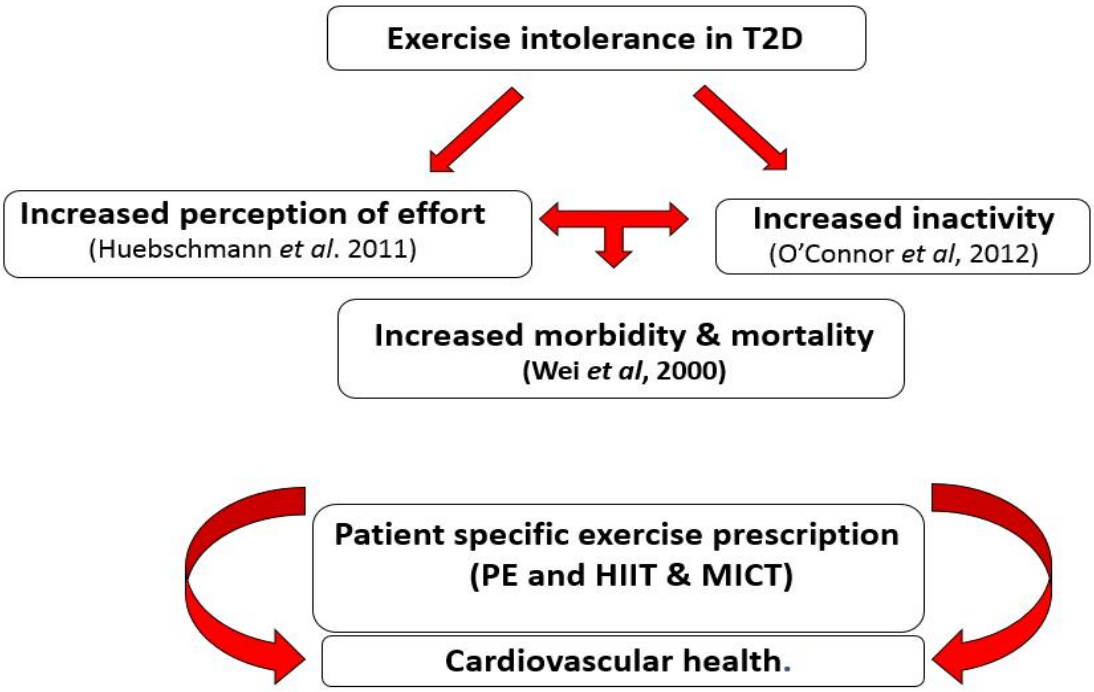

Figure 6.1. Summary of findings.

Chapter 7: Bibliography

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Chapter 8: Appendices

Appendix 8.1

TRINITY COLLEGE DUBLIN | THE UNIVERSITY OF DUBLIN
COLÁISTE NA TRÍONÓIDE

Note: the following recruitment poster was used as part of a larger study

PARTICIPANTS NEEDED FOR A RESEARCH STUDY ON CARDIORESPIRATORY & EXERCISE CAPACITY IN HEALTH AND DISEASE

We are looking to recruit healthy men and women aged between 60 and 70 years who do not exercise regularly.

Assessments (typically costing up to €200) are non-invasive and include:

- Free medical check.
- Body composition.
- Maximal aerobic capacity (VO_{2max}).
- Maximal leg strength and endurance.
- Leg circulation with ultrasound.
- Bloods tests for cholesterol and blood sugar levels

Participants would attend the human performance laboratory in the Department of Physiology of Trinity College Dublin on 6 occasions. For further information, please contact:

Adam McDermott mcdermad@tcd.ie
Aaron Nevin nevinaa@tcd.ie

* Less than 2 bouts of 60 min of moderate-intensity exercise per week for the past 6 months

Appendix 8.2

Note: the following recruitment poster was used as part of a larger study

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE
THE UNIVERSITY OF DUBLIN

Participant Information Sheet

The effect of age and sex on the time course and mechanisms of adaptation in cardiovascular and metabolic health with exercise training in type 2 diabetes.

Q: What is the main purpose of this study?
A: Type 2 Diabetes Mellitus (T2DM) occurs when glucose builds up in the blood due to the body's inability to use insulin effectively. People with T2DM have a lower exercise capacity and a slower uptake of blood oxygen. Currently it is unknown if these diabetes-induced impairments in exercise capacity are different between men and women and/or younger and middle-aged people with T2DM. To test this we propose to quantify exercise performance, muscle oxygen uptake and blood flow in both men and women of different ages with and without T2DM.

Q: Why have I been approached about this study?
A: To investigate the effects of type 2 diabetes on cardiorespiratory health a group of participants without these conditions are required to act as healthy controls. You have been approached to be a member of this control group.

Q: What does being in the 'control group' mean?
A: Being in the 'control group' simply means your results will be compared against those of patients who have type 2 diabetes. This will allow us to assess differences in results brought about by type 2 diabetes.

Q: What is required of me if I decide to take part in this study?
A: All participants who are interested in entering the study will be invited to meet a researcher from the Department of Physiology at Trinity College Dublin. You will have the opportunity to go through this information sheet again and ask any questions you might have about the study. You will be given a consent

form to take home and complete. This is so you can take time to decide whether or not you want to take part in the study.

If you decide to take part you will be asked to attend a preliminary visit where you will undergo a full medical examination in the Department of Physiology, Trinity College Dublin. If approved for participation in this study, you will then undergo four testing days at the Human Performance Laboratory in the Watts Building, Department of Physiology, Trinity College Dublin at least 24 hours apart, where you will perform the necessary calf ergometer and cycling testing (see below).

Please note that preparation for the testing days will entail abstinence from caffeine, alcohol, nutritional supplements and strenuous physical activity for 12 to 24 hours prior to testing. In addition, we ask that you do not consume any food during the 3 hours preceding the tests.

Q: What will each visit to the Human Performance Laboratory consist of?

A:

Visit 1: Calf blood flow and maximal cycling capacity (VO_{2max}) test (session will last approximately 140 min)

You will have your height and weight recorded and you will be familiarised with the cycle ergometer. During this session you will perform moderate-intensity exercises on the calf ergometer (exercise machine). Each contraction will last 1 second, accompanied by a 2 second rest. You will do this 3 times for 5-6 minutes at a time, for a total of 100-120 contractions per bout, with a 10-minute rest between bouts. The calf test requires you to exercise your right calf muscle by pushing your toes against a plate (plantar-flexion). While performing these tests, the investigator will be monitoring your blood pressure, heart rate, and leg blood circulatory data. You will then complete an incremental cycling exercise test to failure in the upright position. During the exercise test you will be required to increase your level of effort in a stepwise manner until you reach, and cannot sustain a maximum effort. The level of effort will increase by approximately 15-25 watts every minute. Five minutes after the end of the incremental test you will perform a constant-load cycling

exercise to volitional fatigue at 85% of the peak power achieved during the incremental test.

Visit 2: Calf and quadriceps muscle deoxygenation, calf blood flow and two submaximal cycling bouts (session will last approximately 121 min).

In this visit we will assess the ability of your calf and quadriceps muscles to extract oxygen during occlusion tests followed by an assessment of the ability of your popliteal artery to dilate and increase blood flow to the calf after an occlusion test as well as measuring several cardiorespiratory variables (i.e. oxygen uptake, heart rate, cardiac output - heart's blood pumping capacity and muscle's oxygen extraction capacity) during two short cycling bouts. For the first two tests we will inflate a cuff to a high pressure (i.e. 250mmHg) around your lower/upper quadriceps to cut off the circulation to the calf/quadriceps muscles. The occlusion in each of these tests should take up to 7 minutes (this time is dependent on each person's individual response and therefore can vary but will not exceed 7 minutes) and will be conducted while a near-infrared lighting device measures the extraction of oxygen in the specified muscles. We will also inflate a cuff to a high pressure (i.e. 250mmHg) around your calf to cut off the circulation to this muscle. After a 5-minute period, the cuff will be released, and blood flow will be measured by observing changes in the diameter of the arteries and blood velocity (these measurements will be made using an ultrasound probe on the back of your leg). Following this, you will perform two 6-minute moderate-intensity bouts of constant-force cycling exercise, with a 15-minute rest between bouts. These intensities of exercise are individualised according to each person's physical fitness and determined from Visit 1.

Visit 3: Four submaximal cycling bouts (session will last approximately 115 min).

In this visit we will assess the rate of increase of oxygen uptake, cardiac output (heart's blood pumping capacity) and muscle oxygen extraction capacity (rate of muscle oxygenation-deoxygenation) during four exercise bouts at a high-

intensity. There will be a period of 15 minutes rest between the first and second bout as well as between the third and fourth bout. A period of 45 minutes rest will also be completed between the second and third bout.

Visit 4: Four submaximal cycling bouts (session will last up to 139 min).

In this visit you will perform four 15-minute submaximal bouts of constant-force cycling exercise at a moderate- to high-intensity. As in the previous visit there will be a period of 15 minutes rest between the first and second bout as well as between the third and fourth bout. A period of 45 minutes rest will also be completed between the second and third bout. In addition, there will be recordings of the rate of increase of oxygen uptake, cardiac output (heart's blood pumping capacity) and muscle oxygen extraction capacity (rate of muscle oxygenation-deoxygenation) during these exercise bouts.

Q: How long will the study last?
A: As previously mentioned, this study will entail the medical check plus the four visits to the lab. Each visit will need to be separated by at least 24 hours, therefore this study can be finished within 10 days (i.e. one visit every second day). However, we appreciate that due to personal and work commitments it might not be feasible to undertake this study in such an intensive manner, therefore, we are able to spread these visits out over a two month period.

Q: What are the possible benefits of taking part in this study?
A: Whilst you will not benefit directly from the study, you are contributing to the advancement of the knowledge on the diabetes-induced impairments in exercise capacity.

Q: What are the possible risks of taking part?
A: The risks associated with this study are minimal and described below.

Blood sampling: Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area. Occasional dizziness and nausea, and bruising are also potential risks. The risks will be minimized or eliminated by having only trained personnel who use sterile techniques to draw blood. Additionally, you

will be monitored while blood is being obtained in a lying down position. All blood samples will be taken in the laboratory under sterile conditions using "one-time use" needles and containers. Proper procedures will be followed for the protection and safety of all people involved, and for the disposal of biohazardous waste in line with College/Faculty policy and best laboratory practices.

Ultrasound: The risk of using ultrasound is minimal. You may experience minor redness at the point where the ultrasound probe is pressed against your skin. This redness is due to the pressure on your skin from the probe. The redness is temporary and dissipates quickly.

Cuff occlusion: A cuff will be inflated in the calf and quadriceps at a pressure of 250 mmHg for up to 7 minutes. The risks associated with this occlusion include slight bruising, tingling and numbness, moderately elevated blood pressure, minor elevation of heart rate, and discomfort from the cuff pressure. The discomfort caused by the cuff pressure quickly disappears after the test. Women on oestrogen replacement therapy have an increased risk of developing blood clots and occlusion of leg blood flow may increase this risk. However, to our knowledge, there have been no actual cases of blood clots reported in studies involving blood flow occlusion. So far, over 1000 tests have been performed in our laboratory both in young and older individuals with no adverse outcomes.

VO_{2max} and constant load cycling tests: The study involves both a VO_{2max} test (maximal aerobic capacity test) and a constant load cycling test to determine VO_{2max}, VO_2 kinetics, and cardiac output. Due to its nature, all participants will experience physical stress which may cause some discomfort such as temporary muscle fatigue and shortness of breath. These feelings go away very quickly once the exercise bout has finished. It is possible that you may also experience light headedness, chest discomfort, cramping in the legs and irregular heartbeats during this test. With a VO_{2max} test there is a very slight

risk that participants may suffer a heart attack (one occurrence per 5000 tests in a population with a low prevalence of known coronary heart disease). For this reason, a qualified medical doctor will deem the participant suitable to complete an exercise test to exhaustion. All VO_{2max} tests will be supervised by a minimum of two exercise physiologists. Other potential risks, including fainting, nausea, muscle strain, and muscle soreness, will be minimised by a proper warm-up, familiarisation procedure, and a cool-down.

Q: How do I know if I am suitable to take part?
A: You will be considered suitable to take part in this study if you are aged between 60 and 70 years, have a BMI $\geq$ 25 kg/m^2, are untrained ($\leq$ 2 bouts of 60 min of moderate-intensity or "somewhat hard" exercise per week and have not participated in a continuous exercise program for the last 6 months) and have been approved for participation following the free full medical examination.

Please note that in order to be considered you must be free from any diagnosis of cardiovascular or otherwise serious medical ailments/comorbid conditions including autonomic insufficiency/dysfunction, symmetrical neuropathy, abnormal cardiac function or evidence of ischaemic heart disease, angina or other cardiac or pulmonary symptoms limiting exercise performance. Absence of comorbid conditions will be established and confirmed by history, physical examination and laboratory testing. Controlled hypertensives (<160/90mmHg at rest) will be admitted to the study.

If you are a woman of childbearing age, you may participate in this study only if you are not pregnant or lactating. If you should become pregnant during the study please inform the investigators immediately.

Q: Do I have to take part?
A: No. Your participation in this study is voluntary and you are free to withdraw at any time, without giving any reason.

Q: What if I change my mind during the study?
A: As stated above, you are free to withdraw at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study. Although unlikely, please be

aware that the investigators may also stop your participation in the study at any time without your consent.

Q: Will my information be kept confidential?
A: Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the study group. The data or material will be retained after the study is completed. This material will not be used in future unrelated studies without further specific permission being obtained. Contact with your General Practitioner will only take place with your full consent. Information will be provided only in the event of an adverse outcome (i.e. cardiac event) during exercise or medical abnormalities discovered during medical examination.

Q: Who is funding the research?
A: This research is being funded by the Health Research Board (HRB).

Q: Who has reviewed this study?
A: The study has been reviewed and approved by the Faculty of Health Sciences Research Ethics Committee from Trinity College Dublin.

Q: What type of insurance covers this study?
A: This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

Q: What if I have further questions?
A: The investigators involved in this study are more than happy to answer any further questions you may have regarding this study. You can contact them on:

Mr Adam McDermott	0860809968	mcdermad@tcd.ie
Mr Aaron Nevin	08605932222	nevinaa@tcd.ie
Dr Mikel Egaña	01-8961770	megana@tcd.ie

Please note: If the study team learns of important new information that might affect your desire to remain in the study, you will be informed at once.

Appendix 8.3

Note: the following recruitment poster was used as part of a larger study

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Participants needed for a research study on cardiorespiratory & exercise capacity in type 2 diabetes

We are looking to recruit men and women with type 2 diabetes aged between 18 and 70 years who **do not** exercise regularly for the possibility to undertake a 12-week supervised training intervention designed by trained exercise physiologists.

If interested in receiving more information, please contact:

Norita Gildea: gildean@tcd.ie / 0872251557

Adam McDermott: mcdermad@tcd.ie / 0860809968

Appendix 8.4

Note: the following recruitment poster was used as part of a larger study

St. Vincent's Healthcare GROUP LIMITED

ELM PARK, DUBLIN 4

DEPARTMENT OF ENDOCRINOLOGY AND DIABETES MELLITUS

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: The effect of age and sex on the time course and mechanisms of adaptation in cardiovascular and metabolic health with exercise training in type 2 diabetes.

NAME OF PRINCIPAL INVESTIGATOR:

Prof Donal O'Shea
Dr. Ronan Canavan
Mr. Adam McDermott
Ms Norita Gildea
Dr Joel Rocha
Prof Simon Green
Dr Mikel Egaña

You are being invited to participate in a research study. Thank you for taking time to read this.

WHAT IS THE PURPOSE OF THIS STUDY?

The aim of the study is to measure the initial responses of blood flow and oxygen consumption during a calf-exercise and cycling-exercise before, during and after a 12-week period. During the 12-week period some participants will complete a supervised exercise training programme training 3 times per week under supervision at the Loughlinstown Leisure Centre, Co. Dublin and the others will continue with their normal lifestyle.

WHY HAVE I BEEN CHOSEN?

You have Type 2 Diabetes Mellitus and as therapy to improve your condition we want to investigate the time course of adaptation in cardiovascular and metabolic health with exercise training.

WHAT WILL HAPPEN IF I VOLUNTEER?

