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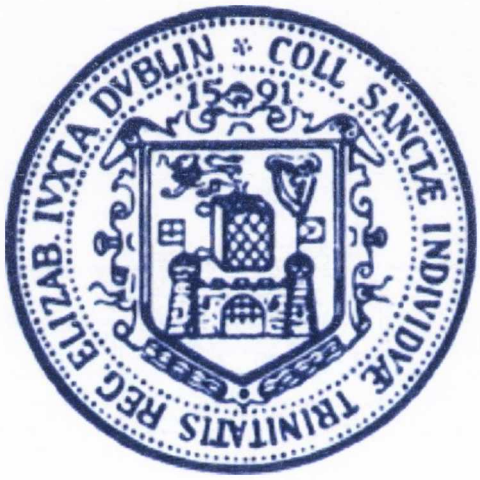
The effect of exercise and pioglitazone on the dynamic responses of oxygen uptake and leg vascular conductance in males with type 2 diabetes.



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

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I Declaration

I declare that the data contained within this thesis is entirely of my own work, with the exception of the reliability study assessing blood pressure readings from the COLIN and the Finometer, which was performed by Ms. Heather Reilly. Assistance in the supervision of study participants during gym sessions was provided by Ms. Catherine Kiely and Mr. Robert Maxwell. This thesis has not been previously submitted as an exercise for a degree in this or any other university. I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

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II Summary

The exercise capacity of an individual with T2D is often reduced due to the effects of T2D upon oxygen consumption- ($\dot{V}O_2$) and vascular conductance (VC)-kinetics. Exercise training has been shown to induce improvements in the $\dot{V}O_2$-kinetic and the VC-kinetic responses of individuals with T2D. Rosiglitazone use in T2D has resulted improvements in $\dot{V}O_2$peak in individuals with T2D, although it did not result in improvements in $\dot{V}O_2$ kinetics. However, no study has yet investigated the effects of treatment with pioglitazone (PIO) on measures of $\dot{V}O_2$peak, $\dot{V}O_2$ kinetic or VC kinetic responses. It has been shown that treatment with PIO induces similar effects on endothelial function as rosiglitazone, therefore PIO treatment may induce similar improvements in exercise performance.

The aims of this study were to assess $\dot{V}O_2$- and VC-kinetics in middle-aged men with T2D and non-diabetic (ND) males, prior to and in response to exercise training and treatment with PIO. Additionally, we collected blood samples for analysis of measures of glycaemic control, as well as determination of levels of known inflammatory and endothelial markers.

Initial baseline testing compared responses from ND men (n = 20) to those of age-, BMI- and activity level-matched males with T2D free from CVD and insulin use (n = 33). Subjects underwent testing on two separate days. On the first day of testing, VC kinetic responses using venous occlusion plethysmography were assessed in response to steady-state low (30% of maximum voluntary contraction (MVC)) and high (70% MVC) intensity exercise. VC responses to an incremental plantar-flexion test and forearm reactive hyperaemia were also assessed. Subjects then completed a graded cycle test to exhaustion to determine $\dot{V}O_2$peak and ventilatory threshold (VT). On the second day of testing, subjects $\dot{V}O_2$ kinetic responses were assessed during four bouts of steady-state submaximal cycling exercise at 80% VT. Subjects performed an additional two bouts during which cardiac output (CO) responses were measured at rest, 30s, and 240s into each bout using an inert gas rebreathe technique.

Subjects were then assigned to either an exercise (EXS) or control (CTL) condition, with some of the subjects with T2D prescribed a 30mg dose of PIO to be taken once daily. The EXS groups completed a twelve-week supervised training intervention exercising three days per week, followed by a sixteen-week unsupervised training period. At the end of each training period, subjects repeated the testing

protocol undertaken at baseline. Statistical analysis compared the ND CTL and EXS groups with the T2D EXS and CTL groups, with a secondary analysis then comparing the T2D EXS and CTL groups with the PIO-treated EXS and CTL groups.

Results indicated no differences in VC kinetic responses between the ND and T2D groups at baseline. $\dot{V}O_2$peak and VT were lower in the T2D group, with $\dot{V}O_2$ kinetic responses also slower in the T2D group. CO responses were also impaired during exercise in the T2D group. Analysis of blood samples indicated no differences between groups for circulating levels of inflammatory and endothelial markers, or mRNA expression of IL-6, TNF-α, and the adipocytokines visfatin and resistin.

Following the exercise intervention, the ND EXS group increased their $\dot{V}O_2$peak to a greater extent that the T2D EXS group. Treatment with PIO did not result in any increase in $\dot{V}O_2$peak. ND groups continued to display faster $\dot{V}O_2$ kinetics following the intervention, and neither ND nor T2D or PIO groups displayed a speeding of the kinetic response following the intervention. This was unexpected. However, all EXS groups improved peak workload and time to failure significantly from baseline, and the EXS groups showed an increased tolerance for higher workloads before reaching VT following the intervention. Following the intervention, there were no significant differences detected in the speed of the VC kinetic response between the ND and T2D groups as a consequence of exercise training. VC kinetic responses in the PIO treatment group following the intervention were similar to their T2D counterparts. Indices of glycaemic control were unchanged in the T2D and PIO groups following the intervention. Measures of IL-6 were increased in the ND exercise group at 3 months, while PIO treatment also resulted in increased levels of IL-6. Within the T2D and PIO groups, exercise resulted in reduced levels of ICAM-1. PIO treatment was also associated with increased mRNA expression of TNF-α and visfatin, and reduced expression of resistin.

Therefore performing exercise at the same relative workload post-intervention does not result in the speeding of either $\dot{V}O_2$ or VC kinetic responses to exercise that have been evident in previous studies. However, the impairment in VC kinetics previously demonstrated in individuals with T2D may be a consequence of exercise intensity. The added treatment of pioglitazone with exercise training in individuals with T2D did not result in any additional improvements in maximal exercise capacity or $\dot{V}O_2$ and VC kinetics that had been anticipated.

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IV Acknowledgements

I wish to thank my supervisor, Dr. Mikel Egaña, for providing me the opportunity to complete this study, and for his support and guidance during the past three years, throughout which he has contributed massively to my learning and development and I am massively grateful.

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This study could not have been completed without the participation of all those individuals who gave up countless hours to perform testing and especially those individuals in the EXS groups who willingly gave up many, many hours to attend the gym. I hope you got as much out of participating as you hoped, I thank you.

Finally, a big thank you to my family, whose unending support has allowed me to remain the 'permanent student'; without their constant love and encouragement I would not be where I am today.

V Abbreviations

A	Amplitude
a.u	Arbitrary unit
a-v O_2 diff	Difference in arterial-venous oxygen content
ABI	Ankle-brachial index
ACSM	American College of Sports Medicine
Adj R^2	Adjusted R-squared value
ANOVA	Analysis of variance
AUC	Area under the curve
BbB	Breath-by-breath
beats.min^{-1}	Beats per minute
BF	Blood flow
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
BRS	Baroreflex sensitivity
c	Decay constant
Ca^{2+}	Calcium
CHD	Coronary heart disease
CI	Cardiac index
CO	Cardiac output
CO_2	Carbon dioxide
CRP	C-reactive protein
CTL	Control
CVD	Cardiovascular disease
e	Exponential
E	Peak velocity of early diastolic filling
E'	Early diastolic mitral annular velocity
ECG	Electrocardiogram

Ed	Diastolic elastance
EDNO	Endothelium-derived nitric oxide
EDV	Endothelium-dependent vasodilation
ELISA	Enzyme-linked immunosorbent assay
End A	Steady-state amplitude
EXS	Exercise
FBF	Forearm blood flow
FBG	Fasting blood glucose
FFA	Free fatty acids
FPG	Fasting plasma glucose
FVC	Forearm vascular conductance
g	Gravity (unit of force)
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT4	Glucose transporter type 4
Hb	Haemoglobin
HbA_{1c}	Glycosylated haemoglobin
HCT	Haematocrit
HDL-C	High-density lipoprotein cholesterol
HHb	Deoxygenated haemoglobin
HR	Heart rate
$hr.wk^{-1}$	Hour per week
ICAM-1	Intercellular adhesion molecule-1
IL-6	Interleukin-6
IL-10	Interleukin-10
K^+	Potassium ion
KCl	Potassium chloride
kg	Kilogram
$kg.m^{-2}$	Kilograms per metre squared
KH_2PO_4	Potassium phosphate

$km.hr^{-1}$	Kilometres per hour
L	Litre
LBF	Leg blood flow
LDL-C	Low-density lipoprotein cholesterol
$L.min^{-1}$	Litres per minute
LVC	Leg vascular conductance
LVDD	Left ventricular diastolic dysfunction
m	Metre
MAP	Mean arterial pressure
MET	Metabolic equivalent
$MET.hr^{-1}.wk^{-1}$	Metabolic hours per week
$mg.day^{-1}$	Milligrams per day
$mg.dl^{-1}$	Milligrams per decilitre
min	Minute
ml	Millilitre
$ml.kg^{-1}.min^{-1}$	Millilitres per kilogram per minute
$ml.min^{-1}$	Millilitres per minute
$ml.min^{-1}.mmHg^{-1}$	Millilitres per minute per millimetre of mercury
$ml.100ml^{-1}.min^{-1}.mmHg^{-1}$	Millilitres per minute per millimetre of mercury
ml O_2.100ml $blood^{-1}$	Millilitres of oxygen per hundred millilitres of blood
$ml.min^{-1}.W^{-1}$	Millilitres per minute per watt
mmHg	Millimetres of mercury
$mmHg.L^{-1}.min^{-1}$	Millimetres of mercury per litre per minute
$mmol.L^{-1}$	Millimoles per litre
$mmol.mol^{-1}$	Millimoles per mole
MMP-9	Matrix metalloproteinase-9
MRT	Mean response time
MSD	Mesoscale Discovery®
MVC	Maximum voluntary contraction

N	Newtons
NaCl	Sodium chloride
Na_2HPO_4	Sodium phosphate, dibasic
ND	Non-diabetics
NEFA	Non-esterified fatty acids
ng	Nanogram
$ng.ml^{-1}$	Nanograms per millilitre
nm	Nanometre
NO	Nitric oxide
N_2O	Nitrous oxide
OD	Optical density
O_2	Oxygen
PAD	Peripheral arterial disease
PAI-1	plasminogen activator inhibitor-1
PCR	Polymerase chain reaction
PCWP	Pulmonary capillary wedge pressure
pg	Picogram
$pg.ml^{-1}$	Picogram per millilitre
PIO	Pioglitazone
PO_2	Partial O_2 pressure
PPAR	Peroxisome proliferator-activated receptor
PRT	Progressive resistance training
QaO_2	Systemic O_2 delivery
RBC	Red blood cells
RER	Respiratory exchange ratio
RH	Reactive hyperaemia
RM	Repeated measures
ROS	Reactive oxygen species
rpm	Revolutions per minute

RSG	Rosiglitazone
RVU	Rebreathing valve unit
s	Second
SAA	Serum amyloid A
sd	Standard deviation
SF_6	Sulphur hexafluoride
SV	Stroke volume
SVI	Stroke volume index
t	Time
$t_{1/2}$	Half-life
T2D	Type 2 diabetes
TD	Time delay
TNF-α	Tumour necrosis factor-alpha
TPR	Total peripheral resistance
TZD	Thiazolidinedione
UCP	Uncoupling protein
VC	Vascular conductance
VCAM-1	Vascular cell adhesion molecule-1
$\dot{V}CO_2$	Rate of carbon dioxide production
$\dot{V}_e$	Minute ventilation
VT	Ventilatory threshold
$\dot{V}O_2$	Rate of oxygen consumption
$\dot{V}O_2$ at VT	Oxygen consumption at the ventilatory threshold
$\dot{V}O_2$ gain	Increase in oxygen consumption per unit of workload
$\dot{V}O_{2m}$	Muscle oxygen consumption
$\dot{V}O_2$peak	Peak oxygen consumption
W	Watt
WHO	World Health Organisation
WHR	Waist-hip ratio

1RM	1 repetition maximum
Δ 50% peak-VT	Midpoint between peak workload and ventilatory threshold
τ	Time constant
μl	Microlitre
$\mu l.well^{-1}$	Microlitres per well
^{o}	Degrees
%	Percent

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CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION TO TYPE 2 DIABETES

1.1.1 General Information

Diabetes mellitus, and in particular type 2 diabetes mellitus (T2D), is becoming increasingly prevalent in both developed and developing nations. In 2003, it was estimated that there were 194 million cases of diagnosed diabetes worldwide, of which T2D accounted for between 85-95% of all cases in the developed world, and almost all cases in developing countries (Waugh *et al.*, 2006). The rising prevalence in developing countries is attributed to increased longevity among the population and the adoption of Western practices in both diet and exercise (Goldhaber-Fiebert *et al.*, 2003). Furthermore it has been predicted that worldwide prevalence will nearly double by the year 2030 to 366 million people (Wild *et al.*, 2004). This epidemic rise in the prevalence of T2D has extremely important consequences for both individuals and society in general.

Increasing rates of T2D will lead to a rise in health costs, both in terms of direct costs associated with the treatment of T2D, and an increase in the treatment of comorbid conditions associated with T2D, such as cardiovascular disease (CVD), of which diabetic patients are at increased risk (Tolman *et al.*, 2004). In the U.S., approximately 14% of total health care expenditure is associated with the direct treatment of diabetes (Wagstaff & Goa, 2002), with the costs of treatment exceeding $100 billion each year (Yach *et al.*, 2006).

In the treatment of T2D, physical activity has been shown to improve glycaemic control and insulin sensitivity, with certain minimum levels of activity recommended in the treatment of T2D (Haskell *et al.*, 2007; Nelson *et al.*, 2007). However, at present, less than half of U.S. adults perform the minimum recommended volume of physical activity (Morrato *et al.*, 2007); and cross-population studies have indicated no significant differences between U.S. and non-U.S. populations. Therefore the general lifestyle of the worldwide population at present is inadequate to deal with the rising epidemic of T2D. With specific regard to individuals with T2D, they typically display both an aversion and intolerance to exercise, with peak exercise capacity (i.e. peak oxygen uptake – $\dot{V}O_2$peak) significantly lower than $\dot{V}O_2$peak values of age-, weight, and activity-level-matched non-diabetic (ND) counterparts

(Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Lalande *et al.*, 2008). Additionally, individuals with T2D also display a slower rate of increase in $\dot{V}O_2$ uptake ($\dot{V}O_2$ kinetics) and blood flow (BF) kinetic responses compared to ND counterparts (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Kingwell *et al.*, 2003; Lalande *et al.*, 2008; Macananey *et al.*, 2011). This would indicate impaired delivery to and/or utilisation of oxygen (O_2) within the muscle. This would cause the individual to increasingly rely on anaerobic metabolism and lead to an increased level of recruitment of type II muscle fibres, which fatigue more quickly (Regensteiner *et al.*, 1998; Bauer *et al.*, 2007).

Exercise training has been shown to significantly speed the $\dot{V}O_2$ kinetic response in a diabetic population (Brandenburg *et al.*, 1999). It was speculated that the improvement in the kinetic response was not solely due to the metabolic effects of the training intervention, but that improvements in the circulatory and cardiovascular responses to exercise may also be involved, which would have obvious benefits for a T2D population.

Additionally, it has been demonstrated that pharmacological intervention with the thiazolidinedione (TZD) rosiglitazone leads to improvements in $\dot{V}O_2$peak in a cohort of individuals with T2D (Regensteiner *et al.*, 2005; Kadoglou *et al.*, 2008). It stands to reason that treatment with TZDs may also have positive effects on $\dot{V}O_2$ kinetics.

The purpose of this introduction is to analyse some of the characteristics of T2D which might impact upon exercise capacity, with specific regard to assessing BF and $\dot{V}O_2$ kinetic responses to constant-load exercise in a ND and T2D population. Additionally existing literature examining the impact of interventions (both exercise and pharmacological) on these kinetic responses will be critically analysed.

1.1.2 Characteristics of Individuals with T2D

T2D is a chronic condition in which the body cannot effectively utilise the insulin produced by the pancreas to fully regulate sugar levels in the blood. This results in an increase in the levels of circulating blood sugar levels, a condition known as hyperglycaemia. At present, diagnosis of T2D is defined (according to the World Health Organisation (WHO)) as fasting plasma glucose (FPG) levels $\geq$ 7.0mmol.L^{-1} or 2-hr plasma glucose levels $\geq$ 11.1mmol.L^{-1} following an oral glucose tolerance test.

Glycosylated haemoglobin (HbA_{1c}) is not considered to be a suitable tool for diagnosis of T2D according to WHO, although the American College of Sports Medicine (ACSM) currently states that HbA_{1c} levels in excess of 48mmol.mol^{-1} is grounds for diagnosis (Colberg *et al.*, 2010).

Initially, subjects who develop T2D display elevated levels of insulin in the circulation. A persistent elevation in insulin levels is known as hyperinsulinaemia. Hyperinsulinaemia is capable of maintaining normal glucose metabolism as long as pancreatic β-cell remain functional (Wagstaff & Goa, 2002). However, as β-cell function deteriorates, plasma glucose levels begin to rise. One measure that is used to determine the overall effect of raised blood glucose levels is HbA_{1c} levels. HbA_{1c} arises when normal haemoglobin (Hb) is continuously exposed to glucose and the glucose then binds with the Hb non-enzymatically forming a glycoprotein. Once the Hb molecule is glycosylated, it remains that way. Thus HbA_{1c} provide an indication of average plasma glucose levels over the previous four weeks to three months, given that the mean lifespan of the red blood cell is approximately 120 days. HbA_{1c} concentrations above 42mmol.mol^{-1} (6.0%) are believed to represent a two-fold increase in the likelihood of progressing from a pre-diabetic state to a diagnosis of T2D (Nathan *et al.*, 2007). In addition, HbA_{1c} levels provide a good indication of the excess mortality risk associated with T2D, as a 12.6mmol.mol^{-1} (1%) increase in HbA_{1c} is associated with a 28% independent increase in risk of mortality (Dunstan *et al.*, 2002); while a 12.6mmol.mol^{-1} (1%) decrease is associated with a 21% drop in risk of death (Rosak *et al.*, 2005; Richter *et al.*, 2007), a 14% decrease in risk of myocardial infarction (Richter *et al.*, 2007), and a 35% reduction in microvascular complications (Dunstan *et al.*, 2002). Diabetic patients also have increased levels of small low-density lipoproteins (LDL), which are known to increase atherogenicity, which can in turn partly explain the increased risk of CVD associated with T2D (Stirban & Tschoepe, 2008).

1.1.3 Risk Factors Associated with Type 2 Diabetes

There are a number of risk factors that have been identified to increase the likelihood of development of T2D. These risk factors have been determined using population-based, epidemiological studies of both T2D and CVD, and include age, ethnicity, obesity, lipid profile, blood pressure (BP), and fasting plasma glucose (FPG) concentration (Abdul-Ghani *et al.*, 2007), with genetic predisposition the most

important risk factor for the development of T2D (Scheen, 2003). Supporting evidence for a genetic influence on development of T2D is found in the concordance rates of T2D in twins, with monozygotic twins displaying higher incidence rates than dizygotic twins (O'Rahilly *et al.*, 2005). Additionally, recurrence rates of T2D are higher for siblings than for the general population (O'Rahilly *et al.*, 2005). A counterargument to this finding is that siblings will lead very similar lifestyles, especially in their youth, and therefore are be more likely to display higher rates of incidence. Proponents of this argument draw support from a study of Pima Indians (Khamaisi & Raz, 2002). The extremely high frequency of T2D in Pima Indians living on the reservation was seen as possible evidence of a genetic/ethnic component to T2D. However, the lack of T2D among non-reservation Pima Indians was considered evidence that the high rates of T2D in reservation Indians reflected changes in the lifestyle of the Indians, and not the expression of a diabetic genotype. However, it is acknowledged that the contribution of heredity differs considerably between different populations and in different environments (O'Rahilly *et al.*, 2005), with diagnosis of T2D among Latinos double that of Caucasians (Castaneda *et al.*, 2002). While there has not, as yet, been identified a specific single 'diabetes gene', it is likely that T2D is heterogeneous, resulting from defects in one or more molecular pathways. These defects are likely caused by single nucleotide polymorphisms, which appear to be involved in increasing risk of T2D (O'Rahilly *et al.*, 2005).

Obesity is an extremely prominent risk factor, with over 80% of diabetics being either overweight or obese (Wagstaff & Goa, 2002). The risk of T2D also increases exponentially with increases in body mass index (BMI), with any moderate excess body weight becoming a much greater risk factor if this excess body weight is in the form of visceral body fat. Visceral fat is more prevalent in men, suggesting an increased risk of diabetes in males versus females (Scheen, 2003). The association between obesity and T2D is due to numerous interactions between the function of adipose tissue as an endocrine organ and the contribution of adipose tissue to insulin resistance. The function of adipose tissue as an endocrine organ is influenced by a number of factors, including the location of the adipose tissue (visceral vs. subcutaneous), the size of the adipocytes, and adipocytes' metabolism of glucose and corticosteroids (Lazar, 2005). In obese individuals, increased lipolysis contributes to high circulating levels of non-esterified fatty acids (NEFA), while insulin resistance of the liver leads to elevated hepatic output of triglyceride-rich particles (Tushuizen *et*

al., 2007). The increase in circulating NEFA and triglycerides leads to increased atherosclerosis and impaired BF in obese individuals. Insulin resistance can further aggravate this, as insulin resistance is associated with an impaired ability of exercise to stimulate muscle BF, O_2 delivery and glucose uptake (Hallsten *et al.*, 2003).

1.2 The Effect of T2D on Acute Exercise Performance

1.2.1 Impact of T2D on the Cardiovascular System

The highest rates of comorbidity associated with T2D arise from cardiovascular complications. Cardiovascular disease (CVD) is responsible for as much as 70% of mortality rates in T2D, with the relative risk for cardiovascular-related mortality increasing 2-3 fold for men with T2D, and 3-4 fold for women (Richter *et al.*, 2006).

T2D is also a predictor for peripheral arterial disease (PAD) and intermittent claudication (Green *et al.*, 2007). The intermittent claudication arises from the atherosclerotic narrowing of the major resistance vessels supplying the lower limbs, resulting in a reduction in maximum BF to the extremities during exercise. This reduction in BF to the extremities during exercise is also evident in diabetic patients without PAD or any other clinical CVD (Kingwell *et al.*, 2003; Lalande *et al.*, 2008), resulting in a reduced rate of O_2 supply to the working muscles, as evident in the significantly longer time constant (τ) for O_2 uptake during submaximal treadmill and cycling exercise (Green *et al.*, 2007), and longer τ values for leg vascular conductance (LVC) during calf plantar-flexion exercise (Macananey *et al.*, 2011) for diabetics versus non-diabetics.

In order to properly determine the effect of T2D-related impairments on the cardiovasculature, it is necessary to discuss BF responses to exercise under normal conditions.

1.2.2 Blood Flow Dynamics

There are a number of contributing factors to the BF response during exercise. Firstly, normal physiological concentrations of insulin have been shown to increase skeletal muscle BF, which in turn leads to increases in insulin and glucose delivery (Steinberg *et al.*, 1994). This increase in BF augments the ability of insulin to stimulate glucose uptake via greater capillary bed and skeletal muscle perfusion.

Insulin's ability to increase BF is directly related to the degree of insulin sensitivity of vascular smooth muscle; and is inversely related to the degree of obesity of an individual (Steinberg *et al.*, 1994). However, insulin is not the only factor that regulates BF and VC, as skeletal muscle BF and VC are regulated by a number of factors controlled by both central and local control mechanisms (Delp, 1999).

Onset of exercise results in a characteristic BF and VC response. Until recently, BF responses were typically characterised by a bi-phasic response, with a third phase sometimes present at high-intensity workrates, with the BF and VC response thought to contain only 'growth' phases, as displayed in figure 1.1 (Saunders *et al.*, 2005; Tschakovsky *et al.*, 2006). However, analysis of the BF and VC responses to plantar-flexion exercise (at both 30% and 70% MVC) in the present thesis indicated that the BF and VC responses were best fitted using either a tri-phasic model (with 2 'growth' phases and 1 'decay' phase) or a quad-phasic model (2 growth & 2 decay phases); which is consistent with recent findings by Reeder & Green (2012) utilising the same exercise model.

The basis for choosing between differing models of BF and VC responses is determined by implementing the model that provides the best 'goodness-of-fit', or the strongest mathematical model on a statistical basis (Lamarra, 1990), and data from Reeder & Green (2012) demonstrate that the quad-phasic model is strongest from a statistical basis for plantar-flexion at 30% MVC, and the tri-phasic model (with 1 decay phase) is strongest for plantar-flexion at 70% MVC (appendix I).

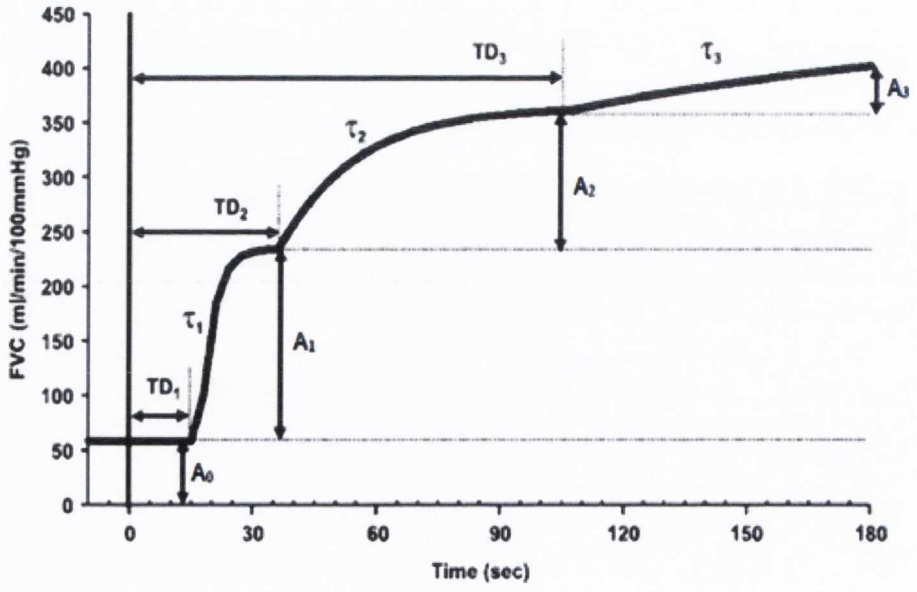


Figure 1.1. The standard tri-phasic model used to characterise leg VC responses to onset of exercise. The third phase (characterised by τ_3 and A_3) is typically only evident at high intensities.

The pattern of BF during exercise can be divided into four distinct phases, with two 'growth' and two 'decay' phases evident (Figure 1.2). Each phase is defined by an amplitude (A), time delay (TD) and time constant (τ), as well as its corresponding number between one and four, according to when each phase appears. For example, TD_2 and A_3 represent the time delay and amplitude of phases 2 and 3, respectively. The initial, or transitional, phase is characterised by a rapid increase (hyperaemia) in VC within the first 1-2 seconds of exercise. The most likely cause of the initial hyperaemia is the muscle pump mechanism either through imparting kinetic energy to the blood or by mechanical deformation of the vascular wall due to muscle contraction, with the increased VC resulting from changes in the myogenic tone of smooth muscle cells in response to increases in intravascular pressure (Delp, 1999; Clifford, 2007).

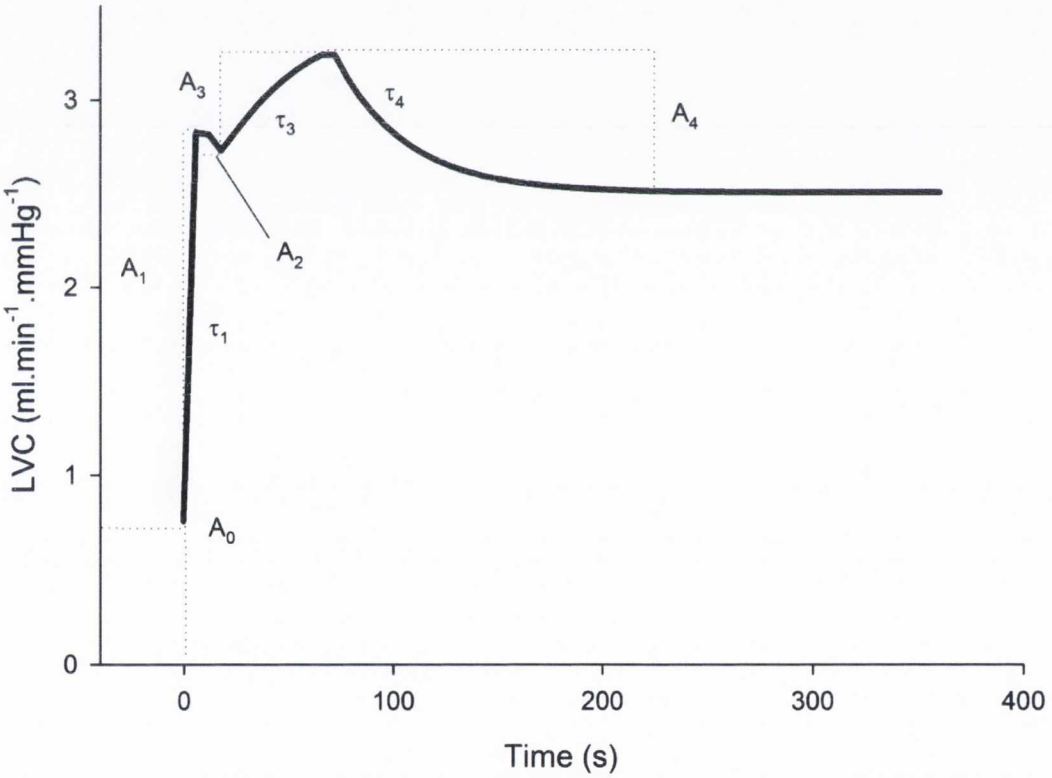


Figure 1.2. Representative LVC kinetic response to exercise in the calf muscle at 30% MVC. TD represents the time delay from the onset of exercise to the increase in flow of a particular phase. τ represents the time constant in seconds (s), the time taken to reach ~63% of the response of that particular phase. A refers to the amplitude, or change in flow. Subscripts refer to the individual phases.

The second phase witnesses a rapid decrease in flow, which has previously been noted, described as an 'overshoot' in the hyperaemic responses during knee extensor (Shoemaker *et al.*, 1996) and forearm exercise (Saunders *et al.*, 2005) at low-

moderate intensities; indicating that previous studies have also noted the first or 'fast' decay, but have not attempted to apply a model that accommodates it. Physiological causes of this decay have yet to be fully elucidated, but both the Bayliss mechanism and/or an increase in muscle sympathetic (noradrenergic) nerve activation offer potential physiological bases for this phenomenon. The length of action of the second phase (fast decay) of the BF/VC response during exercise is primarily determined by the delay in onset of vasodilators that induce a sustained increase in flow. The actions of these vasodilators characterise the third phase, or second increase. The magnitude of vasodilation is dose-dependent (Wunsch *et al.*, 2000), therefore the greater the workload being imparted the greater the increase in BF/VC in this third phase. Power output and BF are linearly coupled up to peak intensities, with BF/VC reaching a stable level within 30-90 seconds depending on intensity (Saltin *et al.*, 1998). The achievement of steady state is believed to be due to the interaction of a number of mechanisms rather than one controlling factor. These mechanisms are thought to include the accumulation of metabolic vasodilators, vasodilation due to shear-stress exerted on the vascular walls due to the increase in BF to the active musculature, and deoxygenation of red blood cells, which has been shown to have local vasodilatory effects (Tschakovsky *et al.*, 2006). The interaction of these factors and their effect on the vasculature around the body would suggest that there isn't a single vasodilatory mechanism responsible for the BF/VC response to exercise, but rather the dynamic BF response is the result of the interaction of different systems (Tschakovsky *et al.*, 2006). Some factors that have been identified as having a strong vasodilatory response include nitric oxide (NO) and insulin. Since individuals with T2D are resistant to insulin, it is logical to assume that this in turn may reduce the vasodilatory capacity of the vasculature, and in turn BF dynamics during exercise.

It has also been shown that the rise in BF/VC appears to be a consequence of the increased demand for O_2 delivery (and in turn BF) being sensed in relation to the capacity of the heart and being integrated into the signalling of the sympathetic nervous system. The activity of the sympathetic nerves of the blood vessels supplying the muscles increases gradually without causing a reduction in BF/VC until a reduction in BF becomes necessary to maintain blood pressure (Saltin *et al.*, 1998). This 'functional sympatholysis' refers to the concept that sympathetic vasoconstriction in muscle may be overridden by local control factors until the signal from the sympathetic nerves overpowers that of local factors (Delp, 1999), and can

result in the presence of a fourth phase in the BF/VC model. The fourth phase of the BF/VC response is not always present, but appears to be more prevalent during exercise at lower intensities. The point of onset of the fourth phase can also vary between individuals. What is clear is that at present, there is a paucity of information describing the dynamics of the BF/VC response at various intensities, and is an area worthy of further investigation. However, findings by Reeder & Green (2012) have demonstrated that the time delay for the fourth phase coincided with the time delay for the slow decay in EMG activity of the active musculature. A decrease in EMG activity during constant workload stimuli has been previously demonstrated (Scheuermann *et al.*, 2001). Potential physiological bases for the declining EMG activity are the increase in force-generating capacity of muscle fibres due to either increasing muscle temperature and/or phosphorylation of myosin light chains (Sargeant, 1987; Oskouei & Herzog, 2009), inducing a reduction in the number of active motor units required to sustain the target force. This leads to a reduced demand for muscle BF, and would offer a physiological basis for the slow decay in the hyperaemic response.

The BF/VC model just discussed relies on healthy endothelial function, which allows vasodilation, increased flow, and decreased resistance. The presence of endothelial dysfunction will result in impaired vasodilation/vasoconstriction (Maiorana *et al.*, 2001a; Stirban & Tschoepe, 2008). A number of the risk factors associated with T2D are also associated with endothelial dysfunction, such as increased BMI, ageing, and body fat content, which lead to stiffening of the arteries and an increase in low-grade inflammation of the vasculature (Wykretowicz *et al.*, 2007). Mortality rates related to T2D are significantly associated with atherosclerotic macrovascular complications, while morbidity rates with T2D are in part often the result of microvascular dysfunction, with its subsequent onset of retinopathy, neuropathy, and nephropathy (Maiorana *et al.*, 2001a).

T2D or insulin resistance may inhibit the ability of the vasculature to dilate to the extent necessary to allow adequate oxygen delivery to the active skeletal muscle (Colberg *et al.*, 2003). If individuals with T2D therefore attempt to exercise with inadequate oxygen supply, they will display a reduced exercise capacity in comparison to healthy subjects. Results of a study by Cleland *et al.* (2000) investigating insulin-mediated vasodilation and endothelium-derived nitric-oxide (EDNO) production in subjects with T2D indicated that a significant positive

relationship exists between the vascular and metabolic effects of insulin. However, while the group with T2D displayed a trend for reduced action of insulin on the vasculature, there was no significant difference compared with the control group. Cleland *et al.* (2000) concluded that the data supported the hypothesis that insulin plays an important role in the maintenance of vascular endothelial function, to the extent that impaired insulin action would cause relative endothelial dysfunction, in addition to its negative impact upon the metabolism. As such, it is possible that T2D may result in an impaired blood flow response compared to the model discussed above.

Endothelial dysfunction associated with the hyperinsulinaemic state is characterised by elevated levels of endothelial and inflammatory markers in response to the rise in reactive oxygen species (ROS) and increased oxidative stress (Shanik *et al.*, 2008). Typical measures of endothelial function include elevated levels of plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin and Endothelin-1 taken from plasma samples (Stirban & Tschoepe, 2008). Increased production of acute-phase reactants results from stimulation by cytokines that are derived from ROS generation (Wright *et al.*, 2006).

The majority of the inflammatory markers of concern are adipocytokines – cytokines released by adipose tissue into systemic circulation. It is established that increased adipose tissue is associated with insulin resistance. Adipose tissue, especially visceral fat, results in increased circulation of free fatty acids (FFA) that inhibit the action of insulin via the Randle cycle (the competition between glucose and fatty acids for substrates) in insulin-sensitive tissues. The reduced use of glucose in muscle stimulates hepatic output of glucose and very low-density lipoprotein-cholesterol (VLDL-C), in turn resulting in glucose-stimulated insulin secretion (Ronti *et al.*, 2006). Glycation and glycoxidation of the VLDL-C has been shown to increase PAI-1 and other endothelial and inflammatory markers (Wright *et al.*, 2006).

1.2.3 Maximal Oxygen Consumption & Oxygen Uptake Kinetics during Acute Exercise

Oxygen uptake kinetics are typically assessed during steady-state submaximal exercise, performed at a percentage of the subject's maximal oxygen uptake ($\dot{V}O_2max$). $\dot{V}O_2max$ is related to a number of metabolic factors, including the volume

of mitochondria, the rate of respiration in individual mitochondrion, and/or the number of active mitochondria within the muscle fibre. The volume of mitochondria within the muscle fibre will also vary according to the muscle fibre type (Dudley *et al.*, 1987). When a 'true' $\dot{V}O_2max$ is achieved, a plateau is often seen with increasing workloads, as $\dot{V}O_2max$ is limited by those factors (thickness of the diffusion barrier, the amount of time the blood spends in capillary, etc) that provide resistance to tissue gas exchange (Roca *et al.*, 1989; Turner *et al.*, 1993). Achievement of $\dot{V}O_2max$ and progression to higher workloads requires increasing levels of ATP hydrolysis, which can be only be sustained for a limited time due to greater reliance on increasing levels of anaerobic metabolism and PCr degradation, which cannot be sustained for very long. As a consequence, achievement of $\dot{V}O_2max$ is typically used as a measure of a person's exercise capacity.

However, physically untrained subjects, or subjects unfamiliar with ergometric testing, do not always display a 'true' $\dot{V}O_2max$, a phenomenon that can also occur when using an exercise protocol that does not utilise whole body muscle mass, or only smaller muscle masses (Noakes, 1988). To overcome this problem, '$\dot{V}O_2peak$' has been adopted as the premier descriptor for highest levels of $\dot{V}O_2$ achieved when a true $\dot{V}O_2max$ may not be determined. Peak $\dot{V}O_2$ values measured in untrained subjects are only a fraction of $\dot{V}O_2max$, but are often used as an indicator of exercise capacity, as other variables such as heart rate and lactate, often suggest that the subject is indeed operating at, or very close to, their maximal aerobic capacity. Exercise training and increased familiarisation with the testing protocol can increase the percentage of $\dot{V}O_2max$ achieved.

Assessment of oxygen uptake ($\dot{V}O_2$) kinetics currently utilises a three-phase model, presented in Figure 1.3 (Stirling *et al.*, 2005; Korzeniewski & Zoladz, 2006). These three phases can be described as the cardiodynamic, the primary, and the slow components. The rate of O_2 consumption at the onset of the cardiodynamic phase is fitted using an exponential term (τ_1) to allow the mathematical modelling of the $\dot{V}O_2$ kinetic response (Barstow *et al.*, 1996; MacDonald *et al.*, 1998). This results in the subject incurring an O_2 deficit (Lador *et al.*, 2006). The primary component can be described as a monoexponential increase in O_2 consumption rising to steady state consumption. The kinetic time constant τ_2 (i.e. the time to reach ~63% of the exponential response) used to describe the primary component ranges between 20-60s

in healthy individuals, and can characterise the training status of skeletal muscle in humans (Korzeniewski & Zoladz, 2004). The third phase, the slow component, is only witnessed during exercise performed at intensities above the ventilatory threshold (VT). However, the correlation between the slow component and the blood lactate profile is coincidental and not causal, and is thought to reflect recruitment of type II muscle fibres, which have slower kinetics due to less efficient oxidative metabolism (Stirling *et al.*, 2005).

The primary component of the $\dot{V}O_2$ kinetic response reflects the increased O_2 transfer from ambient air to the mitochondria. This is achieved via increased O_2 uptake and systemic O_2 delivery (Lador *et al.*, 2006). Assuming that arterial O_2 concentration is the same at rest and during aerobic exercise, the increase in systemic O_2 delivery (QaO_2) is achieved via an increase in BF. However, while the models for $\dot{V}O_2$ and BF kinetics are similar in profile at the onset of exercise, BF kinetics (and therefore also QaO_2 kinetics) are twice as fast as those of $\dot{V}O_2$ (Lador *et al.*, 2006). This increase in BF at the onset of exercise is the dominant cause of the initial increase in $\dot{V}O_2$ at the onset of exercise, hence phase I is referred to as the cardiodynamic component (De Cort *et al.*, 1991). The faster kinetics of BF compared to $\dot{V}O_2$ suggests that O_2 delivery to the working muscles is capable of satisfying demand at the onset of exercise (Tschakovsky & Hughson, 1999).

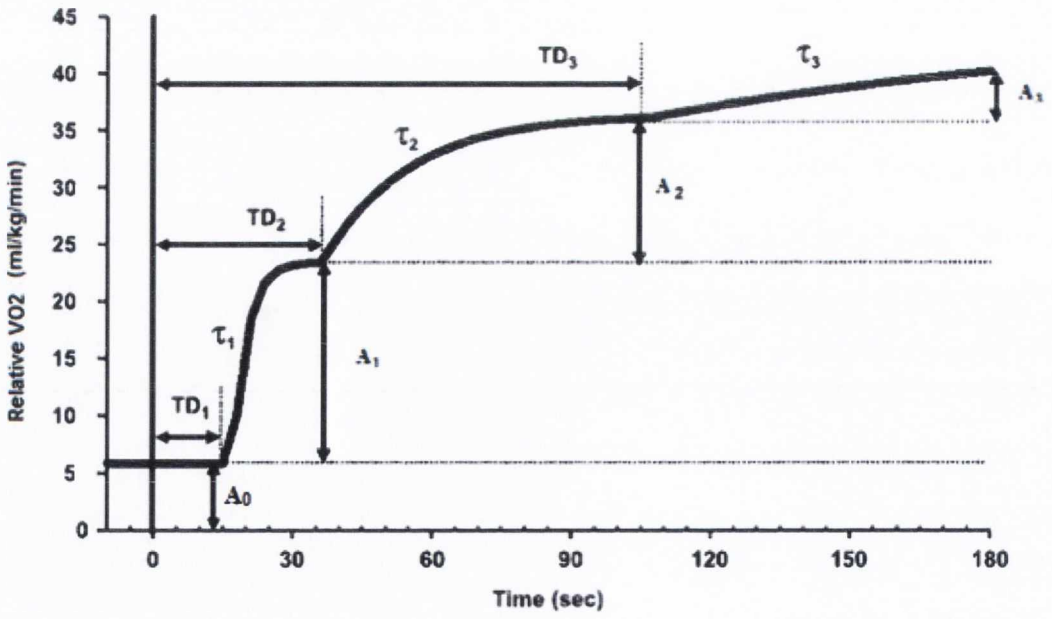


Figure 1.3. A representative model of the $\dot{V}O_2$ kinetic response to exercise. TD represents the time delay from the onset of exercise to the increase in flow of a particular phase. τ, represents the time constant in seconds (s), the time taken to reach ~63% of the response of that particular phase. A refers to the amplitude, or increase in flow. Subscripts refer to the individual phases.

The presence of an O_2 deficit is evidence that there are limitations placed on the efficiency of $\dot{V}O_2$ on-kinetics. The root cause of this limitation has been the subject of much research, and it is believed that $\dot{V}O_2$ kinetics are limited either in the delivery of O_2 to the muscle, or by a rate limitation in the utilisation of O_2, reflecting some form of metabolic inertia (Tschakovsky & Hughson, 1999; Poole *et al.*, 2008).

Previous research has demonstrated that the rate of increase in $\dot{V}O_2$ during the transition from rest to constant-load exercise is slower in females with T2D compared to healthy females, as demonstrated in figure 1.4 (characterised by lengthening of τ) (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011) and in a mixed cohort of males and females (Bauer *et al.*, 2007). Figure 1.4 illustrates the $\dot{V}O_2$ kinetic response to transition from rest to exercise at 30W in a ND and T2D individual in a study performed by Brandenburg *et al.* (1999), which clearly demonstrates the impaired kinetic response in the female with T2D. However, a recent study by Wilkerson *et al.* (2011) did not find any difference in τ values between older males with and without T2D. A longer τ results in an increased O_2 deficit, and an increase in levels of the factors implicated in fatigue, in turn reducing exercise capacity (Poole *et al.*, 2008). Fatigue during dynamic exercise is associated with changes in concentration of ATP, PCr, phosphate (P_i), and lactate. Reduced concentrations of PCr appear to indicate an increased state of fatigue, rather than a direct cause (Lamb & Stephenson, 1991). Exercise that results in a large increase in the concentration of P_i, means that more P_i is able to freely enter the sarcoplasmic reticulum, where it can precipitate and form calcium phosphate, which in turn reduces the amount of free calcium available for release into the sarcoplasm (Fryer *et al.*, 1995; Posterino & Fryer, 1998). This in turn reduces the amount of calcium released for cross-bridge formation, in turn reducing force production and increasing fatigue. While lactate production is associated with fatigue, the release of hydrogen ions (H^+) due to the buffering of lactic acid is erroneously thought to contribute to fatigue. In fact the conversion of lactic acid into lactate with the release of a proton by lactate dehydrogenase has been shown to slow rather than contribute to metabolic acidosis (Juel, 1998). However, the drop in pH (increased acidosis) may play a role in development of fatigue. It appears however, that changing pH values are only a factor in fatigue with changes in body temperature (Westerblad *et al.*, 1997). Thus, a decrease in pH due to H^+ accumulation does not appear to be a major cause of fatigue

during exercise. However, the increase in H^+ ions during exercise is directly related to glycolytic flux and ATP hydrolysis (Busa & Nuccitelli, 1984), which is more likely to be a major contributor to fatigue. A related possible cause is the fact that the majority of ATP stores are in the form of MgATP, which when hydrolysed, releases Mg^{2+}, which can impair calcium release required for cross-bridge formation (Lamb & Stephenson, 1991), which would also induce fatigue.

One possible mechanism suggested by Bauer *et al.* (2007) for an impaired kinetic response during exercise is a reduced capillary density in the skeletal muscle of individuals with T2D. Therefore it is possible that an intervention, such as sustained exercise training that may increase capillary density, would result in a speeding of $\dot{V}O_2$ kinetics in individuals with T2D. Such an intervention was performed on female individuals with T2D (Brandenburg *et al.*, 1999), with the exercise intervention resulting in a speeding of the $\dot{V}O_2$ kinetic response to cycling exercise at varying intensities. However, the post-intervention assessments implemented the same absolute workloads as at baseline. While comparison with baseline data is relevant in the sense that it gives an indication of overall improvement in performance; due to the resulting improvements in $\dot{V}O_2$peak the post-intervention assessments were performed at a relatively easier workload. Therefore the effect of an intervention on the kinetic response at the same relative workload remains unknown, as does the impact on gender, as to this author's knowledge no study has assessed the effects of an exercise intervention in a male population with T2D. This warrants further investigation.

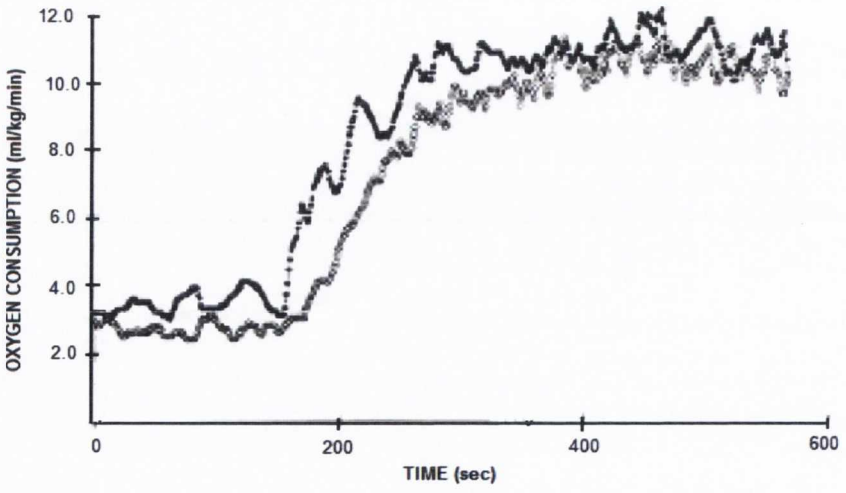


Figure 1.4. $\dot{V}O_2$ kinetic data from two individuals, one ND (black line), and one T2D (grey line). The τ value for the ND individual was 40s, whereas the individual with T2D τ value was slower at 72s. Taken from Brandenburg *et al.* (1999).

A number of other possible mechanisms have been identified as causing the T2D-associated reduction in exercise capacity. Firstly, a diabetes-induced decrease in the oxidative capacity of slow-twitch fibres will require the recruitment of a greater proportion of fast-twitch fibres per workload. The slower $\dot{V}O_2$ kinetics of fast-twitch fibres in turn slows the second phase of the $\dot{V}O_2$ kinetic response (Poole *et al.*, 2008). A second possibility is that the reduced availability of NO, and elevated levels of ROS associated with T2D will result in impaired arteriolar vasodilation, causing a subsequent reduction in microvascular partial O_2 pressure (PO_2). The reduction in PO_2 of slow-twitch fibres will result in a modification of the myocyte's energetics towards those of fast-twitch fibres, and in turn, the slower uptake kinetics of fast-twitch fibres (Poole *et al.*, 2008). A further possibility is that the reduced ability to increase BF in individuals with T2D decreases the individual's capacity to cope with an increase in workload/exercise due to the effect of BF upon the cardiodynamic component of $\dot{V}O_2$ kinetics (De Cort *et al.*, 1991).

$\dot{V}O_2$ kinetic responses are also affected by the same metabolic factors that affect $\dot{V}O_2$max, namely the volume of mitochondria, the rate of respiration in individual mitochondrion, and/or the number of active mitochondria within the muscle fibre. The volume of mitochondria within the muscle fibre will also vary according to the muscle fibre type (Dudley *et al.*, 1987). It has been shown that mitochondria (per unit volume) has a relatively fixed capacity to produce ATP, due to a relatively constant ratio between the inner mitochondrial membrane surface area and mitochondrial volume (Schwerzmann *et al.*, 1989). Therefore the mitochondrial volume will determine the skeletal muscle oxidative ATP rephosphorylation capacity. However, during submaximal steady-state exercise, mitochondrial ATP production is likely to be affected by factors other than volume. Included in these factors are fibre type distributions, as type 1 and 2a fibres contain the vast majority of mitochondria; while type 1 muscle fibres have lower rates of ATP turnover than type 2a and 2b fibres, and as such greater economy. During submaximal steady-state exercise, varying fitness levels and fibre type distribution will result in differing levels of mitochondrial volume and function, thereby affecting $\dot{V}O_2$ kinetic responses.

1.2.4 Cardiac Function during Acute Exercise

Onset of acute exercise results in a rapid rise in cardiac output (CO), the kinetics of which have been shown to be significantly faster than the associated rise in $\dot{V}O_2$ kinetics (De Cort *et al.*, 1991; Lador *et al.*, 2006; Adami *et al.*, 2010, 2011). The profile of CO kinetics can also be characterised by a two-phase model (Lador *et al.*, 2006). The first phase can be described as baroreflex resetting, a decrease in vagal tone, and an increase in preload; while the second phase reflects sympathetic drive stimulation, and has slower kinetics. The profile of the CO kinetic response is mirrored by the kinetic response of QaO_2, which highlights that a dissociation exists between QaO_2 and $\dot{V}O_2$ kinetics (De Cort *et al.*, 1991; Lador *et al.*, 2006; Adami *et al.*, 2010, 2011).

The effect of T2D on cardiac function during exercise is ambiguous. Some studies have shown that uncomplicated T2D results in reduced CO during exercise compared to healthy controls (Roy *et al.*, 1989; Gusso *et al.*, 2008), whereas others have shown similar CO values during exercise (Baldi *et al.*, 2003; Regensteiner *et al.*, 2009; Mac Ananey *et al.*, 2011).

While it remains unclear whether T2D affects CO and SV values during exercise, it is generally accepted that T2D is associated with diastolic dysfunction. It is thought that as many as 60% of individuals with T2D display impaired diastolic function, while an additional 28% demonstrate pseudonormal patterns (Poirier *et al.*, 2000). Regensteiner *et al.* (2009) demonstrated that females with T2D free from CVD presented elevated pulmonary capillary wedge pressure (PCWP) following onset of exercise, despite findings of similar CO, HR and SV at peak exercise. Elevated PCWP is indicative of some degree of left ventricular failure, and is suggestive of cardiac dysfunction. It is however, unknown if similar findings would be replicated in males.

It has also been shown that otherwise healthy individuals with T2D have an elevated ratio of peak velocity of early diastolic filling to early diastolic mitral annular velocity (E/E') at rest, which is used as an estimate of left ventricular filling pressure (Baldi *et al.*, 2006; Ha *et al.*, 2008). Additionally, the rate of increase in E/E' in the transition from rest to exercise is greater in individuals with T2D (Baldi *et al.*, 2006; Ha *et al.*, 2008). Furthermore, diastolic elastance (Ed), calculated by (E/E')/SV, is also elevated during exercise in individuals with T2D, an additional indication of

diastolic dysfunction. There also appears to be a gender related component, as females with T2D show greater increases in E/E' and Ed during exercise compared to males with T2D (Ha *et al.*, 2008). Given that a predominance of the research mentioned was performed on female individuals, it is unknown whether impaired cardiac function during exercise as a consequence of T2D is as prevalent in men.

1.3 Effects of an Exercise Intervention in the Treatment of T2D

1.3.1 Interventions Used in the Treatment of T2D

Treatment protocols for T2D are step-based in design due to the progressive nature of the disease. Initial interventions are modelled on lifestyle modifications, with subsequent additional therapies involving pharmacotherapy (Richter *et al.*, 2006). When treating T2D, the primary measure of efficacy when assessing the treatment is the effect upon HbA_{1c} concentrations. Beyond this, secondary efficacy parameters include fasting plasma glucose (FPG), fasting insulin, C-peptide, and lipid levels (Phillips *et al.*, 2001). On average, within three years of diagnosis a lifestyle intervention alone is not sufficient to maintain target HbA_{1c} concentrations in half of all cases, and combination therapies are needed (Rosak *et al.*, 2005). However, the initial treatment upon diagnosis of T2D will typically involve a lifestyle intervention.

1.3.2 Lifestyle Interventions

Lifestyle interventions can be justified on a number of grounds. The aim of the intervention is to delay the onset of T2D in those individuals displaying impaired fasting glucose or impaired glucose tolerance, or to postpone the requirement for pharmacological treatment in those diagnosed with T2D. Lifestyle interventions aim to preserve β-cell function and to delay/prevent any of the vascular complications associated with T2D (Nathan *et al.*, 2007). A study by Guerra-Juarez *et al.* (2007) on descendants of individuals with T2D (n=60) found that only 15% of subjects had a BMI in normal range, 68% were obese, and 17% were overweight. Additionally, 42% of these descendants were classed as insulin resistant, and 15% were considered glucose intolerant. While this may seem to support a genetic component to T2D, environmental factors were found to be responsible for 98.6% of the conditions diagnosed (Guerra-Juarez *et al.*, 2007). Therefore lifestyle interventions that target

these environmental factors should be successful in reducing complications associated with T2D, obesity and other related conditions.

One factor that should be considered in monitoring a lifestyle intervention is the compliance rate of the individual. Longitudinal studies on the effectiveness of lifestyle interventions have found that they have their maximum impact within the first six months, with diminishing returns thereafter, a consequence of progressively reduced compliance after six months (Stirban & Tschoepe, 2008).

The risk of developing T2D is increased with excess weight/obesity, as a consequence of increased insulin resistance and impaired β-cell function due to obesity. Impaired β-cell function is often a consequence of the increased insulin resistance resulting in hyperglycaemia, due to exhaustion of the β-cell through over-production of insulin (Nilsson, 2008). Therefore weight loss is often prescribed as a lifestyle intervention in order to improve insulin sensitivity. The method of weight loss must be carefully managed, as rapid weight loss due to severe caloric restriction will result in an increase in circulating fatty acids due to increased levels of lipolysis (Tolman *et al.*, 2004). Increased lipolysis due to rapid weight loss increases the risk of cholelithiasis (gall stones) in obese patients, which paradoxically results in an increased mortality risk associated with weight loss (Nilsson, 2008). Therefore exercise is generally prescribed, often in combination with modest dietary restriction, as the preferred method of weight loss. And results of previous studies would support this method, with weight loss due to exercise shown to improve insulin sensitivity to a greater extent than diet-induced weight loss (Sigal *et al.*, 2004); while a combined programme of exercise and dietary caloric restriction resulted in greater loss of adipose tissue than dietary restriction alone (Scheen, 2003). Furthermore, a slight increase in physical activity in conjunction with modest weight loss was shown to reduce the progression from IGT to T2D by 58% (Abdul-Ghani *et al.*, 2007).

Given the apparent effectiveness of exercise in improving insulin sensitivity and health in diabetic subjects, it is necessary to look at the specific mechanisms by which exercise achieves these improvements.

1.3.3 General Benefits of and Recommended Guidelines for Exercise in T2D

It has been shown that people who do not perform habitual physical activity, and in turn have low physical fitness levels, have higher incidences of T2D, and increased risk of CVD and other metabolic abnormalities (Kadoglou *et al.*, 2008). The

American Diabetic Association (ADA) advises diabetic subjects to perform moderate-intensity physical activity for ≥ 30 minutes on most days of the week, with no more than two days separating each individual session (Di Loreto *et al.*, 2005), while the ACSM recommends ≥ 150mins of aerobic exercise per week spread over at least three days per week, with no more than two consecutive days between sessions. The ACSM also recommends moderate to vigorous resistance exercise 2-3 days per week in addition to the aerobic exercise (Colberg *et al.*, 2010). The question remains, however, as to whether the recommendations should suggest greater volumes of exercise. Studies have shown that there exists an inverse linear dose response between the amount of physical activity among diabetic subjects and total CVD, incidence of coronary heart disease (CHD), and mortality (Di Loreto *et al.*, 2005). Additionally, unfit men with T2D have greater than double the risk of mortality compared to men of moderate fitness, with low cardiorespiratory fitness considered to be as strong a risk factor for mortality as smoking, hypertension, and other major risk factors (Sigal *et al.*, 2004). Cardiorespiratory fitness appears to be regulated by left ventricular diastolic function, cardiac autonomic function, metabolic profile, endothelial function, hereditary components and skeletal muscle metabolism; in short, those factors which are impaired in the diabetic state (Kadoglou *et al.*, 2008). Further studies linking physical exercise capacity with wellness and mortality include those of Ekelund *et al.* (2007), who found that aerobic fitness was significantly and inversely related to fasting insulin levels; and Sigal *et al.* (2004), who showed that in individuals both with and without CVD, actual exercise capacity (determined by physiological testing) was a better predictor of mortality risk than age-predicted exercise capacity.

These findings would suggest that increasing daily physical activity, and in turn exercise capacity, should result in reduced metabolic risk factors in sedentary individuals, in particular individuals with T2D (Ekelund *et al.*, 2007), to the extent that a one metabolic equivalent (1-MET) improvement in treadmill performance by diabetic patients was associated with a 12% reduction in mortality (Sigal *et al.*, 2004). Exercise has also been shown to result in a trend towards decreased medication use in individuals with T2D previously treated by pharmacological means only (Goldhaber-Fiebert *et al.*, 2003), which has important benefits on treatment costs for T2D.

However, despite encouragement, many individuals with T2D fail to engage in nutrition and exercise interventions (Goldhaber-Fiebert *et al.*, 2003). This may be due to the prescribed exercise programs being too difficult for (obese) individuals

with T2D to complete (Scheen, 2003) due to the associated exercise intolerance (Regensteiner *et al.*, 1998). It is known that exercise can reduce the risk of developing T2D, possibly by as much as 30% (Bassuk & Manson, 2005). However, when assessing the volume of physical activity among 23,283 members of the general public using the Medical Expenditure Panel Survey, Morrato *et al.* (2007) reported that only 39% of individuals with T2D voluntarily performed exercise (vs. 58% of non-diabetics), despite the fact that over three-quarters of those assessed recalling being told by a medical practitioner to perform exercise (Morrato *et al.*, 2007). Of that 39%, less than a third performed the recommended volume of exercise. In addition, with diabetic subjects, lower levels of physical activity are associated with lower scores on quality of life assessments (Tessier *et al.*, 2000). In non-diabetic individuals, as the number of risk factors for T2D increases, the proportion of individuals reporting to be physically active decreases, with many of the listed risk factors, such as age, male sex, BMI, diastolic function, heart rate recovery, and HbA_{1c} being independent predictors of exercise capacity (Fang *et al.*, 2005). Levels of activity within the diabetic population do not differ between normal-weight individuals and those who are either obese and/or overweight. These findings indicate that the greater amount of sedentary behaviour in an individual's life, the greater the risk of developing T2D (Morrato *et al.*, 2007).

In the population of individuals with T2D that do exercise, it appears that the level of exercise performed is not of an adequate intensity. A study by Johnson *et al.* (2005) assessing walking speed in individuals with T2D determined that the median walking pace was 3.3km.hr^{-1}, well below that necessary (4km.hr^{-1}) to be considered as moderate intensity, although quite how Johnson *et al* (2005) determined the absolute workload required to be classified as moderate intensity was not provided. Indeed, the level of energy expenditure during exercise is independently associated with clustered metabolic risk in healthy individuals, allowing for prediction of risk of metabolic syndrome independent of aerobic fitness and percent body fat (Ekelund *et al.*, 2007).

1.3.4 Exercise-Induced Improvements in Glycaemic Control

A single bout of exercise, either endurance- or resistance-based, has been shown to improve insulin sensitivity and glucose tolerance (Sigal *et al.*, 2006; Praet & van Loon, 2007), with the positive effect of exercise on insulin sensitivity still evident for up to 24-72 hours afterwards, depending upon the duration and intensity of the

bout (Boule *et al.*, 2005; Sigal *et al.*, 2006). This acute response has been attributed to sustained activation of the skeletal muscle glucose transporter system, consumption of glycogen stores within the muscle and liver, and an increase in skeletal muscle BF. Prolonged exercise training can induce structural adaptations that increase insulin sensitivity, including weight loss, the upregulation of glucose transporter type 4 (GLUT4) expression (intracellular molecular transporters that translocate to the cell membrane when activated by insulin and transport glucose from the blood into the cell), improved NO-mediated muscle BF, decreased hormonal stimulation of hepatic glucose production and the normalisation of blood lipid profiles. However, it appears that the improvements in insulin sensitivity are primarily a result of the cumulative effects of each acute exercise bout rather than permanent structural adaptations, as the beneficial effects of training on insulin sensitivity may be lost within two weeks following the cessation of training (Sigal *et al.*, 2006; Praet & van Loon, 2007).

As mentioned above, one structural adaptation that occurs in response to prolonged training is an upregulation of GLUT4 expression. The level of increase in GLUT4 content is correlated with the training-induced improvements in insulin sensitivity (Zierath, 2002). However, when comparing healthy and insulin-resistant individuals prior to training, GLUT4 protein levels are not different between groups. Training results in both increased content and expression of GLUT4 in both populations (O'Gorman *et al.*, 2006). On this evidence, it would seem to suggest that exercise-induced improvements in insulin sensitivity are the consequence of the increased GLUT4 content and expression, and are independent of changes in the insulin-signalling pathway (O'Gorman *et al.*, 2006).

Exercise onset results in a shift in fuel source from primarily NEFA to increased reliance on glucose and the muscle's stores of glycogen, with the scale of this shift increasing with increasing exercise intensity, resulting in greater carbohydrate oxidation (Sigal *et al.*, 2004). In order to maintain supply of carbohydrate, the liver increases gluconeogenesis in order to maintain adequate circulating glucose. The increase in gluconeogenesis and glycogenolysis in response to exercise is stimulated by a rise in glucagon, which requires a drop in the levels of circulating insulin. The rate of increase in hepatic glucose production is closely coupled to the rate of increase in muscle glucose uptake during exercise (Sigal *et al.*, 2004). Therefore, due to the reduction in circulating insulin, it is necessary to increase

glucose uptake at the muscle via increased insulin-sensitivity, as discussed above, and exercise-induced glucose uptake.

The degree of exercise-induced glucose uptake via exercise-stimulated GLUT4 translocation can completely account for the level of glucose transport activity into the active skeletal muscle cells (Ryder *et al.*, 2001). The cause of translocation of the GLUT4 transporters to the cell membrane during exercise is not entirely understood (Beeson *et al.*, 2003).

The onset of insulin resistance within skeletal muscle tissue typically precedes development of T2D, so muscle tissue is often highly insulin resistant by the time T2D is finally diagnosed, which can reduce the level of exercise-induced glucose uptake (Ryder *et al.*, 2001). T2D-related impairments in the signalling cascade can prevent the translocation of GLUT4 transporters to the cell membrane. While individuals with T2D also demonstrate similar exercise-induced improvements in glucose uptake and insulin sensitivity compared to ND individuals, exercise can cause a paradoxical increase in insulin secretion in individuals with T2D (Kilpelainen *et al.*, 2007). A possible mechanism proposed by Kilpelainen *et al.* (2007) to explain this atypical response of insulin secretion is a hyperbolic relationship between β-cell function and both insulin resistance and plasma glucose concentration. Under this hyperbolic relationship, a reduction in plasma glucose levels due to increased uptake in the muscle due to exercise, would result in an increase in insulin secretion if it occurred on the descending limb of the curve, as would be evident in the case of reduced β-cell function, as occurs in T2D. Therefore the ascending limb of the curve would represent the 'typical' relationship between β-cell function, insulin resistance and plasma glucose concentration. Therefore the aim of the training intervention should be to result in a permanent shift in the relationship between exercise and insulin secretion to the ascending limb of the hyperbolic curve.

1.3.5 Improvements in Blood Flow and Endothelial Function

Onset of exercise, as discussed earlier, results in an almost instantaneous increase in BF. This sudden increase in BF results in an increase in the shear stress placed on the conduit vessel walls. Studies have suggested that this increased stress on the vessel walls may act as a stimulus for EDNO, implying that the exercise-induced improvement in endothelial vasodilation is a systemic effect of exercise, and not a local response within the active muscle group (Stewart, 2002). Furthermore,

improvements in endothelial function are not correlated with exercise-associated reductions in BP, indicating that improved endothelial vasodilator function is due to other factors (Stewart, 2002). The implication of this is that hyperaemia induced by chronic exercise may lead to up-regulation in release of EDNO. This in turn will improve insulin sensitivity, given that insulin-mediated vasodilation is EDNO-dependent (Steinberg *et al.*, 1994).

T2D is associated with a reduced exercise capacity (Kjaer *et al.*, 1990; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Fang *et al.*, 2005). Impairments in vascular or endothelial function associated with T2D offer a possible connection between T2D and a reduced exercise capacity. Possible sources include thickening of the medial layer of conduit arteries due to greater levels of connective tissue, glycoproteins, and calcium. Work by Stewart (2002) comparing endothelial function between individuals with T2D and healthy ND individuals after a period of aerobic training displayed improved endothelial function in the T2D group only, despite increases in fitness in both groups. This would suggest that exercise has the capability to restore/improve endothelial function in individuals with T2D. However, the decrease in elasticity seen in the arteries of individuals with T2D due to the structural changes in the medial layer is not reversed with exercise, suggesting that exercise is not a strong enough stimulus to alter the T2D-induced structural changes, limiting the capacity of exercise to improve blood flow/circulation in diabetic subjects (Loimaala *et al.*, 2003).

To this author's knowledge, the effect of an exercise training intervention on BF/VC kinetic responses in males with T2D have not been studied, and are investigated in this thesis.

1.3.6 Improvements in $\dot{V}O_2$ Kinetics

Exercise training has been shown to accelerate $\dot{V}O_2$ kinetics, evident in the reduction in τ that occurs after sustained training, especially in moderate-intensity exercises that rely predominantly on slow-twitch muscle fibres (Brandenburg *et al.*, 1999; Grassi, 2003; Mac Ananey, 2010). The improvement in $\dot{V}O_2$ kinetics can most likely be attributed to training-induced increases in the number and content of mitochondria and capillary beds, allowing for a faster transition to oxidative

phosphorylation (and reduced τ) and increased surface area for O_2 diffusion at the capillary/myocyte interface (Korzeniewski & Zoladz, 2006).

Brandenburg *et al.* (1999) had both ND and T2D females embark on a three-month aerobic training program before reassessing their $\dot{V}O_2$ kinetic response at the same absolute constant-load efforts of 20, 30, and 80W. Following this training program, the T2D subjects showed the greatest improvement in $\dot{V}O_2$peak. Individuals with T2D also displayed significantly faster $\dot{V}O_2$ kinetic response to constant-load exercise intensities at 20 and 30W. This improvement was associated with a reduction in the respiratory exchange ratio (RER) from baseline, which suggests that the individuals with T2D did not find the effort as hard second time around. Furthermore, these individuals with T2D significantly increased their $\dot{V}O_2$peak from baseline. Therefore they performed the post-intervention constant-load cycling at a relatively lower workload. It has yet to be determined if the kinetic response to exercise performed at the same relative intensity as at baseline increases following a training intervention.

1.3.7 Improvements in Cardiac Function

There have been very few studies investigating the effect of a prolonged exercise training intervention on cardiac function in individuals with T2D. Recent data produced by Mac Ananey (2010) failed to show any change in absolute CO or any of its parameters in males or females with T2D during steady-state cycling at various intensities following either three or six months training, despite improvements in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetic responses. However, the rate of adjustment of CO from rest to assessment at 30s was faster post-intervention.

It is well established that individuals with T2D are at risk for diastolic dysfunction, and possibly reduced CO during acute exercise. It is not unreasonable to expect regular training to induce physiological adaptations that would result in improvements in cardiac function, as prolonged training, in particular endurance training, will result in an increase in left ventricular mass. This hypertrophy represents a physiological adaptation to exercise, not a pathological adversity, allowing for increased diastolic filling (Stewart, 2002). Training may be expected to result in improvements in glycaemic control, which have been shown to be closely linked to cardiac function (Loimaala *et al.*, 2003). Indeed, Loimaala *et al.* (2003) were able to

demonstrate that exercise training improved cardiovascular autonomic regulation, recording an increase in baroreflex sensitivity (BRS), used as an indication of reflectory cardiovascular neural regulation. However, the improvements in BRS were correlated with a positive change in HbA_{1c} levels and muscle power, independent of changes in $\dot{V}O_2peak$, systolic blood pressure, heart rate and cardiac function; which would suggest that the improvements in autonomic function are due to improved glucose control rather than improvements in central haemodynamics. Therefore an exercise training intervention may not necessarily result in any significant improvements in central haemodynamics or CO. However, further studies are required to explore if exercise training induces adaptations in the magnitude or dynamic response of cardiac function when the same relative submaximal exercise intensities are employed both pre- and post-training.

1.4 Pharmacological Interventions

At present, there are various types of pharmacological agents used in the treatment of T2D. The majority of these are oral agents, which are aimed at specific mechanisms involved in the insulin pathway or digestive system. Of these, the predominant types are:

- Sulphonylureas (sold as Glibenclamide): insulin secretagogues which act directly upon β-cells;
- Meglitinide analogues (sold as Repglinide): insulin secretagogues, which have an earlier onset and shorter duration than sulphonylureas;
- Biguanides (sold as Metformin): suppress hepatic glucose production;
- α-glucosidase inhibitors (sold as Acarbose): slow the intestinal absorption of complex carbohydrates, in turn reducing circulating glucose levels; and
- Thiazolidinediones (either Rosiglitazone or Pioglitazone): sensitise liver and adipose tissue to the effects of insulin, and stimulate adipogenesis, glucose and lipid metabolism (Scheen, 2003).

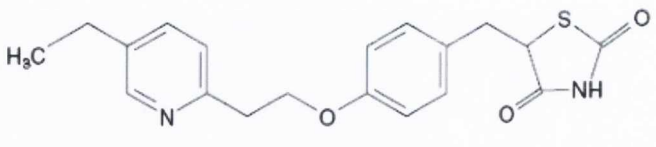
More severe cases of T2D will result in prescription of either inhaled or intramuscular insulin (DeFronzo *et al.*, 2005). Given that the present thesis explored a number of new actions of the oral agent pioglitazone (PIO) of the thiazolidinedione (TZD) family, this section will review the targeted actions of TZDs, the effectiveness

of their actions, risks and benefits to their use, and directions for future use in treatment.

1.4.1 The Background to Thiazolidinediones

The thiazolidinedione (TZD) family consists of four different oral agents aimed at the treatment of T2D: troglitazone, pioglitazone, rosiglitazone, and rivoglitazone. Troglitazone has been implicated in causing hepatotoxicity, and as a result has been withdrawn from the market. Rivoglitazone is the newest member of the TZD family and is currently undergoing research to determine its suitability as a form of treatment for T2D. Rosiglitazone (RSG) has been linked to an increase in risk in incidence of heart failure (Home *et al.*, 2009; Komajda *et al.*, 2010; Nissen & Wolski, 2010), and while remaining in use in the treatment of T2D with existing patients, it is now no longer being prescribed to new patients in the US or in Europe. Pioglitazone (PIO) has recently been linked with a possible increased incidence of bladder cancer in males, yet still remains in widespread use in the treatment of T2D while this claim is further investigated (Lewis *et al.*, 2011; Stephenson, 2011). PIO and RSG have similar methods of action, which will be discussed below, but differ in the side-chain of the main thiazolidine-structure as depicted in figure 1.5. These structural differences are believed to result in the differences in bioavailability, metabolism and antihyperglycaemic potency of each (Wagstaff & Goa, 2002; Richter *et al.*, 2006, 2007).

Rosiglitazone



Pioglitazone

Figure 1.5. The chemical structure of RSG and PIO respectively. Differences in the thiazolidine-structure on the left result in the differing properties of the two compounds. Taken from Wagstaff & Goa (2002).

Any differences in the actions, functions or effectiveness of either PIO or RSG will be discussed as encountered, but for the purposes of this introduction, the actions of PIO are the sole remit of this section.

1.4.2 Methods of Action

The effectiveness of TZDs in attaining glycaemic control is thought to be the result of their stimulatory action upon the peroxisome proliferator-activated receptor gamma (PPARγ) (Richter *et al.*, 2006, 2007). The PPAR family contains three subtypes, each of which is a nuclear receptor ligand-activated transcription factor (Waugh *et al.*, 2006). The first of these, PPARα, is expressed in the liver. Of the TZD family, only PIO has been shown to bind with PPARα, indicating direct action upon the liver (Wagstaff & Goa, 2002; Tan *et al.*, 2005), influencing triglyceride metabolism, which will be discussed below. The second member of the PPAR family, PPARδ, is expressed in a wide range of mammalian tissue, but thus far has not been shown to be a target of PIO expression. The third member, PPARγ, is expressed in high levels in white adipose tissue, as well as PPARγ isoforms found in the heart, vascular smooth muscle, monocytes, spleen, kidney, liver, pancreatic β-cells, intestine, adrenal, and skeletal muscle tissue (Wagstaff & Goa, 2002; Waugh *et al.*, 2006). PIO is a selective and potent activator of PPARγ (Wagstaff & Goa, 2002). PPARγ activators have been shown to regulate cellular proliferation, inflammatory responses and glucose metabolism (Hallsten *et al.*, 2002). Once activated, PPARγ combines with another nuclear receptor, retinoid-X receptor, to form a heterodimer that binds to target DNA sequences to regulate the transcriptional activity of genes that are involved in glucose and lipid metabolism (Wagstaff & Goa, 2002; Waugh *et al.*, 2006).

Because PPARγ are primarily located within adipose tissue, PIO action is primarily concentrated here. PIO-induced activation of PPARγ results in increased expression of uncoupling proteins (UCP)-1 and –2 in subcutaneous preadipocytes. The actions of UCP-1 and UCP-2 result in differentiation of adipocytes, and the generation of greater numbers of smaller adipocytes. These smaller adipocytes are more insulin-sensitive, and produce lower levels of FFA, tumour necrosis factor-alpha (TNF-α) and leptin (Wagstaff & Goa, 2002; Waugh *et al.*, 2006). Due to the PIO-induced reductions of FFA, TNF-α and leptin, there is less competition for glucose use as a substrate in skeletal muscle (the Randle cycle). Therefore glucose uptake into

skeletal muscle is increased, and PIO indirectly impacts upon glucose metabolism in the liver (Wagstaff & Goa, 2002; Tan *et al.*, 2005; Waugh *et al.*, 2006).

Beneficial effects of PIO treatment in T2D can be seen in the activation of PPARγ located in vascular smooth muscle. Activated PPARγ has been shown to induce vasorelaxation due to blockade of potassium ion (K^+) channels, and reduced voltage-gated calcium (Ca^{2+}) current, both of which are responsible for vasoconstriction. PPARγ activation also inhibits angiogenesis, and the proliferation and migration of vascular smooth muscle cells, which often results in cardiovascular complications in T2D (Bakris *et al.*, 2003). One of these mechanisms in particular is thought to involve matrix metalloproteinase-9 (MMP-9). Elevated MMP-9 levels are used as an indicator of atherosclerotic severity and plaque vulnerability. TZDs have, however, been shown to lower levels of MMP-9, thereby slowing the processes involved in vascular degradation in T2D (Kadoglou *et al.*, 2008).

1.4.3 Dosage

PIO is typically administered in an individual dose of 15 or 30mg daily in monotherapy, although if necessary, the dose can be titrated up to 45mg daily. Plasma concentrations of PIO are dose-dependent (Waugh *et al.*, 2006). PIO is metabolised in the liver by cytochrome P450 enzymes, and has a half-life ($t_{1/2}$) of 3-7 hours. However, PIO metabolites have a much longer $t_{1/2}$ of 16-24 hours, the actions of which allow PIO to be administered in a single-daily dose. If PIO is used in a combination therapy that results in hypoglycaemia (typically treatment with a sulphonylurea), the coindicated therapy should be reduced. PIO has also been contraindicated in patients with cardiac failure or hepatic impairment (Waugh *et al.*, 2006).