Your participation is entirely voluntary. If you initially decide to take part you can subsequently change your mind without difficulty. This will not affect your future treatment in any way. Furthermore your doctor may decide to withdraw you from this study if he/she feels it is in your best interest.
If you agree to participate, before the commencement of the project you will be requested to provide 2 blood samples (approx. 2.5 tsp.) and you will undergo a

screening visit to determine if your current health status meets the inclusion criteria for this study. Upon receiving medical clearance to participate in this, you will be randomly allocated into one of the EXERCISE (continuous or interval training) or CONTROL (non-exercise) groups. Participants in the EXERCISE groups will perform a 12-week supervised exercise training programme. Participants in the CONTROL group will continue normal life for the following 12-weeks (If you are allocated to the control group you will be offered the possibility to undertake the 12-week supervised exercise programme only after you finish the 12-weeks of your usual daily routine). Regardless of your group you will, at week 0 and 12, undergo four testing days (three at the human laboratory in the Biotechnology Building, Department of Physiology, Trinity College Dublin, and one at St Columcille's Hospital) at least 48 hours apart, where you will perform the necessary calf ergometer and cycling testing (see below). In addition, you will be asked to give a blood sample and wear a continuous glucose monitor system once during each assessment week (i.e. Weeks 0, 3, 6, 9, 12) and record your energy intake (by filling 3-day food diaries) and physical activity (using a questionnaire and accelerometer). From this point assessments will be undertaken during 2 visits every 3 weeks (i.e. weeks 3, 6 and 9) at TCD. Please note that preparation for the testing days will entail abstinence from caffeine, alcohol, nutritional supplements and strenuous physical activity for 24 hours prior to testing. In addition, we ask that you do not consume any food during the 3 hours preceding the tests.

Below we have included all the information regarding where each visit will be, what will be required from you and how long will it take to complete the individual tests and whole visit.

Visit before the start of the study

Screening visit (Location: St. Columcille's Hospital)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Blood sampling	Allows measurement of the sugar and fats in your blood.	You will need to provide 2 blood samples (approx. 2.5 tsp.).	5	80
Ankle-Brachial Index (ABI)	Allows assessment of the risk of having narrowed or blocked arteries in your legs or arms.	You will need to initially rest and then do 30 heel raises so that your resting and post-exercise blood pressure can be measured at the ankle and in the arm.	15	
Exercise stress test	Allows detection of possible problems in the	You will need to walk on a treadmill whilst connected to a heart	60 (approx. 15 min	

	heart during exercise.	monitoring device to see how far you walk and if you develop chest pain or changes in the activity of your heart.	of exercise)	

Visits during assessment weeks 0 and 12

Visit 1 (Location: St. Collumcille's Hospital)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Pulse wave velocity (PWV)	Allows the measurement of how stiff your arteries are.	You will need to rest whilst the arteries in your neck and groin are monitored using an external pressure sensor.	20	70
Body measurements	Allows the measurement of your physical characteristics.	You will need to stand still and relaxed whilst measures of height, weight, neck, hip and waist circumferences and body fat are taken.	10	
Maximal cycling capacity test	Allows the measurement of the maximum amount of oxygen you can use during a maximal cycling effort.	You will need to cycle in the seated position where the resistance felt on the pedals will go up until you cannot rotate them. During this test you will be required to increase your level of effort until you cannot cycle any further.	30 (approx. 12 min of exercise)	
Cardiac output exercise test	Allows the estimation of the maximum blood your heart can pump during each minute	You will need to cycle at a high-intensity until you cannot rotate the pedals.	10 minutes (approx. 4 min of exercise)	

Visit 2 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Fat tissue thickness by Ultrasound	Allows the measurement of the fat layer above your muscles in the leg.	You will need to rest whilst the fat layer in your leg is measured using an ultrasound machine.	10	175
Calf and quadriceps NIRS occlusion	Allows the assessment of the ability of the muscles of the back of your lower leg and front of the upper thigh to use oxygen.	You will need to be in a lying position whilst a blood pressure cuff is inflated to a high pressure of 250mmHg for up to 7 min for each muscle.	25	
Flow-mediated dilation (FMD)	Allows the assessment of the ability of the artery at the back of your knee to widen and increase the amount of blood that goes to the muscles of the lower leg.	You will need to be in a lying position whilst a blood pressure cuff is inflated around the lower leg to a high pressure of 250mmHg for 5 min.	20	
Calf exercise	Allows the measurement of the amount of blood that goes to the muscles of the lower leg	You will need to push your toes against a force plate that measures the strength of your lower leg. Each push will last 1 second, followed by a 2	80 (18 min of exercise)	

	during moderate-intensity exercise.	second rest and this exercise will continue for 6min. This exercise will then need to be repeated two more times but you will have a 10-minute rest between each 6 min session.		
Constant-load cycling test at moderate-intensity	Allows the measurement of how much oxygen your muscles use during moderate-intensity cycling exercise.	You will need to cycle for 9 min (including 3min warm-up) in the seated position whilst the resistance felt on the pedals will be of a constant moderate-intensity for 6 min. This will be repeated once but you will have 12 min of rest in between.	40 (18 min of exercise)	

Visit 3 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Constant-load cycling test at high-intensity	Allows the measurement of how much oxygen your muscles use during high-intensity cycling exercise.	You will need to cycle for 9 min (including 3 min of warm-up) in the seated position whilst the resistance felt on the pedals will be of a constant high-intensity for 6 min. This will be repeated three more times but there will be a total rest period of 69 min	115 (36 min of exercise)	115

Visit 4 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Constant-load cycling test at moderate and high intensity	Allows the measurement of how much oxygen your muscles use	You will need to cycle for 15 min (including 3 min of warm-up) in the seated position whilst the resistance felt on the	139 (60 min of exercise	139

	during moderate and high intensity cycling exercise.	pedals will be of a moderate-intensity for the first 6 min and a high-intensity for the last 6 min. This will be repeated three more times but there will be a total rest period of 69 min	)	

Visits during the remaining assessment weeks (Weeks 3, 6 and 9)

Visit 1 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Pulse wave velocity (PWV)	Allows the measurement of how stiff your arteries are.	You will need to rest whilst the arteries in your neck and groin are monitored using an external pressure sensor.	20	169
Body measurements	Allows the measurement of your physical characteristics.	You will need to stand still and relaxed whilst measures of height, weight, neck, hip and waist circumferences and body fat are taken.	10	
Constant-load cycling test at moderate and high intensity	Allows the measurement of how much oxygen your muscles use during moderate	You will need to cycle for 15 min (including 3 min of warm-up) in the seated position whilst the resistance felt on the pedals will	139 (60 min of exercise)	

	and high intensity cycling exercise.	be of a moderate-intensity for the first 6 min and a high-intensity for the last 6 min. This will be repeated three more times but there will be a total rest period of 69 min		

Visit 2 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Fat tissue thickness by Ultrasound	Allows the measurement of the fat layer above your muscles in the leg.	You will need to rest whilst the fat layer in your leg is measured using an ultrasound machine.	10	159
Flow-mediated dilation (FMD)	Allows the assessment of the ability of the artery at the back of your knee to widen and increase the amount of blood that goes to the muscles of the lower leg.	You will need to be in a lying position whilst a blood pressure cuff is inflated around the lower leg to a high pressure of 250mmHg for 5 min.	20	
Calf exercise	Allows the measurement of	You will need to push your toes against a force	80	

	the amount of blood that goes to the muscles of the lower leg during moderate-intensity exercise.	plate that measures the strength of your lower leg. Each push will last 1 second, followed by a 2 second rest and this exercise will continue for 6min. This exercise will then need to be repeated two more times but you will have a 10-minute rest between each 6 min session.	(18 min of exercise)	
Constant-load cycling test at moderate-intensity	Allows the measurement of how much oxygen your muscles use during moderate-intensity cycling exercise.	You will need to cycle for 9 min (including 3min warm-up) in the seated position whilst the resistance felt on the pedals will be of a constant moderate-intensity for 6 min.	19 (9 min of exercise)	
Maximal cycling capacity test	Allows the measurement of the maximum amount of oxygen you can use during a maximal cycling effort.	You will need to cycle in the seated position where the resistance felt on the pedals will go up until you cannot rotate them. During this test you will be required to increase your level of effort until you cannot cycle any further.	30 (approx. 12 min of exercise)	

Please note that you will need to provide 1 fasted blood sample (approx. 1.25 tsp.) during each assessment week.

Summary of all visits over the 3 month period			
Week	**Number of visits**	**Location of visits**	**Total time per visit (min)**
Screening	1	St. Columcille's Hospital	80
0	4	St. Columcille's Hospital (1) Trinity College Dublin (3)	70 175 115 139
3	2	Trinity College Dublin (2)	169 159

6	2	Trinity College Dublin (2)	169 159
9	2	Trinity College Dublin (2)	169 159
12	4	Trinity College Dublin (4)	70 175 115 139
		Total (min)	**2062**
		Total (hours)	**34.4**

As indicated in the above table this study will take 3 months to be completed and will involve approximately 34 hours of testing during this period.

- Supervised exercise training programme (Exercise groups only):
During the 12 week exercise training period you will perform three exercise sessions per week of either moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT) depending on which group you were randomised to. Cycling will be the main exercise mode for both groups and all sessions will be supervised by a qualified Personal Trainer.

If you are in the MICT group sessions will last a total of 60 minutes consisting of 10 minutes for warm-up/cool-down (5 min each) and 50 minutes at a moderate training intensity.

If you are in the HIIT group sessions will last a total of 30 minutes consisting of 10 minutes for warm-up/cool-down (5 min each) and an interval training protocol of 10x1min sessions at a high-intensity separated by 10x1min recovery periods at a low-intensity.

The exercise intensities for both groups will be recalculated every 3 weeks to reflect changes in fitness. The programme will be performed in Loughlinstown Leisure Centre, Co. Dublin.

You will have your blood glucose levels measured before and after training. If blood glucose before exercise is <6.8mmmol/l a snack with 25 g carbohydrate and 7 g protein will be provided to you. You will also need to keep a glucose monitoring log sheet, which will be regularly checked by the research team.

During the exercise session you will wear a heart rate monitor and be asked how the exercise feels to ensure adherence with the intensity of training. Blood pressure will also be monitored before and after the exercise sessions.

ARE THERE ANY BENEFITS FROM MY PARTICIPATION?

You may or may not benefit from taking part in this study.

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING?

There are a number of minor risks associated with this study.

Blood sampling: Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area. Occasional dizziness and nausea, and bruising are also potential risks. The risks will be minimized or eliminated by having only trained personnel to draw blood. Additionally, you will be monitored while blood is being obtained in a lying down position. All blood samples will be taken in the laboratory under sterile conditions using "one-time use" needles and containers. Proper procedures will be followed for the protection and safety of all people involved, and for the disposal of biohazardous waste in line with College/Faculty policy and best laboratory practices.

Ultrasound: The risk of using ultrasound is minimal. You may experience minor redness at the point where the ultrasound probe is pressed against your skin. This redness is due to the pressure on your skin from the probe. The redness is temporary and dissipates quickly.

Cuff occlusion: A cuff will be inflated in the calf and quadriceps at a pressure of 250 mmHg for up to 7 minutes. The risks associated with this occlusion include slight bruising, tingling and numbness, moderately elevated blood pressure, minor elevation of heart rate, and discomfort from the cuff pressure. The discomfort caused by the cuff pressure quickly disappears after the test. Women on oestrogen replacement therapy have an increased risk of developing blood clots and occlusion of leg blood flow may increase this risk. However, to our knowledge, there have been no actual cases of blood clots reported in studies involving blood flow occlusion. So far, over 1000 tests have been performed in our laboratory both in young and older individuals with no adverse outcomes.

Maximal and constant load cycling tests: The study involves both maximal and constant load cycling tests to determine the ability of your muscles to use oxygen. Due to its nature, all participants will experience physical tiredness which may cause some discomfort such as temporary muscle fatigue and shortness of breath. These feelings go away very quickly once the exercise session has finished. It is possible that you may also experience light headedness, chest discomfort, cramping in the legs and irregular heartbeats during this test. With a maximal exercise test there is a very slight risk that participants may suffer a heart attack To minimise the risk prior to performing the maximal exercise tests, a stress test using 12-lead ECG and blood pressure measurements will be carried out at St Columcille's hospital.

Exercise training (Exercise groups only): Exercise training may result in muscle tightness, soreness, fatigue and rarely a pulled muscle. However, these will be minimised by a proper warm-up, familiarisation procedure, and a cool-down. In addition, the intensity of exercise will be closely monitored and a qualified personal trainer will supervise each session. The risk of a coronary event, although very minor, does exist.

WHAT HAPPENS IF I DO NOT AGREE TO PARTICIPATE?

If you decide not to participate in this study your treatment will not be affected in any way.

CONFIDENTIALITY

Your identity will remain confidential. A study number will identify you. Your name will not be published or disclosed to anyone.

COMPENSATION

Your doctors are adequately insured by virtue of their participation in the clinical indemnity scheme.

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

This study is a collaborative study between Prof Donal O'Shea (consultant endocrinologist, St Vincent's University) and Dr Mikel Egana (Assistant Professor in Physiology, Trinity College Dublin) and has been funded by the Health Research Board of Ireland.

Will I be paid for taking part in this study? No

Will my expenses be covered for taking part in this study? Yes, expenses related to transport will be covered.

HAS THIS STUDY BEEN REVIEWED BY AN ETHICS COMMITTEE?

The St. Vincent's Healthcare Group, Ethics and Medical Research Committee have reviewed and approved this study.

CONTACT DETAILS

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from any of the investigators: Mr. Adam McDermott (0860809968 / mcdermad@tcd.ie), Ms. Norita Gildea (0872251557/gildean@tcd.ie), Dr Joel Rocha (0873840236 /borgespj@tcd.ie) or Dr Mikel Egaña, (01-8961770/ megana@tcd.ie). If the study team learns of important new information that might affect your desire to remain in the study, you will be informed at once.

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

- I have read and understood the Participant Information. YES □ NO □
- I have had the opportunity to ask questions and discuss the study. YES □ NO □
- I have received satisfactory answers to all my questions. YES □ NO □
- I have received enough information about this study. YES □ NO □
- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care.

YES ☐ NO ☐

- I understand that relevant sections of any of my data collected during the study will be processed and may be looked at by responsible individuals of the research team, where it is relevant to my taking part in this research. I give permission for these individuals to have access to and process this data. YES ☐ NO ☐

- I understand that the data or material will be retained after the study is completed, however, this material will not be used in future unrelated studies without further specific permission being obtained. YES ☐ NO ☐

- I agree to provide blood samples or for the research team to use blood samples (or part of a blood sample) which are taken for clinical reasons. YES ☐ NO ☐

- I freely and voluntarily agree to be part of this research study. YES ☐ NO ☐

_____

Date: _____

Participant's Name in print: _____

Investigator's Signature: _____

Date: _____

Investigator's Name in print: _____

Appendix 8.5

Medical Questionnaire

DEPARTMENT OF PHYSIOLOGY, TRINITY COLLEGE, DUBLIN.

Project Title: The effect of age and sex on the time course and mechanisms of adaptation in cardiovascular and metabolic health with exercise training in type 2 diabetes.

Supervising Researchers: Mr Aaron Nevin and Mr Adam McDermott.

Principal Investigator: Dr Mikel Egaña.

Medical Personnel/Physician:_____

The purpose of this survey is to keep a record of all subject/participant personal, medical and general health details for later comparison and data analysis. It is also essential to ensure any unnecessary risk or injury is avoided to all involved in the experimental series. Please complete all of the personal information at the top of this page and answer all of the questions accurately. All information will be kept as confidential as possible.

Name:_____ Date:_____

Height:_____ Weight:_____

Sex:_____ Age & D.O.B.:_____

Contact Telephone Number and E-mail:_____

Please circle the appropriate answer and provide details in all cases.

1. Are you a smoker? YES NO _____
2. Do you suffer from asthma? YES NO _____
3. Do you drink alcohol? YES NO _____
4. Do you drink tea/coffee? YES NO _____
5. Do you drink Coke/Pepsi etc? YES NO _____
6. Are you a diabetic? YES NO _____
7. Are you lactose intolerant? YES NO _____
8. Have you ever had any soft tissue injuries (ie: broken bones, ligament damage…)?