Given that on average, approximately half of all diabetic require a form of combination therapy within three years of diagnosis (Rosak *et al.*, 2005), it is therefore important to know which therapies work well together. PIO has been indicated for use with metformin (Wagstaff & Goa, 2002; Richter *et al.*, 2006, 2007) given that they have complementary modes of action.

1.4.4 Impact Upon Glycaemic Control & Insulin Sensitivity

PIO use has resulted in significant improvements in glycaemic control and insulin sensitivity in a number of studies, resulting in significant reductions in HbA_{1c}

and FPG after treatment over time courses varying in length from 12 to 26 weeks (Aronoff *et al.*, 2000; Rosenblatt *et al.*, 2001). Only one study has failed to demonstrate a significant improvement in glycaemic control (Scherbaum & Goke, 2002) compared to a placebo. However, subjects participating in this study received a dose of 15mg.day^{-1}, which is generally the initial prescription of PIO, with patients titrated up to a dose of 30 of 45mg.day^{-1}.

A meta-analysis of twenty-two trials by Richter *et al.* (2006) concluded that the PIO-induced improvements in HbA_{1c} were not clinically different from other oral anti-diabetic drugs. However, it seems that PIO treatment induces significantly greater improvements in measures of insulin sensitivity (fasting plasma insulin and fasting serum insulin) than acarbose (Goke, 2002), metformin (Pavo *et al.*, 2003) and sulphonylurea treatments (Charbonnel *et al.*, 2005; Pfutzner *et al.*, 2005; Tan *et al.*, 2005).

1.4.5 Impact on Cardiovascular and Endothelial Function

Treatment with PIO has been linked with improvements in a number of cardiovascular risk factors associated with T2D. Dislipidaemia is one of the biggest risk factors of CVD associated with T2D. Dislipidaemia is characterised by increased plasma concentrations of triglycerides, decreased HDL-C levels accompanied by an increase in plasma LDL-C small particles (Goldberg *et al.*, 2005; Waugh *et al.*, 2006). PIO has been shown to not only influence glucose levels but to also alter lipid metabolism. Goldberg *et al.* (2005) compared the effects of PIO and RSG on lipid levels in T2D patients diagnosed with dislipidaemia over a six-month period. The results of the study conclusively supported the positive impact of TZD treatment with respect to glycaemic control, but displayed contrasting effects of PIO and RSG on lipid levels. Over the six-month period, both PIO and RSG significantly increased circulating HDL-C; but the relative change from baseline was significantly greater in the PIO treatment arm. The effects of PIO and RSG on LDL-C levels also differed. PIO treatment decreased overall levels of LDL-C, and caused a shift in LDL-C particle size from small, dense particles to larger, more buoyant particles; whereas RSG treatment resulted in a net increase in LDL-C concentrations, and while RSG also resulted in an increase in particle size, the magnitude of this increase was smaller than that of PIO treatment (Goldberg *et al.*, 2005). Furthermore, triglyceride levels, a further risk factor for cardiovascular disease, also increased with RSG treatment,

whereas PIO decreased triglyceride levels with respect to baseline measures. It was concluded that while the effects of PIO and RSG on indicators of glycaemic control and insulin sensitivity were similar, PIO treatment was shown to exert greater effects in reducing dislipidaemia and in turn risk of cardiovascular disease. In support of the conclusions drawn by Goldberg *et al.* (2005), Waugh *et al.* (2006) performed a meta-analysis of ten double-blind trials comparing the effects of PIO and RSG on lipid profile parameters such as triglyceride levels, total cholesterol, HDL-C and LDL-C over 12-26 weeks of treatment. The results were conclusive in demonstrating the significantly greater positive effects of PIO over RSG on improving lipid profile in individuals with T2D.

The conditions described above of dislipidaemia, high circulating levels of FFA, glucose, and chronic hypertension associated with T2D all exert significant stress on the vasculature, which can cause chronic vascular inflammation, leading to increased concentrations of C-reactive protein (CRP), TNF-α and interleukin-6 (IL-6) (Martens *et al.*, 2006; Pitocco *et al.*, 2009; Vijay *et al.*, 2009; Nerla *et al.*, 2010). This negatively affects the ability of the vascular endothelium to induce vasodilation. Instead, the increased metabolic stress placed on the endothelial walls leads to increased expression of adhesion molecules that bind macrophages and platelets to the vasculature creating atherosclerotic lesions (Reusch *et al.*, 2003). Under normal conditions, insulin increases release of EDNO and decreases expression of inflammatory cytokines and adhesion molecules. However, in the diabetic state, NO secretion and vasodilation are impaired, resulting in increased expression of adhesion molecules and activation of macrophages and platelets (Reusch *et al.*, 2003). This process is initially mediated by selectins, which weakly bind and roll the macrophages together, while immunoglobulins such as VCAM-1 cause firm adhesion and migration of the macrophages and platelets from the blood to the intima of the vascular wall (Permana *et al.*, 2009). In T2D, elevated levels of serum E-selectin (E-selectin is specific to activated endothelium) and VCAM-1 are indicative of endothelial dysfunction, and are correlated with the degree of insulin resistance. Both E-selectin and VCAM-1 have been shown to decrease to normal levels after restoration of normoglycaemia (Stad & Buurman, 1994). It has been reported that VCAM-1 expression is regulated by PPARα; therefore treatment with PIO may be expected to induce reductions in levels of VCAM-1 and improve endothelial function.

1.4.6 Impact on Adipose Tissue, Fatty Acid, and Triglyceride Metabolism

A decrease in circulating FFA is one of the mechanisms of action of PIO. Glucose and fatty acids compete for use as a substrate in skeletal muscle. This effect, known as the Randle cycle, can explain the onset of insulin resistance. As circulating NEFA increase, muscle glucose uptake decreases and becomes less efficient; overtime causing hyperglycaemia and leading to insulin resistance (Tan *et al.*, 2005). However, this is countered with PIO treatment due to its suppressive effect on NEFA output. As a consequence of this activation, PIO treatment is associated with a 15-25% decrease in both fasting and postprandial triglyceride concentrations.

PIO treatment also significantly impacts upon the adipocytokines produced by adipose tissue, but especially upon production levels of adiponectin. Whereas some adipocytokines, such as IL-6 and TNF-α are produced from multiple sources, adiponectin is produced specifically in adipose tissue. Adiponectin has both anti-inflammatory and antiatherogenic properties, with plasma levels correlating negatively with BMI, plasma glucose, insulin, and triglyceride levels, while also sharing a positive correlation with HDL-C levels (Sharma *et al.*, 2006). This suggests low adiponectin levels could be used as a biomarker to indicate insulin resistance. It can therefore be assumed that increased adiponectin production by adipocytes would confer improvements in insulin sensitivity and glycaemic control. However, a recent study by Sharma *et al.* (2006) found no relationship between insulin sensitivity and adiponectin levels, despite PIO-induced improvements in both, suggesting that increased adiponectin levels are not the sole mechanism of improving insulin sensitivity. Further research into the role of other adipocytokines is warranted.

1.4.7 Impact on Exercise Capacity

It has been well documented that the ability of individuals with T2D to exercise is compromised by both a reduced exercise capacity ($\dot{V}O_2$peak) and slower $\dot{V}O_2$ kinetics (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011). To this author's knowledge no study has yet investigated the effects of PIO treatment on exercise capacity in a T2D population. As such, it remains unknown whether the effect of PIO in combination with an exercise training intervention is synergistic in nature. The results of two intervention studies demonstrating a positive effect of RSG on exercise performance suggest that PIO may

have a similar positive effect. Regensteiner *et al.* (2005) demonstrated that a four-month course of RSG resulted in a significant increase in $\dot{V}O_2$peak, and while the τ values of the $\dot{V}O_2$ kinetic response did not change to constant-load cycling at 30W, there was a significant increase in the amplitude of the second phase of the kinetic response, suggesting improved performance following treatment. In a separate study, Kadoglou *et al.* (2007) demonstrated that while exercise training and RSG individually resulted in increased $\dot{V}O_2$peak after eight months treatment, a combined treatment of exercise and RSG had a synergistic effect, improving $\dot{V}O_2$peak to a greater extent than that expected by their individual actions. Evidence suggests that PIO treatment results in improved cardiac function, demonstrating similar cardiac improvements to those elicited by exercise training (van der Meer *et al.*, 2009), which imply that a course of PIO treatment may result in improvements in exercise performance similar to those seen in studies using RSG.

1.4.8 Risks Associated With Use

The negative consequences associated with TZD use have been well documented, and are generally the result of the drugs method of action. Most recently, the US Food and Drug Administration have suggested that long-term use of PIO (> 1 year) may be associated with an increased risk of bladder cancer (Stephenson, 2011); while Lewis *et al.* (2011) suggested that PIO use for more than 2 years is only weakly associated with an increased risk. Lewis *et al.* (2011) also confirmed that short-term use was not associated with any increased risk. In terms of side-effects of use, PIO is associated with dose-dependent increases in bodyweight (Wagstaff & Goa, 2002; Waugh *et al.*, 2006), an apparent consequence of PPARγ activation leading to differentiation and an increased number of small adipocytes. Furthermore, PIO can lead to dose-related fluid retention, in part the result of the increase in adiposity (Wagstaff & Goa, 2002; Waugh *et al.*, 2006). As a consequence of this oedema, plasma volume expansion may result, leading to reports from clinical trials of anaemia, with decreases in haemoglobin concentrations ranging between 0.5 and 0.75mg.dl^{-1} (Richter *et al.*, 2006).

1.5 Implementation & Recommendations for Exercise Programmes

1.5.1 Implementation of Exercise Program

A positive relationship exists between exercise intensity and improvements in both cardiorespiratory fitness and HbA_{1c} levels (Sigal *et al.*, 2004). However, as already stated, most exercise programs designed to induce metabolic improvements are beyond the exercise capacity of individuals with T2D (Scheen, 2003), thereby rendering the relationship between intensity and metabolic improvements redundant in most cases. Therefore, in constructing a training program for individuals with T2D, it is necessary to consider a number of factors, including the volume and intensity of the desired program.

Despite not being able to perform exercise to the same capabilities as healthy counterparts, studies on individuals with T2D have shown that a training intervention as modest as three hours exercise per week of moderate intensity is sufficient to elicit an improvement in insulin sensitivity in as little as 12 weeks (Jeon *et al.*, 2007). A study by Di Loreto *et al.* (2005) on insulin-treated individuals with T2D found a significant inverse correlation between $MET.hr^{-1}.wk^{-1}$ and daily units of insulin used in the treatment of T2D. This correlation translated itself into a daily reduction of ~11 units of insulin when subjects walked 3 miles daily over a two-year period. In order to achieve this significant reduction in daily insulin, energy expenditure during exercise had to be in excess of 27 $MET.hr^{-1}.wk^{-1}$ when exercising, with significant reductions in HbA_{1c}, total cholesterol, triglycerides, and blood pressure found for exercise expenditure in the range of 11-20 $MET.hr^{-1}.wk^{-1}$. It was not demonstrated by Di Loreto *et al.* (2005) how many of the participants were physically capable of performing exercise that would result in energy expenditure of 11-20 $MET.hr^{-1}.wk^{-1}$. Treatment of T2D with insulin is usually indicative of a more severe form of T2D, and Praet & van Loon (2007) have shown that insulin-treated individuals with T2D generally display severe exercise intolerance due to reduced oxidative capacity, neuropathy-related muscle weakness, muscle sarcopenia, and/or micro- and macrovascular damage, therefore non-insulin dependent individuals with T2D may be better able to tolerate exercise training at the desired workloads. One interesting finding regarding the benefits reported by Di Loreto *et al.* (2005) was that they occurred without any significant weight loss. It was found among participants that to achieve long-term weight loss through exercise required energy expenditure in excess

of 20 $MET.hr^{-1}.wk^{-1}$, which is consistent with other findings showing that the volume of exercise needed to achieve significant, sustained weight loss is much greater than the volume required to improve indices of glycaemic control and cardiovascular health (Sigal *et al.*, 2006), and is demonstrated by the fact that a significant inverse association still exists between moderate-intensity physical activity and progression of T2D after adjusting for BMI (Jeon *et al.*, 2007).

One further consideration regarding the volume and intensity of training interventions for individuals with T2D is that training programs that are of greater volume and intensity have been shown to induce greater increases in HDL-C and decreases in triglycerides, which in turn will improve BF, O_2 and insulin delivery (Sigal *et al.*, 2004). However, the reduced exercise capacity of individuals with T2D may also be attributable to an accelerated decline in muscle mass and strength with ageing, with age-related sarcopenia occurring in proportion to both the decrease in glucose uptake and muscle strength seen in T2D (Praet & van Loon, 2007). A study by Loimaala *et al.* (2003) found that exercise-induced increases in muscle power correlated significantly with improvements in glycaemic control. This implies that exercise-induced increases in muscle mass and performance will result in improvements in the ability to manage glucose levels. Therefore, not only is it important to consider volume and intensity in an exercise program aimed at individuals with T2D, but the type of exercise being performed, as the findings of Praet & van Loon (2007) and Loimaala *et al.* (2003) suggest that resistance training may be more effective in maintaining glycaemic control than aerobic endurance training.

Endurance training has long been promoted for use in management and prevention of T2D; however, there is evidence that the effects of resistance training on insulin sensitivity and glucose homeostasis may be similar (Castaneda *et al.*, 2002), if not better sustained than endurance training (Sigal *et al.*, 2004). Resistance training is associated with a substantial increase in muscle mass (Praet & van Loon, 2007), which in turn results in an increase in the available glucose storage area. This increase in storage area promotes the clearance of glucose from the circulation, in turn reducing the dependency on insulin to maintain normoglycaemia (Ibanez *et al.*, 2005), as it is not thought that the increased mass affects the muscle's response capacity to insulin (Dunstan *et al.*, 2002).

The American Diabetic Association only recommends progressive resistance training (PRT) for young individuals with T2D, but does not suggest PRT for older individuals or individuals with long-standing T2D, due to the effects of PRT upon blood pressure (Ibanez *et al.*, 2005). However, a number of studies investigating the effects of PRT on both older individuals and hypertensives did not elicit any negative side effects. Ibanez *et al.* (2005) implemented a progressive resistance training program with older males with T2D, who underwent a 16-week training program of two sessions per week. Training was a combination of heavy resistance and 'explosive' strength training as it provided a reflection of the requirements of daily activities, and was reported to be an effective strategy to minimise age-related decreases in muscle mass, maximum strength, and muscle power output. The training protocol implemented by Ibanez *et al* (2005) was similar to that described by Izquierdo *et al.* (2001). Briefly, subjects underwent progressive increases in resistance on a weekly basis, with the first eight weeks comprising 3-4 sets of 10-15 repetitions at 50-70% 1RM. During the second eight weeks, subjects performed 3-5 sets of 5-6 repetitions at 70-80% 1RM, as well as 3-4 sets of 6-8 repetitions at ~50% 1RM as fast as possible ('explosive' strength training). Ibanez *et al* (2005) found that PRT resulted in significant increases in strength, decreases in abdominal fat, and improvements in insulin sensitivity (reduced FPG), although HbA_{1c} levels were unchanged. The improvements in insulin sensitivity and glucose control were consistent whether subjects used acute or chronic resistance exercises, and were thought to be related to observed decreases in visceral abdominal fat levels and abdominal obesity.

An additional point in support of resistance training in treatment of T2D is that exercise is often prescribed in conjunction with a dietary-induced weight loss. However, as seen in older non-diabetic adults, weight loss is associated with accelerated bone and muscle loss (Daly *et al.*, 2005). However, Daly *et al.* (2005) found that the addition of a six-month program of high-intensity PRT (3 sets of 8-10 repetitions at 75-85% 1RM) to a dietary weight loss program prevented the loss of bone mineral density (BMD) in individuals with T2D compared to those on the weight loss program alone, as well as resulting in increased lean muscle mass and strength. The preservation of BMD is of critical importance to mobility and general health later in life for diabetic and non-diabetic individuals alike.

A combined resistance training (3 sets of 8-10 repetitions at 75-85% 1RM) and weight loss protocol was shown to achieve significantly greater reductions in

HbA_{1c} levels than in the weight loss intervention alone (Dunstan *et al.*, 2002). This greater reduction in HbA_{1c} is likely a consequence of the negative correlation between HbA_{1c} and muscle cross-sectional area (Sigal *et al.*, 2004). This finding is important to consider when designing exercise programs for individuals with T2D, as aerobic endurance programs are unlikely to elicit any significant increase in muscle mass. This has been illustrated in the work of Sigal *et al.* (2004), who compared the effects of a combined endurance- and resistance-training program to those of an endurance-training program alone. While both interventions resulted in improvements in insulin sensitivity, the intervention resulted in a significantly greater improvement in insulin sensitivity in the combined intervention group. This was notable due to the fact that this intervention resulted in an increase in muscle mass, whereas the endurance intervention did not.

1.5.2 Recommendations for Training Programs Specific for Individuals with T2D

The most recent ACSM guidelines recommends ≥ 150min of aerobic exercise per week spread over at least three days during the week, with no more than two consecutive days between sessions to ensure increases in insulin sensitivity are sustained. They also recommend moderate to vigorous resistance exercise 2-3 days per week in addition to the aerobic exercise (Colberg *et al.*, 2010). Aerobic endurance exercise should be performed with the aim of improving mitochondrial oxidative capacity, which is correlated with $\dot{V}O_2peak$ (Ostergard *et al.*, 2006), therefore aerobic exercise should be performed at a moderate intensity below VT with the aim of increasing $\dot{V}O_2peak$. Resistance exercise should be performed at progressive high intensities, as this induces the greatest increases in muscle mass and strength associated with improvements in glycaemic control; and has been shown to be appropriate for individuals with T2D, even if hypertensive (Dunstan *et al.*, 2002). Within a middle-aged male population with T2D, resistance training has been shown to help reverse age- and T2D-related sarcopenia (Praet & van Loon, 2007), as well as to help maintain BMD, even if weight loss occurs as a consequence of exercising (Daly *et al.*, 2005).

1.6 Study Protocol & General Hypothesis

It has previously been demonstrated that individuals with T2D display reduced exercise capacity compared to healthy controls (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Lalande *et al.*, 2008; Mac Ananey *et al.*, 2011; Wilkerson *et al.*, 2011). Furthermore, it has been demonstrated that females with T2D display slower $\dot{V}O_2$ kinetic responses to cycling exercise (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011). However, a recent investigation in older males with T2D did not demonstrate any T2D-related impairment in $\dot{V}O_2$ kinetics compared to healthy controls (Wilkerson *et al.*, 2011). However, there is a dearth of information on kinetic responses in middle-aged male cohorts. Furthermore, it has recently been shown that female with T2D also display impaired LVC kinetics to high intensity exercise in the calf (Macananey *et al.*, 2011). To this author's knowledge, no studies have attempted to determine whether males with T2D also display this impairment, and whether the impairment exists at low intensities.

Brandenburg *et al.* (1999) demonstrated that an exercise intervention was able to induce a significant improvement in the $\dot{V}O_2$ kinetic response to cycling exercise at 20 and 30W in females with T2D. Similarly, Mac Ananey (2010) demonstrated significant speeding of the $\dot{V}O_2$ kinetic responses to varying workloads in a mixed cohort of males and females with T2D. It is unknown whether an exercise intervention would induce similar responses in a male-only cohort, and whether exercising at a relative workload will impact on the findings. Furthermore, initial evidence suggests that treatment of T2D with TZDs leads to an increase in exercise capacity (Regensteiner *et al.*, 2005; Kadoglou *et al.*, 2007). However, research to date has focused on RSG; it is unknown at this point whether use of PIO will elicit the same results.

This study was conducted with four aims for investigation. The initial aim of the study was to assess $\dot{V}O_2$ and vascular conductance kinetic responses in middle-aged males with and without type 2 diabetes at the same relative intensities to determine the presence (if any) of a diabetes-related exercise impairment in middle-aged males.

The second aim of the study was to determine the effect of exercise-training and pioglitazone on $\dot{V}O_2$ kinetic and CO responses in middle-aged males with and without type 2 diabetes.

The third aim of the study was to determine the effects of the same exercise training and pioglitazone interventions on vascular conductance kinetics and reactive hyperaemia responses in the same group of individuals.

Finally, the fourth aim of the study was to determine the effect of these interventions on circulating levels and expression of various endothelial and inflammatory serum and mRNA markers. It was thought that this analysis would provide insight into the mechanisms of action of both the training intervention and PIO treatment on endothelial and peripheral haemodynamic adaptations, which in turn may offer a molecular basis for any improvement in performance witnessed as a consequence of the intervention.

It was hypothesised that at baseline, individuals with T2D would display significant impairments in both LVC and $\dot{V}O_2$ kinetics, $\dot{V}O_2$peak and performance on the plantar-flexion and cycle incremental tests compared to healthy individuals. Following the supervised phase of the training intervention, it was expected that the exercising T2D group would display significantly greater absolute and relative improvements than the exercising ND group; while it was further expected that PIO would have an added positive impact on these improvements. It was also expected that all exercising groups would be able to maintain the gains made in the supervised phase during the unsupervised phase of training.

CHAPTER 2: THE EFFECT OF T2D ON THE DYNAMIC RESPONSE CHARACTERISTICS OF $\dot{V}O_2$ AND LVC IN MALES

2.1 INTRODUCTION

It is well established that individuals with type 2 diabetes (T2D) have a reduced maximal exercise capacity ($\dot{V}O_2$peak) in comparison with healthy individuals of similar age, weight and activity levels (Kjaer *et al.*, 1990; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011; Wilkerson *et al.*, 2011). In addition, the rate of adjustment of oxygen uptake to steady-state exercise ($\dot{V}O_2$ kinetics) have been shown to be slower in individuals with T2D in many recent studies including young and middle-aged females (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011) as well as in a combined cohort of middle-aged males and females (Bauer *et al.*, 2007). However, a recent study by Wilkerson *et al.* (2011) on older males with T2D (mean age ~ 65yr) did not find any impairment in $\dot{V}O_2$ kinetic responses to submaximal cycling exercise compared with ND subjects, despite the individuals with T2D displaying reduced $\dot{V}O_2$peak responses. To this author's knowledge, no studies have looked at $\dot{V}O_2$ kinetic responses in young or middle-aged men with T2D.

The cause of the impairment in exercise performance is the source of some debate, with results from various studies offering contrasting results. The control of $\dot{V}O_2$ kinetics depends on a number of factors, including O_2 delivery (which in turn is affected by CO and BF), and O_2 extraction. A number of studies have looked at central cardiac function as a potential source of impairment. Roy *et al.* (1989) demonstrated impaired peak cardiac output (CO) in individuals with T2D, while Regensteiner *et al.* (2009) demonstrated tendencies for lower peak CO in females with T2D, although these differences did not reach significance. In contrast, both Mac Ananey *et al.* (2011) and Lalande *et al.* (2008) found no differences in CO during submaximal exercise between individuals with T2D and healthy counterparts. However, Lalande *et al.* (2008) did demonstrate reduced femoral arterial BF in individuals with T2D during steady-state exercise; suggesting that reduced O_2 delivery via impaired vascular function and reduced muscle BF perfusion may be a contributing factor to the T2D-associated impairment in exercise performance. In support of these findings, Kingwell *et al.* (2003) reported reduced leg BF responses to

steady-state cycling exercise, while Macananey *et al.* (2011) demonstrated slower LVC kinetic responses to steady-state plantar-flexion contractions at high intensities (70% MVC) in females with T2D compared with ND controls. However, to this author's knowledge it is unknown if the BF and VC kinetic responses to steady-state exercise are impaired in males with T2D.

A further avenue of investigation for the root cause of impairment in exercise performance is measures of O_2 extraction. It has been shown that muscle fibres of individuals with T2D have lower mitochondrial content than those of healthy individuals (Larsen *et al.*, 2009). Alternatively, Kelley *et al.* (2002) reported no differences between mitochondrial content of individuals with T2D and healthy counterparts, but suggested that mitochondria of different muscle fibre types have differing metabolic characteristics. In support of this, Copp *et al.* (2010) found that diabetic rats have a greater proportion of muscle BF diverted to glycolytic muscle tissues compared to healthy rat models. Similarly, Oberbach *et al.* (2006) described a metabolic shift in muscle fibres towards glycolytic metabolism in individuals with T2D; while Baldi *et al.* (2003) demonstrated that individuals with T2D demonstrated a reduction in the difference in maximal arterial-venous O_2 content (a-v O_2 diff) during exercise, which was associated with reduced $\dot{V}O_2$peak compared to healthy controls. These findings suggest that impaired metabolic function in the muscle may impair the O_2 extraction capabilities of individuals with T2D, revealing a potential source of the slower $\dot{V}O_2$ kinetics and reduced exercise performance in individuals with T2D.

The aim of the study was to determine the presence (if any) of an impairment of the $\dot{V}O_2$ kinetic and cardiac output responses to steady-state sub-maximal cycling exercise in a large group of middle-aged males with T2D. A secondary aim was to determine if peripheral O_2 delivery was impaired through analysis of BF and VC during steady-state plantar-flexion exercise at low (30% MVC) and high intensities (70% MVC) in the calf, and also through forearm reactive hyperaemia (RH). LVC kinetic responses to calf exercise in males with T2D are unknown. It was hypothesised that middle-aged males with T2D would demonstrate a reduced $\dot{V}O_2$peak, and slowed $\dot{V}O_2$ and LVC kinetic responses compared to healthy counterparts.

2.2. METHODS

2.2.1 Subjects

The study was comprised of two groups of participants, one group of individuals with T2D, and a healthy ND group.

2.2.1.1 Recruitment of subjects

All participants in the ND group (n=20) were recruited from the general population via a series of advertisements placed locally and distributed among local businesses. Contact details for the principal investigator were provided and interested candidates were asked to contact the investigator for further information.

All participants with T2D (n=33) were recruited from patients attending the Diabetes Day Care Centres of St. Columcille's Hospital, Loughlinstown, Co. Dublin, and St. Vincent's University Hospital, Dublin. Suitable candidates were identified via chart review performed by the investigator prior to the patients' attendance. These candidates were provided with a short information sheet outlining the study details and those interested in participation were asked to contact the investigator.

2.2.1.2 Inclusion/Exclusion Criteria

ND participants were initially considered eligible for participation if between the ages of 30 and 75, free from CVD or any other serious medical conditions that would affect exercise performance. Participants had to be sedentary (defined as $<1 hr.wk^{-1}$ of moderate exercise over the previous six months). Further grounds for exclusion included either a resting systolic BP in excess of 170mmHg and/or diastolic BP above 95mmHg.

Individuals with T2D were identified as being suitable for participation if between the ages of 30 and 75; if the date of diagnosis of T2D had occurred within the previous 10 years; and if their HbA_{1c} levels were $<86 mmol.mol^{-1}$ (indicating reasonable levels of glycaemic control). Additionally, candidates had to be sedentary, and treating their T2D through a dietary intervention or use of oral agents other than TZDs due to their potential effect on endothelial function (Albertini *et al.*, 2007; Kadoglou *et al.*, 2008; Harashima *et al.*, 2009). Furthermore, patients currently using insulin were not considered eligible, as it suggests a more severe progression of T2D. Additional grounds for exclusion included existence of persistent proteinuria (urinary

protein >200mg.dl^{-1}) or excess urinary creatinine (>2mg.dl^{-1}). Diagnosis of peripheral arterial disease (PAD) or coronary heart disease (CHD) was also grounds for exclusion. Absence of these conditions was confirmed by a twelve-lead electrocardiogram (ECG) stress test (described below). Hypertensive patients (systolic BP >140mmHg) were admitted to both ND and T2D groups as long as resting systolic BP did not exceed 170mmHg, and were spread evenly between the two groups. However, subjects who used beta-blockers in treatment of hypertension were not considered in the analysis, due to the effects of this class of drug on sympathetic activation of the heart (Francesconi *et al.*, 1999).

2.2.1.3 Participant information form

Prior to admission to the study, all subjects were provided with the participant information form (see appendix II), which explicitly stated the aims of the study, the procedures and requirements for participation, as well as the potential risks and benefits of participation. Having read the information form and had any questions answered by the principal investigator, willing participants then provided written consent (appendix II) before beginning participation in the study. The study was conducted in accordance to the principles outlined by the Declaration of Helsinki, and was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College Dublin.

2.2.1.4 Stress Test for Individuals with T2D

All individuals with T2D were also required to undergo a stress test (Bruce protocol) on a treadmill supervised by a qualified medical practitioner in St. Columcille's Hospital, Loughlinstown, Co. Dublin. Subjects performed the stress test while undergoing a 12-lead ECG. Pulse waveforms were assessed at rest, during the exercise, and during the recovery to ensure that the heart responded adequately to exercise and to exclude the possible presence of PAD and/or CHD. BP and heart rate (HR) were assessed continuously during the stress test protocol, with a systolic BP in excess of 220mmHg during exercise considered grounds for exclusion. Absence of PAD was determined via the absence of claudication/pain in the limbs during the stress test, and was confirmed at a later date by measuring the ankle:brachial index (see below).

2.2.1.5 Medical Examination

All ND participants were required to undergo a medical examination by a medical doctor. Subjects were required to complete a medical questionnaire (appendix III) and undergo a physical examination.

2.2.1.6 Blood Sample Collection

Blood samples were collected from participants following a 12-hour fast. Full blood counts, including haemoglobin (Hb) and haematocrit (HCT) were determined using a haematological particle analyser (Coulter Ac Tdiff: Coulter Ltd., United Kingdom). Fasting blood glucose (FBG), HbA_{1c} and lipid profile (total cholesterol, HDL-C, LDL-C, triglycerides) were determined at the haematology laboratory of St. Columcille's Hospital, Loughlinstown.

2.2.1.7 Determination of Physical Activity Levels

In order to confirm that participants were sedentary, subjects were equipped with an RT3 accelerometer (Stayhealthy Inc, California). The RT3 unit is a triaxial accelerometer, which assesses motion in the mediolateral (X), anteroposterior (Y), and vertical (Z) axes. The acceleration is recorded at intervals, converted to a digital representation, and processed to obtain an "activity count," which is stored in the unit's memory chip. The RT3 has four modes of operation; mode 1 samples and stores activity counts on individual axes at 1 second intervals; mode 2 samples and stores vector magnitude (a measure combining all three axes of motion, calculated as $[(X^2 + Y^2 + Z^2)^{0.5}]$) activity counts at 1s intervals; mode 3 samples and stores accumulated activity counts on individual axes at 1 minute intervals; and mode 4 samples and stores accumulated vector magnitude activity counts over 1-min intervals (Eston *et al.*, 1998; Powell *et al.*, 2003). Triaxial accelerometers have been shown to estimate energy expenditure during low-level activities with greater accuracy than heart rate, and are highly-associated with energy intake during 1 week under free-living conditions (Eston *et al*, 1998). The RT3 unit in particular has been validated against oxygen uptake in both children and adults over a range of activities (Powell *et al*, 2003), while also having been validated to detect changes in exercise intensity in overweight/obese individuals, ensuring that the activity levels of the subjects in the current study would be correctly assessed (Jacobi *et al.*, 2007).

Subjects were requested to wear the RT3 unit for a minimum of 5 days, including one day from a weekend during all waking hours (except for swimming or bathing). Subjects were asked to place the unit on their belt loop. It has previously been demonstrated that activity counts do not differ between left or right hip placement (Powell & Rowlands, 2004). It has been shown that longer wear times does not significantly increase reliability of the data, with four days of data with 6 hours wear time per day shown to optimise the balance between intraclass correlation and participant burden in overweight and obese adults (Jerome *et al.*, 2009). Therefore, subject's who compiled a minimum of four days data were accepted; however, any subject's who completed less than four days data had the unit returned to them and asked to complete it again. All data recording points were made in mode 4 (vector magnitude with counts recorded every minute).

2.2.2 Experimental Design

2.2.2.1 Study Overview

Subjects were required to attend the cardiovascular laboratory in the Department of Physiology, Trinity College Dublin on two separate occasions. The first visit to the laboratory began with collection of anthropometric data. Subsequently, subjects were familiarised with the calf ergometer, and underwent assessment of steady-state LVC kinetic responses to calf exercise. Following this, LVC responses were assessed as subjects underwent a graded incremental plantar-flexion test to failure. Following a rest period, forearm blood flow (FBF) responses to RH were assessed. Subjects completed the first visit to the lab with a graded incremental cycle test to exhaustion to determine $\dot{V}O_2$peak and VT. On the second visit to the laboratory, subjects' $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT were assessed over four bouts; CO responses during steady-state cycling were then assessed during a fifth and sixth bout.

Visits to the lab were separated by a minimum of 72 hours and no more than one week. Subjects were asked to refrain from performing any exercise in the 24-hour period preceding both test dates. Every effort was made to replicate the test conditions for both test dates, including the time of day the test was performed, and room

temperature; while subjects were asked to refrain from alcohol and caffeine consumption for 24 hours prior to testing.

2.2.2.2 Anthropometry

2.2.2.2.1 Mass, Height, Body Mass Index

Each subject's first visit to the laboratory began with the collection of anthropometric data. Body mass was determined on a set of platform beam scales (AVERY, United Kingdom), with height measured using a SECA^{TM} Stadiometer (SECA Ltd., Germany). Body mass index (BMI) was then determined by dividing the subject's mass (kg) by their height squared (m^2).

2.2.2.2.2 Leg Volume

Leg volume was determined by a regression equation formulated by Clarys & Marfell-Jones (1986) based upon measures of calf skinfold thickness, calf girth, tibial malleolar length, and the bimalleolar breadth:

Tibial malleolar length x [38.20851 + maximal calf girth – (π x (medial calf skinfold/10))] x 80.24423 – 2467 [1]

Calf skinfold thickness was assessed using Harpenden skinfold callipers (Baty Ltd., United Kingdom). Calf girth and tibial malleolar length were determined using a measuring tape, while bimalleolar breadth was measured using dual scale electronic Vernier callipers (Mitutoya, Japan).

2.2.2.2.3 Waist:Hip Ratio

The subject's waist measurement was assessed at the level of the umbilicus, with the hip measurement collected in line with the greater trochanter. Both measurements were determined using a measuring tape. The waist:hip ratio (WHR) was then calculated by dividing the waist measurement (cm) by the subject's hip measurement (cm).

2.2.2.2.4 Ankle:Brachial Index

Assessment of the ankle:brachial index (ABI) was performed to determine the relative condition of the peripheral arteries of the legs to the brachial artery, as a further determination to ensure the absence of PAD. The technique for assessing ABI

has been described elsewhere (Fleck, 2007). Briefly, measurements were collected by initially placing a blood pressure cuff (Hokanson, United States) 2.5cm above the antecubital fossa of the left arm with the subject having been in the supine position for a period of five minutes. The brachial artery was located and an MD6 Bidirectional Doppler (Hokanson, USA) was applied at a 45° angle towards the subject's head. The cuff was then inflated to suprasystolic pressures (~220mmHg), and gradually deflated until the pulse could be detected via Doppler. The pressure (mmHg) at which this occurred was recorded, and the procedure was then repeated on the right arm. Repeating the measurement on both arms has been shown to reduce standard error and bias by 30-40% (Espeland *et al.*, 2008). Following this, the cuff was then placed 2.5cm above the medial malleolus of the left leg, and the posterior tibialis artery located (Anderson, 2002). The procedure from above was repeated for detection of the pulse in the posterior tibialis artery, as well as the dorsalis pedis artery, and then again on the right leg. The ABI was then calculated by dividing the highest of the four readings from the posterior tibialis and dorsalis pedis arteries by the higher of the two brachial artery recordings. A reading of between 0.9 and 1.3 indicated absence of PAD.

2.2.3 First Visit to the Cardiovascular Laboratory

2.2.3.1 Familiarisation with Calf Ergometer

Having collected the anthropometric data, the investigator determined resting BF measurements. Following this, subjects were then asked to perform a series of low-intensity isometric plantar-flexions of the right calf muscle while supine to familiarise themselves with the protocol.

2.2.3.2 Maximum Voluntary Contractions

Having completed the familiarisation process, subjects were then required to perform a series of maximum voluntary contractions (MVC) to determine the peak force (Newtons, N) produced via plantar-flexion. This was repeated until the investigator was satisfied that the subject had achieved their true MVC, which typically took between six and ten contractions. The investigator then calculated the workload corresponding to 30% and 70% of the peak force produced, as these would

be the workloads the subject would perform during constant-load exercise aimed at assessing LVC kinetics.

2.2.3.3 LVC Kinetics

Subjects were required to complete three six-minute bouts of constant-load contractions at 30% MVC, operating a 2:4s contraction:relaxation ratio. This contraction:relaxation ratio (i.e., 1:2) was selected because it has previously been shown to reflect to the relative period of activation of the calf muscle during walking on flat and inclined terrain (Egana & Green, 2005). The low workload of 30% MVC was chosen as previous investigations had looked at effects of higher intensity plantar-flexion exercise only (Egana & Green, 2005, 2007; Macananey *et al.*, 2011). A ten-minute rest period separated each bout to ensure full recovery prior to the onset of subsequent bouts. For the duration of the six-minute bouts, a cuff around the upper thigh was inflated at a constant pressure of 50mmHg (Engelke *et al.*, 1996; Egana & Green, 2005). As a consequence, during the relaxation phase of each plantar-flexion, pooling of the blood in the veins was detected via strain gauge and used to determine the BF response to each individual contraction. During contraction, the blood that had accumulated in the veins was driven past the cuff back into venous circulation via skeletal muscle pump action on the veins.

A subset of individuals in each group (n = 8 in the ND group; n = 15 in the T2D group) were asked to perform an additional two bouts at 70% MVC. The recovery period after each bout was extended to 15 minutes to account for the increased level of exertion, but the 6s duty cycle was the same as the bouts at 30% MVC. The bouts at 70% MVC were performed in an attempt to replicate the findings of Macananey *et al.* (2011), who demonstrated that females with T2D displayed impaired LVC kinetic responses at 70% MVC in the upright posture relative to healthy age- and BMI-matched individuals, which suggests that their findings are also applicable to males with T2D.

2.2.3.4 Calf Incremental Plantar-Flexion Test and Forearm Reactive Hyperaemia

Following the conclusion of the constant-load bouts and a suitable rest period (~20 minutes), subjects then underwent a graded calf incremental test. Using the same 2:4s contraction:relaxation ratio, subjects began by exerting a force of 100N per contraction for two minutes. Following completion of each two-minute phase, the

workload was increased in increments of 200N every two minutes. The increments were chosen based on previous work performed in this laboratory that suggested subjects would achieve failure within 10-12 minutes, reflecting typical time to failure in VO_2max testing (Egana & Green, 2005, 2007; Mac Ananey, 2010). The test continued until subjects were unable to achieve the desired force output for three consecutive contractions. During the incremental test, calf LBF was recorded during the relaxation phase between each contraction, and mean arterial pressure (MAP) and HR (on a beat-by-beat basis) were also recorded.

After completion of the incremental plantar-flexion test, subjects underwent assessment of forearm blood flow (FBF) responses to ischaemic reactive hyperaemia. Following measurement of resting FBF values, circulation to the right forearm was occluded by inflating a pneumatic cuff to 220mmHg for a five-minute period (Wascher *et al.*, 1998). It has previously been demonstrated that peak FBF responses are dose-dependent up to five minutes ischaemia, therefore occlusion beyond this is unnecessary (Engelke *et al.*, 1996; Francesconi *et al.*, 1999). Following the release of the cuff, FBF was assessed at 10s intervals for five minutes, and once per minute for an additional five minutes.

2.2.3.5 Incremental Cycle Test to Exhaustion ($\dot{V}O_2$peak test)

The test commenced with a three-minute rest period where the subject sat stationary on the bike. The subjects then began to cycle at 40watts (W), maintaining a cadence between 60-65 revolutions per minute (rpm). At three-minute intervals, the workload was increased by an increment of 30W until volitional exhaustion (defined as the inability to maintain the required power output for 3s at a cadence of 60rpm (Egana *et al.*, 2007)). The three-minute increment in workload has been previously validated as an effective protocol to achieve VO_2max (Zhang *et al.*, 1991; Pierce *et al.*, 1999; Roffey *et al.*, 2007). Peak workload was determined according to the point of termination of the test. Each subject was required to complete a minimum of one minute of the final stage to have been considered to achieve said workload. During the resting and exercise periods, HR was continuously monitored with recordings made every five seconds; while pulmonary minute ventilation ($\dot{V}_e$), $\dot{V}O_2$ and $\dot{V}CO_2$ were recorded on a breath-by-breath (BbB) basis. VT was established using the V-slope method (Beaver *et al.*, 1986; Amann *et al.*, 2006). The V-slope method has been

validated as an appropriate method for the detection of metabolic acidosis, even in individuals where other gas exchange indices are insensitive for a variety of reasons (Wasserman *et al.*, 1990). The V-slope method compared volumes of $\dot{V}O_2$ and $\dot{V}CO_2$, and has good reliability as changes in these volumes are independent of chemoreceptor sensitivity or the ventilatory response to exercise. The V-slope method detects the $\dot{V}O_2$ at which CO_2 is generated as a result of HCO_3^- buffering of lactic acid. Above the VT, the rate of increase in $\dot{V}O_2$ and $\dot{V}CO_2$ increases at a steeper rate, creating a clear inflection point, defining VT (Wasserman *et al*, 1990).

2.2.4 Second Visit to the Cardiovascular Laboratory

2.2.4.1 $\dot{V}O_2$ Kinetics Analysis and Cardiac Output Responses

After a minimum period of 72 hours following completion of the first visit to the laboratory, the subjects returned to the cardiovascular lab for assessment of $\dot{V}O_2$ kinetics during steady state cycling. Subjects were required to complete three minutes semi-unloaded cycling (resistance on the wheel of 10W) transitioning into a six-minute bout of cycling at an intensity equivalent to 80% VT, determined from the incremental test performed during their first visit. This was repeated for a total of four bouts, with each bout separated by a twelve-minute recovery period. The 12-minute duration was determined in a pilot study as the time required for lactate and HR to return to resting values. This finding is in agreement with prior research (Timmons *et al.*, 1997; Koppo *et al.*, 2009; Whipp, 2009). During these four bouts, BbB $\dot{V}O_2$ responses and HR values (every five seconds) were recorded.

After completion of these four bouts, subjects performed an additional two bouts with the same protocol described above. However, during these final two bouts, cardiac output (CO) measurements were taken prior to each bout, 30s and 240s into the 80% VT workload using an inert gas rebreathe technique. Figure 2.1 demonstrates a subject performing the CO rebreathe technique.

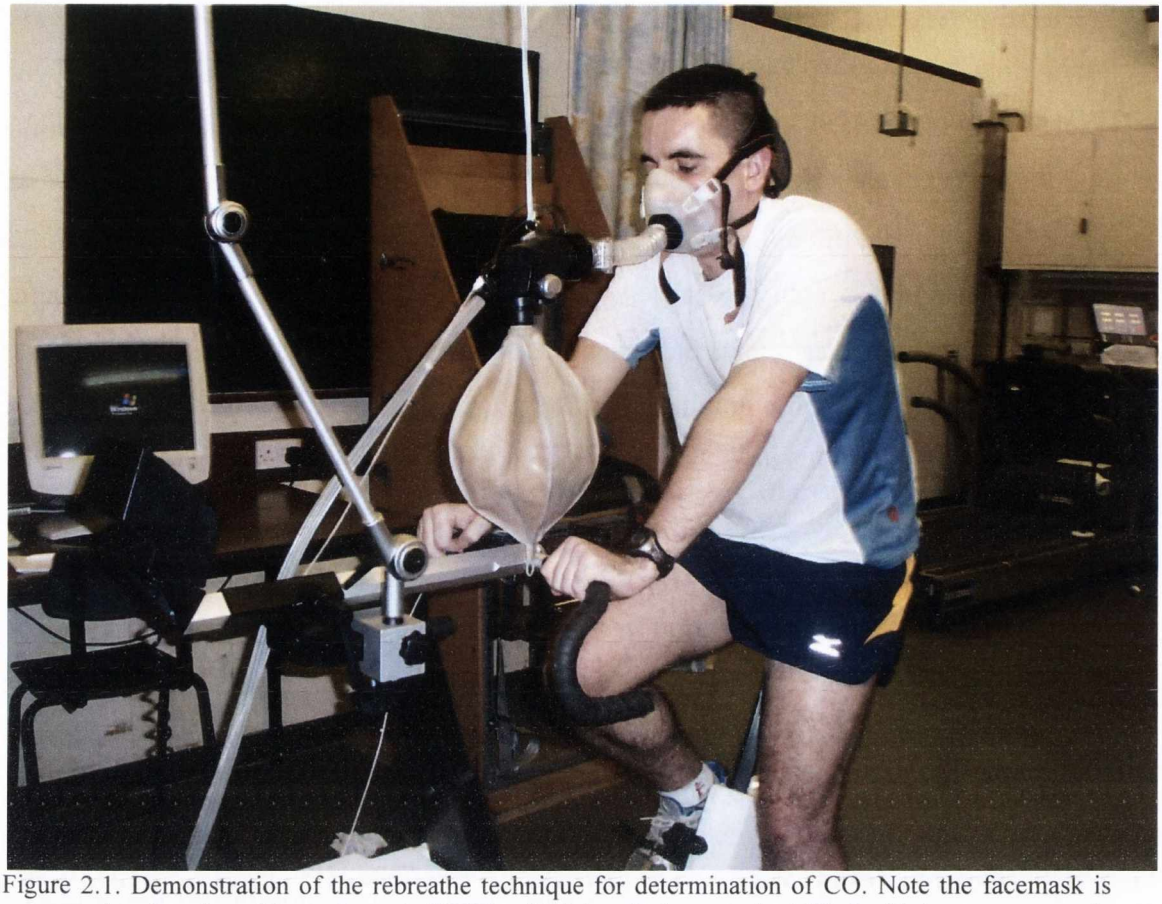


Figure 2.1. Demonstration of the rebreathe technique for determination of CO. Note the facemask is connected to the rebreathing valve unit (RVU), which has 5L bag attached filled with inert gaseous mix for determination of CO.

2.2.5 Equipment and Techniques

2.2.5.1 Calf Ergometer

To assess VC responses during exercise, subjects were placed supine into a custom-built calf ergometer (figure 2.2). The calf ergometer consists of a tilt table and two immobilised footplates, each of which are connected to an analogue-digital converter (Powerlab ML795, AD Instruments, USA), which provides an electronic read-out (sampled at 40Hz) of the force applied to the foot-plates (figure 2.3). Assessments were taken from the right foot only, with the left foot resting on a padded cushion parallel to the right leg. To prevent backward movement of the subject away from the footplate following plantar-flexion, subjects were secured to the table via a body harness. Calibration data for the calf ergometer demonstrating the coefficient of variation are given in appendix IV. An intraclass coefficient (ICC) value of 0.8 for the calibration data over the duration of the testing period indicates that the foot-plate readings have excellent repeatability.

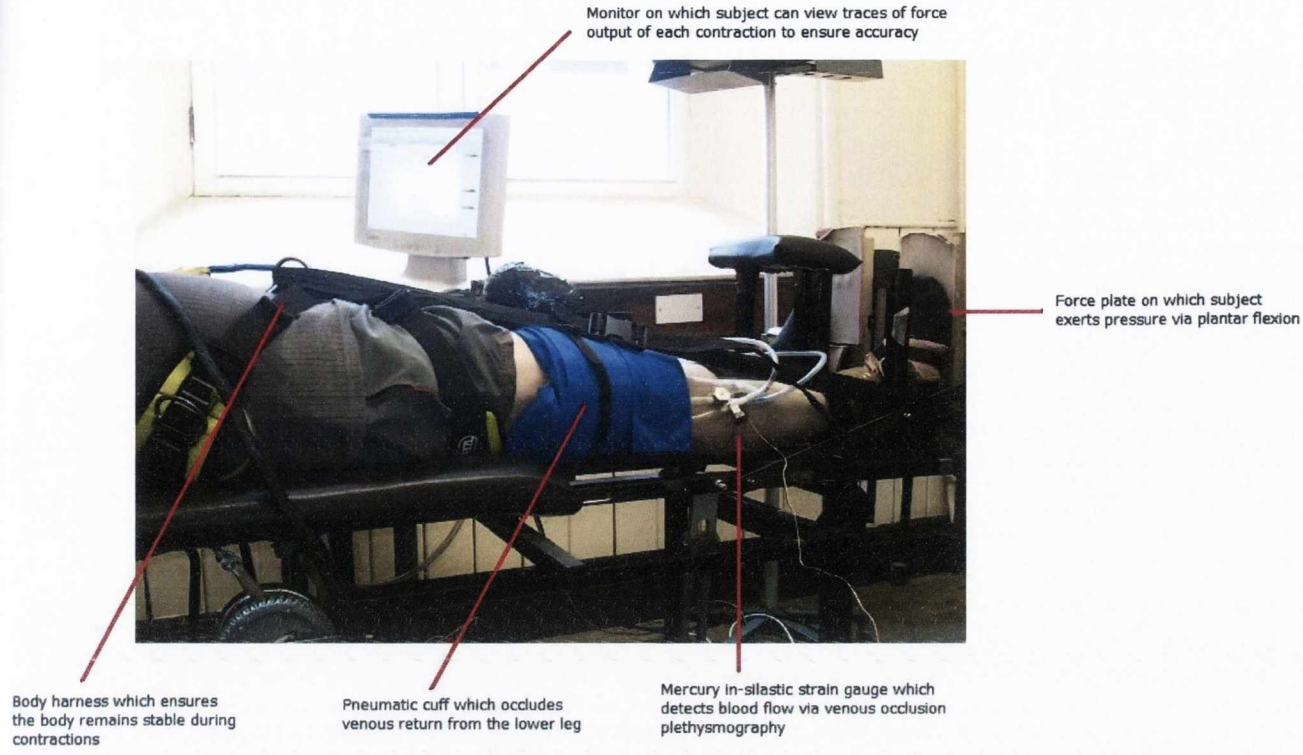


Figure 2.2. A subject strapped into the calf ergometer performing constant-load exercise at 30% of MVC.

2.2.5.2 Venous Occlusion Plethysmography

BF was assessed using venous occlusion plethysmography (Hokanson EC-6, USA). VOP has been recently validated as an accurate tool for measurement of blood flow in comparison to the gold standard method of Doppler ultrasound (Green *et al.*, 2011). VOP assesses BF by placing a mercury-silastic strain gauge (Hokanson, USA) (measuring 2cm less than the widest part of the calf) around the subject's right calf and connecting to the plethysmograph. The strain gauge was calibrated prior to each use in percent volume change allowing for normalisation of results, with their accuracy guaranteed as long as electrical continuity is maintained within the electrodes (Hokanson, USA). A cuff (Hokanson, USA) was placed around the upper right thigh of the subject, and inflated to 50mmHg. The pressure of 50mmHg was chosen to occlude venous return without interfering with arterial BF (Egana & Green, 2005).

The cuff used in testing was a standard large adult thigh cuff utilised for determination of thigh blood pressure and leg venous plethysmography (Hokanson model CC22^{TM}). The cuff houses a 'bladder' which receives pressurised airflow from

a custom-built rapid cuff inflator causing inflation of the bladder to a fixed pressure (50mmHg). Inflation pressure was controlled by an attached sphygmomanometer used to monitor the degree of pressure of the cuff.

As blood pooled in the veins, the strain gauge detected the change in calf girth, as displayed in figure 2.3. Resting BF measurements were determined by rapidly inflating and deflating the leg cuff to 50mmHg at five-second intervals on 8-10 separate occasions. BF during exercise was continuously assessed by measuring the change in girth over time during the 4s relaxation period between contractions.

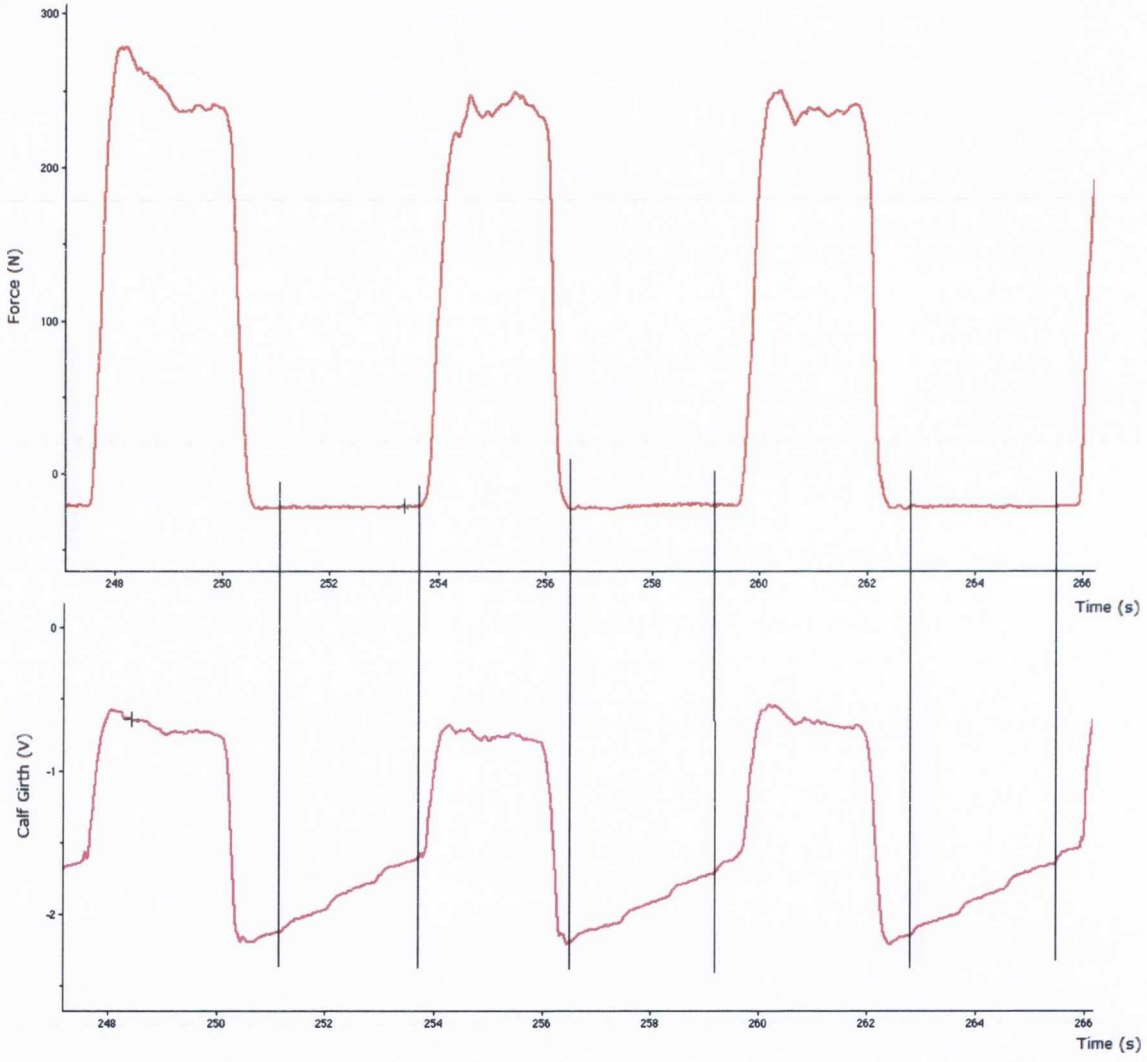


Figure 2.3. Example of electronic read-out provided during plantar-flexion exercise. The top panel reflects force production (denoted by the red line (-)). The bottom panel reflects BF as determined by VOP, with the change in calf girth denoted by the pink line (-). The black lines indicate the relaxation period in which BF has been assessed. Calf BF was calculated as the rate of change of calf girth (v) over time (s).

2.2.5.3 Blood Pressure Tonometry

Blood pressure (and in turn HR) was measured on a beat-to-beat basis using applanation tonometry (COLIN CBM7000, Japan). Applanation tonometry has been previously validated in both adult and children populations (Zorn *et al.*, 1997), with excellent inter-observer reproducibility (Siebenhofer *et al.*, 1999), and repeatability (Crilly *et al.*, 2007). The left arm was extended away from the body level with the heart, with an oscillometric cuff placed around the upper arm. A tonometric blood pressure sensor was placed over the radial artery on the left wrist and held in place with a Velcro strap. The sensor was calibrated to the pressure recorded by the cuff at five-minute intervals to ensure accuracy of the recordings. MAP was then calculated from the recordings by the following formula:

$$\text{MAP} = 1/3(\text{systolic BP}) + 2/3(\text{diastolic BP}) \quad [2]$$

2.2.5.4 Metronome

A metronome (Metronome MA-330; Korg, Japan) was used to provide an electronic count allowing the subjects to exert a contraction:relaxation ratio of 2:4s. The forces produced by each contraction (figure 2.3) were visible to the subjects on the computer monitor (figure 2.2).

2.2.5.5 Vascular Conductance Kinetics

Having recorded the absolute BF ($ml.100ml^{-1}.min^{-1}$) from the change in calf girth over time, the BF response was then divided by the corresponding MAP to give vascular conductance (VC) units of $ml.100ml.^{-1}min^{-1}.mmHg^{-1}$. Each response was then further adjusted for leg volume (ml) providing final units of $ml.min^{-1}.mmHg^{-1}$.

The mean VC responses of the three bouts were curvefitted using the software TableCurve (TableCurve 2D v5.01, SYSTAT Software INC, USA), using a quad-exponential function described by the following equation:

$$y(t) = A_0 + A_1(1 - e^{-(t-TD1/\tau1)})\mu_1 - A_2(1 - e^{-(t-TD2/\tau2)})\mu_2 + A_3(1 - e^{-(t-TD3/\tau3)})\mu_3 - A_4(1 - e^{-(t-TD4/\tau4)})\mu_4 \quad [3]$$

y represents the VC at time t, A_0 is the resting VC, A_1 and A_3 are the increases in VC during the primary and tertiary phase of the kinetic response respectively. A_2 and A_4 represent the decrease in amplitude during the secondary fast decay and the slow decay of the fourth phase respectively. TD_1, TD_2, TD_3, and TD_4 represent the time

delays for the four individual phases respectively; while τ_1, τ_2, τ_3 and τ_4 representing the time constants for the corresponding phases. μ_1, μ_2, μ_3 and μ_4 represent binary variables where $\mu_1 = 0$ for $t < TD_1$, and $\mu_1 = 1$ for $t \geq TD_1$; $\mu_2 = 0$ for $t < TD_2$, and $\mu_2 = 1$ for $t \geq TD_2$; $\mu_3 = 0$ for $t < TD_3$, and $\mu_3 = 1$ for $t \geq TD_3$; and $\mu_4 = 0$ for $t < TD_4$, and $\mu_4 = 1$ for $t \geq TD_4$.

The steady-state amplitude, referred to as End A, was calculated using the following formula:

$$y(t) = A_0 + A_1(1 - e^{-(360-TD1/\tau1)}) - A_2(1 - e^{-(360-TD2/\tau2)}) + A_3(1 - e^{-(360-TD3/\tau3)}) - A_4(1 - e^{-(360-TD4/\tau4)}) \quad [4]$$

2.2.5.6 Incremental Test Parameters and Slopes

LBF and LVC responses during the incremental test were calculated in the same manner as that described in section 2.2.5.5. Following calculation of the BF and VC responses, these responses were then plotted and the slope of the individual response calculated. Two plots were formed. Initially, BF and VC responses were plotted on the *y*-axis, with the workload (N) plotted on the *x*-axis. The slope was then taken as the coefficient (a) of the x-value in the following equation:

$$y = ax + b \quad [5]$$

Following this, the workloads achieved were expressed as a percentage, with the highest workload achieved expressed as 100%. The BF and VC value were then plotted against the percentage workload using equation [5] above.

2.2.5.7 Reactive Hyperaemia Response

During the RH protocol, baseline FBF was assessed six times during a one-minute period by rapidly inflating (5s) to 50mmHg and deflating to 0mmHg (5s) a pneumatic cuff (Hokanson, USA) secured around the upper right arm. Inflation of the cuff caused a change in the volume of the forearm, which was detected by a strain gauge placed around the widest part of the right forearm, in a manner similar to that described above (see section 2.2.5.2). Since volume of the forearm was not assessed, FBF responses were absolute ($ml.100ml^{-1}.min^{-1}$). Ischaemia was then induced by inflating the cuff around the upper right arm to supra-systolic pressures (~220mmHg) for a five-minute period (Wascher *et al.*, 1998). Following release of the arterial occlusion, FBF was assessed at ten-second intervals for a five-minute period, with a

further FBF assessment at one-minute intervals for an additional five minutes after this to ensure a return to resting FBF levels. FBF responses in the post-ischaemic phase were assessed during the second complete cardiac cycle following release of the cuff (figure 2.4). This was done to ensure venous leakage did not result in underestimating the FBF (Tschakovsky *et al.*, 1995). To determine the rate of decline from peak values back to steady-state post-ischaemic FBF, responses were fitted by applying a mono-exponential function (TableCurve 2D v5.01, USA)) to the data points (Wascher *et al.*, 1998):

$$y(t) = a + b(e^{-(c*t)}) \quad [6]$$

b represents the amplitude of the FBF response, the rate of decline (decay constant) is characterised by c, and steady-state post-ischaemic flow is represented by a; while the area under the curve (AUC) was calculated based on the mono-exponential fit assessed. FBF responses were adjusted for the MAP associated with the cardiac cycle in which FBF was calculated in order to give forearm VC ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$).

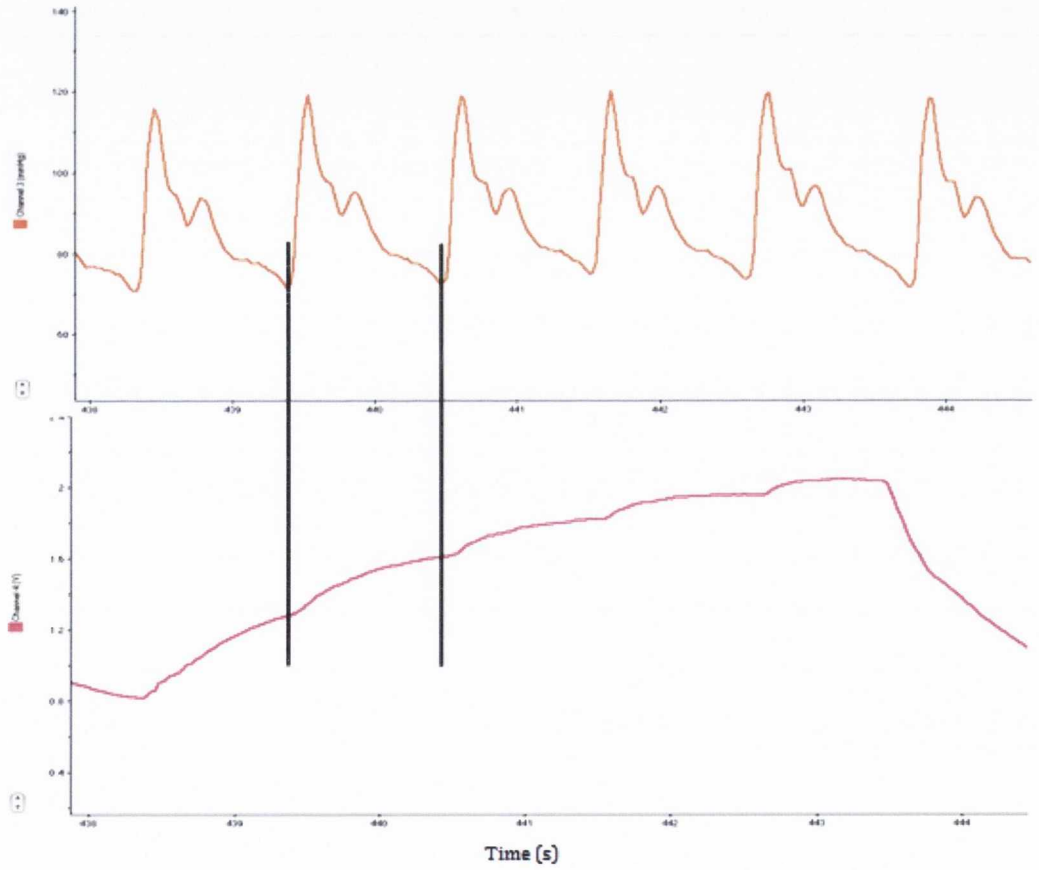


Figure 2.4. A sample trace from a subject undergoing forearm RH. The pink trace (-) represents the change in arm girth over time, with the orange trace (-) representing MAP on a beat-by-beat basis. The black lines represent the time period in which the RH response was assessed (the second clear heart-beat following release of the venous occlusion).

2.2.5.8 Cycle Ergometer and Innocor Metabolic Unit

Participants performed all cycle-based exercise testing on an isokinetic cycle ergometer (Excalibur Sport, LODE Ltd., Netherlands). The LODE ergometer is considered the gold standard when performing laboratory-based cycle ergometry testing, demonstrating excellent repeatability (Earnest *et al.*, 2005). Subjects were fitted to the bike, with recordings made of saddle height, handle bar length, and height for future recordings. A HR monitor (Polar S610i, Polar Ltd., Finland) was fitted to the subject's chest. A pulse oximeter (Innocor, Innovision, Denmark) was fitted to the middle finger of the right hand. A nose-clip (Speedo®, United Kingdom) and silicone face mask (Hans Rudolph., USA, 7900) were also fitted and connected to a rebreathing valve unit (RVU) that allowed for BbB determination of $\dot{V}_e$, $\dot{V}O_2$, and $\dot{V}CO_2$ (Innocor, Innovision, Denmark). The Innocor has demonstrated excellent reliability for gas exchange measurements (Fontana *et al.*, 2009).

2.2.5.9 $\dot{V}O_2$ Kinetics Analysis

BbB data was determined for each individual bout. Any individual breaths lying outside four standard deviations of the mean were excluded from analysis, being attributed to erratic breathing due to events such as coughing or swallowing. In order to effectively compare the data between individuals, BbB data were interpolated to determine per-second values of $\dot{V}O_2$. The time-aligned data for the four bouts were subsequently averaged to determine mean per-second $\dot{V}O_2$ values for the six-minute period. The data were further smoothed using a five-point centred moving average to accentuate the underlying physiological response. Baseline $\dot{V}O_2$ was set as the average of the $\dot{V}O_2$ values during the three minutes of semi-unloaded cycling (10W) preceding the six minutes at 80% VT.

The $\dot{V}O_2$ kinetic responses to exercise below VT were fitted using TableCurve graphing software (TableCurve 2D v5.01, USA). A number (5 ND individuals, 10 individuals with T2D) of the $\dot{V}O_2$ kinetic responses on visual inspection appeared to contain a third phase (slow component). Therefore all data for these 15 individuals were fitted using both bi-exponential [7] and tri-exponential [8] models, with the remainder of subjects fitted with the bi-exponential model [7] only:

$$\dot{V}O_2(t) = A_0 + A_1(1 - e^{-(t-TD1/\tau1)})\mu_1 + A_2(1 - e^{-(t-TD2/\tau2)})\mu_2 \qquad [7]$$

$$\dot{V}O_2(t) = A_0 + A_1(1 - e^{-(t-TD1/\tau1)})\mu_1 + A_2(1 - e^{-(t-TD2/\tau2)})\mu_2 + A_3(1 - e^{-(t-TD3/\tau3)})\mu_3 \quad [8]$$

Where $\mu_1 = 0$ for $t < TD_1$, and $\mu_1 = 1$ for $t \geq TD_1$; $\mu_2 = 0$ for $t < TD_2$, and $\mu_2 = 1$ for $t \geq TD_2$, and $\mu_3 = 0$ for $t < TD_3$, and $\mu_3 = 1$ for $t \geq TD_3$. $\dot{V}O_2(t)$ is the $\dot{V}O_2$ at any given time point; A_0 is the baseline $\dot{V}O_2$; A_1, A_2, and A_3 are the asymptotic amplitudes for the exponential terms for phases I, II and III; τ_1, τ_2, and τ_3 are the time constants for phases I, II and III; and TD_1, TD_2, and TD_3 are the time delays for phases I, II and III. With regard to the 15 individuals displaying a visual slow component, parameter estimates were drawn from the model providing the best 'goodness-of-fit' (appendix XV; (Wilkerson *et al.*, 2004), with 12 individual responses best characterised by the tri-exponential model. Analysis of 95% confidence intervals (CI) for the primary parameters of interest (τ_2 and A_2) revealed that those responses best characterised by the tri-exponential model fit had narrower 95% CI for both τ_2 (4.4 ± 1.7s vs. 2.5 ± 0.7s) and A_2 (0.18 ± 0.13 vs. 0.09 ± 0.06) compared to the bi-exponential model, confirming the appropriate use of this model in these cases. Only parameter estimates for the cardiodynamic and primary phases from the tri-exponential model are presented in this manuscript (Motulsky & Ransnas, 1987).

The steady-state $\dot{V}O_2$ response, referred to as End A was calculated using the following formula:

$$y(t) = A_0 + A_1(1 - e^{-(360-TD1/\tau1)}) + A_2(1 - e^{-(360-TD2/\tau2)}) \quad [9]$$

The mean response time (MRT) for each subject (the time to reach ~63% of the exercise plateau from baseline) was calculated using the following equation (MacDonald *et al.*, 1998):

$$MRT = (A_1/(A_1 + A_2))*(TD_1 + \tau_1) + (A_2/(A_1 + A_2))*(TD_2 + \tau_2) \quad [10]$$

$\dot{V}O_2$ gain, defined as the increase in O_2 consumption per unit of workload (W), was calculated using the following equation:

$$\dot{V}O_2 \text{ gain } (ml.min^{-1}.W^{-1}) = (End\ A - A_0)/(Workload\ @\ 80\%\ VT - 10W)*1000 \quad [11]$$

2.2.5.10 Heart Rate Kinetics Analysis

Heart rate (HR) was measured and recorded every five seconds during each bout. Time-aligned data for bouts 1-4 were averaged to determine the average HR response to steady-state cycling at 80% VT. Once averaged the data were curvefitted to the following monoexponential equation using TableCurve (TableCurve 2D v5.01, USA):

$$HR(t) = A_0 + A_1(1 - e^{-(t-TD/\tau)}) \quad [12]$$

Where HR(t) is the HR at any given time point during the six-minute cycling bout; A_0 represents the average HR (beats.min^{-1}) during the three minutes semi-unloaded cycle prior to the onset of cycling at 80% VT; A_1 is the asymptotic amplitude for the mono-exponential rise in HR (beats.min^{-1}) over the six-minute bout; τ represents the time constant (s) for the rise in HR, and TD representing the delay (s) before HR begins to increase following the increase in workload (W).

The steady-state HR response, referred to as End A, was calculated using the following formula:

$$y(t) = A_0 + A_1(1 - e^{-(360-TD/\tau)}) \quad [13]$$

2.2.5.11 Cardiac Output Analysis

Prior to the onset of the final two bouts of constant-load cycling designed to assess CO, subjects completed two solitary rebreathes to familiarise themselves with the rebreathe technique. Rebreathing requires the subject to maintain a constant breathing frequency by keeping rhythm with a graphical tachymeter on the screen of the Innocor, aided by verbal prompts from the investigator. The typical rhythm was set at a rate of twenty breaths per minute.

CO was determined using the Innocor (Innovision, Denmark) via the inert gas rebreathe technique, which shows close agreement with cardiovascular MRI values (Saur *et al.*, 2009), thermodilution and direct Fick measurements (Peyton & Thompson, 2004). CO values derived via the Innocor are also highly reproducible (Peyton *et al.*, 2009). The subject breathing through a closed loop system, in which the RVU the subject is connected to has a five-litre rebreathe bag attached (figure 2.1). This is connected to an infrared photoacoustic gas analyser (Innocor, Innovision, Denmark). The rebreathe bag is filled from the Innocor with a concentrated gas

mixture containing 0.5% nitrous oxide (N_2O, blood-soluble), 0.1% sulphur hexafluoride (SF_6, blood-insoluble), 15% O_2 and 5% CO_2. The subject is required to fully inhale the contents of the bag with each inspiration, with each exhalation refilling the bag. The N_2O is absorbed into the bloodstream, with the infrared photoacoustic gas analyser detecting changes in the gas mixture composition to calculate pulmonary BF and CO. The rate at which N_2O is taken up by the bloodstream is proportional to the BF in the perfused lungs. In the absence of an intrapulmonary shunt, this pulmonary blood flow is equivalent to CO. It can require 3-6 breaths to achieve full N_2O clearance. Assuming the quality of the rebreathes performed by the individual were adequate, the results of CO at each time point were averaged, as were the factors of HR and stroke volume (SV), where SV was defined as the volume of blood ejected by the left ventricle into the aorta per heart beat. SV was calculated using the following equation:

$$SV\ (ml) = (CO / HR)*1000 \quad [14]$$

a-v O_2 diff was estimated using the Fick principle. The Fick principle states:

$$CO = \dot{V}O_2 / \text{a-v } O_2 \text{ diff} \quad [15]$$

Via rearrangement of this equation a-v O_2 diff can be estimated

$$\text{Estimated a-v } O_2 \text{ diff} = \dot{V}O_2 / CO \quad [16]$$

Both CO and $\dot{V}O_2$ values are given by the Innocor, allowing determination of a-v O_2 diff.

Immediately following performance of the CO rebreathe, the subject then had their BP measured via sphygmanomometer at the brachial artery of the left arm. MAP was then calculated using equation [2]. Having determined these parameters, total peripheral resistance (TPR) was then calculated:

$$TPR = MAP / CO \quad [17]$$

2.2.5.12 CO Rate of Increase

The rate of increase in CO response was calculated to determine the percentage of increase in CO during steady-state cycling that occurred during the first 30s of cycling at 80% VT. The rate of increase was calculated using the following equation:

$$[CO(30s) - CO(rest)]/[CO(240s) - CO(rest)] * 100 \quad [18]$$

2.2.6 Statistical Analysis

All statistical analyses were performed using PRISM software (Version 5.03, GraphPad Software Inc, USA). Data was compared between groups using unpaired t-tests. Significance for all tests was set at $P<0.05$ and all results are presented as mean ± standard deviation (sd). Data that was not normally distributed (as determined by an F-test) were analysed assuming a non-Gaussian distribution via the Mann-Whitney U test.

2.3 Results

2.3.1 Subjects

2.3.1.1 Physical Characteristics

Physical characteristics for subjects are presented in table 2.1 below. Subjects were matched for age, BMI and activity levels, as determined by the LOPAR questionnaire (Regensteiner *et al.*, 1996) and use of RT3 accelerometers (Rowlands *et al.*, 2004; Perry *et al.*, 2010) (figure 2.5). The LOPAR questionnaire is presented in appendix V, while individual anthropometric measurements are found in appendix VI. Subjects in the ND group were taller than their T2D counterparts, but this did not result in any differences in BMI values between groups. No differences were detected in measures of the ABI, with the findings indicative of healthy function of the peripheral arteries in both groups. Both groups also had similar measures of leg volume. One area in which the two groups did differ was with respect to the WHR, with individuals with T2D displaying a significantly greater WHR than their healthy counterparts.

Table 2.1. Anthropometric and physical activity data for non-diabetic subjects and individuals with T2D. Data presented as mean ± sd. ** P<0.001.

	Non-Diabetic Group (n=20)	Type 2 Diabetic Group (n=33)
Age (yrs)	55.3 ± 11.1	57.5 ± 7.3
Height (m)	1.79 ± 0.07**	1.74 ± 0.06
Body Mass (kg)	91.6 ± 11.5	92.6 ± 12.1
BMI ($kg.m^{-2}$)	28.5 ± 3.4	30.5 ± 3.5
ABI (a.u)	1.17 ± 0.06	1.17 ± 0.09
WHR (a.u)	0.98 ± 0.1	1.02 ± 0.0**
Leg Volume (ml)	2843 ± 322	2675 ± 356
LOPAR ($MET.hr.wk^{-1}$)	183 ± 71	182 ± 56

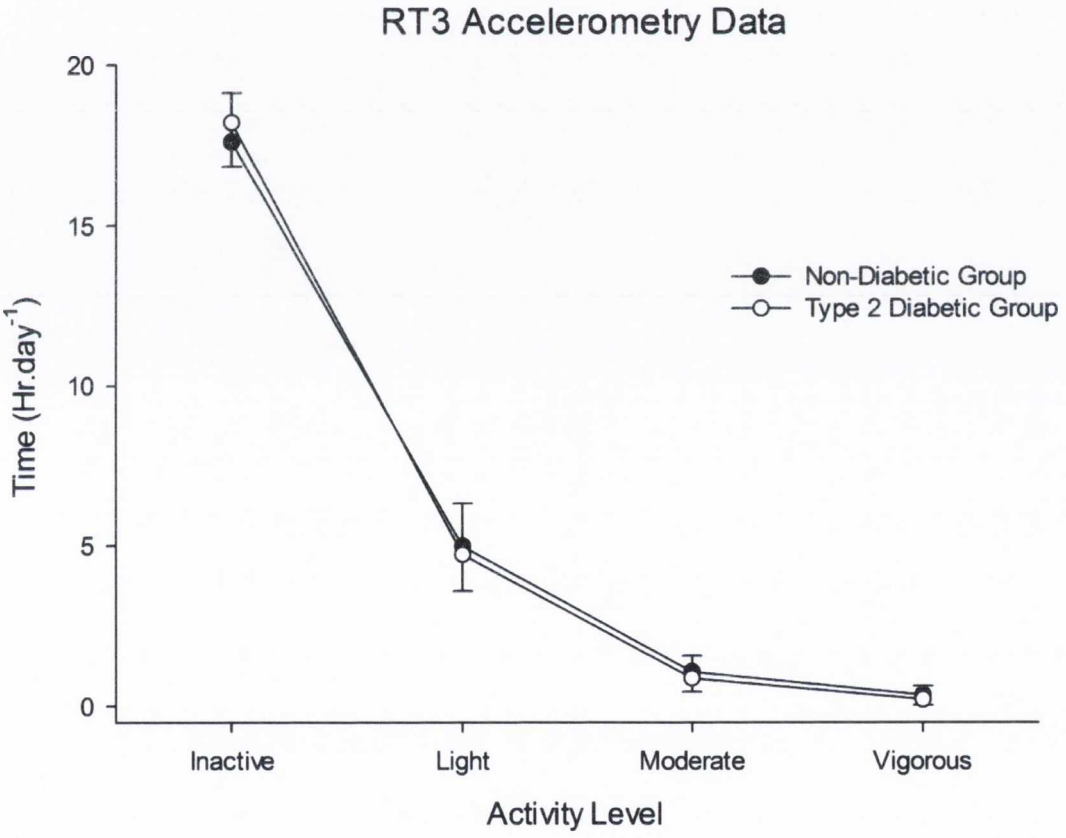


Figure 2.5. Mean activity levels for each group based on the number of hours per day in each category of activity as determined by RT3 accelerometers (Stay Healthy Inc, Monrovia, California). No difference was found between groups at any activity level.