   YES NO _____

   _____
9. Does your family have a history of stroke and/or heart disease?

   YES NO _____

   _____

10. Do you have any allergies? YES NO _____

_____

11. Do you have any other medical/health related complaints that should be made aware to the investigators? YES NO _____

_____

12. Do you perform any regular physical activity? YES NO

If YES, please indicate type, duration and frequency._____

_____

_____

13. Are you currently taking any prescribed medication? YES NO

If YES, please indicate which drugs, and reasons for prescription._____

_____

_____

14. Have you ever knowingly or unknowingly taken any performance enhancing agents (eg: anabolics, steroids, β-blockers…)? YES NO

If YES, please indicate which agents, and why._____

_____

_____

15. Are you currently taking any other dietary supplements (eg: vitamins, iron, proteins…)?

YES NO

If YES, please indicate which supplements, and why._____

_____

_____

Please sign and date this survey below if the answers you have given are, to the best of your knowledge, true and correct. If you are unsure of any questions or have any information you think may be important, but not specifically addressed by these questions, please make it known to the principal investigator of the study.

Signature of Participant:_____ Date:_____

Signature of Supervising Researcher:_____ Date:_____

Signature of Principal Investigator:_____ Date:_____

Following completion of this survey and a physical assessment of the above listed volunteer/subject, I concluded that there are no evident contraindications to participation in the study entitled above and according to the study proposal which has received appropriate ethical approval from the School of Medicine, Faculty Research Ethics Committee.

Signature of Physician:_____Date:_____

General Examination (Doctors Use Only)

Obs:	Pulse ……beats.min^{-1}	Reg. / Irreg.	
	BP ……../……..mmHg		
Head:	Nose	Throat	FBC Result: ……
Neck:	Nodes	Thyroid	……
CVS:	Apex beat	Heart Sounds	PFT Result: ……
RS:	Exp^{n}	Perc. / Ausc.	……

Medical Summary

Fit for Exercise Test to Exhaustion Y N Signature: ……..

Appendix 8.6

Low activity physical activity recall questionnaire (LOPAR)

Instructions for Administration: Determine for each major category (sleep, work, house or yard, recreation or leisure) the estimated number of hours/week spent within that category during the preceding week. Then using the cards as prompts, ask about specific activities within each intensity of activity (heavy to very light). It is not expected that every hour of the week can be accounted for. However, asking the subject to estimate their total sleep hours, and the total expected hours within each major category of activity (168 hours/week), as compared to the break-down of activities within each major category of activity, helps the subject more reliably remember their activities. Instructions for question 2 pertain to all three major categories of activity.

Scoring: For each activity (heavy to very light), calculate the number of hours/week spent in that activity (days/week x hours/day). Sum hours/week in each category to determine total hours per week. The amount of energy expenditure for each activity is expressed as metabolic equivalents (MET's). One MET equals 3.5 ml/kg/min of oxygen consumption. Activities are classified scoring in the following scale: very light (0.9 – 2.0 MET's), light (2.1 – 3.0 MET's), moderate (3.1 – 5.0 MET's) and heavy (5.1 – 7.0 MET's). Data are reported in MET hours/week (hours/week x the MET value of the activity).

1. How many hours do you sleep a night, on average? ___ hours x 7 Sleep hours/week ___

2. Explain to subject that you are going to ask about typical WORK activities performed during the past week (includes work for pay or regular volunteer activities). If subject not employed, go to question # 3.
   How many total hours did you work per week on average? Work hours/week ___

Here is a listing of typical work activities (Show participant Card A). Activities are classified as heavy, moderate, light and very light depending on their average energy demands. With your job, time may be spent in more than one category of activity. Let's start with heavy activities and then go on to moderate, light, and then very light activities. a) Please tell me the average number of days during the last week you performed heavy activities at work. b) Please tell me the average length of time you performed heavy activities in a day. Then, repeat above directions for all intensities of activity.

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1–7.0 MET's)				
MODERATE (3.1–5.0 MET's)				
LIGHT (2.1–3.0 MET's)				
VERY LIGHT (0.9–2.0 MET's)				
TOTAL				

3. Did you perform HOUSEHOLD CHORES OR YARD WORK around the home during the past week (Follow instructions given above, except refer to Card B)? If yes, how many total hours did you spend in household chores?

Household or yard hours/week _____

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1–7.0 MET's)				
MODERATE (3.1–5.0 MET's)				
LIGHT (2.1–3.0 MET's)				
VERY LIGHT (0.9–2.0 MET's)				
TOTAL				

Total hours did you spend in leisure activities? Recreation or leisure hours/week _____

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1–7.0 MET's)				
MODERATE (3.1–5.0 MET's)				
LIGHT (2.1–3.0 MET's)				
VERY LIGHT (0.9–2.0 MET's)				
TOTAL				

Selected List of Activities with MET values (in parentheses)*

CARD A PHYSICAL ACTIVITIES AT WORK

HEAVY	MODERATE	LIGHT	VERY LIGHT
Heavy power tools (6.0)	Locksmith (3.5)	Cashier (2.5)	Sitting (1.5)
Coal mining (7.0)	Carrying < 20 Ibs (5.0)	Light assembly (2.5)	Standing (2.0)
Loading truck (6.5)	Farming (4.5)	Physician (2.5)	Typing (1.5)
Shoveling (7.0)	Machine tooling (4.0)	Teacher (2.5)	Computer work (1.5)
Heavy carpentry (7.0)	Forestry, chain saw (4.5)	Tailoring, machine (2.5)	Receptionist (1.5)

CARD B HOUSEHOLD CHORES AND YARD WORK

HEAVY	MODERATE	LIGHT	VERY LIGHT
Roofing (6.0)	Food shopping (3.5)	Preparing meals (2.5)	Sitting (1.5)
Digging (6.0)	Heavy cleaning (4.5)	Sweeping (2.5)	Standing/ laundry (1.5)
Chopping wood (6.0)	Laying carpet (4.5)	Making bed (2.5)	Fold, hang clothes (1.5)
Shoveling snow (6.0)	Weeding (4.5)	Fertilizing (2.5)	Sewing (1.5)
Manual lawn mowing (6.0)	Power lawn mowing (4.5)	Ironing (2.5)	

CARD C RECREATIONAL ACTIVITIES

HEAVY	MODERATE	LIGHT	VERY LIGHT
Walking/hiking uphill (6.0)	Locksmith (3.5)	Cashier (2.5)	Sitting (1.5)
Moderate canoeing (7.0)	Carrying < 20 Ibs (5.0)	Light assembly (2.5)	Standing (2.0)
Bicycling 10-12 mph (6.0)	Farming (4.5)	Physician (2.5)	Typing (1.5)
Light stationary cycle (5.5)	Machine tooling (4.0)	Teacher (2.5)	Computer work (1.5)
Aerobic dance (7.0)	Forestry, chain saw (4.5)	Tailoring machine (2.5)	Receptionist (1.5

*MET values for many activities can be obtained from Ainsworth BE, *et al*. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sport Exerc 1993;25:71-80.

Appendix 8.7

RPE

6	least effort
7	very, very light
8	
9	very light
10	
11	fairly light
12	
13	somewhat hard
14	
15	hard
16	
17	very hard
18	
19	very, very hard
20	maximum effort

Appendix 8.8

Experiment 1

Physical characteristics: T2D and ND

T2D Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis
			Systolic	Diastolic						
1	M	11.9	146	90	64.8	1.73	109.0	36.42	1.15	7.7
7	M	12.4	140	90	59.8	1.78	95.0	29.98	0.98	6.0
8	F	5.8	112	70	62.5	1.70	60.0	20.76	0.75	7.6
11	F	7.0	120	78	64.0	1.64	75.0	27.89	0.87	13.2
14	M	9.4	128	68	67.4	1.64	74.5	27.70	0.96	3.7
19	M	12.9	110	70	64.2	1.84	107.9	31.87	1.00	3.8
20	M	13.7	124	70	64.8	1.69	90.0	31.51	1.09	4.5
23	F	6.8	100	70	63.9	1.63	75.7	28.67	0.79	12.5
26	F	5.7	138	70	62.3	1.60	66.5	25.98	0.92	8.1
27	M	9.2	132	72	61.1	1.71	88.3	30.20	1.09	4.8
35	M	10.3	102	62	58.8	1.78	79.0	24.93	0.91	3.2
36	M	5.6	124	78	59.1	1.75	96.7	31.58	1.05	5.7
38	F	9.0	130	75	60.2	1.65	74.5	27.36		6.1
46	M				67.1	1.78	100.0	31.56	1.07	4.5
Mean		**9.21**	**123.54**	**74.08**	**62.9**	**1.71**	**85.2**	**29.03**	**0.97**	**6.5**
S.D.		2.89	14.33	8.21	2.8	0.07	15.2	3.77	0.12	3.1

ND Participants	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis
			Systolic	Diastolic						
3	M	9.9	124	70	64.1	1.75	93.4	30.50	1.00	3.7
4	M	9.3	136	84	68.0	1.64	85.3	31.71	1.01	4.4
5	M	8.6	110	64	65.1	1.75	87.7	28.64	0.96	4.9
6	M	9.4	144	90	66.0	1.58	77.2	30.92	0.96	4.3
7	M	7.1	112	64	67.4	1.77	79.0	25.22	0.91	4.2
9	F		120	60	71.6	1.58	58.9	23.59		8.1
10	M		122	85	58.6	1.75	82.0	26.78	0.98	4.1
13	F		125	70	67.4	1.57	56.8	23.04	0.82	4.0
14	M		128	86	62.1	1.76	78.9	25.47	0.91	2.8
16	M		125	80	67.3	1.73	88.2	29.47	1.17	3.7
17	M		140	90	58.8	1.77	85.0	27.13	1.04	5.6
18	F		130	85	59.0	1.63	65.8	24.77	0.91	6.7
19	F				66.2	1.6	65.8	25.70		
20	M				65.3	1.8	85.6	26.42	0.98	
Mean		**8.86**	**126.33**	**77.33**	**64.8**	**1.69**	**77.8**	**27.10**	**0.97**	**4.7**
S.D.		1.09	10.22	10.99	3.9	0.09	11.5	2.75	0.08	1.5

T2D VO_2 and workload data during Ramp test

	Oxygen Uptake (VO2)									% of VO2	
T2D Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	RCP (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	Ve (L/min)	VCO2 (L/min)	RER	RCP	VT
1	2.0	18.5	1.8	16.6	1.5	13.4	81.6	2.2	1.1	90.1	72.4
7	2.1	21.6	1.7	18.4	1.4	14.3	61.0	2.2	1.1	85.0	66.4
8	1.5	25.2	1.5	24.4	1.2	20.4	61.9	2.0	1.3	96.6	81.1
11	1.3	16.8	1.2	16.4	1.0	13.9	52.8	1.4	1.1	97.5	82.5
14	2.0	27.3	1.9	25.9	1.5	19.7	68.7	2.1	1.0	94.6	72.2
19	2.6	23.7	2.1	19.0	1.5	14.1	106.9	2.8	1.1	80.3	59.5
20	2.4	26.7	2.1	23.8	1.9	20.6	65.7	2.5	1.1	89.2	77.2
23	1.5	20.2	1.4	18.4	1.1	14.7	72.9	2.0	1.3	91.4	72.8
26	1.9	29.2	1.7	25.3	1.6	23.9	64.0	2.1	1.1	86.8	81.9
27	1.9	21.1	1.6	18.4	1.4	15.5	69.7	1.9	1.0	87.1	73.3
35	2.3	28.7	2.2	27.8	1.7	21.2	88.2	2.7	1.2	96.7	73.9
36	2.6	26.8	2.5	26.0	2.2	22.5	82.3	2.9	1.1	97.0	83.8
38	1.5	20.2	1.5	19.5	1.1	14.2	60.8	1.8	1.2	96.5	70.2
46	2.1	20.7	1.8	17.8	1.4	14.3	54.9	1.6	0.8	85.9	69.0
Mean	**2.0**	**23.3**	**1.8**	**21.3**	**1.5**	**17.3**	**70.8**	**2.2**	**1.1**	**91.1**	**74.0**
S.D.	0.4	4.0	0.4	4.0	0.3	3.8	14.6	0.4	0.1	5.5	6.8

		Workloads (Watts)						% of Workload		
T2D Participant	Peak Heart Rate (Bpm)	Peak	85% Peak	RCP	50% Delta	VT	80% VT	RCP	VT	Time of Ramp Test
1	165.0	156.0	132.6	119.0	124.0	92.0	73.6	76.3	59.0	704.0
7	136.0	162.0	137.7	130.0	125.0	77.1	70.4	80.2	47.6	728.0
8	168.0	116.0	98.6	97.0	88.0	60.0	48.0	83.6	51.7	544.0
11	173.0	65.0	55.3	54.0	55.5	46.0	36.8	83.1	70.8	450.0
14	132.0	142.0	120.7	124.0	115.5	89.0	71.2	87.3	62.7	648.0
19	153.0	183.0	155.6	141.0	137.8	92.5	74.0	77.0	50.5	812.0
20	169.0	152.0	129.2	130.0	128.3	104.5	83.6	85.5	68.8	688.0
23	152.0	120.5	102.4	97.5	96.5	55.5	58.0	80.9	46.1	562.0
26	144.0	159.0	135.2	143.2	137.0	100.0	92.0	90.1	62.9	716.0
27	143.0	141.0	119.9	108.0	105.5	58.0	56.0	76.6	41.1	644.0
35	154.0	176.0	149.6	159.2	144.0	95.0	89.0	90.5	54.0	618.0
36	157.0	198.0	168.3	152.0	158.0	114.8	95.0	76.8	58.0	684.0
38	178.0	97.0	82.5	92.7	72.9	40.8	39.0	95.5	42.0	642.2
46	133.0	131.0	111.4	112.5	104.3	62.8	62.1	85.9	47.9	604.0
Mean	**154.1**	**142.8**	**121.3**	**118.6**	**113.7**	**84.8**	**67.8**	**83.5**	**59.5**	**646.0**
S.D.	15.1	35.3	30.0	27.8	28.6	23.5	18.7	6.0	7.6	89.5

ND VO_2 and workload data during Ramp test

	Oxygen Uptake (VO2)									% of VO2	
ND Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	RCP (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	Ve	VCO2	RER	RCP	VT
3	2.2	23.6	2.0	21.3	1.4	14.9	78.1	2.5	1.1	90.4	63.2
4	1.7	19.3	1.6	18.3	1.3	14.8	74.8	1.9	1.1	94.8	76.3
5	1.8	20.8	1.6	18.8	1.2	14.1	79.0	2.1	1.1	90.5	68.1
6	2.3	29.9	2.2	29.1	1.4	18.6	81.4	2.4	1.1	97.4	62.1
7	2.0	24.8	1.9	24.3	1.5	19.1	64.5	2.0	1.0	97.9	77.0
9	1.4	23.1	1.3	22.1	1.1	18.6	43.0	1.5	1.1	95.7	80.6
10	3.2	39.0	2.9	35.4	2.2	27.1		3.6	1.1	90.8	69.7
13	1.3	23.5	1.3	22.4	1.0	17.1	56.4	1.7	1.3	95.1	72.8
14	2.7	34.2	2.5	31.1	1.8	23.1	75.0	2.9	1.1	90.8	67.5
16	2.3	25.6	2.2	25.0	1.6	18.2	69.7	2.7	1.2	97.7	71.1
17	2.9	33.7	2.7	31.8	2.0	23.3	108.0	3.4	1.2	94.3	69.2
18	1.5	22.5	1.4	21.4	1.0	15.5	57.5	1.8	1.2	95.3	68.9
19	2.0	30.0	1.9	28.9	1.4	21.0	66.3	2.4	1.2	96.6	69.9
20	2.5	29.3	2.3	27.4	1.7	20.1			0.0	93.3	68.5
Mean	2.1	27.1	2.0	25.5	1.5	19.0	71.1	2.4	1.1	94.3	70.4
S.D.	0.6	5.7	0.5	5.2	0.4	3.7	16.1	0.6	0.3	2.7	5.0

ND Participant	Peak Heart Rate (Bpm)	Workloads (Watts)			% of Workload		Time to failure (s)
		Peak	RCP	VT	RCP	VT	
3	130.0	155.0	140.0	93.8	90.3	60.5	700.0
4	128.0	135.0	123.6	84.9	91.6	62.9	620.0
5	149.0	138.0	121.0	78.0	87.7	56.5	632.0
6	164.0	173.0	159.0	84.0	91.9	48.6	772.0
7	165.0	220.0	164.5	111.0	74.8	50.5	960.0
9	133.0	95.0	83.0	65.0	87.4	68.4	630.0
10	156.0	275.0	233.0	177.0	84.7	64.4	915.0
13	160.0	108.0	91.0	57.0	84.3	52.8	512.0
14	158.0	185.0	168.0	117.0	90.8	63.2	820.0
16	154.0	141.0	129.0	71.0	91.5	50.4	644.0
17	151.0	200.0	187.0	126.0	93.5	63.0	880.0
18	162.0	128.0	107.0	65.0	83.6	50.8	592.0
19	159.0	164.0	152.0	95.0	92.7	57.9	736.0
20	141.0	188.0	167.0	110.0	88.8	58.5	832.0
Mean	**150.7**	**164.6**	**144.7**	**95.3**	**88.1**	**57.7**	**731.8**
S.D.	12.7	47.4	39.9	31.6	5.0	6.3	134.9