2.3.1.2 Haematological Parameters

Mean haematological parameters are presented below in table 2.2. Individuals with T2D unsurprisingly had significantly greater levels of HbA_{1c} and FBG (P<0.0001). Measures of cholesterol, HDL-C, LDL-C and triglycerides were similar

between groups. Individual data is found for haematological parameters in appendix VII.

Table 2.2 Haematological parameters for ND subjects and individuals with T2D. Data presented as mean ± sd. *** P<0.0001.

	Non-Diabetic Group (n=20)	Type 2 Diabetic Group (n=31)
HbA_{1c} ($mmol.mol^{-1}$)	36.32 ± 4.20	51.14 ± 9.81***
HbA_{1c} (%)	5.47 ± 2.53	6.83 ± 3.05***
FBG ($mmol.L^{-1}$)	4.74 ± 0.66	7.37 ± 1.36***
Cholesterol ($mmol.L^{-1}$)	4.75 ± 0.71	4.47 ± 0.97
HDL-C ($mmol.L^{-1}$)	1.30 ± 0.28	1.24 ± 0.25
LDL-C ($mmol.L^{-1}$)	3.23 ± 0.72	2.87 ± 0.82
Triglycerides ($mmol.L^{-1}$)	1.48 ± 0.82	1.97 ± 0.94

2.3.2 Maximal Voluntary Contractions & Peak Calf Incremental Data

Performance data for MVCs and the incremental plantar-flexion test are presented in table 2.3 (individual data is found in appendices VIII & IX). MVCs were similar for both groups (1115 ± 284 ND; 1196 ± 262 T2D; figure 2.6). Similarly, groups did not differ with regard to the peak force (N) achieved during the incremental plantar-flexion test. As a consequence, both groups had similar times to failure (TTF) for the test. However, when the peak force achieved during the incremental test was expressed relative to each individual's MVC (figure 2.7), ND individuals achieved a significantly greater percentage of their MVC (67.9 ± 13.8%) compared with the T2D group (59.4 ± 12.6%; P<0.001).

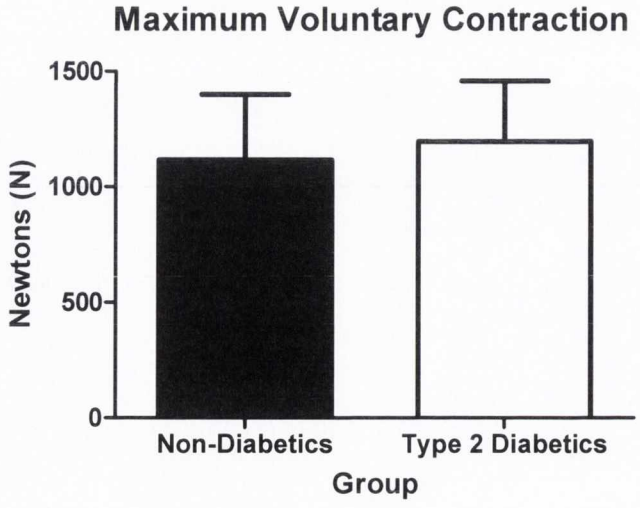


Figure 2.6. Mean values for MVCs for non-diabetics and individuals with T2D.

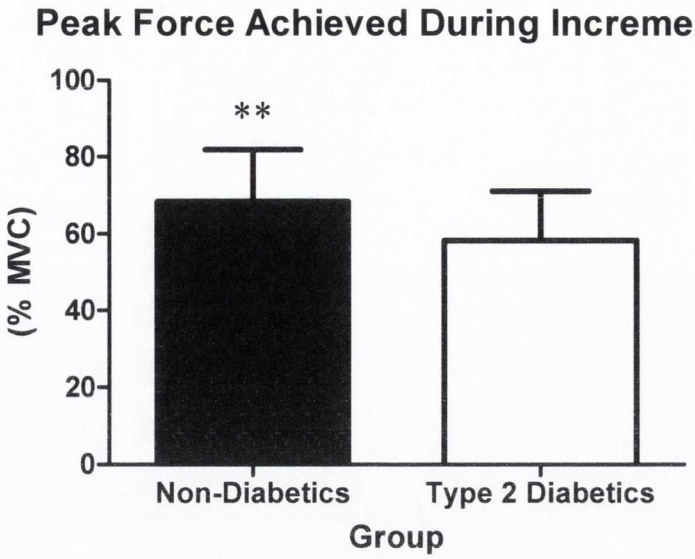


Figure 2.7. The percentage of MVC achieved during the incremental Test for ND and T2D groups. * indicates a significantly greater mean in the ND group (** P<0.001).

ND individuals displayed numerically higher mean peak LBF and LVC responses than individuals with T2D, however, these differences were not statistically significant (LBF: P=0.20; LVC: P=0.32). Similar trends were seen for the slopes of the LBF and LVC responses, with the ND group displaying steeper slope values, whether expressed as a function of workload (N) or as a function of their peak workload (expressed as %Max), but again, these differences were not significant (LBF f(N): P=0.31; LBF f(%): P=0.60; LVC f(N): P=0.64; f(%) P=0.57). Mean LBF and LVC responses at each workload for the two groups are presented in figures 2.8 and 2.9 below.

Table 2.3. Mean peak parameters and slopes of BF and VC during the incremental plantar-flexion test. Data presented as mean ± sd.

	Non-Diabetic Group (n = 20)	**Type 2 Diabetic Group (n = 33)**
Peak during Incremental (N)	750 ± 204	706 ± 203
Time to Failure (min)	8.5 ± 2.1	7.9 ± 2.0
Peak LBF ($ml.min^{-1}$)	707 ± 337	602 ± 255
BF Slope (function of Wkld (N))	0.82 ± 0.33	0.77 ± 0.35
BF Slope (function of Wkld (%Max))	635 ± 343	547 ± 273
Peak LVC ($ml.min^{-1}.mmHg^{-1}$)	6.63 ± 3.54	5.41 ± 2.28
VC Slope (function of Wkld (N))	0.0074 ± 0.0036	0.0068 ± 0.0034
VC Slope (function of Wkld (%Max))	5.72 ± 3.75	4.80 ± 2.48

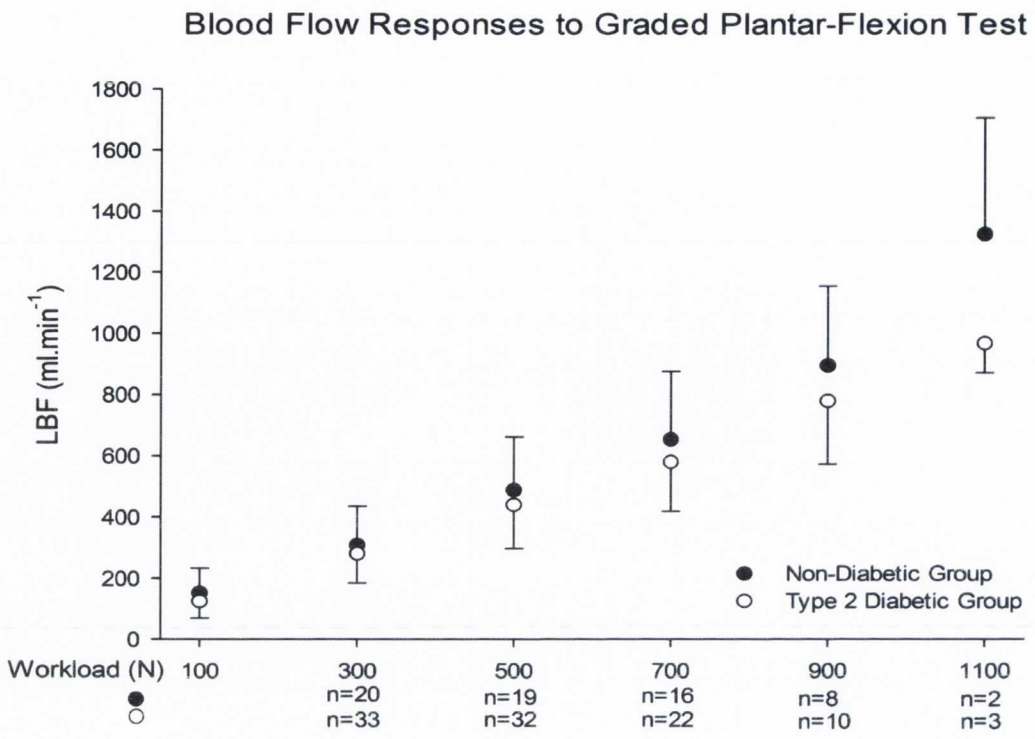


Figure 2.8. Mean LBF responses for each group at each workload. The n number for each group is presented below the x-axis.

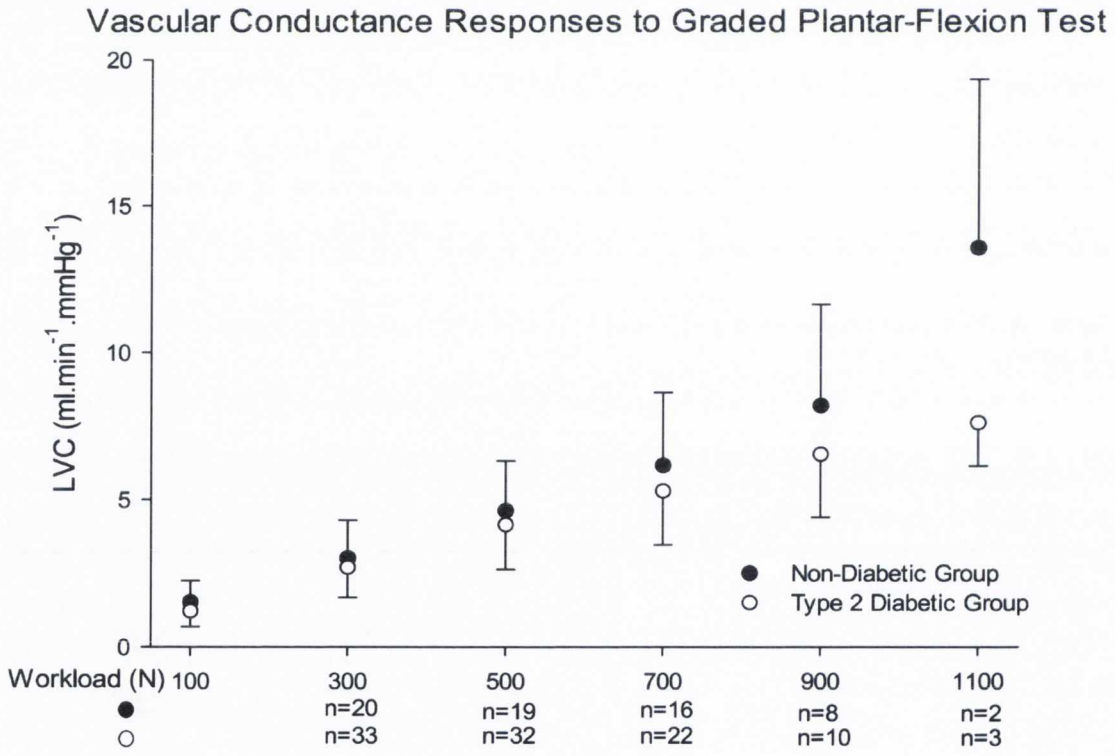


Figure 2.9. Mean LVC responses for each group at each workload.

2.3.3 Leg Vascular Conductance Kinetic Responses to Plantar-Flexion at 30% MVC

Mean LVC parameter estimates for the two groups are presented in table 2.4. Individual curve-fit parameters are listed in appendix X. No significant differences were found between ND individuals and individuals with T2D for any of the fitted parameters. The mean value for the primary outcome variable, the time constant of the third phase, τ_3, was numerically faster in the ND individuals, but the high degree of variability in τ_3 values meant that the differences between groups was not significant (P=0.30). Sample kinetic responses for an individual from both the ND and T2D groups are presented in figure 2.10.

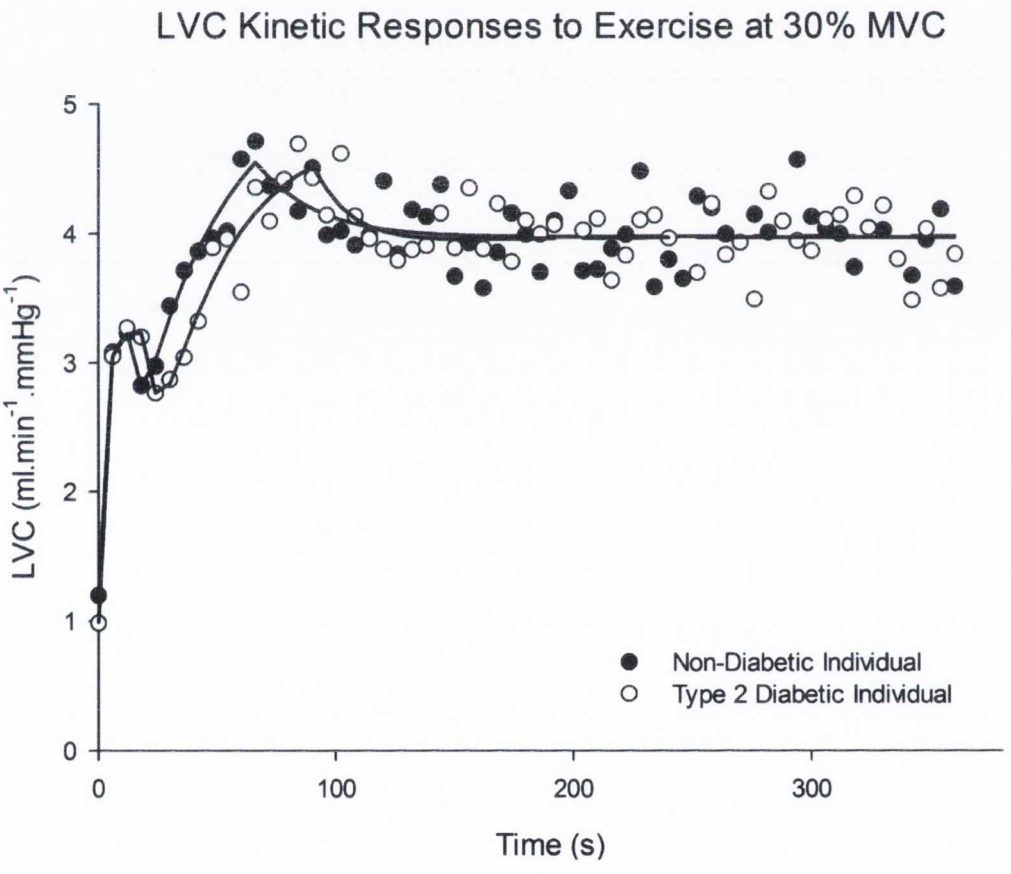


Figure 2.10. Sample kinetic responses for a representative individual from each group at 30% MVC. Closed circles (•) represent averaged data points for the ND individual, open circles (°) represent the individual with T2D. The solid lines represent the fitted models generated in TableCurve.

Table 2.4. Parameter estimates for the fitted LVC kinetic responses at 30% MVC.

	Non-Diabetic Group (n = 20)	**Type 2 Diabetic Group (n = 33)**
Baseline A ($ml.min^{-1}.mmHg^{-1}$)	0.87 ± 0.29	0.75 ± 0.24
A_1 ($ml.min^{-1}.mmHg^{-1}$)	2.74 ± 1.99	2.26 ± 1.30
TD_1 (s)	0.8 ± 1.1	0.8 ± 0.8
τ_1 (s)	2.6 ± 1.0	3.2 ± 2.6
A_2 - Fast Decay ($ml.min^{-1}.mmHg^{-1}$)	1.14 ± 0.91	0.86 ± 0.59
TD_2 - Fast Decay (s)	10.6 ± 3.4	11.6 ± 8.0
τ_2 - Fast Decay (s)	8.7 ± 5.6	8.9 ± 6.5
A_3 ($ml.min^{-1}.mmHg^{-1}$)	1.69 ± 1.24	1.54 ± 0.97
TD_3 (s)	22.6 ± 8.7	21.3 ± 9.1
τ_3 (s)	23.9 ± 22.7	32.9 ± 25.8
A_4 - Slow Decay ($ml.min^{-1}.mmHg^{-1}$)	0.67 ± 0.37	0.62 ± 0.45
TD_4 - Slow Decay (s)	76.0 ± 22.1	87.5 ± 29.8
τ_4 - Slow Decay (s)	39.9 ± 59.5	35.1 ± 36.2
End A (ml.min-1.mmHg-1)	3.50 ± 1.72	3.07 ± 1.17

2.3.4 Leg Vascular Conductance Kinetic Responses to Plantar-Flexion at 70% MVC

This comparison was performed in a subset of males in each group to determine if workload affected the kinetics of LVC responses of ND individuals and individuals with T2D. Individual responses are presented in appendix XI. Significant differences were detected for the amplitude of the first phase of the LVC response (A_1), with ND individuals having a greater increase in LVC in this first phase ($P<0.05$). In contrast, individuals with T2D displayed a faster rate of increase in flow in this first phase (τ_1). Individuals with T2D tended to have a reduced level of decay in LVC during the second phase (A_2) ($P=0.06$), which contributed to the finding of similar End A values between the two groups. The primary outcome variable, τ_3, tended to be slower in individuals with T2D ($P=0.076$), however, the large standard deviations ensured that this difference did not attain significance. Figure 2.11 illustrates representative sample responses from an individual in each group. It was determined that the LVC responses to steady-state contractions at 70% MVC did not elicit the slow-decay component (4^{th} phase) of the fitted model. As a consequence the response to 70% MVC was considered tri-phasic.

Table 2.5. Fitted LVC kinetic responses for a subset of individuals at 70% MVC. * indicates significant differences detected between groups ($P<0.05$).

	Non-Diabetic Group (n = 8)	**Type 2 Diabetic Group (n = 15)**
Baseline A ($ml.min^{-1}.mmHg^{-1}$)	0.87 ± 0.34	0.98 ± 0.36
A_1 ($ml.min^{-1}.mmHg^{-1}$)	5.28 ± 1.64*	3.84 ± 1.40
TD_1 (s)	1.8 ± 1.1	1.3 ± 1.1
τ_1 (s)	5.1 ± 1.8	3.6 ± 1.4*
A_2 - Fast Decay ($ml.min^{-1}.mmHg^{-1}$)	1.57 ± 0.87	0.90 ± 0.41
TD_2 - Fast Decay (s)	13.5 ± 3.9	12.1 ± 4.9
τ_2 - Fast Decay (s)	8.9 ± 11.4	10.9 ± 20.2
A_3 ($ml.min^{-1}.mmHg^{-1}$)	2.05 ± 0.69	2.47 ± 1.59
TD_3 (s)	22.5 ± 6.5	18.4 ± 4.5
τ_3 (s)	12.3 ± 11.4	30.0 ± 29.8
End A ($ml.min^{-1}.mmHg^{-1}$)	6.63 ± 1.70	6.38 ± 2.48

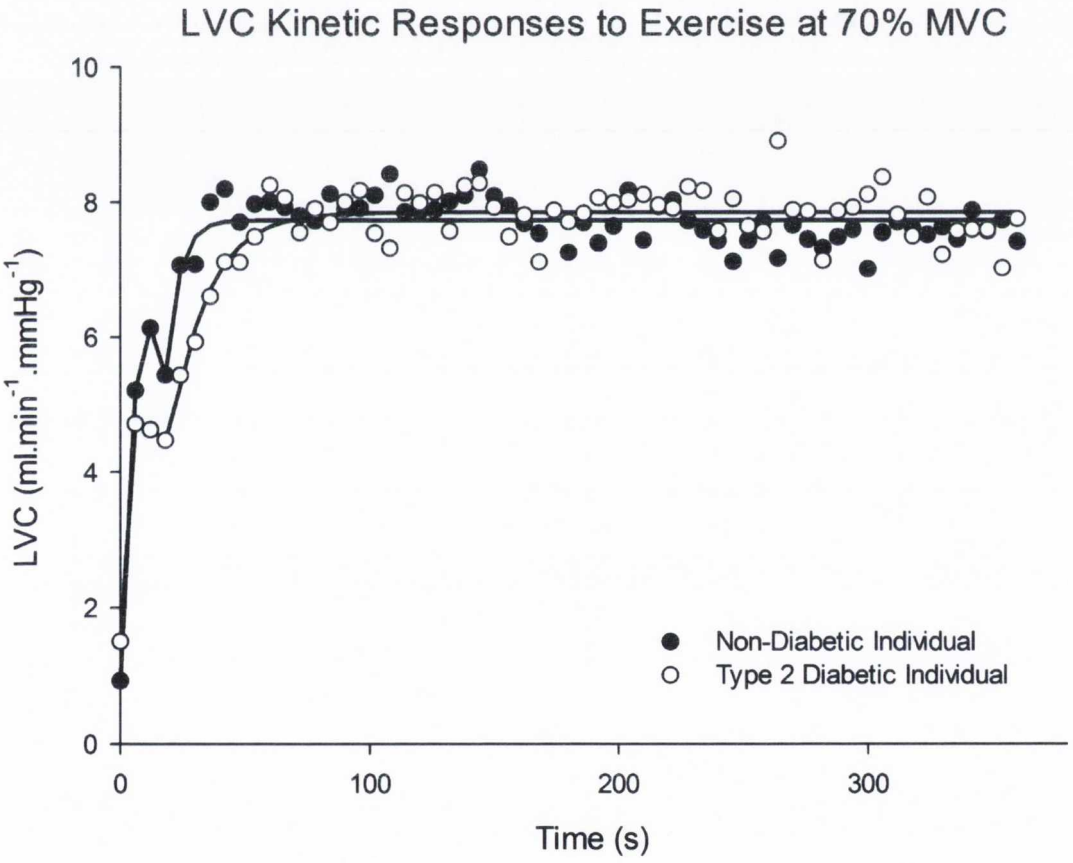


Figure 2.11. Sample LVC responses of two representative individuals to steady-state contractions at 70% MVC. The ND individual response is represented by closed circles (•), with the T2D response characterised by open circles (°). The solid lines represent the fitted models generated in TableCurve. τ_3 for the ND individual was 5.0s, and 14.3s for the individual with T2D.

2.3.5 Forearm Reactive Hyperaemia Responses to Ischaemia

Mean forearm VC (FVC) responses from the fitted model are listed in table 2.6 below, with individual response parameters listed in appendix XII. Figure 2.12 displays the time-aligned group average FBF responses from both groups. No differences between healthy individuals and individuals with T2D were detected for any of the models' parameters, when fitted for either FBF or FVC responses. Additionally, comparisons of either peak FBF or peak forearm VC following the release of ischaemia and the sum of the fitted models amplitude (b) and steady-state post-ischaemic flow (a) (which provides the models estimate of peak flow) were similar.

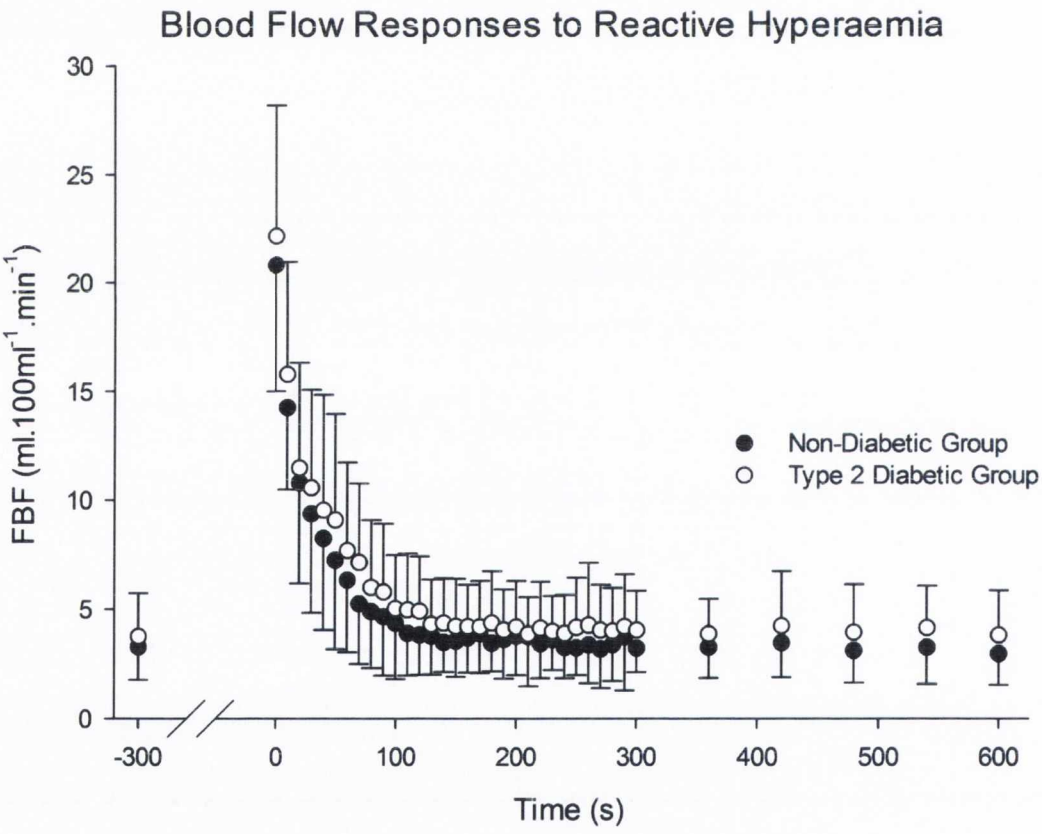


Figure 2.12. Mean time-aligned FBF responses for the non-diabetic group (•) and individuals with T2D (°). Values at -300s represent resting FBF prior to the five-minute period of ischaemia, with time at 0s representing the release of the cuff.

Table 2.6. Mean fitted parameter responses from mono-exponential model of forearm VC responses to ischaemic reactive hyperaemia protocol. Peak VC refers to experimentally measured values. a + b represents the model's estimate of peak FVC.

	Non-Diabetic Group (n = 20)	**Type 2 Diabetic Group (n = 33)**
Resting VC ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	3.55 ± 1.90	3.67 ± 1.95
a ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	3.37 ± 1.50	4.12 ± 2.07
b ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	18.55 ± 5.56	18.26 ± 5.39
Decay Constant	0.04 ± 0.03	0.04 ± 0.03
AUC (a.u)	2557 ± 995	3049 ± 1444
Peak VC ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	21.98 ± 5.92	22.16 ± 6.16
a + b ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	21.92 ± 5.81	22.38 ± 6.48

2.3.6. Performance Data from Maximal Incremental Cycle Test

Mean data relating to the parameters assessed during the graded incremental test are presented below in Table 2.7, with individual responses in appendix XIII. $\dot{V}O_2$peak was significantly greater in ND individuals ($P<0.001$), whether expressed in absolute terms ($L.min^{-1}$) or relative to body mass ($ml.min^{-1}.kg^{-1}$; 32.7 ± 6.9 ND; 26.8 ± 4.4 T2D, figure 2.13).

Similar to the impairment in $\dot{V}O_2$peak, the oxygen consumption at ventilatory threshold ($\dot{V}O_2$ at VT) was also impaired in the individuals with T2D ($P<0.05$), as determined by the V-slope method (Amann *et al.*, 2006). When $\dot{V}O_2$ at VT was expressed as a percentage of $\dot{V}O_2$peak, both groups were seen to reach VT at a similar relative level of oxygen consumption.

Individuals with T2D also achieved $\dot{V}O_2$peak and $\dot{V}O_2$ at VT at lower workloads than their ND counterparts ($P<0.0001$), and consequently had a faster TTF for the test as well. No differences were detected between groups with respect to respiratory-exchange ratio (RER), peak HR or $\dot{V}_e$.

Table 2.7. Contains peak performance data from incremental cycle test to exhaustion. Data presented as mean ± sd.* P<0.05. ** P<0.001. *** P<0.0001.

	Non-Diabetic Group (n = 20)	Type 2 Diabetic Group (n = 33)
$\dot{V}O_2$peak (L.min^{-1})	2.96 ± 0.51**	2.46 ± 0.39
$\dot{V}O_2$ at VT (ml.min^{-1}.kg^{-1})	24.4 ± 6.4*	20.5 ± 4.1
$\dot{V}O_2$ at VT (% $\dot{V}O_2$peak)	74.5 ± 11.7	76.3 ± 9.1
Peak Workload (W)	199 ± 29***	166 ± 27
Workload at VT (W)	165 ± 28***	130 ± 21
Workload at VT (% Peak)	82.5 ± 5.0***	78.3 ± 5.0
Time To Failure (Min)	18.5 ± 3.1***	15.1 ± 2.7
Peak RER	1.08 ± 0.05	1.10 ± 0.06
Peak HR (beats.min^{-1})	163 ± 13	156 ± 14
Peak HR (% Pred Max)	99.1 ± 6.4	95.1 ± 8.8
Peak $\dot{V}_e$ (L.min^{-1})	97.3 ± 9.4	89.6 ± 15.5

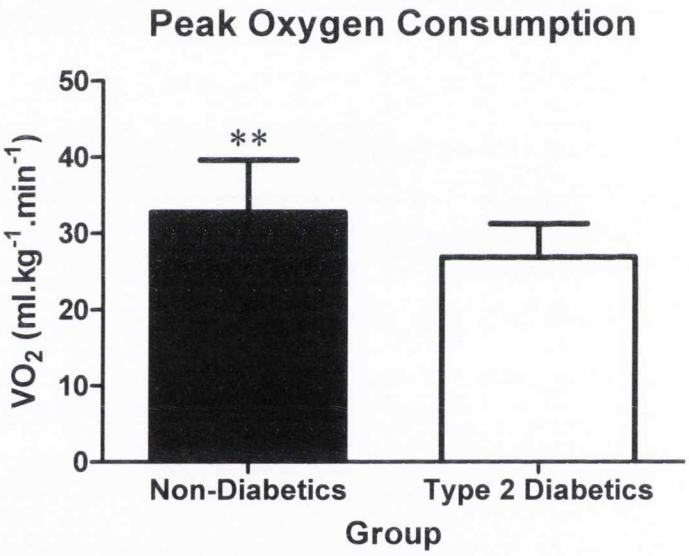


Figure 2.13. Peak oxygen consumption ($\dot{V}O_2$peak) relative to body mass (ml.min^{-1}.kg^{-1}). Data presented as mean ± sd. ** P<0.001.

2.3.7. $\dot{V}O_2$ Kinetic Responses to Constant-Load Cycling at 80% VT

Sample $\dot{V}O_2$ kinetic responses for a representative individual from both the ND and T2D groups are presented in figure 2.14, with mean parameter values listed in table 2.8 and individual responses in appendix XIV. Goodness-of-fit data for the model fits are presented in appendix XV. Subjects transitioned from cycling at a semi-unloaded workload (10W) to the workload that corresponded to 80% VT. The workload corresponding to 80% VT was significantly greater in ND individuals than individuals with T2D (132 ± 22 vs. 104 ± 17; P<0.0001). The mean amplitude of the

first phase response (A_1) was significantly greater in the ND group. The time delay to the onset of the second phase (TD_2) tended to be shorter in the ND group compared to individuals with T2D (P=0.06). The primary outcome variable in $\dot{V}O_2$ kinetic analysis, τ_2, was significantly faster in ND individuals. The amplitude of the 2nd phase (A_2) of the kinetic response was also greater in ND individuals. Given that ND individuals had higher amplitudes in both the first and second phase of the response, it is unsurprising that they also displayed significantly higher steady-state $\dot{V}O_2$ consumption (End A) than individuals with T2D. Additionally, ND individuals had faster mean response times (MRT – the time to achieve 63% of the total kinetic response) than individuals with T2D, primarily as a consequence of the faster τ_2 values. No differences were detected between groups for $\dot{V}O_2$ gain ($ml.min^{-1}.W^{-1}$; ND: 11.1 ± 0.7, T2D: 11.4 ± 2.3).

Table 2.8. Mean parameter values for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT. Data is presented as mean ± sd. * P<0.05; ** P<0.001; *** P<0.0001.

	Non-Diabetic Group (n = 20)	**Type 2 Diabetic Group (n = 33)**
Baseline A ($L.min^{-1}$)	0.77 ± 0.07	0.76 ± 0.14
A_1 ($L.min^{-1}$)	0.45 ± 0.20*	0.34 ± 0.14
TD_1 (s)	5.0 ± 4.8	5.8 ± 4.5
τ_1 (s)	10.9 ± 7.8	14.1 ± 7.7
A_2 ($L.min^{-1}$)	0.87 ± 0.15**	0.69 ± 0.22
TD_2 (s)	28.0 ± 5.8	31.6 ± 6.76
τ_2 (s)	33.5 ± 10.7**	45.3 ± 11.7
End A ($L.min^{-1}$)	2.09 ± 0.27**	1.79 ± 0.29
MRT (s)	47.3 ± 9.4***	58.2 ± 9.7
$\dot{V}O_2$ Gain ($ml.min^{-1}.W^{-1}$)	10.84 ± 0.79	10.99 ± 1.91

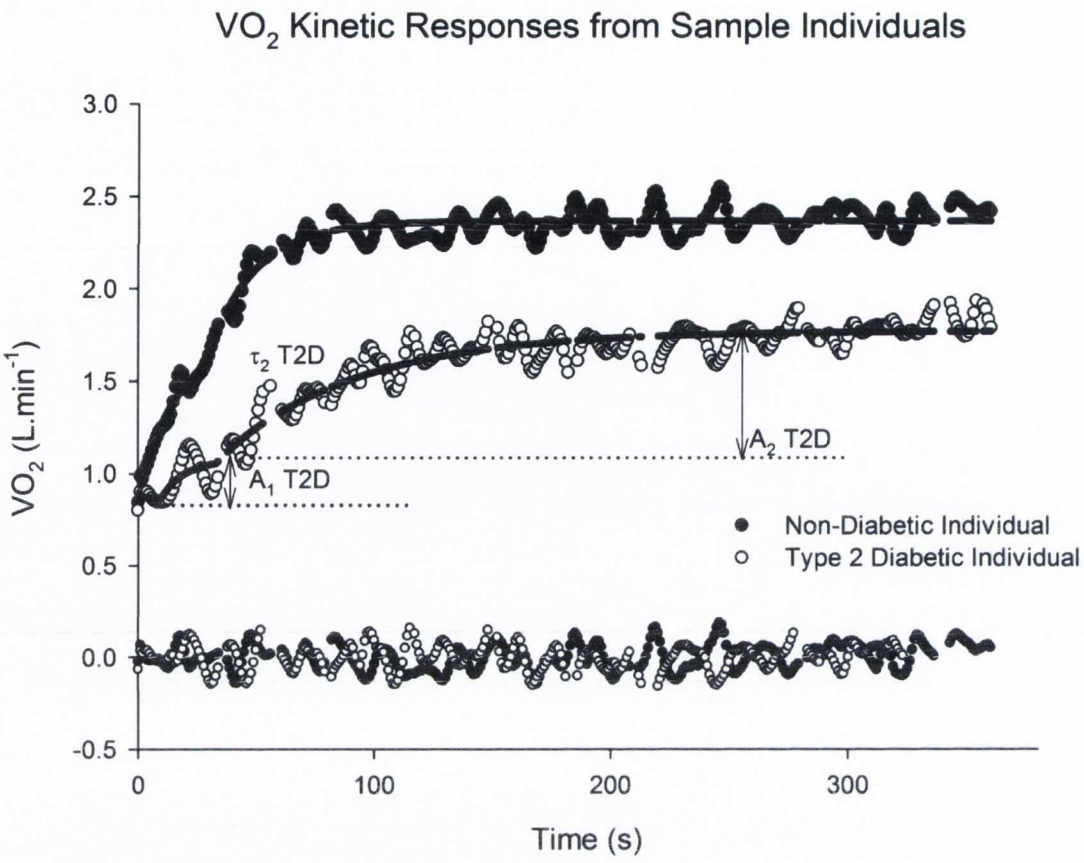


Figure 2.14. Sample $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT are presented. ND individuals (•) display greater amplitudes for both first and second phases, while the rate of adjustment of the 2nd phase (τ_2) is also faster in ND individuals than individuals with T2D (°). Residuals for model fits are presented at 0.0 on the Y-axis demonstrating model fits.

2.3.8. Heart Rate Kinetic Responses to Constant-Load Cycling at 80% VT

Mean parameters for the mono-exponential fit of HR responses of participants are presented in table 2.9, and individual data in appendix XVI. ND individuals displayed a greater increase in HR (A_1) over the six-minute bout ($P<0.001$), resulting in a higher steady-state HR during the exercise (End A, $P<0.05$). Furthermore, the rate of increase in HR (τ) was significantly faster in the ND individuals ($P<0.05$). Sample HR kinetic responses from a representative individual in each group are presented in figure 2.15.

Table 2.9. Mean HR kinetic parameters for both groups. Data presented as mean ± sd. * P<0.05, ** P<0.001.

	Non-Diabetic Group (n=18)	Type 2 Diabetic Group (n=33)
A_0 (beats.min^{-1})	90 ± 10	91 ± 10
A_1 (beats.min^{-1})	42 ± 9**	35 ± 7
Time Delay (s)	3.8 ± 4.8	6.6 ± 5.9
τ (s)	64.1 ± 18.2*	82.0 ± 27.1
End A (beats.min^{-1})	132 ± 11*	125 ± 11

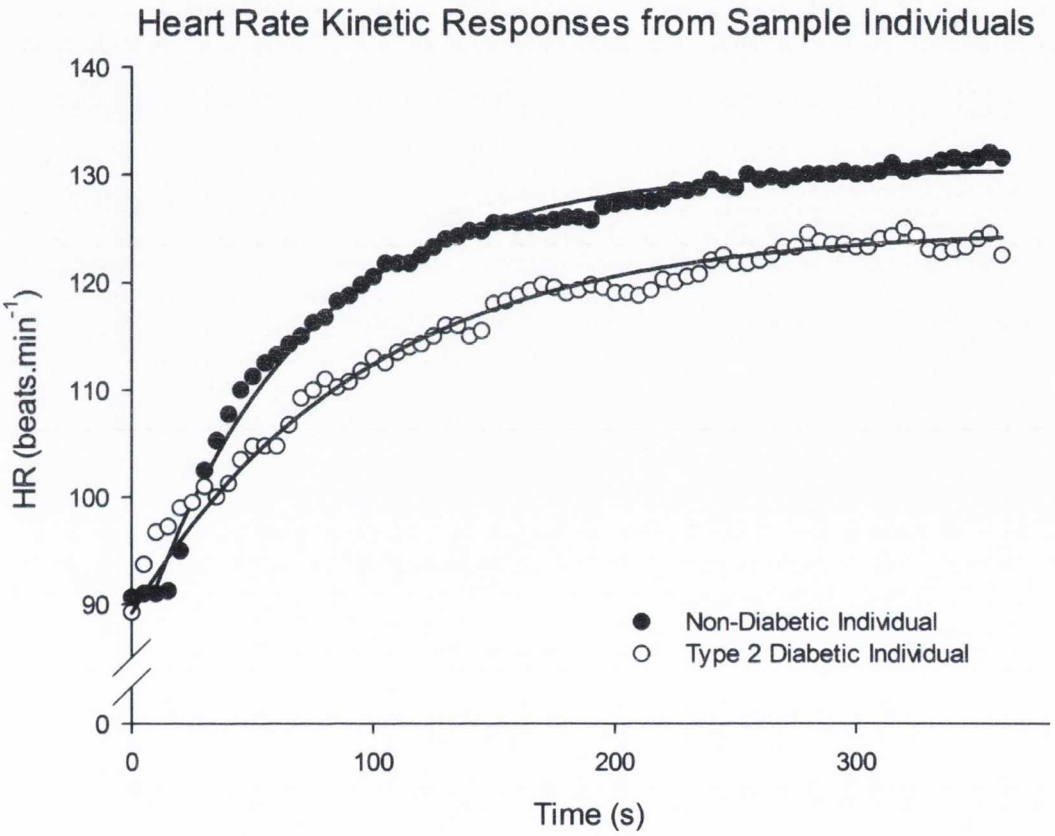


Figure 2.15. Sample HR kinetics for a representative ND individual (•) and individual with T2D (o). These mono-exponential fits clearly represent the lower amplitude and slower rate of increase within the T2D groups.

2.3.9. Cardiac Responses to Constant-Load Cycling at 80% VT

CO and related parameters were calculated at rest (appendix XVII), 30s (appendix XVIII) and 240s (appendix XIX) into steady-state cycling at 80% VT, and are presented below in figure 2.16. At rest, only one parameter, a-v O_2 diff (ml O_2.100ml blood^{-1}), presented a difference between groups, with ND individuals displaying a greater level of O_2 extraction than individuals with T2D (7.6 ± 1.8 vs. 6.3 ± 1.4; P<0.001). Following onset of the cycling exercise, ND individuals recorded

higher CO ($L.min^{-1}$) readings at 30s (11.1 ± 2.3 vs. 9.7 ± 1.3; $P<0.001$) and 240s (13.6 ± 2.4 vs. 11.6 ± 1.4; $P<0.001$) than individuals with T2D. The primary cause for the difference in CO readings was impaired SV (ml) readings in individuals with T2D at both 30s (ND: 106.1 ± 18.5, T2D: 94.5 ± 13.2; $P<0.05$) and 240s (ND: 105.9 ± 17.0, T2D: 94.4 ± 12.5; $P<0.001$). However, at least at 240s, the vasculature may have also contributed to the impaired CO readings in individuals with T2D, as this group displayed elevated MAP (mmHg) (124.5 ± 8.9 vs. 116.2 ± 6.5; $P<0.001$), as well as higher TPR ($mmHg.L^{-1}.min^{-1}$) values (10.9 ± 1.5 vs. 8.6 ± 1.6; $P<0.0001$).

Comparison of the magnitude of increase in CO from rest to 30s showed a significantly greater increase in ND individuals ($P<0.05$). Surprisingly, when the increase in CO from 30s to 240s was analysed, no significant difference between groups was found. Furthermore, when the increase in CO from rest to 30s was expressed as a percentage of the total increase from rest to 240s, no difference between the groups was found.

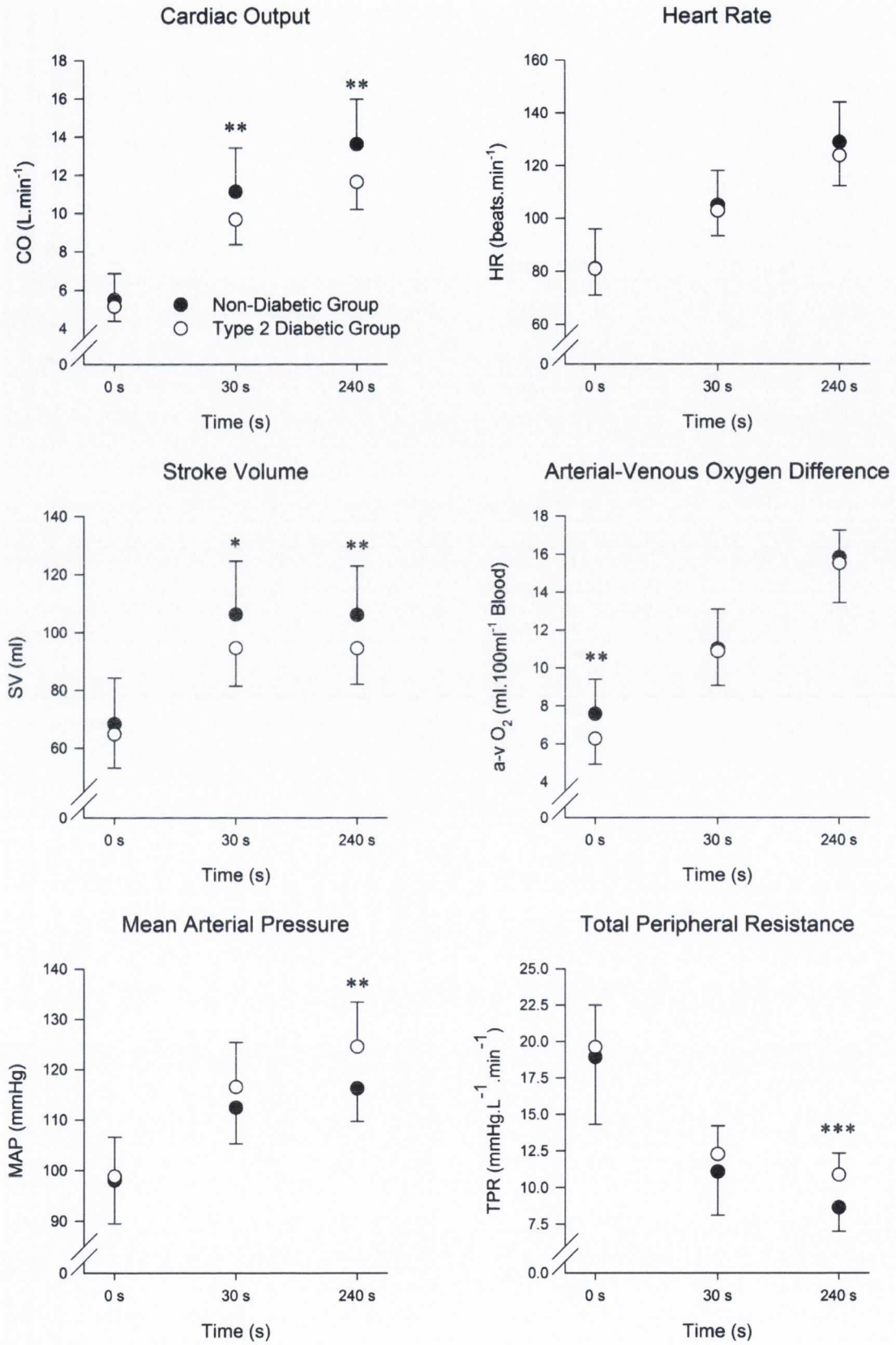


Figure 2.16. Cardiac responses prior to onset, 30s and 240s into steady-state cycling at 80% VT. Dots represent group means, with error bars representing standard deviations. * P<0.05, ** P<0.001, ***P<0.0001.

2.4 DISCUSSION

The majority of past research investigating a diabetes-related impairment in exercise performance and particularly $\dot{V}O_2$ kinetic adaptations has been performed in female populations (Regensteiner *et al.*, 1995). As such there is a dearth of research investigating the impact of T2D on exercise performance in a male-only population. Recently, some male-only studies (Lalande *et al.*, 2008; Wilkerson *et al.*, 2011) demonstrated a similar sized impairment in $\dot{V}O_2$peak (~20%) during a graded cycle test to exhaustion compared to previous studies on females and mixed populations (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003; Mac Ananey *et al.*, 2011). The magnitude of impairment in $\dot{V}O_2$peak in individuals with T2D detected in this study, both in absolute (16.9%) and relative (18%) terms, is also in line with those previously documented (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Lalande *et al.*, 2008; Wilkerson *et al.*, 2011).

The main studies that formed the basis for comparison with the present study were those by Lalande *et al* (2008) and Wilkerson *et al* (2011). However, the aims of the studies by Lalande *et al* (2008) and Wilkerson *et al* (2011) differed from the current study. While both studies detected a T2D-related $\dot{V}O_2$peak (~20%) during a graded cycle test to exhaustion, the present study differed from both studies in a number of respects. The study by Wilkerson *et al* (2011) also investigated the impact of T2D on $\dot{V}O_2$ kinetic responses in males with T2D, however, the age of participants in that study was greater than that in the present study, opening the possibility that age played a factor in the different kinetic responses of the two studies. Similarly, the work by Lalande *et al* (2011) investigating femoral blood flow during submaximal exercise demonstrated impaired CO responses in middle-aged males with T2D during cycling, which corresponds to the findings in the present study. However, the method of assessment of CO differed between the two studies, while the current study also assessed peripheral blood flow responses during plantar-flexion exercise. The results of the plantar-flexion exercise suggest that peripheral vasodilation is intact, therefore muscle perfusion may not be affected and in turn suggest that O_2 delivery may not be the contributing factor to the impaired $\dot{V}O_2$peak responses in middle-aged males with T2D.

Surprisingly, Wilkerson *et al.* (2011) did not detect any difference in $\dot{V}O_2$ kinetic responses to steady-state submaximal exercise in the same group of males.

This finding was in contrast to prior studies (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003; Mac Ananey *et al.*, 2011) and the finding of the present study. It is possible that the differing experimental design and/or age of the participants in this study and that of Wilkerson *et al.* (2011) may have resulted in the disparity in the $\dot{V}O_2$ kinetic responses of the two studies. One of the novel aspects of the study by Wilkerson *et al.* (2011) was the older age of their participants (mean age ~ 65yr); whereas the participants in this study were substantially younger (table 2.1) than those of Wilkerson *et al.* (2011). It is possible that the aged control subjects that participated in the study by Wilkerson *et al.* (2011) were suffering from age-related impairments in $\dot{V}O_2$ kinetics (Bell *et al.*, 1999), whereas those with T2D may have suffered a T2D-related impairment in $\dot{V}O_2$ kinetics much earlier in life, given their duration of diabetes exceeded ten years.

The other difference in methodology between this study and that of Wilkerson *et al.* (2011) was the workload at which submaximal steady-state cycling occurred. Subjects in the present study cycled at the workload corresponding to 80% VT, whereas Wilkerson *et al.* (2011) utilised a workload corresponding to 50% of the subject's $\dot{V}O_2$peak. With regard to these subjects, it was evident that the ND subjects did not hit VT until they were operating at a higher relative percentage of their maximum workload (table 2.7). If the same principle would apply to the subjects in the study by Wilkerson *et al.* (2011), then it may have impacted on the subjects kinetic responses, given that if they all operated at 50% $\dot{V}O_2$peak then the two groups would have been operating at different relative workloads.

Having established that a diabetes-related impairment in $\dot{V}O_2$peak exists, and that middle-aged males with T2D display slower $\dot{V}O_2$ kinetic responses to steady-state cycling, it is necessary to try to elucidate some of the causes of these findings. Most significantly, males with T2D exhibited impaired CO responses as early as 30s into steady-state submaximal exercise, which persisted at 240s. The source of the reduced CO was significantly lower SV values in this population. In contrast to the findings of Lalande *et al.* (2008), no increase in HR occurred to compensate for the reduced SV, resulting in reduced CO values. The impairment in CO and SV values in individuals with T2D mirrors some (Roy *et al.*, 1989; Gusso *et al.*, 2008) but not all (Mac Ananey *et al.*, 2011) previous studies. T2D has been associated with left ventricular diastolic dysfunction (LVDD), which is suggestive of either reduced

compliance or prolonged relaxation and is characterised by early filling (Stewart, 2002; Fang *et al.*, 2005), which may be responsible for impaired cardiac functioning in individuals with T2D. This impairment to normal heart function may result in a reduced left ventricular ejection fraction in response to exercise, even if normal systolic function has been preserved in individuals with T2D (Stewart, 2002), which would explain the reduction in SV values detected here.

Evidence suggests that the impairment in $\dot{V}O_2$ kinetics in individuals with T2D may be due in part to reductions in O_2 delivery. The slowed HR kinetics observed in the present study in individuals with T2D (table 2.9) may suggest a reduction in O_2 delivery. However, further studies are needed to elucidate this given that previous studies did not report differences in HR kinetic responses in individuals with T2D despite the presence of impaired $\dot{V}O_2$ kinetic responses (Regensteiner *et al.*, 1998; Mac Ananey *et al.*, 2011). This would suggest that the regulatory mechanisms of HR and peripheral BF are independent of each other.

Further support for a reduction in O_2 delivery can be seen in the results of estimated a-v O_2 diff. At rest, individuals with T2D displayed a significantly reduced a-v O_2 diff compared to healthy individuals. However, by 30s into steady-state cycling at 80% VT, individuals with T2D had recovered to display similar a-v O_2 diff values to healthy individuals, which would suggest increased levels of O_2 extraction over the first 30s in individuals with T2D compared to healthy individuals. This would provide indirect support for the findings of Bauer *et al.* (2007), who showed that the rate of change in deoxygenated haemoglobin/myoglobin concentrations ([HHb]) was characterised by an "overshoot" in the initial stages of steady-state exercise in individuals with T2D. This "overshoot" was interpreted to represent an increase in O_2 extraction to compensate for an impaired increase in muscle BF, and hence impaired O_2 delivery. The increase in a-v O_2 diff in the first 30s in individuals with T2D mirrors the change in [HHb] concentrations seen during steady-state exercise, and could be interpreted to contribute to the potential impairment in O_2 delivery during steady-state submaximal exercise evident in the findings of both this study and that of Bauer *et al.* (2007).

The results of the plantar-flexion exercise bouts would, however, seem to contradict the conclusion that impaired LBF responses are behind the impaired exercise performance. No significant differences were recorded in either peak LBF,

peak LVC, or LBF and LVC at submaximal workloads recorded during the graded plantar-flexion protocol. Additionally, no significant differences were found in FBF or FVC during the forearm reactive hyperaemia, which would indicate that peripheral vasodilatory mechanisms are intact. Furthermore, the rate of change (τ_3) and End A of the LBF and LVC responses to steady-state plantar-flexion exercise at both low (30% MVC) and high (70% MVC) intensities did not significantly differ between groups. This is in contrast to the findings of previous work (Kingwell *et al.*, 2003; Lalande *et al.*, 2008), which have indicated significantly reduced LBF responses in the diabetic state during steady-state exercise, and slowed LVC kinetics to steady-state exercise (Macananey *et al.*, 2011). However, two points of note should be made regarding these findings. Firstly, while not significantly different, τ_3 values in the T2D group at 70% MVC tended to be slower than their healthy counterparts (P=0.076). This would also appear to be the case with data from the incremental plantar-flexion test, as again, while not achieving significance, BF and VC responses to higher workloads (700N and higher) were numerically lower in the T2D group. This may indicate that LBF responses to exercise are slowed at higher workloads in individuals with T2D. Secondly, consideration needs to be given to the position and modality of exercise performed when comparing these results to past studies. While Kingwell *et al.* (2003) and Lalande *et al.* (2008) also performed exercise in the supine position, both studies had exercise modalities that employed large muscle masses (quadricep muscle group) and resulted in an increase in the central component of BF (Parker *et al.*, 2008), and in turn increased CO. The plantar-flexion exercise protocol employed in the current study was designed to utilise a small muscle mass and therefore derive an increase in BF through peripheral vasodilation as opposed to an increase in CO (Parker *et al.*, 2008). Macananey *et al.* (2011) used the same exercise modality as this study; however, the two studies differed in the workload employed during the steady-state exercise (30% MVC vs. 70% MVC) and also the posture of the subjects. To effectively compare the two studies with respect to workload, a subset of subjects in each group performed bouts at 70% MVC. While numerically slower, the τ_3 values in the T2D group in this study were not significantly slower than those of the ND group, unlike the findings of Macananey *et al.* (2011). However, these subjects performed the plantar-flexion exercise in the supine position, whereas in the study by Macananey *et al.* (2011) subjects were upright at a 67^{o} angle. This upright postural position has

previously been shown to increase LBF (Egana & Green, 2005), the effect of which is seen in the initial contractions. This postural effect may have been significant enough to accentuate diabetes-induced differences on cardiovascular reflexes associated with tilting the body from the supine to upright position, leading to the slowed kinetic response of Macananey *et al.* (2011) in upright testing, and the absence of significance in the findings of this study.

Similar to the findings of Macananey *et al.* (2011), no difference in the End A of LVC responses to steady-state contractions were observed in the present study at either 30% or 70% MVC. However, the amplitude of the first phase (A_1) of the LVC response to steady-state contractions at 70% MVC was significantly lower in individuals with T2D. The first phase response has been suggested to result from vascular compression of the smooth muscle lining the arterioles (Clifford, 2007). The impaired response of A_1 in individuals with T2D may suggest impaired autonomic innervation of the smooth muscle of vascular walls of individuals with T2D, which may impact upon LBF during the initial stages of exercise. Related to this, individuals with T2D achieved a lower % of their MVC during the graded plantar-flexion test. This would indicate that individuals with T2D suffered faster rates of fatigue than their healthy counterparts, which would suggest a greater reliance upon glycolytic muscle fibres. Aside from faster rates of fatigue and differing metabolic properties from type 1 fibres, the large motor neurons that innervate glycolytic fibres have sparse afferent innervation and are less likely to fire in response to descending inputs. These factors might suggest that in addition to attenuated O_2 delivery, the impairment in exercise performance associated with T2D may also be related to altered autonomic function of motor neurons innervating both skeletal and smooth muscle.

In conclusion, it was demonstrated that males with T2D display impaired $\dot{V}O_2$peak and slowed $\dot{V}O_2$ kinetics during cycling exercise. CO and SV values were also reduced in these same individuals, while HR kinetics were also slower. Combining these results with the finding that estimated a-v O_2 diff increased to a significantly greater extent from rest to 30s in individuals with T2D would seem to indicate that impairments in LBF and O_2 delivery contribute to the exercise impairment evident in individuals with T2D. The leg haemodynamic results of the plantar-flexion exercise tests would suggest that vasodilatory mechanisms in individuals with T2D might be attenuated; but that the main reductions in O_2 delivery

are the consequence of a reduced central component to BF, i.e. CO. However, muscle fibre type and autonomic innervation of both smooth and skeletal muscle may also influence BF responses in the initial stages of exercise and the metabolic properties of skeletal muscle, which may well indeed differ between individuals with T2D and healthy counterparts.

CHAPTER 3: THE EFFECT OF EXERCISE AND PIOGLITAZONE ON PEAK $\dot{V}O_2$ AND $\dot{V}O_2$ KINETIC RESPONSES IN MALES WITH T2D.

3.1 INTRODUCTION

Exercise is usually one of the first prescribed treatments following diagnosis of T2D, due to the known benefits of exercise on measures of glycaemic control, insulin sensitivity, abdominal fat, and lipid profile (Castaneda *et al.*, 2002; Dunstan *et al.*, 2002; Di Loreto *et al.*, 2005; Ibanez *et al.*, 2005; Trenell *et al.*, 2008). However, less than 40% of individuals with T2D exercise (Morrato *et al.*, 2007); and of those individuals with T2D that do exercise, the majority do not exercise at an intensity considered sufficient to elicit the desired improvements in glycaemic control (Johnson *et al.*, 2005; Morrato *et al.*, 2007). One possible reason for the lack of exercise within the diabetic population is the presence of an exercise impairment, as demonstrated in the initial baseline study and other studies (Regensteiner *et al.*, 1998; Bauer *et al.*, 2007), even in individuals with T2D that are free from any microvascular complications. Thus, individuals with T2D are more likely to find exercise a more difficult challenge than healthy counterparts, and as such face a greater deterrent to implementing a training program.

To this author's knowledge, there have been two studies to date that have examined the effect of an exercise training intervention on $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics during submaximal exercise in individuals with T2D. Firstly, Brandenburg *et al.* (1999) demonstrated that a 3-month training intervention was successful in improving both $\dot{V}O_2$peak and the speeding of $\dot{V}O_2$ kinetics at low and moderate intensities of 20 and 30W in females with T2D, and that the T2D group derived greater benefits from the training program than the ND controls. Secondly, Mac Ananey (2010) demonstrated that a 3-month supervised training intervention resulted in significant speeding of $\dot{V}O_2$ kinetics at 50 and 80% VT, and also VT plus 50% of the difference between VT and peak workload (Δ 50% peak-VT) in a mixed group of males and females with T2D. Furthermore, when these individuals continued to exercise for an additional 3 months in an unsupervised capacity, they successfully maintained the gains in performance attained during the supervised phase (Mac Ananey, 2010). When reassessing the $\dot{V}O_2$ kinetic responses following the training interventions, both Brandenburg *et al.* (1999) and Mac Ananey (2010) used the same workloads that

subjects performed during the baseline assessment. Given that these subjects demonstrated significant improvements in $\dot{V}O_2$peak and in the case of Mac Ananey (2010), improvements in the workload at which VT occurred, the subsequent post-training intervention assessments meant that subjects were working at a relatively easier workload, which may have been a factor contributing to the faster kinetic responses. Additionally, Mac Ananey (2010) did not have a ND cohort in the study to determine if the training had a greater effect in individuals with T2D, as found by Brandenburg *et al.* (1999).

In addition, two intervention studies investigated the effect of rosiglitazone (RSG), which like PIO is a member of the thiazolidinedione (TZD) family, on $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics in individuals with T2D. Initially, Regensteiner *et al.* (2005) demonstrated that treatment with RSG for four months resulted in a significant increase in $\dot{V}O_2$peak, but the speed of the $\dot{V}O_2$ kinetic response did not change as a consequence of RSG treatment (Regensteiner *et al.*, 2005). However, there was a significant increase in the amplitude of the second phase of the kinetic response, suggesting improved performance following treatment. In a separate study, Kadoglou *et al.* (2007) demonstrated that a combined treatment of exercise and RSG for eight months resulted in an increase in $\dot{V}O_2$peak greater than that obtained by their individual actions. Research also suggests that treatment with PIO improves cardiac function in individuals with T2D free from diabetes-related CVD, demonstrating similar cardiac improvements to those elicited by exercise training (van der Meer *et al.*, 2009). These findings imply that a course of PIO treatment may result in improvements in exercise performance and $\dot{V}O_2$ kinetics similar to those seen in studies using RSG.

The aims of this intervention study were as follows. Initially, to compare the effect of a supervised exercise training intervention on $\dot{V}O_2$peak and $\dot{V}O_2$ kinetic responses in a ND and T2D group compared to non-exercising controls, and to determine if subjects in the exercise treatment groups could maintain the benefits gained in an unsupervised training intervention. Secondly, to determine if PIO treatment alone resulted in improvements in exercise performance and $\dot{V}O_2$ kinetics, and if the combination of PIO and exercise had any synergistic effect on exercise performance in a sample of individuals with T2D. In comparing $\dot{V}O_2$ kinetic responses to baseline, it was decided to assess $\dot{V}O_2$ kinetics at 80% of the VT

determined at that specific point in time to ensure that subjects were assessed at the same relative workload at each visit. CO was measured during cycling at 80% VT to determine if the improvements (if any) in exercise performance were related to improvements in central cardiac component and BF delivery.

3.2 METHODS

3.2.1 Subjects

Fifty-two male subjects (33 individuals with T2D, 19 non-diabetics) took part in this intervention study. All subjects were deemed sedentary (<1hr.wk^{-1} of intense exercise) in the previous six months, as determined by use of RT3 accelerometers (Rowlands *et al.*, 2004; Perry *et al.*, 2010) and completion of the LOPAR questionnaire (Regensteiner *et al.*, 1996). All subjects provided written consent (appendix II) before beginning participation in the study. The study was conducted in accordance to the principles outlined by the Declaration of Helsinki, and was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College, Dublin.

Subjects were divided into one of six groups, four T2D groups and two ND groups. Within the T2D cohort, subjects were assigned to either the exercise or non-exercising control arms of the study. Within each of the exercise and non-exercising arms of the study, some subjects were prescribed pioglitazone (PIO), the effect of which was a target of this investigation. Therefore, of the T2D groups, two groups were assigned to a 28-week exercise protocol (T2D EXS, n = 11; PIO EXS, n = 6); the other two groups were designated as non-exercising controls (T2D CTL, n = 11; PIO CTL, n = 5). Of the two ND groups, one group was assigned to a 28-week exercise protocol (ND EXS; n = 10), while the other group was designated the non-exercising control condition (ND CTL = 9).

3.2.1.1 Recruitment of subjects

See Chapter 2 section 2.2.1.1 for recruitment of members of ND and T2D groups.

Recruitment of participants for the PIO groups required additional chart assessment by a registrar in the Diabetes Day Care Centres of St. Columcille's and St' Vincent's Hospitals to confirm suitability of the patients for receipt of PIO. Participants who used beta-blockers in the treatment of hypertension were admitted to

the intervention study (n=6; 5 of whom had T2D). However, due to the effect of this class of medication on the sympathetic activation of the heart, data for these subjects was not considered in the analysis of peak HR, CO and related parameters during steady-state cycling.

3.2.1.2 Inclusion/Exclusion Criteria

See Chapter 2 section 2.2.1.2.

3.2.1.3 Participant information form

See Chapter 2 section 2.2.1.3.

3.2.1.4 Stress Test for individuals with T2D

See Chapter 2 section 2.2.1.4.

3.2.1.5 Medical Examination

See Chapter 2 section 2.2.1.5.

3.2.1.6 Blood Sample Collection

See Chapter 2 section 2.2.1.6.

3.2.1.7 Determination of Physical Activity Levels

See Chapter 2 section 2.2.1.7.

3.2.2 Experimental Design

3.2.2.1 Study Overview

Subjects were required to attend the cardiovascular laboratory in the Department of Physiology, Trinity College Dublin, on two separate days at baseline, and after 3 and 7 months respectively. These visits were to be separated by a minimum of 72 hours. Following baseline testing, the exercise groups (ND EXS, T2D EXS, PIO EXS) began a twelve-week supervised exercise intervention. Exercise was to be performed at Monkstown Health & Fitness Centre, Monkstown, Co. Dublin. The aim over the 12 weeks was to achieve 36 sessions (an average of 3 per week). Compliance was determined as completing 32 of the desired 36 sessions. Following

completion of the 12 weeks training, laboratory assessments were repeated. Subjects in the exercise conditions then continued to exercise for an additional 16 weeks in an unsupervised capacity, and were asked to record the frequency and volume of exercise performed. After the 16 weeks unsupervised training, the final assessment in the cardiovascular laboratory was performed. For the control conditions (T2D CTL, ND CTL), subjects were asked to continue with their normal daily routine between assessments. Subjects who were also assigned pioglitazone treatment (PIO EXS, PIO CTL) were required to take a 30mg dose once daily for the duration of the study.

At each phase of the study venous blood samples were also collected. Subjects were required to fast for a 12-hour period and to have not performed any exercise in the preceding 24 hours for collection of venous blood samples.

3.2.2.2 Anthropometry

3.2.2.2.1 Mass, Height, Body Mass Index

See Chapter 2 section 2.2.2.2.1.

3.2.2.2.2 Waist:Hip Ratio

See Chapter 2 section 2.2.2.2.3.

3.2.2.2.3 Ankle:Brachial Index

See Chapter 2 section 2.2.2.2.4.

3.2.3 First Visit to the Cardiovascular Laboratory

3.2.3.1 Graded Incremental Cycle Test to Exhaustion ($\dot{V}O_2$peak test)

Having collected the anthropometric data, subjects were required to perform an incremental cycle test to exhaustion to determine $\dot{V}O_2$peak and VT. A full description of which can be found in Chapter 2 section 2.2.3.5.

3.2.4 Second Visit to the Cardiovascular Laboratory

3.2.4.1 $\dot{V}O_2$ Kinetics Analysis and Cardiac Output Responses

See Chapter 2 section 2.2.4.1.

3.2.5 Exercise Intervention

3.2.5.1 Gym protocol: 12-week supervised training intervention

Following the completion of baseline testing, the exercise treatment groups attended Monkstown Health & Fitness Centre, Monkstown, Co. Dublin, three days per week for a period of twelve weeks. If participants missed a session at any timepoint they were expected to make it up at a later date. Compliance was set at completion of 32 out of a possible 36 sessions. These exercise sessions were supervised by either the principal investigator, a co-investigator from TCD working on a related research study, or an external personal trainer hired by the investigative team for the duration of the study. The participants were given a prescribed workout to follow, which consisted of the following.

Weeks 1 – 4: Session duration ~60 minutes.

- Cardio workout: 5 minute warm-up followed by 15 minutes @ 70% peak HR performed on a choice of either treadmill, cycle ergometer, rowing ergometer or elliptical cross trainer. Total duration = 20 minutes.
- Stretching and core strengthening – these exercises targeted the calf muscles, quadriceps and hamstrings, gluteal muscles, abdominals, back and obliques. Total duration = 10 minutes.
- Resistance training: subjects performed two sets of ten repetitions at 60% of their one-repetition maximum (1RM) determined on their first attendance at the gym. Training sessions for weeks 0 – 4 targeted the upper body (chest press, lateral pull down, upright row and shoulder press) on the first session of the week; the lower body (leg extension, leg curl and leg press) on the second session; and a combination of upper and lower body for the third session (chest press, lateral pull down, upright row, shoulder press, leg extension, leg curl and leg press). Resistance training exercises are listed in table 3.1 below. Total duration = 15 minutes.
- A second bout of 15 minutes cardiovascular exercise at a similar level to the first bout performed on a choice of either treadmill, cycle ergometer, rowing ergometer or elliptical cross trainer. However, at least one of the two cardiovascular bouts had to be performed on a cycle ergometer.

Weeks 4 – 8: Session duration ~70 minutes.

After four weeks of training, the subjects 1RM were reassessed, as well as increasing the intensity of the first cardiovascular bout to 75% peak HR. Additionally, the second bout of cardiovascular exercise was adjusted to include three sets of 90s intervals performed at 75% peak HR, 85% peak HR and 90%+ peak HR, which was to be performed on the cycle ergometer.

- 1st cardiovascular bout: 20-minute duration.
- Stretching and core strengthening: 10-minute duration.
- Resistance training: 20-minute duration.
- 2nd cardiovascular bout: 20-minute duration.

Weeks 8 – 12: Session duration ~75-80 minutes.

After an additional four weeks training, the subject's 1RM were assessed for a third time. Subjects now performed two sets of ten repetitions at 70% of their 1RM. The first cardiovascular bout was increased in intensity to 80% peak HR and extended in duration to 25 minutes. The intervals in the final bout of cardiovascular exercise were increased in duration from 90s to two minutes; however the intensity remained the same as the previous month.

- 1st cardiovascular bout: 25-minute duration.
- Stretching and core strengthening: 10-minute duration.
- Resistance training: 20-minute duration.
- 2nd cardiovascular bout: 20-25 minute duration.

Table 3.1: Table of resistance exercise performed by exercise groups during the supervised phase of the training intervention

Time Period	Resistance Training	Intensity	Sets	Repetitions	Frequency
Weeks 1-4:	Bench Press	60% 1RM	2	10	2 days.wk^{-1}
	Lateral Pulldown	60% 1RM	2	10	2 days.wk^{-1}
	Overhead Press	60% 1RM	2	10	2 days.wk^{-1}
	Upright Row	60% 1RM	2	10	2 days.wk^{-1}
	Leg Curl	60% 1RM	2	10	2 days.wk^{-1}
	Leg Extension	60% 1RM	2	10	2 days.wk^{-1}
	Leg Press	60% 1RM	2	10	2 days.wk^{-1}
Weeks 5-8:					
	Bench Press	60% 1RM	2	10	3 days.wk^{-1}
	Lateral Pulldown	60% 1RM	2	10	3 days.wk^{-1}
	Overhead Press	60% 1RM	2	10	3 days.wk^{-1}
	Upright Row	60% 1RM	2	10	3 days.wk^{-1}
	Leg Curl	60% 1RM	2	10	3 days.wk^{-1}
	Leg Extension	60% 1RM	2	10	3 days.wk^{-1}
	Leg Press	60% 1RM	2	10	3 days.wk^{-1}
Weeks 9-12:					
	Bench Press	70% 1RM	2	10	3 days.wk^{-1}
	Lateral Pulldown	70% 1RM	2	10	3 days.wk^{-1}
	Overhead Press	70% 1RM	2	10	3 days.wk^{-1}
	Upright Row	70% 1RM	2	10	3 days.wk^{-1}
	Leg Curl	70% 1RM	2	10	3 days.wk^{-1}
	Leg Extension	70% 1RM	2	10	3 days.wk^{-1}
	Leg Press	70% 1RM	2	10	3 days.wk^{-1}

3.2.5.2 16-week unsupervised exercise

Following the completion of the 12-week supervised phase and the 3-month assessment of $\dot{V}O_2$peak and VT, subjects were asked to continue to exercise in an unsupervised capacity. Prior to the onset of this phase, all subjects were educated as to how to continue to progress with training. All subjects were informed that they would continue to receive free membership to Monkstown Fitness & Leisure centre. If subjects expressed a desire to exercise in a home-based setting, they were given target intensities and exercises to perform to attain the necessary cardiovascular and resistance targets. Subjects were informed of the physiological bases for trying to exercise at least three times per week, and were asked to record the frequency and volume of exercise performed.

At all stages during the exercise interventions subjects were equipped with a heart rate monitor (Cardiosport, USA), while periodic measurements of blood pressure were taken during exercise with an oscillometric BP monitor (Omron, Japan).

3.2.6 Equipment and Techniques

3.2.6.1 Cycle Ergometer and Innocor Metabolic Unit

See Chapter 2 section 2.2.5.8.

3.2.6.2 $\dot{V}O_2$ Kinetics Analysis

See Chapter 2 section 2.2.5.9.

3.2.6.3 Heart Rate Kinetics Analysis

See Chapter 2 section 2.2.5.10.

3.2.6.4 Cardiac Output Analysis

See Chapter 2 section 2.2.5.11.

3.2.7 Statistical Analysis

All statistical analyses were performed using Datadesk software (Version 6.2.1 OS X, Data Description Inc, USA). Data was compared at baseline, 3 months, and 7 months, in a two-step process. The initial comparison was made between the ND and T2D groups using a three-factor [diabetic status x treatment (exercise, control) x time (baseline, 3-months, 7-months) interaction] repeated-measures (RM) ANOVA with one repeated measure (time). This comparison was to determine the effects of T2D on the effects of an exercise intervention. A second comparison was then performed between the T2D and PIO groups using a three-factor [PIO use x treatment (exercise, control) x time interaction] RM ANOVA. Differences were observed using Bonferroni *post-hoc* test. Significance for all tests was set at $P<0.05$ and all results are presented as means ± standard deviation (sd). Analysis of CO and related parameters were performed in the same way, but with the addition of an additional factor (time of measurement: rest, 30s, 240s) to allow comparison of CO readings within each bout. Therefore a four-factor (diabetic status x treatment x time

x measurement) RM ANOVA was used to compare ND and T2D groups; while a different four-factor (PIO use x treatment x time x measurement) RM ANOVA was used to compare T2D and PIO groups. Differences in the four-factor RM ANOVA were also observed using Bonferroni *post-hoc* test.

3.3 RESULTS

3.3.1 Training Adherence Rates

For subjects in the exercise groups continued participation in the study, they were required to maintain an adherence rate of 32 of 36 sessions during the supervised phase of the intervention. One subject in the T2D EXS group was eliminated from the study on the basis of a low adherence rate (22 completed workouts out of the required 32).

During the home-based phase of the intervention, there were no minimum requirements for adherence rates for continued participation; however, subjects were asked to try to adhere to a minimum of three workouts per week, and to keep a record of the number of workouts and the workout's composition. Mean adherence rates are presented in table 3.1. Despite the ND EXS group having a numerically greater adherence rate during the home-based phase, there was no significant difference in adherence rates between the groups.

Table 3.2. Mean (±sd) adherence rates for exercise groups during the supervised and home-based phases of the exercise intervention.

	Adherence Rates (number of workouts	
	Supervised (out of 36)	Home-based (out of 48)
ND EXS	34.1 ± 1.5	38.1 ± 8.9
T2D EXS	34.5 ± 1.6	29.1 ± 13.6
PIO EXS	34.5 ± 1.4	30.5 ± 1.4

3.3.2 Subjects

3.3.2.1 Physical Characteristics

Physical characteristics at baseline for subjects are presented in table 3.2 below, with individual data listed in appendix XX. Subjects were matched for age and activity levels, as determined by use of RT3 accelerometers. No significant differences were detected for any of the parameters listed in table 3.2 in either

comparison, although when comparing height between the ND and T2D groups, there was a tendency for lower values in the T2D groups (P=0.082). No differences were detected in measures of the ankle-brachial index (ABI), with the findings indicative of healthy function of the peripheral arteries in all groups. ABI was also assessed at 3 and 7 months. However, no significant changes in values were detected in any groups as a consequence of the various interventions.

Table 3.3. Anthropometric and activity-level data for participants in the six groups recorded during subjects baseline visit. Data presented as mean ± sd.

	ND EXS Group (n=10)	**ND CTL Group (n=9)**	**T2D EXS Group (n=11)**	**T2D CTL Group (n=11)**	**PIO EXS Group (n=6)**	**PIO CTL Group (n=5)**
Age (yrs)	58.7 ± 7.4	51.1 ± 14.0	60.5 ± 6.3	56.5 ± 10.2	59.4 ± 11.2	54.5 ± 4.2
Height (m)	1.81 ± 0.06	1.76 ± 0.06	1.75 ± 0.06	1.76 ± 0.05	1.72 ± 0.07	1.77 ± 0.06
ABI (a.u)	1.16 ± 0.07	1.18 ± 0.06	1.15 ± 0.08	1.17 ± 0.10	1.19 ± 0.06	1.11 ± 0.12
RT3 Data ($hr.day^{-1}$)						
Inactive	17.5 ± 1.8	17.7 ± 1.2	18.8 ± 1.5	17.7 ± 1.4	17.5 ± 1.1	17.2 ± 1.3
Light	5.0 ± 1.4	4.9 ± 1.4	4.1 ± 0.9	5.2 ± 1.4	5.2 ± 1.5	5.6 ± 0.9
Moderate	1.2 ± 0.6	1.0 ± 0.3	0.9 ± 0.7	0.9 ± 0.2	0.8 ± 0.3	0.9 ± 0.3
Vigorous	0.4 ± 0.3	0.4 ± 0.3	0.2 ± 0.1	0.2 ± 0.3	0.4 ± 0.4	0.4 ± 0.3

One area in which the groups did differ was with respect to the waist-hip ratio (WHR), the values of which are presented in figure 3.1. Comparing the ND and T2D groups, a significant interaction between treatment and time was evident ($P<0.05$), with post-hoc tests revealing that the EXS groups had a significantly larger WHR at 3 and 7 months compared to baseline; while the CTL groups had a significantly greater WHR compared to EXS groups at 3 months. Main effects for all three factors were detected, with values at 3 and 7 months greater than baseline ($P<0.05$); individuals with T2D displaying a greater WHR than ND individuals ($P<0.05$); and the EXS treatment displaying a lower WHR than the CTL treatment ($P<0.05$).