VO_2 slopes from Ramp test: T2D and ND

T2D	
Slope	
	ml.min.W
1	9.4
7	8.1
8	6.2
11	7.3
14	11.5
19	9.2
20	9.9
23	9.6
26	11.0
27	8.8
35	12.0
36	10.2
38	9.7
46	9.1
Mean	9.4
SD	1.6

ND	
Slope	
	ml.min.W
3	11.9
4	4.9
5	8.0
6	10.0
7	9.8
9	9.8
10	10.6
13	12.4
16	9.0
17	10.2
18	12.5
19	10.5
20	9.2
Mean	9.9
SD	2.0

Δ%[HHb+Mb]/ Δ%PO data from Ramp test: T2D and ND

T2D RAMP HHb VL				
	Slope 1	Slope 2	BP	r^2
1	1.68	-1.05	72.05	0.96
7	1.77	0.22	95.00	0.96
8	1.59	0.06	55.87	0.93
11	2.49	0.09	24.95	0.86
14	0.88	0.47	90.12	0.96
19	2.04	-0.10	52.39	0.97
20	1.18	0.14	75.00	0.96
23	3.45	-0.09	44.00	0.94
26	1.66	0.13	56.00	0.96
35	1.50	0.32	94.10	0.92
36	1.38	-0.73	77.84	0.97
38	1.20	-0.01	71.04	0.97
46	3.14	-2.28	78.86	0.91
Mean	1.84	-0.22	68.25	
SD	0.76	0.75	20.72	
Median	1.66	0.06	72.05	
SEM	0.87	0.86	4.55	

ND RAMP VL (HHb+Mb)				
ID OAN	Slope 1	Slope 2	BP	r^2
3	1.61	-0.80	83.71	0.98
4	0.92	0.04	85	0.92
7	1.32	-1.49	82.63	0.98
6	1.60	0.10	87.07	0.99
7	1.33	-1.50	82.98	0.96
9	2.81	0.06	70.44	0.89
10	1.45	0.13	72.80	0.93
13	2.64	-0.44	47.87	0.96
14	1.83	0.01	66.82	0.97
16	1.60	-2.50	94.09	0.93
17	1.97	0.04	76.74	0.95
18	1.36	-0.88	91.09	0.97
19	1.23	-1.44	85.12	0.87
20	1.12	0.02	87.99	0.99
Mean	1.63	-0.62	79.60	
SD	0.54	0.84	12.01	
Median	1.52	-0.22	83.35	
SEM	0.73	0.91	3.46	

Haematological results: T2D

	T2D Bloods
	HbA1c (%)
1	5.9
7	6.6
8	6.0
11	9.0
14	6.0
19	
20	7.5
23	7.5
26	7.9
27	
35	7.5
36	8.4
38	9.9
46	
Mean	7.5
SD	1.3

RT3 accelerometry data: T2D and ND

	T2D RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
8	18.26	5.29	0.39	0.05
20	15.99	6.16	1.45	0.4
23	14.14	5.39	3.38	1.09
26	16.70	6.12	1.08	0.10
27	16.66	5.58	1.55	0.21
35	20.06	3.55	0.37	0.02
36	17.41	5.31	0.85	0.43
Mean	17.03	5.34	1.30	0.33
SD	1.85	0.87	1.03	0.37

	ND RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
3	19.55	3.59	0.67	0.18
4	13.20	6.51	2.90	1.39
5	19.33	3.83	0.79	0.05
6	22.11	1.89	0.00	0.00
16	23.33	0.67	0.00	0.00
18	23.30	0.70	0.00	0.00
Mean	20.14	2.87	0.73	0.27
SD	3.83	2.25	1.12	0.55

Appendix 8.9

Experiment 2

Physical characteristics: T2D and ND

T2D Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Fat layer (mm)
			Systolic	Diastolic						Vastus Lateralis
1	M	11.9	146	90	64.8	1.73	109.0	36.42	1.15	7.7
7	M	12.4	140	90	59.8	1.78	95.0	29.98	0.98	6.0
8	F	5.8	112	70	62.5	1.70	60.0	20.76	0.75	7.6
14	M	9.4	128	68	67.4	1.64	74.5	27.70	0.96	3.7
19	M	12.9	110	70	64.2	1.84	107.9	31.87	1.00	3.8
20	M	13.7	124	70	64.8	1.69	90.0	31.51	1.09	4.5
27	M	9.2	132	72	61.1	1.71	88.3	30.20	1.09	4.8
35	M	10.3	102	62	58.8	1.78	79.0	24.93	0.91	3.2
36	M	5.6	124	78	59.1	1.75	96.7	31.58	1.05	5.7
Mean		**10.13**	**124.22**	**74.44**	**62.5**	**1.74**	**88.9**	**29.44**	**1.00**	**5.2**
S.D.		2.95	14.33	9.74	3.0	0.06	15.8	4.51	0.12	1.6

ND Participant	Sex	Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Resting Blood Pressure (mmHg)		Waist-To-Hip Ratio	Vastus Lateralis
						Systolic	Diastolic		
1	M	68.2	1.70	77.0	26.64	130	82	1.05	7.4
3	M	64.1	1.75	93.4	30.50	124	70	1.00	3.7
4	M	68.0	1.64	85.3	31.71	136	84	1.01	4.4
5	M	65.1	1.75	87.7	28.64	110	64	0.96	4.9
6	M	66.0	1.58	77.2	30.92	144	90	0.96	4.3
10	M	58.6	1.75	82.0	26.78	122	85	0.98	4.1
11	M	62.2	1.77	90.0	28.73	122	70	0.96	5.2
17	M	58.8	1.77	85.0	27.13	140	90	1.04	5.6
18	F	59.0	1.63	65.8	24.77	130	85	0.91	6.7
Mean		**63.32**	**1.70**	**82.60**	**28.42**	**128.67**	**80.00**	**0.99**	**5.15**
SD		3.88	0.07	8.32	2.30	10.49	9.53	0.04	1.24

Ramp data: T2D and ND

	Oxygen Uptake (VO2)						Workloads (Watts)			
T2D Participant	Maximum (L/min)	Maximum (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	RER	Peak Heart Rate (Bpm)	Peak	50% Delta	VT	TTF (s)
1	2.0	18.5	1.5	13.4	1.1	165.0	156.0	124.0	92.0	704.0
7	2.1	21.6	1.4	14.3	1.1	136.0	162.0	125.0	88.0	728.0
8	1.5	25.2	1.2	20.4	1.3	168.0	116.0	88.0	60.0	544.0
14	2.0	27.3	1.5	19.7	1.0	132.0	142.0	115.5	89.0	648.0
19	2.6	23.7	1.5	14.1	1.1	153.0	183.0	137.8	92.5	812.0
20	2.4	26.7	1.9	20.6	1.1	169.0	152.0	128.3	104.5	688.0
27	1.9	21.1	1.4	15.5	1.0	143.0	141.0	105.5	70.0	644.0
35	2.3	28.7	1.7	21.2	1.2	154.0	176.0	144.0	112.0	618.0
36	2.6	26.8	2.2	22.5	1.1	157.0	198.0	158.0	118.8	684.0
Mean	**2.1**	**24.4**	**1.6**	**18.0**	**1.1**	**153.0**	**158.4**	**125.1**	**91.9**	**674.4**
S.D.	0.3	3.4	0.3	3.6	0.1	13.6	24.8	20.8	18.7	74.8

	Oxygen Uptake (VO2)						Workloads (Watts)			
ND Participant	Maximum (L/min)	Maximum (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	RER	Peak Heart rate (BPM)	Peak	50% Delta	VT	TTF (s)
1	1.9	24.8	1.5	19.4	1.3	159.0	158.0	127.1	96.3	712.0
3	2.2	23.6	1.4	14.9	1.1	130.0	155.0	124.4	93.8	700.0
4	1.7	19.3	1.3	14.8	1.1	128.0	135.0	110.0	84.9	620.0
5	1.8	20.8	1.2	14.1	1.1	149.0	138.0	108.0	78.0	632.0
6	2.3	29.9	1.4	18.6	1.1	164.0	173.0	128.5	84.0	772.0
10	3.2	39.0	2.2	27.1	1.1	156.0	275.0	226.0	177.0	915.0
11	2.4	26.5	1.6	17.7	1.1	141.0	185.0	142.5	100.0	820.0
17	2.9	33.7	2.0	23.3	1.2	151.0	200.0	163.0	126.0	880.0
18	1.5	22.5	1.0	15.5	1.2	160.0	128.0	96.5	65.0	592.0
Mean	**2.2**	**26.7**	**1.5**	**18.4**	**1.1**	**148.7**	**171.9**	**136.2**	**100.5**	**738.1**
SD	0.6	6.4	0.4	4.4	0.1	13.1	45.4	39.0	33.3	116.2

VO_2 kinetics response: T2D

T2D UP DELTA VO2								
ID	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base + A2	SC AMP
1	0.74	0.96	16.44	35.82	98.55	56.00	1.70	0.37
7	1.09	0.73	19.90	36.33	157.86	91.02	1.82	0.35
8	1.34	0.40	27.00	39.75	146.40	46.10	1.73	0.26
14	0.67	0.98	15.95	42.60	132.41	79.88	1.65	0.31
19	0.90	1.39	13.64	42.00	124.80	50.00	2.29	0.21
20	1.16	1.14	14.16	43.53	129.54	29.00	2.30	0.35
27	0.84	0.87	9.76	41.36	148.00	42.03	1.71	0.17
35	0.77	1.15	9.74	39.75	98.40	30.50	1.92	0.14
36	1.19	1.24	11.36	43.56	118.00	52.16	2.42	0.19
Mean	0.97	0.98	15.33	40.52	128.22	52.97	1.95	0.26
Std Dev	0.23	0.30	5.49	2.88	20.93	20.73	0.30	0.09

T2D Primed Delta VO2								
ID	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base + A2	SC AMP
1	1.11	1.10	20.00	30.00	139.20	30.50	2.21	0.19
7	0.87	0.98	25.00	30.00	124.80	61.70	1.85	0.23
8	1.18	0.81	13.29	33.91	148.95	29.00	1.99	0.14
14	0.84	1.04	19.54	35.86	133.46	95.18	1.87	0.12
19	0.91	1.51	22.61	31.76	145.00	23.50	2.42	0.16
20	1.18	1.09	17.00	30.00	142.60	36.35	2.27	0.07
27	0.93	0.98	21.00	37.80	134.40	26.60	1.91	0.08
35	0.86	1.22	17.94	28.01	117.58	26.20	2.07	0.30
36	1.29	1.23	18.96	28.06	110.40	26.70	2.53	0.19
Mean	1.02	1.11	19.48	31.71	132.93	39.53	2.12	0.16
Std Dev	0.17	0.20	3.35	3.45	12.98	23.87	0.25	0.07

T2D UP MRT VO2	
ID	MRT
1	61.00
7	91.00
8	102.49
14	73.65
19	60
20	64.02
27	64.00
35	47.00
36	51.16
Mean	68.26
Std Dev	18.09

T2D Primed MRT VO2	
ID	MRT
1	49.98
7	52.00
8	55.00
14	52.00
19	51.98
20	51.00
27	52
35	53.00
36	54.00
Mean	52.33
Std Dev	1.50

VO_2 Kinetics responses: ND

ND VO2 UP Delta								
ID	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base + A2	SC AMP
1	0.86	0.87	12.96	46.12	105.86	199.70	1.73	0.24
3	0.80	0.90	14.46	47.77	187.12	199.89	1.70	0.29
4	0.78	0.92	13.30	42.36	140.21	31.41	1.70	0.13
5	0.67	0.65	24.60	34.74	150.65	63.71	1.32	0.23
6	0.72	1.20	14.88	47.40	128.74	197.03	1.92	0.21
10	0.89	2.19	13.27	42.75	136.83	60.00	3.08	0.42
11	0.89	1.19	20.48	48.16	173.99	199.27	2.08	0.44
17	0.90	1.56	33.82	36.69	146.71	58.16	2.46	0.44
18	0.65	0.76	11.35	39.08	164.87	199.94	1.41	0.27
Mean	0.80	1.14	17.68	42.79	148.33	134.35	1.93	0.30
Std Dev	0.10	0.48	7.38	5.02	24.57	77.41	0.55	0.11

ND VO2 PRIM Delta								
ID	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base + A2	SC AMP
1	0.90	0.89	10.30	49.74	134.18	199.77	1.78	0.20
3	0.86	0.89	22.00	33.90	120.00	46.10	1.75	0.26
4	0.83	0.96	21.00	34.93	146.45	28.78	1.79	0.10
5	0.87	0.85	23.94	34.88	115.68	37.11	1.72	0.24
6	0.81	1.26	23.96	30.99	141.83	31.09	2.07	0.13
10	0.95	2.28	21.00	31.00	127.20	42.20	3.23	0.28
11	0.85	1.48	19.99	31.18	125.50	69.70	2.33	0.31
17	0.97	1.60	35.80	32.88	130.11	35.61	2.57	0.43
18	0.68	0.80	20.97	31.07	129.64	46.25	1.48	0.16
Mean	0.86	1.22	22.11	34.51	130.06	59.62	2.08	0.23
Std Dev	0.08	0.49	6.54	5.95	9.75	53.92	0.55	0.10

ND MRT VO2 UP Delta	
ID	MRT
1	63.99
3	60.00
4	52.00
5	91.69
6	65.87
10	57.00
11	66.00
17	73.80
18	59.00
Mean	65.48
Std Dev	11.64

ND MRT VO2 PRIM Delta	
ID	MRT
1	65.63
3	62.00
4	54.00
5	51.00
6	45.98
10	39.59
11	41.00
17	65.03
18	51.67
Mean	52.88
Std Dev	9.78

[HHb+Mb] kinetic responses: T2D

T2D UP DELTA VL HHb+Mb								
ID (DSP)	Base	Amp2	TD2	TAU2	Amp 3	TD3	TAU3	Base + A2
1	-105.51	165.00	10.00	20.95	0.00	125.00	5.15	59.49
7	-105.12	182.00	9.00	14.40	24.22	123.80	42.20	76.88
8	20.19	34.00	8.08	26.28	36.36	89.79	44.08	54.19
14	-108.47	345.00	9.00	13.15	32.16	134.00	44.15	236.53
19	-83.18	108.02	10.00	10.20	42.90	115.22	75.35	24.85
20	29.69	83.39	8.00	14.40	1.22	112.80	18.80	113.08
27	-101.20	109.22	8.00	11.20	0.00	82.00	5.00	8.02
35	10.79	275.00	8.00	14.40	6.40	49.65	50.00	285.79
36	-68.85	155.14	9.00	7.02	3.01	146.59	6.00	86.29
Mean	-56.85	161.86	8.79	14.67	16.25	108.76	32.30	105.01
Std Dev	59.34	96.81	0.82	5.78	17.53	29.97	24.75	94.65

T2D Primed VL HHb+Mb								
ID (DSP)	Base	Amp 2	TD2	TAU2	Amp 3	TD3	TAU3	Base + A2
1	-62.02	183.00	8.00	18.05	0.00	100.00	5.15	120.98
7	-111.67	213.23	8.00	17.02	16.10	148.76	26.49	101.56
8	15.88	31.01	4.00	36.55	3.72	148.84	26.60	46.89
14	-79.34	364.00	8.00	24.85	23.16	146.44	40.25	284.66
19	-209.02	295.02	9.00	15.45	1.38	150.00	12.80	86.00
20	24.11	96.02	8.00	22.90	4.14	155.94	14.75	120.14
27	-64.19	113.22	8.00	24.85	0.00	100.72	5.00	49.04
35	-27.78	96.40	7.01	28.08	59.06	81.66	69.44	68.61
36	46.60	185.22	9.00	19.95	0.16	151.14	6.95	231.82
Mean	-51.94	175.24	7.67	23.08	11.97	131.50	23.05	123.30
Std Dev	79.00	105.31	1.50	6.55	19.45	28.66	21.07	82.19

T2D UP HHb MRT	
	MRT
1	20.00
7	27.99
8	28.30
14	18.00
19	25.99
20	20.00
27	12.00
35	14.00
36	10.00
Mean	19.59
SD	6.81

T2D PRIM HHb MRT	
	MRT
1	22.00
7	20.00
8	41.28
14	16.00
19	10.00
20	14.00
27	14.00
35	12.01
36	14.50
Mean	18.20
SD	9.42

[HHb+Mb] kinetic responses: ND

ND UP Delta VL HHb+Mb								
ID	Base	Amp 2	TD2	TAU2	Amp 3	TD3	TAU3	Base + A2
1	-66.22	74.38	12.00	22.20	4.28	136.80	5.15	8.16
3	-109.30	207.55	10.13	12.54	16.30	110.25	22.85	98.25
4	-38.09	265.99	7.00	12.45	2.25	124.86	11.20	227.90
5	-12.12	131.00	9.00	10.50	4.28	144.00	18.80	118.88
6	-105.59	286.00	9.00	8.86	20.28	158.40	61.70	180.41
10	-168.64	258.00	13.00	11.31	14.28	151.20	18.80	89.36
11	-53.61	186.00	11.00	6.41	32.28	165.60	36.35	132.39
17	-26.09	187.50	9.00	12.26	0.28	132.00	59.75	161.41
18	-18.42	79.50	10.00	24.96	0.28	86.40	50.00	61.08
Mean	-66.45	186.21	10.02	13.50	10.50	134.39	31.62	119.76
Std Dev	52.04	78.16	1.80	6.08	11.02	24.78	21.15	65.72

ND PRIM Delta VL HHb+Mb								
ID	Base	Amp 2	TD2	TAU2	Amp 3	TD3	TAU3	Base + A2
1	14.58	78.87	12.00	20.25	0.28	120.00	12.95	93.45
3	-161.04	249.00	10.00	18.30	0.28	153.60	18.80	87.97
4	22.73	254.00	2.00	13.45	0.28	148.80	18.80	276.73
5	42.67	121.04	2.21	14.50	1.73	157.03	64.53	163.71
6	-85.52	310.00	8.00	10.50	22.28	150.00	69.50	224.48
10	-110.66	270.00	12.00	18.30	36.27	144.00	81.20	159.34
11	-44.14	202.50	10.00	14.40	0.28	153.60	77.30	158.36
17	-15.39	240.50	10.36	20.25	10.28	150.00	42.20	225.11
18	-33.22	90.50	9.00	45.60	0.28	97.00	16.85	57.28
Mean	-41.11	201.82	8.40	19.51	7.99	141.56	44.68	160.71
Std Dev	67.19	84.28	3.79	10.33	12.97	19.94	28.58	72.63

ND UP HHb MRT	
	MRT
1	25.99
3	17.98
4	16.00
5	8.00
6	12.00
10	14.00
11	11.00
17	14.00
18	29.99
Mean	16.55
SD	7.16

ND PRIM HHb MRT	
	MRT
1	14.00
3	21.99
4	18.00
5	16.00
6	10.00
10	21.99
11	10.00
17	20.03
18	33.99
Mean	18.44
SD	7.40

ΔHeart rate responses: T2D and ND

T2D UP HR Delta			
	Base	END HR	Delta HR
1	104	151	47
7	87	133	46
8	119	171	52
14	80	124	44
19	93	130	37
20	107	177	70
27	94	143	49
35	101	135	34
36	105	158	53
Mean	99	147	48
SD	12	19	10

T2D PRIM HR Delta			
	Base	END HR	Delta HR
1	122	151	29
7	90	136	46
8	122	171	49
14	84	127	43
19	102	136	34
20	129	185	56
27	107	147	40
35	106	158	52
36	117	162	45
Mean	109	153	44
SD	15	19	9

ND UP HR Delta			
	Base	END HR	Delta HR
1	89	141	51
3	83	123	40
4	78	142	64
5	98	149	51
6	89	148	59
10	77	153	76
11	93	152	59
17	113	166	53
18	82	158	76
Mean	89	148	59
SD	11	12	12

ND PRIM HR Delta			
	Base	END HR	Delta HR
1	107	151	44
3	90	131	41
4	93	136	43
5	112	158	46
6	112	160	48
10	99	159	60
11	101	162	61
17	120	169	49
18	84	159	75
Mean	102	154	52
SD	12	13	11

Haematological results: T2D

	T2D bloods
	HbA1c (%)
1	5.9
7	6.6
8	6.0
14	6.0
19	
20	7.5
27	
35	7.5
36	8.4
Mean	6.8
SD	1.0

RT3 accelerometry results: T2D and ND

	T2D RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
8	18.26	5.29	0.39	0.05
20	15.99	6.16	1.45	0.4
27	16.66	5.58	1.55	0.21
35	20.06	3.55	0.37	0.02
36	17.41	5.31	0.85	0.43
Mean	17.68	5.18	0.92	0.22
SD	1.58	0.98	0.56	0.19

	ND RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
1	13.21	8.27	1.96	0.56
3	19.55	3.59	0.67	0.18
4	13.20	6.51	2.90	1.39
5	19.33	3.83	0.79	0.05
6	22.11	1.89	0.00	0.00
18	23.30	0.70	0.00	0.00
Mean	18.45	4.13	1.05	0.36
SD	4.33	2.83	1.15	0.54

Appendix 8.10

Experiment 3

Physical characteristics: T2D and ND

T2D Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis
			Systolic	Diastolic						
1	M	11.9	146	90	64.8	1.73	109.0	36.42	1.15	7.7
7	M	12.4	140	90	59.8	1.78	95.0	29.98	0.98	6.0
20	M	13.7	124	70	64.8	1.69	90.0	31.51	1.09	4.5
26	F	5.7	138	70	62.3	1.60	66.5	25.98	0.92	8.1
27	M	9.2	132	72	61.1	1.71	88.3	30.20	1.09	4.8
19	M	12.9	110	70	64.2	1.84	107.9	31.87	1.00	3.8
35	M	10.3	102	62	58.8	1.78	79.0	24.93	0.91	3.2
36	M	5.6	124	78	59.1	1.75	96.7	31.58	1.05	5.7
38	F	9.0	130	75	60.2	1.65	74.5	27.36		6.1
46	M				67.1	1.78	100.0	31.56	1.07	4.5
Mean		**10.08**	**127.33**	**75.22**	**62.2**	**1.73**	**90.7**	**30.14**	**1.03**	**5.4**
S.D.		2.98	14.21	9.43	2.9	0.07	14.0	3.34	0.08	1.6

ND Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis Fat Layer (mm)
			Systolic	Diastolic						
1	M	10.3	130	82	68.2	1.70	77.0	26.64	1.05	7.4
3	M	9.9	124	70	64.1	1.75	93.4	30.50	1.00	3.7
4	M	9.3	136	84	68.0	1.64	85.3	31.71	1.01	4.4
6	M	9.4	144	90	66.0	1.58	77.2	30.92	0.96	4.3
10	M		122	85	58.6	1.75	82.0	26.78	0.98	4.1
12	M		125	75	69.4	1.64	72.1	26.81	1.04	
14	M		128	86	62.1	1.76	78.9	25.47	0.91	2.8
17	M		140	90	58.8	1.77	85.0	27.13	1.04	5.6
18	F		130	85	59.0	1.63	65.8	24.77	0.91	6.7
19	F				66.2	1.6	65.8	25.70		
Mean		**9.73**	**131.00**	**83.00**	**64.0**	**1.68**	**78.3**	**27.64**	**0.99**	**4.9**
S.D.		0.46	7.52	6.61	4.2	0.07	8.8	2.47	0.05	1.6

Ramp data: T2D and ND

	Oxygen Uptake (VO2)							Peak Heart Rate (BPM)	Workloads (Watts)				TTF (s)
T2D Participant	Maximum (L/min)	Maximum (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	Ve (L/min)	VCO2 (L/min)	RER		Peak	50% Delta	VT	80% VT	
1	2.012	18.46	1.457	13.37	81.6	2.205	1.10	165	156.0	124.0	92.0	73.6	704.0
7	2.054	21.62	1.363	14.35	61.0	2.211	1.08	136	162.0	125.0	88.0	70.4	728.0
20	2.399	26.66	1.851	20.57	65.7	2.538	1.06	169	152.0	128.3	104.5	83.6	688.0
26	1.941	29.19	1.590	23.91	64.00	2.135	1.10	144	159.0	137.0	115.0	92.0	716.0
27	1.864	21.11	1.367	15.48	69.7	1.903	1.02	143	141.0	105.5	70.0	56.0	644.0
19	2.558	23.71	1.522	14.11	106.90	2.789	1.09	153	183.0	137.8	92.5	74.0	812.0
35	2.270	28.73	1.677	21.23	88.20	2.703	1.19	154.0	176.0	144.0	112.0	89.0	618.0
36	2.593	26.81	2.173	22.47	82.30	2.921	1.13	157.0	198.0	158.0	118.8	95.0	684.0
38	1.508	20.24	1.058	14.20	60.80	1.841	1.22	178	97.0	72.9	48.8	39.0	642.2
46	2.071	20.71	1.430	14.30	54.90	1.570	0.76		131.0	104.3	77.7	62.1	604.0
Mean	**2.13**	**23.72**	**1.55**	**17.40**	**73.51**	**2.28**	**1.07**	**155.44**	**155.5**	**123.7**	**91.9**	**73.5**	**684.02**
S.D.	0.33	3.85	0.30	4.12	16.00	0.45	0.13	13.50	28.4	24.2	22.0	17.6	61.34

	Oxygen Uptake (VO2)							Peak Heart Rate (BPM)	Workloads (Watts)				TTF (s)
ND Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	RCP (mL/kg/min)	VT (L/min)	VT (mL/kg/min)	RER		Peak	50% Delta	VT	80% VT	
1	1.906	24.75	1.800	23.38	1.497	19.44	1.29	159	158.0	127.1	96.3	77.0	712
3	2.201	23.57	1.989	21.30	1.392	14.90	1.12	130	155.0	124.4	93.8	75.0	700
4	1.650	19.34	1.565	18.35	1.259	14.76	1.14	128	135.0	110.0	84.9	67.9	620
6	2.309	29.91	2.249	29.13	1.435	18.59	1.06	164	173.0	128.5	84.0	67.2	772
10	3.194	38.95	2.899	35.35	2.226	27.15	1.12	156	275.0	226.0	177.0	141.6	915
12	2.001	27.75	1.890	26.21	1.450	20.11	1.20	160	149.0	120.5	92.0	73.6	676
14	2.702	34.25	2.454	31.10	1.824	23.12	1.07	158	185.0	151.0	117.0	93.6	820
17	2.862	33.67	2.700	31.76	1..98	23.29	1.18	151	200.0	163.0	126.0	100.8	880
18	1.480	22.49	1.410	21.43	1.020	15.50	1.20		128.0	96.5	65.0	52.0	828
19	1.972	29.97	1.904	28.94	1.379	20.96	1.20		164.0	129.5	95.0	76.0	736
Mean	**2.23**	**28.47**	**2.09**	**26.70**	**1.50**	**19.78**	**1.16**	**150.8**	**172.2**	**137.6**	**103.1**	**82.5**	**765.9**
S.D.	0.55	6.07	0.48	5.47	0.35	4.06	0.07	13.9	42.1	36.2	31.0	24.8	94.0

Elevated baseline (WtoW) VO_2 kinetics response: T2D

T2D WtoW UP VO2									
	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base+AMP	S.C Amp	MRT
1	1.72	0.50	15.87	43.65	160.80	141.65	2.22	0.16	54.97
7	1.30	0.49	8.14	63.29	147.88	40.56	1.79	0.27	89.89
19	1.68	0.49	13.98	47.51	127.23	44.21	2.17	0.17	65.70
20	2.02	0.49	3.13	53.22	179.82	81.47	2.51	0.16	65.00
26	1.67	0.43	13.00	49.50	160.80	92.90	2.10	0.13	67.97
27	1.38	0.47	5.82	50.40	149.32	31.24	1.84	0.19	93.96
35	1.73	0.35	8.96	47.51	78.36	81.74	2.08	0.10	38.98
36	2.15	0.40	12.02	47.15	128.55	54.18	2.55	0.14	65.97
38	1.07	0.21	17.00	45.60	125.00	106.55	1.28	0.12	69.97
46	1.25	0.45	16.12	40.76	80.00	9.72	1.70	0.16	49.96
Mean	1.60	0.43	11.40	48.86	133.78	68.42	2.02	0.16	66.24
SD	0.34	0.09	4.71	6.15	33.55	39.66	0.38	0.05	16.60

T2D WtoW PRIM VO2									
	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base+AMP	S.C Amp	MRT
1	1.93	0.48	16.99	35.00	148.00	46.71	2.41	0.10	53.20
7	1.49	0.54	18.99	30.00	151.00	72.92	2.03	0.03	39.99
19	1.55	0.62	16.00	31.95	112.80	20.75	2.17	0.13	53.19
20	2.12	0.52	20.00	39.79	150.03	36.50	2.64	0.11	73.97
26	1.66	0.46	16.90	33.81	150.66	64.53	2.12	0.14	60.94
27	1.45	0.52	20.00	37.80	148.80	46.10	1.96	0.11	77.97
35	1.73	0.40	19.00	28.05	67.84	57.80	2.13	0.11	39.99
36	1.93	0.60	12.48	32.08	116.99	21.88	2.54	0.15	49.98
38	1.03	0.23	14.00	31.95	125.00	98.75	1.27	0.07	43.98
46	1.38	0.45	18.70	40.66	69.50	5.00	1.82	0.03	31.97
Mean	1.63	0.48	17.31	34.11	124.06	47.09	2.11	0.10	52.52
SD	0.32	0.11	2.55	4.17	32.67	27.83	0.39	0.04	14.89

Elevated baseline (WtoW) VO_2 kinetics response: ND

ND UP VO2									
	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base+AMP	S.C Amp	MRT
1	1.37	0.42	8.15	55.42	140.58	86.61	1.79	0.17	91.96
3	1.40	0.42	19.91	51.43	151.00	81.93	1.82	0.23	67.97
4	1.56	0.33	19	44.9	124	19.4	1.89	0.06	41.98
6	1.46	0.69	17.87	51.45	160.80	46.10	2.15	0.09	69.97
10	2.40	0.78	18.87	51.45	129.60	57.80	3.18	0.24	63.97
12	1.70	0.40	20.87	59.25	148.00	44.15	2.10	0.11	67.97
14	1.97	0.46	11.92	49.00	172.82	54.98	2.43	0.25	83.96
17	1.80	0.52	20.00	51.45	112.00	42.00	2.32	0.21	63.00
18	1.02	0.40	20.00	42.00	139.20	48.05	1.42	0.15	64.00
19	1.21	0.42	26.00	34.00	76.80	114.35	1.63	0.17	53.98
Mean	1.59	0.48	18.26	49.03	135.48	59.54	2.07	0.17	66.88
SD	0.40	0.14	4.93	7.16	27.15	27.35	0.50	0.07	13.95

ND PRIM VO2									
	Base	Amp 2	TD2	TAU2	TD3	TAU3	Base+AMP	S.C Amp	MRT
1	1.44	0.43	20.24	39.81	130.00	44.64	1.87	0.12	71.97
3	1.41	0.49	21.00	35.85	115.00	36.35	1.90	0.17	45.98
4	1.58	0.40	19.67	37.49	92.72	8.31	1.98	0.04	57.93
6	1.48	0.69	20.28	30.00	103.20	42.20	2.17	0.09	55.98
10	2.62	0.75	31.10	49.47	121.61	40.70	3.37	0.12	49.98
12	1.69	0.49	21.00	49.50	148.00	36.00	2.18	0.10	49.98
14	2.05	0.47	11.82	37.06	133.08	20.99	2.52	0.22	43.98
17	2.03	0.57	24.95	45.51	112.54	65.36	2.61	0.17	71.97
18	1.06	0.49	20.00	34.85	138.00	34.40	1.54	0.09	65.97
19	1.30	0.44	24.00	20.25	79.20	5.15	1.74	0.01	43.00
Mean	1.67	0.52	21.41	37.98	117.33	33.41	2.19	0.11	55.67
SD	0.45	0.12	4.87	8.93	21.30	17.88	0.53	0.06	11.05