Comparing the T2D and PIO groups, there was a significant interaction between treatment and time ($P<0.05$), with post-hoc tests revealing that the EXS groups had a significantly lower WHR than the CTL groups at baseline. A time effect was also noted ($P<0.05$), with WHR values at 3 months greater than baseline (not shown).

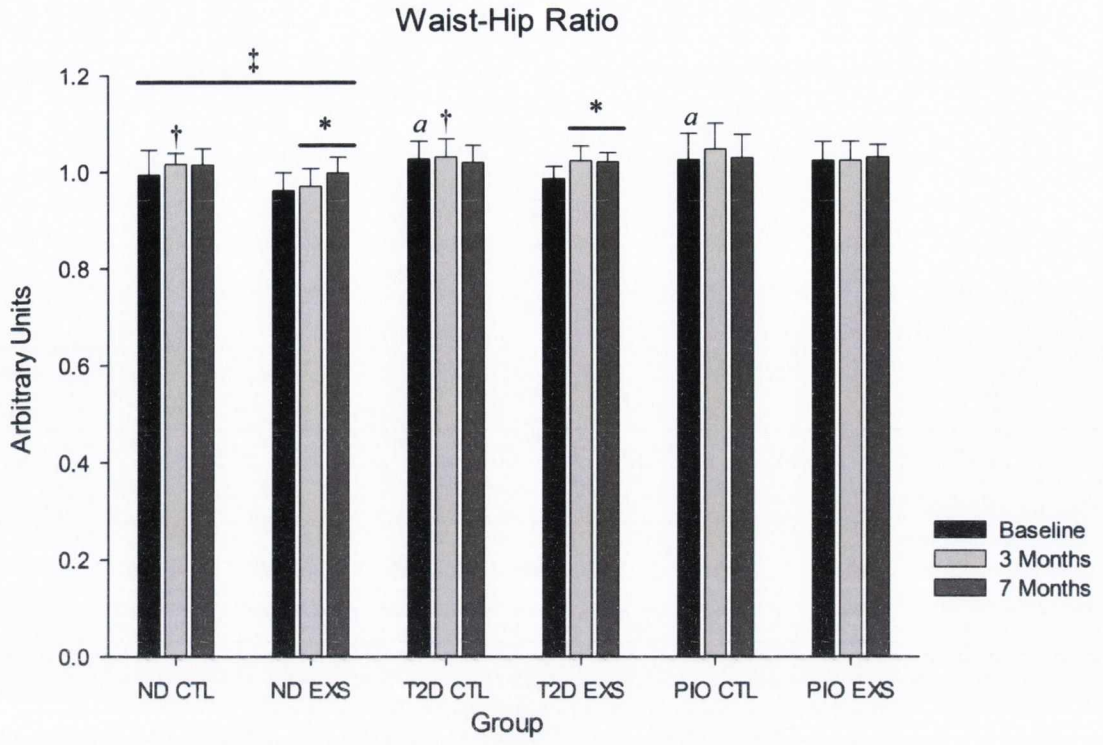


Figure 3.1. Waist-hip ratio values during participation. Data presented as mean ± sd. Symbols are used to represent significant differences in the analysis between the ND and T2D groups; letters are used to indicate significant differences exist between the T2D and PIO groups. * indicates that EXS values at 3 and 7 months were significantly larger than at baseline ($P<0.05$). † indicates that CTL values are greater than EXS values at 3 months ($P<0.05$). ‡ represents a significant effect of T2D on WHR ($P<0.05$). *a* indicates that the EXS groups had a smaller WHR than CTL groups at baseline ($P<0.05$).

Body mass and body mass index (BMI) were also recorded during each visit to the cardiovascular laboratory. Data relating to body mass is presented in table 3.3, while BMI values are illustrated in figure 3.2. In comparing body mass values of the ND and T2D groups, a significant interaction between diabetic status and time was detected ($P<0.05$), however, no significant post-hoc tests were detected. The likely cause of the interaction is that the ND EXS group had a non-significant decrease in mean body mass values as a consequence of the exercise intervention, while the T2D EXS group had a non-significant increase in body mass during the training period. The same interaction was found when comparing BMI values, with post-hoc tests revealing that the T2D groups had a significantly larger BMI than the ND groups at equivalent time points.

In comparing the T2D and PIO groups for body mass values, a significant interaction was detected between PIO status and time ($P<0.05$), with post-hoc tests revealing that the PIO groups had greater body mass than the T2D groups at each assessment; while the PIO groups also had a significant increase in body mass at 7

months compared to baseline. A time effect ($P \leq 0.0001$) was also noted, with values at 3 and 7 months significantly greater than at baseline, while values at 7 months also tended to be greater than 3-month values ($P=0.074$).

Analysis of BMI results indicate that there was a trend towards a three-way interaction ($P=0.0554$), with both PIO groups displaying larger BMI values than both T2D groups and also larger than their baseline values. This was also reflected in a significant interaction between PIO status and time ($P<0.05$). The PIO groups had a larger BMI than the T2D groups at baseline, 3, and 7 months ($P<0.0001$), while the PIO values at 3 ($P<0.05$) and 7 months ($P<0.0001$) were greater than at baseline. Similar to body mass values, a significant time effect was detected, with BMI at baseline significantly lower than at 3 and 7 months.

Table 3.4. Body mass values for each group when attending the laboratory for assessment. Data presented as mean ± sd. Significant differences were detected between the T2D and PIO groups (*aaa* $P<0.0001$), while both PIO groups displayed a significant increase in body mass at 7 months compared to baseline (*bbb* $P<0.0001$). *c* indicates a time effect (*c* $P<0.05$, *ccc* $P<0.0001$).

	Body Mass (kg)		
	Baseline	3 Months^{c}	7 Months^{ccc}
ND CTL	89.1 ± 8.7	89.2 ± 8.9	89.4 ± 8.5
ND EXS	94.3 ± 13.7	92.2 ± 13.4	93.0 ± 13.2
T2D CTL	94.1 ± 11.2^{aaa}	94.2 ± 11.7^{aaa}	94.5 ± 13.1^{aaa}
T2D EXS	88.6 ± 8.0^{aaa}	89.0 ± 7.5^{aaa}	89.3 ± 7.9^{aaa}
PIO CTL	97.0 ± 16.5	98.7 ± 16.7	101.0 ± 18.0^{bbb}
PIO EXS	94.7 ± 16.8	96.1 ± 17.6	96.6 ± 18.8^{bbb}

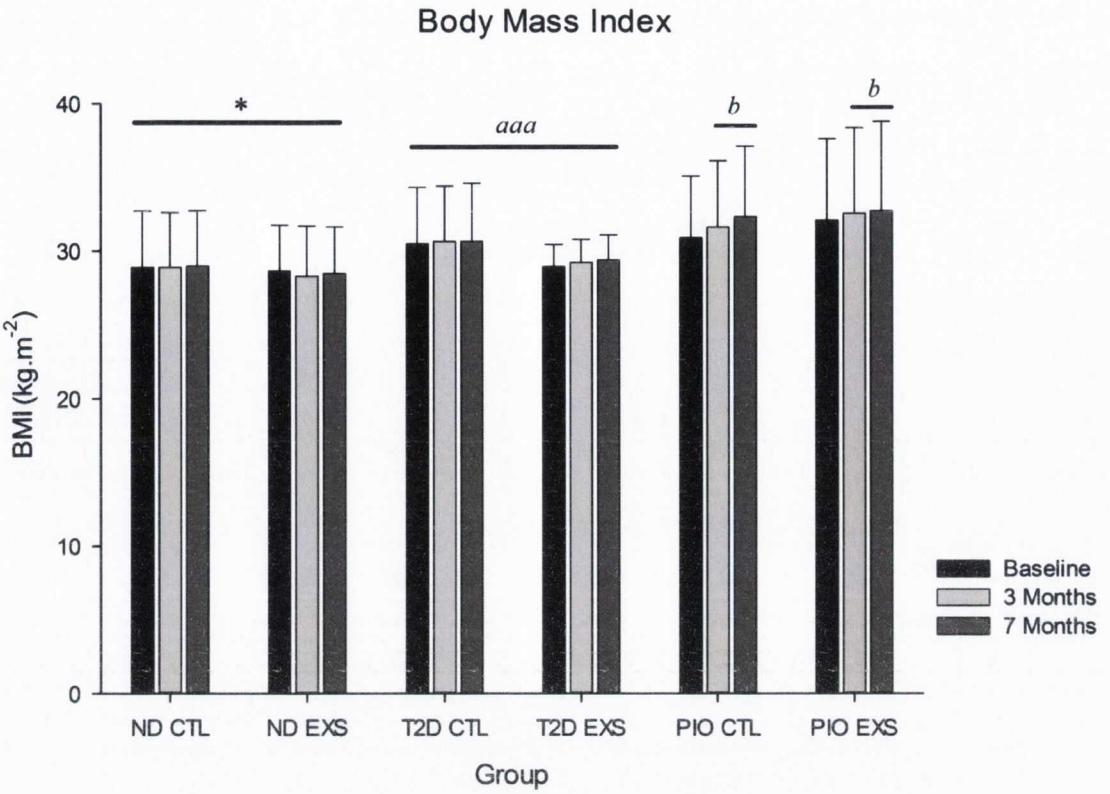


Figure 3.2: Changes in Body Mass Index during participation. Data presented as mean ± sd. Symbols are used to represent significant differences in the analysis between the ND and T2D groups; letters are used to indicate significant differences exist between the T2D and PIO groups. * indicates that ND values were significantly lower than T2D values at matching time points ($P<0.05$). *a* indicates that the T2D groups had a lower BMI than PIO groups at equivalent time points (*aaa* $P<0.0001$). *b* indicates that PIO group values at 3 and 7 months were significantly greater than baseline ($P<0.05$).

3.3.2.2 Haematological Parameters

Indices of glycaemic control form the basis for treatment of T2D. Measures of fasting blood glucose (FBG) and HbA_{1c} are presented in tables 3.4 and 3.5 respectively, with individual data presented in appendix XXI. In comparing FBG levels between the ND and T2D groups, an effect of T2D was found ($P<0.001$), with greater levels in the T2D groups. A time effect was also found ($P<0.05$), however analysis of post-hoc tests surprisingly revealed that FBG at 3 months were significantly higher than at baseline. No differences in FBG were found between the T2D and PIO groups.

Comparison of HbA_{1c} between the ND and T2D groups revealed a significant effect of T2D, with the T2D groups displaying greater HbA_{1c} concentrations ($P<0.05$). No effect of exercise was noted on levels of HbA_{1c}. In comparing HbA_{1c} levels between the PIO and T2D groups, an interaction between PIO status and time was detected ($P<0.05$). Post-hoc tests did not reveal any significant comparisons, but

the PIO groups had numerically lower HbA_{1c} values by 3 months than the T2D groups.

A significant interaction between diabetic status and time ($P<0.05$) was found when comparing haemoglobin concentrations ([Hb]) of the ND and T2D groups. Post-hoc tests revealed that the T2D groups showed non-significant decreases in [Hb], while the ND groups showed non-significant increases in [Hb]. Comparison of [Hb] between the T2D and PIO groups did not reveal any significant differences. No differences in red blood cell concentration [RBC], haematocrit levels, cholesterol, triglycerides, HDL-C or LDL-C levels were found at any point in time between either the ND and T2D groups or the T2D and PIO groups.

Table 3.5. Fasting blood glucose (FBG) levels for each group when attending the laboratory for assessment. Data presented as mean ± sd. Significant differences were detected between the ND and T2D groups, with T2D displaying higher FBG, ** $P<0.001$. † indicates a time effect ($P<0.05$), with values at 3 months greater than at baseline.

	Fasting Blood Glucose ($mmol.L^{-1}$)		
	Baseline	3 Months†	7 Months
ND CTL	4.81 ± 0.62	5.09 ± 0.63	5.26 ± 0.60
ND EXS	4.71 ± 0.63	5.32 ± 0.37	5.26 ± 0.68
T2D CTL	6.82 ± 0.91**	7.55 ± 1.66**	7.04 ± 1.15**
T2D EXS	7.34 ± 1.95**	7.60 ± 2.57**	7.25 ± 2.14**
PIO CTL	7.33 ± 0.56	6.88 ± 0.86	6.62 ± 0.19
PIO EXS	7.95 ± 1.00	7.62 ± 1.28	7.44 ± 0.97

Table 3.6. Fasting glycosylated haemoglobin concentrations (HbA_{1c}) for each group when attending the laboratory for assessment. Data presented as mean ± sd. Significant differences were detected between the ND and T2D groups, with T2D displaying higher HbA_{1c} concentrations, * $P<0.05$.

	Fasting HbA_{1c} ($mmol.mol^{-1}$)		
	Baseline	3 Months	7 Months
ND CTL	38.5 ± 8.3	38.0 ± 7.4	39.3 ± 7.0
ND EXS	36.4 ± 2.7	35.9 ± 2.8	37.7 ± 2.8
T2D CTL	46.4 ± 5.6*	47.3 ± 6.1*	46.3 ± 4.6*
T2D EXS	50.1 ± 13.3*	51.3 ± 13.8*	50.6 ± 13.6*
PIO CTL	48.9 ± 5.4	46.0 ± 4.6	45.4 ± 5.5
PIO EXS	50.8 ± 7.2	48.0 ± 8.5	49.5 ± 7.8

3.3.3. Performance Data from Maximal Incremental Cycle Test

$\dot{V}O_2$peak ($ml.kg^{-1}.min^{-1}$) values from the graded incremental test are presented in Table 3.6. A significant interaction between treatment, diabetic status and time was detected ($P<0.05$). Analysis of post-hoc tests revealed that the ND CTL and ND EXS groups had significantly greater relative $\dot{V}O_2$peak values at baseline compared to both the T2D CTL and T2D EXS groups. Furthermore, treatment with exercise had a significant effect, with the ND EXS group displaying a significant increase in relative $\dot{V}O_2$peak values at 3 and 7 months over baseline, and also a further increase in relative $\dot{V}O_2$peak at 7 months over 3 months. The exercise intervention did not elicit as dramatic response in the T2D EXS group, with $\dot{V}O_2$peak values at 7 months significantly greater than at baseline. Significant interactions were also detected between diabetic status and time ($P<0.05$), and between treatment and time ($P\leq0.0001$). Main effects revealed that time ($P\leq0.0001$) had a significant effect on the findings, as well as diabetic status ($P<0.001$), with individuals with T2D displaying lower relative $\dot{V}O_2$peak values.

In comparing relative $\dot{V}O_2$peak values between the T2D and PIO groups, one subject from the PIO EXS group was excluded from the analysis as physical discomfort caused by the saddle prevented the subject from achieving a true measure of $\dot{V}O_2$peak. Analysis of the remaining subjects revealed a significant interaction between PIO status and time ($P<0.05$). Post-hoc results indicated that relative $\dot{V}O_2$peak values in the PIO groups tended to be lower at 7 months than at 3 months ($P=0.059$). There was also a significant treatment effect, with the EXS groups displaying significantly greater $\dot{V}O_2$peak values compared to the CTL groups ($P<0.05$).

Table 3.7. Peak $\dot{V}O_2$ data from incremental cycle test to exhaustion. Data presented as mean ± sd. Symbols are used to indicate differences between ND and T2D groups, with letters representing differences between T2D and PIO groups. * indicates that values are greater than ND EXS values at baseline (* P<0.05, *** P<0.0001). † indicates that values are lower than ND CTL group at same point in time (††† P≤0.0001). ‡ indicates that values are lower than ND EXS group at same point in time (‡ P<0.05, ‡‡‡ P≤0.0001). § indicates that values are greater than ND EXS group at 3 months (§ P<0.05). ¥ indicates that values are greater than T2D CTL group at same point in time (¥ P<0.05, ¥¥ P<0.001). # indicates that T2D EXS values at 7 months are significantly greater than at baseline (# P<0.05). *a* represents the presence of a treatment effect, with the EXS groups demonstrating greater $\dot{V}O_2$peak values than the CTL groups (*a* P<0.05).

	$\dot{V}O_2$peak ($ml^{-1}.kg^{-1}.min^{-1}$)		
	Baseline	3 Months	7 Months
ND CTL	$34.84 \pm 7.64^{***}$	32.97 ± 6.65	35.05 ± 6.72
ND EXS	30.26 ± 5.96	$34.48 \pm 5.96^{*}$	$38.02 \pm 5.77^{***\,\S}$
T2D CTL	$26.62 \pm 4.23^{\dagger\dagger\dagger\,\ddagger}$	$26.92 \pm 5.56^{\dagger\dagger\dagger\,\ddagger\ddagger\ddagger}$	$26.88 \pm 4.94^{\dagger\dagger\dagger\,\ddagger\ddagger\ddagger}$
T2D EXS[a]	$28.01 \pm 3.24^{\dagger\dagger\dagger}$	$30.27 \pm 3.52^{\ddagger\ddagger\ddagger\,¥}$	$30.90 \pm 3.42^{\dagger\dagger\,\ddagger\ddagger\ddagger\,¥¥\,\#}$
PIO CTL	26.91 ± 4.21	28.04 ± 5.48	24.87 ± 4.32
PIO EXS[a]	30.47 ± 4.42	31.50 ± 4.25	29.04 ± 5.11

Mean data for absolute $\dot{V}O_2$peak ($L.min^{-1}$) is presented in figure 3.3. A three-way interaction was detected (P<0.05) when comparing the ND and T2D groups. Analysis of post-hoc tests revealed that the T2D CTL group had a significantly lower $\dot{V}O_2$peak than both the ND groups at each time point. The T2D EXS group had a lower $\dot{V}O_2$peak than both the ND groups at baseline; however, the exercise intervention was successful in increasing their $\dot{V}O_2$peak to similar levels to the ND CTL group at 3 months. However, a significant difference between the T2D EXS group and the ND CTL group was again evident at 7 months (P<0.05). The ND EXS group had a significantly greater $\dot{V}O_2$peak than the T2D EXS group at all time points (P≤0.0001). The ND EXS group also displayed a significant increase in $\dot{V}O_2$peak at 3 months compared to baseline, and at 7 months compared to both baseline and 3 months. At 7 months the ND EXS group displayed a significantly greater $\dot{V}O_2$peak than the ND CTL group. Between the two T2D groups, the T2D EXS group had a significantly greater $\dot{V}O_2$peak than the T2D CTL group at 7 months, and their $\dot{V}O_2$peak at 7 months was also significantly greater than baseline. Significant interactions were also detected between diabetic status and time, and between treatment and time. Main effects were detected for time and diabetic status. When comparing the T2D and PIO groups, no significant interactions or main effects were detected for absolute $\dot{V}O_2$peak.

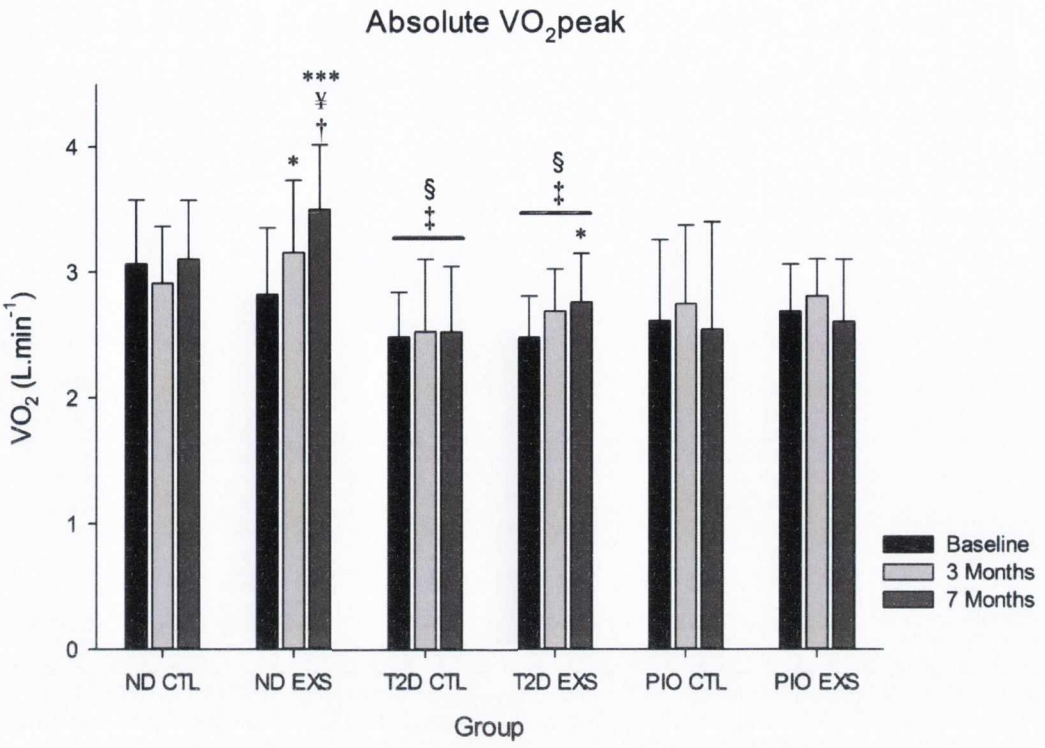


Figure 3.3: Mean group values for $\dot{V}O_2$peak. Data presented as mean ± sd. Symbols are used to represent significant differences in the analysis between the ND and T2D groups; no significant differences were detected after analysis between the T2D and PIO groups. * represents values that are significantly greater than the within-group baseline values (* $P<0.05$, *** $P<0.0001$). ¥ indicates that values are significantly greater than within-group values at 3 months. † indicates values significantly greater than the ND CTL group at same time point ($P<0.05$). ‡ indicates values are significantly lower than equivalent time points in the ND CTL group (‡ $P<0.05$). § indicates that values are significantly lower than equivalent time points in the ND EXS group (§ $P<0.05$). See text for full description of interactions between factors.

Peak workload and time to failure (TTF) were also measured in assessing peak performance. Mean peak workload data are shown in table 3.7 below, while time to failure data is illustrated in figure 3.4. With respect to peak workload data, it is apparent that the supervised exercise training phase was successful in increasing peak workload. A treatment x time interaction was detected when comparing the ND and T2D groups ($P<0.05$), as was an interaction between diabetic status and time. Significant post-hoc tests are indicated in table 3.7. Main effects were also found for time ($P\leq0.0001$), with values at 3 and 7 months greater than baseline, and diabetic status, as individuals with T2D were found to achieve a lower peak workload than ND individuals ($P<0.001$).

A treatment x time interaction was found when comparing the PIO and T2D groups. The EXS group values were greater at 3 months than compared to baseline, but were then found to have significantly decreased at 7 months compared to 3 months.

Table 3.8. Peak workload data from incremental cycle test to exhaustion. Data presented as mean ± sd. Symbols are used to represent significant differences in the analysis between ND and T2D groups; letters are used to indicate significant differences between T2D and PIO groups. * indicates that values are greater in the EXS groups compared to baseline (*** P<0.0001). † indicates that values in the EXS groups are greater than the CTL groups at same point in time († P<0.05 †† P≤0.001). ‡ indicates that values are greater in the ND groups compared to the T2D groups at same point in time (‡‡‡ P≤0.0001). *a* indicates that peak workload values for the EXS groups are greater than at baseline (*aaa* P<0.0001). *b* indicates that the CTL groups are lower than the EXS groups at the same point in time (*bbb* P<0.0001). *c* indicates that the EXS group values at 7 months are lower than the EXS group values at 3 months (*cc* P<0.001).

	Peak Workload (W)		
	Baseline	3 Months	7 Months
ND CTL	200 ± 34‡‡‡	210 ± 37‡‡‡	213 ± 33‡‡‡
ND EXS	196 ± 28‡‡‡	220 ± 32$^{***\ ††\ ‡‡‡}$	229 ± 43$^{***\ †\ ‡‡‡}$
T2D CTL	163 ± 25	168 ± 30^{bbb}	165 ± 29^{bbb}
T2D EXS	163 ± 28	195 ± 35$^{***\ ††\ aaa}$	182 ± 38$^{***\ †\ cc}$
PIO CTL	172 ± 34	178 ± 46^{bbb}	166 ± 33^{bbb}
PIO EXS	184 ± 25	220 ± 30^{aaa}	190 ± 21^{cc}

Analysing the TTF during the graded cycle test to exhaustion revealed a significant treatment x time interaction when comparing the ND and T2D groups, with post-hoc tests revealing that the EXS groups had a significantly greater TTF at 3 and 7 months compared to baseline. The EXS groups also had a longer TTF at 3 and 7 months compared to the CTL groups at the same point in time. Main effects were found for time (P≤0.0001), with TTF at 3 and 7 months greater than baseline, and for diabetic status (P<0.001), with individuals with T2D displaying a reduced TTF compared to ND individuals.

A treatment x time interaction was found when comparing the T2D and PIO groups (P≤0.0001). The EXS groups displayed a significantly longer TTF at 3 and 7 months compared to baseline; however, TTF at 7 months was significantly shorter than at 3 months. The EXS groups also had a significantly longer TTF than the CTL groups at 3 and 7 months. A main effect was found for treatment (P<0.05), with the EXS groups displaying a significantly longer TTF. There was also a main effect for time (P≤0.0001), with TTF at 3 months greater than baseline, and TTF at 7 months significantly shorter than at 3 months.

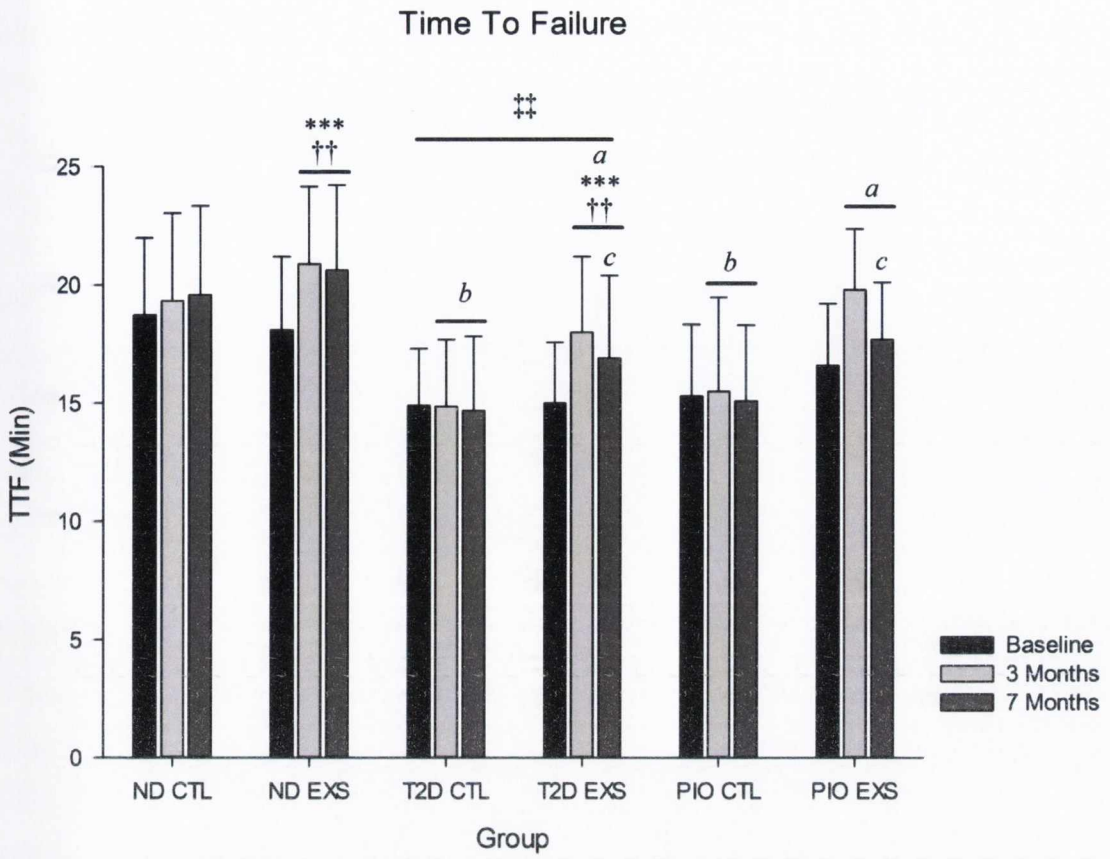


Figure 3.4: Mean group values for Time to Failure during graded cycle test to exhaustion. Data presented as mean ± sd. Symbols are used to represent significant differences in the analysis between the ND and T2D groups; letters are used to represent significant differences between the T2D and PIO groups. * represent an interaction between exercise and time, with the EXS group values at 3 and 7 months greater than baseline (*** $P<0.0001$). † indicates values in the EXS groups are significantly greater than the CTL groups at same time point (†† $P<0.001$). ‡ indicates the presence of a significant T2D effect (‡ $P<0.001$). A significant interaction between exercise and time was detected between the T2D and PIO groups, with *a* signifying values at 3 and 7 months were greater than at baseline in the EXS groups ($P<0.05$), and *b* indicating that CTL values at 3 and 7 months were lower than EXS values at the same point ($P<0.0001$). *c* indicates that EXS values at 7 months were lower than EXS values at 3 months ($P<0.05$). See text for full description of interactions between factors.

A treatment x time interaction was found comparing $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$) at VT for the ND and T2D groups ($P\leq0.0001$). Post-hoc tests revealed that the EXS groups significantly increased VT at 3 and 7 months compared to at baseline, while the EXS groups' VT at 3 months was also greater than the CTL groups at 3 months (figure 3.5). A main effect was found for diabetic status ($P<0.05$), with individuals with T2D reaching VT at a lower $\dot{V}O_2$ than ND individuals. A main effect was also found for time ($P\leq0.0001$), with $\dot{V}O_2$ values at VT significantly greater at 3 and 7 months compared to baseline (not shown).

A significant interaction between PIO use and time was found when comparing the T2D and PIO groups ($P<0.05$). Post-hoc tests revealed that the T2D groups displayed a greater $\dot{V}O_2$ at VT at 3 and 7 months compared to baseline. A

significant interaction was also found between treatment and time ($P<0.05$), with the EXS group values at 3 months greater than baseline, while the EXS group values at 3 and 7 months were greater than the CTL groups at the same point in time ($P<0.001$). A main treatment effect was also found ($P<0.05$), with the EXS groups having a higher $\dot{V}O_2$ at VT than the CTL groups.

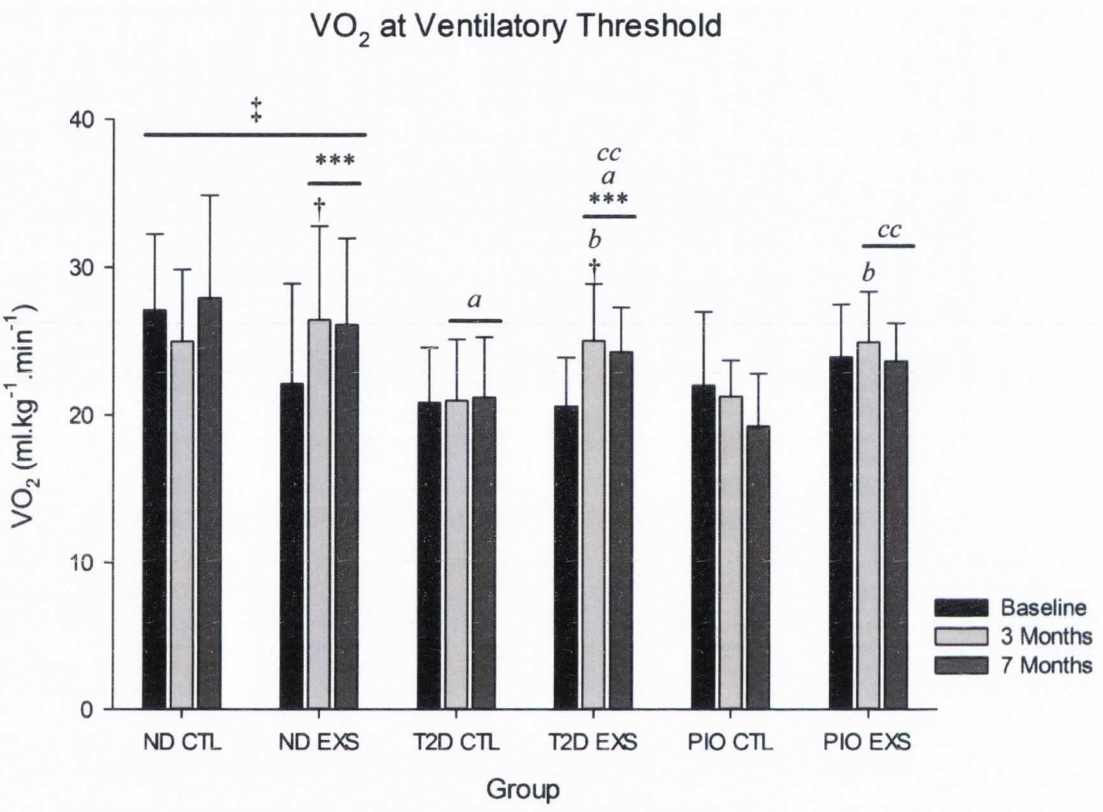


Figure 3.5: $\dot{V}O_2$ values ($ml.kg^{-1}.min^{-1}$) at which subjects reached ventilatory threshold. Data presented as means ± sd. Symbols are used to represent significant differences in the analysis between the ND and T2D groups; letters are used to represent significant differences between the T2D and PIO groups. * indicates that EXS group values at 3 and 7 months are greater than baseline ($P<0.0001$). † indicates that EXS group values are greater than CTL values at the same time point ($P<0.05$). ‡ represents a significant effect of T2D ($P<0.05$). *a* indicates that T2D group values at 3 and 7 months are greater than baseline ($P<0.05$). *b* indicates that EXS group values at 3 months are greater than baseline ($P<0.05$), while *c* indicates that EXS group values are greater than CTL group values at the equivalent point in time ($P<0.001$).

Analysis of peak HR achieved during the incremental cycle test did not reveal any significant differences between the ND and T2D groups, or between the PIO and T2D groups; while analysis of peak $\dot{V}_e$ revealed that individuals with T2D had a lower peak $\dot{V}_e$ than ND individuals ($P<0.05$; appendix XXI). Peak RER data revealed a time effect for the ND and T2D groups, with values at 7 months greater than 3-month values ($P<0.05$). Comparison of the PIO and T2D groups' RER values

revealed a significant interaction between PIO status and time ($P<0.001$), with post-hoc tests revealing that RER values in the PIO groups at 3 months were greater than the T2D groups at the same point in time, as well as PIO group values at 7 months. Additionally, T2D group values at 7 months were lower than at baseline. A main time effect ($P<0.05$) was noted, with 7-month values lower than 3-month values ($P<0.05$). Individual responses for all parameters assessed during the graded cycle test are listed in appendix XXII.

3.3.4 $\dot{V}O_2$ Kinetic Responses to Constant-Load Cycling at 80% VT

Sample $\dot{V}O_2$ kinetic responses from individual representatives of each group to steady-state cycling at 80% VT are presented in figure 3.6 below, mean τ_2 and MRT values are listed in table 3.8 below, with fitted parameters for each individual listed in appendix XXIII. Group goodness-of-fit data is presented in appendix XXIV. Three model fits at baseline and at 7 months in the ND EXS group; one at baseline, four at 3 months, and two at 7 months in the ND CTL group; 4 at baseline, 3 at both 3 and 7 months in the T2D EXS group; four at baseline, two at 3 months, and seven at 7 months in the T2D CTL group; and two at baseline, 3 months and 7 months in both the PIO CTL and PIO EXS groups were fitted using the tri-exponential model. Analysis of the workload (W) which corresponds to 80% VT revealed a significant treatment x time interaction ($P\leq0.001$) between the ND and T2D groups, with post-hoc tests revealing that the EXS groups had a significant increase in workload at 80% VT at 3 and 7 months compared to baseline, while they also cycled at a significantly greater workload at 3 and 7 months compared to the CTL groups at the same point in time. A main effect was found for diabetic status ($P<0.001$), with individuals with T2D operating at a lower workload than ND individuals. A main effect was also found for time ($P\leq0.0001$), with the workloads at 3 and 7 months greater than at baseline.

A similar treatment x time interaction was also found for workload at 80% VT when comparing the PIO and T2D groups ($P\leq0.0001$), with the EXS groups displaying a significant increase in workload at 3 and 7 months compared to baseline, and greater values at 3 and 7 months when compared to the CTL groups at the same point in time. A main treatment effect was also found ($P<0.05$), with the EXS groups

cycling at a higher workload than the CTL groups. A time effect was also noted ($P \leq 0.0001$), with 3 and 7 months higher than at baseline.

Analysis of the model parameters from the bi-exponential model fitted to the $\dot{V}O_2$ kinetic responses revealed that diabetic status had an effect on the amplitude of the first (A_1) and second phase (A_2) of the kinetic response, and End A ($P<0.05$), with individuals with T2D displaying a lower amplitude for each of these parameters compared to ND individuals. A_2 values were numerically greater in the EXS groups than the CTL groups ($P=0.058$). At treatment x time effect was detected for End A values, with the EXS group values at 3 and 7 months greater than baseline, and also greater than the CTL group values at equivalent points in time. Diabetic status also affected the speed of the kinetic response, with individuals with T2D displaying slower τ_2 values and slower MRT ($P<0.05$) than ND individuals (table 3.8). Exercise appeared to have speeded up the onset of the second phase of the kinetic response, with the EXS groups displaying a shorter time delay of the second phase ($P<0.05$). However, exercise did not have any effect on τ_2 or MRT. In comparison of the T2D and PIO groups, a treatment x time interaction was detected with respect to τ_2 values ($P<0.05$). Post-hoc tests indicate that τ_2 values in the EXS group were faster than the CTL groups at 3 months, while the CTL group values at 7 months were also significantly faster than the CTL group values at 3 months. There was also a trend towards faster MRT values in the EXS groups than the CTL groups ($P=0.057$).

Table 3.9. Mean τ_2 responses for each group during steady-state cycling at 80% of VT. Symbols are used to represent differences between the ND and T2D groups, while letters indicate differences between the T2D and PIO groups. A significant effect of diabetic status was found with ND individuals displaying faster τ_2 responses compared to individuals with T2D (* $P<0.05$). *a* indicates that the EXS group value at 3 months are significantly faster than the CTL groups (*a* $P<0.05$). *b* indicates that the CTL group values at 7 months are faster than the CTL group values at 3 months (*b* $P<0.05$).

	τ_2 (s)		
	Baseline	3 Months	7 Months
ND CTL	34.1 ± 12.8*	38.3 ± 25.2*	37.8 ± 8.1*
ND EXS	33.8 ± 10.0*	36.4 ± 6.8*	37.6 ± 6.3*
T2D CTL	44.8 ± 11.4	51.1 ± 16.6	41.8 ± 7.8*b*
T2D EXS	44.9 ± 12.8	39.9 ± 9.4*a*	44.4 ± 11.2
PIO CTL	47.2 ± 15.4	54.9 ± 20.7	34.1 ± 10.2*b*
PIO EXS	47.0 ± 11.2	35.6 ± 4.4*a*	40.1 ± 16.0

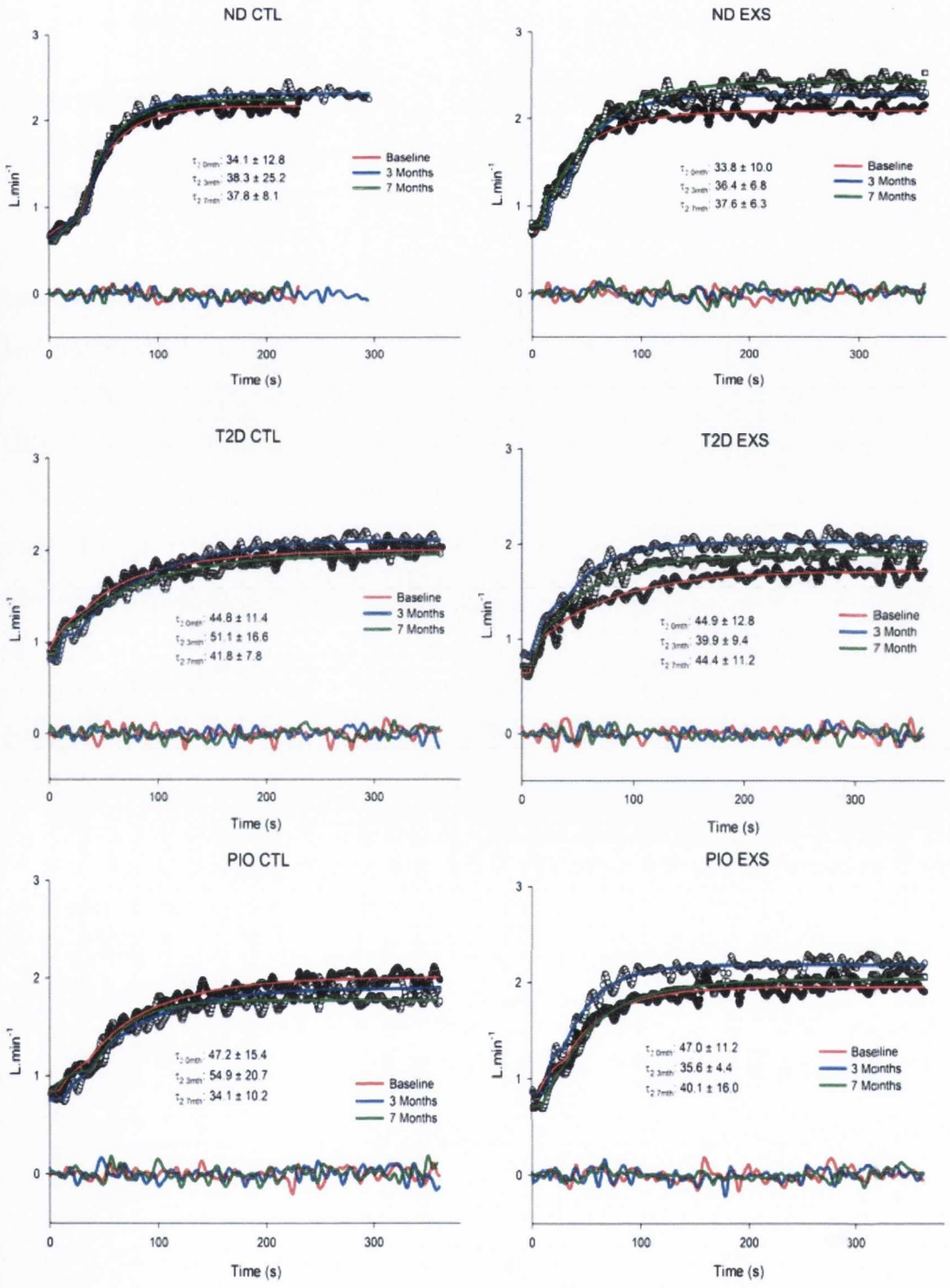


Figure 3.6. $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT from a representative individual in each group are presented. Data points are plotted from averaged $\dot{V}O_2$ data from bouts 1-4, with the coloured lines representing the fitted responses from the bi-exponential model used.

3.3.5. Heart Rate Kinetic Responses to Constant-Load Cycling at 80% VT

Sample HR kinetic responses from an individual in each group are presented in figure 3.7 below. Tau (τ) responses and End A for the mono-exponential fit of HR responses for each group are presented in table 3.9. Individual model responses are listed in appendix XXV.

τ values displayed a treatment x time interaction when comparing the ND and T2D groups ($P<0.05$). Analysis of post-hoc results revealed that the EXS groups had a significant speeding of the HR kinetic response at 3 months compared to baseline. A main effect was witnessed for diabetic status ($P<0.05$), with individuals with T2D displaying significantly slower τ values than ND individuals. A main effect was also evident for time ($P<0.05$), with post-hoc tests indicating that τ at 7 months were significantly longer than at 3 months. No other model parameters displayed any significant differences.

In comparing the HR kinetic responses between the T2D and PIO groups, a significant difference in increase in amplitude of the kinetic response was found between the T2D and PIO groups, with the PIO groups having a lower HR than the T2D groups ($P<0.05$). No other significant differences were detected.

Table 3.10. Mean τ values and End A from HR kinetic responses. Data presented as mean ± sd. Symbols represent significance from comparison of the ND and T2D groups. * indicates that the ND groups have a significantly faster τ response than the T2D groups * $P<0.05$. † indicates that τ at 3 months in the EXS groups was significantly faster than at baseline. No difference was detected for End A values.

	τ (s)			**End A (beats.min^{-1})**		
	Baseline	3 Months	7 Months	Baseline	3 Months	7 Months
ND CTL	61.0 ± 15.7*	54.3 ± 28.9*	59.2 ± 20.5*	133 ± 9	131 ± 12	134 ± 18
ND EXS	68.8 ± 21.0*	51.9 ± 13.1*††	59.8 ± 16.2*	135 ± 9	124 ± 14	127 ± 15
T2D CTL	85.4 ± 30.9	69.2 ± 25.3	89.8 ± 36.0	124 ± 11	125 ± 16	130 ± 11
T2D EXS	82.0 ± 21.4	57.3 ± 11.2††	69.1 ± 11.4	126 ± 12	131 ± 23	129 ± 7
PIO CTL	74.0 ± 28.4	63.7 ± 6.6	75.2 ± 16.7	126 ± 10	131 ± 20	131 ± 20
PIO EXS	76.8 ± 39.2	70.5 ± 17.4	73.9 ± 19.2	128 ± 5	133 ± 4	128 ± 10

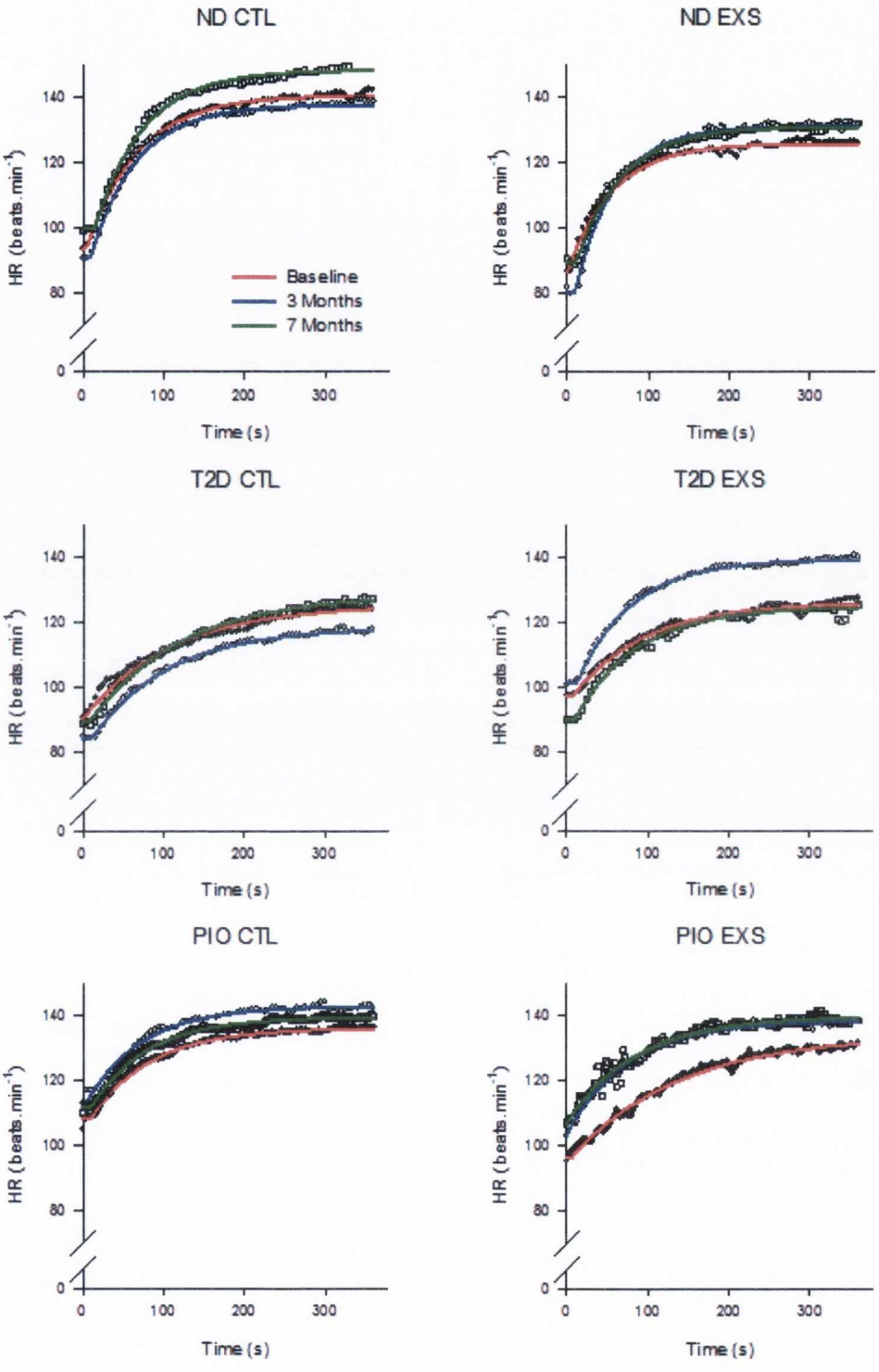


Figure 3.7. Sample HR kinetics from a representative individual in each group. Data presented as mean ±sd. Data points are plotted from averaged HR responses from bouts 1-4, with the coloured lines representing the fitted responses from the mono-exponential model used.

3.3.6. Cardiac Responses to Constant-Load Cycling at 80% VT

Cardiac output (CO) and related parameters were calculated at rest (appendix XXVI), and 30s (appendix XXVII) and 240s (appendix XXVIIII) into steady-state cycling at 80% VT, with mean CO responses presented below in figure 3.8. A four-way interaction was found (treatment x diabetic status x time x measurement) when comparing CO responses between the ND and T2D groups ($P<0.05$). Analysis of post-hoc tests revealed that CO values at rest were significantly lower than CO values recorded at both 30s and 240s ($P\leq0.0001$) at all time points. Furthermore, CO at 30s was significantly lower than CO at 240s at all time points ($P<0.05$). The ND EXS group also displayed greater CO at 240s compared to both T2D groups at baseline, but this difference was not evident at 3 or 7 months.

Additional interactions were detected between time and measurement ($P<0.05$), and diabetic status and measurement ($P<0.05$); while main effects were evident for diabetic status ($P<0.05$), with individuals with T2D displaying lower CO responses, and also for measurement ($P\leq0.0001$). However, no treatment effect was detected, meaning that the exercise intervention did not result in any significant increase in CO responses.

A number of interactions were detected when comparing CO responses of the T2D and PIO groups. A treatment x time interaction ($P<0.05$) was found, with post-hoc tests indicating that CO responses in the EXS groups were greater at 3 and 7 months compared to baseline. A PIO status x time interaction was also detected ($P<0.05$), with post-hoc tests revealing that the PIO groups displayed a greater CO response than the T2D groups at baseline, 3 and 7 months, while the PIO group values were greater at 7 months compared to baseline and 3 month values. The PIO-treated groups also displayed greater CO responses at rest and 240s compared to the T2D groups. A final interaction was detected between time and measurement ($P<0.05$), with post-hoc tests revealing that CO responses at 240s at 3 and 7 months were greater than CO responses at 240s at baseline, and additionally that CO responses at 30s recorded at 7 months were greater than the CO responses at 30s at baseline.

Main effects were detected for measurement ($P\leq0.0001$), and PIO status, with individuals receiving PIO displaying greater CO responses than individuals with T2D ($P<0.05$). A main effect was also evident for time ($P\leq0.0001$), with post-hoc tests

indicating that values at 3 months were significantly greater than at baseline, and that 7-month values were significantly greater than both baseline and 3-month values.

The differences detected in CO responses from both comparisons appear to be primarily driven by differences in SV responses, as HR values were reasonably similar. A time x measurement interaction ($P<0.05$) was detected when comparing HR responses of the ND and T2D groups, with post-hoc tests indicating that HR values at 240s recorded at 7 months were significantly greater than the HR values at 240s at both baseline and 3 months. The same interaction was detected when comparing the PIO and T2D groups ($P<0.05$), with HR at 240s recorded at 7 months significantly greater than HR values at 240s recorded at baseline. In both comparisons, HR at rest was significantly lower than HR at 30s and 240s at all time points, while HR at 30s was also significantly lower than HR at 240s at all time points.

A comparison of SV values between the ND and T2D groups showed that a four-way interaction exists (treatment x diabetic status x time x measurement), with post- hoc tests indicating that resting SV values were lower than SV at 30s and 240s at all time points. However, analysis of post-hoc tests did not reveal any effects of the training intervention on SV values.

An interaction of PIO status and measurement ($P<0.05$) was found when comparing the T2D and PIO groups, with SV values at rest and 240s significantly larger in the PIO groups. An interaction between PIO status and time ($P<0.05$) was also evident, with SV values at 7 months for the PIO-treated groups significantly greater than both baseline and T2D groups at 7 months.

The exercise intervention also appears to have had a positive impact on estimated a-v O_2 diff. Comparison of the ND and T2D groups revealed the presence of a three-factor interaction (treatment x time x measurement, $P<0.05$). Analysis of post-hoc tests revealed that the EXS groups demonstrated a significant increase in a-v O_2 diff at 240s at 3 and 7 months compared to at baseline. Values at 240s from 3 and 7 months in the EXS groups were also significantly greater than the CTL groups at the same point in time.

When comparing the T2D and PIO groups for estimated a-v O_2 diff, a significant interaction between PIO status, treatment and measurement was found ($P<0.05$). Post-hoc test indicate that a-v O_2 diff at 240s was greater at 3 months than at baseline in the T2D groups. Furthermore the PIO-treated groups displayed impaired

a-v O_2 diff at 240s at 7 months compared to the T2D groups at the same point in time and compared to the baseline values of the PIO-treated groups.

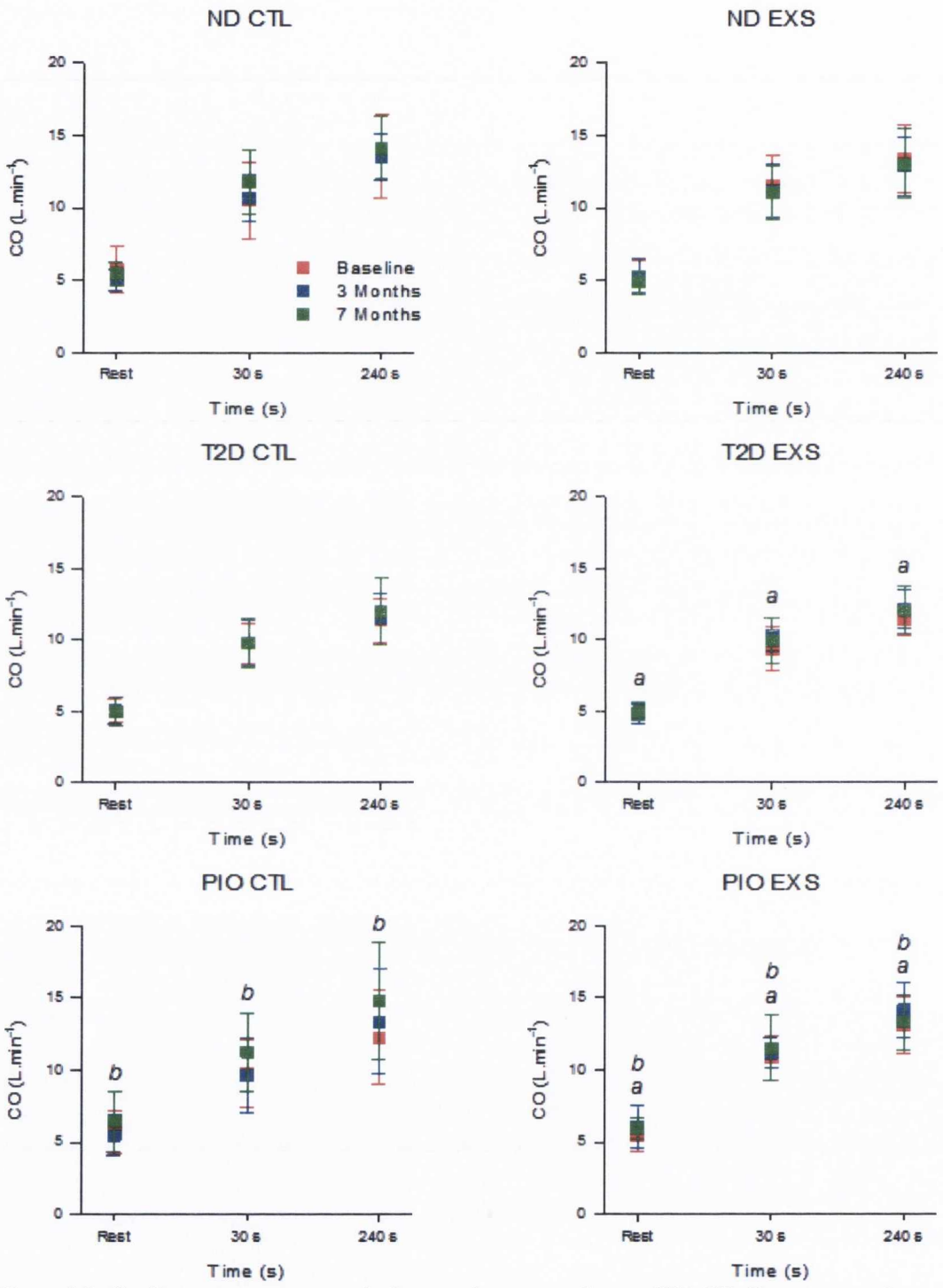


Figure 3.8. Cardiac output responses during steady-state cycling at 80% VT. Data presented as mean ±sd. Data points are plotted from averaged CO responses from bouts 5 & 6. Letters are used to indicate differences between the T2D and PIO groups. *a* represents a treatment and time interaction, with CO values at 3 and 7 months in the EXS groups greater than at baseline (*a* $P<0.05$). *b* indicates a PIO status and time interaction, with the PIO group values at 3 and 7 months greater than their baseline values. For additional interactions and main effects refer to the text.

3.4 Discussion

The main findings from this intervention-based study were that the exercise intervention was successful in improving exercise performance (as determined by $\dot{V}O_2$peak and peak workload) in ND males as early as 3 months, with further improvements in $\dot{V}O_2$peak also evident at 7 months; but an increase in $\dot{V}O_2$peak wasn't seen in males with T2D until 7 months, despite an improvement in peak workload evident at 3 months. The PIO EXS group did not display any significant increase in $\dot{V}O_2$peak, despite increasing their peak workload at 3 months compared to baseline. Furthermore, when assessing the $\dot{V}O_2$ kinetic response to steady-state cycling, the exercise intervention only had a marginal effect on the speed of the kinetic response (as determined by τ_2 and MRT values) when assessed at the same relative intensity (80% VT), with the T2D EXS and PIO EXS groups displaying faster kinetic responses compared to the CTL groups following the supervised intervention, but no significant speeding compared to their own baseline values.

The studies of Brandenburg *et al.* (1999) and Mac Ananey (2010) provide the main basis for comparison with the present study. The most noticeable difference between the findings of this study and those of Brandenburg *et al.* (1999) are that increases in exercise performance (determined by $\dot{V}O_2$peak) were larger in the ND group, whereas Brandenburg *et al.* (1999) demonstrated a greater magnitude of increase in performance in the T2D group. It is possible that differences in methodology, both in testing and in the training intervention, played a role in this outcome. To begin with, the present study looked at responses in male individuals, whereas Brandenburg *et al.* (1999) used females due to an observation that females may suffer a greater level of exercise impairment due to T2D (Regensteiner *et al.*, 1995). Secondly, the training intervention employed in this study differed from that of Brandenburg *et al.* (1999) in that this study utilised both aerobic and resistance-based training, and also divided the intervention phase into a supervised and unsupervised component. All participants in the three EXS groups completed a minimum of 32 sessions out of a possible 36 during the 12-week supervised intervention, ensuring that all subjects had similar adherence rates. Therefore the fact that only the ND EXS group demonstrated an increase in $\dot{V}O_2$peak would suggest that T2D might be the contributing factor to the lack of improvement in $\dot{V}O_2$peak in these groups. Unfortunately adherence to training was lower in all of the EXS groups during the

unsupervised phase. The individuals in the ND EXS group all continued to exercise, but reported that they averaged only 2 sessions per week. The individuals in the T2D EXS and PIO EXS groups were not as compliant, with four and two individuals in each respective group failing to exercise for at least 4 weeks out of the 16 week period, with the remainder of the individuals estimating that they completed an average of 2 sessions a week. However, adherence rates between the groups during the unsupervised phase did not achieve significance. None of the exercising individuals in any of the three groups claimed to have further increased their training workloads beyond those being performed at the end of the 12-week supervised phase, despite it being suggested that they try to increase the volume of aerobic activity to get to 30 minutes in the first cardiovascular bout and to maintain the intervals during the second cardiovascular bout. They were also requested to try to increase the resistance training to begin incorporating a third set of repetitions. Therefore it is difficult to discern if the lack of significant improvement in peak workload following the unsupervised training phase is due to the effect of T2D or simply that the volume of exercise performed was not enough to elicit the desired improvement, in particular in the PIO EXS group. Additionally, the lack of compliance in the unsupervised phase appears to have had a larger effect on performance in the T2D EXS and PIO EXS groups, with 7-month values for peak workload and TTF significantly lower than 3-month values, an effect that wasn't seen in the ND EXS group despite similar adherence rates to the other EXS groups.

Despite the modest improvement in $\dot{V}O_2$peak as a consequence of the training intervention, it was apparent that exercise training led to improved performance, with the exercise groups displaying increases in peak workload, TTF, and an increase in the level of oxygen consumption at which subjects reached ventilatory threshold. These findings would suggest that the lack of a substantial improvement in $\dot{V}O_2$peak might indeed be a consequence of T2D, as the exercise intervention was able to elicit significant increases in these other parameters. Since the improvement in exercise performance does not seem to be associated with an improved $\dot{V}O_2$peak, it is necessary to consider some of the other parameters measured to try to gauge the source of the improved exercise performance.

It has already been described how the exercise interventions of Brandenburg *et al.* (1999) and Mac Ananey (2010) resulted in speeding of the $\dot{V}O_2$ kinetic responses.

However, the varying methodologies employed meant that neither study assessed the kinetic response at the same relative workload following the intervention. In the present study, subjects cycled at 80% of their current VT, which resulted in a significant increase in the workloads at which subjects in the exercise groups operated following the training intervention. What was clearly apparent was that the negative impact of T2D on the $\dot{V}O_2$ kinetic response compared to the ND groups was still evident following the intervention. Furthermore, the kinetic responses (as determined by τ_2 and MRT) were not quickened as a consequence of the intervention. These findings contradict those of Brandenburg *et al.* (1999) and Mac Ananey (2010). It is possible that gender plays a role for the lack of improvement in the kinetic response in males, as Brandenburg *et al.* (1999) demonstrated speeding of the kinetic response due to the exercise intervention in females. However, at present, to this author's knowledge no study has yet investigated any difference in responses to exercise training due to gender. The treatment x time interaction detected in τ_2 values between the T2D and PIO groups appears to be a consequence of slower τ_2 values in the CTL groups at 3 months compared to baseline, as the exercise intervention did not result in a treatment effect.

One consequence of the training intervention was that the End A was increased at 80% VT at 3 and 7 months compared to baseline. One possible reason for the increase in $\dot{V}O_2$ consumption could be the effect of the exercise intervention upon cardiac function. To begin with, the exercise intervention resulted in a significant speeding of the HR kinetic response in the ND and T2D EXS groups after 3 months. It is noticeable that the T2D groups displayed slower HR kinetic responses to exercise at all time points compared to healthy ND counterparts. This would suggest that the slowed HR kinetic response is associated with the impairments in exercise performance in individuals with T2D, but that training is an effective method of improving the kinetic response and cardiac function.

It has been shown that T2D has a negative impact on left ventricular diastolic function (Poirier *et al.*, 2000; Baldi *et al.*, 2006). It has also been postulated that left ventricular diastolic dysfunction (LVDD) may cause reduced SV during exercise in individuals with T2D (Brassard & Poirier, 2009), as found in the work of Gusso *et al.* (2008). Therefore it is possible that the slower HR kinetics and impaired $\dot{V}O_2$ performance of individuals with T2D are a consequence of LVDD. It is known that

exercise training can normalise LVDD in individuals with T2D (Brenner *et al.*, 2001; Brassard *et al.*, 2007), therefore the improvements in HR kinetics evident here in the individuals with T2D might be a consequence of normalisation of LVDD. The speeding of HR kinetics in the ND individuals is more difficult to explain, but given the age of the participants in this study, it is not unreasonable to assume that some degree of age-related LVDD may be present in some of the ND participants that was corrected as a consequence of the training intervention.

Given that it has already demonstrated impaired CO and SV responses in males with T2D, it is possible that there is some association between CO and the impaired $\dot{V}O_2$ kinetics. This was further supported in the present study, as individuals in both T2D groups displayed impaired CO responses compared to the ND groups at baseline, with the ND CTL group also displaying greater CO readings at 240s during assessment at 3 and 7 months, and also higher CO at 30s at 7 months compared to both T2D groups. Analysis of HR and SV values during CO measurements indicate that the T2D-related impairment in CO values is a consequence of reduced SV. This would support the contention of Brassard & Poirier (2009). However, if LVDD is responsible for the impairment in CO and SV in individuals with T2D then exercise could be expected to correct the impairment. While the comparison between the ND and T2D groups did not show any significant change in CO values between groups, comparison of the CO readings from the T2D and PIO groups would appear to support this argument, as the EXS groups displayed greater CO responses at 3 and 7 months compared to baseline. Treatment with PIO also appeared to improve cardiac function, with the PIO groups displaying greater CO responses at rest and 240s compared to the T2D groups. The higher CO in the PIO groups was a consequence of greater SV than T2D groups, while the PIO groups also demonstrated greater SV at 7 months compared to their baseline values. Given that PIO seems to have a positive effect on cardiac function (van der Meer *et al.*, 2009), it is a little surprising that exercise performance was not improved in these individuals. However, it is possible that the improvement in CO was a consequence of the PIO-associated increase in BMI, which would explain the lack of speeding of the $\dot{V}O_2$ kinetic response. However, analysis of cardiac index (CI) readings confirmed an increase in CI values due to PIO treatment compared to T2D groups ($P<0.05$; appendices XXIV-XXVI).

This would suggest the improvement in cardiac function might be associated with the improvements in exercise performance.

Aside from the improvements in cardiac function due to exercise training, exercise-induced adaptations at the mitochondrial level would also appear to influence the results of the current intervention. Comparison of the ND and T2D groups revealed that the exercise intervention resulted in a significant increase in a-v O_2 diff at 3 and 7 months compared to baseline, and compared to the CTL groups at equivalent points in time. When comparing the T2D and PIO groups, the T2D groups demonstrated a significant increase at 3 months over baseline, while the PIO groups displayed impaired a-v O_2 diff at 7 months compared to baseline. It has previously been demonstrated that individuals with T2D display reduced a-v O_2 diff compared to healthy controls (Baldi *et al.*, 2003), with it also having been shown that the kinetics of deoxygenated haemoglobin concentrations ([HHb]) can be characterised by an overshoot as a consequence of the reduced a-v O_2 diff (Bauer *et al.*, 2007). There has been debate in the past as to whether T2D is associated with mitochondrial dysfunction (Kelley *et al.*, 2002; Mogensen *et al.*, 2007) or not (Boushel *et al.*, 2007; De Feyter *et al.*, 2008). While the impairment in resting a-v O_2 diff at baseline would suggest that mitochondrial dysfunction is present, it is also possible that impairments in a-v O_2 diff are due to altered fibre type distribution and differing glycolytic and oxidative capacities of skeletal muscle of individuals with T2D (Simoneau & Kelley, 1997; Oberbach *et al.*, 2006). Skeletal muscle of individuals with T2D was shown by Oberbach *et al.* (2006) to have 16% smaller oxidative fibre fraction, and 49% greater glycolytic fibre fraction compared to healthy tissue. The greater reliance on glycolytic muscle fibres in a T2D population would explain both the impaired a-v O_2 diff, and also the slowed $\dot{V}O_2$ kinetic response to steady-state exercise. It is generally accepted that exercise, and in particular aerobic training results in an increased fraction of oxidative muscle fibres in whole muscle (Salmons & Vrbova, 1969). Therefore, the current intervention may have resulted in increased expression of oxidative fibres in the exercise-treated groups, which would explain the ability to operate at a higher workload at 80% VT and also the increases in a-v O_2 diff and End A in the EXS groups.

It was expected at the outset of this intervention that treatment with PIO would elicit similar benefits in exercise capacity to those seen after treatment with RSG

(Regensteiner *et al.*, 2005; Kadoglou *et al.*, 2007). Despite eliciting improvements in cardiac function, with increases in CO and SV evident following the intervention, individual treatment with PIO or a combined treatment of PIO and exercise did not elicit any additional improvements in exercise performance. It is unknown to this author whether or not PIO treatment has any effect on muscle function or fibre type expression, but PIO treatment had no positive impact on a-v O_2 diff, with values at 7 months actually significantly lower than baseline values. One possible reason for the decrease in a-v O_2 diff in the PIO groups could be a reduction in the O_2 carrying-capacity of the blood, as PIO treatment is known to cause fluid retention and oedema, which can result in anaemia. This would in turn result in reduced O_2 delivery despite the increases in CO evident in this group. The significant increases in body mass and BMI in these groups would suggest that the possibility of fluid retention-based anaemia remains a potential cause for the lack of significant improvement in exercise performance. The presence of a significant interaction between diabetic status and time ($P<0.05$), indicating a decrease in [Hb] in the T2D groups may support this indirectly. However, the lack of any significant effect of PIO treatment on [Hb], [RBC], or haematocrit levels would seem to suggest that fluid retention-based anaemia does not play any role in the decrease in a-v O_2 diff evident in the PIO group.

One aspect that needs to be considered when interpreting the data following the exercise intervention is the volume of work that was performed by the individuals in the exercise groups. As discussed in the first chapter, the ACSM recommends that individuals with T2D perform $\geq$ 150mins of aerobic exercise per week spread over a minimum of three sessions. Additionally, it is recommended by the ACSM that these individuals also perform moderate to vigorous resistance exercise 2-3 times per week in addition to the aerobic exercise (Colberg *et al*, 2010). The addition of resistance training to training recommendations for individuals with T2D has only been recently established, as it was previously thought that it may exacerbate hypertension (Ibanez *et al.*, 2005). In designing the exercise training intervention, the aim was to satisfy the aim of performing $\geq$ 150mins exercise per week. However, the exercise intervention did not satisfy these most recent recommendations of the ACSM until the third month of the supervised intervention. Therefore the training-induced stimuli for improving exercise performance may not have been sufficient in the diabetic exercise groups, therefore explaining the greater level of response in the ND EXS group. Additionally, with respect to reassessment of 1RM every 4 weeks, evidence suggests that training-

induced adaptations may occur faster than this, therefore indicating the resistance–training stimulus should have been adjusted more frequently to obtain greater levels of physiological improvement.

The lack of further improvement in exercise performance during the unsupervised phase of the intervention has troubling implications for exercise-based treatment programs for individuals with T2D. It has previously been shown that a home-based resistance-training program did not maintain improvements in glycaemic control that were made following a supervised training program (Dunstan *et al.*, 2005). Similar to the current study, Dunstan *et al.* (2005) found that reductions in adherence and volume of training in the unsupervised capacity meant reduced levels of glycaemic control. Given that the subjects employed in the current study were educated as to the benefits of exercise, and provided with free gym access for the unsupervised portion of the study, it is disappointing that they failed to maintain the routine developed during the supervised phase. It also suggests that a re-think in how exercise is prescribed to patients in a clinical setting, as it would appear that when left to their own devices, individuals with T2D will fail to exercise at an intensity that will generate significant improvements in glycaemic control and exercise performance (Di Loreto *et al.*, 2005; Morrato *et al.*, 2007; Johnson *et al.*, 2005). It could be argued in the present case that the supervised intervention was also unsuccessful with regard to improvements in glycaemic control, as exercise did not elicit any difference in either FBG or HbA_{1c} levels. However, this author would contend that the subjects participating in this study already demonstrated excellent levels of glycaemic control at baseline, therefore it was always unlikely that further significant decreases in HbA_{1c} due to the addition of exercise alone would occur. The only significant effect on HbA_{1c} was the addition of PIO to treatment of the individuals in those groups, who demonstrated a larger, but non-significant decrease in HbA_{1c} compared to the T2D groups.

CHAPTER 4: THE EFFECT OF EXERCISE AND PIOGLITAZONE ON PEAK LVC AND LVC KINETIC RESPONSES IN MALES WITH T2D.

4.1 INTRODUCTION

It is well established that individuals with T2D display an impairment in peak $\dot{V}O_2$ and slower $\dot{V}O_2$ kinetic responses to submaximal exercise when compared to healthy counterparts (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011). One of the proposed sources of this impairment is reduced peripheral O_2 delivery to the active musculature due to impaired blood flow (BF) responses during exercise in this population (Behnke *et al.*, 2002; Kingwell *et al.*, 2003; Padilla *et al.*, 2006; Lalande *et al.*, 2008; Joshi *et al.*, 2010). The impairment in BF could arise due to a deficient central component to BF, manifesting itself as a reduction in CO values (Gusso *et al.*, 2008; Brassard & Poirier, 2009); or it could be a consequence of reduced peripheral BF delivery due to impaired vasodilatory responses. Evidence for reduced peripheral vasodilation can be found in a number of studies investigating BF and VC responses of individuals with T2D during exercise (Kingwell *et al.*, 2003; Joshi *et al.*, 2010;) and during assessment of NO-mediated vasodilation (McVeigh *et al.*, 1992; Williams *et al.*, 1996; Makimattila *et al.*, 1999).

A review of studies measuring RH responses indicated that 30 out of 31 studies demonstrated reduced forearm RH in individuals with T2D (Yki-Jarvinen, 2003); which would imply that the findings of impaired BF and VC in individuals with T2D are a consequence of impaired vasodilation. However, Yki-Jarvinen (2003) found that these studies did not control for blood pressure, BMI or macrovascular disease. This could in turn indicate that endothelium-dependent vasodilation (EDV) is a consequence of the attendant cardiovascular risk factors associated with T2D, rather than T2D itself. Indeed, when controlling for BMI and blood pressure between healthy controls and individuals with T2D, Sonne *et al.* (2007) displayed similar levels of forearm BF during RH, while (Sarabi *et al.*, 1999) have shown that risk factors may differ between genders for impaired forearm BF during RH, with waist-hip ratio (WHR) and fasting plasma glucose (FPG) being related to impaired EDV in males only.

In healthy individuals, it has been shown that age-associated impairments in EDV can be reversed as a consequence of exercise training (Martin *et al.*, 1990;

Tanaka *et al.*, 1998; Beere *et al.*, 1999; Spier *et al.*, 2004). To this author's knowledge, only two studies have investigated whether an exercise training intervention has the same effect on EDV in individuals with T2D. Maiorana *et al.* (2001a) demonstrated that EDV was enhanced in both resistance and conduit arteries following an eight-week training intervention in a group of individuals with T2D. In a similar intervention performed on healthy individuals, similar improvements in EDV were not seen (Maiorana *et al.*, 2001b). It may be that EDV may only respond to exercise training if an impairment is initially present. However, in these studies BF responses were only assessed in response to infusion of dilators in the forearm. In a study by Mac Ananey (2010), a supervised exercise intervention resulted in a speeding of LVC kinetics in individuals with T2D during high intensity steady-state plantar-flexion exercise (70% MVC) following twelve weeks supervised training, indicating improvements in EDV. The improvements gained during the supervised phase were maintained following an unsupervised gym-based training phase. However, in the study by Mac Ananey (2010), the value corresponding to 70% MVC at baseline was the workload used to assess the LVC kinetic response post-intervention. If MVC was to significantly increase following exercise training, then post-intervention assessment could not be considered to have been performed at 70% MVC. It is also unknown whether the findings of Mac Ananey (2010) extend to exercise performed at lower intensities, or whether an exercise training intervention results in significant speeding of the LVC kinetic response in males with T2D at low-intensity workloads (e.g. 30% MVC)

It is well established that treatment of T2D with PIO is associated with improvements in arterial stiffness (Harashima *et al.*, 2009), slower progression of carotid intima-media thickness (Mazzone *et al.*, 2006), increased levels of adiponectin and HDL-C, and decreases in hsCRP, IL-6 monocyte production, total cholesterol, LDL-C and triglyceride levels (Maegawa *et al.*, 2007; Pitocco *et al.*, 2009; Tsuchiya *et al.*, 2009; Vijay *et al.*, 2009; Nerla *et al.*, 2010). These findings are indicative of improvements in endothelial function, which should in turn result in improved EDV. Therefore a period of PIO treatment should result in improvements in BF responses during exercise in a T2D population. However, to this author's knowledge, the effect of treatment with PIO, either with or without exercise training, on the dynamic responses of LBF and LVC to exercise has yet to be investigated.

To properly assess whether changes in LBF or LVC are due to changes in endothelial function it is important to assess VC at the level of small muscle mass that is not impacted by the central component (Parker *et al.*, 2008). In the present study, the plantar-flexion exercise model in the supine position was chosen to ensure the absence of the central component in the results.

The aim of this study was to determine the effects of a training intervention on submaximal and peak LVC responses and exercise tolerance during an incremental plantar-flexion test in males with T2D. Additionally, the effect of the same training intervention on LVC kinetic responses to low-intensity steady-state plantar-flexion contractions and forearm RH responses in the supine position were assessed. Additionally, the added effect of PIO treatment on these parameters within the T2D groups was assessed, as well as any possible synergistic effect of a combined exercise and PIO treatment on peripheral BF and EDV responses.