Moderate-intensity (80% VT) VO_2 kinetics: T2D and ND

T2D UnPrimed VO2 VT				
ID (DSP)	Base	Amp	TD	Tau
1	1.06	0.70	23.40	41.98
7	0.58	0.70	10.30	38.90
19	0.85	0.70	11.15	36.33
20	1.32	0.71	15.53	55.83
26	0.73	0.93	12.00	32.00
27	0.87	0.50	15.00	39.99
35	0.67	0.93	10.00	34.00
36	1.49	0.46	32.92	35.96
38	0.65	0.40	21.84	37.91
46	0.94	0.40	29.08	66.12
Mean	0.92	0.64	18.12	41.90
Std Dev	0.30	0.20	8.24	10.73

T2D Primed VO2 VT				
ID (DSP)	Base	Amp	TD	Tau
1	1.09	0.85	20.93	31.96
7	0.67	0.81	13.79	31.80
19	0.87	0.70	22.00	31.99
20	1.36	0.74	20.91	40.95
26	0.70	0.94	11.99	26.00
27	0.95	0.40	14.00	26.10
35	0.70	0.93	13.09	27.37
36	1.34	0.61	24.74	42.28
38	0.81	0.24	27.00	35.99
46	0.87	0.44	29.00	41.98
Mean	0.93	0.67	19.75	33.64
Std Dev	0.25	0.24	6.18	6.38

ND VO2 UP VT				
	Base	Amp	TD	Tau
1	0.90	0.49	12.81	44.86
3	0.79	0.56	20.62	43.06
4	0.84	0.64	19.57	39.29
6	0.83	0.61	18.37	37.78
10	0.84	1.55	17.95	36.99
12	1.01	0.66	15.60	45.00
14	0.82	0.99	24.97	31.90
17	0.91	0.81	20.63	43.00
18	0.55	0.46	19.97	35.89
19	0.58	0.62	20.00	35.99
Mean	0.81	0.74	19.05	39.38
SD	0.14	0.33	3.24	4.42

ND VO2 PRIM VT				
	Base	Amp	TD	Tau
1	0.90	0.56	31.16	31.90
3	0.83	0.56	31.40	31.99
4	0.84	0.66	25.00	31.99
6	0.84	0.65	18.99	32.00
10	0.94	1.53	23.00	25.99
12	1.06	0.63	28.00	27.99
14	0.95	1.02	18.00	37.99
17	0.88	0.90	22.81	27.61
18	0.65	0.40	23.00	25.99
19	0.60	0.65	19.98	27.82
Mean	0.85	0.76	24.13	30.13
SD	0.14	0.32	4.76	3.74

Elevated baseline (WtoW) [HHb+Mb] kinetic responses: T2D

T2D WtoW UP HHb VL								
	Base	Amp 2	TD2	TAU2	TD+Tau	Amp 3	TD3	TAU3
1	31.30	76.40	6.00	26.10	32.10	0.02	88.80	44.15
7	-7.86	136.39	9.00	17.40	26.40	21.96	150.00	44.15
19	29.79	34.41	7.00	19.23	26.23	0.47	103.20	16.85
20	69.43	3.92	8.00	18.30	26.30	11.47	225.60	77.30
26	-39.28	7.92	5.00	20.25	25.25	11.47	220.80	42.20
27	11.00	8.92	15.00	26.10	41.10	8.47	122.40	32.45
35	139.40	84.90	1.00	26.10	27.10	4.47	42.80	5.15
36	232.15	32.42	1.00	18.92	19.92	0.47	34.00	5.15
38	-65.14	19.00	10.03	31.85	41.87	8.47	146.57	30.60
Mean	44.53	44.92	6.89	22.69	29.59	7.47	126.02	33.11
SD	92.29	45.06	4.40	4.98	7.42	7.13	68.20	22.68

T2D WtoW PRIM HHb VL								
	Base	Amp 2	TD2	TAU2	TD+Tau	Amp 3	TD3	TAU3
1	86.50	81.20	2.00	28.92	30.92	17.46	101.60	30.50
7	-36.53	115.19	7.00	28.92	35.92	10.47	145.00	55.85
19	93.78	30.21	1.00	30.90	31.90	5.47	146.40	26.60
20	192.30	13.21	17.00	29.04	46.04	2.97	138.00	30.50
26	29.84	10.22	7.82	28.48	36.30	10.47	157.64	61.67
27	49.53	14.21	9.00	36.92	45.92	10.97	121.20	61.70
35	385.20	37.21	8.00	24.92	32.92	0.47	79.20	22.70
36	302.91	26.21	0.97	18.87	19.84	0.42	32.83	11.47
38	-2.04	11.01	14.00	33.48	47.48	1.97	132.40	30.50
Mean	122.39	37.63	7.42	28.94	36.36	6.74	117.14	36.83
SD	142.86	36.58	5.60	5.07	8.97	5.90	39.92	18.25

T2D WtoW UP HHb MRT	
	MRT
1	23.99
7	23.99
19	14.00
20	x
26	6.00
27	76.06
35	28.09
36	14.10
38	52.07
Mean	29.79
SD	23.20

T2D WtoW PRIM HHb MRT	
	MRT
1	24.09
7	15.00
19	15.00
20	x
26	118.95
27	64.98
35	14.97
36	16.05
38	36.08
Mean	38.14
SD	36.93

Elevated baseline (WtoW) [HHb+Mb] kinetic responses: ND

ND UP HHb VL								
	Base	Amp 2	TD2	TAU2	TD+Tau	Amp 3	TD3	TAU3
1	-0.77	61.40	3.00	20.25	23.25	0.01	140.00	42.20
3	-0.77	61.40	3.00	20.25	23.25	0.01	140.00	42.20
4	224.77	38.39	2.00	26.06	28.06	0.01	110.00	97.94
6	129.39	53.42	4.02	41.68	45.70	13.12	158.28	41.94
10	219.54	78.40	5.00	16.35	21.35	0.03	136.00	40.25
12	40.00	120.39	6.00	53.40	59.40	24.02	148.80	50.00
14	63.93	122.39	1.00	22.20	23.20	0.03	148.80	20.75
17	104.89	94.39	8.00	26.10	34.10	2.03	124.80	22.70
18	-19.76	82.51	5.00	51.45	56.45	13.61	145.76	9.05
19	78.99	19.52	2.99	22.18	25.17	3.96	43.12	17.12
Mean	84.02	73.22	4.00	29.99	33.99	5.68	129.56	38.42
SD	87.04	33.29	2.06	13.64	14.56	8.38	33.27	24.94

ND PRIM HHb VL								
	Base	Amp 2	TD2	TAU2	TD+Tau	Amp 3	TD3	TAU3
1	69.45	23.41	14.00	24.15	38.15	0.10	148.80	42.20
3	84.40	88.50	3.00	33.90	36.90	0.01	140.94	11.00
4	238.76	35.41	1.00	26.10	27.10	0.10	110.00	5.15
6	132.26	67.40	10.00	35.85	45.85	3.70	148.80	32.45
10	218.51	105.00	3.00	30.00	33.00	-0.71	148.17	24.65
12	63.92	120.37	5.98	53.41	59.39	0.01	136.12	30.50
14	237.31	106.50	4.00	14.40	18.40	8.20	148.17	30.50
17	242.04	66.01	5.00	14.40	19.40	0.01	124.00	14.90
18	10.44	46.52	5.00	22.20	27.20	0.01	145.76	14.90
19	69.41	21.03	1.00	12.45	13.45	1.62	49.57	14.90
Mean	136.65	68.02	5.20	26.69	31.88	1.30	130.03	22.12
SD	89.05	36.11	4.05	12.44	13.90	2.73	31.05	11.67

ND UP WtoW MRT	
	MRT
1	47.99
3	20.00
4	29.04
6	69.97
10	16.00
12	81.96
14	23.99
17	25.99
18	53.98
19	18.50
Mean	38.74
SD	23.37

ND PRIM WtoW MRT	
	MRT
1	18.50
3	29.99
4	25.95
6	35.99
10	27.99
12	49.98
14	16.00
17	16.00
18	23.99
19	20.00
Mean	26.44
SD	10.48

Moderate-intensity (80% VT) [HHb+Mb] kinetics: T2D and ND

T2D UP VT WtoW HHb				
	Base	AMP	TD	Tau
1	-90.86	130.32	15.00	9.91
7	-94.74	117.89	12.01	7.89
19	-53.91	104.38	13.02	9.92
20	12.49	67.50	8.00	21.91
26	-60.68	29.99	12.00	7.91
27	-21.90	35.00	2.00	11.91
35	-158.73	273.99	8.00	15.91
36	7.09	205.01	11.01	19.92
38	-72.74	11.82	16.00	13.91
46	-61.32	64.98	15.00	27.90
Mean	-59.53	104.09	11.20	14.71
SD	50.90	82.72	4.24	6.68

T2D PRIM VT WtoW HHb				
	Base	AMP	TD	Tau
1	-39.60	126.69	11.00	18.00
7	-143.31	130.04	11.00	13.91
19	-44.52	111.88	11.00	15.00
20	56.39	92.40	8.00	19.91
26	0.30	39.13	11.00	12.00
27	1.30	51.48	10.00	15.01
35	68.10	270.98	8.00	15.91
36	94.70	190.01	9.00	24.91
38	-22.75	24.51	10.00	29.90
46	-4.91	90.46	13.00	21.50
Mean	-3.43	112.76	10.20	18.60
SD	67.74	74.07	1.55	5.54

ND HHb UP VT WtoW				
	Base	A2	TD2	TAU2
1	-40.49	38.79	13.00	25.99
3	-179.18	128.33	11.20	15.58
4	36.05	176.75	3.00	17.21
6	-2.36	158.75	9.00	9.21
10	-33.24	203.74	9.00	13.21
12	-53.24	170.75	9.00	17.21
14	-189.51	245.73	10.00	10.21
17	-19.65	125.77	15.00	10.21
18	-85.82	56.79	10.00	32.20
19	7.10	47.79	7.00	16.21
Mean	-56.03	135.32	9.62	16.72
SD	75.50	69.66	3.25	7.31

ND HHb PRIM VT WtoW				
	Base	A2	TD2	TAU2
1	37.61	62.79	11.00	20.00
3	-110.26	194.74	7.03	23.99
4	23.79	203.74	7.00	24.99
6	-21.64	179.75	9.00	17.00
10	42.38	185.75	10.00	14.00
12	32.65	197.74	11.00	16.00
14	-86.34	316.01	9.00	13.98
17	67.49	152.76	6.00	26.20
18	-58.27	47.79	2.00	56.19
19	-10.18	56.79	5.00	18.21
Mean	-8.28	159.78	7.70	23.05
SD	59.90	83.45	2.87	12.45

Elevated baseline (WtoW) ΔHeart rate: T2D and ND

T2D UP HR WtoW			
	Base	End	Delta
1	119	151	32
7	109	128	19
19	114	139	25
20	137	171	34
26	116	146	30
27	105	141	36
35	143	158	15
36	123	157	34
38	130	163	33
46	125	133	8
Mean	122	149	27
SD	12	14	10

T2D PRIM HR WtoW			
	Base	End	Delta
1	120	154	34
7	115	141	26
19	116	140	24
20	152	175	23
26	123	147	24
27	118	145	27
35	147	161	14
36	137	162	25
38	138	163	25
46	128	137	9
Mean	129	153	23
SD	13	12	7

ND UP HR WtoW			
	Base	End	Delta
1	109	136	27
3	100	128	28
4	108	133	25
6	113	149	36
10	120	153	33
12	134	159	25
14	123	156	33
17	122	152	30
18	110	159	49
19	129	143	14
Mean	117	147	30
SD	11	11	9

ND PRIM HR WtoW			
	Base	End	Delta
1	117	141	24
3	107	130	23
4	111	131	20
6	119	148	29
10	127	156	29
12	140	159	19
14	136	156	20
17	145	159	14
18	121	158	37
19	131	154	23
Mean	125	149	24
SD	13	11	6

Moderate-intensity (80% VT) ΔHeart rate: T2D and ND

T2D UP HR 80% VT			
	Base	End	Delta
1	99	119	20
7	90	109	19
19	97	114	17
20	107	137	30
26	81	116	35
27	87	105	18
35	102	143	41
36	100	123	23
38	107	130	23
46	110	125	15
Mean	98	122	24
SD	9	12	9

T2D PRIM HR 80% VT			
	Base	End	Delta
1	96	120	24
7	98	115	17
19	101	116	15
20	128	152	24
26	93	123	30
27	100	118	18
35	116	147	31
36	110	137	27
38	125	138	13
46	113	128	15
Mean	108	129	21
SD	12	13	7

ND UP HR 80% VT			
	Base	End	Delta
1	84	109	25
3	80	100	20
4	80	108	28
6	88	113	25
10	87	120	33
12	100	134	34
14	82	123	41
17	90	122	32
18	84	110	26
19	77	129	52
Mean	85	117	32
SD	7	11	9

ND PRIM HR 80% VT			
	Base	End	Delta
1	106	117	11
3	90	107	17
4	90	111	21
6	99	119	20
10	97	127	30
12	114	140	26
14	110	136	26
17	115	145	30
18	91	121	30
19	79	131	52
Mean	99	125	26
SD	12	13	11

Haematological responses: T2D

	HbA1c (%)
1	5.9
7	6.6
19	
20	7.5
26	7.9
27	
35	7.5
36	8.4
38	9.9
46	
Mean	7.7
SD	1.3

RT3 accelerometry data: T2D and ND

	T2D RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
20	15.99	6.16	1.45	0.4
26	16.70	6.12	1.08	0.10
27	16.66	5.58	1.55	0.21
35	20.06	3.55	0.37	0.02
36	17.41	5.31	0.85	0.43
Mean	17.36	5.34	1.06	0.23
SD	1.59	1.07	0.48	0.18

	ND RT3 Data (h.day)			
	Inactive	Light	Mod	Vigorous
1	13.21	8.27	1.96	0.56
3	19.55	3.59	0.67	0.18
4	13.20	6.51	2.90	1.39
6	22.11	1.89	0.00	0.00
18	23.30	0.70	0.00	0.00
Mean	18.27	4.19	1.11	0.43
SD	4.82	3.16	1.28	0.58

Appendix 8.11

Experiment 4

Physical characteristics:

Control group physical characteristics:

Control Wk 0										
Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis Fat Layer (mm)
			Systolic	Diastolic						
3	M	12.6	146	76	55.2	1.74	94.5	31.21	1.06	7.8
10	F	8.6	128	60	71.3	1.61	73.0	28.16	0.81	12.3
12	M	9.4	132	74	48.3	1.82	108.8	32.85	1.02	5.0
18	F	9.4	118	70	42.8	1.58	65.0	26.04	0.87	7.5
21	F	8.1	114	76	51.4	1.58	76.0	30.44	0.96	9.9
22	F				63.1	1.55	86.0	35.80	0.86	12.7
31	M		129	77	55.8	1.86	92.5	26.74	0.95	5.6
33	M	10.5	140	80	55.4	1.88	99.0	28.01	0.95	4.7
41	F		118	78	42.0	1.5	79.1	35.16	0.92	11.6
42	F		124	72	58.7	1.55	87.0	36.21	0.89	13.7
Mean		**9.77**	**127.67**	**73.67**	**54.4**	**1.67**	**86.1**	**31.06**	**0.93**	**9.1**
S.D.		1.61	10.58	5.96	8.9	0.14	13.2	3.81	0.08	3.4

Control Week 12						
Participant	Sex	Resting Blood Pressure (mmHg)		Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio
		Systolic	Diastolic			
3	M	126	62	93.2	30.43	1.05
10	F	140	68	73.5	28.71	0.81
12	M	136	80	109.9	33.18	1.00
18	F	102	70	59.0	23.63	0.89
21	F	142	81	76.5	30.64	0.92
22	F	118	56	88.0	36.63	0.88
31	M	136	78	96.7	27.95	0.92
33	M	124	70	100.4	28.41	0.94
41	F	118	78	80.6	35.82	1.13
Mean		**126.89**	**71.44**	**86.4**	**30.60**	**0.95**
S.D.		13.00	8.62	15.6	4.10	0.10

MICT group physical characteristics:

MICT Week 0										
Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis Fat Layer (mm)
			Systolic	Diastolic						
4	M	8.9	152	80	52.1	1.83	116.5	34.79	1.00	5.4
7	M	12.4	140	90	59.8	1.78	95.0	29.98	0.98	6.0
8	F	5.8	112	70	62.5	1.70	60.0	20.76	0.75	7.6
15	M	12.7	138	88	41.4	1.74	115.0	37.98	1.07	5.8
20	M	13.7	124	70	64.8	1.69	90.0	31.51	1.09	4.5
23	F	6.8	100	70	63.9	1.63	75.7	28.67	0.79	12.5
24	F	X	114	66	36.9		116.0		1.03	
28	M	7.8	118	78	54.1	1.74	94.7	31.28	1.04	7.3
30	M	9.7	122	88	52.7	1.88	94.1	26.62	0.97	6.1
39	M	6.2	110	62	42.2	1.7	72.5	25.09	0.96	5.9
Mean		**9.73**	**124.44**	**77.78**	**54.2**	**1.75**	**95.2**	**30.20**	**0.97**	**6.8**
S.D.		2.93	16.18	9.24	9.9	0.08	19.1	5.19	0.12	2.3