4.2 Methods

4.2.1 Subjects

The same fifty-two subjects (33 individuals with T2D, 19 non-diabetics) that were assessed in the previous chapter were also assessed for their LVC responses in this intervention study in conjunction with the previous study described in chapter 3. The study was conducted in accordance to the principles outlined by the Declaration of Helsinki, and was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College, Dublin.

Subjects were in the same treatment groups as in the previous study. As with the previous study, subjects in receipt of beta-blockers were admitted to the study, as the main focus of this study was on peripheral circulation. The absence of a strong central component to the exercise requirement meant that it was considered that this class of drug would not adversely affect the outcome.

4.2.1.1 Recruitment of subjects

See Chapter 3 section 3.2.1.1.

4.2.1.2 Inclusion/Exclusion Criteria

See Chapter 2 section 2.2.1.2.

4.2.1.3 Participant information form

See Chapter 2 section 2.2.1.3.

4.2.1.4 Stress Test for Individuals with T2D

See Chapter 2 section 2.2.1.4.

4.2.1.5 Medical Examination

See Chapter 2 section 2.2.1.5.

4.2.1.6 Blood Sample Collection

See Chapter 2 section 2.2.1.6.

4.2.1.7 Determination of Physical Activity Levels

See Chapter 2 section 2.2.1.7.

4.2.2 Experimental Design

4.2.2.1 Study Overview

Measurements were collected during the subject's first visit to the cardiovascular laboratory in the Department of Physiology, Trinity College, Dublin baseline, and after 3 and 7 months respectively for assessment following the exercise training intervention. Following baseline testing, the study protocol was identical to that described in Chapter 3 section 3.2.2.1.

4.2.2.2 Anthropometry

4.2.2.2.1 Mass, Height, Body Mass Index

See Chapter 2 section 2.2.2.2.1.

4.2.2.2.2. Leg Volume

See Chapter 2 section 2.2.2.2.2.

4.2.2.2.3 Waist:Hip Ratio

See Chapter 2 section 2.2.2.2.3.

4.2.2.2.4 Ankle:Brachial Index

See Chapter 2 section 2.2.2.2.4.

4.2.3 Visit to the Cardiovascular Laboratory

4.2.3.1 Familiarisation with Calf Ergometer

See Chapter 2 section 2.2.3.1.

4.2.3.2 Maximum Voluntary Contraction

See Chapter 2 section 2.2.3.2.

4.2.3.3 LVC Kinetics

See Chapter 2 section 2.2.3.3.

4.2.3.4 Calf Incremental Plantar-Flexion Test and Arm Reactive Hyperaemia

See Chapter 2 section 2.2.3.4.

4.2.4 Exercise Intervention

4.2.4.1 Gym protocol: 12-week supervised exercise

See Chapter 3 section 3.2.5.1.

4.2.4.2 16-week unsupervised exercise

See Chapter 3 section 3.2.5.2.

4.2.5 Equipment & Techniques

4.2.5.1 Calf Ergometer

See Chapter 2 section 2.2.5.1.

4.2.5.2 Venous Occlusion Plethysmography

See Chapter 2 section 2.2.5.2.

4.2.5.3 Blood Pressure Measurements

At baseline, all blood pressure (BP) recordings were determined by applanation tonometry (COLIN CBM7000, Japan) as described more fully in Chapter 2 section 2.2.5.3. However, mid-way through the present intervention, the laboratory acquired a Finometer® Model-2 (Finapres Medical Systems B.V., the Netherlands), which provides a continuous assessment of BP at the level of the finger using an arterial volume-clamp method and physiological calibration (Physiocal) criteria for accurate unloading of the finger arteries. To compensate for the differing models, a reliability study was performed to ensure that the readings attained for each method were accurate, with the inter-correlation coefficient value indicating this is the case (appendix XXIX). The Finometer has is capable of detecting acute changes in blood pressure (Schutte *et al.*, 2003), as well as being validated for use with overweight, obese, and hypertensive patients (Schutte *et al.*, 2004).

4.2.5.4 Metronome

See Chapter 2 section 2.2.5.4.

4.2.5.5 Vascular Conductance Kinetics

See Chapter 2 section 2.2.5.5.

4.2.5.6 Incremental Test Parameters and Slopes

See Chapter 2 section 2.2.5.6.

2.2.5.7 Reactive Hyperaemia Response

See Chapter 2 section 2.2.5.7.

4.2.6 Statistical Analysis

All statistical analyses were performed using Datadesk software (Version 6.2.1 OS X, Data Description Inc, USA). Data was compared at baseline, 3 months, and 7 months, in a two-step process. The initial comparison was made between the ND and T2D groups using a three-factor (Diabetic status x treatment x time interaction) RM ANOVA with one repeated measure (time). This comparison was to determine the effects of an exercise intervention on individuals with T2D. A second

comparison was then performed between the T2D and PIO groups using a three-factor (PIO use x treatment x time interaction) RM ANOVA with time as a repeated measure. This was to determine if PIO treatment alone or a combined exercise and PIO treatment improved VC responses in individuals with T2D. All significant differences were observed using Bonferroni *post-hoc* test. Significance for all tests was set at $P<0.05$ and all results are presented as mean ± standard deviation (sd).

4.3 Results

4.3.1 Training Adherence Rates

See Chapter 3 section 3.3.1.

4.3.2 Subjects

4.3.2.1 Physical Characteristics

Physical characteristics for subjects are described in detail in Chapter 3 section 3.3.3.2. Subjects were matched for age, and activity levels, as determined by use of RT3 accelerometers. No differences were detected in measures of the ankle-brachial index (ABI), with the findings indicative of healthy function of the peripheral arteries in all groups. ABI was also assessed at 3 and 7 months. However, no significant changes in ABI values were detected in any groups as a consequence of the interventions. Leg volume was also assessed and is presented below in table 4.1. A significant interaction was detected between treatment and time ($P<0.05$) when comparing the PIO and T2D groups, with post-hoc tests revealing that the CTL groups had a greater leg volume than the EXS groups at 7 months ($P<0.05$). As a consequence, all data relating to LBF and LVC are adjusted for leg volume.

Table 4.1. Leg volume (ml) for participants in the six groups recorded at each time point. Data presented as mean ± sd. Letters indicate significant differences between PIO and T2D groups, with *a* indicating that CTL groups had a greater leg volume than EXS groups ($P<0.05$) at 7 months.

	Leg Volume (ml)		
	Baseline	3 Months	7 Months
ND CTL	2721 ± 224	2714 ± 227	2702 ± 290
ND EXS	2931 ± 380	2879 ± 386	2896 ± 372
T2D CTL	2663 ± 402	2663 ± 395	2730 ± 405[a]
T2D EXS	2543 ± 240	2575 ± 261	2507 ± 244
PIO CTL	2600 ± 434	2611 ± 484	2759 ± 430[a]
PIO EXS	2710 ± 325	2688 ± 475	2699 ± 375

4.3.2.2 Haematological Parameters

Haematological parameters are fully presented in Chapter 3 section 3.3.2.2.

4.3.3 Maximum Voluntary Contractions & Peak Calf Incremental Data

Peak MVC values are presented in figure 4.1 below. Individual MVC responses and peak parameters from the incremental test are listed in appendix XXX. In comparing the ND and T2D groups, a significant interaction was found between treatment and time ($P<0.001$), with the EXS groups showing significant increases in MVC at 3 and 7 months compared to baseline, and compared to the CTL groups at 3 and 7 months. A significant interaction between diabetic status and time was also found ($P<0.001$), with post-hoc tests revealing that at baseline, the ND groups had lower MVC values than the T2D groups. The opposite finding was true at 3 and 7 months however, with the ND groups displaying greater MVC values than their T2D counterparts and compared to baseline ND values. A main effect for time was found ($P\leq0.0001$), with values at 3 and 7 months significantly greater than baseline.

Comparison of the T2D and PIO groups revealed a significant three-way interaction between treatment, PIO status and time. Post-hoc tests revealed that at baseline, the T2D CTL group had greater mean MVC values than both the T2D EXS and PIO CTL groups. At 7 months, the T2D EXS group had significantly greater MVC values than at baseline, and compared to the PIO CTL group values at 7 months.

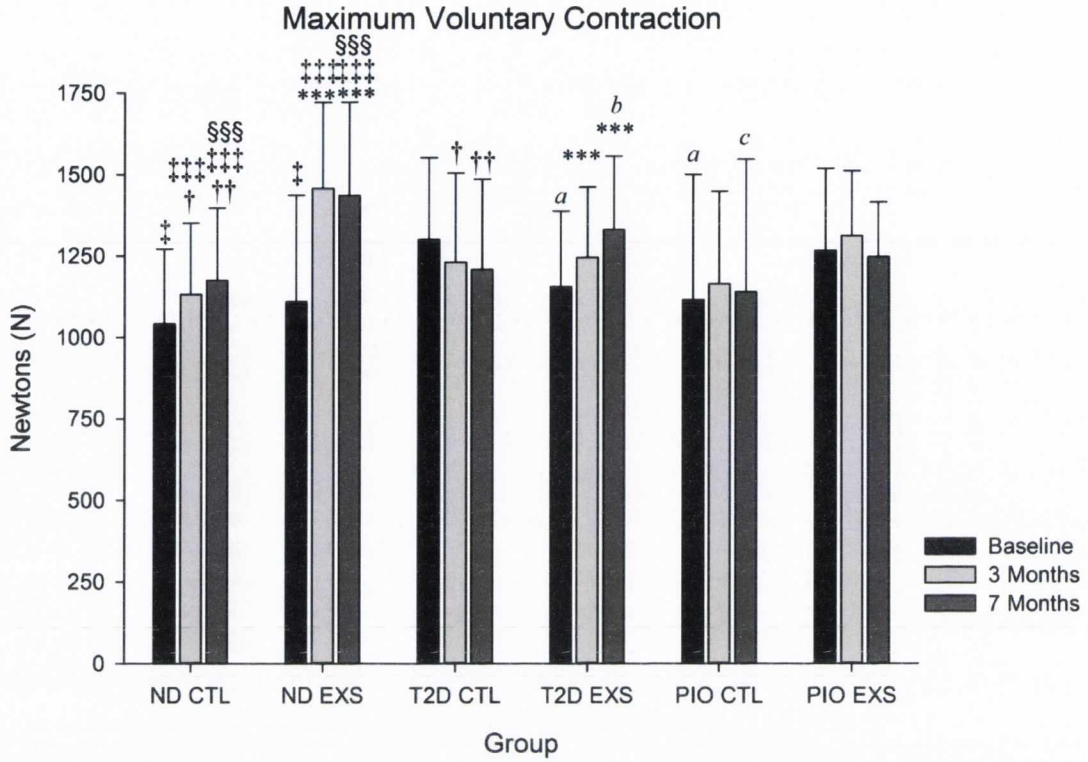


Figure 4.1. MVC data from participants during the intervention study. Data presented as mean ± sd. Symbols are used to signify significant differences between the ND and T2D groups. Letters are used to indicate significant differences between the T2D and PIO groups. * indicates that EXS group values at 3 and 7 months were significantly greater than baseline (*** P<0.0001). † indicates that EXS group values are significantly greater than CTL groups at the same point in time († P<0.05 †† P<0.001). ‡ indicates that significant differences between the ND and T2D groups exist at equivalent time points (‡ P<0.05 ‡‡‡ P<0.0001). § indicates that the ND group values at 7 months were significantly greater than baseline (§§§ P<0.0001). *a* indicates that values are significantly lower than the T2D CTL group at baseline (*a* P<0.05). *b* indicates that the T2D EXS group values at 7 months are significantly greater than at baseline (*b* P<0.05). *c* indicates that the PIO CTL group at 7 months was significantly lower than the T2D EXS group values at 7 months (*c* P<0.05).

Figure 4.2 illustrates subjects' time to failure (TTF) during the incremental plantar-flexion test. When comparing the ND and T2D groups, a significant interaction was detected between treatment and time (P<0.05), with post-hoc tests revealing that the EXS groups had a significant increase in TTF at 3 and 7 months compared to baseline. The EXS groups' performance at 3 months also tended to be greater than the CTL groups (P=0.056). In comparing both the ND and T2D groups and the T2D and PIO groups, a main effect for time was found, with TTF at 3 and 7 months greater than baseline.

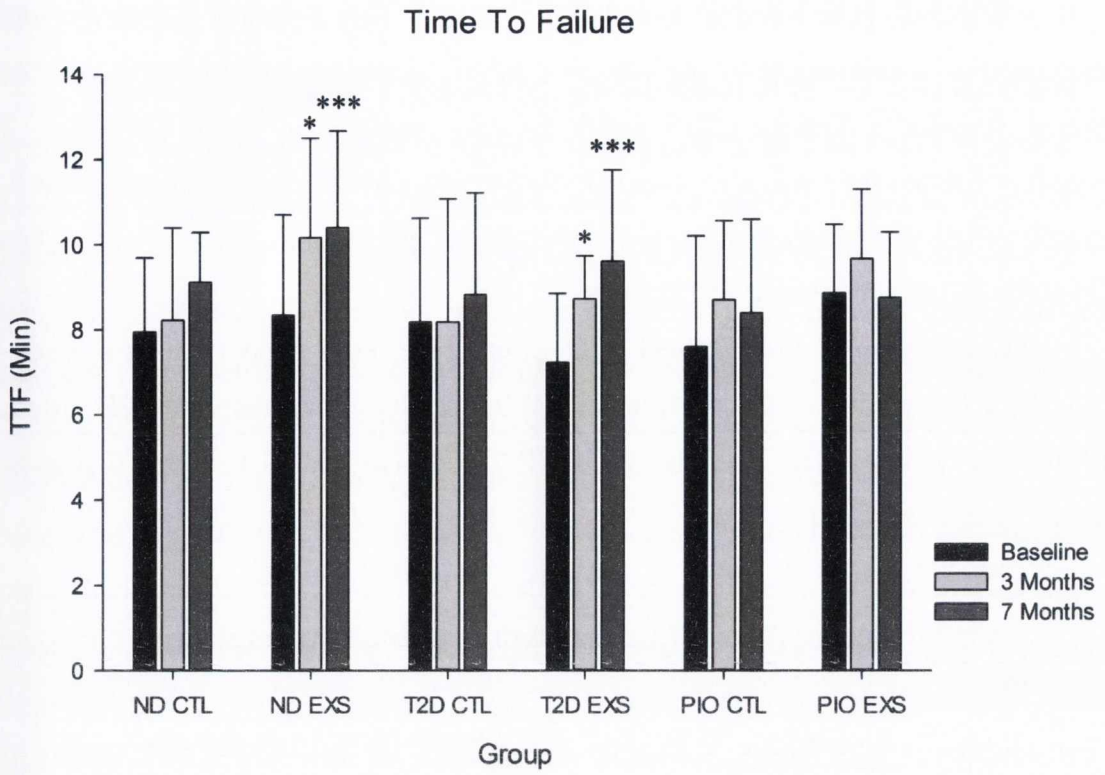


Figure 4.2. Time to failure (minutes) for subjects during the incremental plantar-flexion test. Data presented as means ± sd. * indicates a significant increase in TTF in the EXS groups compared to baseline when comparing the ND and T2D groups (* P<0.05 *** P<0.0001).

The peak force achieved (N) during the incremental plantar-flexion test are presented in table 4.2 below. Time effects were noted in both the ND and T2D and the T2D and PIO group comparisons, with performances improving at 3 and 7 months compared to baseline.

Table 4.2. Mean (±sd) peak force achieved during the incremental plantar-flexion test. * indicates presence of a time effect, with values in the ND and T2D groups greater than at baseline (* P<0.05 *** P<0.0001). *a* represents a time effect between the T2D and PIO groups, with values at 3 and 7 months greater than at baseline (*a* P<0.05).

	Peak Force (N)		
	Baseline	3 Months$^{*\,a}$	7 Months$^{***\,a}$
ND CTL	700 ± 173	744 ± 219	833 ± 100
ND EXS	740 ± 227	920 ± 257	940 ± 227
T2D CTL	718 ± 244	718 ± 289	791 ± 259
T2D EXS	645 ± 157	773 ± 101	864 ± 216
PIO CTL	660 ± 261	780 ± 179	740 ± 219
PIO EXS	800 ± 167	900 ± 179	833 ± 163

When the peak force achieved during the incremental plantar-flexion test was expressed as a percentage of the subject's MVC, comparison of the ND and T2D groups revealed a diabetes effect , with the data presented in figure 4.3 below. A significant time effect was also found ($P<0.05$), with post-hoc tests indicating that 7-month values were numerically greater, though not significantly so, than at baseline ($P=0.08$) and at 3 months ($P=0.12$).

Comparison of the PIO and T2D groups revealed a trend towards a greater %MVC achieved during the incremental test in the PIO groups than the T2D groups ($P=0.057$). A time effect was also found ($P<0.05$), with values at 3 and 7 months greater than at baseline.

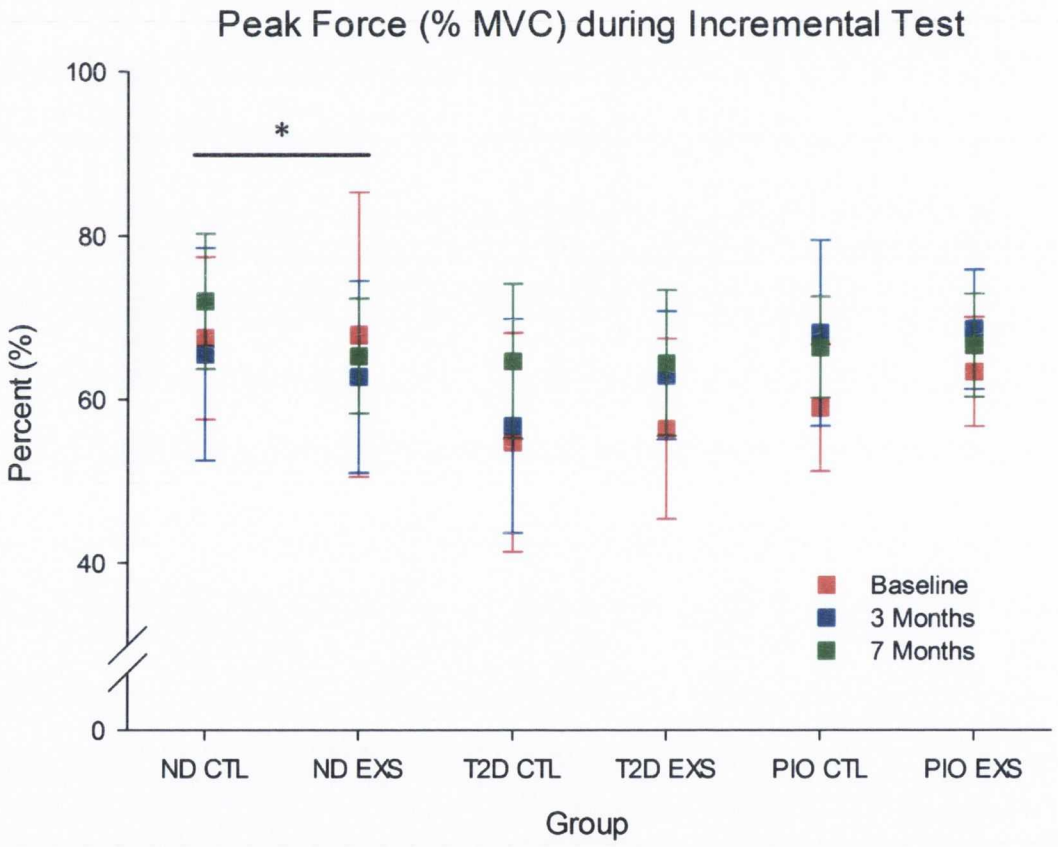


Figure 4.3. Percentage of MVC achieved during the incremental test. Data presented as means ±sd. In comparison of the ND and T2D groups, * indicates an effect of T2D ($P<0.05$).

4.3.4 Vascular Conductance Responses During the Incremental Plantar-Flexion Test

Vascular conductance responses at each workload of the incremental plantar-flexion test are presented below in figure 4.4, with individual data found in appendix XXXI. In comparing the LVC responses of the ND and T2D groups, there were trends towards a three-way interaction between diabetic status, treatment, and time due to slightly lower LVC responses at rest (P=0.0598) and 100N (P=0.082) in the T2D groups compared to the ND groups; however these did not achieve significance. In comparison of the LVC responses at various workloads no further differences were detected between the ND and T2D groups. However, differences were detected when comparing the T2D and PIO groups. At 300N, a significant interaction between PIO status and treatment was detected ($P<0.05$). No significant post-hoc tests were detected, but there was a trend towards greater LVC responses in the PIO EXS group compared to the PIO CTL group (P=0.073). Treatment also tended towards an effect on results (P=0.0505), with the EXS groups displaying greater LVC responses than the CTL groups.

Because some participants attained different maximum workloads during each visit, statistical analysis at higher intensities was not always possible due to missing values. To overcome this, a second analysis was performed, measuring LVC responses at each workload up to the smallest maximum workload attained during the three visits. Even allowing for this, no differences in workloads were detected between the ND and T2D groups. When the same statistical analysis was performed for the T2D and PIO groups, a significant interaction between treatment and PIO status was found at 500N ($P<0.05$), with post-hoc tests revealing that the PIO EXS group had greater LVC responses than the PIO CTL group ($P<0.05$), and tended to have greater LVC responses than the T2D CTL group (P=0.088) and the T2D EXS group (P=0.074). A significant treatment effect was also detected at 500N, with the EXS groups displaying greater LVC responses than the CTL groups at 500N.

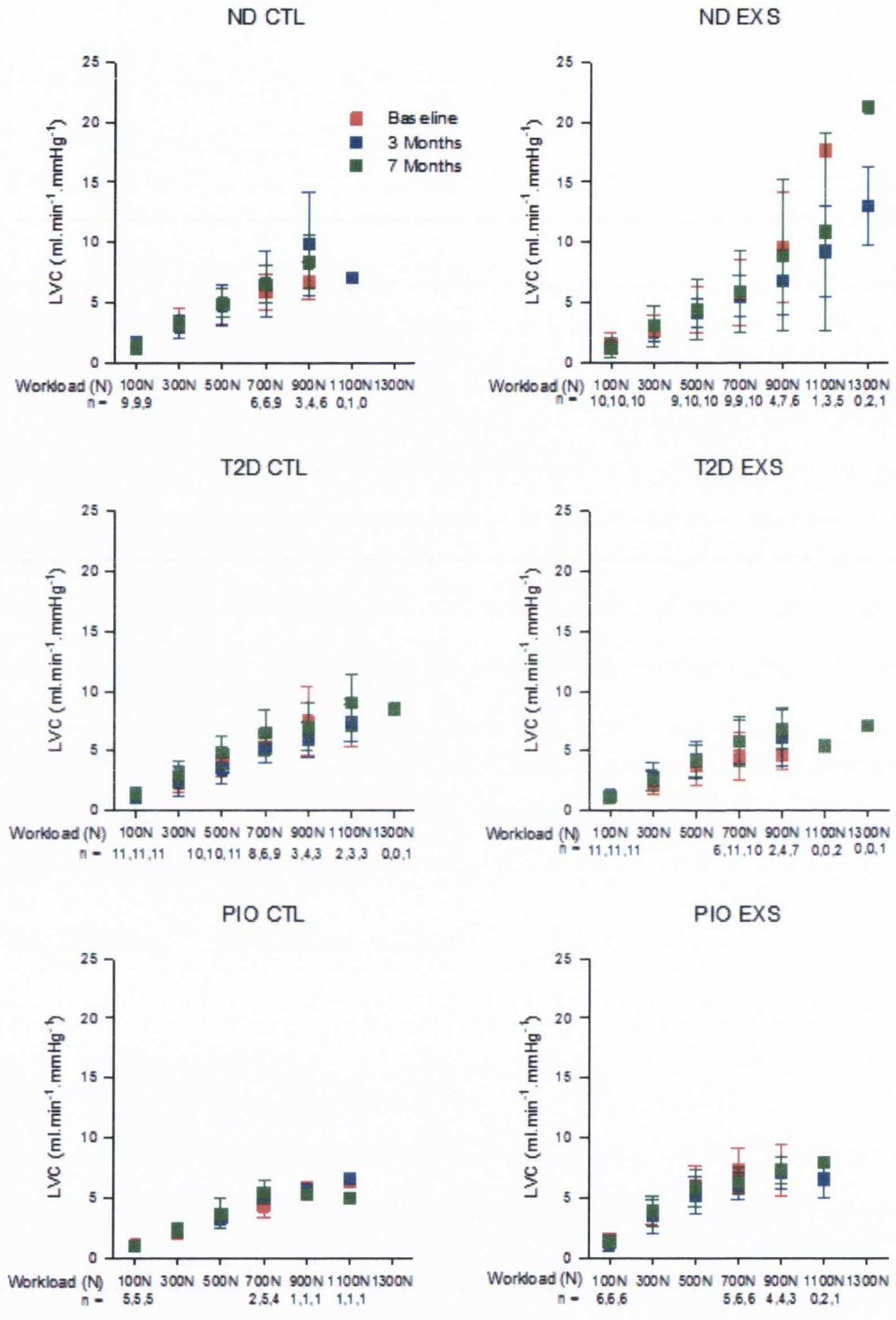


Figure 4.4. Mean (±sd) LVC responses at each workload during the incremental plantar-flexion test. Attrition rates during the test can be seen below the x-axis, with the n-number completing each workload given. See the text for significant interactions and differences between the groups, and for details about the two approaches used to detect statistical effects.

As an additional measure to overcome the missing values that occurred due to differing performances at each visit, the slopes of the individual LVC responses were calculated to determine an effective comparison between groups, and also compared peak LVC attained during the incremental plantar-flexion test.

The slopes of individual LVC responses were calculated based on two plots. In the first instance, the slope was calculated when LVC responses were linearly plotted against absolute workloads (N). In this case, no significant differences were detected when the ND groups were compared to the T2D groups, or when the PIO and T2D groups were compared (table 4.3).

Table 4.3. Slopes of the LVC responses during the incremental plantar-flexion test (mean ±sd). Slopes were plotted against workload (N).

	Slope		
	Baseline	3 Months	7 Months
ND CTL	0.0073 ± 0.0026	0.0077 ± 0.0041	0.0087 ± 0.0021
ND EXS	0.0069 ± 0.0038	0.0077 ± 0.0026	0.0073 ± 0.0042
T2D CTL	0.0069 ± 0.0034	0.0071 ± 0.0022	0.0082 ± 0.0026
T2D EXS	0.0098 ± 0.0089	0.0075 ± 0.0025	0.0074 ± 0.0030
PIO CTL	0.0063 ± 0.0022	0.0064 ± 0.0011	0.0056 ± 0.0035
PIO EXS	0.0084 ± 0.0031	0.0069 ± 0.0018	0.0074 ± 0.0016

In the second instance, the slope was calculated when each individuals' LVC responses were plotted against relative workload (%), with the highest workload attained by an individual expressed as 100%. Group responses for these findings are presented in table 4.4. In comparing the ND and T2D groups, a time effect was detected ($P \leq 0.0001$), with post-hoc tests revealing that slopes at 3 months ($P < 0.05$) and 7 months ($P \leq 0.0001$) were steeper than at baseline, and 7-month values also significantly greater than those at 3 months ($P < 0.05$). A three-way interaction between treatment, PIO status, and time was detected when comparing the T2D and PIO groups ($P < 0.05$). Post-hoc tests indicate that the PIO EXS group had a steeper slope to their LVC responses than the T2D EXS and the PIO CTL groups at baseline ($P < 0.05$), while the T2D EXS group had a significant increase in the slope of their LVC responses at 7 months compared to their baseline responses ($P < 0.05$). A significant interaction between PIO status and time was also detected ($P < 0.05$), with the slopes at 7 months in the T2D groups steeper than at baseline ($P < 0.001$) and at 3 months ($P < 0.05$). A main effect of time was also noted ($P < 0.05$), with 7-month values greater than at baseline.

Table 4.4. Slopes of the LVC responses during the incremental plantar-flexion test (mean ±sd). Slopes were plotted against the highest workload achieved expressed as 100%. Symbols represent differences between the ND and T2D groups, with letters used to indicate differences between the T2D and PIO groups. An effect of time was found between the ND and T2D groups, with * indicating that values at 3 and 7 months were greater than baseline (* P<0.05 *** P<0.0001). † indicates that 7-month values were greater than values at 3 months († P<0.05). *a* signifies that the T2D EXS and PIO CTL group values were lower than the PIO EXS group values at baseline (*a* P<0.05). *b* indicates that the T2D EXS group values at 7 months were greater than baseline (*b* P<0.05). *c* signifies a time effect, with values at 7 months greater than at baseline (*c* P<0.05).

	Slope (peak Wkld expressed as %)		
	Baseline	3 Months*	7 Months*** † *c*
ND CTL	5.02 ± 1.71	6.04 ± 4.27	7.23 ± 2.08
ND EXS	5.49 ± 4.78	7.12 ± 3.59	7.26 ± 5.99
T2D CTL	5.17 ± 2.84	4.92 ± 1.99	6.30 ± 2.36
T2D EXS	4.25 ± 2.10*a*	5.69 ± 1.66	6.53 ± 1.86*b*
PIO CTL	3.59 ± 1.60*a*	4.94 ± 1.02	4.64 ± 2.02
PIO EXS	6.64 ± 2.69	6.13 ± 1.77	6.15 ± 1.58

Significant differences in the peak LVC attained were found between groups, with mean data (± sd) presented in table 4.5. A significant time effect was detected between the ND and T2D groups (P≤0.0001), with peak LVC values at 7 months significantly greater than at baseline (P≤0.0001), and at 3 months (P<0.05). Between the T2D and PIO groups, a significant interaction between treatment, PIO status and time was found (P<0.05), with the PIO EXS group displaying greater peak LVC responses at baseline compared to the T2D EXS group (P<0.05) and the PIO CTL group (P<0.001). At 7 months, the T2D EXS group displayed a significant increase in peak LVC over baseline (P<0.05). While not attaining significance, the PIO CTL groups peak LVC responses tended to be lower at 7 months than both the T2D CTL group (P=0.067) and the PIO EXS group (P=0.0797). A significant interaction between PIO status and time (P<0.05) was found, with post-hoc tests revealing that the T2D groups showed greater peak LVC responses at 7 months compared to both baseline (P≤0.0001) and 3 months (P<0.05). A main effect for time was noted (P<0.05), with 7-month values greater than baseline values.

Table 4.5. Peak LVC responses recorded during the incremental plantar-flexion test (mean ±sd). Symbols represent differences between the ND and T2D groups, with letters used to indicate differences between the T2D and PIO groups. An effect of time was found between the ND and T2D groups, with * indicating that values at 7 months were greater than baseline (*** P<0.0001), while. † indicates that 7 month values were greater than values at 3 months († P<0.05). *a* signifies that the T2D EXS and PIO CTL groups were lower than the PIO EXS group at baseline (*a* P<0.05). *b* indicates that the T2D EXS group values at 7 months were greater than baseline (*b* P<0.05). *c* signifies a time effect, with values at 7 months greater than at baseline (*c* P<0.05).

	Peak LVC ($ml.min^{-1}.mmHg^{-1}$)		
	Baseline	3 Months	7 Months*** † *c*
ND CTL	5.83 ± 1.73	6.79 ± 4.06	7.87 ± 2.23
ND EXS	6.41 ± 4.46	7.34 ± 3.60	7.82 ± 6.49
T2D CTL	5.62 ± 2.69	5.22 ± 1.97	6.92 ± 2.59
T2D EXS	4.71 ± 1.70*a*	6.21 ± 1.83	6.83 ± 1.78*b*
PIO CTL	3.98 ± 1.33*a*	5.32 ± 0.86	4.93 ± 1.84
PIO EXS	7.25 ± 2.17	6.79 ± 1.54	7.14 ± 1.33

4.3.5 Leg Vascular Conductance Kinetic Responses to Plantar-Flexion at 30% MVC

Figure 4.5 presents both the LVC kinetic responses to constant-load plantar-flexion at 30% of MVC for representative individuals in each group, and the predicted LVC responses resulting from fitting the LVC responses using the quad-phasic model described in Chapter 2 section 2.2.3.4. τ_3 and End A values are presented in tables 4.6 and 4.7 respectively, with the rest of the parameter values from the individual model fits presented in appendix XXXII.

In comparison of the ND and T2D groups, a significant time effect was seen for the amplitudes of the second and third phases of the kinetic response, with values at 7 months exceeding those at 3 months and at baseline (P<0.05). For the amplitude of the third phase, individuals with T2D displayed a tendency for a smaller increase in LVC than ND individuals (P=0.096). In comparing the T2D and PIO groups, a similar time effect was found for the amplitude of third phase, with values at 7 months exceeding baseline (P<0.05). An effect of PIO treatment was also seen on the amplitude of the third phase, with the PIO-treated groups displaying a greater amplitude than the T2D groups (P<0.05).

The rate of adjustment of the kinetic response of the third phase, τ_3, was the primary outcome variable when assessing LVC kinetic responses. Mean data (±sd) are presented below in table 4.6. No significant differences were detected in either the comparison of the ND and T2D groups or the comparison between the T2D and PIO groups.

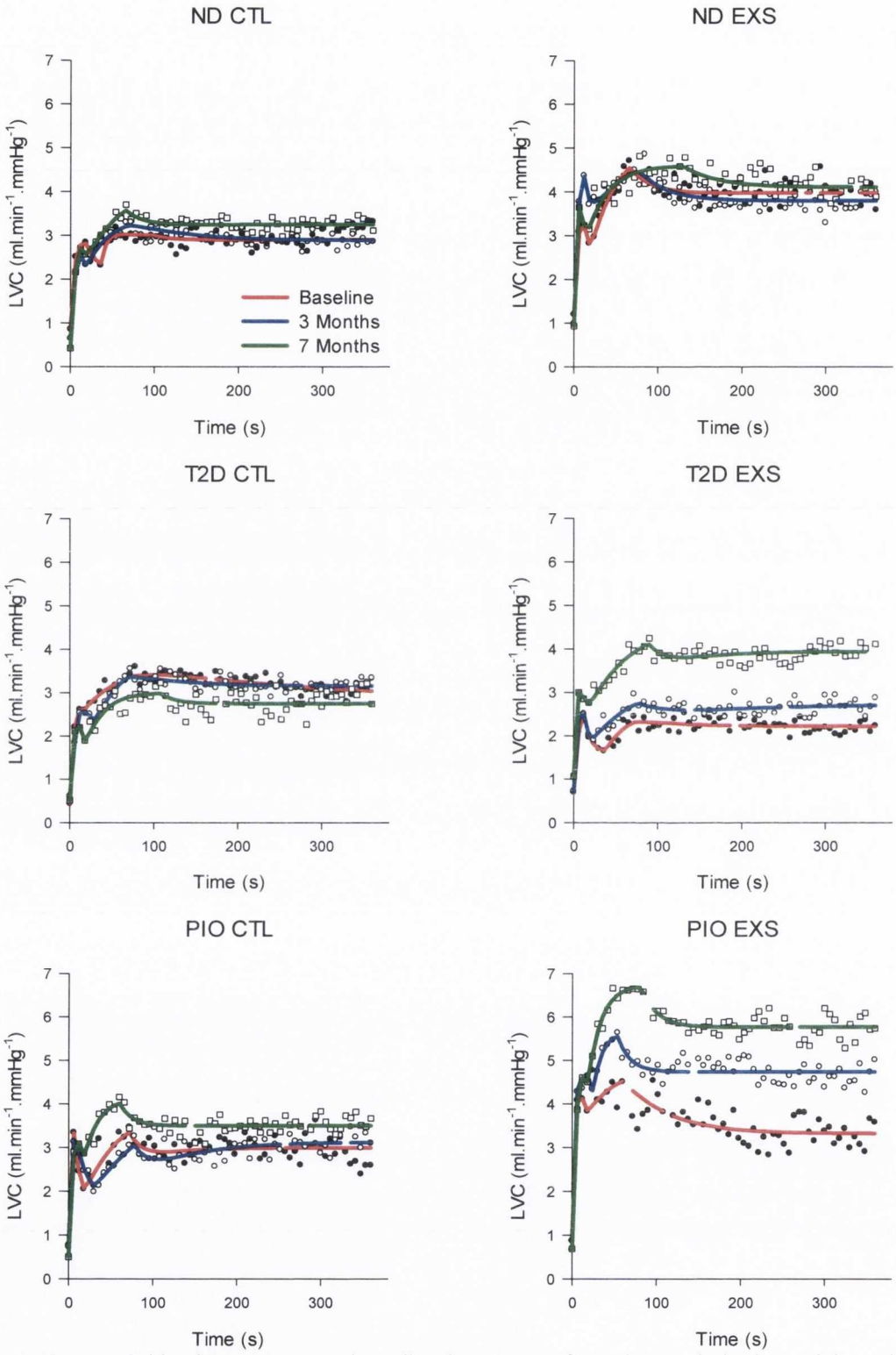


Figure 4.5. LVC kinetic responses and predicted responses from the quad-phasic model to plantar-flexion at 30% MVC for representative individuals. Breaks in the plot (e.g. PIO EXS) represent excluded data points that lay outside of the 95% prediction interval. See the text for significant interactions and differences between the groups.

Table 4.6. Group means (±sd) for the primary outcome variable τ_3 of the LVC kinetic response to constant-load plantar-flexion exercise at 30% MVC. No significant interactions or main effects were detected in comparison of the groups.

	τ_3 (s)		
	Baseline	3 Months	7 Months
ND CTL	26.3 ± 32.4	23.4 ± 22.6	27.2 ± 22.5
ND EXS	26.8 ± 15.0	30.4 ± 22.9	26.4 ± 13.1
T2D CTL	42.4 ± 29.3	32.5 ± 31.9	46.0 ± 33.5
T2D EXS	23.3 ± 16.2	44.2 ± 28.5	37.2 ± 30.1
PIO CTL	37.8 ± 25.7	30.2 ± 16.9	27.1 ± 9.8
PIO EXS	31.4 ± 21.1	34.6 ± 31.1	41.4 ± 19.2

Steady-state amplitude values (End A) during the constant-load plantar-flexion bout are presented in table 4.7 below. In comparing the ND and T2D groups, an interaction between treatment and time was discovered ($P<0.05$), with post-hoc analysis revealing that the EXS groups displayed greater End A values at 3 and 7 months than at baseline ($P<0.05$). Time was found to have an effect on responses, with 7-month values higher than baseline ($P<0.05$), with a trend for 3-month values to exceed baseline values ($P=0.063$). While not achieving statistical significance, individuals with T2D also tended to display lower End A values than ND subjects ($P=0.074$).

Comparison of End A values in the T2D and PIO groups revealed a significant interaction between treatment and time ($P<0.05$), with post-hoc tests revealing that the EXS group values at 3 and 7 months were greater than baseline values ($P<0.05$), while the EXS group values at 3 and 7 months also exceeded the CTL group values at the same point in time ($P\leq0.0001$). A significant interaction was also found between PIO status and treatment ($P<0.05$), with the PIO EXS group displaying greater amplitudes than both the T2D CTL and PIO CTL groups. Main effects were found for treatment, with the EXS groups displaying End A values greater than the CTL groups ($P<0.05$); and for time, with 3- and 7-month values exceeding baseline ($P<0.05$).

Table 4.7. End A values of the LVC responses recorded during the constant-load plantar-flexion bouts at 30% MVC (mean ±sd). Symbols represent differences between the ND and T2D groups, with letters used to indicate differences between the T2D and PIO groups. * indicates that End A values in the EXS groups were significantly greater at 3 and 7 months compared to baseline ($P<0.05$). † indicates a time effect, with 7 month values significantly greater than at baseline ($P<0.05$). *a* indicates that the EXS group values at 3 and 7 months were significantly greater than at baseline (*a* $P<0.05$; *aa* $P<0.001$). *b* indicates that the EXS group values at 3 and 7 months were significantly greater than the CTL groups at the same point in time ($P\leq0.0001$). *c* indicates that the PIO EXS group had greater End A values than the T2D CTL and PIO CTL groups ($P<0.05$). *d* represents a time effect, with 3 and 7 month values greater than at baseline. *e* represents a treatment effect ($P<0.05$), with EXS groups displaying a higher End A than CTL groups.

	End A ($ml.min^{-1}.mmHg^{-1}$)		
	Baseline	3 Months[d]	7 Months[† d]
ND CTL	3.51 ± 1.44	3.57 ± 1.32	3.78 ± 1.31
ND EXS	3.17 ± 1.91	3.71 ± 1.41[*]	3.99 ± 1.75[*]
T2D CTL	3.05 ± 0.93	2.84 ± 0.76	2.74 ± 0.66
T2D EXS[e]	2.59 ± 0.99	3.41 ± 1.16[* a bbb]	3.15 ± 0.93[* aa bbb]
PIO CTL	2.48 ± 0.70	2.71 ± 0.64	2.99 ± 1.01
PIO EXS[c e]	3.76 ± 1.12	4.23 ± 1.39[a bbb]	4.67 ± 1.08[aa bbb]

4.3.6 Reactive Hyperaemic Responses to Forearm Ischaemia

Representative reactive hyperaemic responses and predicted responses using the mono-phasic model (Chapter 2 section 2.2.5.7) to forearm ischaemia are presented in figure 4.6 below, with individual parameters listed in appendix XXXIII. No differences in resting FVC were found between any of the groups. Following release of the inflated cuff responsible for inducing ischaemia, peak FVC was assessed and compared between groups, with mean data (±sd) for peak FVC presented in table 4.8. In comparison of the ND and T2D groups, an interaction between diabetic status and time was found for peak FVC ($P<0.05$). Post-hoc analysis revealed that peak FVC responses in the T2D groups at 3 months were lower than the ND groups at 3 months ($P<0.05$), and also less than the T2D group responses at 7 months ($P<0.05$). Peak FVC responses for the T2D and PIO groups were also compared. A significant interaction between PIO status and treatment was detected ($P<0.05$), with the PIO EXS group displaying significantly greater peak FVC than the T2D CTL and PIO CTL groups ($P<0.05$), and a trend towards greater peak FVC than the T2D EXS group ($P=0.077$). A treatment effect was noted, with the EXS groups displaying greater peak FVC than the CTL groups ($P<0.05$); while a time effect ($P<0.001$) was also discovered, with peak FVC responses at 7 months greater than at 3 months, and tending to be greater than baseline peak values ($P=0.063$).

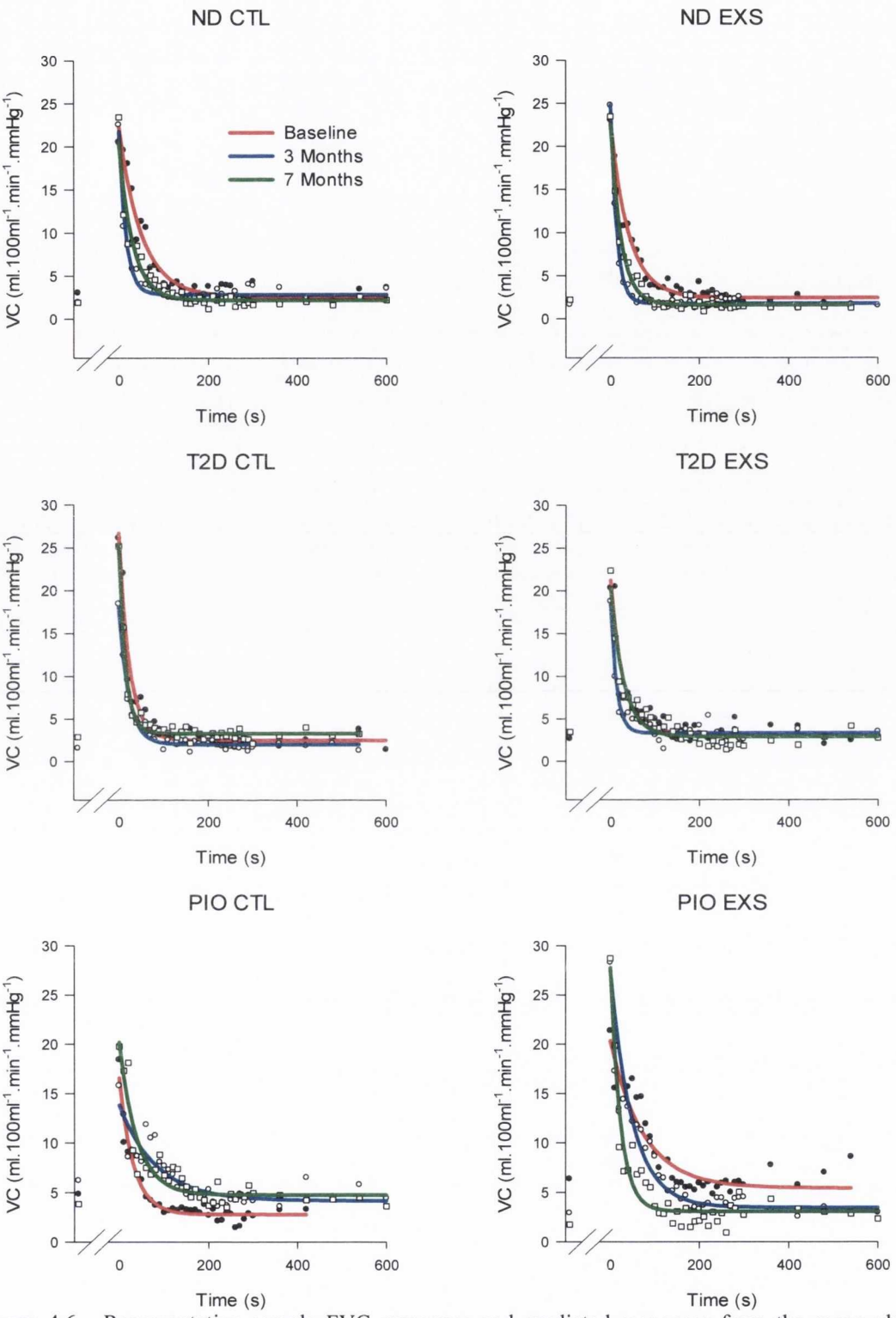


Figure 4.6. Representative sample FVC responses and predicted responses from the mono-phasic model from each group to five minutes of ischaemia. Resting FVC prior to ischaemia is presented to the left of the break in the x-axis, with fitted responses applied following release of ischaemia.

Table 4.8. Peak FVC responses recorded following release of forearm ischaemia (mean ±sd). Symbols represent differences between the ND and T2D groups, with letters used to indicate differences between the T2D and PIO groups. * indicates that values are significantly greater than the T2D group values at 3 months (* P<0.05). *a* indicates that the CTL group values are significantly lower than values in the PIO EXS group (*a* P<0.05). *b* represents a treatment effect, with the EXS group values greater than the CTL groups (*b* P<0.05). *c* indicates that values at 7 months are greater than values at 3 months (*cc* P<0.001).

	Peak FVC ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)		
	Baseline	3 Months	7 Months^{cc}
ND CTL	24.9 ± 4.3	26.9 ± 15.9*	25.6 ± 12.4
ND EXS	20.5 ± 5.8	22.2 ± 6.8*	21.0 ± 7.2
T2D CTL^{a}	21.6 ± 6.2	17.7 ± 4.6	25.0 ± 6.7*
T2D EXS^{b}	21.7 ± 4.0	20.2 ± 4.7	24.9 ± 5.3*
PIO CTL^{a}	19.3 ± 2.7	19.5 ± 4.5	20.8 ± 5.1
PIO EXS^{b}	26.7 ± 6.2	24.0 ± 7.8	31.0 ± 7.3

The fitted model determined the rate of decrease in FVC following release of ischaemia from peak FVC to steady-state FVC, termed the decay constant. Mean (±sd) values for the decay constant are presented in table 4.9 below. In comparing the ND and T2D groups, a significant interaction between diabetic status and time was detected (P<0.05). No significant post-hoc tests were detected; however, analysis of the results suggests that mean values for the decay constant are larger in the ND groups. A time effect was also noted (P<0.05), with values at 3 months greater than baseline (P<0.001).

With respect to the comparison of decay constant values between the T2D and PIO groups, no significant differences or interactions were detected. However, there was a strong trend towards lower decay constant values in the PIO groups, with the T2D groups displaying greater values (P=0.0597).

Table 4.9. Decay constant values determining the rate of decline in FVC following release of ischaemia from peak to steady-state (mean ±sd). A significant interaction between diabetic status and time was detected between the ND and T2D groups, but no significant post-hoc tests were noted; however, a time effect was noted, with 3-month values greater than at baseline (** P<0.001). No significant differences were detected between the T2D and PIO groups.

	Decay Constant		
	Baseline	3 Months**	7 Months
ND CTL	0.035 ± 0.023	0.047 ± 0.028	0.043 ± 0.009
ND EXS	0.044 ± 0.032	0.069 ± 0.034	0.054 ± 0.029
T2D CTL	0.040 ± 0.014	0.064 ± 0.036	0.060 ± 0.017
T2D EXS	0.039 ± 0.031	0.051 ± 0.025	0.037 ± 0.020
PIO CTL	0.043 ± 0.016	0.036 ± 0.017	0.035 ± 0.015
PIO EXS	0.028 ± 0.021	0.040 ± 0.021	0.037 ± 0.019

4.4 Discussion

The main finding from the analysis of LVC kinetics during steady-state plantar-flexion exercise at low intensities (30% MVC) was that the rate of adjustment of the third phase of the kinetic response (τ_3) did not differ between either the ND and T2D groups or the T2D and PIO groups at any stage of the training intervention. Neither the exercise intervention nor treatment with PIO had any significant effect on τ_3 values. This was unexpected given that previous research by Mac Ananey (2010) in this lab demonstrated that following a supervised training intervention, individuals with T2D demonstrated a speeding of LVC kinetics (faster τ values) to plantar-flexion at 70% MVC. Additionally, the gains made during the supervised intervention were maintained twelve weeks later, following an additional unsupervised intervention (Mac Ananey, 2010).

There are a number of considerations that could offer a suitable explanation for the differences in the findings between the two studies. Firstly, Mac Ananey (2010) used the workload that equated to 70% MVC at baseline in the subsequent assessments after the supervised and unsupervised interventions. Given that the subjects in the ND EXS and T2D EXS groups significantly increased their MVC following exercise training, if this study had utilised the same protocol as Mac Ananey (2010), then it is possible that a speeding of the LVC kinetic response may have occurred, as the relative workload would have been lower post-intervention. Evidence in support of this finding is the increase in the End A values of the LVC kinetic response in the EXS groups. Mac Ananey (2010) did not display any change in End A values post-intervention, likely a reflection of using the same absolute workload as at baseline.

Secondly, it may be a result of postural differences, with those exercising in the current study performed exercise in the supine position, compared to the upright position utilised in the other study (Mac Ananey, 2010; Macananey *et al.*, 2011). This has previously been shown to impact on LBF and LVC responses (Egana & Green, 2005) and may contribute to the lack of difference between the groups.

It is also possible that the difference in findings is a consequence of gender, as this study only examined responses in male subjects, whereas Mac Ananey (2010) employed a combined group of males and females. It has previously been shown that in older individuals, healthy males exhibit relatively preserved exercising LBF

responses, while women display reduced hyperaemic and vasodilatory responses (Parker *et al.*, 2008). This may suggest that even in the diabetic state, LBF and LVC kinetic responses are preserved in males, at least at low intensities, therefore explaining the lack of difference between τ_3 values at baseline, and the lack of change in τ_3 values post-intervention.

Finally, the two studies applied different models when fitting the data. Mac Ananey (2010) applied a bi-exponential model to their data, while data from the present study was fitted using a quad-exponential model, which accounted for two decay phases following each exponential increase in flow. Applying goodness-of-fit measures to the bi-exponential and quad-exponential fits of the current data revealed that 100 of the 156 total fits were best qualified by the quad-phasic model (appendix I), while comparisons of the adjusted R-squared (Adj R^2) responses revealed that the quad-exponential model provided significantly greater Adj R^2 values ($P<0.05$). This is in line with unpublished data that suggests that LBF and LVC kinetic responses at low-intensities are best categorised by the quad-exponential function used here (Reeder & Green, 2012). This again may hint that the different outcomes of this study and that of Mac Ananey (2010) are a consequence of the workload employed as the data from the present study revealed that the fourth phase (slow decay) was not present at 70% MVC (Chapter 2, section 2.3.5).

To this author's knowledge, in addition to the study by Mac Ananey (2010), only one previous study directly assessed the effect of a training intervention on the τ value of the femoral VC kinetic response to knee-extension exercise in ND individuals (Shoemaker *et al.*, 1996). This study demonstrated that in healthy young men, a training intervention as short as ten days was sufficient to result in significant speeding of the LVC response to onset of exercise. The fact that this study did not replicate these findings would therefore suggest that possibly either age or diabetic status has a significant impact upon LVC adaptations to exercise training. One other potential reason for the different findings between the two studies is the fact that the results of Shoemaker *et al.* (1996) are affected by the central component, as they demonstrated higher amplitudes of the adaptive and steady-state phases of the CO response following training, which may have contributed to the faster LVC kinetics (Parker *et al.*, 2008). In contrast, since this study was designed to remove the impact of the central component to isolate the EDV impact on peripheral LVC, it can be

concluded that the training-induced improvements in the HR kinetics (chapter 3 section 3.3.5) did not affect LVC kinetic measurements.

The amplitude of the steady-state LVC responses (End A) did show differences as a consequence of the interventions. In comparing the ND and T2D groups, the End A values in the EXS groups were greater at 3 and 7 months compared to baseline, while there was a trend towards lower End A values in the T2D groups. Similarly, the comparison between the T2D and PIO groups revealed the same effect of exercise on End A values at 3 and 7 months. LVC responses at 30% MVC of the current visit were assessed, and MVC values significantly increased at 3 and 7 months, meaning that workloads were higher during the later assessments compared to baseline, hence explaining the increased End A given that BF increases linearly as a consequence of power output (Saltin *et al.*, 1998). This would imply that the exercise intervention was successful in improving MVC and subsequently LVC kinetic responses during steady-state exercise, suggesting that vasodilatory capacity was increased as a consequence of exercise training, which is in line with expectations based on previous findings (Martin *et al.*, 1990; Shoemaker *et al.*, 1996; Maiorana *et al.*, 2001a; Spier *et al.*, 2004).

In comparing the ND and T2D groups' time to failure (TTF) during the incremental plantar-flexion test, the EXS groups showed significant improvements at 3 and 7 months compared to baseline, indicating that the EXS intervention was successful in improving exercise performance. The comparison between the T2D and PIO groups demonstrated significant increases in TTF at 3 and 7 months compared to baseline, but the improvement was not specific to either treatment, but rather a main effect. One interesting finding was the comparison of subject's peak force achieved during the incremental test expressed as a percentage of their MVC. Comparison of the ND and T2D groups revealed that the T2D groups achieved a lower percentage of their MVC during the test. As discussed in chapter 2, this may reflect fibre type differences in the muscle composition of individuals with T2D. It has been demonstrated that submaximal exercise in diabetic rats resulted in a significantly greater distribution of BF to the glycolytic fibres, suggesting that individuals with T2D have greater reliance on glycolytic muscle fibres during exercise, and therefore have faster rates of fatigue (Copp *et al.*, 2010). It might have been expected that the training intervention would therefore have resulted in conversion of some glycolytic fibres to more oxidative fibres, thus allowing for subjects to attain a greater

percentage of their MVC during the incremental test (Salmons & Vrbova, 1969). However, it is also possible that the level of stimulation provided by the training intervention was not sufficient enough to result in change in the muscle isoform expression, despite the improvements in TTF (Rutherford & Jones, 1988). This would explain the lack of difference in the percentage of MVC achieved during the incremental test at 3 and 7 months in the exercising groups. It is not surprising that there was no effect of PIO treatment on percentage MVC achieved, as despite PIO's known effects on endothelial function (Harashima *et al.*, 2009; Mazzone *et al.*, 2006; Maegawa *et al.*, 2007; Tsuchiya *et al.*, 2009; Vijay *et al.*, 2009; Nerla *et al.*, 2009; Pitocco *et al.*, 2008), to this author's knowledge it has not been shown that PIO has any specific effects on muscle fibre type or expression.

Previous research has shown that exercise training can be expected to elicit significant increases in LBF and LVC in an ageing cohort (Martin *et al.*, 1990; Vaitkevicius *et al.*, 1993; Tanaka *et al.*, 1998; Beere *et al.*, 1999). To this author's knowledge there have been no studies directly measuring the effect of a training intervention upon peak LVC in a diabetic population. However, Maiorana *et al.* (2001a) did demonstrate that training resulted in improved vascular function in response to infused dilators in a T2D group. As a consequence, it was surprising that no specific treatment effect of exercise upon peak LVC findings was detected. However, there was a significant main effect of time in comparison of the ND and T2D groups, with 7-month values greater than baseline. It is possible that higher peak LVC values at 7 months in the ND CTL and T2D CTL groups have masked the beneficial effect of training; although the reason for the higher values in the CTL groups is more difficult to explain. Given the linear nature of LBF and LVC in response to power output (Saltin *et al.*, 1998) it appears that the higher peak LVC values are due to improved performance in the incremental test; despite the CTL groups not increasing TTF, they did demonstrate an increase in peak force values at 7 month compared to baseline.

Comparison of the T2D and PIO groups highlighted the positive effect of the exercise intervention in the T2D EXS group, with peak LVC values at 7 months greater than at baseline. What was surprising was that the positive effect of PIO on endothelial function (Harashima *et al.*, 2009; Mazzone *et al.*, 2006; Maegawa *et al.*, 2007; Tsuchiya *et al.*, 2009; Vijay *et al.*, 2009; Nerla *et al.*, 2009; Pitocco *et al.*, 2008) did not result in improvements in the peak LVC response of the PIO-treated groups.

This was all the more surprising due to the fact that a time effect was found for TTF and peak force achieved during the incremental test, with 7-month values greater than at baseline when comparing the T2D and PIO groups. Therefore, it may have been expected that these groups would have demonstrated an increase in peak LVC (Saltin *et al.*, 1998), but this was not found to be the case.

LVC responses were assessed at each workload during the incremental protocol. No differences were detected at any workload when comparing the ND and T2D groups. One possible reason for this may be due to increasing levels of motor unit recruitment as subjects' progress to higher workloads during the incremental protocol. It has previously been demonstrated that the impairments in EDV associated with ageing were muscle specific, with EDV-related impairments related to the slow-twitch soleus feed arteries only, with no differences in gastrocnemius feed arteries detected (Woodman *et al.*, 2002). While the present study did not examine fibre-type BF differences, if T2D-related EDV impairments affected BF in a similar manner to ageing-related EDV impairments, then it is possible that a greater reliance on glycolytic fibres to which BF is not impaired would explain the lack of significant difference in LBF or LVC at the higher workloads during the incremental test (Henneman *et al.*, 1974), while also possibly explaining the trends towards lower VC in the T2D groups at rest and the initial workload (100N).

Despite not severely impacting on peak LVC values, it appears that PIO treatment in combination with exercise training resulted in significant increases in LVC at submaximal workloads, as the PIO EXS group showed greater LVC responses at 300N and 500N. Again, if T2D-related EDV impairments impacts upon LVC in the same manner as ageing-related impairments, then PIO-induced improvements in EDV could be expected to significantly improve LVC at the lower workloads in which slow-twitch fibres are the most prevalent recruited fibres (Henneman *et al.*, 1974; Woodman *et al.*, 2002), while having diminishing returns as the proportion of glycolytic fibres being recruited increases at higher workloads. The results of comparison of the slopes of the LVC responses (plotted against workloads expressed as %) would support these contentions, both with respect to the ND vs. T2D and the T2D vs. PIO group comparison; with time effects noted at 3 and 7 months for an increase in the steepness of the slope response in the ND and T2D group comparison, with the T2D and PIO group comparison yielding a similar result at 7 months only. This may suggest that the rate of increase in LVC responses

quickened as a result of the interventions. However, this conclusion should be interpreted with caution, as significance was due to a time effect, not a treatment effect.

Analysis of the results from the RH protocol did not reveal too many differences between groups. Peak FVC responses following the release of ischaemia were lower in the T2D CTL group at 3 months compared to the ND groups, but otherwise no differences in peak values were detected between the ND and T2D groups. It may have been expected that greater levels of impairment in peak FVC values would have been evident in the T2D groups given the results of previous studies following infusion of known EDV and EIDV vasodilators (Williams *et al.*, 1996; McVeigh *et al.*, 1992; Makimattila *et al.*, 1999). However, in a study using ischaemia as the method of determining RH, Francesconi *et al.* (1999) did not find any significant differences between individuals with T2D and controls after five minutes of ischaemia. Rather, they found that RH was influenced by plasma glucose levels, a finding replicated by Sarabi *et al.* (1999), who found that in males, FPG levels and WHR were the only significant predictors of EDV. While significant differences were detected between the ND and T2D groups for FBG levels and WHR, the good level of glycaemic control displayed by the subjects with T2D in this study likely contributed to the absence of differences in RH responses between the groups.

In comparing the peak FVC responses between T2D and PIO groups, the PIO EXS group had greater peak FVC values than both control groups, and trended towards greater values than the T2D EXS groups. The fact that the PIO CTL group did not show any improvement in peak FVC values would suggest that the greater values in the PIO EXS group were a consequence of the exercise intervention, which is supported by the presence of a treatment effect, and in agreement with previous findings (Olive *et al.*, 2002; Yki-Jarvinen, 2003). The positive effect of the exercise intervention on peak FVC should be interpreted with caution, as the increase in peak FVC values evident in both T2D EXS and PIO EXS groups was not evident until 7 months, with peak FVC values following the supervised phase not different from baseline.

Comparison of the decay constant (measuring the rate of decline from peak FVC to steady-state FVC post-ischaemia) revealed no significant differences between groups, although there were trends towards lower values (longer recoveries) in the T2D groups compared to the ND groups. This would suggest that EDV is

significantly intact in the T2D groups to allow sufficient O_2 delivery to the ischaemic tissues following cuff release. It could be expected that individuals with T2D would display significantly longer recovery time of FVC values post-ischaemia based on findings of T2D-associated impairments in EDV (Williams *et al.*, 1996; McVeigh *et al.*, 1992; Makimattila *et al.*, 1999). However, it has previously been demonstrated that impairments in capillary BF and capillary recruitment were only present in individuals with T2D that also presented with microvascular complications (Womack *et al.*, 2009). Given that the subjects with T2D were screened for presence of these complications, and also demonstrated excellent control of their HbA_{1c} levels, it is possible that previous findings of impairments in EDV in individuals with T2D are not due to the presence of T2D but rather the associated microvascular complications that are often attendant with T2D.

Analysis of the results of the incremental plantar-flexion test and reactive hyperaemia protocols should be interpreted with caution. The increased VC responses in the PIO EXS group irrespective of test modality could also be due to the effects of pioglitazone on fluid retention, causing expansion of the plasma volume, hence causing artificial increases in VC responses over time. Additionally, given the low *n* numbers in the PIO-treated groups, generalisation to a wider population is difficult; in particular with regard to the RH results. Given the disparity in peak FVC and decay constant in the groups during the RH protocol over time irrespective of the treatment arm, it is possible that the results from the RH protocol are subjected to lower levels of repeatability and greater intraday variation than those results gained from assessment of LVC responses, which appear to be much more consistent over time.

The lack of significant differences in the rate of adjustment of steady-state LVC responses, peak LVC responses during the incremental plantar-flexion test, and peak FVC following ischaemic RH suggests that VC responses are reasonably preserved in males with T2D. However, the baseline trends towards slower τ_3 values at 70% MVC (Chapter 2 section 2.3.5) and incremental test results at baseline (Chapter 2 section 2.3.2) would hint at possible impairments at higher intensity workloads. These findings would support the contention that this cohort of males with T2D has reasonably healthy endothelial function compared with participants in previous studies (Mac Ananey, 2010), with exercise typically only improving endothelial function in individuals with a definite impairment in endothelial function

(Sonne *et al.*, 2007). The modest improvements in LVC responses (peak LVC during the incremental plantar-flexion test, End A values during the constant-load bouts following the training intervention suggest the training induced improvements in peripheral vasodilation, which offer a potential mechanism for the improvements in cycling exercise evident in chapter 3.

CHAPTER 5: THE EFFECTS OF EXERCISE AND PIOGLITAZONE ON ENDOTHELIAL AND INFLAMMATORY MARKERS

5.1 INTRODUCTION

Type 2 diabetes (T2D) is associated with increased levels of inflammation and endothelial dysfunction in the vasculature (el-Mesallamy *et al.*, 2007; Alghasham & Barakat, 2008; Hovens *et al.*, 2008; Lozano-Nuevo *et al.*, 2011). The pro-inflammatory state is associated with changes in circulating levels of certain cytokines that impact directly and indirectly on endothelial function. Among the cytokines that are elevated in the event of an inflammatory response and in the diabetic state are interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). IL-6 is typically secreted by T-cells and macrophages to stimulate an immune response, and is evident in the circulation earlier than other cytokines. However, T-cells and macrophages are not the only source of IL-6, with smooth muscle cells within blood vessels and adipocytes also known to produce IL-6. IL-6 is also known to have anti-inflammatory properties, displaying inhibitory effects upon TNF-α and activation of interleukin-10 (IL-10). IL-10 is an anti-inflammatory cytokine that is known to inhibit synthesis of pro-inflammatory cytokines such as TNF-α, and has been shown to display reduced levels in the circulation of individuals with T2D (van Exel *et al.*, 2002; Bluher *et al.*, 2005).

TNF-α is also produced in a number of different locations, including endothelial cells and adipose tissue. Increased levels of IL-6 and TNF-α produced by adipocytes are known to stimulate production of C-reactive protein (CRP), a positive acute-phase protein produced in the liver and found in plasma. As a consequence of its association with adipocyte-derived IL-6 and TNF-α, CRP is typically elevated in individuals that are obese and/or have T2D (Alghasham & Barakat, 2008; Bluher *et al.*, 2005; el-Mesallamy *et al.*, 2007).

Similar to CRP, the apolipoprotein serum amyloid A (SAA) is produced in the liver in response to IL-6 and TNF-α, and is a positive acute-phase protein. Recent evidence suggests that SAA is also produced by adipocytes, and that serum concentrations in the blood are related to BMI levels. It is thought that pro-inflammatory increases in SAA levels are of a greater magnitude than increases in

CRP. Circulating levels of SAA have also previously been shown to be elevated in individuals with T2D (Kumon *et al.*, 1994; Hatanaka *et al.*, 2007).

The role of adipose tissue as a source of cytokines has come under increasing levels of scrutiny in the treatment of T2D, given the association of obesity and in particular visceral fat with T2D. Of the many cytokines produced by adipocytes, two that are thought to play a significant role in the progression of T2D are visfatin and resistin. Visfatin is enriched in the visceral fat, and plasma levels of visfatin have been shown to be elevated in individuals with T2D (Alghasham & Barakat, 2008; Zhu *et al.*, 2008; Kang *et al.*, 2010; Esteghamati *et al.*, 2011). It is possible however, that this association is a consequence of body mass, as Chen *et al.* (2006) demonstrated that the independent association between visfatin levels and T2D was lost after adjusting for BMI and WHR. Furthermore, research has still to determine the physiological relevance of visfatin, as plasma concentrations are as much as 100-fold lower than those of insulin, despite similar receptor-binding affinity and its insulin-mimicking actions (Arner, 2006).

Resistin is another adipocytokine whose role in the development of obesity and T2D is not yet fully understood. A number of studies have shown increasing levels of resistin with increasing levels of obesity (Fujinami *et al.*, 2004; Hasegawa *et al.*, 2005; Hui-Bing *et al.*, 2006; Gharibeh *et al.*, 2010); however, others have shown contradictory findings (Pfutzner *et al.*, 2003; Chen *et al.*, 2006). Resistin has been shown to initiate signalling events causing increased expression of pro-inflammatory cytokines including IL-6 and TNF-α (Stofkova, 2010).

T2D is associated with the pro-inflammatory state, while elevated insulin levels are known to induce endothelial dysfunction leading to development of conditions such as atherosclerosis and peripheral arterial disease (PAD), and in severe cases causing diabetic ulcers and neuropathy. Markers in the blood that indicate endothelial dysfunction include inter-cellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). ICAM-1 is expressed by endothelial cells in the pro-inflammatory state, and allows migration of leucocytes across the vascular endothelium. VCAM-1 is expressed by endothelial cells in response to stimulation by cytokines including TNF-α. Individuals with T2D display elevated levels of ICAM-1 (el-Mesallamy *et al.*, 2007; Bruno *et al.*, 2008) and VCAM-1 (Matsumoto *et al.*, 2002; Urso *et al.*, 2010), although it appears that circulating levels

of both ICAM-1 and VCAM-1 are related to the degree of insulin resistance associated with T2D (El Amine *et al.*, 2010) or presence of associated comorbidities (Bruno *et al.*, 2008; Hellemons *et al.*, 2011). Furthermore, resistin has been shown to upregulate VCAM-1 (Lozano-Nuevo *et al.*, 2011; Hui-Bing *et al.*, 2006), while ICAM-1 has a positive association with CRP levels (el-Mesallamy *et al.*, 2007).

One of the main areas of interest in this study was the effect that these interventions would have on these markers. Previous research has shown that a home-based exercise intervention had no impact on circulating levels of CRP, ICAM-1 or VCAM-1 in individuals with T2D (Scheede-Bergdahl *et al.*, 2009), while aerobic exercise has been shown to reduce levels of visfatin in young individuals with T2D (Brema *et al.*, 2008), while intensive glycaemic control was also seen to reduce visfatin levels (Zhu *et al.*, 2008). Exercise is also known to result in a significant increase in circulating levels of IL-6 produced by the contracting muscle (Febbraio & Pedersen, 2005), with this 'myokine'-derived IL-6 exerting anti-inflammatory effects via suppression of TNF-α. This would suggest that the exercise intervention should result in further reductions of the acute-phase reactants CRP and SAA, with possible further effects on ICAM-1 and VCAM-1, as well as increases in levels of IL-10 (Petersen & Pedersen, 2005).

Furthermore, treatment of T2D with PIO has been shown to reduce CRP in most (Heliovaara *et al.*, 2007; Maegawa *et al.*, 2007; Nerla *et al.*, 2010; Park *et al.*, 2011), but not all studies (Pitocco *et al.*, 2009). Despite the reductions in CRP and associated improvements in endothelial function (Maegawa *et al.*, 2007), treatment with PIO does not seem to reduce levels of IL-6, TNF-α or resistin (Martens *et al.*, 2006; Shadid *et al.*, 2006), although this too is not conclusive (Vijay *et al.*, 2009). Furthermore, visfatin levels appear to be unaffected by PIO in a combined group of men and women with T2D (Hammarstedt *et al.*, 2006; Takebayashi *et al.*, 2007). However, the effect may be one of gender, as Takebayashi *et al.* (2007) found a significant elevation in visfatin levels due to PIO when measuring a subset of female samples.

Therefore the aims of this study were to determine if type 2 diabetes in middle-aged males resulted in any significant differences in circulating plasma levels of IL-6, IL-10, TNF-α, CRP, SAA, ICAM-1 and VCAM-1. Additionally, these markers were assessed following the exercise and PIO interventions (described in

chapters 3 and 4), with the objective of determining if these interventions significantly affected expression of these markers. Furthermore, mRNA expression levels of IL-6, TNF-α, visfatin and resistin from whole blood were analysed to determine if T2D had a significant impact upon expression of these markers at baseline, and following the exercise and T2D interventions.

5.2 METHODS

5.2.1 Subjects

A total of fifty-eight males were recruited to participate in the study, thirty-nine of whom have T2D and nineteen healthy age-matched individuals. All subjects were classified as sedentary (<1hr.wk^{-1} of moderate intensity exercise) for the previous six months, as determined by use of RT3 accelerometers (Stay Healthy Inc, Monrovia, California) (Rowlands *et al.*, 2004; Perry *et al.*, 2010) and completion of the LOPAR questionnaire (Regensteiner *et al.*, 1996).

All subjects provided written consent (appendix II) before beginning participation in the study. The study was conducted in accordance to the principles outlined by the Declaration of Helsinki, and was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College, Dublin.

5.2.1.1 Subject recruitment

See chapter 3 section 3.2.1.1.

5.2.1.2 Inclusion/Exclusion Criteria

See chapter 2 section 2.2.1.2.

5.2.1.3 Participant Information Form

See Chapter 2 section 2.2.1.3.

5.2.1.4 Blood Sample Collection

Venous blood samples were drawn from the antecubital vein in arm into 6ml lithium heparin vacutainers (BD, Oxford, UK) for the determination of plasma levels of IL-6, IL-10, TNF-α, CRP, SAA, ICAM-1, and VCAM-1. The vacutainers were centrifuged at 5,000rpm for 10min at room temperature and the plasma extracted and

stored at -80°C for later use. In addition venous blood samples were collected in 2.5ml PAXgene Blood RNA tubes (PreAnalytix, Qiagen, UK) for the determination of mRNA levels of IL-6, TNF-α, visfatin, and resistin. The blood RNA tubes were stored at -80°C until RNA extraction was performed.

5.2.2 Experimental Design

5.2.2.1 Study Overview

Subjects were required to provide fasting blood samples at baseline, and after 3 and 7 months respectively for assessment of blood samples. Non-diabetic subjects had their samples collected at the cardiovascular laboratory in the Department of Physiology, Trinity College, Dublin. Individuals with T2D provided their samples at the Diabetes Day Care Centre where they received treatment for their T2D (either St. Columcille's Hospital, Loughlinstown; or St. Vincent's Hospital, Dublin). During the periods between blood sample collections, subjects underwent the interventions previously described in Chapter 3 section 3.2.2.1. Plasma samples collected for determination of levels of IL-6, IL-10 and TNF-α were analysed using enzyme-linked immunosorbent assay (ELISA). Plasma samples collected for measures of CRP, SAA, ICAM-1 and VCAM-1 were analysed using MesoScale Discovery® (MSD) custom-designed vascular injury panel. Finally whole blood samples collected in PAXgene Blood RNA tubes were used to determine mRNA levels of IL-6, TNF-α, visfatin, and resistin.

5.2.2.2 Anthropometry

5.2.2.2.1 Mass, Height, Body Mass Index

See Chapter 2 section 2.2.2.2.1.

5.2.2.2.2 Waist:Hip Ratio

See Chapter 2 section 2.2.2.2.3.

5.2.2.2.3 Ankle:Brachial Index

See Chapter 2 section 2.2.2.2.4.

5.2.2.2.4 Medication Usage among Participants

As described in Chapter 2 section 2.2.1.2, subjects with T2D were admitted if they were using an oral medication for the treatment of T2D that was not a member of the TZD family. As is standard practice with the treatment of T2D, prescription of medication often targets secondary co-morbidities associated with T2D. Subjects were therefore also admitted to the study if they displayed controlled hypertension. In a number of cases, this meant that subjects were in receipt of anti-hypertensive medication. Additionally, a number of subjects were in receipt of cholesterol-lowering medications and non-steroidal anti-inflammatory drugs (Nu-Seal Aspirin). Table 5.1 below describes the medication use within each group.

Table 5.1: Medication usage within each participating group. NSAID = Non-steroidal anti-inflammatory drugs; PPI = Proton pump inhibitor; CCB = calcium channel blocker; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CAI = Cholesterol absorption inhibitor; XOI = Xanthine Oxidase inhibitor; AAB = alpha-adrenergic receptor blocker; IM = Incretin mimetic.

	Medication			
	T2D	Blood Pressure	Cholesterol	Other
ND CTL (n=9)		ß-blocker (n=1)	Statin (n=1)	NSAID (n=1) PPI (n=1)
ND EXS (n=10)			Statin (n=2) CCB (n=1)	NSAID (n=3) PPI (n=1)
T2D CTL (n=11)	Metformin (n=8) Sulphonylurea (n=2)	ß-blocker (n=2) ACEI (n=3) ARB (n=2)	CAI (n=1) Statins (n=5)	NSAIDs (n=7) PPI (n=1) XOI (n=1)
T2D EXS (n=11)	Metformin (n=7) Sulphonylurea (n=4)	ARB (n=2) ACEI (n=4) ß-blocker (n=2)	Statins (n=7) CCB (n=1) CAI (n=1)	NSAIDs (n=8) PPI (n=1) XOI (n=1)
PIO CTL (n=6)	Metformin (n=5) IM (n=1)	ACEI (n=4) ARB (n=1) ß-blocker (n=1)	Statins (n=5)	NSAIDs (n=5) XOI (n=1)
PIO EXS (n=7)	Metformin (n=4) Sulphonylurea (n=1)	ACEI (n=1) ARB (n=2) ß-blocker (n=1) CCB (n=1)	Statins (n=4)	NSAIDs (n=4)

5.2.3 Enzyme-linked Immunosorbent Assay

5.2.3.1 Materials

5.2.3.1.1 ELISA kits

Human IL-6 ELISA kit (BioLegend, Cambridge, UK);
Human IL-10 ELISA kit (BioLegend, Cambridge, UK);
Human TNF-α ELISA kit (BioLegend, Cambridge, UK).

5.2.3.1.2 General laboratory materials & chemicals

Potassium Chloride (KCl) (Sigma, Wicklow, Ireland);
Potassium Phosphate (KH_2PO_4) (Sigma, Wicklow, Ireland);
Sodium Chloride (NaCl) (Sigma, Wicklow, Ireland);
Sodium phosphate, dibasic (Na_2HPO_4) (Sigma, Wicklow, Ireland);
2N Sulphuric Acid Solution (2N H_2SO_4) (Sigma, Wicklow, Ireland);
Tween®-20 (Sigma, Wicklow, Ireland).

5.2.3.1.3 General laboratory products

Biosphere filter pipette tips (Sarstedt, Nümbrecht, Germany);
Falcon tubes (15ml, 50ml) (Sarstedt, Nümbrecht, Germany);
Microcentrifuge tube (1.5ml) (Sarstedt, Nümbrecht, Germany);
Optical adhesive covers (Applied Biosystems, Warrington, UK);
96-well optical reaction plates (Applied Biosystems, Warrington, UK);
Pipette tips (Sarstedt, Nümbrecht, Germany).

5.2.3.2 Analysis of IL-6, IL-10, and TNF-α

All ELISA measurements were carried out by the principal investigator, with samples measured singularly. The day prior to analysis, a 96-well plate (BioLegend, Cambridge, UK) was coated with kit-specific capture antibody (1:200 dilution with coating buffer (5x coating buffer diluted to 1x with deionised water) and incubated overnight at 4°C. Additionally, phosphate-buffered saline (PBS) solution was prepared by adding 8.01g NaCl, 1.15g Na_2HPO_4, 0.204g KH_2PO_4, and 0.201g KCL per litre of deionised water.

The day of the analysis, the incubated plate was washed with wash buffer (PBS; 0.05% Tween-20 in PBS), and the residual buffer was blotted by tapping the plate upside down on absorbent paper. The plate was blocked with a reagent diluent (5x assay diluent diluted to 1x with PBS; 200µl.well^{-1}), sealed and incubated for 1hr at room temperature with shaking at 200rpm. After washing, kit-specific standards (appropriately diluted in reagent diluent) and samples were added (50µl.well^{-1}). The plate was sealed and allowed to incubate at room temperature for 2hrs with shaking at 200rpm. After washing the plate, biotinylated detection antibody (1:200 dilution in reagent diluent; 50µl.well^{-1}) was added and the plate sealed and incubated at room temperature for 1hr with shaking at 200rpm. The plate was again washed and reacted with Avidin-Horseradish Peroxidase (1:1000 dilution in reagent diluent; 50µl.well^{-1}) for 30min at room temperature with shaking at 200rpm. After washing, Tetramethylebenzidine substrate solution (1:1 mixture of colour reagent A and colour reagent B) was added (50µl.well^{-1}) and the plate incubated in the dark for a minimum of 15min or until such time as sufficient colour development had taken place. The reaction was stopped using 1M H_2SO_4 (50µl.well^{-1}). The optical density (OD) was measured at 450nm using a 96-well plate reader (EL_X800 Universal Microplate Reader, Biotek). A standard curve was generated and the regression equation of the curve was used to determine the concentration of IL-6, IL-10, and TNF-α in each sample. Values are expressed as pg.ml^{-1}.

5.2.4 HUMAN MSD® 96-WELL MULTI-SPOT® VASCULAR INJURY PANEL II ASSAY

5.2.4.1 Materials

5.2.4.1.1 MSD® Vascular Injury Panel Kit

Read Buffer T (4x), with surfactant;
Blocker A Kit;
MULTI-SPOT 96-well 4-Spot Vascular II Plates;
SULFO-TAG™ Anti-Human Vascular Injury II Detection Antibody Blend (50x);
Diluent 15;
Human Vascular Injury II Calibrator Blend.

5.2.4.1.2 General laboratory materials & chemicals

Potassium Chloride (KCl) (Sigma, Wicklow, Ireland);
Potassium Phosphate (KH_2PO_4) (Sigma, Wicklow, Ireland);
Sodium Chloride (NaCl) (Sigma, Wicklow, Ireland);
Sodium phosphate, dibasic (Na_2HPO_4) (Sigma, Wicklow, Ireland);
Tween®-20 (Sigma, Wicklow, Ireland).

5.2.4.1.3 General laboratory products

Biosphere filter pipette tips (Sarstedt, Nümbrecht, Germany);
Falcon tubes (15ml, 50ml) (Sarstedt, Nümbrecht, Germany);
Microcentrifuge tube (2ml) (Sarstedt, Nümbrecht, Germany);
Optical adhesive covers (Applied Biosystems, Warrington, UK);
Pipette tips (Sarstedt, Nümbrecht, Germany).

5.2.4.2 Analysis of Vascular Injury Markers (CRP, SAA, ICAM-1, VCAM-1)

All measurements were carried out by the principal investigator, with samples measured singularly. A MULTI-SPOT 96-well 4-spot vascular plate was coated with 5% blocking solution (150µl.well^{-1}) and incubated at room temperature for 1hr with shaking. The plate was washed with wash buffer (PBS; 0.05% Tween-20 in PBS) and the residual buffer was blotted by tapping the plate upside down on absorbent paper. The plate was blocked with diluent 15 (40µl.well^{-1}). Following this, standards (appropriately diluted in diluent 15) and samples (1:200 dilution with 1% blocker A solution (5% blocker A solution diluted to 1% with PBS)) were added (10µl.well^{-1}). The plate was sealed and allowed to incubate at room temperature for 2hrs with shaking. After washing the plate, SULFO-TAG detection antibody blend (1:50 dilution in diluent 15; 25µl.well^{-1}) was added and the plate sealed and incubated at room temperature for 1hr with shaking. The plate was again washed and reacted with 1x Read Buffer T (4x Read Buffer T diluted to 1x with distilled water; 150µl.well^{-1}). The plates were read immediately using a Sector Imager 2400 (MSD, USA) and analysed using Discovery Workbench 3.0 software (MSD, USA). Values are expressed as ng.ml^{-1}.

5.2.5 Polymerase Chain Reaction (PCR) Analysis

5.2.5.1 Materials

5.2.5.1.1 mRNA Extraction Kit

PAXgene™ Blood RNA Kit v2 (PreAnalytiX, Switzerland).

5.2.5.1.2 General laboratory products

Biosphere filter pipette tips (Sarstedt, Nümbrecht, Germany);
Falcon tubes (15ml, 50 ml) (Sarstedt, Nümbrecht, Germany);
Microcentrifuge tube (1.5ml) (Sarstedt, Nümbrecht, Germany);
Optical adhesive covers (Applied Biosystems, Warrington, UK);
96-well optical reaction plates (Applied Biosystems, Warrington, UK);
PCR tubes (Sarstedt, Nümbrecht, Germany);
Pipette tips (Sarstedt, Nümbrecht, Germany).

5.2.5.1.3 Molecular reagents

Absolute ethanol (Sigma, Wicklow, Ireland);
High capacity cDNA reverse transcription kit (Applied Biosystems, Warrington, UK);
Taqman gene expression assays (Applied Biosystems, Warrington, UK);
Taqman universal PCR master mix (Applied Biosystems, Warrington, UK).

5.2.5.2 Total RNA Extraction

All RNA extractions and PCR analysis were carried out by the principal investigator. Initially, blood samples were incubated in the RNA tube at room temperature for 2 hours to achieve complete lysis of blood cells and then centrifuged for 10 minutes at 3000-5000 x *g*. Following decanting of the supernatant blood RNA tube, RNase-free water (4ml) was added to the pellet and the tube was closed using a new BD Hemogard closure. The RNA tube was vortexed until the pellet was visibly dissolved and then centrifuged for 10 minutes at 3000-5000 x *g*. Following this the supernatant was completely removed. Resuspension buffer (350µl) was added to the RNA tube and the tube was vortexed until the pellet was visibly dissolved. The sample was pipetted into a 1.5ml microcentrifuge tube, into which binding buffer

(300µl) and proteinase K (40µl) were added. The microcentrifuge tube was vortexed for 5s and incubated for 10min at 55°C in a shaker-incubator at 400rpm. The lysate from the microcentrifuge tube was pipetted into a shredder spin column placed in a 2ml processing tube and then centrifuged for 3min at a speed of 13000 x *g*. The supernatant of the flow-through fraction was pipetted to a new 1.5ml microcentrifuge tube without disturbing the pellet in the processing tube, and ethanol (350µl) was added to the sample, which was then vortexed. 700µl of the sample was pipetted into an RNA spin column placed in a 2ml processing tube and centrifuged for 1min at 13000 x *g*. The spin column was placed in a new 2ml processing tube and the remaining sample was pipetted into the RNA spin column and centrifuged for 1min at 13000 x *g*. The spin column was placed in a new 2ml processing tube into which 350µl of wash buffer 1 was added, and the RNA spin column centrifuged for 1min at 13000 x *g*. The spin column was again placed in a new 2ml processing tube. DNase reaction mixture (80µl; 1:7 dilution of reconstituted rDNase in reaction buffer rDNase) was pipetted directly onto the centre of the silica membrane of each column. Samples were incubated with this solution at room temperature for 15min to digest DNA. Wash buffer 1 was then added (350µl) to the spin column, and the spin column was centrifuged for 1min at 13000 x *g*. The spin column was placed in a new 2ml processing tube; 500µl of wash buffer 2 (1:4 dilution with ethanol) was added the spin column and centrifuged for 1min at 13000 x *g*. After placing the spin column in a new 2ml processing tube, wash buffer 2 was added (500µl) and the spin column was centrifuged for 3min at 13000 x *g*. The spin column was placed in a new 2ml processing tube and centrifuged for 1min at 13000 x *g*. Following this, the spin column was placed in a 1.5ml microcentrifuge tube and elution buffer (40µl) was pipetted directly onto the spin column membrane. The spin column was centrifuged for 1min at 13000 x *g* to elute the RNA. This step was again repeated. The eluate was incubated for 5min at 65°C in a shaker-incubator (Excella E24 Incubator Shaker Series, New Brunswick Scientific) without shaking. After incubation, the samples were chilled immediately on ice and stored at -80°C.

5.2.5.3 RNA quantification and reverse transcription

The optical density (OD) of the extracted RNA was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific) to determine RNA

concentration and purity. The concentration of RNA can be determined via the degree of light absorbance. An OD of 1.0 at 260nm represents an RNA concentration of 44μg.ml^{-1}. This allows the RNA concentration in each sample of extracted RNA to be determined using the following equation:

$$RNA = OD_{260} \text{ x dilution factor x } 44\mu g.ml^{-1}$$

The absorbance is also measured at a wavelength of 280nm, which allows the purity of RNA to be determined. A ratio of OD_{260}:OD_{280} of approximately 1.8-2.2 ensures the purity of the RNA sample. Individual sample RNA concentrations and OD_{260}:OD_{280} ratios are listed in appendix XXXIV. RNA concentrations were equalised with RNase-free H_2O so that equal concentrations of RNA could be used as a template for reverse transcription.

The ABI High Capacity cDNA archive kit (Applied Biosystems) was used to reverse-transcribe the equalised RNA samples. Equalised RNA (17μl) was mixed with 2x master mix (17μl, containing 1:5 dilution of 10x reverse transcription buffer; 1:12.5 dilution of 25x dNTPs; 1:5 dilution of random primers; 1:10 dilution of multiScribe reverse transcriptase and 1:2.38 dilution of H_2O) in a PCR minitube. The samples were placed in a thermal cycler (PTC-200 Peltier Thermal Cycler, Biosciences, Dublin, Ireland) and incubated at 25°C for 10min, then at 37°C for 120min. The cDNA was frozen at -20°C prior to real-time polymerase chain reaction (RT-PCR) analysis.

5.2.5.4 RT-PCR

RT-PCR was performed using Taqman gene expression assays (Applied Biosystems), which contained specific target primers and FAM-labelled MGB target probes (table 5.1).

Table 5.2: List of the gene assays employed during PCR
*Gene reference as listed on the National Centre for Biotechnology Information (NCBI). Entrez-Nucleotide website: http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=nucleotide

Gene name	**Assay number**	**NCBI gene reference***
TNF-α	Hs00174128_ml	NM_001124357
IL-6	Hs00985639_ml	NM_000600.3
Resistin	Hs00220767_ml	NM_001193374.1
Visfatin	Hs00237184_ml	NM_005746.2

5.2.5.5 Multi-target (multiplex) Q-PCR

Initially cDNA was diluted with RNase-free H_2O (1:4 dilution) and 4µl of the dilute was added to each well on a PCR plate. Following this, target primers (0.5µl), GAPDH (0.5µl), and Taqman master mix (5µl) were mixed and added to each well (10µl reaction volume). The solution was pipetted using an electronic pipette (EDP3 10-100µl) to ensure accurate pipetting.

A StepOnePlus Real-Time PCR System (Applied Biosystems) was used to perform RT-PCR measurements. The PCR plates were placed in a RT-PCR thermocycler and measured according to the following protocol: Holding stage: 50°C for 2min, followed by 95°C for 20s; Cycling state step 1: 95°C for 1s, and step 2: 60°C for 20s. Steps 1 and 2 were repeated 40 times and the fluorescence was read during the annealing and extension phase (60°C) for the duration of the programme.

5.2.5.6 RT-PCR analysis (IL-6, TNF-α, visfatin, resistin)

The ΔΔCT method (StepOne Software v2.1 Applied Biosystems) was used to assess gene expression for all RT-PCR analysis. Relative gene expression was determined by comparing the gene expression of experimental samples to the mean of control samples. The fold-difference was compared initially between the T2D and ND groups, followed by comparison of the T2D and PIO groups. The fold-difference is assessed using the cycle number (CT) difference between samples. The threshold for fluorescence is set and the CT is measured against this value. In order to accurately assess the difference between gene expressions, the threshold was set when the PCR reaction was in the exponential phase.

5.2.6 Statistical Analysis

Analyses of the results from the ELISA assays, the Vascular Injury Panel Assays, and from RT-PCR were performed in three stages. The initial analysis was a comparison of baseline values assessing differences between the ND and T2D groups. All statistical analyses relating to this comparison were performed using PRISM software (Version 5.03, GraphPad Software Inc, USA). Data was compared between ND and T2D groups using unpaired t-tests. Significance for all tests was set at $P<0.05$ and all results are presented as mean ± standard deviation (sd). Data that were not

normally distributed (as determined by an F-test) were analysed assuming a non-Gaussian distribution via the Mann-Whitney U test.

The second analysis compared the ND and T2D groups using a three-factor (Diabetic status x treatment x time) RM ANOVA with one repeated measure (time). The third comparison was then performed between the T2D and PIO groups using a three-factor (PIO use x treatment x time) RM ANOVA with time as a repeated measure. All statistical analyses were performed using Datadesk software (Version 6.2.1 OS X, Data Description Inc, USA). Data was compared at baseline, 3 months, and 7 months. Differences were observed using Bonferroni *post-hoc* test. Significance for all tests was set at $P<0.05$ and all results are presented as mean ± standard deviation (sd).

5.3 Results

5.3.1 Baseline Comparisons

5.3.1.1 ELISA Results (IL-6, IL-10, TNF-α)

Plasma samples from those subjects who completed all three phases of the study were analysed for levels of IL-6, IL-10, and TNF-α using Enzyme-Linked Immunosorbent Assays (ELISAs). Group means (±sd) for the assays at baseline are presented below in figure 5.1. Analyses were performed on samples from nineteen ND subjects, and from 31 individuals with T2D who were matched for BMI, age and activity levels. With respect to IL-6, seven of the ND samples did not contain detectable levels of IL-6 in the analysis, while one sample was also excluded as analysis of the residuals showed that this sample did not display normal distribution. Two samples from individuals with T2D did not contain traceable levels of IL-6. Of the remaining samples that were included in the analysis (11 ND individuals, 29 individuals with T2D), while the T2D group displayed greater mean levels of IL-6, no significant differences were detected between groups (figure 5.1 top panel).

In the comparing IL-10 levels, one sample from a ND individual was excluded from analysis based on analysis of residuals. IL-10 was detected in significant levels in all other samples to allow for comparison (ND = 18, T2D = 31). No significant differences were detected between groups, but similar to IL-6, the T2D group had a larger mean value than the ND group (figure 5.1 middle panel).

Analysis of TNF-α levels proved more difficult, as the ELISA protocol only discovered detectable levels of TNF-α in four of the ND individuals, and eleven individuals with T2D. The data presented in figure 5.1 (bottom panel) reflects the means (±sd) of those samples in which TNF-α was detected. No significant differences were detected between the two groups.

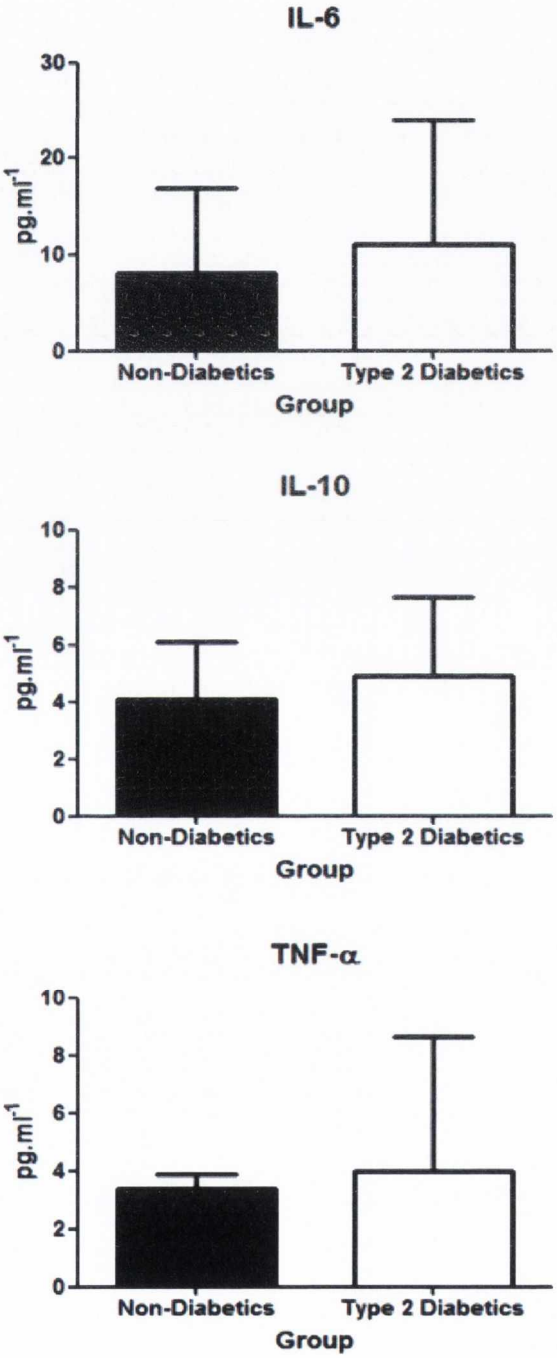


Figure 5.1. Mean (±sd) data for the ND and T2D groups for plasma levels of IL-6 (top panel), IL-10 (middle panel), and TNF-α (bottom panel). No significant differences were detected for any of the markers as determined using ELISA.

5.3.1.2 Vascular Injury Panel Assay (CRP, SAA, ICAM-1, VCAM-1)

Plasma samples from those subjects who completed all three phases of the study were analysed for levels of C-reactive protein (CRP), Serum Amyloid A (SAA), Inter Cellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) using MesoScale Discovery's (MSD, USA) 96-Well MULTI-SPOT® Vascular Injury Panel II Assay. All four markers were compared between nineteen ND samples and thirty-one T2D samples from individuals matched for age, BMI and activity levels. Mean data (±sd) are presented below in figure 5.2. No significant differences were detected for CRP (upper left panel); SAA (upper right panel); ICAM-1 (lower left panel); or VCAM-1 (lower right panel).

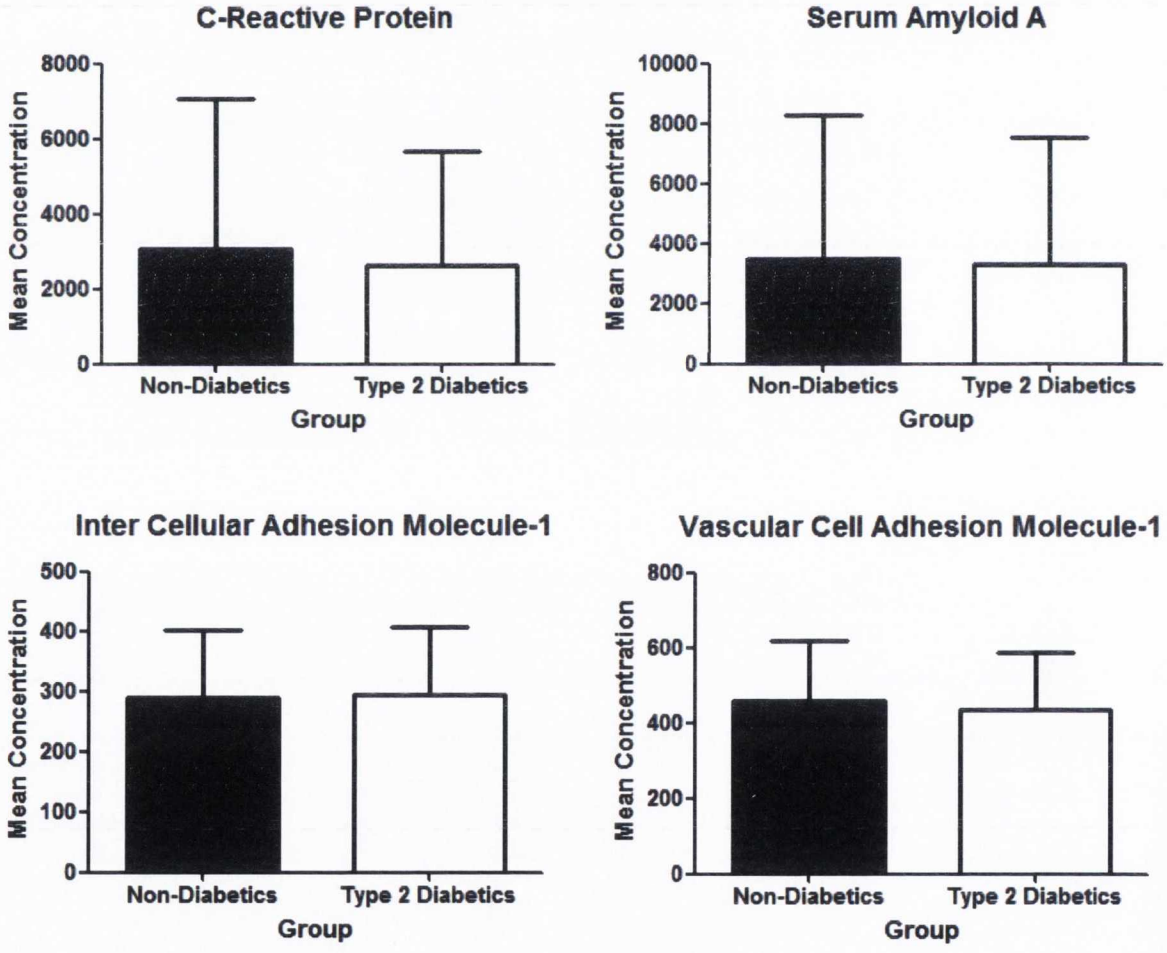


Figure 5.2. Mean (±sd) data for measures of vascular injury. Levels of CRP (upper left), SAA (upper right), ICAM-1 (lower left), and VCAM-1 (lower right) were similar between the two groups (significance set at 0.05).

5.3.1.3 RT-PCR Results (IL-6, TNF-α, Visfatin, Resistin)

Real-time polymerase chain reaction (RT-PCR) was performed on cDNA samples that were derived from extracted mRNA, which were collected from all subjects who completed at least baseline testing. Mean data (±sd) are presented in figure 5.3 below. With respect to IL-6, sixteen samples from ND individuals and thirty-seven samples from individuals with T2D matched for age and BMI were compared, with results revealing no significant difference in levels of gene expression of IL-6 between the two groups (upper left panel).

Levels of TNF-α gene expression were compared between nineteen ND individuals and thirty-nine individuals with T2D. Groups were matched for age, but a slight difference was noted for BMI ($kg.m^{-2}$), with the T2D group expressing greater values (ND: 28.6 ± 3.4; T2D: 30.5 ± 3.5). No significant difference was detected between the ND and T2D groups (upper right panel).

Gene expression levels for the adipocytokine visfatin (lower left panel) was compared between seventeen ND individuals and thirty-eight individuals with T2D matched for age and BMI, with no significant differences detected between the two groups. Similarly, comparison of resistin levels (lower right panel) between eighteen ND individuals and thirty-eight individuals with T2D matched for age and BMI again revealed no differences between the ND and T2D groups.

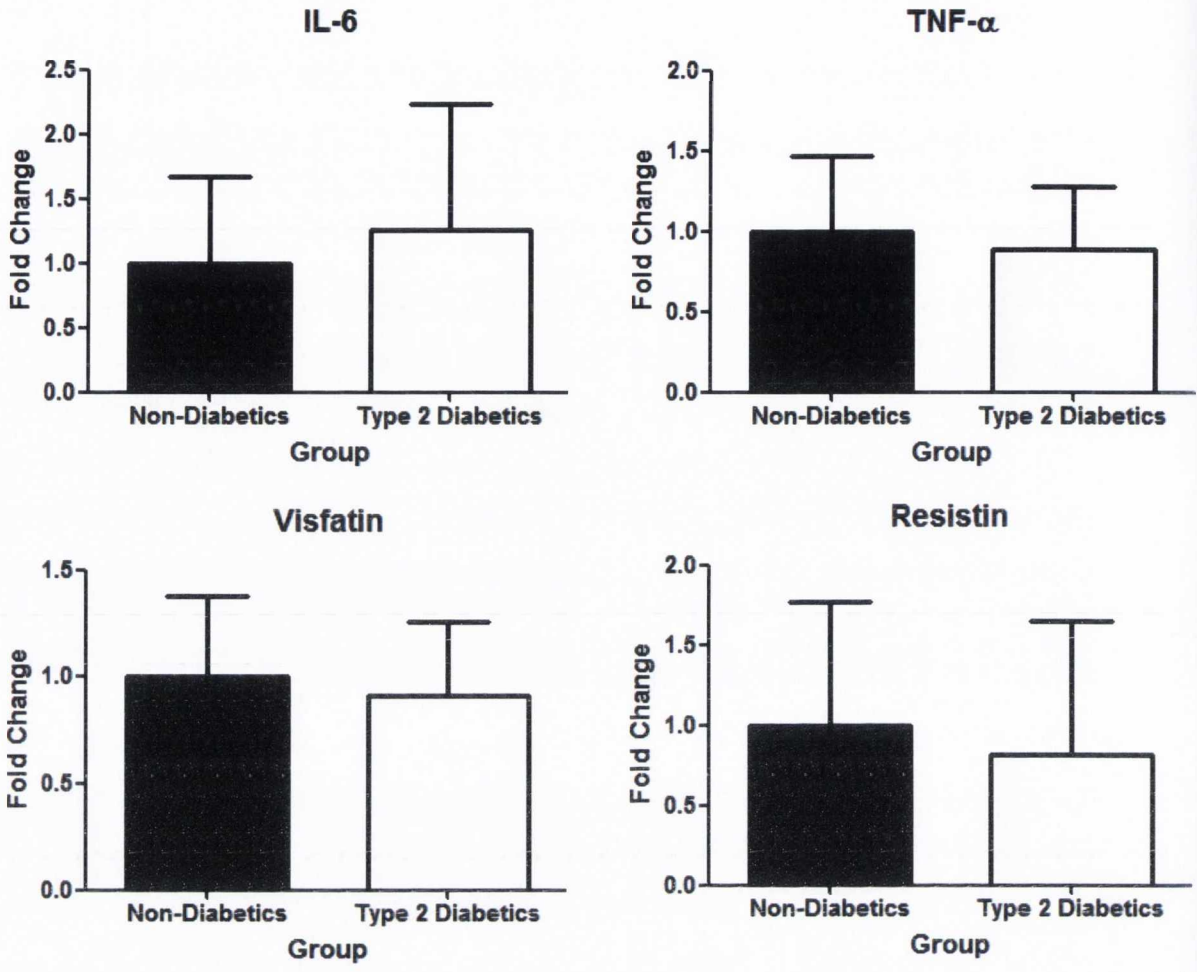


Figure 5.3. Comparison of gene expression levels in whole blood for IL-6 (upper left), TNF-α (upper right), visfatin (lower left), and resistin (lower right). Data is presented as mean ± sd. No significant differences were detected for any of the four markers.

5.3.2 Intervention-based Data Comparison

5.3.2.1 ELISA Results (IL-6, IL-10, TNF-α)

Comparison of the ND CTL (n = 9), ND EXS (n = 10), T2D CTL (n = 11), and T2D EXS (n = 11) groups, and comparison of the T2D CTL, T2D EXS, PIO CTL (n = 6), and PIO EXS (n = 7) groups were performed for plasma levels of IL-6, IL-10, and TNF-α at baseline, 3 months and 7 months. The addition of an extra subject in each of the PIO-treated groups did not result in any significant difference in the physical characteristics to those presented in Chapter 3 section 3.3.1.1. Mean data (±sd) are presented in figure 5.4. One subject from the ND EXS group was excluded from comparison of IL-10 levels between the ND and T2D groups on the basis of residual analysis. While no significant differences were detected between the two groups, there was a trend towards significantly higher levels of IL-10 in the

individuals with T2D, with mean values just failing to achieve significance (P=0.064). No significant differences were found in the comparison between the T2D and PIO groups. Data for IL-10 is presented in the upper panel of figure 5.4.

With respect to IL-6, only five subjects from the ND CTL group, nine subjects from the ND EXS group, and five subjects from the PIO EXS group provided samples that had a determinable concentration of IL-6 for analysis, with the other groups providing the full complement of samples. Furthermore, one subject in the ND EXS group was excluded as analysis of residuals indicated that results from the subject in question did not conform to normal distribution. Analysis of the remaining samples indicated that a three-way interaction existed between the ND and T2D groups ($P<0.05$), with post-hoc tests revealing that levels of IL-6 in the ND EXS group were greater at 3 months compared to baseline, and were also greater than both T2D groups at 3 months. The ND EXS group also showed a reduction in IL-6 at 7 months compared to at 3 months. An interaction between diabetic status and time was also found ($P<0.05$), with the ND group values at 3 months greater than at baseline and the T2D group values at 3 months. A main effect of time was also found ($P<0.05$), with values at 3 months greater than at baseline (figure 5.4 middle panel). Comparison of T2D and PIO samples revealed a treatment x time interaction ($P<0.05$), with CTL group values at 3 months greater than at baseline. A PIO x time interaction was also found, with PIO-treated group values at 3 months greater than T2D group values at 3 months. A main effect of PIO treatment was also found ($P<0.05$), with PIO treatment associated with increased levels of IL-6.

Statistical analysis of plasma TNF-α could not be performed due to the fact that a large number of samples did not contain determinable levels to allow for comparison between groups. Mean data for the viable samples are presented in the lower panel of figure 5.4, with the n number for each group is listed below the x-axis. However, the large number of missing values makes statistical analysis and interpretation of these results impossible.

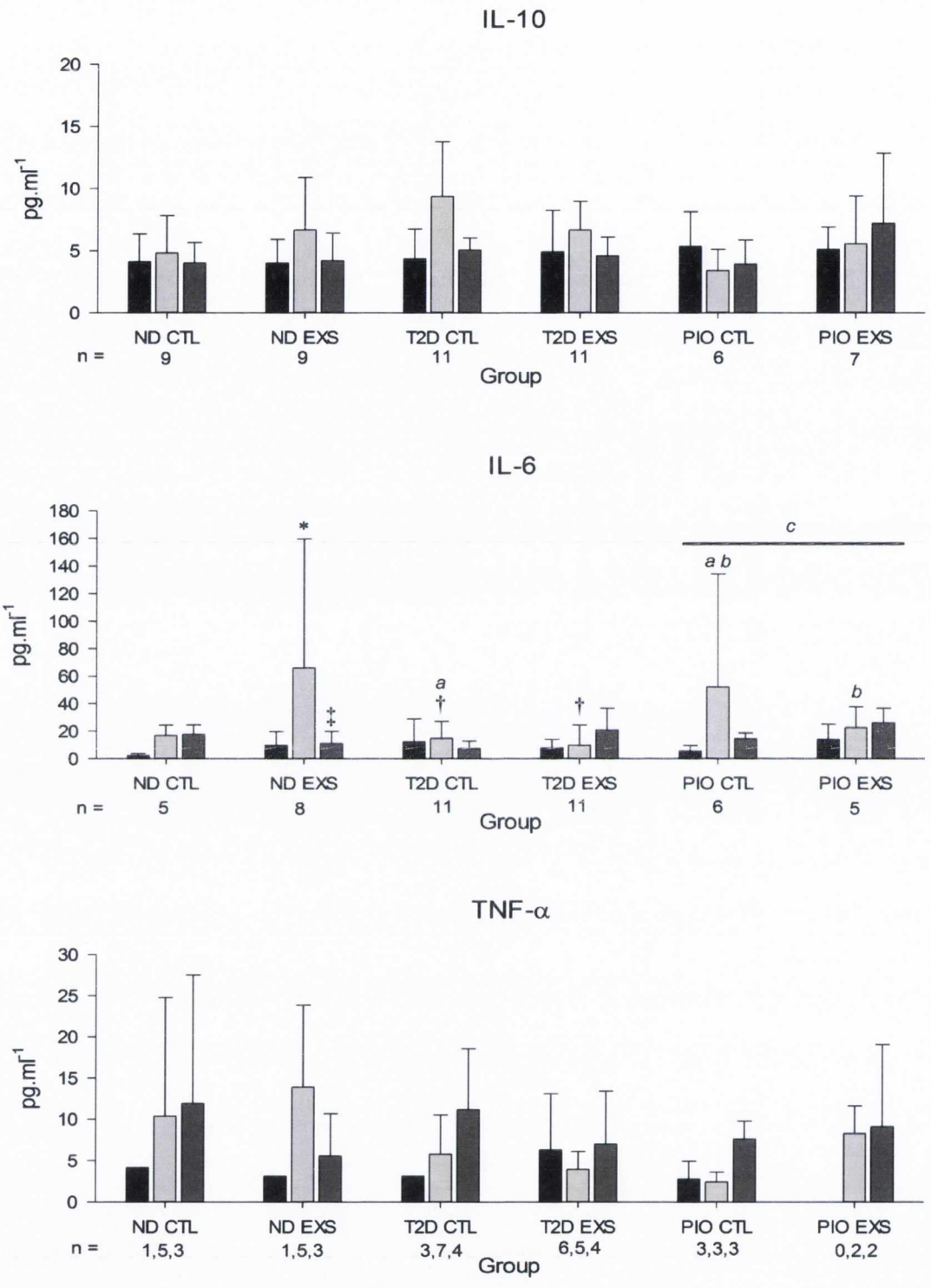


Figure 5.4 Intervention-based ELISA results for IL-10 (upper panel), IL-6 (middle panel), and TNF-α (lower panel). Symbols represent significant differences between the ND and T2D groups, while letters are used to indicate significant differences between the T2D and PIO groups. * indicates that the ND EXS group values at 3 months are significantly greater than at baseline (* $P<0.05$). † indicates that the T2D group values at 3 months were lower than the ND EXS group values at 3 months († $P<0.05$). ‡ indicates that the ND EXS group values at 7 months were lower than at 3 months. *a* indicates that the CTL group values at 3 months are greater than at baseline. *b* indicates that the PIO group values at 3 months are greater than the T2D groups at the same point in time (*b* $P<0.05$). *c* indicates that the PIO groups have greater levels of IL-6 than the T2D groups (*c* $P<0.05$). Data presented as mean ± sd.

5.3.2.2 Vascular Injury Panel Assay Results (CRP, SAA, ICAM-1, VCAM-1)

Comparison of the ND and T2D groups, and comparison of the T2D and PIO groups were performed for plasma markers of vascular injury at baseline, 3 months and 7 months. Mean data (±sd) for CRP and SAA are presented in figure 5.5 below. In comparison of the ND and T2D groups, no significant interactions or main effects were found for CRP levels, although there was a trend towards a time effect (P=0.058), with post-hoc tests indicating that levels at 7 months were higher than at the other two time points. No effects were noted when comparing the T2D and PIO groups. Similarly, comparison of SAA did not indicate any significant differences between either the ND and T2D groups or the T2D and PIO groups.

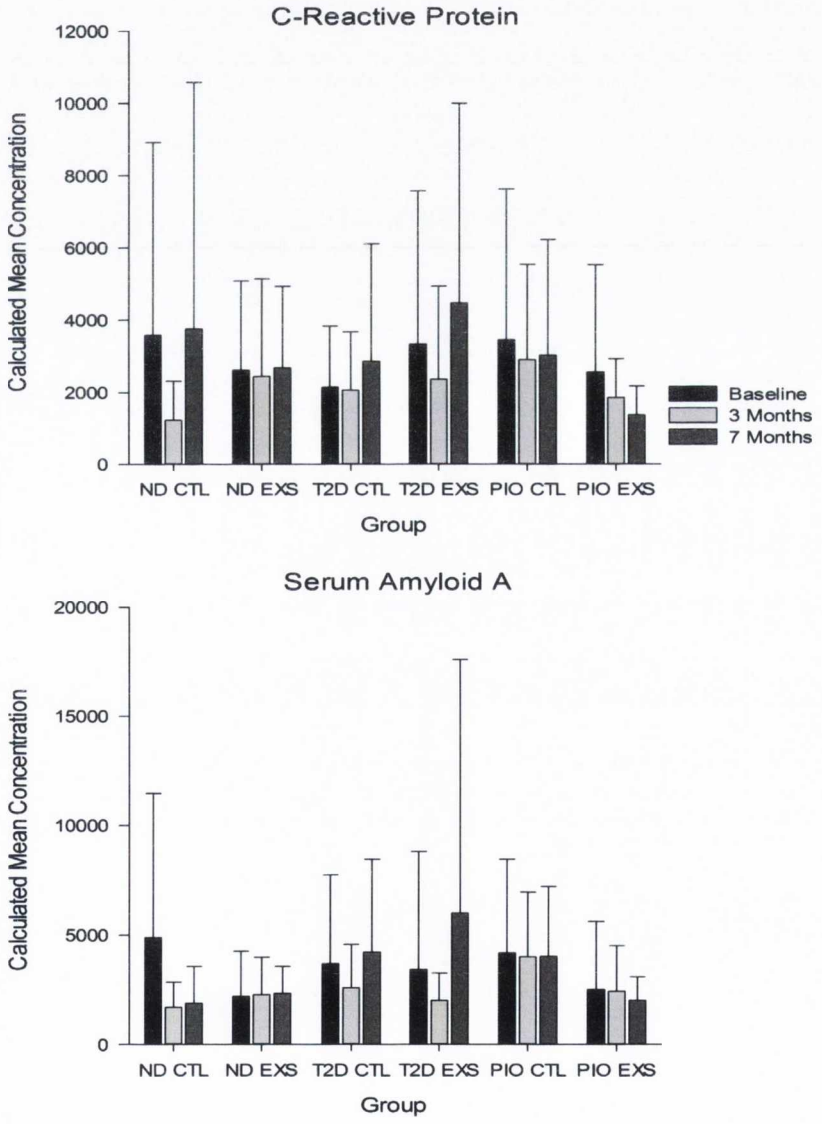


Figure 5.5. Mean (±sd) data for CRP and SAA determined from plasma samples. No significant differences were detected between groups for either marker.

Data from the comparison of ICAM-1 and VCAM-1 values are presented below in figure 5.6. Comparison of mean ICAM-1 values (upper panel) did not reveal any statistically significant differences between the ND and T2D groups. However, comparison of the T2D and PIO groups revealed a significant treatment effect ($P<0.05$), with the EXS groups displaying lower levels of ICAM-1 than the CTL groups.

When comparing VCAM-1 levels, no differences were detected between either the ND and T2D groups, or the T2D and PIO groups. However, comparison of the T2D and PIO groups did reveal a trend towards lower levels of VCAM-1 in the PIO-treated groups ($P=0.076$).

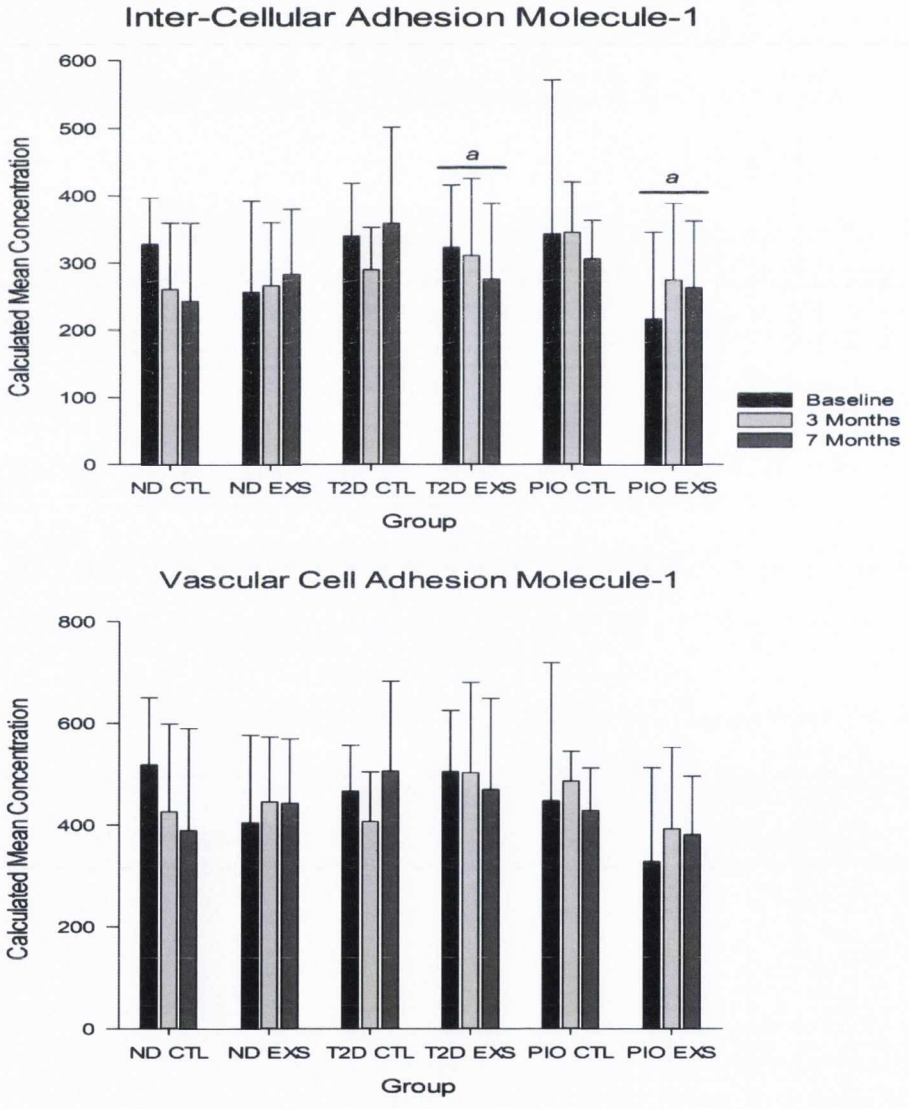


Figure 5.6. Mean (±sd) data for ICAM-1 (upper panel) and VCAM-1 (lower panel). Letters indicate significant differences between the T2D and PIO groups ($P<0.05$). *a* indicates a significant treatment effect, with the EXS groups displaying lower ICAM-1 levels that the CTL groups. No effects were noted for VCAM-1.

5.3.2.3. RT-PCR Results (IL-6, TNF-α, visfatin, resistin)

With regard to the four target genes, the *n* number for each analysis varied slightly due to exclusion of certain samples due to failure of the RT-PCR protocol to significantly detect phosphorescence. With respect to the ND CTL group, the *n* number was seven for all markers, with the exception of analysis of IL-6, which was reduced to six subjects. The ND EXS group had samples for each phase from six subjects in the IL-6 and resistin analyses, and seven for measurement of visfatin and TNF-α. Both the T2D CTL and T2D EXS group had nine subjects in analyses of all four markers; while both PIO groups had six subjects for all markers, with the exception of the PIO EXS group for measurement of IL-6, which was reduced to five subjects.

Results for IL-6 (upper panel) and TNF- α (lower panel) are presented in figure 5.7. Comparison of the ND and T2D groups for levels of IL-6 did not reveal any significant differences between the groups. Similarly, no effects were noted when the T2D and PIO groups were compared.

Significant differences were detected between the ND and T2D groups with respect to TNF-α levels, with a three-way interaction (treatment x time x diabetic status) found ($P<0.05$). Analysis of post-hoc results revealed that a significant difference was found at baseline between the T2D EXS and ND EXS groups, with the ND EXS group displaying greater levels of TNF-α gene expression. An additional interaction between diabetic status and time was also noted ($P<0.05$). No significant post-hoc tests were noted, however analysis of the results indicate that over time the ND groups displayed decreasing levels of TNF-α, while the T2D groups displayed increasing levels of TNF-α.

In comparing the PIO and T2D groups levels of TNF-α, a significant effect of PIO status was noted ($P<0.05$), with the PIO groups displaying greater levels of TNF-α than the T2D groups.

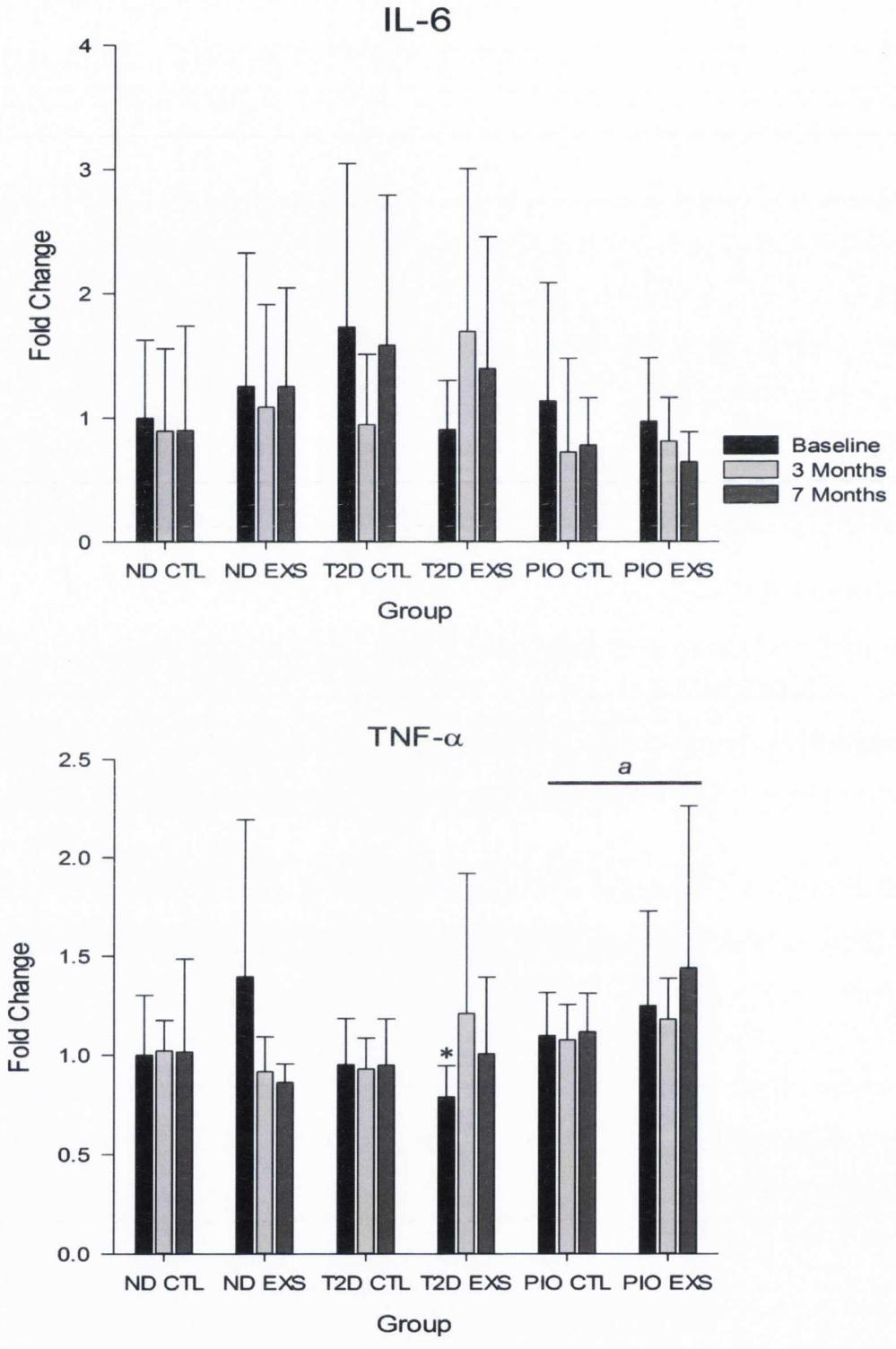


Figure 5.7. Mean (±sd) data for IL-6 (upper panel) and TNF-α (lower panel) gene expression determined by RT-PCR. No significant differences were detected for levels of IL-6 expression. With respect to TNF-α, symbols represent differences between the ND and T2D groups, while letters represent differences between the T2D and PIO groups. * indicates that the T2D EXS group display lower mean values that the ND EXS group at baseline (* $P<0.05$). *a* indicates a significant effect of PIO treatment, with the PIO groups displaying greater levels of TNF-α expression than the T2D groups (*a* $P<0.05$).

Comparison of visfatin (upper panel) and resistin (lower panel) levels of gene expression are presented in figure 5.8. With respect to visfatin, comparison of the ND and T2D group values revealed a significant interaction between diabetic status and time ($P<0.05$). While no significant differences were revealed via post-hoc analysis, the ND groups showed a reduction in mean levels of visfatin expression over time, while the T2D group showed a general increase in mean visfatin levels. No other significant interactions or effects were detected between the ND and T2D groups. Analysis of the results from the T2D and PIO groups revealed a significant interaction between PIO status and time, with post-hoc tests indicating that values in the PIO groups at 7 months were significantly greater than the T2D group values at 7 months, and also tended to be larger than PIO group values at 3 months ($P=0.067$). In comparing these groups, there was also a trend towards a significant interaction between PIO status and treatment ($P=0.069$), with the T2D EXS group displaying non-significantly lower values than the PIO EXS group.

Resistin levels did not differ between the ND and T2D groups. However, comparison of the T2D and PIO groups did reveal a significant interaction between PIO status and time ($P<0.05$). Analysis of post-hoc results revealed that at baseline, the PIO-treated groups displayed significantly greater levels of resistin expression than the T2D groups ($P<0.05$), while PIO-treated groups showed a significant reduction in resistin expression levels at 7 months compared to baseline ($P<0.05$). The reduction in resistin in the PIO groups was also evident at 3 months, with both groups showing a non-significant decrease in levels from baseline ($P=0.058$).

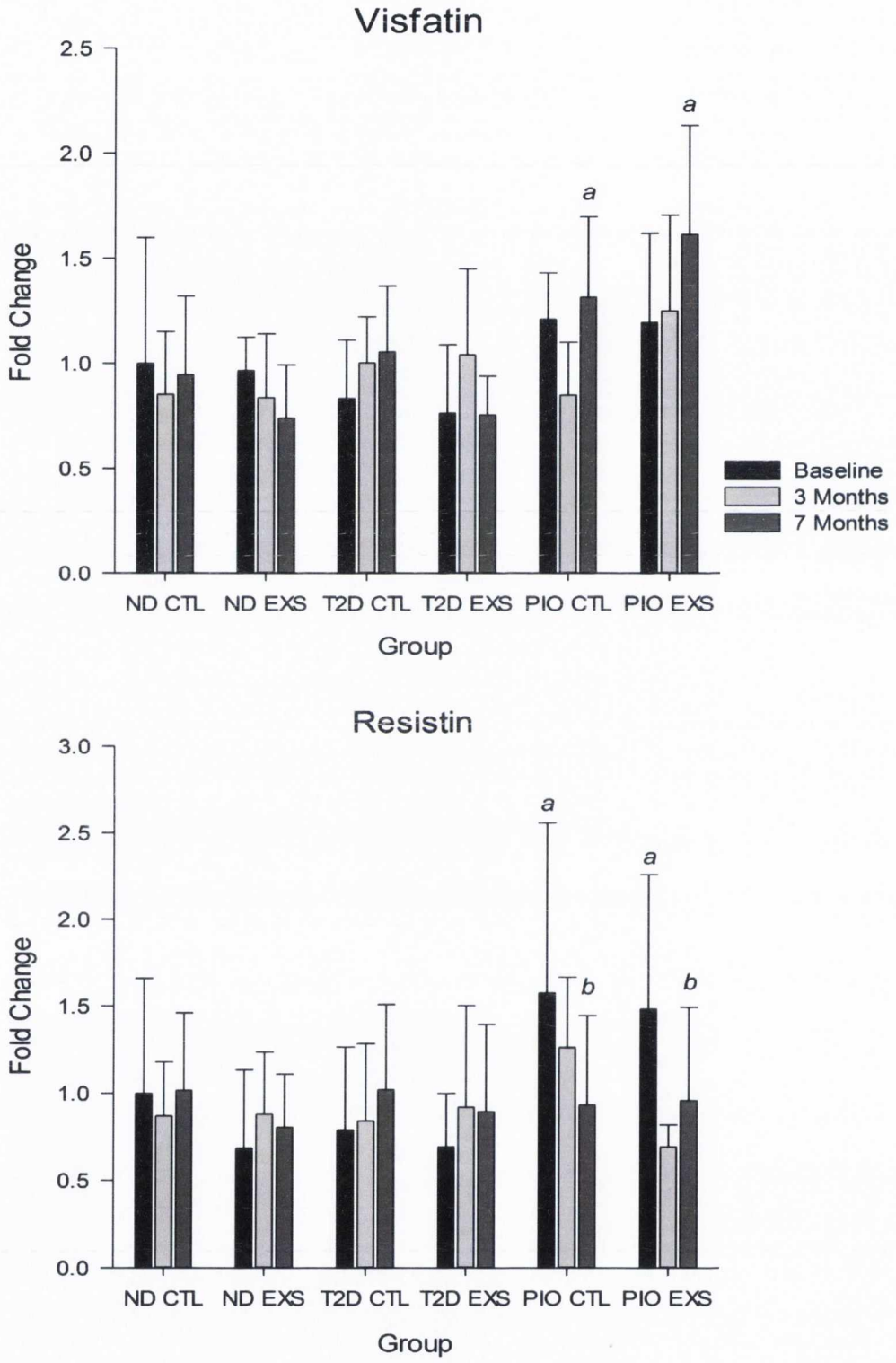


Figure 5.8. Mean (±sd) values for visfatin (upper panel) and resistin (lower panel) gene expression. No significant differences were detected between the ND and T2D groups for either marker. With regard to visfatin, the T2D and PIO groups displayed a significant interaction between PIO status and time, with PIO values at 7 months greater than T2D values (*a* $P<0.05$). For resistin, *a* indicates that the PIO groups at baseline display higher levels of resistin expression than the T2D groups (*a* $P<0.05$), while PIO values at 7 months were significantly lower than at baseline (*b* $P<0.05$).

5.4 Discussion

Analysis of the baseline ELISA results revealed that there were no significant differences between non-diabetics and individuals with T2D with respect to circulating levels of IL-6, IL-10, or TNF-α. These findings were in contrast to previous results, with other studies displaying elevated levels of IL-6 and TNF-α (Bluher *et al.*, 2005; Alghasham & Barakat, 2008; Koleva-Georgieva *et al.*, 2011) and reduced levels of IL-10 in individuals with T2D (Bluher *et al.*, 2005; van Exel *et al.*, 2002). Furthermore, no differences were detected when comparing markers of vascular injury, with circulating baseline levels of the endothelial markers CRP, SAA, ICAM-1 and VCAM-1 similar between non-diabetics and individuals with T2D, which is also in contrast with previous findings (Alghasham & Barakat, 2008; Bluher *et al.*, 2005; Bruno *et al.*, 2008; el-Mesallamy *et al.*, 2007; Hatanaka *et al.*, 2007; Kumon *et al.*, 1994; Matsumoto *et al.*, 2002; Urso *et al.*, 2010). While it may appear that these findings are unexpected, critical analysis of published evidence would suggest that the findings achieved in this study tend to agree with those of prior investigations. For example, previous studies have shown that circulating levels of inflammatory markers in individuals with T2D are linked to measures of insulin sensitivity (Bluher *et al.*, 2005), while CRP levels have been shown to be positively correlated with both fasting plasma glucose (FPG) levels (Bluher *et al.*, 2005), HbA_{1c} and insulin levels (Heliovaara *et al.*, 2007), with TNF-α levels also correlated with HbA_{1c} (Koleva-Georgieva *et al.*, 2011). VCAM-1 is another marker that shows a positive correlation with both FPG and insulin sensitivity (Matsumoto *et al.*, 2002). While baseline data between groups reveals significantly greater FBG and HbA_{1c} levels between non-diabetics and individuals with T2D (Chapter 2, table 2.2), the individuals with T2D display excellent glycaemic control, with HbA_{1c} and FBG levels at the lower end of the target range; while measures of total cholesterol and HDL-C are not different between groups, which have been positively associated with VCAM-1 levels (Matsumoto, 2002).

Additionally, CRP and ICAM-1 levels demonstrate a positive relationship (el-Mesallamy *et al.*, 2007), therefore if CRP is not significantly different between groups it is to be expected that ICAM-1 levels would not significantly differ either. One result that is difficult to account for is the lack of difference in SAA levels, as SAA levels are not significantly correlated with glycaemic control (Kumon *et al.*, 1994),

therefore some additional factor that influences SAA must account for the lack of difference between the two groups that is beyond the scope of this study.

Comparison of PCR results for levels of gene expression of IL-6, TNF-α, visfatin and resistin did not reveal any significant baseline differences between non-diabetics and individuals with T2D. With respect to IL-6 and TNF-α, this is in contrast to previous findings (Navarro-Gonzalez *et al.*, 2010), which demonstrated significantly increased mRNA expression of IL-6 and TNF-α in peripheral blood mononuclear cells (PBMC) in individuals with T2D. While a number of the present subjects were hypertensive, the entire cohort of T2D participants in the study by Navarro-Gonzalez *et al.* (2010) were hypertensive; therefore it is possible that the increased expression of IL-6 and TNF-α is a consequence of elevated blood pressure causing inflammation of the vasculature. It is also possibly a consequence of method of sampling mRNA, as it has been shown that mRNA expression profiles vary depending on whether the RNA is sampled from PBMC's or from whole blood (Gaarz *et al.*, 2010).

With respect to visfatin and resistin, the lack of difference between non-diabetics and individuals with T2D is also not as surprising as may first appear. In a study comparing young obese adults with age-matched individuals with T2D, no difference in baseline levels of circulating visfatin was found (Brema *et al.*, 2008). Furthermore, while visfatin has been shown to be an independent predictor of T2D (Esteghamati *et al.*, 2010), elevated levels of visfatin in individuals with T2D are primarily determined by FPG and triglycerides. As shown in chapter 2 (table 2.2), FBG levels were relatively low in the individuals with T2D, while triglycerides were similar between the two groups. A further finding that lends credence to the lack of a significant difference between the two groups is that visfatin levels are negatively associated with endothelial function (Takebayashi *et al.*, 2007). As seen in chapter 2, individuals with T2D displayed similar LVC kinetics, as well as similar peak LBF and LVC responses during the graded incremental plantar-flexion test (table 2.3) and reactive hyperaemia responses to ischaemia between ND and T2D groups (table 2.6) would suggest that endothelial function might be relatively healthy in this cohort of individuals with T2D, which would offer a credible explanation for the lack of difference in visfatin levels between the two groups at baseline.

The lack of difference between resistin mRNA expression between the ND and T2D group is not surprising given that no direct relationship between resistin and

either susceptibility to T2D (Gharibeh *et al.*, 2010) or any clinical measure of insulin resistance has been established (Pfutzner *et al.*, 2003). HDL-C has been shown to be an independent predictor of serum resistin levels (Hasegawa *et al.*, 2005); and given the lack of difference between HDL-C levels (chapter 2, table 2.2), this would support the absence of difference in resistin expression. A modest positive correlation between BMI and resistin levels has been shown irrespective of diabetic status (Fujinami *et al.*, 2004), while a correlation between resistin levels and VCAM-1 levels have been shown (Lozano-Nuevo *et al.*, 2011; Hui-Bing *et al.*, 2006). Both BMI and VCAM-1 levels are similar between this study's ND subjects and individuals with T2D, so it is not surprising that resistin levels are similar between the groups.

Exercise is known to stimulate anti-inflammatory responses through the production of the myokine IL-6, which inhibits the pro-inflammatory effects of TNF-α, and stimulates production of IL-10 (Febbraio & Pedersen, 2005). It has been suggested that TNF-α is a cause of insulin resistance, with IL-6 rather a marker of insulin resistance, and not a protagonist (Petersen & Pedersen, 2005). Therefore, it could be expected that exercise-induced suppression of TNF-α may result in improvements in both insulin resistance and reduction in pro-inflammatory markers such as CRP, SAA and cellular adhesion molecules. Previous research has shown that an exercise intervention has produced mixed results with respect to circulating inflammatory markers (Zoppini *et al.*, 2006; Yakeu *et al.*, 2010). In comparing the ND and T2D groups for ELISA recordings of IL-6, IL-10, and TNF-α, the differences detected were within the ND groups, in particular the ND EXS group, with IL-6 values at 3 months significantly greater than their 7-month value, as well as the T2D groups at 3 months, which is contrary to what would have been expected, given the association of insulin resistance and the pro-inflammatory state. More noticeable was the absence of any effect of the exercise intervention on reducing IL-6 levels or increasing circulating IL-10, which is in contrast to some (Yakeu *et al.*, 2010) prior findings but not others (Zoppini *et al.*, 2006). However, the ability to infer the effect of exercise on the pro-inflammatory state is limited in this case due to the lack of results with respect to TNF-α due to the small number of viable samples. It is possible that the absence of a significant concentration of TNF-α in these samples is indicative of exercise-induced suppression of TNF-α to trace levels; however, given that the control samples were equally affected this is most likely not the case.

Results from the MSD Multi-Spot™ Vascular Injury Panel did not reveal any significant differences between the ND and T2D groups for measures of CRP, SAA, ICAM-1, or VCAM-1. It had been expected that exercise would have resulted in reductions in circulating levels of these markers, as had been shown previously (Okada *et al.*, 2010; Jorge *et al.*, 2011). However, the lack of significant difference in these markers is in agreement with previous findings that indicated that a three-month exercise intervention had no effect on CRP, ICAM-1 or VCAM-1 in young adults with T2D (Hatunic *et al.*, 2007). Therefore it is possible that the relatively intact insulin sensitivity in the T2D groups (as evident by the lack of effect of exercise on FBG or HbA_{1c} in chapter 3, tables 3.4 and 3.5) suggests that the pro-inflammatory state typically associated with T2D may be reduced in this cohort of individuals with T2D.

With respect to PCR analysis, the T2D EXS group demonstrated a lower baseline level of expression of TNF-α than the ND EXS group, which would further strengthen the argument that the current diabetic cohort had good levels of insulin sensitivity and a reduction in the pro-inflammatory state. No effect of exercise was noted in mRNA expression levels of IL-6, TNF-α, visfatin or resistin between the ND and T2D groups. Yakeu *et al.* (2010) had previously shown that exercise resulted in a downregulation of expression of pro-inflammatory cytokines including IL-6 and TNF-α, as well as an upregulation of anti-inflammatory markers in leucocytes derived from whole blood. Additionally, Jorge *et al.* (2011) had demonstrated an increase in visfatin levels following exercise, irrespective of the modality of exercise. The absence of any effect on visfatin expression may be due to sampling from the circulation, with visfatin expression likely to be concentrated in the visceral adipose tissue. Jorge *et al.* (2011) also demonstrated that exercise had no effect on resistin levels, which would seem to confirm the present findings, and also those of Pfutzner *et al.* (2003), who failed to establish a link between resistin levels and insulin resistance.

The effect of the addition of PIO treatment on these markers was also determined. Results from ELISA comparison between the T2D and PIO groups did not reveal any effects of PIO treatment on IL-6 or IL-10, which is in agreement with the majority of prior research (Martens *et al.*, 2006; Shadid *et al.*, 2006; Park *et al.*, 2011). Given that PIO acts predominantly upon adipose tissue, the lack of effect of PIO on these measures of IL-6 or IL-10 would suggest that the circulating levels of

these cytokines were derived from elsewhere. However, as with the comparison between the ND and T2D groups, it was not possible to carry out analysis of TNF-α levels due to the low *n* number of samples.

Comparison of markers of vascular injury did not reveal any significant differences between the T2D and PIO groups with respect to CRP or SAA. This is in contrast to previous studies, which found that treatment with PIO resulted in a reduction in CRP levels (Heliovaara *et al.*, 2009; Park *et al.*, 2011, Maegawa *et al.*, 2007; Nerla *et al.*, 2010; Vijay *et al.*, 2009). In contrast, Pitocco *et al.* (2009) failed to detect a difference in CRP levels following treatment with PIO. However, Heliovaara *et al.* (2007) found that CRP levels were correlated with both HbA_{1c} and insulin. Given the absence of any effect of either PIO or EXS on HbA_{1c} it is unsurprising that CRP levels were unaffected.

Treatment with PIO did result in differences in circulating levels of ICAM-1. However, in comparison of the T2D and PIO groups, it was the exercise intervention that resulted in a significant lowering of ICAM-1 compared to controls. This would support the finding of Zoppini *et al.* (2006), who found that exercise, despite having no impact on CRP or TNF-α, resulted in a reduction in ICAM-1 independent of variations in HbA_{1c} or HDL-C. The absence of any effect of PIO on VCAM-1 levels supports the contentions of Bruno *et al.* (2008) and Lozano-Nuevo *et al.* (2011), that these cellular adhesion molecules have independent actions, offering differing insights into the relationship between the pro-inflammatory response and its effect on the microvasculature.

The effect of PIO on mRNA expression of IL-6, TNF-α, visfatin and resistin was also assessed. No effect of PIO was noted on IL-6 expression, which is unsurprising given the lack of significance in difference in circulating levels already noted. More surprisingly, the PIO-treated groups displayed greater levels of TNF-α mRNA expression than the T2D groups. Given that PIO's site of action is adipose tissue, resulting in preferential differentiation for subcutaneous vs. visceral adipose tissue, it seems contradictory to suggest that the action of PIO is directly responsible for the increased levels of TNF-α, especially given that it has been linked with a reduction in low-grade inflammation (Maegawa *et al.*, 2007). Rather it may be an indirect consequence of the increase in body mass indices (chapter 3 table 3.2 & figure 3.2), with previous research demonstrating an association between obesity and TNF-α expression (Hansen *et al.*, 2010; He *et al.*, 2010; Popko *et al.*, 2010).

Visfatin mRNA expression was greater at 7 months in the PIO-treated groups compared to the T2D groups, which is in contrast to previous findings (Hammarstedt *et al.*, 2006; Takebayashi *et al.*, 2007). However, given the site of action of PIO, it is unsurprising that visfatin expression should increase. Furthermore, the length of treatment with PIO in the study by Hammarstedt *et al.* (2006) was only 3-4 weeks. Given that it took a period of 28 weeks for the difference between these two groups to become apparent, it is not necessarily surprising that Hammarstedt *et al.* (2006) failed to discover any significant difference in visfatin expression. The study by Takebayashi *et al.* (2007) also revealed that circulating visfatin was significantly increased in a subgroup of eleven females ($P<0.05$), which would suggest that gender possibly plays a role in the effect of PIO; however, the finding of increased expression of visfatin in males treated with PIO in the present study would suggest that the possibility of a gender effect is reduced. Rather it may be concluded that PIO has a modest effect on visfatin, given that the increase in expression at 7 months was found when directly compared to the T2D groups. The level of expression at 7 months in the PIO groups, while numerically greater, was not significantly elevated from baseline.

Within the PIO-treated groups, resistin expression at 7 months was significantly lower than at baseline, which is in contrast to the findings of Shadid *et al.* (2006), who found no change in plasma resistin levels as a consequence of PIO treatment for 18-20 weeks. However, the baseline values for resistin expression in the PIO groups were significantly greater than the T2D groups, which would not be expected given that at baseline the T2D and PIO groups were made as uniform as possible. This would suggest that resistin is not directly related to measures of insulin resistance or adiposity (Pfutzner *et al.*, 2003; Hasegawa *et al.*, 2005; Iqbal *et al.*, 2005; Gharibeh *et al.*, 2010), given that in this study body mass indices significantly increased in the PIO groups, yet resistin levels significantly decreased.

Despite the weight of evidence in support of the conclusions drawn in the above arguments, there is a strong possibility that the results of the investigations described are heavily influenced by the medications used by participating subjects in the treatment of their diabetes. It has previously been shown that metformin results in improved vascular and endothelial function (Mather *et al.*, 2001). While the mechanism of this improvement remains to be fully elucidated, it has been suggested that metformin may have antioxidant effects (Faure *et al.*, 1999), which would reduce

ROS-associated levels of inflammation. Given the high level of metformin use among the diabetic participants, it is possible that the lack of significance in the findings is a result of metformin use.

It is also possible that the blood pressure medication in use by participants also played a role. Use of ACE inhibitors and angiotensin receptor blockers have been shown to improve endothelium-dependent vasodilation in individuals with T2D (Bragulat *et al.*, 2003; Wang *et al.*, 2005; Schmieder *et al.*, 2007). However, the evidence of the effect of blocking drugs on the renin-angiotensin-aldosterone system resulting in improvements in endothelial function are not conclusive, as a longitudinal study examining the effects of these drugs did not discover any improvement in endothelial function, suggesting that endothelial dysfunction may be a an irreversible feature of hypertension (Sozen *et al.*, 2009). If these medications have a positive effect on endothelial function, it would be expected that medication usage would translate into reduced levels and expression of the inflammatory and endothelial markers assessed; whereas if the findings of Sozen *et al.* (2009) are applicable at a population level, then use of these anti-hypertensive medications would not be expected to have any effect on endothelial function.

Use of statins has also been suggested to reduce inflammation, oxidative stress and improve endothelial function (Ludwig & Shen, 2006); but again, the effects are yet to be fully elucidated. One study demonstrated that statin use resulted in reduced monocyte release of TNF-α, IL-6, and reduced lymphocyte release of TNF-α, which was accompanied by a decrease in circulating plasma levels of CRP (Krysiak *et al.*, 2011). However, despite these potential effects of statins on the reduction in inflammatory levels, residual macrovascular morbidity and mortality persist in hypertensive individuals with T2D (Libby & Plutzky, 2007), thereby suggesting that the effect of statins on cardiovascular inflammation and endothelial function may be minimal.

In conclusion, circulating levels of inflammatory cytokines and markers of endothelial dysfunction, as well as mRNA expression of some of these markers did not differ between non-diabetics and individuals with T2D, in contrast to the majority of current literature. This would imply that associated factors such as insulin resistance and sensitivity are relatively well preserved in this cohort of individuals with T2D. Interventions in the form of exercise and pharmacological treatment with

PIO had only minor effects, further supporting the contention that similar interventions are most effective in uncontrolled populations. However, the effect of existing medication usage in the treatment of T2D should be considered, as they may have a significant impact in the circulating levels and expression of the inflammatory markers and adipocytokines measured, which would impact upon the results demonstrated.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION

Prior research shows that individuals with type 2 diabetes (T2D) display reduced exercise capacity ($\dot{V}O_2$peak) compared to healthy controls (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Lalande *et al.*, 2008; Mac Ananey *et al.*, 2011; Wilkerson *et al.*, 2011), as well as impaired $\dot{V}O_2$ kinetic responses to steady-state submaximal exercise (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011). The impairment in exercise capacity in individuals with T2D has important clinical implications, as performing exercise usually forms one aspect of the initial treatment prescription following diagnosis of T2D (Colberg *et al.*, 2010). Determining the source of the T2D-related impairments in exercise performance has been widely researched but definitive answers remain elusive. The source of the impairment has been attributed to impaired cardiac output (CO) responses and central dysfunction by some (Roy *et al.*, 1989; Regensteiner *et al.*, 2009, Gusso *et al.*, 2008; Baldi *et al.*, 2006), while this has been disputed elsewhere (Lalande *et al.*, 2008; Mac Ananey *et al.*, 2011). Alternatively, the T2D-related impairment during exercise has been thought to be a result of endothelial dysfunction impairing blood flow (BF) to the active musculature (Kingwell *et al.*, 2003; Macananey *et al.*, 2011); while impairments in O_2 extraction due to mitochondrial dysfunction have also been implicated in impaired exercise performance in individuals with T2D (Baldi *et al.*, 2003).

Part of the reason for the variation in findings between studies may be due to differing subject characteristics. The majority of studies investigating the T2D-related exercise impairment utilised female-only cohorts (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011) based on the results of a study including only 4 males and 6 female per group that demonstrated that the diabetes-induced exercise impairment may be larger in females than males with T2D (Regensteiner *et al.*, 1995). To this author's knowledge, only one study has investigated if the T2D-related impairment in exercise capacity and $\dot{V}O_2$ kinetics is present in a male-only cohort (Wilkerson *et al.*, 2011). Surprisingly, despite displaying similar levels of impairment in $\dot{V}O_2$peak to previous studies (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Lalande *et al.*, 2008; Mac Ananey *et al.*, 2011),

males with T2D did not display any impairment in $\dot{V}O_2$ kinetic responses compared with healthy age-matched controls. However, this study only investigated responses in older males (mean age ~ 65yrs), therefore leaving open the possibility that the $\dot{V}O_2$ kinetic responses in the healthy controls were affected by age-related impairments in the kinetic response, thereby ameliorating any difference in the $\dot{V}O_2$ kinetic response due to T2D (Bell *et al.*, 1999).

The lack of difference in $\dot{V}O_2$ kinetic responses during submaximal steady-state cycling in males with T2D in the study of Wilkerson *et al.* (2011) was all the more surprising given that two previous studies investigating leg blood flow (LBF) responses during submaximal exercise in middle-aged males with T2D demonstrated impaired LBF responses in the T2D groups (Kingwell *et al.*, 2003; Lalande *et al.*, 2008). The presence of impaired LBF responses in males with T2D would be expected to reduce O_2 delivery to the active musculature and therefore result in impaired $\dot{V}O_2$ kinetic responses during cycling exercise. The purpose of the study in chapter 2 was to investigate measures of $\dot{V}O_2$peak and $\dot{V}O_2$ kinetic responses to steady-state cycling in middle-aged males with T2D. Furthermore, this study sought to determine the potential source of the impairment in exercise performance of individuals with T2D by investigating CO responses during cycling, and also leg vascular conductance (LVC) responses during plantar-flexion exercise and forearm vascular conductance (FVC) responses during reactive hyperaemia (RH) to determine if the exercise-related impairment was due to either central or peripheral dysfunction.

The current findings confirmed the presence of a T2D-related impairment in $\dot{V}O_2$peak, and in contrast to the findings of Wilkerson *et al.* (2011), analysis revealed a T2D-related impairment in $\dot{V}O_2$ kinetics during cycling exercise performed at moderate intensities. These findings suggest that the age of the participants in the study of Wilkerson *et al.* (2011) may have contributed to the lack of difference in $\dot{V}O_2$ kinetics, as the current findings matched those of Regensteiner *et al.* (1998), Brandenburg *et al.* (1999), Bauer *et al.* (2007), and Mac Ananey *et al.* (2011), whose participants were of a similar age profile to those in this study.

CO responses during exercise were impaired during steady-state cycling in males with T2D, suggesting that central dysfunction likely played a role in the exercise-related impairment. This is supported by some (Roy *et al*, 1989; Baldi *et al.*, 2006; Gusso *et al.*, 2008; Regensteiner *et al.*, 2009) but not all (Lalande *et al.*, 2008;

Mac Ananey *et al.*, 2011) previous studies; but the combined presence of impaired HR kinetic responses in males with T2D in the present study would suggest that central dysfunction is a potential source of the impairment in exercise performance.

Attempts to determine any possible effect of endothelial dysfunction and impaired peripheral BF did not provide any significant evidence of a role for these factors in the impaired exercise performance in males with T2D, which is in contrast to previous findings (Kingwell *et al.*, 2003; Lalande *et al.*, 2008; Macananey *et al.*, 2011). The current plantar-flexion exercise protocol, utilising only small muscle mass, removed any effects of the central component on peripheral BF, therefore isolating peripheral circulation. Leg vascular conductance (LVC) kinetic responses to low-intensity (30% MVC) and high-intensity (70% MVC) plantar-flexion exercise were not significantly different between males with T2D and healthy controls. Furthermore, LBF and LVC responses during a graded incremental plantar-flexion test were not significantly different between groups, a finding in line with that of Womack *et al.* (2009), who found that an incremental handgrip exercise protocol only resulted in impaired responses in individuals with T2D that displayed concomitant microvascular complications compared to both individuals with T2D free from microvascular complications and healthy ND individuals. However, during the high-intensity (70% MVC) exercise, the T2D group did tend to display a slower τ_3 response than healthy males (P=0.076), which is in line with the findings of Macananey *et al.* (2011). Furthermore, while not significant, LBF and LVC responses were numerically lower at higher workloads during the incremental test in males with T2D (figures 2.8 and 2.9). Therefore while endothelial dysfunction and peripheral circulation do not seem to impact on submaximal or low-intensity exercise, it seems likely that they may contribute at least in part to any T2D-related exercise impairment at higher intensities. The lack of any difference in either peak FVC or the decay constant during RH between males with T2D and healthy controls provides further support for the relatively healthy endothelial function in this male cohort. This finding in particular is in contrast to a number of previous studies, with a meta-analysis by Yki-Jarvinen (2003) revealing that 30 out of 31 studies investigating RH responses in individuals with T2D found T2D-related impairments in the RH response. However, it has been suggested that impairments in RH responses were due to improper matching of individuals with T2D and healthy controls. The age-, BMI-, and activity-

level matched cohort resembled that utilised by Sonne *et al.* (2007) who also failed to detect any difference in the RH response between individuals with T2D and healthy ND individuals.

The absence of any T2D-related endothelial dysfunction in response to plantar-flexion or RH is less surprising when the levels of circulating inflammatory and endothelial markers are considered. The lack of any significant elevation in pro-inflammatory cytokines, adhesion molecules or adipocytokines associated with the inflammatory state in T2D would suggest that in this cohort of males with T2D, the vasculature is reasonably intact, which would contribute to the lack of impairment in LVC kinetics or responses to the incremental plantar-flexion test. These findings should not be generalised to the general T2D population, but rather are likely a reflection of the good levels of glycaemic control displayed by the participants in this study.

The final possible factor that comes under consideration when trying to determine the source of the T2D-related impairment in exercise performance is whether or not an impairment in O_2 extraction at the capillary exists, a contention supported by Baldi *et al.* (2003). Using the Fick principle, the difference in arterial-venous oxygen content (a-v O_2 diff) was estimated. Despite lower resting a-v O_2 diff values in the T2D group, no difference was noted during exercise between the T2D group and healthy controls. This would seem to suggest that O_2 extraction was intact during exercise, a contention that is supported by the findings of Bauer *et al.* (2007), who in measuring microvascular kinetics via deoxygenated haemoglobin concentrations [HHb], found a characteristic overshoot in [HHb] levels following the onset of exercise. This overshoot would appear to be a compensatory mechanism to overcome the impaired O_2 delivery associated with slowed $\dot{V}O_2$ kinetics. Therefore, these findings suggest that in middle-aged men with well-controlled T2D, endothelial function and O_2 extraction remain relatively well preserved; with the T2D-related impairment in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics seemingly a consequence of impaired cardiac function.