MICT Week 12						
Participant	Sex	Resting Blood Pressure (mmHg)		Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio
		Systolic	Diastolic			
4	M	142	80	115.0	34.34	1.02
7	M	136	76	91.9	28.67	0.94
8	F	110	60	57.2	20.03	0.74
15	M	124	72	108.5	35.43	X
20	M	136	72	85.0	30.12	1.08
23	F	107	60	73.4	27.63	0.88
24	F	116	75	110.0	38.06	X
28	M	126	74	92.9	30.68	0.99
30	M	118	78	93.0	26.31	0.90
39	M	118	64	73.0	25.26	0.98
Mean		**123.30**	**71.10**	**90.0**	**29.65**	**0.94**
S.D.		11.70	7.25	18.4	5.33	0.10

HIIT group physical characteristics:

HIIT Week 0										
Participant	Sex	Aortic PWV (m/s)	Resting Blood Pressure (mmHg)		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio	Vastus Lateralis Fat Layer (mm)
			Systolic	Diastolic						
26	F	5.7	138	70	62.3	1.60	66.5	25.98	0.92	8.1
34	M	9.5	112	64	51.0	1.88	99.0	28.01	1.07	5.3
35	M	10.3	102	62	58.8	1.78	79.0	24.93	0.91	3.2
36	M	5.6	124	78	59.1	1.75	96.7	31.58	1.05	5.7
38	F	9.0	130	75	60.2	1.65	74.5	27.36		6.1
45	M	7.6	124	70	56.1	1.88	100.4	28.41	0.94	4.4
47	M				46.1	1.82	96.4	29.10	0.99	4.1
48	F		118	78	42.0	1.5	80.6	35.82	1.13	
49	M	7.7	124	74	32.4	1.83	94.0	28.07	0.86	8.4
Mean		**7.91**	**121.50**	**71.38**	**52.0**	**1.74**	**87.5**	**28.81**	**0.98**	**5.7**
S.D.		1.82	10.99	6.02	10.1	0.13	12.4	3.23	0.10	1.8

HIIT Week 12						
Participant	Sex	Resting Blood Pressure (mmHg)		Body Mass (kg)	BMI (kg.m2)	Waist-To-Hip Ratio
		Systolic	Diastolic			
26	F	118	68	65.7	25.66	0.92
34	M	115	70	94.0	26.60	1.01
35	M	110	70	78.0	24.62	0.87
36	M	122	67	93.8	29.94	1.04
38	F	142	70	73.0	26.81	0.88
45	M	124	82	98.9	27.98	0.93
47	M	115	79	100.0	30.19	0.99
48	F	141	78	82.0	36.44	0.89
49	M	120	72	93.0	27.77	0.90
Mean		**123.00**	**72.89**	**86.5**	**28.45**	**0.94**
S.D.		11.28	5.37	12.2	3.51	0.06

Ramp data Week 0 and 12:

Control Ramp data:

	Control Week 0													
	Oxygen Uptake (VO2)								Peak Heart Rate (BPM)	Workloads (Watts)				Time of Ramp Test (s)
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)	Ve (L/min)	VCO2 (L/min)	RER		Peak	RCP	50% Delta	VT	
3	1.791	18.95	1.551	1.331	14.08	73.4	2.063	1.15	149	139.0	108.0	110.0	81.0	636.0
10	1.214	16.63	1.104	0.900	12.33	43.5	1.394	1.15	148	101.0	80.0	78.0	55.0	484.0
12	2.426	22.30	2.183	1.394	12.81	83.8	2.822	1.16	158	200.0	168.0	143.5	87.0	690.0
18	1.405	21.62	1.401	1.109	17.06	52.3	1.407	1.00	160	107.0	100.0	88.3	69.5	508.0
21	1.658	21.82	1.395	1.190	15.66	67.7	1.868	1.13	169	120.0	83.4	92.3	64.5	780.0
22	1.551	18.03	1.424	1.119	13.01	60.8	1.735	1.12	180	120.0	94.7	92.5	64.9	780.0
31	2.298	24.84	2.105	1.722	18.62	76.0	2.534	1.10	188	175.0	158.6	141.5	108.0	615.0
33	2.787	28.15	2.743	1.835	18.54	88.5	3.121	1.12	160.0	246.8	238.4	189.9	133.0	830.4
41	1.605	20.29	1.442	1.198	15.15				167.0	126.0		103.0	80.0	584.0
42	1.619	18.61	1.527	1.191	13.69				135.0	91.0	79.7	85.5	80.0	606.0
Mean	**1.84**	**21.12**	**1.69**	**1.30**	**15.09**	**68.25**	**2.12**	**1.12**	**161.40**	**142.6**	**123.4**	**112.4**	**82.3**	**651.34**
S.D.	0.50	3.44	0.50	0.29	2.32	15.41	0.65	0.05	15.59	49.6	54.1	35.3	23.1	117.08

	Control Week 12							
	Oxygen Uptake (VO2)					Peak Heart Rate (BPM)	Workloads (Watts)		TTF (s)
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)		Peak	VT	
3	1.947	20.89	1.725	1.434	15.39	133	138.5	98.5	634.1
10	1.397	19.00	1.364	1.086	14.78	132	103.0	54.5	492.0
12	2.240	20.38	2.062	1.406	12.79	174	193.0	93.6	669.0
18	1.238	20.98	1.132	0.948	16.07	154	103.0	64.5	492.0
21	1.554	20.31	1.482	1.172	15.32	147.0	128.8	75.9	595.1
22	1.678	19.07	1.577	1.307	14.85	157	119.0	75.7	774.0
31	2.659	27.50	2.426	1.911	19.76	174	195.0	112.6	675.0
33	2.800	27.89	2.708	1.928	19.20	160	252.0	142.3	846.0
41	1.803	22.37	1.671	1.339	16.61	183	144.0	91.8	656.0
Mean	**1.92**	**22.04**	**1.79**	**1.39**	**16.09**	**157.11**	**152.9**	**89.9**	**648.13**
S.D.	0.54	3.36	0.51	0.34	2.20	17.93	50.2	26.6	116.18

MICT Ramp data:

	MICT Week 0													
	Oxygen Uptake (VO2)									Workloads (Watts)				
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)	Ve (L/min)	VCO2 (L/min)	RER	Peak Heart Rate (BPM)	Peak	RCP	50% Delta	VT	TTF (s)
4	3.181	27.30	2.986	2.216	19.02	105.9	3.871	1.22	157	231.0	207.0	182.0	133.0	1004.0
7	2.054	21.62	1.746	1.363	14.35				136	162.0	130.0	125.0	88.0	728.0
8	1.512	25.20	1.461	1.226	20.43				168	116.0	97.0	88.0	60.0	544.0
15	3.222	28.02	2.951	2.558	22.24	126.7	3.758	1.17	174	245.0	190.0	197.5	150.0	825.0
20	2.399	26.66	2.141	1.851	20.57	65.7	2.538	1.06	169	152.0	130.0	128.3	104.5	688.0
23	1.528	20.18	1.396	1.112	14.69	72.9	2.014	1.32	152	120.5	97.5	96.5	72.5	562.0
24	2.069	17.84	2.001	1.828	15.76	72.8	2.233	1.08	178	155.0	140.0	123.8	92.5	700.0
28	1.785	18.85	1.712	1.426	15.06	72.8	1.981	1.11	167	186.0	157.0	137.8	89.6	648.0
30	2.445	25.98	2.283	1.597	16.97	89.7	2.681	1.10	144	213.0	190.8	168.5	124.0	729.0
39	1.155	15.93	1.091	0.994	13.71	32.0	1.159	1.00	143	96.3	71.5	77.4	58.6	465.0
Mean	**2.24**	**23.52**	**2.08**	**1.69**	**17.68**	**79.81**	**2.53**	**1.15**	**158.80**	**175.6**	**141.1**	**138.6**	**101.6**	**714.22**
S.D.	0.63	3.91	0.58	0.48	2.95	28.30	0.92	0.09	14.51	46.3	45.2	37.2	29.2	138.78

	MICT Week 12							
	Oxygen Uptake (VO2)					Peak Heart Rate (BPM)	Workloads (Watts)	
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)		Peak	VT	TTF (s)
4	3.746	32.57	3.526	3.067	26.67	170	295.0	200.0	1260.0
7	1.886	20.53	1.792	1.691	18.41	136	196.0	133.0	864.0
8	1.792	31.33	1.731	1.476	25.80	176	142.0	74.8	648.0
15	3.656	33.70	2.353	2.847	26.24	167.0	310.0	192.0	1320.0
20	2.695	31.71	2.599	2.408	28.33	160.0	203.0	154.2	892.0
23	1.790	24.39	1.612	1.400	19.07	140.0	147.0	107.5	668.0
24	2.365	21.50	2.133	1.839	16.72	183.0	175.0	124.9	780.0
28	3.020	32.51	2.491	2.165	23.30	173.0	244.0	155.4	822.0
30	2.882	30.99	2.529	2.119	22.78	138.0	259.0	168.8	867.0
39	1.881	25.77	1.624	1.474	20.19	150.0	132.0	94.3	608.0
Mean	**2.57**	**28.50**	**2.24**	**2.05**	**22.75**	**159.30**	**210.3**	**140.5**	**872.90**
S.D.	0.75	4.96	0.60	0.58	3.99	17.19	64.1	41.1	241.33

HIIT Ramp data:

	HIIT Week 0										
	Oxygen Uptake (VO2)					Peak Heart Rate (BPM)	Workloads (Watts)				TTF (s)
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)		Peak	RCP	50% Delta	VT	
26	1.941	29.19	1.685	1.590	23.91	144.0	159.0	143.0	137.0	115.0	716.0
34	2.133	21.55	2.031	1.495	15.10	150.0	179.0	165.2	145.5	112.0	627.0
35	2.270	28.73	2.196	1.677	21.23	154.0	176.0	159.2	144.0	112.0	618.0
36	2.593	26.81	2.514	2.173	22.47	157.0	198.0	152.0	158.0	118.8	684.0
38	1.508	20.24	1.455	1.058	14.20	178	97.0	92.7	72.9	48.8	642.2
45	2.800	27.89	2.708	1.908	19.00	158	252.0	223.0	197.1	142.2	846.0
47	2.797	29.01	2.640	2.066	21.43	174.0	232.0	205.6	189.1	146.2	786.0
48	1.803	22.37	1.671	1.339	16.61	183.0	144.0	131.3	117.9	91.8	656.0
49	2.988	31.79	2.803	2.225	23.67	164.0	247.0	224.8	199.7	152.3	831.0
Mean	**2.31**	**26.40**	**2.19**	**1.73**	**19.74**	**162.44**	**187.1**	**166.3**	**151.2**	**115.4**	**711.80**
S.D.	0.51	4.02	0.51	0.40	3.67	13.31	51.1	44.2	41.0	31.7	88.36

	HIIT Week 12								
	Oxygen Uptake (VO2)					Peak Heart Rate (BPM)	Workloads (Watts)		TTF (s)
Participant	Maximum (L/min)	Maximum (mL/kg/min)	RCP (L/min)	VT (L/min)	VT (mL/kg/min)		Peak	VT	
26	2.124	32.33	2.022	1.861	28.33	149.0	187.0	130.2	828.0
34	2.573	27.37	2.345	2.063	21.95	144.0	210.0	152.0	720.0
35	2.502	32.08	2.196	1.817	23.29	158.0	220.0	130.3	750.0
36	2.537	27.05	2.403	2.037	21.72	152	230.0	142.5	780.0
38	1.839	25.19	1.577	1.391	19.05	185	130.0	82.6	840.0
45	3.189	32.24	2.897	2.625	26.54	169	292.0	226.5	966.0
47	3.167	31.67	2.826	2.297	22.97	171.0	280.0	181.4	930.0
48	2.020	24.63	1.889	1.591	19.40	180.0	150.0	105.5	680.0
49	3.335	35.86	2.982	2.225	23.92	151.0	252.0	177.0	846.0
Mean	**2.59**	**29.83**	**2.35**	**1.99**	**23.02**	**162.11**	**216.8**	**147.6**	**815.56**
S.D.	0.54	3.86	0.48	0.37	3.02	14.63	54.8	43.1	93.86

VO_2 kinetic responses Week 0 and 12:

Control group VO_2 kinetic responses:

Control UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
3	0.9	0.7	18.2	36.5	112.3	26.3	1.5	0.2
10	0.6	0.5	9.0	27.5	140.4	39.8	1.1	0.2
12	1.4	0.9	29.0	30.0	153.6	42.2	2.2	0.3
18	0.6	0.7	17.0	31.9	160.8	42.5	1.3	0.1
21	0.8	0.8	24.0	33.9	150.0	53.9	1.6	0.2
22	0.7	0.4	12.5	30.1	96.0	18.0	1.1	0.1
31	1.1	1.2	23.0	33.9	151.2	57.8	2.3	0.3
33	0.9	1.8	22.7	30.0	156.0	36.4	2.7	0.3
41	0.8	0.7	20.0	39.8	124.8	106.6	1.5	0.1
42	0.9	0.5	17.0	30.8	111.0	114.6	1.4	0.1
Mean	0.9	0.8	19.2	32.4	135.6	53.8	1.7	0.2
Std Dev	0.2	0.4	5.8	3.6	22.8	32.1	0.5	0.1

Control UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
3	0.7	0.8	16.8	37.6	117.4	27.0	1.5	0.2
10	0.7	0.7	20.4	30.1	153.0	34.9	1.4	0.1
12	1.0	1.1	17.5	33.9	129.6	55.9	2.0	0.4
18	0.6	0.5	21.0	24.2	115.2	59.8	1.1	0.2
21	0.8	0.6	26.0	30.2	153.8	49.7	1.4	0.2
22	0.7	0.8	24.0	22.2	134.4	38.3	1.5	0.2
31	1.0	1.3	24.0	30.0	151.2	26.6	2.3	0.2
33	0.7	1.7	21.0	35.9	156.0	92.9	2.4	0.4
41	0.8	0.7	20.9	37.6	122.6	70.4	1.5	0.1
42	0.8	0.4	23.8	30.0	79.2	22.7	1.2	0.1
Mean	0.8	0.9	21.5	31.2	131.2	47.8	1.6	0.2
Std Dev	0.1	0.4	3.0	5.2	24.1	22.4	0.5	0.1

Control UnPrimed Wk 0

ID (DSP)	MRT
3	63.97
10	61.92
12	58.15
18	49.85
21	70.79
22	54.00
31	75.77
33	60.70
41	64.00
42	43.71
Mean	60.3
Std Dev	9.5

Control UnPrimed Wk 12

ID (DSP)	MRT
3	64.00
10	54.37
12	83.90
18	63.00
21	65.80
22	59.00
31	71.50
33	82.83
41	61.86
42	52.00
Mean	65.8
Std Dev	10.8

MICT group VO_2 kinetic responses:

MICT UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
4	0.9	1.5	20.0	35.9	150.0	53.9	2.5	0.4
7	1.0	0.8	22.0	32.0	168.0	55.9	1.8	0.4
8	1.3	0.4	28.5	43.3	154.2	70.7	1.7	0.3
15	1.1	1.6	12.0	40.6	148.5	44.2	2.7	0.3
20	1.2	1.2	22.9	33.9	127.2	34.6	2.3	0.3
23	0.7	0.8	20.0	33.9	137.4	30.4	1.6	0.1
24	0.9	0.7	13.0	35.9	123.9	34.7	1.6	0.1
28	1.2	0.6	21.0	33.9	138.0	87.1	1.8	0.4
30	0.9	1.2	28.0	32.0	134.4	77.3	2.0	0.3
39	0.6	0.6	21.0	33.9	150.0	75.4	1.2	0.3
Mean	1.0	0.9	20.8	35.5	143.2	56.4	1.9	0.29
Std Dev	0.2	0.4	5.3	3.7	13.4	20.3	0.5	0.1