The subsequent studies in chapters 3, 4, and 5 sought to determine the effect of an exercise intervention on these measures of exercise performance. The intervention was divided into two phases; a supervised twelve-week intervention with the intention of improving exercise performance and glycaemic control, and an

unsupervised home-based phase designed to maintain or further improve on the gains made during the supervised phase (Dunstan *et al.*, 2005; Mac Ananey, 2010). It is well established that training results in improvements in $\dot{V}O_2$peak in healthy individuals and diabetics alike (Brandenburg *et al.*, 1999; Loimaala *et al.*, 2003; Ostergard *et al.*, 2006; Kadoglou *et al.*, 2007; Mac Ananey, 2010). Two previous studies have also demonstrated speeding of the $\dot{V}O_2$ kinetic response in individuals with T2D following a supervised exercise intervention (Brandenburg *et al.*, 1999; Mac Ananey, 2010). However, methodologies differed between the present study and those mentioned, with Brandenburg *et al.* (1999) and Mac Ananey (2010) assessing the post-intervention responses at the same absolute workload that the pre-intervention assessment was performed at. This presents a problem, as the present results indicate that the ND EXS group had a greater magnitude of response to the exercise intervention than the T2D EXS group. If this study had therefore employed the same protocol as Brandenburg *et al.* (1999) and Mac Ananey (2010), then the T2D EXS group would have been operating at a relatively greater intensity than the ND EXS group following the exercise intervention. Therefore the current protocol was adjusted so that subjects performed steady-state cycling at the workload that corresponded to 80% of their ventilatory threshold (VT) at that visit, i.e. the workload corresponding to 80% VT was recalculated after the supervised and home-based training phases. Consequently, the exercise intervention did not result in a significant speeding of $\dot{V}O_2$ kinetics in any of the exercise groups following either phase of the exercise intervention. However, there was a significant increase in the workload at which the assessment was made following both phases of the intervention compared to baseline, and in turn the steady-state amplitude (End A) of the $\dot{V}O_2$ kinetic response was also greater after both phases of the intervention. To accommodate an increase in the amplitude of the $\dot{V}O_2$ kinetic response without any change in the time constant requires physiological adaptations to increase either O_2 delivery or extraction. HR kinetics were significantly faster in the exercise groups as a consequence of the supervised exercise intervention, suggesting that central function was improved. In the T2D and PIO groups, CO responses during steady-state cycling were greater in the exercising groups compared to the control groups after 3 months, which was maintained following the home-based phase. Estimates of a-v O_2 diff and $\dot{V}O_2$ gain did not reveal any difference in values as a consequence of the exercise

intervention, which would suggest that improved O_2 extraction was not the source of the increase in amplitude of the $\dot{V}O_2$ kinetic response.

Similar to the protocol employed for the $\dot{V}O_2$ kinetic responses, LVC kinetic responses were assessed at the workload corresponding to 30% MVC at the time of assessment. To this author's knowledge, the only prior study investigating the effect of a training intervention on LVC kinetics was performed by Mac Ananey (2010), who assessed LVC kinetic responses at 70% MVC. However, as with the $\dot{V}O_2$ kinetics intervention, Mac Ananey (2010) used the same workload from the baseline intervention during subsequent assessments. This therefore presents the same issue as the $\dot{V}O_2$ kinetic analysis, i.e. that differing levels of improvement between groups would result in differing relative workloads post-intervention. Therefore, by assessing LVC kinetics at 30% MVC at the time of assessment it ensured that the relative workload was the same between each group. Similar to the $\dot{V}O_2$ kinetic responses, LVC kinetics were not quicker after either the supervised or the home-based intervention compared with baseline, contrary to the findings of Mac Ananey (2010). As with the $\dot{V}O_2$ kinetics findings, the End A of the LVC response was increased following the supervised exercise intervention, and this increase was maintained during the home-based phase, suggesting an improvement in LVC at 30% MVC.

Supporting this finding is the increase in peak LVC during the incremental plantar-flexion test following the supervised exercise intervention, which was further increased following the home-based phase of the intervention. Additionally, there was a steepening of the slope of the LVC response (plotted relative to peak workload (expressed as 100%)) after the home-based intervention. These increases, combined with the increase in End A values of the LVC kinetic responses, suggest that the exercise intervention resulted in significant improvements in peripheral circulation, offering a potential source for the increase in exercise capacity following the exercise intervention.

The exercise intervention was successful in increasing End A of the $\dot{V}O_2$ response to cycling at 80% VT, speeding of HR kinetics; along with increases in peak LVC during the incremental plantar-flexion test and increasing End A of LVC response to steady state plantar-flexion at 30% MVC. Yet the question remains as to why the T2D-related impairment in $\dot{V}O_2$ kinetic responses to steady-state exercise at the same relative workload compared with healthy ND controls persists post-

intervention. Clearly the difference in methodology discussed from those studies of Brandenburg *et al.* (1999) and Mac Ananey (2010) is likely to have played a role in the different findings of the studies. But the physiological basis for a reduced $\dot{V}O_2$peak and slower $\dot{V}O_2$ kinetics in individuals with T2D still remains. By process of elimination, it would seem that the improvements in central cardiac function and peripheral circulation do not eliminate the impairment in either $\dot{V}O_2$peak or the $\dot{V}O_2$ kinetic response. Therefore the lack of improvement in the $\dot{V}O_2$ kinetic response may be related to the fact that the magnitude of the improvement in central cardiac function and peripheral circulation was not significant enough to bring these responses in line with those of ND individuals. Alternatively, the persistence of slower $\dot{V}O_2$ kinetic responses may be due to a lack of improvement in $\dot{V}O_2$ gain and a-v O_2 diff. This would suggest that the T2D-related impairment in exercise due to T2D is of a metabolic nature. Since metabolic responses were not assessed in this study, the potential causes of metabolic dysfunction discussed are only speculative. Previous research has indicated that males with T2D display lower mitochondrial content (Larsen *et al.,* 2009; Boushel *et al.,* 2007); while it has also been shown that individuals with T2D display reduced electron transport chain (ETC) activity and respiration when corrected for mitochondrial content (Mogensen *et al.*, 2007). Furthermore, individuals with T2D have a greater proportion of type 2x muscle fibres (Mogensen *et al.*, 2007; Larsen *et al.*, 2009), which has been linked to mitochondrial dysfunction (Conley et al., 2007), due to the different metabolic characteristics of the different fibre types (Mogensen *et al.*, 2007). The combination of these two findings provides a potential basis for the impairment in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics associated with T2D.

Exercise training has been shown to increase mitochondrial function in healthy males by ~30%, while it has also been demonstrated to restore mitochondrial function in males with T2D to equivalent levels to healthy controls (Meex *et al.*, 2010; Phielix *et al.*, 2010). Therefore if mitochondrial dysfunction was the source of the impairment in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics in males with T2D, it should be expected that the training intervention would have eliminated these differences. Since mitochondrial content and function were not directly assessed, it cannot be definitively stated that the training intervention did increase mitochondrial content. Indeed, this may not be the case, as Phielix *et al.* (2010) demonstrated a positive

relationship between mitochondrial function and insulin sensitivity. The lack of improvement in HbA_{1c} in the T2D EXS and the PIO EXS groups may indicate that the training intervention did not result in a change in mitochondrial function. But it is more likely that the lack of change in HbA_{1c} is due to the good glycaemic control evident in the individuals with T2D at the onset of exercise training. It seems safe to assume that exercise training would have successfully increased mitochondrial content as expected, but that mitochondrial function is not the sole contributing factor towards the impairment in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetic responses in males with T2D. Therefore despite the noted improvements in central cardiac function and peripheral circulation, T2D-related deficits in these parameters as well as metabolic dysfunction at the level of the mitochondria are all likely to contribute to the T2D-induced impairments in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetics.

An additional focus of the intervention studies was the effect of treatment with pioglitazone (PIO) on measures of exercise performance and endothelial function. Addition of PIO treatment did not result in any improvement in $\dot{V}O_2$peak, which contrasts with previous studies demonstrating that treatment with another drug from the thiazolidinedione family, rosiglitazone (RSG), resulted in improvements in $\dot{V}O_2$peak (Regensteiner *et al.*, 2005; Kadoglou *et al.*, 2007). This finding was surprising given that the site and method of action (PPARγ activator) are the same for both PIO and RSG. However, the lack of improvement in $\dot{V}O_2$ kinetics with PIO treatment is consistent with the findings of Regensteiner *et al.* (2005), who found that treatment with RSG, without any additional exercise intervention, failed to result in faster τ_2 values. The increase evident in CO and CI values as a consequence of PIO treatment is suggestive of a positive effect of PIO on central cardiac function, which is in agreement with previous findings (van der Meer *et al.*, 2009).

Treatment with PIO did not elicit any effect on the LVC kinetic response, with τ_3 unaffected in the PIO-treated groups. The PIO EXS group did display an increase in the End A of the LVC kinetic response, but it appears that this effect was a consequence of the exercise intervention, not the addition of PIO. This effect was replicated in the comparison of LVC responses to the incremental plantar-flexion test, as treatment with PIO did not result in any significant effect on peak LVC or the slope of the LVC profile (expressed against either N or % peak workload). Therefore, despite the known positive effects of PIO on endothelial function (Harashima *et al.*,

2009; Mazzone *et al.*, 2006; Maegawa *et al.*, 2007; Tsuchiya *et al.*, 2009; Vijay *et al.*, 2009; Nerla *et al.*, 2009; and Pitocco *et al.*, 2008), it does not appear that PIO induces any additional benefits on peripheral circulation during exercise.

Addition of PIO did not result in any change in $\dot{V}O_2$ gain, but did result in an decrease in a-v O_2 diff values at 7 months compared to baseline ($P<0.05$), suggesting that PIO treatment did not induce positive metabolic adaptations in individuals with T2D. This contention is not supported by the findings of (Rabol *et al.*, 2010) who demonstrated that treatment with PIO resulted in an increase in mitochondrial respiration per milligram of muscle. But these findings offer a possible answer to the question as to why improvements in central cardiac function and metabolic function with PIO treatment did not translate into improvements in $\dot{V}O_2$peak and $\dot{V}O_2$ kinetic responses. One possible reason for these findings contradicting the current hypothesis is the influence of gender; as in the studies that demonstrated a positive effect of RSG on $\dot{V}O_2$peak, 50% of the participants were middle-aged females (mean age ~56yrs) in the study by Regensteiner *et al.* (2005); while in the study by Kadoglou *et al.* (2007), 30 out of 49 subjects receiving RSG were middle-aged females (mean age ~58yrs). It has been shown that the greater level of vascular dysfunction in healthy post-menopausal women compared to age-matched men (Perregaux *et al.*, 1999) is exacerbated in the diabetic state, with females with T2D displaying greater levels of aortic stiffening than diabetic males (De Angelis *et al.*, 2004) and increased left-ventricular stiffness during exercise among patients with T2D (Ha *et al.*, 2008). Therefore improvements in endothelial function associated with a TZD may have a greater magnitude of impact in post-menopausal females with T2D, resulting in the improved $\dot{V}O_2$peak values evident in the studies of Regensteiner *et al.* (2005) and Kadoglou *et al.* (2007).

The smaller impact of treatment with PIO on the endothelial function of males with T2D appears to be supported by the lack of any significant difference in the majority of inflammatory and endothelial markers assessed in chapter 5. No difference in circulating levels of inflammatory markers IL-6, IL-10, or TNF-α were detected, while mRNA expression of IL-6 was also unaffected by PIO treatment. Surprisingly, mRNA expression of TNF-α was greater in the PIO-treated groups than the T2D groups, perhaps an indication of the reduced effect of PIO treatment on the endothelial function of males with T2D. It appears safe to conclude that treatment

with PIO does not elicit large enough improvements in endothelial function that will translate into an increase in $\dot{V}O_2$peak in males with T2D.

When considering the findings of the various studies, brief consideration needs to be given to the methodology employed and the equipment utilised during the testing procedures. Firstly, the duty cycle employed during the plantar-flexion exercise was set with a 2:4 s contraction:relaxation ratio to imitate the active phase of walking on a flat or inclined surface (Egana & Green, 2005). However, it is likely that a shorter duty cycle (3 s, 1:2 s contraction:relaxation ratio) would have resulted in increased accuracy in the fitting of the LVC response due to a greater number of data points, and may have further accentuated any differences between groups in the speed of the kinetic response. However, given the importance of trying to implement exercise programs in the diabetic population, it was thought that the duty cycle employed would be of more clinical relevance.

Similarly, there is some debate over whether the V-slope method can be utilised with an incremental test protocol with three minutes protocol (Amann *et al*, 2006), as it is thought that it may result in over-estimation of the VT due to the low sensitivity of the $\dot{V}O_2$ response to longer increments (Wasserman *et al*, 1990), which may in turn have resulted in overestimation of the VT in some individuals, leading to the tri-phasic response to steady-state exercise evident in some individuals. However, it has also been demonstrated that the efficiency of the V-slope response is intact with longer stage duration (Davis *et al.*, 2006), with studies utilising the V-slope method of VT detection with 3 minute increments also displaying similar results in diabetic individuals (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011; Sales *et al.*, 2011), and ~10% lower than the responses of trained individuals under the same protocol (Green *et al.*, 2010), which would be expected under typical testing conditions.

In conclusion, in chapter 2 it was demonstrated that middle-aged males with well-controlled T2D display impaired exercise performance compared to healthy age-, BMI-, and activity-level matched ND controls. This impairment was manifested as reduced $\dot{V}O_2$peak values during the graded cycle test to exhaustion, slowed τ_2 responses in $\dot{V}O_2$ kinetics and reduced CO and SV values during moderate-intensity steady-state cycling exercise. LVC responses to plantar-flexion exercise were assessed during an incremental test and during steady-state exercise at both 30% and

70% MVC; and these responses were not impaired in males with T2D; as was the case with RH responses to forearm ischaemia, indicating that the T2D-related impairment in exercise performance was likely associated with impaired central cardiac function, with a possible contribution from metabolic sources.

The exercise intervention was successful in improving $\dot{V}O_2$peak in the ND EXS and T2D EXS group, but did not result in improvements in $\dot{V}O_2$peak in the PIO EXS group, which was also the case in the PIO CTL group. In addition, the exercise intervention resulted in an increase in the End A of the $\dot{V}O_2$ kinetic responses assessed at 80% VT, while the τ_2 responses were similar, thus suggesting that a positive adaptation in either O_2 delivery or O_2 extraction was evident. PIO treatment did result in significant improvements in CO responses, but this did not translate into improvements in either $\dot{V}O_2$peak or $\dot{V}O_2$ kinetic responses. Similar to the results for $\dot{V}O_2$ kinetic response, LVC kinetic responses were not quickened as a consequence of the exercise or PIO intervention, but the End A values of the responses in the exercise groups were increased. Moreover, exercise did result in an increase in peak LVC responses and the slope of the LVC response during the incremental plantar-flexion protocol, indicating that peripheral circulation did respond to exercise training. However, treatment with PIO did not significantly improve endothelial function, and the combination of exercise and PIO, nor exercise alone, was not effective in improving the glycaemic control of these subjects. These findings were supported by the lack of change in plasma levels and mRNA expression of inflammatory and endothelial markers.

6.2 Limitations to Methodology and Analysis

One limiting factor that weakens the strength of the conclusions made is the small *n* numbers in the PIO-treated groups. In an attempt to a) satisfy the inclusion & exclusion criteria, and b) match participants for age, BMI, activity levels, and measures of glycaemic control; the study was severely limited in the number of potential candidates capable for addition of PIO to the medications already employed in their treatment of T2D. This may indeed be a contributing factor to the lack of significance in measured parameters, particularly $\dot{V}O_2$peak, as the PIO EXS group significantly improved cycling performance (expressed as peak workload and TTF) without a concomitant increase in $\dot{V}O_2$peak, in contrast with the findings of the ND EXS and T2D EXS groups. However, peak HR and RER values would indicate that $\dot{V}O_2$peak values were indeed accurate (appendix XXI).

In a similar vein, interpretation of circulating TNF-α levels were impeded by lack of samples exhibiting measurable levels of plasma TNF-α. While it therefore does not allow for interpretation of circulating levels, the fact that levels of mRNA expression of TNF-α were detected in most participants, as well as levels of other inflammatory and endothelial markers, ensures that the conclusions drawn regarding the effects of an exercise and PIO intervention on measures of endothelial function can be drawn with confidence.

Associated with the difficulties in accurately assessing TNF-α and the other markers is the potential impact of the medications being utilised in the patients' treatment of T2D prior to enrolment in the study. Evidence suggests that metformin has a positive impact on endothelial function (Mather *et al.*, 2001), while statins and various anti-hypertensives may also influence circulating levels and expression of markers of inflammation and endothelial function. There is a dearth of information describing the specific impact of these medications on the markers that were investigated in this study. As such, it is impossible to state definitively what impact these medications had on the results.

A further limitation in the methodology of the investigation was that the exercise program was designed to satisfy the criteria of >150 minutes exercise per week. However, following the onset of the study, the ACSM released further exercise directives suggesting the addition of 2-3 resistance sessions in addition to 150 minutes of aerobic exercise (Colberg *et al*, 2010). As a result, the participant's did not meet

the ACSM recommended guidelines during the supervised phase until the 8th week of the intervention. Consequently, the intervention may not have provided adequate stimulus for the physiological adaptations to occur that would have resulted in the improvements in $\dot{V}O_2$peak and τ_2 of the $\dot{V}O_2$ kinetic responses that were expected in the T2D groups. However, given that the ND EXS group did display the expected responses, it is possible that the intervention was adequate to achieve the expected responses, therefore indicating that the underlying root cause of the results is the effect of T2D.

Finally, the results of the home-based phase of the intervention are likely affected by the decrease in adherence rates during this phase. In particular, the T2D EXS group was most affected, as four participants stopped exercising altogether for a period ranging between four and thirteen weeks. Consequently, the improvements made in exercise performance by a number of subjects that maintained good compliance are masked by a reversion towards baseline values in those individuals with poor compliance. However, the main parameters of interest, such as $\dot{V}O_2$peak and τ_2 of the $\dot{V}O_2$ kinetic response, were not dramatically different at 7 months than the 3-month values when compliance was good within these groups. Therefore, the conclusion that home-based exercise is capable of maintaining the gains made during a supervised intervention is accurate for individuals that exercise for as little as two days per week.

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Appendices

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the ND EXS group.

Baseline									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	2.4226	7	58	2.1019	13	59	2.26	1.3225	**Bi-Phasic**
2	1.4512	7	58	0.9870	13	60	2.25	4.1545	**Quad-Phasic**
3	3.1104	7	58	2.1180	13	59	2.26	4.0606	**Quad-Phasic**
4	4.2293	7	59	4.7374	13	60	2.25	-0.9475	**Bi-Phasic**
5	2.9939	7	59	2.3836	13	59	2.26	2.2187	**Bi-Phasic**
6	14.6345	7	59	13.6626	13	59	2.26	0.6165	**Bi-Phasic**
7	1.1944	7	58	1.0602	13	59	2.26	1.0969	**Bi-Phasic**
8	4.9336	7	58	2.7999	13	56	2.27	6.2236	**Quad-Phasic**
9	24.4695	7	59	7.1290	13	57	2.26	20.2697	**Quad-Phasic**
10	3.4019	7	58	2.7450	13	59	2.26	2.0739	**Bi-Phasic**

3 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	2.6107	7	58	2.2477	13	59	2.26	1.3994	**Bi-Phasic**
2	2.3990	7	58	1.1561	13	57	2.26	8.9581	**Quad-Phasic**
3	6.4438	7	60	2.6128	13	59	2.26	12.7077	**Quad-Phasic**
4	3.6069	7	57	3.3053	13	58	2.26	0.7756	**Bi-Phasic**
5	0.9831	7	59	0.7207	13	59	2.26	3.1564	**Quad-Phasic**
6	5.2615	7	59	4.5291	13	59	2.26	1.4014	**Bi-Phasic**
7	2.3411	7	59	0.3712	13	57	2.27	44.2274	**Quad-Phasic**
8	3.7074	7	57	2.3463	13	59	2.26	5.0277	**Quad-Phasic**
9	10.2566	7	59	2.4460	13	57	2.27	26.6095	**Quad-Phasic**
10	5.7128	7	60	3.0537	13	59	2.26	7.5468	**Quad-Phasic**

7 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	1.0729	7	58	0.9132	13	58	2.26	1.4866	**Bi-Phasic**
2	1.2513	7	58	1.2356	13	58	2.26	0.1077	**Bi-Phasic**
3	4.5710	7	56	1.0989	13	58	2.26	26.8575	**Quad-Phasic**
4	9.5478	7	58	11.4817	13	59	2.26	-1.4597	**Bi-Phasic**
5	0.7536	7	59	0.4781	13	58	2.26	4.8979	**Quad-Phasic**
6	18.7446	7	56	15.6184	13	55	2.27	1.6013	**Bi-Phasic**
7	2.5883	7	58	1.1164	13	57	2.26	10.9858	**Quad-Phasic**
8	4.6787	7	59	3.8051	13	59	2.26	1.9898	**Bi-Phasic**
9	11.2258	7	58	2.8220	13	59	2.26	25.8090	**Quad-Phasic**
10	4.0371	7	58	1.7743	13	58	2.26	10.8395	**Quad-Phasic**

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the ND CTL group.

Baseline									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	3.0299	7	59	2.8963	13	58	2.26	0.3920	Bi-Phasic
2	1.8182	7	59	1.6765	13	58	2.26	0.7184	Bi-Phasic
3	21.6158	7	59	17.0718	13	58	2.26	2.2624	Quad-Phasic
4	1.1614	7	57	1.1782	13	59	2.26	-0.1237	Bi-Phasic
5	2.9761	7	56	3.8097	13	58	2.26	-1.8597	Bi-Phasic
6	10.1799	7	60	1.2411	13	58	2.26	61.2216	Quad-Phasic
7	8.8183	7	58	1.2113	13	58	2.26	53.3817	Quad-Phasic
8	4.9039	7	59	2.2763	13	60	2.25	10.1963	Quad-Phasic
9	5.0239	7	58	2.8162	13	58	2.26	6.6636	Quad-Phasic

3 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	4.1128	7	59	0.6166	13	57	2.26	47.2543	Quad-Phasic
2	1.6481	7	60	1.1231	13	59	2.26	4.0510	Quad-Phasic
3	10.4590	7	58	7.0150	13	58	2.26	4.1731	Quad-Phasic
4	2.5868	7	59	2.2515	13	59	2.26	1.2908	Bi-Phasic
5	3.5121	7	59	2.7073	13	60	2.25	2.6258	Quad-Phasic
6	3.0477	7	58	1.5150	13	58	2.26	8.5991	Quad-Phasic
7	26.8667	7	60	2.2147	13	59	2.26	96.4710	Quad-Phasic
8	6.7383	7	58	1.2899	13	59	2.26	36.6082	Quad-Phasic
9	4.4710	7	57	4.8565	13	58	2.26	-0.6747	Bi-Phasic

7 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	7.2704	7	60	2.2789	13	59	2.26	18.9830	Quad-Phasic
2	0.8601	7	58	0.6839	13	59	2.26	2.2330	Bi-Phasic
3	23.9069	7	60	12.5364	13	60	2.26	8.0118	Quad-Phasic
4	1.2166	7	58	0.5472	13	57	2.26	10.1930	Quad-Phasic
5	4.9807	7	60	5.3374	13	60	2.25	-0.5903	Bi-Phasic
6	1.4933	7	56	1.3935	13	57	2.26	0.5974	Bi-Phasic
7	30.5400	7	60	2.5728	13	58	2.26	92.3975	Quad-Phasic
8	3.0779	7	59	0.7159	13	60	2.25	29.1453	Quad-Phasic
9	10.1728	7	57	7.9516	13	56	2.27	2.2812	Quad-Phasic

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the T2D EXS group.

Baseline									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	4.6639	7	59	1.3471	13	58	2.26	20.9281	Quad-Phasic
2	4.4672	7	58	2.4808	13	58	2.26	6.8062	Quad-Phasic
3	1.5699	7	59	1.0538	13	59	2.26	4.2444	Quad-Phasic
4	0.3406	7	57	0.3406	13	58	2.26	-0.0003	Bi-Phasic
5	2.3039	7	58	1.3415	13	56	2.26	5.8591	Quad-Phasic
6	6.0373	7	58	5.0866	13	58	2.26	1.5887	Bi-Phasic
7	9.5364	7	60	3.1100	13	59	2.26	17.9089	Quad-Phasic
8	1.1990	7	59	0.6964	13	58	2.26	6.1334	Quad-Phasic
9	0.3826	7	58	0.2211	13	58	2.26	6.2123	Quad-Phasic
10	1.9640	7	59	1.5287	13	59	2.26	2.4674	Quad-Phasic
11	3.5649	7	59	1.1801	13	57	2.26	16.8396	Quad-Phasic

3 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	6.0060	7	60	1.2450	13	60	2.26	33.7813	Quad-Phasic
2	3.8858	7	59	3.0748	13	58	2.26	2.2418	Bi-Phasic
3	2.3007	7	59	1.2598	13	58	2.26	7.0234	Quad-Phasic
4	2.5284	7	57	1.8549	13	58	2.26	3.0863	Quad-Phasic
5	1.4321	7	59	0.8591	13	58	2.26	5.6701	Quad-Phasic
6	2.7716	7	58	2.5937	13	59	2.26	0.5946	Bi-Phasic
7	11.3937	7	58	3.8071	13	58	2.26	16.9387	Quad-Phasic
8	9.5451	7	57	6.4228	13	57	2.26	4.0511	Quad-Phasic
9	0.9115	7	59	0.8231	13	58	2.26	0.9126	Bi-Phasic
10	1.8636	7	58	1.1957	13	59	2.26	4.8411	Quad-Phasic
11	3.2273	7	60	1.6596	13	59	2.26	8.1862	Quad-Phasic

7 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
1	3.2854	7	59	1.3995	13	59	2.26	11.6782	Quad-Phasic
2	1.8410	7	59	1.5219	13	59	2.26	1.8173	Bi-Phasic
3	1.5009	7	57	1.3437	13	60	2.26	1.0331	Bi-Phasic
4	2.9669	7	57	4.0475	13	57	2.26	-2.2249	Bi-Phasic
5	2.2813	7	59	0.8946	13	59	2.26	13.4350	Quad-Phasic
6	2.4322	7	59	1.6937	13	60	2.26	3.8518	Quad-Phasic
7	5.4463	7	58	2.4771	13	58	2.26	10.1886	Quad-Phasic
8	5.6220	7	60	3.7193	13	57	2.26	4.2632	Quad-Phasic
9	0.8488	7	59	0.5246	13	57	2.26	5.1504	Quad-Phasic
10	1.7783	7	57	1.8262	13	57	2.26	-0.2184	Bi-Phasic
11	4.6739	7	58	3.0512	13	58	2.26	4.5206	Quad-Phasic

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the T2D CTL group.

Baseline									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	3.5281	7	60	3.2267	13	60	2.25	0.8252	**Bi-Phasic**
2	4.0646	7	58	3.5688	13	58	2.26	1.1809	**Bi-Phasic**
3	1.7402	7	57	1.4777	13	59	2.26	1.5394	**Bi-Phasic**
4	2.5904	7	58	1.1813	13	57	2.26	9.9401	**Quad-Phasic**
5	1.1001	7	57	0.8070	13	58	2.26	3.0878	**Quad-Phasic**
6	7.5042	7	60	0.9409	13	59	2.26	60.4516	**Quad-Phasic**
7	1.2970	7	59	0.9365	13	59	2.26	3.3368	**Quad-Phasic**
8	10.5911	7	59	4.2131	13	59	2.26	13.1203	**Quad-Phasic**
9	6.8057	7	58	3.2653	13	58	2.26	9.2160	**Quad-Phasic**
10	2.7717	7	59	1.7933	13	58	2.26	4.6375	**Quad-Phasic**
11	1.3595	7	58	0.8376	13	59	2.26	5.4004	**Quad-Phasic**

3 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	3.1443	7	58	3.1900	13	59	2.26	-0.1241	**Bi-Phasic**
2	3.1704	7	59	1.6193	13	58	2.26	8.1421	**Quad-Phasic**
3	2.3602	7	59	1.9179	13	59	2.26	1.9987	**Bi-Phasic**
4	1.3254	7	57	1.2065	13	57	2.26	0.8215	**Bi-Phasic**
5	0.7272	7	58	0.5791	13	58	2.26	2.1743	**Bi-Phasic**
6	0.7510	7	57	0.4503	13	58	2.26	5.6755	**Quad-Phasic**
7	0.4898	7	59	0.2692	13	59	2.26	7.1037	**Quad-Phasic**
8	2.0391	7	57	1.8926	13	57	2.26	0.6452	**Bi-Phasic**
9	3.2339	7	56	1.6160	13	57	2.26	8.3432	**Quad-Phasic**
10	2.8634	7	58	2.6710	13	58	2.26	0.6124	**Bi-Phasic**
11	0.9330	7	56	0.8504	13	58	2.26	0.8258	**Bi-Phasic**

7 Months									
	Bi-Phasic			Quad-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	7.2228	7	60	5.0276	13	59	2.26	3.7840	**Quad-Phasic**
2	7.9711	7	58	6.8027	13	59	2.26	1.4885	**Bi-Phasic**
3	4.2380	7	59	2.6588	13	59	2.26	5.1476	**Quad-Phasic**
4	5.7617	7	59	3.8563	13	58	2.26	4.1999	**Quad-Phasic**
5	1.5142	7	58	1.0154	13	57	2.26	4.0936	**Quad-Phasic**
6	3.6124	7	59	1.6786	13	58	2.26	9.7929	**Quad-Phasic**
7	2.7084	7	59	0.9088	13	60	2.26	17.4924	**Quad-Phasic**
8	2.3010	7	58	1.1450	13	58	2.26	8.5818	**Quad-Phasic**
9	11.0042	7	58	7.4647	13	58	2.26	4.0304	**Quad-Phasic**
10	1.7720	7	59	1.1461	13	58	2.26	4.6424	**Quad-Phasic**
11	2.8527	7	58	2.9609	13	59	2.26	-0.3167	**Bi-Phasic**

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the PIO EXS group.

Baseline									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	2.9082	7	59	2.9119	13	59	2.26	-0.0109	**Bi-Phasic**
2	3.3535	7	57	2.2564	13	58	2.26	4.1331	**Quad-Phasic**
3	15.7155	7	59	7.1536	13	57	2.26	9.9739	**Quad-Phasic**
4	18.1516	7	58	9.8859	13	58	2.26	7.1069	**Quad-Phasic**
5	14.2270	7	59	5.9230	13	58	2.26	11.9168	**Quad-Phasic**
6	4.5540	7	58	4.6332	13	58	2.26	-0.1452	**Bi-Phasic**

3 Months									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	2.3726	7	59	2.0232	13	59	2.26	1.4970	**Bi-Phasic**
2	2.0359	7	58	1.3893	13	59	2.26	4.0332	**Quad-Phasic**
3	9.1474	7	59	9.2065	13	59	2.26	-0.0556	**Bi-Phasic**
4	11.7868	7	58	8.6156	13	58	2.26	3.1286	**Quad-Phasic**
5	3.7437	7	58	2.6681	13	59	2.26	3.4940	**Quad-Phasic**
6	5.8079	7	58	6.0423	13	58	2.26	-0.3298	**Bi-Phasic**

7 Months									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	5.6053	7	58	2.1833	13	59	2.26	13.5833	**Quad-Phasic**
2	2.3256	7	58	1.5638	13	58	2.26	4.1403	**Quad-Phasic**
3	16.4493	7	59	12.5105	13	59	2.26	2.7286	**Quad-Phasic**
4	7.1034	7	58	6.8782	13	59	2.26	0.2837	**Bi-Phasic**
5	6.9852	7	59	2.3182	13	58	2.26	17.1119	**Quad-Phasic**
6	3.1381	7	59	2.1712	13	59	2.26	3.8597	**Quad-Phasic**

Appendix I: Goodness of fit data for comparison of the bi-phasic and quad-phasic models used to fit LVC kinetic responses to contractions at 30% MVC in the PIO CTL group.

Baseline									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	2.7084	7	59	0.9088	13	60	2.25	4.2421	**Quad-Phasic**
2	2.8527	7	58	2.9609	13	59	2.26	-1.2651	**Bi-Phasic**
3	5.8092	7	59	4.9448	13	59	2.26	1.5150	**Bi-Phasic**
4	4.2380	7	59	2.6588	13	59	2.26	5.1476	**Quad-Phasic**
5	5.7617	7	59	3.8563	13	58	2.26	4.1999	**Quad-Phasic**

3 Months									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	0.5939	7	58	0.4101	13	59	2.26	3.8835	**Quad-Phasic**
2	1.7339	7	60	0.3308	13	58	2.26	36.0553	**Quad-Phasic**
3	3.3122	7	58	3.2890	13	59	2.26	0.0611	**Bi-Phasic**
4	3.3947	7	59	1.3557	13	57	2.26	12.5328	**Quad-Phasic**
5	4.5873	7	58	1.8920	13	58	2.26	12.1088	**Quad-Phasic**

7 Months									
	Bi-Phasic			**Quad-Phasic**					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	***Model***
1	0.6801	7	57	0.3858	13	57	2.26	6.3568	**Quad-Phasic**
2	1.4076	7	57	1.4638	13	58	2.26	-0.3264	**Bi-Phasic**
3	1.7927	7	58	1.6145	13	58	2.26	0.9385	**Bi-Phasic**
4	4.2324	7	59	4.0670	13	59	2.26	0.3526	**Bi-Phasic**
5	1.9014	7	56	1.5372	13	58	2.26	2.0139	**Bi-Phasic**

Appendix II: Participant Information and consent form for provided to individuals with T2D.

PARTICIPANT INFORMATION FORM FOR DIABETICS

1. Title of study: The effect of combined treatment with exercise and Pioglitazone versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2D) and non-diabetic controls.

2. Introduction: The current proposal aims to investigate the effect of combined treatment with exercise and Pioglitazone versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with T2D and non-diabetic controls. The short-term objectives are to measure blood vessel and inflammatory markers and the initial responses of blood flow and oxygen consumption during a calf-exercise and cycling-exercise before and after a 12-week supervised exercise programme. As a long-term objective, we will explore the effectiveness of a home-based training programme in maintaining the benefits acquired during the supervised training programme.

3. Procedures: You will be one of the 60 volunteers recruited for this study and you must be between 30 and 75 years old. Out of 60, 30 will receive Pioglitazone therapy), and the other 30 will not. Before the commencement of the project, you will undergo a full medical examination and a stress test. If approved for participation in this study, you will be assigned into an EXERCISE or CONTROL (non-exercise) group. Participants in the EXERCISE group will perform a 12-week supervised exercise-training programme. This will be followed by a 4-month period of prescribed and periodically monitored home-based training programme. Participants in the CONTROL group will continue normal life for the following 7 months.

ALL PARTICIPANTS (both EXERCISE and CONTROL groups) will measure their energy consumption (by filling 3-day food diaries) and will visit the Human Laboratory in the Department of Physiology in Trinity College Dublin on 2 days (separated by 72 hours) on 3 OCCASIONS: prior to and at completion of the supervised programme, as well as at the completion of the home-based training programme.

- Visits to the laboratory (2 visits Trinity College Dublin):

Visit 1: Cycling $\dot{V}O_2$ max (maximal cycling test to failure) & Calf blood flow (visit will last up to 180 min)

During this session you will perform low intensity exercises on the calf ergometer (exercise machine). Each contraction will last 2 seconds, accompanied by a 4 second rest. You will do this 4 times for 5-6 minutes at a time, for a total of 50-60 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).

After a period of recovery (~20mins), you will then perform an incremental test on the calf ergometer. During this test, the force of contraction will be increased every 2 minutes, requiring you to increase your level of effort until you reach and cannot sustain a maximum effort.

While performing these tests, the investigator will be monitoring your blood pressure, heart rate, and leg blood circulatory data.
In addition, prior to the calf incremental test, we will cut off the circulation to your forearm and calf muscle by inflating a cuff to a high pressure around your upper arm and your thigh. After a 5-minute period, the cuff will be released, and blood flow will be measured by observing changes in the girth of the forearm and calf muscle respectively (Reactive hyperaemia).
You will have your height and weight recorded and you will be familiarised with the cycle ergometer. 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 40watts every 3 minutes.

Visit 2: Submaximal cycling trials (session will last up to 140 min)
You will perform four 6-minute submaximal trials of constant-load cycling exercise, with a 15-minute rest between bouts.
After a sufficient recovery period, you will perform an additional 2 bouts of 6 minute constant-load cycling. Recordings of cardiac output will be made at rest, and after the first and fourth minute of cycling.

- Supervised exercise training programme (Exercise Group Only):
You will be asked to perform a 12 week supervised exercise programme, training three times per week for 1 hour. The programme will include a combination of progressive aerobic and resistance training exercises. The supervised training programme will be carried out in Monkstown Pool & Fitness Center (or surrounding center). During the training sessions you will wear a heart rate monitor to adhere to the target intensity of training. Blood pressure will be monitored before and after the exercise sessions.

- Home-based training programme:
When the 12-week supervised training programme has been completed, you will be asked to attend a workshop with the investigators of the study and Dr. Mikel Egaña based on creating a home-based exercise programme. Then you will be prescribed a programme that incorporates your own exercise preferences and you will perform all exercises without supervision at your home, outdoor track, or in a fitness club. The same guidelines for exercise will be applied to all subjects as follows: a) at least 3 exercise sessions per week (either aerobic, resistance or combined sessions including aerobic and resistance training) b) at least 2 aerobic exercise sessions per week c) at least 2 resistance training session per week and d) at least 25 minutes of exercise (excluding rest periods) should be performed. You will be given a training diary and instructed on how to record your training mode, frequency, duration, intensity and comfort.

4. Benefits:
Lack of exercise and general physical inactivity can frequently incapacitate inactive people with and without diabetes to the point where they become socially isolated and

depressed. Consequently, improving exercise tolerance following the exercise intervention, can increase physical activity, and lessen social isolation and depression. Understanding the magnitude and causes of the cardiovascular effects to exercise training is very important to potentially target appropriate treatment interventions, such as optimal exercise prescriptions and pharmacological agents. An optimal exercise prescription will help to improve exercise performance (even at very low workloads) and thereby prevent increasing disability and improve quality of life in inactive men and women with and without diabetes.
This study should also help to provide greater insight into the mechanisms of Pioglitazone's effect on treating diabetes. In addition, it is expected that we will gain insights into the effectiveness of a prescribed, home-based exercise programme in maintaining any benefits acquired during a supervised exercise programme, after intensive supervision has ceased. This will help in educating and motivating participants to take greater responsibility for their own exercise.

5. Risks:
Reactive hyperemia: Endothelial function will be determined using forearm and calf muscle reactive hyperemic blood flow by Venous Occlusion Plethysmography. An arm cuff will be inflated at a pressure of 220 mmHg for 5 minutes. After this arterial occlusion, changes in girth of the forearm will be measured respectively. The test may create a feeling of numbness during arterial occlusion although it is very well tolerated. So far, over 1000 tests have been performed both in young and older individuals in our lab with no adverse outcomes.

$\dot{V}O_2$ max and constant-load cycle test: The study involves both a $\dot{V}O_2$max test and a constant-load cycling test to determine $\dot{V}O_2$max, $\dot{V}O_2$ kinetics, and cardiac output both prior to and following the intervention. Due to its nature, there will be physical stress experienced by all subjects. With a $\dot{V}O_2$max test there is always a very slight risk that participants may suffer a coronary attack (approximately one occurrence per 5,000 tests). All $\dot{V}O_2$max tests will be preceded by a stress test using 12-lead ECG and blood pressure measurements and will be supervised by an exercise physiologist. A qualified medical doctor will deem the subject suitable to complete an exercise test to exhaustion.

Exercise training: Exercise training may result in muscle tightness, soreness, fatigue and rarely a pulled muscle. Though the intensity of exercise will be closely monitored and an exercise physiologist will supervise each session, the risk of a coronary event, although very minor, does exist.

Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area. All contaminated materials will be disposed of in Sharps containers in line with college / faculty policy and best laboratory practices.

Pioglitazone: One recent study has highlighted an increased risk of coronary heart failure with use of Pioglitazone in some subjects. A qualified medical doctor will deem the subject suitable for prescription of Pioglitazone prior to testing. In some cases, prolonged use has been associated with small increases in bodyweight, due to an increase in subcutaneous body fat and increased fluid retention.

6. Exclusion from participation:

Inclusion criteria:

You will be **included** in this study if you **are 30 to 75 years old** the length of time since diagnosis of your diabetes is less than 10 years, your glycosylated haemoglobin levels (HbA_{1c}) are <10% (adequate control on therapy), you are sedentary ($\leq$ 1 bout of exercise per week and have not participated in a continuous exercise program for the last 6 months) and if your diabetes is treated by diet or oral agents (hypoglycaemic and anti-platelets will be allowed).

Exclusion criteria:

You will be **excluded** if your diabetes is treated with an antidiabetic drug of the thiazolidinedione (TZD) family at the start of the testing period; or by insulin (suggests more advanced diabetes). You will also be excluded if you suffer from persistent proteinuria (urine protein >200mg/dl) or have high creatinine levels (suggestive of renal disease, which can alter exercise performance); if your systolic blood pressure is higher than 170mmHg at rest or 220mmHg with exercise; or if your diastolic blood pressure is higher than 95mmHg at rest or 105mmHg with exercise. If you are a controlled hypertensive you can still be admitted to the study. Additional grounds for your exclusion include the presence of a comorbid condition and/or diabetes complications. Absence of comorbid conditions will be established and confirmed by history, physical examination and laboratory testing. To confirm coronary arterial disease and established peripheral arterial disease (PAD), you will undergo a stress test in either St. Columcille's Hospital, St. Vincent's Hospital or the Human Laboratory of the Department of Physiology TCD with a 12-lead electrocardiogram and blood pressure measurements and the resting ankle:brachial index will be measured.

7. Confidentiality:
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.

8. Compensation:
This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

9. Voluntary Participation:
You have volunteered to participate in this study. You may quit 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.

10. Stopping the study:
You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This trial has Research Ethics Committee approval from Trinity College Dublin and from St. Vincent's Hospital (Irishtown, Dublin).

12. Further information:
You can get more information or answers to your questions about the study, your participation in the study, and your rights, from the lead investigator (Mr Eamonn O'Connor (087-9631348 / oconnoeg@tcd.ie), or Dr Mikel Egaña, (01-8963728 / 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.

All travel expenses to attend all lab sessions in Trinity College (i.e. car petrol/parking or bus/train/taxi fares) will be covered by the research team

DECLARATION:

I have read, or had read to me, the information leaflet for this project and I understand the contents. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I understand that I may withdraw from the study at any time and I have received a copy of this agreement.

PARTICIPANT'S NAME: .....

CONTACT DETAILS: .....

PARTICIPANT'S SIGNATURE:.....

Date:.....

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE:.....

Date:.....

Appendix II: Participant Information and consent form for provided to ND individuals.

PARTICIPANT INFORMATION FORM FOR NON-DIABETICS

1. Title of study: The effect of combined treatment with exercise and Pioglitazone (PIO) versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2D) and non-diabetic controls.

2. Introduction: The current proposal aims to investigate the effect of combined treatment with exercise and pioglitazone versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with T2D and non-diabetic controls. The short-term objectives are to measure blood vessel and inflammatory markers and the initial responses of blood flow and oxygen consumption during a calf-exercise and cycling-exercise before and after a 12-week supervised exercise programme. As a long-term objective, we will explore the effectiveness of a home-based training programme in maintaining the benefits acquired during the supervised training programme.

3. Procedures: You will be one of the 30 non-diabetic participants recruited for this study and you must be between 30 and 75 years old. Before the commencement of the project, you will undergo a full medical examination. If approved for participation in this study, you will be assigned into an EXERCISE or CONTROL (non-exercise) group. Participants in the EXERCISE group will perform a 12-week supervised exercise-training programme. This will be followed by a 4-month period of prescribed and periodically monitored home-based training programme. Participants in the CONTROL group will continue normal life for the following 7 months.

ALL PARTICIPANTS (both EXERCISE and CONTROL groups) will measure energy consumption (by filling 3-day food diaries) and will visit the Human Laboratory in the Department of Physiology in Trinity College Dublin on 2 days (separated by 72 hours) on 3 OCCASIONS: prior to and at completion of the supervised programme, as well as at the completion of the home-based training programme.

- Visits to the laboratory (2 visits Trinity College Dublin):

Visit 1: Cycling $\dot{V}O_2$ max (maximal cycling test to failure) & Calf blood flow (visit will last up to 180 min)

During this session you will perform low intensity exercises on the calf ergometer (exercise machine). Each contraction will last 2 seconds, accompanied by a 4 second rest. You will do this 4 times for 5-6 minutes at a time, for a total of 50-60 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).

After a period of recovery (~20mins), you will then perform an incremental test on the calf ergometer. During this test, the force of contraction will be increased every 2 minutes, requiring you to increase your level of effort until you reach and cannot sustain a maximum effort.

While performing these tests, the investigator will be monitoring your blood pressure, heart rate, and leg blood circulatory data.
In addition, prior to the calf incremental test, we will cut off the circulation to your forearm and calf muscle by inflating a cuff to a high pressure around your upper arm and your thigh. After a 5-minute period, the cuff will be released, and blood flow will be measured by observing changes in the girth of the forearm and calf muscle respectively (Reactive hyperaemia).
You will have your height and weight recorded and you will be familiarised with the cycle ergometer. 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 40watts every 3 minutes.

Visit 2: Submaximal cycling trials (session will last up to 180 min)
You will perform four 6-minute submaximal trials of constant-load cycling exercise, with a 15-minute rest between bouts. The intensity of the exercise bouts will be set at 80% of the subject's ventilatory threshold, determined from Visit #1. Recordings of $\dot{V}O_2$ kinetics will be made during these bouts.
After a sufficient recovery period, you will perform an additional 2 bouts of 6 minute constant-load cycling. Recordings of cardiac output will be made at rest, and after the first and fourth minute of cycling.

- Supervised exercise training programme (Exercise group only):
You will be asked to perform a 12-week supervised exercise programme, training three times per week for 1 hour. The programme will include a combination of progressive aerobic and resistance training exercises. The supervised training programme will be carried out in Monkstown Pool & Fitness Center (or surrounding center). During the training sessions you will wear a heart rate monitor to adhere to the target intensity of training. Blood pressure will be monitored before and after the exercise sessions.

- Home-based training programme:
When the 12-week supervised training programme has been completed, you will be asked to attend a workshop with the investigators of the study and Dr. Mikel Egaña based on creating a home-based exercise programme. Then you will be prescribed a programme that incorporates your own exercise preferences and you will perform all exercises without supervision at your home, outdoor track, or in a fitness club. The same guidelines for exercise will be applied to all subjects as follows: a) at least 3 exercise sessions per week (either aerobic, resistance or combined sessions including aerobic and resistance training) b) at least 2 aerobic exercise sessions per week c) at least 2 resistance training session per week and d) at least 25 minutes of exercise (excluding rest periods) should be performed. You will be given a training diary and instructed on how to record your training mode, frequency, duration, intensity and comfort.

4. Benefits:
Lack of exercise and general physical inactivity can frequently incapacitate inactive people to the point where they become socially isolated and depressed. Consequently, improving exercise tolerance following the exercise intervention, can increase

physical activity, and lessen social isolation and depression. An optimal exercise prescription will help to improve exercise performance (even at very low workloads) and thereby improve quality of life in inactive men and women.
In addition, it is expected that we will gain insights into the effectiveness of a prescribed, home-based exercise programme in maintaining any benefits acquired during a supervised exercise programme, after intensive supervision has ceased. This will help in educating and motivating participants to take greater responsibility for their own exercise.

5. Risks:
Reactive hyperemia: Endothelial function will be determined using forearm and calf muscle reactive hyperemic blood flow by Venous Occlusion Plethysmography. An arm cuff will be inflated at a pressure of 220 mmHg for 5 minutes. After this arterial occlusion, changes in girth of the forearm will be measured respectively. The test may create a feeling of numbness during arterial occlusion although it is very well tolerated. So far, over 1000 tests have been performed both in young and older individuals in our lab with no adverse outcomes.

$\dot{V}O_2$ max and constant-load cycle test: The study involves both a $\dot{V}O_2$max test and a constant-load cycling test to determine $\dot{V}O_2$max, $\dot{V}O_2$ kinetics, and cardiac output both prior to and following the intervention. Due to its nature, there will be physical stress experienced by all subjects. With a $\dot{V}O_2$max test there is always a very slight risk that participants may suffer a coronary attack (approximately one occurrence per 5,000 tests). All $\dot{V}O_2$max tests will be preceded by a stress test using 12-lead ECG and blood pressure measurements and will be supervised by an exercise physiologist. A qualified medical doctor will deem the subject suitable to complete an exercise test to exhaustion.

Exercise training: Exercise training may result in muscle tightness, soreness, fatigue and rarely a pulled muscle. Though the moderate intensity of exercise will be closely monitored and an exercise physiologist will supervise each session, the risk of a coronary event, although very minor, does exist.

Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area. All contaminated materials will be disposed of in Sharps containers in line with college / faculty policy and best laboratory practices.

6. Exclusion from participation:
Inclusion criteria:
You will be **included** in this study if you **are 30 to 75 years old.** Additionally, you must be sedentary ($\leq$ 1 bout of exercise per week and have not participated in a continuous exercise program for the last 6 months), not be taking any medications, and have normal HbA_{1c} to be considered for inclusion,
Exclusion criteria:
You must be free of any active medical problems.

7. Confidentiality:
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.

8. Compensation:
This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

9. Voluntary Participation:
You have volunteered to participate in this study. You may quit 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.

10. Stopping the study:
You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This trial has Research Ethics Committee approval from Trinity College Dublin and from St. Vincent's Hospital (Irishtown, Dublin).

12. Further information:
You can get more information or answers to your questions about the study, your participation in the study, and your rights, from the investigator (Mr Eamonn O'Connor (087-9631348 / oconnoeg@tcd.ie), or Dr Mikel Egaña, (01-8963728 / 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.

All travel expenses to attend all lab sessions in Trinity College (i.e. car petrol/parking or bus/train/taxi fares) will be covered by the research team

DECLARATION:

I have read, or had read to me, the information leaflet for this project and I understand the contents. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I understand that I may withdraw from the study at any time and I have received a copy of this agreement.

PARTICIPANT'S NAME: .....

CONTACT DETAILS: .....

PARTICIPANT'S SIGNATURE:.....

Date:.....

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE:.....

Date:.....

Appendix III: Medical Questionnaire to be filled out by ND individuals during their medical examination prior to participation.

DEPARTMENT OF PHYSIOLOGY, TRINITY COLLEGE, DUBLIN.

MEDICAL QUESTIONNAIRE

Project Title:_____

Supervising Researcher:_____

Principal Investigator:_____

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.

Subject Name:_____ Date:_____

Height:_____ Weight:_____

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

Contact Telephone Numbers:_____

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 Subject:_____ Date:_____

Signature of Supervising Researcher:_____ Date:_____

Signature of Principal Investigator:_____ Date:_____

Following completion of this survey and a physical assessment of he 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:_____

Appendix IV: Calibration data for the Calf Ergometer. Coefficient of Variation values are presented for 20kg and 80kg loads at the bottom right of the spreadsheet.

Test day	20kg mV	80kg mV	Test day	20kg mV	80kg mV	Test day	20kg mV	80kg mV	Test day	20kg mV	80kg mV
1	752.126	2888.126	41	732.602	2811.602	81	768.4485	2892.449	121	787.9595	2977.96
2	729.65	2766.65	42	780.621	2970.621	82	756.4485	2871.449	122	795.63	3033.63
3	712.5325	2581.533	43	780.702	2748.702	83	796.8475	3091.848	123	783.1815	2922.182
4	765.186	2889.186	44	749.1065	2825.107	84	768.5955	2880.596	124	772.257	2929.257
5	796.635	2938.635	45	750.8805	2904.881	85	737.4835	2771.484	125	805.8085	3082.809
6	769.367	2971.367	46	738.996	2835.996	86	763.7275	2902.728	126	787.596	2953.596
7	799.228	2992.228	47	791.3115	2984.312	87	773.1755	2996.176	127	784.789	2977.789
8	786.124	3033.124	48	756.391	2901.391	88	784.4755	3019.476	128	776.4515	2978.452
9	747.7365	2790.737	49	749.5735	2873.574	89	777.2895	2928.29	129	813.0835	3096.084
10	696.1105	2637.111	50	763.426	2914.426	90	714.618	2628.618	130	763.8635	2893.864
11	795.3805	3036.381	51	807.897	3102.897	91	794.505	3059.505	131	800.563	3056.563
12	809.1365	3125.137	52	785.7465	2987.747	92	825.2135	3156.214	132	786.0295	3012.03
13	817.3765	2998.377	53	796.085	3040.085	93	799.4245	3013.425	133	765.2195	2838.22
14	777.1735	2922.174	54	759.508	2937.508	94	788.275	2990.275	134	775.8175	2908.818
15	808.288	3013.288	55	749.5455	2798.546	95	788.511	3014.511	135	776.7265	2885.727
16	768.2355	2919.236	56	815.571	3107.571	96	761.2785	2924.279	136	750.032	2784.032
17	744.903	2847.903	57	733.7195	2665.72	97	773.075	2957.075	137	847.888	3232.888
18	784.761	3028.761	58	783.2135	2913.214	98	780.857	2976.857	138	848.0755	3275.076
19	845.9795	3221.98	59	754.2525	2854.253	99	775.1745	2956.175	139	770.6695	2897.67
20	794.7925	3047.793	60	753.7465	2829.747	100	757.0375	2860.038	140	789.0035	2988.004
21	740.394	2876.394	61	796.541	3013.541	101	806.944	3068.944	141	848.647	3201.647
22	764.8645	2969.865	62	759.356	2844.356	102	770.6965	2939.697	142	810.561	3036.561
23	737.8845	2837.885	63	781.4645	2983.465	103	783.368	3015.368	143	835.431	3163.431
24	740.7035	2855.704	64	730.2995	2749.3	104	848.5445	3272.545	144	838.066	3181.066
25	786.3645	3018.365	65	773.497	2936.497	105	798.8325	3048.833	145	800.1635	3074.164
26	762.988	2919.988	66	769.403	2872.403	106	781.984	2983.984	146	794.405	3008.405
27	740.5275	2819.528	67	723.6835	2691.684	107	786.2855	2985.286	147	750.3625	2802.363
28	720.4775	2703.478	68	736.4455	2758.446	108	800.871	3053.871	148	792.9495	3045.95
29	767.6365	2921.637	69	751.55	2851.55	109	814.9805	3160.981	149	839.7475	3209.748
30	768.9265	2961.927	70	783.728	2916.728	110	779.3045	2981.305	150		
31	707.2035	2687.204	71	782.857	2969.857	111	800.7595	3068.76	151		
32	739.5265	2848.527	72	766.613	2905.613	112	780.6465	2973.647	152		
33	755.328	2900.328	73	823.2325	3196.233	113	793.128	3031.128	153		
34	759.843	2985.843	74	746.965	2771.965	114	803.715	3017.715	154		
35	733.9685	2773.969	75	812.839	3086.839	115	799.13	3058.13	155		
36	734.845	2831.845	76	722.6755	2588.676	116	802.902	3055.902	156		
37	745.5425	2881.543	77	806.9275	3107.928	117	796.0175	3064.018	157		
38	716.5965	2786.597	78	776.287	2984.287	118	831.819	3159.819	158		
39	740.427	2873.427	79	781.8725	2971.873	119	799.051	3061.051	159		
40	741.181	2832.181	80	761.434	2861.434	120	805.5295	3046.53	160		
									Mean	776.41	2948.95
									SD	31.14	133.87
									CV	4.01	4.54

Appendix V: LOPAR questionnaire and scorecard

LOW-LEVEL PHYSICAL ACTIVITY RECALL (LO-PAR)

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 (METs). One MET equals 3.5 ml/kg/min or oxygen consumption. Activities are classified according to the following scale: very light (0.9-2.0 METs), light (2.1-3.0 METs), moderate (3.1-5.0 METs) and heavy (5.1-7.0 METs). 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 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
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 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
TOTAL				

4. Did you perform **RECREATIONAL OR LEISURE-TIME ACTIVITIES** during the past week (**refer to Card C**)? If yes, how many 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 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
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 lbs. (5.0)	Light assembly (2.5)	Standing (2.0)
Loading truck (6.5)	Farming (4.5)	Physician (2.5)	Typing (1.5)
Shovelling (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 (5.0)	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)
Shovelling snow (6.0)	Weeding (4.5)	Fertilizing (2.5)	Sewing (1.5)
Manual lawn mowing (6.0)	Power lawn mowing (4.5)	Ironing (2.3)	

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 lbs. (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)
Leisurely swimming (6.0)			

*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 VI: Anthropometric data for subjects in the baseline comparison between ND and T2D groups.

Non-Diabetic Group									
	Age	**ABI**	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	yr	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	61	1.19	0.93		1.77	82.55	26.35	25.70	2.00
2	43	1.14	0.95	85.25	1.82	89.50	27.02	22.80	2.11
3	69	1.20	0.97	210	1.83	77.00	22.99	21.50	1.99
4	63	1.16	0.96	264	1.80	97.10	29.97	28.00	2.17
5	54	1.16	0.94		1.80	97.85	30.20	33.30	2.17
6	57	1.25	1.02	35	1.79	90.00	28.09	32.30	2.09
7	62	1.22	0.98		1.93	113.25	30.56	32.00	2.43
8	52	1.09	0.89	203.5	1.89	112.50	31.66	28.66	2.39
9	65	1.20	0.98		1.87	96.40	27.57	30.70	2.22
10	64	1.02	0.97	119.25	1.79	107.40	33.52	33.75	2.25
11	62	1.20	1.01	178.5	1.71	76.00	26.14	25.75	1.88
12	33	1.10	0.90		1.81	77.00	23.50	15.50	1.97
13	31	1.10	0.95	212	1.77	75.00	23.54	21.50	1.92
14	65	1.10	0.99	173	1.87	95.55	27.32	23.66	2.21
15	57	1.16	1.01		1.84	83.55	24.68	23.60	2.07
16	64	1.24	1.04	263.5	1.75	86.20	28.15	30.94	2.02
17	53	1.21	1.03	233	1.63	89.85	33.82	35.52	1.95
18	58	1.26	1.02	110	1.77	89.45	28.71	28.42	2.06
19	36	1.22	1.02	259.25	1.73	93.25	31.16	26.66	2.07
20	56	1.19	1.05	214	1.74	102.50	34.05	32.44	2.16
Mean	**55.25**	**1.17**	**0.98**	**162.88**	**1.79**	**91.60**	**28.45**	**27.64**	**2.11**
sd	11.13	0.06	0.05	70.86	0.07	11.52	3.40	5.15	0.15

Type 2 Diabetic Group									
	Age	**ABI**	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	yrs	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	64	1.06	0.96	158.5	1.72	86.85	29.53	33.30	2.00
2	61	1.15	0.95	159.5	1.78	97.95	30.91	30.70	2.16
3	68	1.00	0.96	99	1.72	88.70	30.16	33.75	2.01
4	57	1.18	0.98	198.25	1.84	106.00	31.48	32.70	2.28
5	62	1.29	0.96	167.5	1.77	94.80	30.43	30.00	2.12
6	58	1.09	1.01	135	1.68	80.50	28.52	25.06	1.90
7	62	1.15	0.96	152	1.80	86.25	26.62	31.85	2.06
8	59	1.15	1.00	182	1.71	84.20	28.80	29.71	1.97
9	62	1.25	1.08	319.5	1.85	121.75	35.57	28.11	2.43
10	64	1.27	0.99	164.75	1.82	90.35	27.28	26.66	2.12
11	56	1.29	1.06	156.875	1.68	88.65	31.41	29.71	1.98
12	46	1.12	1.01	208.75	1.70	79.00	27.34	31.15	1.91
13	57	1.2	1.03	152.125	1.68	81.75	28.96	29.20	1.92
14	64	1.24	1.05		1.66	86.25	31.49	33.75	1.94
15	61	1.15	0.96	190.5	1.84	103.15	30.47	31.00	2.26
16	55	1.36	1.04	128	1.71	103.30	35.53	33.75	2.14
17	65	1.03	1.01	142.25	1.76	86.60	27.96	25.72	2.03
18	63	1.20	1.01	174.625	1.76	91.10	29.58	28.11	2.07
19	64	1.15	1.07	283	1.69	103.95	36.61	33.75	2.13
20	41	1.26	1.08	157.75	1.85	112.85	32.97	29.80	2.36
21	37	1.08	1.06	257	1.72	99.75	33.91	26.90	2.12
22	68	1.23	1.06	121.25	1.65	83.20	30.56	33.75	1.91
23	57	1.13	1.02	340.75	1.76	84.25	27.20	28.11	2.01
24	49	1.24	0.98	117	1.79	78.50	24.64	20.06	1.97
25	62	1.13	1.05	157.125	1.72	107.30	36.27	38.20	2.19
26	47	1.08	1.03	189.5	1.71	97.75	33.63	35.30	2.09
27	52	1.12	1.04	194	1.77	125.15	39.95	40.00	2.39
28	59	1.08	1	204	1.74	82.15	27.13	25.72	1.97
29	60	1.15	0.98	248.5	1.82	89.40	26.99	33.35	2.11
30	56	1.23	1.04	149.25	1.71	79.50	27.19	26.35	1.92
31	58	1.28	1.08	213	1.63	88.50	33.31	35.20	1.94
32	49	1.18	0.98	168.25	1.72	83.20	28.12	24.96	1.96
33	55	1.05	1.02	130.75	1.75	84.00	27.43	29.63	2.00
Mean	**57.52**	**1.17**	**1.02**	**181.88**	**1.74**	**92.62**	**30.54**	**30.46**	**2.07**
sd	7.33	0.09	0.04	56.14	0.06	12.14	3.52	4.15	0.14

Appendix VII: Haematological data for subjects in the baseline comparison between ND and T2D groups.

Non-Diabetic Group								
	RBC	**Hb**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.53	14.2	4.7	36.61	4.13	1.46	2.67	1.4
2	4.92	15.1	4.2	34.43	5.17	1.26	3.69	2.19
3	5.14	16.1	3.8	36.61	4.14	1.27	2.92	1.14
4	4.99	15.6	4.2	36.61	4.48	1.44	3.11	0.66
5	4.98	15.4	4.5	39.89	4.52	0.97	3.49	1.6
6	4.11	13.3	5.6	40.98	5.87	1.25	4.37	2.67
7	4.38	13.8	5.3	32.24	4.88	1.21	3.51	1.87
8	5.06	16.2	4.9	34.43	4.18	1.94	2.4	0.87
9	5.6	16.7	5.3	38.80	5.48	1.42	4.03	1.14
10	4.62	15.6	5.6	37.71	3.31	0.93	2.4	1.06
11	4.49	15.00	4.3	34.43	4.67	1.33	3.48	0.78
12	5.32	17.8	5.4	34.43	4.22	1.87	2.55	0.62
13	4.23	13.6	3.9	28.96	4.23	1.49	2.89	0.7
14	5.14	14.3	5.7	46.45	5.37	1.19	4	1.72
15	5.33	16.9	3.8	32.24	5.29	1.29	3.99	0.75
16	4.09	13.8			5	1.12	2.44	3.17
17	4.26	14.4			5	1.27	2.32	3.1
18	4.52	15	5		4.1	1.12	2.63	0.78
19	4.34	15.4	4.3		6.2	0.85	4.48	1.92
20	4.48	15.4	4.6		4	1.02	2.16	1.81
Mean	**4.73**	**15.18**	**4.74**	**36.32**	**4.75**	**1.30**	**3.23**	**1.48**
sd	0.45	1.19	0.66	4.20	0.71	0.28	0.72	0.82

Type 2 Diabetic Group								
	RBC	**Hb**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.32	13.5	8	46.45	3.8	1.08	2.38	2.73
2	5.1	16.5	6.7	40.98	5.1			1.57
3	5.19	16.5	8.2	54.10	3.5	1.09	2.33	0.93
4	5.17	15.6	5.6	38.80	4.7	1.27	3.31	1.65
5	4.27	14.2	6.5	44.26	4	1.34	2.4	1.98
6	5.66	16.5	6.9	38.80	6.1	1.49	4.33	1.3
7	4.8	15.2	7.8	56.28	5.1	1.22	3.55	3.17
8	4.57	14.4	7.4		2.8	1.18	1.49	1.19
9	4.3	14.7	5.4	46.45	4	1.45	2.31	2.79
10	4.83	14.2	6.7	57.38	2.2	0.78	1.32	1.77
11	5.41	16.2	8.2	63.93	6.2	1.34	4.15	4.99
12	4.88	14.9	12.3	84.70	3.3	1.3	1.87	0.86
13	4.66	14.6	8.2	60.66	4.7	1.29	3.01	1.56
14	4.37	13.1	5.3	40.98	6.6	1.78	4.55	1.54
15	4.81	15.2	6.6	56.28	3.5	0.89	2.32	1.72
16	5.06	15.9	7.9	55.19	5.6	1.23	4.34	1.97
17	4.18	14	7	42.08	4.3	1.07	3.1	3.23
18	4.95	14.9	7.4	48.63	3.6	1.12	2.3	1.73
19	5.28	16.6	5.6	40.98	4	1.53	2.16	3.68
20	4.96	15.6	5.7	42.08	4.7	1.06	3.29	1.98
21	5.07	15.2	7.6	48.63	4.9	1.36	3.35	1.48
22								
23	4.85	15.6	6.9	44.26	4.8	1.08	3.16	1.76
24	5.2	15.5	7.3	45.36	5	1.08	3.6	2.61
25	4.81	13.6		56.28				
26	4.56	14.3	7.4	57.38	4.9	1.76	2.6	1.19
27	5.17	15.8	8.3	55.19	4.3	1.59	2.9	1.52
28	4.38	13.5	8.3	51.91	4.1	1.22	2.85	1.16
29	5.24	15.8	7.4	46.45	5.1	0.92	3.29	1.96
30	4.84	14.2	6.9	44.26	4.9	1.03	2.39	3.26
31	5.04	15.6	8.8	50.82	5	1.26	2.98	1.67
32	4.6	14.4	9.3	62.84	3.7	1.5	1.92	0.61
33	4.94	15.4	6.8	62.84	4.1	0.84	2.62	1.4
Mean	**4.86**	**15.04**	**7.37**	**51.14**	**4.47**	**1.24**	**2.87**	**1.97**
sd	0.36	0.97	1.36	9.81	0.97	0.25	0.82	0.94

Appendix VIII: Peak MVC and incremental plantar-flexion data for subjects in the baseline comparison between ND and T2D groups.

Non-Diabetic Group				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	**N**	**min**	**N**	**% MVC**
1	937	7.0	700	74.71
2	1035	10.5	900	86.96
3	1190	10.0	900	75.63
4	936	6.0	500	53.42
5	842	8.0	700	83.14
6	1033	8.0	700	67.76
7	1657	10.0	900	54.32
8	1730	12.0	1100	63.58
9	1620	12.0	1100	67.90
10	924	4.0	300	32.47
11	807	8.0	700	86.74
12	1024	8.0	700	68.36
13	852	6.3	500	58.69
14	947	6.0	500	52.80
15	863	8.0	700	81.11
16	1085	8.0	700	64.52
17	1312	10.0	900	68.60
18	1042	7.5	700	67.18
19	1426	10.0	900	63.11
20	1039	10.0	900	86.62
Mean	**1115.05**	**8.47**	**750.00**	**67.88**
St. Dev	283.96	2.08	203.91	13.76

Type 2 Diabetic Group				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	**N**	**min**	**N**	**% MVC**
1	933	6.0	500	53.59
2	1133	7.2	700	61.78
3	1080	8.0	700	64.81
4	1586	7.0	700	44.14
5	1425	6.0	500	35.09
6	870	6.0	500	57.47
7	1160	6.0	500	43.10
8	1184	10.0	900	76.01
9	1630	11.3	1100	67.48
10	1317	6.0	500	37.97
11	1052	8.0	700	66.54
12	1470	10.0	900	61.22
13	1125	8.0	700	62.22
14	1018	8.7	900	88.41
15	1612	12.0	1100	68.24
16	1050	6.0	500	47.62
17	1231	8.0	700	56.86
18	1002	8.0	700	69.86
19	1294	8.0	700	54.10
20	1759	12.0	1100	62.54
21	1048	8.0	700	66.79
22	1008	6.0	500	49.60
23	1078	4.0	300	27.83
24	1504	10.0	900	59.84
25	744	6.0	500	67.20
26	1030	8.0	700	67.96
27	1316	8.0	700	53.19
28	915	8.0	700	76.50
29	1506	10.0	900	59.76
30	1255	9.2	900	71.71
31	788	6.0	500	63.45
32	1432	10.0	900	62.85
33	898	6.0	500	55.68
Mean	**1195.55**	**7.92**	**706.06**	**59.44**
St. Dev	261.96	1.95	203.01	12.55

Appendix IX: LVC responses to each workload during the incremental plantar-flexion data for subjects in the baseline comparison between ND and T2D groups.

Non-Diabetic Group								
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.12	7.17	1.48	3.58	4.32	7.17		
2	1.02	5.80	1.37	3.00	5.80			
3	0.76	2.06	0.94	1.43	2.06			
4	0.82	11.34	1.53	3.76	5.68	11.34		
5	1.35	6.56	1.23	3.98	6.56	5.91		
6	0.82	7.10	1.26	5.68	6.89	6.87	7.10	
7	1.17	5.67	2.75	3.56	4.92	5.67		
8	0.64	5.15	0.67	1.78	2.85	3.24	5.15	
9	0.98	8.22	1.60	3.69	5.33	6.62	8.22	
10	1.18	9.21	0.93	2.27	4.80	6.64	9.21	
11	0.57	5.66	1.01	1.40	2.89	4.70	5.66	
12	0.94	6.04	1.77	2.29	4.45	6.04		
13	1.17	4.91	1.97	2.56	4.43	4.91		
14	0.37	2.93	0.94	1.61	2.65	2.93		
15	1.26	7.32	3.52	5.26	6.90	6.53	7.32	
16	1.31	17.65	2.62	5.08	7.80	12.24	16.09	17.65
17	1.13	4.36	1.43	1.96	2.63	4.36		
18	0.52	2.15	0.80	2.15				
19	0.36	3.83	1.10	2.10	3.13	3.83		
20	0.84	9.51	1.54	3.14	3.85	5.73	6.80	9.51
Mean	**0.92**	**6.63**	**1.52**	**3.01**	**4.63**	**6.16**	**8.19**	**13.58**
sd	0.31	3.54	0.72	1.29	1.68	2.47	3.44	5.75

Type 2 Diabetic Group								
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.19	6.29	1.25	2.66	4.52	6.29		
2	0.42	1.21	1.07	1.21				
3	0.81	6.67	1.14	2.88	4.48	6.67		
4	0.67	4.31	1.71	2.76	3.27	4.31		
5	1.22	8.76	1.00	3.53	4.47	6.08	6.41	8.76
6	0.49	5.89	1.12	2.90	5.89			
7	0.38	5.96	0.72	1.10	2.48	3.85	5.29	5.96
8	0.61	10.78	0.94	3.06	4.36	6.47	10.78	
9	1.35	5.74	0.84	2.61	3.88	5.74		
10	0.60	4.09	0.97	1.94	4.09			
11	0.35	2.68	0.44	1.54	1.62	2.68		
12	0.99	7.40	0.89	4.21	7.40			
13	0.79	6.80	1.64	2.59	4.74	6.80		
14	1.21	3.66	0.71	0.89	2.10	3.66		
15	0.50	5.36	1.11	1.82	2.33	4.86	5.36	
16	0.75	4.77	1.53	2.94	4.77			
17	0.73	2.47	1.16	1.81	2.47			
18	0.63	4.77	1.33	3.04	4.77			
19	0.92	3.77	2.09	2.83	2.82	2.47	3.77	
20	0.83	8.11	1.35	3.28	4.82	5.81	6.50	8.11
21	0.96	3.23	0.54	1.57	3.23			
22	0.63	6.75	1.31	2.90	4.88	6.75		
23	0.91	6.74	3.04	4.61	6.12	5.67	6.74	
24	0.87	3.15	1.25	2.34	2.59	3.15		
25	0.76	3.09	0.99	1.71	3.09			
26	0.62	2.30	0.56	1.81	1.95	2.30		
27	0.66	7.60	0.57	2.36	4.82	6.39	7.60	
28	1.77	9.56	2.04	5.35	7.87	9.56		
29	0.74	4.52	1.77	3.29	4.06	4.52	4.09	
30	1.07	4.62	1.61	3.49	4.62			
31	1.25	8.85	1.19	4.56	6.59	7.78	8.85	
32	0.95	4.00	1.59	3.15	4.00			
33	0.65	4.60	0.75	2.45	3.77	4.60		
Mean	**0.83**	**5.41**	**1.22**	**2.70**	**4.15**	**5.29**	**6.54**	**7.61**
sd	0.31	2.28	0.53	1.03	1.52	1.84	2.14	1.47

Appendix X: LVC kinetic responses from fitting of quad-phasic model to steady-state contractions at 30% MVC.