MICT UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
4	1.0	1.8	21.0	28.1	147.8	46.1	2.8	0.1
7	0.9	1.2	22.0	30.0	148.8	40.3	2.0	0.1
8	1.0	0.6	20.0	18.3	188.8	30.5	1.6	0.1
15	1.1	1.9	21.0	26.9	150.0	21.1	3.0	0.2
20	1.0	0.7	21.0	20.3	147.8	24.7	1.8	0.1
23	0.7	0.9	18.0	20.3	126.2	16.7	1.6	0.1
24	0.8	1.1	18.0	26.1	138.2	20.8	1.9	0.1
28	0.9	1.4	23.0	24.2	130.0	28.6	2.3	0.1
30	0.8	1.4	21.0	22.2	151.2	38.3	2.2	0.2
39	0.8	0.6	24.0	28.1	148.0	52.0	1.4	0.1
Mean	0.9	1.2	20.9	24.4	147.7	31.9	2.1	0.12
Std Dev	0.1	0.5	1.9	4.0	16.9	11.8	0.5	0.0

MICT UnPrimed Wk 0	
ID (DSP)	MRT
4	82.40
7	91.00
8	102.49
15	72.00
20	74.90
23	60.00
24	69.50
28	75.70
30	58.70
39	66.70
Mean	75.3
Std Dev	13.6

MICT UnPrimed Wk 12	
ID (DSP)	MRT
4	46.00
7	52.00
8	37.00
15	44.00
20	41.00
23	35.00
24	48.00
28	42.00
30	44.00
39	44.00
Mean	43.3
Std Dev	5.0

HIIT group VO_2 kinetic responses:

HIIT UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
26	0.8	1.2	10.0	30.0	134.4	40.3	2.0	0.2
34	0.8	0.9	25.0	30.0	127.4	42.6	1.7	0.3
35	0.8	1.1	18.0	30.0	98.4	30.5	1.9	0.1
36	1.2	1.2	15.6	34.8	128.3	56.1	2.4	0.3
38	0.8	0.4	26.0	43.7	150.0	63.7	1.3	0.1
45	1.5	1.1	29.0	33.9	143.5	85.1	2.6	0.2
47	1.0	1.6	19.6	33.8	124.8	58.1	2.6	0.3
48	0.8	0.7	20.3	27.5	112.7	70.4	1.5	0.2
49	1.0	1.8	14.0	35.6	138.9	100.8	2.8	0.3
Mean	1.0	1.1	19.7	33.3	128.7	60.8	2.1	0.23
Std Dev	0.3	0.4	6.1	4.8	15.8	22.3	0.5	0.1

HIIT UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	TD3	TAU3	Base + A2	AMP SC
26	0.8	1.2	15.0	22.2	105.6	63.7	1.9	0.1
34	0.9	1.2	21.0	30.0	108.0	14.9	2.1	0.0
35	0.6	1.7	15.0	26.1	146.4	14.9	2.4	0.1
36	0.9	1.3	21.0	30.0	112.8	22.7	2.2	0.1
38	0.4	0.5	28.0	28.1	182.9	38.3	0.9	0.1
45	0.8	1.8	30.0	26.1	203.8	57.8	2.7	0.2
47	0.6	1.7	27.6	25.8	125.2	46.0	2.4	0.5
48	0.8	0.8	12.5	23.3	115.6	56.2	1.6	0.2
49	1.0	1.6	24.5	25.9	119.9	42.4	2.6	0.3
Mean	0.8	1.3	21.6	26.4	135.6	39.7	2.1	0.182
Std Dev	0.2	0.5	6.4	2.7	35.3	18.5	0.5	0.1

HIIT UnPrimed Wk 0	
ID (DSP)	MRT
26	48.45
34	81.00
35	47.00
36	51.00
38	66.30
45	63.35
47	58.70
48	61.95
49	47.98
Mean	58.4
Std Dev	11.2

HIIT UnPrimed Wk 12	
ID (DSP)	MRT
26	32.80
34	44.00
35	31.60
36	41.98
38	43.98
45	45.93
47	48.00
48	41.80
49	42.00
Mean	41.3
Std Dev	5.6

[HHb+Mb] kinetic responses Week 0 and 12:

Control group [HHb+Mb] kinetic responses:

Control UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	Amp3	TD3	TAU3	Base + A2
3	-162.66	88.99	6.01	16.36	22.18	118.93	38.37	-73.67
10	-65.32	7.71	22.95	14.24	7.52	97.02	55.70	-57.61
12	-191.38	254.00	10.00	14.40	4.01	106.00	5.15	62.62
18	-83.18	110.00	8.00	14.40	37.22	124.80	59.75	26.83
21	-78.32	46.50	9.00	14.40	14.22	124.80	34.40	-31.82
22	-61.12	23.42	12.00	14.40	17.50	105.60	61.70	-37.71
31	-23.11	155.42	9.00	18.30	45.67	150.00	67.55	132.31
33	-75.29	283.00	11.00	8.10	42.67	122.40	81.20	207.71
41	-102.47	5.99	11.81	14.00	9.22	128.11	34.48	-96.48
42	-39.88	5.50	13.97	14.20	5.15	129.00	31.60	-34.38
Mean	-88.3	98.1	11.4	14.3	20.5	120.7	47.0	9.8
Std Dev	52.2	103.0	4.7	2.6	15.8	15.0	22.2	97.8

Control UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	A3	TD3	TAU3	Base + A2
3	-74.23	115.00	8.02	16.38	9.99	100.75	16.85	40.77
10	-34.11	5.00	19.00	14.40	2.00	99.85	42.20	-29.106
12	-155.40	128.00	13.20	10.50	18.72	146.40	92.90	-27.4
18	-75.92	105.42	6.00	14.40	29.38	137.50	87.90	29.495
21	-124.70	64.41	11.00	12.37	56.35	130.17	61.54	-60.2939
22	-48.30	22.42	9.66	16.35	8.40	152.50	49.55	-25.887
31	-120.72	197.00	8.00	10.50	0.01	140.00	5.30	76.28
33	-133.09	297.50	12.00	11.00	44.38	150.00	79.05	164.41
41	-58.20	10.00	9.00	14.40	8.22	100.00	34.40	-48.204
42	-107.72	5.01	12.89	12.21	16.23	100.33	46.05	-102.711
Mean	-93.2	95.0	10.9	13.3	19.4	125.8	51.6	1.7
Std Dev	40.6	95.8	3.7	2.2	18.6	22.8	29.1	77.6

Control Wk 0

ID (DSP)	MRT
3	24.99
12	15.24
18	28.99
21	17.00
22	14.00
31	17.00
33	19.00
Mean	19.5
Std Dev	5.5

Control Wk 12

ID (DSP)	MRT
3	24.99
12	15.00
18	19.00
21	13.00
22	53.49
31	7.00
33	19.00
Mean	21.6
Std Dev	15.1

MICT group [HHb+Mb] kinetic responses:

MICT UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	A3	TD3	TAU3	Base + A2
4	-118.61	234.00	12.00	12.45	44.22	126.20	81.20	115.39
7	-105.12	184.00	8.00	16.35	22.22	126.20	59.75	78.88
8	20.19	34.00	8.08	26.28	36.36	89.79	44.08	54.19
15	-9.87	117.53	9.00	14.40	22.22	187.20	98.75	107.67
20	29.69	83.39	8.00	14.40	1.22	112.80	18.80	113.08
24	-75.40	118.50	5.00	13.40	2.90	74.40	16.85	43.10
28	-231.86	26.92	18.00	22.20	63.67	115.20	67.55	-204.94
30	-139.35	204.42	12.00	14.40	12.67	150.00	36.35	65.07
39	-5.37	123.00	13.00	12.45	47.67	158.40	85.10	117.63
Mean	-70.6	125.1	10.3	16.3	28.1	126.7	56.5	54.5
Std Dev	87.0	71.9	3.8	4.8	21.3	34.7	29.4	101.3

MICT UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	A3	TD3	TAU3	Base + A2
4	-61.13	154.00	14.00	33.90	1.00	141.60	9.05	92.875
7	-141.74	251.01	11.98	26.05	9.99	148.86	34.71	109.2673
8	-104.89	97.50	12.00	39.75	16.00	115.20	34.40	-7.39
15	-19.04	132.42	7.00	29.05	22.38	167.50	67.25	113.381
20	-66.14	111.42	7.00	31.95	22.38	140.00	73.15	45.282
24	-66.76	176.00	7.30	21.40	18.00	150.00	28.90	109.237
28	-60.22	163.00	5.00	24.15	8.00	150.00	17.10	102.781
30	-89.61	216.00	8.00	24.15	22.38	124.00	34.80	126.393
39	-30.25	158.00	11.00	43.65	44.27	146.00	59.75	127.754
Mean	-71.1	162.1	9.3	30.5	18.3	142.6	39.9	91.1
Std Dev	37.3	48.4	3.0	7.6	12.3	15.3	22.1	44.3

MICT Wk 0	
ID (DSP)	MRT
4	24.99
7	34.99
15	19.00
20	15.00
28	110.95
30	19.00
39	15.00
Mean	34.1
Std Dev	34.6

MICT Wk 12	
ID (DSP)	MRT
4	28.99
7	13.00
15	19.00
20	38.99
28	13.00
30	24.99
39	34.99
Mean	24.7
Std Dev	10.3

HIIT group [HHb+Mb] kinetic responses:

HIIT UnPrimed Wk 0								
ID (DSP)	Base	Amp2	TD2	TAU2	A3	TD3	TAU3	Base + A2
26	-39.65	61.82	7.00	12.45	9.22	93.60	44.15	22.166
34	-12.22	298.00	11.00	10.50	0.00	150.00	5.15	285.78
35	10.79	275.00	8.00	12.45	0.00	98.00	50.00	285.791
36	-68.85	155.14	9.00	7.02	3.01	146.59	6.00	86.294
38	-48.01	37.00	11.00	22.20	38.67	88.00	112.40	-11.014
45	-132.30	294.00	12.00	9.55	46.67	153.60	71.45	161.7
47	-52.25	338.40	8.00	12.45	0.01	157.91	0.39	286.155
48	-58.20	10.00	8.00	12.45	8.80	98.40	46.10	-48.204
49	-77.40	143.93	11.80	25.97	24.49	113.09	39.23	66.53354
Mean	-53.1	179.3	9.5	13.9	14.5	122.1	41.7	126.1
Std Dev	40.5	125.6	1.9	6.1	17.8	29.3	35.9	133.7

HIIT UnPrimed Wk 12								
ID (DSP)	Base	Amp2	TD2	TAU2	A3	TD3	TAU3	Base + A2
26	4.638	73	9	20.25	5.0009	127.5	25.95	77.638
34	3.88	341.50	8.00	14.10	18.38	102.50	20.05	345.382
35	-62.61	469.92	8.00	16.35	17.28	150.00	36.35	407.307
36	-63.86	267.92	10.00	10.50	8.28	150.00	34.40	204.061
38	-70.217	55	9	24.15	25.2757	117.6	63.65	-15.217
45	-30.29	243.99	12.00	16.69	22.26	144.13	42.46	213.6999
47	-142.84	318	10	14.4	46.2736	132	75.35	175.16
48	-58.20	11.00	8.00	20.25	9.28	156.00	36.35	-47.204
49	5.17	145.00	10.00	22.20	15.28	148.80	30.50	150.168
Mean	-46.0	213.9	9.3	17.7	18.6	136.5	40.6	167.9
Std Dev	48.3	153.1	1.3	4.4	12.3	17.9	17.9	150.2

HIIT Wk 0	
ID (DSP)	MRT
26	11.00
34	15.00
36	10.00
38	118.02
45	15.00
47	9.00
49	29.00
Mean	29.6
Std Dev	39.6

'HIIT Wk 12	
ID (DSP)	MRT
26	11.99
34	18.00
36	14.61
38	106.05
45	11.00
47	15.00
49	21.00
Mean	28.2
Std Dev	34.5

ΔHeart rate responses Week 0 and 12:

Con UP WK 0

ID (DSP)	Base	End Amp	Delta HR
3	109	131	22
10	98	143	46
12	98	152	54
18	126	168	42
21	92	136	44
22	99	164	65
31	107	175	68
33	100	153	53
41	113	158	45
42	97	127	30
Mean	104	151	47
Std Dev	10	16	14

Con UP WK 12

ID (DSP)	Base	End Amp	Delta HR
3	95	120	25
10	97	135	38
12	120	173	53
18	108	156	48
21	82	124	42
22	96	154	58
31	119	169	50
33	90	153	63
41	119	172	53
42	95	124	29
Mean	102	148	46
Std Dev	13	21	12

MICT UP WK 0

ID (DSP)	Base	End Amp	Delta HR
4	90	151	61
7	87	133	46
8	119	170	51
15	117	167	50
20	107	172	65
23	93	141	48
24	131	172	41
28	96	156	60
30	88	129	41
39	113	134	21
Mean	104	153	48
Std Dev	15	17	13

MICT UP WK 12

ID (DSP)	Base	End Amp	Delta HR
4	77	120	43
7	80	124	44
8	104	152	48
15	94	138	44
20	88	126	38
23	89	122	33
24	121	158	37
28	99	138	39
30	87	126	39
39	114	129	15
Mean	96	133	38
Std Dev	14	13	9

HIIT UP WK 0

ID (DSP)	Base	End Amp	Delta HR
26	82	141	59
34	102	144	42
35	101	153	52
36	105	147	42
38	115	162	47
45	90	154	64
47	108	165	58
48	119	172	53
49	111	154	43
Mean	104	155	51
Std Dev	12	10	8

HIIT UP WK 12

ID (DSP)	Base	End Amp	Delta HR
26	97	138	41
34	98	136	38
35	84	141	58
36	98	136	38
38	92	143	51
45	87	142	55
47	79	144	65
48	119	163	44
49	104	148	44
Mean	95	143	48
Std Dev	12	8	9

HbA_{1c} data pre and post intervention

Week 0	
MICT	HbA1c (%)
4	6
7	6.6
8	6.2
15	7.6
20	7.1
23	7.5
24	6.5
28	6.7
30	6.5
39	7.4
Mean	6.8
SD	0.6

Week 12	
MICT	HbA1c (%)
4	5.8
7	6.4
8	6.1
15	6.7
20	6.7
23	7.3
24	6.3
28	6.9
30	6
39	6.7
Mean	6.5
SD	0.5

Week 0	
HIIT	HbA1c (%)
26	7.9
34	7.2
35	7.5
36	7.3
47	7.4
48	6.3
49	7.7
Mean	7.3
SD	0.5

Week 12	
HIIT	HbA1c
26	6.2
34	5.3
35	6.7
36	6.9
47	7.6
48	6
49	7.5
Mean	6.6
SD	0.8

Week 0	
CON	HbA1c (%)
3	7.1
10	5.5
18	7.3
21	6.4
22	6.7
31	8.6
41	6.3
Mean	6.8
SD	1.0

Week 12	
CON	HbA1c
3	7.8
10	5.9
18	7.1
21	6.5
22	6.5
31	8.6
41	6.3
Mean	7.0
SD	0.9

RT3 accelerometry data: Week 0

Control Baseline RT3				
	Sed	Light	Mod	Vig
3	17.7	5.91	0.39	0
10	16.72	4.72	1.75	0.81
12				
18	17.35	5.91	0.74	0
21	19.03	4.7	0.25	0.01
22	18.98	4.86	0.14	0.02
31	17.85	4.63	1.37	0.15
33	17.65	4.84	1.18	0.34
41	20.28	3.12	0.52	0.07
42				
Mean	18.20	4.84	0.79	0.18
SD	1.15	0.87	0.58	0.28

MICT Baseline RT3				
	Sed	Light	Mod	Vig
4	17.61	4.58	1.6	0.21
7				
8	18.26	5.29	0.39	0.05
15	17.09	6.06	0.8	0.05
20	15.99	6.16	1.45	0.4
23	14.14	5.39	3.38	1.09
24	19.3	4.12	0.48	0.11
28				
30	18.39	5.24	0.31	0.06
39	17.35	4.72	0.88	0.28
Mean	17.27	5.20	1.16	0.28
SD	1.60	0.71	1.01	0.35

HIIT Baseline RT3				
	Sed	Light	Mod	Vig
26	16.7	6.12	1.08	0.1
34	14.25	7.49	1.98	0.29
35	20.06	3.55	0.37	0.02
36	17.41	5.31	0.85	0.43
38				
45	17.65	4.84	1.18	0.34
47	15.83	6.85	1.05	0.27
48	20.28	3.12	0.52	0.07
49				
Mean	17.45	5.33	1.00	0.22
SD	2.17	1.63	0.52	0.15