Non-Diabetic Group

ID	a ml.min^{-1}.mmHg^{-1}	A_1 ml.min^{-1}.mmHg^{-1}	TD_1 s	Tau_1 s	A_2 (decay) ml.min^{-1}.mmHg^{-1}	TD_2 (decay) s	Tau_2 (decay) s	A_3 ml.min^{-1}.mmHg^{-1}	TD_3 s	Tau_3 s	A_4 (decay) ml.min^{-1}.mmHg^{-1}	TD_4 (decay) s	Tau_4 (decay) s	End A ml.min^{-1}.mmHg^{-1}	Adj R^2 %
1	1.15	0.92	3.06	3.26	0.88	11.68	2.05	2.05	18.00	46.30	0.74	87.78	22.57	2.48	82.64
2	1.01	1.49	1.02	4.03	1.22	10.53	3.71	0.98	13.02	6.93	0.25	111.78	26.87	2.01	57.92
3	0.85	2.69	0.00	2.42	0.70	5.16	3.99	1.05	24.65	24.48	0.64	65.93	117.33	3.32	77.61
4	0.83	0.46	0.00	2.44	0.57	6.07	13.20	1.44	17.81	41.18	0.61	71.00	8.00	1.55	15.87
5	0.34	2.03	1.76	3.66	0.43	13.17	13.71	0.55	29.81	30.60	0.35	95.81	5.00	2.13	51.05
6	0.98	1.98	0.01	3.17	2.14	6.51	16.00	4.95	11.93	41.42	1.03	113.73	7.81	4.73	61.27
7	0.40	2.35	0.99	3.00	1.10	6.00	7.07	0.70	28.70	12.59	0.38	64.42	19.00	1.97	68.18
8	1.19	2.06	0.00	2.43	0.45	11.99	2.04	2.49	20.54	37.45	1.31	66.00	31.00	3.99	78.56
9	0.94	8.71	0.00	0.81	3.95	12.49	5.35	2.92	17.37	5.25	0.87	79.55	32.35	7.74	87.53
10	0.43	1.44	1.84	3.10	1.00	12.13	7.62	2.07	18.00	21.31	1.13	45.82	19.74	1.81	44.79
11	0.91	6.28	2.00	3.29	1.70	12.90	17.34	1.20	42.67	20.51	0.70	88.00	19.36	5.99	78.30
12	0.86	2.03	0.00	3.97	0.73	16.96	13.70	0.84	35.73	6.86	0.10	87.21	20.00	2.90	69.73
13	0.87	4.94	1.27	3.11	2.88	15.26	9.55	4.29	22.06	19.72	0.82	60.40	1.53	6.40	85.76
14	0.98	1.01	0.51	3.52	0.82	5.33	8.63	0.59	10.43	7.39	0.25	96.01	264.88	1.61	26.15
15	1.01	2.84	0.00	1.37	0.71	13.70	14.41	0.60	36.47	9.22	0.24	102.54	15.00	3.50	73.67
16	0.75	1.67	0.00	1.39	0.13	9.67	18.58	1.90	29.94	20.14	0.25	77.62	5.00	3.93	82.00
17	1.51	3.10	0.00	1.36	1.10	11.08	5.09	0.92	19.80	11.09	0.90	70.62	13.05	3.51	87.40
18	1.19	4.50	3.24	2.50	0.87	11.96	1.20	0.75	17.24	7.61	1.30	43.37	51.05	4.27	93.93
19	0.47	1.95	0.00	1.55	0.45	7.44	8.28	0.89	18.42	6.42	0.91	35.00	49.59	1.95	22.67
20	0.76	2.40	1.00	1.00	0.91	11.68	1.65	2.58	19.48	102.00	0.51	57.98	69.42	4.23	87.79
Mean	**0.87**	**2.74**	**0.83**	**2.57**	**1.14**	**10.59**	**8.66**	**1.69**	**22.60**	**23.91**	**0.67**	**76.03**	**39.93**	**3.50**	**64.64**
sd	0.29	1.99	1.05	1.01	0.91	3.42	5.62	1.24	8.67	22.68	0.37	22.05	59.50	1.72	22.58

Type 2 Diabetic Group

ID	a ml.min^{-1}.mmHg^{-1}	A_1 ml.min^{-1}.mmHg^{-1}	TD_1 s	Tau_1 s	A_2 (decay) ml.min^{-1}.mmHg^{-1}	TD_2 (decay) s	Tau_2 (decay) s	A_3 ml.min^{-1}.mmHg^{-1}	TD_3 s	Tau_3 s	A_4 (decay) ml.min^{-1}.mmHg^{-1}	TD_4 (decay) s	Tau_4 (decay) s	End A ml.min^{-1}.mmHg^{-1}	Adj R^2 %
1	0.53	3.08	1.70	6.47	0.48	30.01	5.56	0.63	41.08	3.54	0.53	140.06	26.22	3.22	88.66
2	0.95	0.37	0.00	5.80	0.14	36.76	8.45	0.75	41.37	14.98	0.10	71.71	4.50	1.83	63.53
3	0.99	2.24	1.99	1.58	1.02	21.08	4.86	2.60	23.98	30.37	0.84	90.46	17.55	3.97	83.28
4	0.69	2.01	0.00	5.04	1.07	11.89	6.65	0.99	37.94	29.11	0.41	71.00	39.38	2.21	72.21
5	0.56	2.50	0.00	1.25	0.81	4.78	7.48	0.98	23.43	25.01	0.41	78.07	9.00	2.82	77.28
6	0.58	1.34	0.00	2.64	1.32	4.23	8.76	1.24	4.03	15.53	0.14	128.25	20.00	1.71	9.33
7	0.82	4.72	0.54	2.36	2.22	9.65	4.11	2.19	19.64	11.16	0.63	72.00	74.37	4.88	79.94
8	0.82	3.54	1.48	2.89	0.42	12.50	6.39	0.49	23.44	2.00	0.74	104.41	28.92	3.69	77.70
9	0.91	1.97	1.80	2.98	1.04	12.22	7.66	1.93	17.96	27.92	0.59	90.00	10.92	3.19	88.83
10	0.51	1.79	1.98	1.71	0.90	15.42	6.98	0.97	16.36	46.00	0.51	66.00	11.90	1.86	71.82
11	0.71	3.51	0.27	2.52	1.02	6.20	30.13	3.79	11.97	39.96	1.11	59.96	17.69	5.68	88.09
12	0.50	0.37	0.00	13.54	0.24	12.87	9.30	0.82	18.00	43.76	0.30	99.65	46.14	1.14	86.69
13	0.74	2.12	0.00	1.94	1.10	8.47	7.00	1.15	17.93	15.20	0.14	84.00	15.01	2.78	72.84
14	1.00	3.78	0.00	0.83	1.21	5.65	7.03	0.44	12.00	5.05	0.38	68.04	147.95	3.69	87.04
15	1.07	2.10	1.29	2.92	0.74	15.08	4.52	4.56	17.45	64.03	2.34	54.00	55.23	4.63	85.79
16	0.50	3.94	0.61	1.88	0.76	5.79	8.61	0.60	12.16	6.16	0.20	52.00	15.97	4.08	73.66
17	0.51	2.41	0.00	1.89	0.94	5.81	1.01	1.92	21.19	71.30	0.35	198.00	15.16	3.54	92.08
18	0.44	1.84	0.00	3.77	0.55	5.73	7.00	1.26	19.97	55.51	0.60	106.71	6.60	2.38	79.79
19	1.06	0.50	0.00	0.82	0.31	12.07	8.55	2.61	20.48	72.24	1.30	48.00	47.33	2.53	86.53
20	0.51	0.96	0.92	1.97	0.24	6.75	6.50	0.40	17.20	82.49	0.12	98.84	15.00	1.52	50.07
21	0.65	4.16	0.00	4.67	0.64	32.27	7.70	0.51	41.36	3.00	0.65	96.32	13.33	4.02	78.34
22	0.80	2.12	0.00	5.33	1.06	11.71	11.73	1.79	23.38	20.39	1.15	90.00	146.21	2.68	72.40
23	1.48	1.84	0.00	1.83	0.95	6.50	18.36	1.35	24.16	40.44	0.80	77.24	40.51	2.92	54.48
24	0.68	1.30	2.00	6.56	0.67	1.95	30.00	2.01	31.65	94.72	0.91	60.92	53.46	2.34	77.73
25	0.80	1.03	1.40	2.02	0.84	5.95	9.65	1.38	24.28	19.20	0.43	52.00	49.68	1.95	30.18
26	0.51	0.84	1.13	1.85	0.49	13.20	9.00	1.37	17.39	20.79	0.17	87.77	5.35	2.05	53.71
27	0.64	2.15	0.73	1.53	0.76	6.21	6.30	1.52	11.00	46.49	0.13	90.00	1.11	3.41	76.53
28	0.67	1.73	0.00	1.34	0.13	11.16	6.19	0.98	25.87	20.21	0.74	68.94	9.53	2.51	63.31
29	0.72	1.10	2.00	7.25	0.38	12.82	3.40	1.34	24.68	17.02	0.58	95.44	20.00	2.20	64.67
30	1.24	5.94	0.95	1.24	3.24	7.27	15.44	2.22	23.95	11.91	0.69	100.05	20.00	5.47	64.29
31	0.87	3.25	0.99	1.25	0.94	13.38	13.27	0.95	16.63	6.00	0.84	101.07	42.48	3.28	68.44
32	0.78	2.50	1.37	2.21	0.85	6.02	3.19	3.00	12.04	50.10	0.65	68.00	29.56	4.77	82.88
33	0.54	1.39	2.00	4.88	0.94	11.95	0.82	2.12	9.78	75.18	1.15	120.00	100.93	2.05	84.91
Mean	**0.75**	**2.26**	**0.76**	**3.23**	**0.86**	**11.62**	**8.84**	**1.54**	**21.33**	**32.93**	**0.62**	**87.48**	**35.06**	**3.07**	**71.67**
sd	0.24	1.30	0.79	2.58	0.59	8.02	6.54	0.97	9.13	25.76	0.45	29.80	36.24	1.17	17.25

Appendix XI: LVC kinetic responses from fitting of quad-phasic model to steady-state contractions at 70% MVC.

Non-Diabetic Group

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	End A	MRT	Adj R^2
	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	%
1	0.86	5.69	3.08	6.38	1.58	12.90	1.50	2.62	11.63	36.37	7.58	30.06	73.76
2	0.70	5.75	1.14E-05	7.46	2.23	11.04	36.51	2.39	25.23	19.94	6.62	43.40	81.65
3	0.62	8.32	2.8702	6.37	2.99	14.13	3.04	1.40	21.82	7.50	7.35	25.17	82.53
4	0.72	3.18	3.0603	5.37	0.39	22.41	7.10	1.31	31.30	15.31	4.82	24.21	86.11
5	0.93	5.36	1.0616	3.08	1.19	13.91	3.21	2.61	17.94	5.03	7.72	15.10	92.31
6	1.66	6.08	1.79	5.14	1.72	11.96	8.00	1.88	23.99	3.17	7.90	20.47	81.28
7	0.59	3.42	1.56	4.92	1.95	11.98	6.94	1.20	18.00	4.96	3.27	32.39	56.67
8	0.89	4.44	0.95	1.96	0.51	9.45	5.05	2.98	29.67	5.79	7.80	18.26	74.65
Mean	**0.87**	**5.28**	**1.80**	**5.08**	**1.57**	**13.47**	**8.92**	**2.05**	**22.45**	**12.26**	**6.63**	**26.13**	**78.62**
sd	0.34	1.64	1.13	1.81	0.87	3.92	11.38	0.69	6.53	11.35	1.70	9.05	10.67

Type 2 Diabetic Group

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	End A	MRT	Adj R^2
	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	s	$ml.min^{-1}.mmHg^{-1}$	s	%
1	0.74	4.46	4.38	4.46	1.64	14.15	3.37	2.06	20.90	6.95	5.63	25.67	75.59
2	0.82	2.99	2.52	6.85	0.96	12.00	4.00	1.14	16.59	14.34	4.00	24.76	83.81
3	0.97	4.60	0.95	2.00	0.73	19.00	82.83	1.69	18.97	11.15	6.55	24.91	55.58
4	0.74	2.72	0.00	3.23	0.67	18.19	5.59	0.78	23.88	4.83	3.57	16.63	52.56
5	0.94	6.49	1.94	4.90	1.47	12.00	6.09	2.89	18.89	16.62	8.85	21.93	71.45
6	0.41	2.25	0.90	2.95	0.31	7.00	17.11	1.79	18.25	91.49	4.10	57.00	85.70
7	0.75	2.01	2.32	5.22	0.94	12.31	5.50	1.02	19.26	84.47	2.82	65.93	38.93
8	0.83	1.95	0.97	2.93	0.50	12.27	5.50	2.50	17.80	40.44	4.78	41.02	64.20
9	0.53	4.75	0.92	3.43	0.60	13.00	5.19	1.84	17.60	8.73	6.52	13.38	70.84
10	1.46	2.57	0.00	3.97	0.93	5.63	2.48	2.47	11.49	74.66	5.53	56.19	76.50
11	1.17	5.36	0.98	1.38	0.57	16.13	2.32	2.33	21.05	10.32	8.29	13.53	90.12
12	1.52	3.52	1.26	2.73	0.48	5.50	9.89	2.47	11.38	26.13	7.03	20.70	71.32
13	0.82	5.08	0.59	4.18	1.16	20.55	3.70	3.00	28.27	8.40	7.73	23.51	83.96
14	1.44	5.07	1.22	2.57	1.58	7.61	3.04	7.52	12.43	36.38	12.44	38.64	91.90
15	1.50	3.73	0.11	2.98	0.94	6.45	6.87	3.55	19.77	14.33	7.85	22.87	92.59
Mean	**0.98**	**3.84**	**1.27**	**3.59**	**0.90**	**12.12**	**10.90**	**2.47**	**18.44**	**29.95**	**6.38**	**30.98**	**73.67**
sd	0.36	1.40	1.14	1.38	0.41	4.93	20.24	1.59	4.48	29.76	2.48	16.69	15.62

Appendix XII: RH responses from fitting of mono-phasic model.

Non-Diabetic Group							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.53	15.42	0.0133	17.95	2521.34	18.13	93.90
2	2.87	16.65	0.0290	19.52	1951.66	19.68	97.18
3	1.20	14.27	0.0666	15.47	935.72	15.30	97.44
4	5.33	9.49	0.0248	14.82	3579.65	13.33	75.61
5	3.74	28.99	0.0581	32.73	2516.99	32.99	96.11
6	4.20	9.75	0.0336	13.95	2028.26	15.36	62.37
7	3.02	22.33	0.1232	25.35	1992.05	25.34	98.16
8	3.72	17.76	0.0336	21.48	2762.13	22.65	92.48
9	2.36	20.65	0.0233	23.01	2444.83	23.07	98.77
10	4.38	14.22	0.0371	18.60	3012.70	19.09	88.30
11	2.28	9.33	0.0214	11.61	1804.10	10.98	90.87
12	4.32	26.97	0.0855	31.28	2905.11	30.70	96.77
13	1.52	22.67	0.0485	24.19	787.16	24.32	96.70
14	1.66	15.32	0.0121	16.97	2358.69	17.07	96.69
15	2.66	22.36	0.0597	25.02	2132.51	24.61	97.52
16	4.61	20.00	0.0462	24.61	3198.47	26.26	87.90
17	7.75	20.26	0.0170	28.00	5373.65	27.22	90.39
18	2.68	19.84	0.0200	22.52	2597.37	20.55	94.84
19	3.10	19.48	0.0335	22.59	2443.07	23.31	96.12
20	3.45	25.18	0.01	28.63	3791.43	29.53	94.83
Mean	**3.37**	**18.55**	**0.04**	**21.92**	**2556.84**	**21.98**	**92.15**
sd	1.50	5.56	0.03	5.81	995.46	5.92	8.80

Type 2 Diabetic Group							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	4.26	28.47	0.0982	32.73	2590.13	33.35	95.04
2	3.28	16.51	0.0322	19.79	2478.40	20.33	90.00
3	4.79	19.86	0.0253	24.66	3642.10	25.27	95.92
4	2.77	14.10	0.0186	16.88	2423.86	17.77	89.00
5	2.59	20.31	0.0303	22.90	2224.06	23.20	98.46
6	4.34	21.41	0.0264	25.75	3418.19	26.41	95.78
7	1.83	17.52	0.0562	19.36	715.64	20.04	95.27
8	1.67	15.09	0.0537	16.75	1282.95	16.81	96.97
9	6.20	19.77	0.0150	25.97	5043.04	26.15	92.94
10	4.53	13.24	0.0189	17.77	3686.97	17.87	92.67
11	3.47	11.73	0.0206	15.20	2653.86	16.45	94.96
12	3.90	22.54	0.0404	26.44	2899.32	25.90	95.72
13	2.83	21.70	0.1239	24.52	1870.66	24.63	96.96
14	5.46	23.52	0.0140	28.98	4954.94	24.96	89.01
15	4.98	29.09	0.0707	34.07	3098.77	34.00	99.29
16	3.05	18.70	0.0423	21.75	2273.59	22.03	93.13
17	3.43	13.78	0.0378	17.21	2628.55	18.01	92.35
18	4.44	13.51	0.0219	17.95	3547.05	16.99	91.06
19	4.09	17.39	0.0248	21.48	3155.75	21.96	95.55
20	3.13	19.39	0.0475	22.52	2474.60	23.05	95.23
21	4.75	21.29	0.0312	26.04	3817.87	27.69	95.28
22	3.34	8.63	0.0286	11.97	2303.60	11.80	88.54
23	2.47	23.22	0.0389	25.69	2077.59	26.14	95.75
24	10.78	23.48	0.0249	34.25	6763.80	20.88	72.92
25	4.59	12.91	0.0224	17.51	3057.80	16.17	77.11
26	1.46	13.27	0.1008	14.73	1007.45	14.79	96.19
27	1.70	5.62	0.0337	7.32	982.72	7.57	56.08
28	4.01	26.85	0.0693	30.85	2550.69	30.96	98.03
29	2.32	15.57	0.0161	17.89	2496.05	16.49	94.44
30	5.43	14.93	0.0134	20.36	4051.25	21.35	84.19
31	7.37	22.89	0.0248	30.25	5342.25	31.56	96.62
32	9.67	20.31	0.0220	29.97	6723.10	30.16	97.40
33	3.00	16.15	0.0280	19.15	2375.14	20.64	96.60
Mean	**4.12**	**18.26**	**0.04**	**22.38**	**3048.84**	**22.16**	**91.95**
sd	2.07	5.39	0.03	6.48	1443.85	6.16	8.61

Appendix XIII: Peak individual responses to graded cycle test to exhaustion.

Non-Diabetic Group											
	Peak Wkld	TTF	VT	VO_2peak	VO_2peak	VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	beats.min^{-1}	% Age-Predicted Max	a.u
1	190	18	160	2.62	31.68	1.63	19.67	87.36	160	100.63	1.09
2	220	21	190	3.50	39.14	3.03	33.64	110.62	179	101.13	1.13
3	160	15	130	2.10	27.31	1.21	15.68	90.32	130	88.09	1.08
4	190	16	160	2.33	23.95	1.50	15.45	105.63	159	101.27	1.15
5	190	16	160	2.52	25.77	1.44	14.74	103.15	170	102.41	1.16
6	190	18	160	2.58	28.64	2.32	25.72	85.90	173	106.13	1.00
7	220	21	190	3.21	28.35	2.63	23.24	105.90	167	105.70	1.03
8	250	24	220	3.72	33.03	3.21	28.57	96.79	161	95.83	1.04
9	220	21	190	3.20	33.24	1.80	18.66	90.41	143	92.26	1.06
10	160	14	130	2.55	23.77	1.70	15.86	105.05	162	103.85	1.11
11	190	18	160	3.11	40.95	2.67	28.55	110.93	165	104.43	1.07
12	220	21	190	3.38	43.90	2.81	36.43	104.05	164	87.70	0.99
13	220	21	190	3.49	46.56	2.46	32.86	108.59	189	100.00	1.16
14	160	15	130	2.38	24.86	2.04	21.37	83.34	151	97.42	1.11
15	190	18	130	3.02	36.13	2.14	25.42	90.80	159	97.55	1.06
16	160	15	130	2.58	29.94	2.29	26.62	101.58	171	109.62	1.14
17	160	14	130	2.38	26.50	2.10	23.37	85.88	161	98.41	1.08
18	220	21	160	3.40	38.00	2.63	29.35	84.47	148	91.36	1.12
19	250	22.5	190	3.73	39.97	2.47	26.48	95.28	177	95.20	1.07
20	220	21	190	3.33	32.52	2.72	26.54	99.63	175	106.71	1.01
Mean	**199.00**	**18.53**	**164.50**	**2.96**	**32.71**	**2.24**	**24.41**	**97.28**	**163.20**	**99.13**	**1.08**
sd	29.36	3.08	28.00	0.51	6.87	0.56	6.35	9.35	13.40	6.38	0.05

Type 2 Diabetic Group											
	Peak Wkld	TTF	VT	VO_2peak	VO_2peak	VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	beats.min^{-1}	% Age-Predicted Max	a.u
1	130	12	100	2.29	26.39	1.50	17.31	86.73	138	88.46	1.09
2	190	18	160	2.92	29.76	2.25	22.99	114.72	159	100.00	1.14
3	160	15	130	2.54	28.60	1.57	17.74	68.99	163	107.24	1.06
4	160	15	130	2.85	26.87	1.80	16.93	99.39	133	81.60	1.06
5	130	12	100	1.70	17.95	0.94	9.92	72.23	131	82.91	1.13
6	130	12.5	100	2.11	26.22	1.54	19.12	81.97	161	99.38	1.10
7	160	13	100	2.03	23.55	1.46	16.91	74.70	170	107.59	1.17
8	160	15	130	2.40	28.49	1.82	21.60	111.03	163	101.24	1.15
9	220	20	160	2.78	22.83	2.65	21.75	97.12	154	97.47	1.12
10	220	19.5	160	2.99	33.08	2.20	24.35	102.05	177	113.46	1.13
11	190	18	160	2.72	30.67	2.10	23.63	94.65	151	92.07	1.16
12	190	18	130	2.56	32.39	2.03	25.71	98.95	173	99.43	1.16
13	160	15	130	2.44	29.79	1.99	24.33	91.00	148	90.80	1.07
14	160	13.5	130	2.00	23.23	1.32	15.28	77.28	144	92.31	1.19
15	190	18	160	2.73	26.51	1.86	18.06	58.15	156	98.11	1.16
16	160	15	130	2.77	26.77	1.81	17.51	100.91	160	95.97	1.00
17	130	12	100	1.98	22.84	1.91	22.00	56.72	137	88.39	1.04
18	160	14.5	130	2.19	24.03	2.02	22.15	94.55	161	103.21	1.10
19	130	12	100	2.00	19.25	1.65	15.90	81.33	136	87.18	1.14
20	190	18	130	2.97	26.34	2.11	18.68	91.87	167	93.30	1.07
21	160	13.5	130	2.51	25.16	1.87	18.77	91.91	156	85.25	1.22
22	130	10.5	100	2.08	24.94	1.53	18.44	72.21	127	83.55	1.05
23	190	18	130	2.70	32.02	2.12	25.11	93.75	157	95.32	1.19
24	190	18	160	2.71	34.51	2.27	28.87	109.47	175	102.34	1.07
25	160	15	130	2.43	22.63	1.88	17.55	84.47	163	103.16	1.03
26	160	13	130	2.18	22.28	1.76	18.02	102.32	172	99.42	1.16
27	160	13	130	3.09	24.71	2.68	21.41	91.27	138	82.14	0.95
28	160	15	130	1.84	22.34	1.42	17.37	64.88	168	104.35	1.07
29	220	21	160	3.18	35.55	2.49	27.87	114.91	168	105.00	1.09
30	160	14.25	130	2.32	29.16	1.81	22.77	104.37	168	102.44	1.05
31	160	15	130	2.32	26.23	1.71	19.31	99.61	154	95.06	1.16
32	190	16.25	160	2.89	34.76	2.26	27.17	97.45	150	87.72	1.05
33	130	12	100	2.08	24.75	1.73	20.61	76.04	162	98.18	1.08
Mean	**166.36**	**15.11**	**130.00**	**2.46**	**26.81**	**1.88**	**20.46**	**89.61**	**155.76**	**95.94**	**1.10**
sd	26.79	2.66	21.21	0.39	4.38	0.38	4.08	15.51	13.73	8.21	0.06

Appendix XIV: Individual bi-exponential model fits for the $\dot{V}O_2$ kinetic response to steady-state cycling at 80% VT.

Non-Diabetic Group

ID	Workload W	a L.min^{-1}	A_1 L.min^{-1}	TD_1 s	Tau_1 s	A_2 L.min^{-1}	TD_2 s	Tau_2 s	End A L.min^{-1}	MRT s	VO_2 Gain ml.min^{-1}.W^{-1}
1	152	0.70	0.80	1.77	15.02	0.80	26.41	21.41	2.30	32.30	11.25
2	104	0.68	0.23	2.93	3.75	0.85	26.78	34.54	1.76	49.62	11.47
3	128	0.72	0.35	0.00	2.46	0.93	14.68	42.14	2.00	41.93	10.84
4	128	0.76	0.46	5.44	6.24	0.76	30.51	30.02	1.98	42.08	10.37
5	128	0.78	0.27	5.25	9.49	0.85	22.74	30.57	1.89	44.04	9.46
6	128	0.70	0.50	5.63	5.37	0.84	20.99	30.97	2.04	36.71	11.37
7	128	0.70	0.35	9.99	4.66	0.97	33.19	33.25	2.02	52.78	11.16
8	152	0.89	0.59	3.00	8.22	0.95	29.08	31.18	2.43	41.43	10.87
9	176	0.70	0.66	13.77	5.01	1.15	32.41	26.14	2.50	44.06	10.88
10	104	0.83	0.18	0.00	5.52	0.78	17.78	57.76	1.78	62.65	10.18
11	152	0.70	0.55	8.50	14.94	1.02	30.75	36.45	2.27	51.87	11.06
12	152	0.68	0.40	0.13	29.99	1.05	30.99	24.93	2.13	48.79	10.21
13	152	0.87	0.80	0.01	14.41	0.69	27.35	19.85	2.36	29.60	10.53
14	104	0.80	0.35	15.50	14.68	0.64	39.87	37.68	1.79	60.79	10.55
15	104	0.83	0.20	5.92	4.96	0.65	27.17	29.90	1.68	46.15	9.07
16	104	0.73	0.18	0.00	10.59	0.86	30.46	41.40	1.77	61.02	11.05
17	104	0.78	0.37	1.93	12.79	0.78	32.18	44.92	1.93	57.18	12.25
18	128	0.86	0.57	1.19	29.99	0.70	33.65	10.22	2.13	38.16	10.79
19	152	0.90	0.40	10.36	6.04	1.15	27.85	37.85	2.45	52.98	10.92
20	152	0.71	0.74	7.59	14.53	1.03	26.00	48.34	2.48	52.50	12.48
Mean	**131.60**	**0.77**	**0.45**	**4.95**	**10.93**	**0.87**	**28.04**	**33.48**	**2.09**	**47.33**	**10.84**
sd	22.40	0.07	0.20	4.74	7.78	0.15	5.78	10.69	0.27	9.37	0.79

Type 2 Diabetic Group

ID	Workload W	a L.min^{-1}	A_1 L.min^{-1}	TD_1 s	Tau_1 s	A_2 L.min^{-1}	TD_2 s	Tau_2 s	End A L.min^{-1}	MRT s	VO_2 Gain ml.min^{-1}.W^{-1}
1	128	0.77	0.28	15.00	5.49	1.14	34.26	36.83	2.18	61.21	11.97
2	80	0.87	0.17	14.72	1.14	0.71	33.82	49.12	1.74	70.07	12.52
3	80	0.97	0.32	8.05	8.65	1.00	33.82	58.40	2.29	73.75	18.80
4	104	0.57	0.48	4.00	20.67	0.65	36.86	48.21	1.71	59.41	12.07
5	80	0.72	0.13	0.00	2.01	0.55	16.93	29.04	1.40	37.65	9.67
6	80	0.70	0.28	9.23	13.64	0.42	45.00	34.54	1.40	56.86	10.04
7	128	0.91	0.45	3.30	15.44	0.48	35.25	33.84	1.84	44.69	7.92
8	128	0.74	0.50	1.06	19.18	0.86	28.99	41.67	2.10	52.21	11.50
9	128	0.64	0.33	6.00	9.20	0.93	24.70	34.11	1.91	47.39	10.73
10	104	0.85	0.28	0.00	14.71	0.75	30.23	35.25	1.89	51.66	10.97
11	104	0.67	0.41	2.46	20.82	0.51	43.91	43.63	1.59	58.96	9.79
12	104	0.64	0.50	7.31	7.94	0.58	28.00	72.00	1.72	60.82	11.44
13	104	0.56	0.61	9.99	28.39	0.51	29.50	43.99	1.68	53.30	11.87
14	104										
15	128	0.73	0.40	1.78	16.13	0.95	35.06	34.10	2.08	53.99	11.44
16	104	0.95	0.42	2.64	12.52	0.61	34.40	51.20	1.97	57.06	10.93
17	80	0.69	0.25	7.96	17.50	0.46	44.90	33.15	1.40	59.49	10.15
18	104	0.90	0.27	12.03	2.59	0.75	26.00	34.16	1.92	48.11	10.86
19	104	0.72	0.22	0.00	10.67	0.63	30.04	37.59	1.56	52.91	9.04
20	80	0.65	0.26	6.99	19.33	0.58	32.00	64.20	1.48	74.64	11.93
21	104	0.81	0.37	6.90	24.68	0.72	35.16	54.41	1.89	69.77	11.57
22	80	0.75	0.31	9.56	30.00	0.26	38.74	39.31	1.32	57.17	8.17
23	128	0.71	0.50	13.20	11.05	0.72	35.20	45.85	1.94	57.74	10.40
24	104	0.72	0.10	0.00	5.79	0.80	22.21	60.36	1.62	74.11	9.53
25	104	0.85	0.24	2.22	12.22	0.62	29.39	31.88	1.71	48.34	9.09
26	104	0.89	0.55	7.00	22.82	0.57	25.85	60.19	2.00	58.41	11.85
27	104	1.22	0.18	0.00	1.74	0.85	15.82	44.97	2.25	50.44	11.01
28	104	0.55	0.56	7.87	23.59	0.37	29.88	55.12	1.48	52.55	9.89
29	128	0.83	0.32	10.10	10.00	1.00	32.02	34.74	2.15	55.43	11.20
30	104	0.69	0.20	4.38	8.02	0.66	35.10	38.67	1.56	59.38	9.20
31	104	0.84	0.46	5.67	16.72	0.65	29.08	44.34	1.95	52.35	11.82
32	128	0.70	0.42	5.91	22.59	1.18	32.00	65.87	2.30	79.62	13.55
33	80	0.63	0.15	1.00	18.36	0.60	25.60	59.66	1.38	72.01	10.72
Mean	**104.00**	**0.76**	**0.34**	**5.82**	**14.11**	**0.69**	**31.55**	**45.33**	**1.79**	**58.17**	**10.99**
sd	16.97	0.14	0.14	4.45	7.73	0.22	6.76	11.66	0.29	9.70	1.91

Appendix XV: Goodness-of-fit data for model fits of the $\dot{V}O_2$ kinetic responses to determine whether the bi- or tri-exponential model fit was applied to the data.

ND	Bi-Phasic			Tri-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
2	1.800531	7	344	1.049647	10	343	2.63	80.12119879	Tri
5	0.705747	7	344	0.574201	10	346	2.63	25.88766243	Tri
6	0.413539	7	341	0.258424	10	337	2.63	66.02618986	Tri
15	0.806434	7	338	0.792164	10	342	2.63	2.011499093	Bi
20	0.634903	7	340	0.746363	10	336	2.63	-16.37736566	Bi

T2D	Bi-Phasic			Tri-Phasic					
ID	*RSS*	*p*	*N*	*RSS*	*p*	*N*	*Critical Value*	*F-value*	*Model*
6	0.682055	7	341	0.53601	10	340	2.63	30.24372004	Tri
7	0.458259	7	336	0.424864	10	338	2.63	8.672546975	Tri
8	0.977855	7	345	0.748876	10	343	2.63	34.24545917	Tri
9	0.682848	7	342	0.544471	10	346	2.63	28.71889793	Tri
13	0.725269	7	332	0.764701	10	332	2.63	-5.586243349	Bi
14	0.336048	7	349	0.265595	10	344	2.63	29.79818724	Tri
15	0.563721	7	337	0.613043	10	340	2.63	-8.930434578	Bi
17	0.812409	7	341	0.587137	10	340	2.63	42.58852335	Tri
19	0.447277	7	346	0.407648	10	341	2.63	10.82316844	Tri
21	0.592065	7	345	0.414919	10	346	2.63	48.24405464	Tri
27	1.774387	7	341	1.567983	10	338	2.63	14.52395239	Tri

Appendix XVI: Individual mono-exponential model fits for HR kinetic response to steady-state cycling at 80% VT.

Non-Diabetic Group						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1						
2	111	34	0.00	65.58	145	98.45
3	73	38	10.10	44.98	111	99.17
4	98	49	0.00	63.05	146	98.00
5	81	60	0.99	90.28	140	99.83
6	91	40	6.99	68.36	130	99.22
7	89	52	1.49	70.64	141	99.47
8	77	45	17.37	33.07	122	99.77
9	87	40	0.00	55.33	126	99.34
10	105	25	1.00	108.14	129	99.23
11	89	48	1.86	64.47	137	98.83
12	93	47	3.68	63.96	141	99.82
13	87	50	3.63	41.45	137	99.00
14	89	27	7.60	70.70	116	99.18
15	87	30	0.00	62.88	117	95.63
16	79	46	0.00	68.35	125	98.92
17	98	38	3.40	83.78	135	99.00
18	97	45	9.97	57.54	142	99.76
19	96	40	1.00	40.93	136	97.31
20						
Mean	**90.37**	**41.81**	**3.84**	**64.08**	**131.93**	**98.89**
sd	9.59	9.03	4.80	18.21	10.56	1.04

Type 2 Diabetic Group						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	72	33	0.00	90.64	104	98.71
2	85	27	12.99	54.77	112	97.25
3	95	29	2.00	109.29	123	98.11
4	88	42	1.00	100.04	129	98.08
5	84	42	11.05	64.62	126	99.74
6	99	32	1.00	124.70	129	95.55
7	79	36	14.00	64.94	115	99.49
8	87	41	0.00	97.63	127	99.02
9	98	29	8.84	89.98	126	99.04
10	77	28	7.84	68.41	105	99.04
11	86	45	14.91	84.83	129	99.89
12	91	35	1.00	68.60	125	99.04
13	96	56	0.00	66.68	152	98.95
14	86	35	11.32	55.17	120	98.57
15	91	43	5.00	115.69	132	99.63
16	94	24	5.74	91.04	118	98.70
17	89	24	9.00	78.53	112	98.75
18	66	45	2.00	126.82	108	98.46
19	83	37	12.00	129.36	118	99.62
20	89	36	0.00	45.22	125	98.90
21	89	36	0.00	95.76	124	98.35
22	85	29	16.00	46.76	114	99.07
23	102	45	17.00	55.76	147	99.56
24	91	35	0.00	110.17	124	97.88
25	108	27	11.16	74.43	136	99.62
26	112	38	13.56	65.23	149	99.04
27	109	26	0.00	105.30	134	98.20
28	94	28	13.04	41.87	122	99.20
29	96	37	6.98	129.59	131	99.23
30	96	29	0.00	42.62	125	98.51
31	98	30	0.00	64.68	128	96.44
32	100	41	10.00	96.68	139	98.97
33	85	35	11.74	51.53	120	99.27
Mean	**90.87**	**34.97**	**6.64**	**82.04**	**125.14**	**98.72**
sd	9.97	7.29	5.93	27.06	11.43	0.92

Appendix XVII: Individual CO values and related parameters at rest.

Non-Diabetic Group								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	5.49	82	66.89	33.47	2.74			9.30
2	6.01	94	63.20	29.94	2.85			8.46
3	4.53	58	78.64	39.56	2.28	90.0	2.28	9.28
4	5.70	84	67.86	31.30	2.63			7.12
5	3.75	56	66.90	30.76	1.72	96.0	3.12	8.01
6	5.70	89	64.02	30.62	2.72			8.61
7	7.36	111	67.17	27.64	3.03	112.9	4.08	9.92
8	5.73	70	81.82	34.29	2.40	102.5	2.99	8.34
9	5.74	73	79.27	35.68	2.58	93.3	2.62	8.47
10	3.55	104	34.29	15.22	1.58	85.7	5.63	9.48
11	4.11	90	45.64	24.30	2.19	93.3	3.84	9.71
12	8.57	72	114.10	57.85	4.34	100.0	1.73	7.29
13	7.05	87	81.46	42.45	3.67	99.0	2.33	5.54
14	4.94	76	65.01	29.37	2.23	84.7	2.88	7.30
15	4.08	66	61.81	29.91	1.97			9.12
16	4.29	79	54.32	26.90	2.13	96.7	3.59	4.15
17	6.29	91	69.15	35.43	3.22	98.3	2.77	6.66
18	3.75	60	62.51	30.29	1.82	11.3	0.37	4.69
19	5.61	91	61.56	29.73	2.71	11.7	0.39	5.10
20	7.29	92	79.20	36.67	3.37	14.5	0.39	4.97
Mean	**5.48**	**81.13**	**68.24**	**32.57**	**2.81**	**79.33**	**2.60**	**7.58**
sd	1.38	14.86	15.97	8.26	0.69	35.25	1.47	1.83

Non-Diabetic Group								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	4.15	71	76.77	38.46	2.08	105.0	2.73	7.05
2	4.85	90	53.94	24.99	2.25	100.0	4.00	6.37
3	4.99	84	59.45	29.52	2.48	105.0	3.56	6.24
4	5.53	75	73.49	32.21	2.43	95.5	2.97	5.60
5	4.92	60	82.09	38.80	2.32	90.0	2.32	6.34
6	5.40	87	62.02	32.57	2.83	105.0	3.22	5.97
7	4.20	87	48.57	23.56	2.04	100.0	4.24	6.76
8	6.03	82	74.05	37.67	3.07	103.3	2.74	5.44
9	5.11	91	56.11	23.05	2.10	102.5	4.45	9.66
10	4.32	85	50.80	23.97	2.04	98.3	4.10	8.31
11	4.52	78	57.92	29.20	2.28	99.2	3.40	4.67
12	5.03	79	63.70	33.43	2.64	104.2	3.12	5.24
13	4.36	70	62.23	32.47	2.27	100.0	3.08	7.32
14	4.75	71	67.36	34.73	2.45	80.8	2.33	4.89
15	5.35	82	66.07	29.24	2.37	106.2	3.63	6.39
16	5.34	75	71.22	33.28	2.50	99.2	2.98	7.56
17	4.51	77	58.93	29.01	2.22	82.5	2.84	7.02
18	5.10	87	58.69	28.33	2.46	95.8	3.38	7.31
19	5.58	77	72.45	34.06	2.62	90.8	2.67	6.31
20	6.09	69	88.84	37.69	2.58	116.7	3.10	5.54
21	5.05	83	61.12	28.87	2.39	93.5	3.24	4.36
22	3.90	59	66.04	34.65	2.04	93.3	2.69	6.99
23	6.15	84	73.23	36.47	3.06	107.5	2.95	3.85
24	4.19	97	43.36	22.03	2.13	102.5	4.65	7.31
25	6.99	95	73.79	33.72	3.20	95.8	2.84	3.57
26	5.92	103	57.50	27.51	2.83	11.1	0.40	6.67
27	6.33	78	81.18	34.03	2.65	91.7	2.69	7.49
28	6.10	69	88.45	44.90	3.10	93.7	2.09	7.28
29	4.54	91	49.87	23.63	2.15	95.3	4.03	8.35
30	4.45	83	53.59	27.93	2.32	99.3	3.55	4.86
31	6.00	78	76.88	39.64	3.09	96.7	2.44	5.88
32	4.46	85	52.44	26.70	2.27	90.0	3.37	4.91
33	5.08	92	55.27	27.68	2.54	10.9	0.39	5.47
Mean	**5.13**	**80.94**	**64.77**	**31.33**	**2.48**	**92.77**	**3.04**	**6.27**
sd	0.76	9.95	11.66	5.55	0.34	22.26	0.92	1.35

Appendix XVIII: Individual CO values and related parameters after 30s steady-state cycling at 80% VT.

Non-Diabetic Group								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	12.67	95	133.49	66.79	6.34			11.61
2	13.62	114	119.90	56.80	6.45			10.25
3	7.89	78	101.80	51.21	3.97	105.0	2.05	12.53
4	11.94	114	104.71	48.30	5.51			10.18
5	13.03	109	119.56	54.97	5.99			9.28
6	10.97	115	95.39	45.63	5.25			10.45
7	13.07	126	104.13	42.85	5.38	122.8	2.87	10.99
8	13.62	107	127.24	53.32	5.71	114.2	2.14	9.40
9	11.69	94	125.07	56.29	5.26	105.0	1.87	9.49
10	8.11	113	71.78	31.85	3.60	105.0	3.30	14.09
11	10.23	114	89.77	47.80	5.45	108.3	2.27	10.32
12	14.87	110	135.83	68.87	7.54	116.7	1.69	11.19
13	11.40	107	107.07	55.80	5.94	103.3	1.85	8.13
14	6.53	96	67.99	30.72	2.95	120.0	3.91	16.68
15	9.91	88	113.52	54.94	4.80			10.88
16	9.10	100	91.03	45.08	4.51	113.3	2.51	9.08
17	10.67	111	96.13	49.25	5.47	111.7	2.27	9.97
18	8.12	78	104.16	50.47	3.94	19.4	0.39	14.59
19	13.66	116	117.79	56.89	6.60	23.4	0.41	11.75
20	11.25	117	96.17	44.53	5.21	18.3	0.41	9.30
Mean	**11.12**	**104.93**	**106.13**	**50.62**	**5.29**	**91.89**	**1.99**	**11.01**
sd	2.29	13.16	18.45	9.44	1.10	39.21	1.05	2.10

Non-Diabetic Group								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L\ min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg\ L^{-1}\ min^{-1}$	$ml\ O_2\ 100ml\ blood^{-1}$
1	9.37	92	101.86	51.03	4.69	110.0	2.16	14.84
2	10.77	100	107.72	49.91	4.99	110.0	2.20	9.80
3	9.66	103	94.25	46.79	4.80	123.3	2.64	10.72
4	9.87	100	98.73	43.27	4.33	119.2	2.75	10.17
5	9.95	85	117.09	55.35	4.70	102.5	1.85	8.66
6	8.34	106	79.05	41.51	4.38	115.0	2.77	12.66
7	8.92	106	84.15	40.82	4.33	118.3	2.90	11.01
8	10.96	98	111.88	56.91	5.57	130.0	2.28	9.87
9	8.98	108	83.16	34.16	3.69	117.5	3.44	14.75
10	10.74	103	104.24	49.18	5.07	116.7	2.37	10.67
11	10.45	104	100.50	50.66	5.27	120.0	2.37	10.06
12	8.88	103	86.18	45.23	4.66	117.5	2.60	10.23
13	9.60	97	98.97	51.64	5.01	116.7	2.26	10.67
14	8.01	92	97.04	50.03	4.13	100.8	2.01	11.05
15	11.66	108	108.48	48.00	5.16	123.3	2.57	9.13
16	8.69	100	87.31	40.80	4.06	122.5	3.00	15.83
17	7.85	92	85.75	42.21	3.86	112.5	2.67	9.72
18	9.31	107	83.41	40.27	4.49	96.7	2.40	10.14
19	9.32	96	97.21	45.70	4.38	111.7	2.44	10.00
20	11.48	96	120.16	50.97	4.87	130.8	2.57	10.23
21	10.95	98	112.46	53.12	5.17	113.3	2.13	8.09
22	6.983	83	84.19	44.17	3.66	116.7	2.64	11.28
23	11.37	102	111.96	55.76	5.66	125.8	2.26	8.99
24	9.42	121	78.19	39.72	4.79	121.7	3.06	12.00
25	10.017	122	82.30	37.60	4.58	123.3	3.28	10.75
26	10.64	122	87.21	41.72	5.09	17.3	0.41	11.53
27	11.52	118	97.62	40.92	4.83	103.3	2.52	14.01
28	8.837	97	91.56	46.48	4.49	117.5	2.53	9.83
29	9.22	105	87.78	41.60	4.37	107.5	2.58	11.84
30	9.511	106	89.73	46.77	4.96	115.0	2.46	8.69
31	11.399	109	104.58	53.92	5.88	123.3	2.29	10.01
32	9.772	114	85.72	43.64	4.97	105.0	2.41	10.29
33	6.328	107	59.14	29.61	3.17	12.0	0.40	11.34
Mean	**9.66**	**102.88**	**94.53**	**45.74**	**4.67**	**109.60**	**2.40**	**10.87**
sd	1.30	9.50	13.22	6.33	0.59	25.80	0.62	1.81

Appendix XIX: Individual CO values and related parameters after 240s steady-state cycling at 80% VT.

Non-Diabetic Group

ID	CO L min^{-1}	HR beats.min^{-1}	SV ml	SVI ml m^{-2}	CI L.min^{-1} m^{-2}	MAP mmHg	TPR mmHg L^{-1} min^{-1}	a-v O_2 ml O_2 100ml blood^{-1}
1	16.10	123	131.34	65.71	8.06			12.99
2	15.79	145	108.92	51.60	7.48			15.80
3	10.13	99	102.39	51.50	5.10			18.05
4	13.86	140	99.01	45.67	6.39			14.97
5	14.08	146	96.47	44.36	6.48			15.61
6	12.29	140	87.72	41.96	5.88			15.99
7	14.29	143	100.30	41.28	5.88	123.3	2.99	16.56
8	15.80	126	125.41	52.56	6.62	115.8	2.20	16.15
9	14.73	114	129.75	58.40	6.63	105.0	1.80	16.44
10	10.05	131	76.71	34.04	4.46	113.3	3.33	16.81
11	11.47	133	86.19	45.89	6.11	110.8	2.42	18.59
12	17.47	138	127.10	64.44	8.86	120.8	1.87	14.01
13	15.06	141	106.83	55.67	7.85	111.7	2.01	15.48
14	12.00	113	102.00	46.08	5.42	121.7	2.64	14.46
15	11.81	114	103.56	50.12	5.71			14.51
16	11.48	126	91.14	45.14	5.69	115.0	2.55	14.33
17	11.49	137	83.84	42.95	5.88	113.3	2.64	16.76
18	11.29	94	120.14	58.21	5.47	23.1	0.40	17.56
19	17.79	134	132.78	64.13	8.59	27.1	0.42	16.70
20	15.12	141	107.21	49.64	7.00	21.2	0.43	14.74
Mean	**13.61**	**128.78**	**105.94**	**50.47**	**6.48**	**94.01**	**1.98**	**15.82**
sd	2.36	15.15	16.95	8.53	1.18	40.35	0.99	1.42

Type 2 Diabetic Group

ID	CO L.min^{-1}	HR beats.min^{-1}	SV ml	SVI ml.m^{-2}	CI L.min^{-1}.m^{-2}	MAP mmHg	TPR mmHg.L^{-1}.min^{-1}	a-v O_2 ml O_2.100ml blood^{-1}
1	10.92	111	98.42	49.31	5.47	113.3	2.3	18.74
2	13.24	133	99.91	46.29	6.14	116.7	2.5	18.28
3	11.56	116	99.68	49.49	5.74	125.0	2.5	12.13
4	11.52	114	101.52	44.49	5.05	120.8	2.7	14.08
5	11.36	102	111.91	52.90	5.37	120.0	2.3	15.56
6	10.10	124	81.48	42.79	5.31	128.3	3.0	14.55
7	9.73	123	79.09	38.37	4.72	125.0	3.3	14.56
8	12.63	117	107.98	54.93	6.43	136.7	2.5	16.60
9	11.37	119	95.51	39.23	4.67	130.8	3.3	17.35
10	12.50	125	100.41	47.38	5.90	120.0	2.5	17.21
11	11.48	118	97.27	49.03	5.79	126.7	2.6	15.44
12	11.66	137	85.10	44.66	6.12	118.3	2.6	14.29
13	11.35	115	98.73	51.51	5.92	128.3	2.5	13.55
14	10.73	118	90.92	46.88	5.53	112.5	2.4	15.70
15	14.25	123	115.85	51.26	6.30	133.3	2.6	14.58
16	10.30	127	81.08	37.89	4.81	135.0	3.6	18.44
17	10.97	110	99.73	49.09	5.40	121.7	2.5	12.48
18	10.28	134	76.68	37.02	4.96	109.2	2.9	16.01
19	10.10	108	93.61	44.00	4.75	117.5	2.7	13.53
20	13.32	116	114.84	48.72	5.65	135.0	2.8	14.01
21	12.59	124	101.34	47.87	5.95	126.7	2.6	13.69
22	9.29	109	85.63	44.93	4.87	131.7	2.9	15.92
23	12.06	116	103.98	51.79	6.01	130.8	2.5	15.53
24	11.40	147	77.76	39.50	5.79	126.7	3.2	17.10
25	14.753	144	102.81	46.98	6.74	126.7	2.7	11.56
26	12.13	149	81.38	38.93	5.80	16.8	0.4	15.71
27	13.81	131	105.44	44.20	5.79	110.0	2.5	18.03
28	10.83	121	89.87	45.62	5.49	124.2	2.7	13.72
29	11.36	126	90.12	42.71	5.38	118.3	2.8	19.38
30	11.03	135	81.67	42.57	5.75	120.0	2.8	15.60
31	14.16	125	113.29	58.42	7.30	126.7	2.2	13.58
32	12.07	134	90.08	45.86	6.15	115.0	2.5	19.46
33	8.67	137	63.25	31.67	4.34	13.4	0.4	15.37
Mean	**11.62**	**123.76**	**94.43**	**45.64**	**5.62**	**117.01**	**2.56**	**15.51**
sd	1.43	11.48	12.48	5.64	0.64	27.26	0.63	2.07

Appendix XX: Anthropometric data for the ND EXS group at each assessment during the intervention study.

Baseline								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.19	0.93		1.77	82.55	26.35	25.7	2.00
2	1.14	0.95	85.3	1.82	89.50	27.02	22.8	2.11
3	1.20	0.97	210.0	1.83	77.00	22.99	21.5	1.99
4	1.16	0.96	264.0	1.80	97.10	29.97	28.0	2.17
5	1.16	0.94		1.80	97.85	30.20	33.3	2.17
6	1.25	1.02	35.0	1.79	90.00	28.09	32.3	2.09
7	1.22	0.98		1.93	113.25	30.56	32.0	2.43
8	1.09	0.89	203.5	1.89	112.50	31.66	28.7	2.39
9	1.02	0.97	119.3	1.79	107.40	33.52	33.8	2.25
10	1.20	1.01	178.5	1.71	76.00	26.14	25.8	1.88
Mean	**1.16**	**0.96**	**156.50**	**1.81**	**94.32**	**28.65**	**28.38**	**2.15**
sd	0.07	0.04	79.94	0.06	13.73	3.11	4.40	0.17

3 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.22	0.93	325.0	1.78	87.35	27.72	27.3	2.05
2	1.03	0.91	208.0	1.82	83.65	25.39	21.0	2.05
3	1.07	0.97	218.0	1.83	73.55	22.08	20.4	1.95
4	1.10	0.95	143.8	1.78	93.80	29.77	29.7	2.11
5	1.10	0.97	210.0	1.80	96.35	29.90	31.0	2.16
6	1.27	1.04	139.0	1.79	88.95	27.92	30.1	2.08
7	1.14	0.97	255.0	1.91	108.55	29.76	29.5	2.37
8	1.10	1.00	240.9	1.87	109.75	31.55	28.1	2.34
9	1.07	0.97	173.5	1.78	106.90	33.74	33.8	2.24
10	1.24	1.00	241.0	1.71	73.50	25.14	24.0	1.86
Mean	**1.14**	**0.97**	**215.41**	**1.80**	**92.24**	**28.30**	**27.48**	**2.12**
sd	0.08	0.04	55.48	0.05	13.40	3.41	4.37	0.16

7 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.20	0.97		1.77	86.40	27.58	27.0	2.04
2	1.15	1.01		1.81	83.90	25.61	19.3	2.05
3	1.12	1.02		1.83	74.35	22.32	19.8	1.95
4	1.15	1.03		1.79	96.15	30.01	28.1	2.15
5	1.10	1.03		1.80	98.40	30.37	31.6	2.18
6	1.11	1.05		1.78	90.30	28.50	30.7	2.08
7	1.15	0.95	188.0	1.91	109.45	30.16	30.7	2.38
8	1.12	0.98	269.8	1.88	109.25	30.91	27.8	2.35
9	1.12	0.98	196.3	1.79	106.55	33.25	32.0	2.25
10	1.23	0.97	219.3	1.70	74.80	25.88	25.1	1.86
Mean	**1.15**	**1.00**	**218.31**	**1.81**	**92.96**	**28.46**	**27.21**	**2.13**
sd	0.04	0.03	36.75	0.06	13.21	3.18	4.61	0.17

Appendix XX: Anthropometric data for the ND CTL group at each assessment during the intervention study.

Baseline								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.10	0.90		1.81	77.00	23.50	15.5	1.97
2	1.22	0.96		1.77	93.05	29.70	29.5	2.10
3	1.10	0.95	212.0	1.77	75.00	23.54	21.5	1.92
4	1.10	0.99	173.0	1.87	95.55	27.32	23.7	2.21
5	1.24	1.04	263.5	1.75	86.20	28.15	30.9	2.02
6	1.21	1.03	233.0	1.63	89.85	33.82	35.5	1.95
7	1.26	1.02	110.0	1.77	89.45	28.71	28.4	2.06
8	1.22	1.02	259.3	1.73	93.25	31.16	26.7	2.07
9	1.19	1.05	214.0	1.74	102.50	34.05	32.4	2.16
Mean	**1.18**	**0.99**	**209.25**	**1.76**	**89.09**	**28.88**	**27.13**	**2.05**
sd	0.06	0.05	53.54	0.06	8.72	3.83	6.12	0.10

3 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.11	0.99	450.8	1.82	77.00	23.25	15.1	1.98
2	1.19	0.99	126.3	1.77	93.05	29.70	25.7	2.10
3	1.09	0.99	200.0	1.77	75.10	24.11	20.7	1.92
4	1.15	1.05	198.5	1.88	95.80	27.11	23.7	2.22
5	1.25	1.03	220.5	1.75	85.60	27.95	29.0	2.01
6	1.01	1.01	198.0	1.63	87.95	33.10	34.6	1.93
7	1.17	1.04	111.5	1.77	91.40	29.17	26.7	2.08
8	1.18	1.02	168.4	1.73	93.90	31.37	27.4	2.08
9	1.2	1.03	249.0	1.735	102.9	34.18	29.6	2.16
Mean	**1.15**	**1.02**	**213.65**	**1.76**	**89.19**	**28.88**	**25.82**	**2.05**
sd	0.07	0.02	98.93	0.07	8.91	3.73	5.61	0.10

7 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.13	0.98	243.8	1.82	78.20	23.61	16.8	1.99
2	1.19	1.00		1.77	94.70	30.23	24.7	2.12
3	1.07	0.95	224.5	1.77	75.70	24.16	18.4	1.93
4	1.00	1.04	174.5	1.87	95.25	27.24	26.4	2.21
5	1.30	1.02	244.0	1.75	85.40	27.89	31.5	2.01
6	1.24	1.02	178.0	1.63	90.20	33.95	35.4	1.96
7	1.17	1.04	133.5	1.77	90.30	28.99	29.8	2.07
8	1.11	1.04	169.6	1.73	91.90	30.71	25.2	2.06
9	1.16	1.05	173.5	1.74	102.80	34.15	32.6	2.16
Mean	**1.15**	**1.02**	**192.67**	**1.76**	**89.38**	**28.99**	**26.75**	**2.06**
sd	0.09	0.03	40.00	0.07	8.51	3.75	6.28	0.10

Appendix XX: Anthropometric data for the T2D EXS group at each assessment during the intervention study.

Baseline								
	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.06	0.96	158.50	1.72	86.85	29.53	33.30	2.00
2	1.15	0.95	159.50	1.78	97.95	30.91	30.70	2.16
3	1.00	0.96	99.00	1.72	88.70	30.16	33.75	2.01
4	1.18	0.98	198.25	1.84	106.00	31.48	32.70	2.28
5	1.26	1.00	77.00	1.81	93.00	28.54	33.75	2.13
6	1.09	1.01	135.00	1.68	80.50	28.52	25.06	1.90
7	1.15	0.96	152.00	1.80	86.25	26.62	31.85	2.06
8	1.15	1.00	182.00	1.71	84.20	28.80	29.71	1.97
9	1.27	0.99	164.75	1.82	90.35	27.28	26.66	2.12
10	1.12	1.01	208.75	1.70	79.00	27.34	31.15	1.91
11	1.20	1.03	152.13	1.68	81.75	28.96	29.20	1.92
Mean	1.15	0.99	153.35	1.75	88.60	28.92	30.71	2.04
sd	0.08	0.03	39.02	0.06	8.03	1.52	2.87	0.12

3 Months								
	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.16	1.01	209.8	1.70	88.50	30.62	33.4	2.00
2	1.21	1.02	139.8	1.77	95.80	30.58	32.5	2.13
3	1.08	1.08	145.5	1.71	89.05	30.35	32.9	2.02
4	1.12	1.05	208.0	1.83	106.15	31.87	28.7	2.27
5	1.29	1.00	135.0	1.80	92.00	28.40	32.3	2.12
6	1.16	1.05	206.8	1.68	82.80	29.51	27.3	1.92
7	1.13	0.98	145.8	1.80	87.00	26.85	30.1	2.07
8	1.31	1.04	185.0	1.71	85.40	29.38	28.9	1.97
9	1.14	1.00	190.8	1.82	91.40	27.59	28.7	2.13
10	1.13	0.99	204.3	1.70	79.20	27.40	30.8	1.91
11	1.12	1.04	217.3	1.68	81.70	28.95	31.5	1.92
Mean	1.17	1.02	180.69	1.74	89.00	29.23	30.62	2.04
sd	0.07	0.03	32.42	0.06	7.48	1.57	2.06	0.11

7 Months								
	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.17	1.01	235.5	1.71	88.85	30.39	35.8	2.01
2	1.18	1.03	161.8	1.77	98.10	31.31	29.2	2.15
3	1.05	1.05	214.0	1.71	90.55	30.97	33.4	2.03
4	1.07	1.02		1.82	106.40	32.12	30.3	2.27
5	1.25	1.00	143.8	1.80	92.20	28.46	34.4	2.12
6	1.18	1.05	165.5	1.67	81.90	29.37	28.7	1.91
7	1.17	1.03	190.5	1.79	87.10	27.18	29.2	2.06
8	1.20	1.02	233.3	1.70	84.45	29.39	29.2	1.96
9	1.17	0.99	185.3	1.82	91.00	27.47	24.7	2.13
10	1.11	1.01	145.0	1.70	78.80	27.27	29.7	1.90
11	1.13	1.03	156.3	1.68	82.90	29.37	32.5	1.93
Mean	1.15	1.02	183.08	1.74	89.30	29.39	30.63	2.04
sd	0.06	0.02	34.58	0.06	7.86	1.69	3.13	0.12

Appendix XX: Anthropometric data for the T2D CTL group at each assessment during the intervention study.

Baseline

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.15	0.96	190.5	1.84	103.15	30.47	31.0	2.26
2	1.36	1.04	128.0	1.71	103.30	35.53	33.8	2.14
3	1.03	1.01	142.3	1.76	86.60	27.96	25.7	2.03
4	1.20	1.01	174.6	1.76	91.10	29.58	28.1	2.07
5	1.15	1.07	283.0	1.69	103.95	36.61	33.8	2.13
6	1.09	1.04	354.8	1.73	80.20	26.80	33.8	1.94
7	1.26	1.08	157.8	1.85	112.85	32.97	29.8	2.36
8	1.08	1.06	257.0	1.72	99.75	33.91	26.9	2.12
9	1.24	1.03		1.76	90.95	29.53	27.0	2.07
10	1.13	1.02	340.8	1.76	84.25	27.20	28.1	2.01
11	1.24	0.98	117.0	1.79	78.50	24.64	20.1	1.97
Mean	**1.17**	**1.03**	**214.56**	**1.76**	**94.05**	**30.47**	**28.90**	**2.10**
sd	0.10	0.04	88.00	0.05	11.21	3.84	4.16	0.12

3 Months

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.19	1.00	199.0	1.83	103.70	30.97	32.5	2.26
2	1.27	1.04	296.3	1.71	101.75	35.00	32.7	2.13
3	1.07	1.02	168.3	1.76	86.40	27.89	26.4	2.03
4	1.11	1.05	221.5	1.76	91.10	29.58	27.6	2.07
5	1.17	1.03	272.3	1.69	102.75	35.98	34.3	2.12
6	1.02	1.03	364.5	1.73	81.10	27.10	34.1	1.95
7	1.20	1.13	209.9	1.85	116.75	34.11	31.2	2.39
8	1.06	1.04	145.3	1.70	99.70	34.50	27.4	2.10
9	1.24	1.01	120.8	1.75	90.95	29.70	31.0	2.07
10	1.20	0.99	325.3	1.76	84.25	27.35	28.4	2.00
11	1.25	1.01	132.3	1.78	78.00	24.90	18.9	1.96
Mean	**1.16**	**1.03**	**223.19**	**1.76**	**94.22**	**30.64**	**29.48**	**2.10**
sd	0.08	0.04	81.70	0.05	11.72	3.75	4.48	0.13

7 Months

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.19	1.01	188.3	1.83	105.85	31.61	33.9	2.28
2	1.22	1.03	248.3	1.71	102.70	35.12	34.8	2.14
3	0.91	1.02	159.5	1.75	84.80	27.69	25.7	2.01
4	1.05	1.00	227.8	1.76	92.15	29.75	26.7	2.09
5	1.19	1.02	186.0	1.68	104.35	36.97	35.7	2.13
6	1.18	1.01	269.3	1.73	80.80	27.00	33.7	1.95
7	1.19	1.10	419.5	1.87	120.60	34.30	34.1	2.44
8	1.17	1.05	302.3	1.70	97.30	33.67	28.5	2.08
9	1.10	1.01	113.8	1.75	90.45	29.53	32.0	2.06
10	1.20	0.95	270.0	1.75	81.60	26.64	27.6	1.97
11	1.26	1.02	180.8	1.78	78.65	24.82	22.1	1.97
Mean	**1.15**	**1.02**	**233.20**	**1.76**	**94.48**	**30.65**	**30.44**	**2.10**
sd	0.10	0.04	83.12	0.06	13.05	3.97	4.50	0.15

Appendix XX: Anthropometric data for the PIO EXS group at each assessment during the intervention study.

Baseline								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.12	1.04	194.0	1.77	125.15	39.95	40.0	2.39
2	1.18	1.03	203.5	1.67	102.45	36.96	37.6	2.10
3	1.15	0.98	248.5	1.82	89.40	26.99	33.4	2.11
4	1.23	1.04	149.3	1.71	79.50	27.19	26.4	1.92
5	1.28	1.08	213.0	1.63	88.50	33.31	35.2	1.94
6	1.18	0.98	168.3	1.72	83.20	28.12	25.0	1.96
Mean	**1.19**	**1.03**	**196.08**	**1.72**	**94.70**	**32.09**	**32.90**	**2.07**
sd	0.06	0.04	34.82	0.07	16.83	5.53	6.06	0.18

3 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.12	1.01	198.3	1.77	127.20	40.60	38.6	2.40
2	1.27	1.09	215.3	1.67	106.00	38.01	33.8	2.13
3	1.26	1.02	190.0	1.82	89.20	26.93	25.1	2.11
4	1.15	1.02	169.0	1.71	80.20	27.43	25.2	1.93
5	1.29	1.04	233.3	1.63	89.45	33.67	36.6	1.95
6	1.17	0.97	196.0	1.72	84.60	28.60	25.0	1.98
Mean	**1.21**	**1.03**	**200.29**	**1.72**	**96.11**	**32.54**	**30.71**	**2.08**
sd	0.07	0.04	21.99	0.07	17.56	5.82	6.33	0.18

7 Months								
	ABI	**WHR**	**LOPAR**	**Height**	**Weight**	**BMI**	**Body Fat**	**BSA**
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.17	1.04	179.8	1.77	131.30	41.91	41.3	2.43
2	1.21	1.04	188.0	1.67	102.90	36.90	31.7	2.10
3	1.15	1.05	154.8	1.82	90.00	27.32	26.7	2.11
4	1.22	1.03	156.0	1.71	78.80	26.95	27.1	1.91
5	1.22	1.05	179.8	1.63	91.90	34.59	39.1	1.97
6	1.14	0.98	216.8	1.72	84.90	28.70	25.1	1.98
Mean	**1.19**	**1.03**	**179.17**	**1.72**	**96.63**	**32.73**	**31.80**	**2.09**
sd	0.04	0.03	22.91	0.07	18.78	6.07	6.88	0.19

Appendix XX: Anthropometric data for the PIO CTL group at each assessment during the intervention study.

Baseline

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.19	1.10	419.5	1.87	120.60	34.30	34.1	2.44
2	1.10	1.01	113.8	1.75	90.45	29.53	32.0	2.06
3	0.91	1.02	159.5	1.75	84.80	27.69	25.7	2.01
4	1.20	0.95	270.0	1.75	81.60	26.64	27.6	1.97
5	1.13	1.05	157.1	1.72	107.30	36.27	38.2	2.19
Mean	**1.11**	**1.03**	**223.98**	**1.77**	**96.95**	**30.89**	**31.51**	**2.13**
sd	0.12	0.06	123.62	0.06	16.52	4.20	5.02	0.19

3 Months

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.22	1.14	400.8	1.85	122.60	35.82	30.7	2.44
2	1.16	1.02	115.8	1.75	89.20	29.13	31.6	2.05
3	1.03	1.02	164.8	1.75	86.80	28.34	25.9	2.03
4	1.20	1.01	268.5	1.75	84.90	27.72	28.3	2.01
5	1.11	1.05	144.9	1.72	109.90	37.15	39.2	2.21
Mean	**1.14**	**1.05**	**218.93**	**1.76**	**98.68**	**31.63**	**31.13**	**2.15**
sd	0.08	0.05	116.81	0.05	16.73	4.48	5.05	0.18

7 Months

	ABI	WHR	LOPAR	Height	Weight	BMI	Body Fat	BSA
ID	a.u.	a.u.	METhr.wk^{-1}	m	kg	kg.m^{-2}	%	m^2
1	1.19	1.11	238.8	1.85	127.65	37.30	32.1	2.48
2	1.31	1.01	136.3	1.75	89.90	29.36	32.3	2.06
3	0.96	0.98	145.5	1.75	86.75	28.17	26.1	2.02
4	1.23	1.02	264.5	1.75	88.90	29.03	27.8	2.05
5	1.12	1.03	170.6	1.72	111.80	37.79	39.5	2.23
Mean	**1.16**	**1.03**	**191.13**	**1.76**	**101.00**	**32.33**	**31.54**	**2.17**
sd	0.13	0.05	57.37	0.05	18.02	4.78	5.19	0.19

Appendix XXI: Haematological data for the ND EXS group at each assessment during the intervention study.

Baseline									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.53	14.2	41.9	4.7	36.61	4.13	1.46	2.67	1.4
2	4.92	15.1	44.9	4.2	34.43	5.17	1.26	3.69	2.19
3	5.14	16.1	48.5	3.8	36.61	4.14	1.27	2.92	1.14
4	4.99	15.6	45.7	4.2	36.61	4.48	1.44	3.11	0.66
5	4.98	15.4	46.1	4.5	39.89	4.52	0.97	3.49	1.6
6	4.11	13.3	38.8	5.6	40.98	5.87	1.25	4.37	2.67
7	4.38	13.8	39.4	5.3	32.24	4.88	1.21	3.51	1.87
8	5.06	16.2	47.4	4.9	34.43	4.18	1.94	2.4	0.87
9	5.6	16.7	49.5	5.6	37.71	3.31	0.93	2.4	1.06
10	4.62	15.6	44.9	4.3	34.43	4.67	1.33	3.48	0.78
Mean	**4.83**	**15.20**	**44.71**	**4.71**	**36.39**	**4.54**	**1.31**	**3.20**	**1.42**
sd	0.43	1.11	3.63	0.63	2.67	0.69	0.28	0.62	0.66

3 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.44	14.3	41.6	5.3	38.80	3.92	1.56	2.42	0.72
2	5.45	17.1	49.7	5.3	34.43	4.14	1.45	2.74	1.11
3	4.67	15.8	44.5	5.5	37.71	4.2	1.2	2.81	2.15
4	5.02	16.4	47.1	5.1	35.52	4.57	1.54	3.22	0.62
5	4.85	15.5	45.2	6	38.80	4.25	0.94	3.49	1.88
6	4.12	14.1	39.9	5.5	37.71	5.7	1.38	4.28	1.45
7	4.5	14.5	41.1	5.1	31.15	4.72	1.27	3.33	2.21
8	5.39	16.7	49.6	4.6	32.24	4.28	2.06	2.41	0.97
9	5.52	17.2	48.1	5.6	36.61	2.78	0.85	1.9	1.04
10	5.2	17.9	51.2	5.2		6.1	1.52	3.99	1.29
Mean	**4.92**	**15.95**	**45.80**	**5.32**	**35.88**	**4.47**	**1.38**	**3.06**	**1.34**
sd	0.48	1.33	3.98	0.37	2.79	0.92	0.34	0.74	0.57

7 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.74	15.4	43.3		38.80	4.41	1.41	2.78	2.71
2	4.87	15.2	43.6	5.3	34.43	4.44	1.35	3.08	1.32
3	4.4	15.1	42.9	5.1	36.61				
4	5.18	17.8	48.3		35.52	4.61	1.45	3.13	0.75
5	4.77	16	45	5.5	38.80	4.78	0.92	3.61	2.35
6	5.13	16.7	48.1	6.5	42.08	5.91	1.34	4.44	1.77
7	5.39	17.1	48.6	5.3		5.1	1.05	2.67	3.04
8	5.08	15.8	47.9	4.3		4.7	1.87	2.22	1.35
9	5.39	15.9	48.5			3.2	0.86	2.01	0.73
10	5.1	17.5	49.7	4.8		5.6	1.2	3.97	0.94
Mean	**5.01**	**16.25**	**46.59**	**5.26**	**37.71**	**4.75**	**1.27**	**3.10**	**1.66**
sd	0.31	0.97	2.59	0.68	2.76	0.78	0.31	0.80	0.86

Appendix XXI: Haematological data for the ND CTL group at each assessment during the intervention study.

Baseline									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.32	17.8	51.3	5.4	34.43	4.22	1.87	2.55	0.62
2	4.39	13.5	40.6	4.8	44.26	4.43	0.97	3.05	2.99
3	4.23	13.6	38.6	3.9	28.96	4.23	1.49	2.89	0.7
4	5.14	14.3	41.2	5.7	46.45	5.37	1.19	4	1.72
5	4.09	13.8	39.9			5	1.12	2.44	3.17
6	4.26	14.4	41.9			5	1.27	2.32	3.1
7	4.52	15	44.2	5		4.1	1.12	2.63	0.78
8	4.34	15.4	46.2	4.3		6.2	0.85	4.48	1.92
9	4.48	15.4	43.0	4.6		4	1.02	2.16	1.81
Mean	**4.53**	**14.80**	**42.99**	**4.81**	**38.52**	**4.73**	**1.21**	**2.95**	**1.87**
sd	0.42	1.34	3.87	0.62	8.25	0.73	0.31	0.79	1.04

3 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.23	18.3	49.8	6	33.33	4.26	1.47	2.9	0.8
2	5.38	16.7	49.8	5.7	43.17	3.85	1.02	2.65	2.15
3	4.99	16.2	45.2	4.7	30.05	3.23	1.24	2.1	0.51
4	5.54	16	44.6	5.3	45.36	5.52	1.25	4.18	1.91
5	4.04	13.8	40.0	5.2		5.9	1.03	1.75	3.87
6	3.97	13.2	39.7	4.4		4.1	1.76	1.75	1.3
7	4.99	15.7	48.0	5.4		4.9	1.31	3.18	0.91
8	4.02	14.1	43.5	4		5.4	0.78	3.66	2.13
9	4.46	14.2	41.5	5.1		4	1.03	2.26	1.56
Mean	**4.74**	**15.36**	**44.68**	**5.09**	**37.98**	**4.57**	**1.21**	**2.71**	**1.68**
sd	0.62	1.65	3.90	0.63	7.43	0.90	0.29	0.85	1.01

7 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.08	16.4	50.0	4.7	34.43				
2	5.01	15.7	46.0	5.9	44.26	4.59	1.13	3.39	1.99
3	4.16	13.8	37.6	5.3		3.7	1.22	2.14	0.75
4	5.21	17.4	47.9	6.3		5.4	1.04	3.55	1.79
5	4.21	13.7	40.9	4.7		5.2	1.14	2.76	2.87
6	4.01	13.4	41.0	5.5		4.5	1.27	2.78	0.99
7	4.54	14.9	44.6	4.8		4.5	1.2	2.91	0.86
8	4.31	15.5	45.8	4.9		5.7	0.85	3.99	1.86
9				4.6		4.5	1.14	2.43	2.05
Mean	**4.57**	**15.10**	**44.23**	**5.26**	**39.34**	**4.80**	**1.12**	**3.07**	**1.59**
sd	0.47	1.42	4.11	0.60	6.96	0.68	0.14	0.61	0.76

Appendix XXI: Haematological data for the T2D EXS group at each assessment during the intervention study.

Baseline									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.32	13.5	39.7	8	46.45	3.8	1.08	2.38	2.73
2	5.1	16.5	48.3	6.7	40.98	5.1			1.57
3	5.19	16.5	48.2	8.2	54.10	3.5	1.09	2.33	0.93
4	5.17	15.6	46.3	5.6	38.80	4.7	1.27	3.31	1.65
5	6	18	51.4	7.8	46.45	3.9	1.01	2.71	1.77
6	5.66	16.5	48.5	6.9	38.80	6.1	1.49	4.33	1.3
7	4.8	15.2	45.0	7.8	56.28	5.1	1.22	3.55	3.17
8	4.3	14.7	41.7	5.4	46.45	4	1.45	2.31	2.79
9	4.83	14.2	42.9	6.7	57.38	2.2	0.78	1.32	1.77
10	4.88	14.9	43.5	12.3	84.70	3.3	1.3	1.87	0.86
11	4.37	13.1	41.8	5.3	40.98	6.6	1.78	4.55	1.54
Mean	**4.97**	**15.34**	**45.21**	**7.34**	**50.12**	**4.39**	**1.25**	**2.87**	**1.83**
sd	0.54	1.47	3.63	1.95	13.29	1.28	0.28	1.05	0.76

3 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.37	13.7	40.2	7	45.36	4.3	1.19	2.7	3.3
2	5.27	16.4	47.8	6.5	43.17	5.2			1.79
3	4.96	15.7	46.9	7.5	53.01	3.1	0.97	1.94	1.1
4	5.12	15.8	45.7	5.8		5.3	1.5	3.53	1.92
5	5.63	16.8	48.7	8.5	47.54	3.7	0.93	2.42	2.16
6	5.39	15.3	45.8	5.7	39.89	5.7	1.49	4.18	1.49
7	4.47	14.1	43.8	7.2	53.01	4.8	1.01	3.23	2.91
8	4.4	15.2	43.6		45.36				
9	4.7	14	41.8	8.4	54.10	2.7	0.91	1.67	1.43
10	5.17	15.5	45.7	14.2	87.98	4.1	1.57	2.52	0.93
11	4.73	14.5	43.6	5.2	43.17	5.2	1.69	3.3	1.19
Mean	**4.93**	**15.18**	**44.87**	**7.60**	**51.26**	**4.41**	**1.25**	**2.83**	**1.82**
sd	0.43	1.00	2.55	2.57	13.77	1.01	0.31	0.80	0.78

7 Months									
	RBC	Hb	Hct	FBG	HbA_{1c}	Cholesterol	HDL-c	LDL-c	Triglycerides
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.38	13.5	38.0	9.4	47.54	4.5	1.09	2.84	4.23
2	5.14	16.2	47.6	6.6	43.17	4.7	1.06	3.32	2.07
3	5.07	15.9	47.2	6.2	48.63	2.7	0.87	1.74	1.28
4	5.2	15.9	48.0	5.3	45.36	4.1	1.35	2.53	1.83
5	5.65	16.6	49.1	7.5	46.45	3.4	0.92	2.37	1.82
6	5.25	15.3	44.6	5.4	37.71	5.4	1.46	3.69	1.29
7	4.65	14.5	44.6	7.4	55.19	3.8	0.98	2.7	2.01
8	4.41	15.1	43.6	6.6	44.28	4.2	1.42	2.46	2.93
9	4.58	13.9	40.3	7.4	50.82	1.8	0.83	1.06	0.87
10	5.15	15.3	45.8	12.6	86.89	3.8	1.52	2.33	1.02
11	4.64	14.4	43.1	5.4		6.3	1.66	4.38	1.1
Mean	**4.92**	**15.15**	**44.72**	**7.25**	**50.60**	**4.06**	**1.20**	**2.67**	**1.86**
sd	0.41	0.98	3.38	2.14	13.57	1.22	0.29	0.90	0.99

Appendix XXI: Haematological data for the T2D CTL group at each assessment during the intervention study.

Baseline									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.81	15.2	45.8	6.6	56.28	3.5	0.89	2.32	1.72
2	5.06	15.9	44.7	7.9	55.19	5.6	1.23	4.34	1.97
3	4.18	14	41.0	7	42.08	4.3	1.07	3.1	3.23
4	4.95	14.9	43.4	7.4	48.63	3.6	1.12	2.3	1.73
5	5.14	16.1	46.7	5.3	38.80	4.6	1.14	3.2	2.51
6	5.28	16.6	50.4	5.6	40.98	4	1.53	2.16	3.68
7	4.96	15.6	46.3	5.7	42.08	4.7	1.06	3.29	1.98
8	4.87	16.1	45.0	7.7	47.54	2.8	1.2	1.52	0.81
9	5.07	15.2	44.1	7.6	48.63	4.9	1.36	3.35	1.48
10	4.85	15.6	44.7	6.9	44.26	4.8	1.08	3.16	1.76
11	5.2	15.5	46.9	7.3	45.36	5	1.08	3.6	2.61
Mean	**4.94**	**15.52**	**45.36**	**6.82**	**46.35**	**4.35**	**1.16**	**2.94**	**2.13**
sd	0.29	0.70	2.37	0.91	5.63	0.81	0.17	0.79	0.82

3 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.85	15.2	45.8	7.9	58.47	3.8	0.94	2.52	1.08
2	5.16	15.9	44.7	7.3	54.10	5.2	1.01	3.67	1.6
3	4.35	14.7	41.0	7.9	40.98	4.3	1.08	2.87	3.25
4	5.18	15.4	43.4	7.2	42.08	4.2	1.24	2.72	1.75
5	5.18	15.9	46.7	11.9	39.89	4.5	1.19	3.07	1.97
6	4.95	15.7	50.4	6.5	40.98	4.3	1.47	2.52	3.71
7	4.9	15.5	46.3	5	45.36	3.9	0.95	2.64	1.64
8	4.93	15.9	45.0	7.2	51.91	2.6	1.31	1.32	0.85
9	5.26	15.4	44.1	6.8	48.63	4.7	1.39	3.08	1.5
10	5.08	15.4	44.7	7.6	49.73	4.9	1.01	3.22	3.77
11	5.3	15.9	46.9	7.7	48.63	4.5	1.06	3.1	2.46
Mean	**5.01**	**15.54**	**45.36**	**7.55**	**47.34**	**4.26**	**1.15**	**2.79**	**2.14**
sd	0.27	0.38	2.37	1.66	6.06	0.69	0.18	0.60	1.02

7 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.94	15.3	46.2	4.8	49.73	3.2	0.94	2.31	0.95
2	4.85	15.1	45.8	9.2	54.10	4.8	1.07	3.47	2.07
3	4.17	14.1	43.3	7.6	43.17				
4	4.89	14.3	45.5	7.7	43.17	3.4	1.16	2.29	1.85
5	5.3	16.3	47.3	6.9	39.89	5.2	1.32	3.67	2.71
6	4.92	15.3	47.7	6.5	44.26	3.6	1.34	2.13	3.19
7	5.04	15.9	45.2	5.8	39.89	6.1	1.16	4.79	1.95
8	4.98	15.7	45.1	7.7	49.73	2.9	1.37	1.5	0.86
9	5.07	14.7	45.8	7.5	48.63	4.3	1.11	2.75	2.31
10	5.19	16	47.3	6.6	46.45	5.3	1.24	3.62	1.43
11	5.09	15.2	48.8	7.1	49.73	4.8	1.04	3.31	2.04
Mean	**4.95**	**15.26**	**46.18**	**7.04**	**46.25**	**4.36**	**1.18**	**2.98**	**1.94**
sd	0.29	0.70	1.51	1.15	4.55	1.05	0.14	0.97	0.73

Appendix XXI: Haematological data for the PIO EXS group at each assessment during the intervention study.

Baseline									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.17	15.8	45.3	8.3	55.19	4.3	1.59	2.9	1.52
2	5.24	15.8	45.3	7.4	46.45	5.1	0.92	3.29	1.96
3	4.58	14	40.7	7	45.36	4.3	1.23	2.37	1.55
4	4.84	14.2	41.8	6.9	44.26	4.9	1.03	2.39	3.26
5	5.04	15.6	47.0	8.8	50.82	5.0	1.26	2.98	1.67
6	4.60	14.4	41.7	9.3	62.84	3.7	1.5	1.92	0.61
Mean	**4.91**	**14.97**	**43.63**	**7.95**	**50.82**	**4.55**	**1.26**	**2.64**	**1.76**
sd	0.28	0.85	2.55	1.00	7.15	0.54	0.26	0.50	0.86

3 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.13	15.4	45.7	8.6		3.9	1.46	2.21	1.91
2	5.31	15.8	45.8	7	44.26	4.4	1.05	2.62	1.6
3				6.6	44.26	6.1	1.33	3.84	2.05
4	4.52	13.2	47.0	6	39.89	5.9	1.32	3.11	3.24
5	5.04	15.6	41.7	8.2	49.73	5.8	1.23	3.62	2.09
6	4.60	14.4	45.0	9.3	61.75	3.8	1.7	1.8	0.66
Mean	**4.92**	**14.88**	**45.04**	**7.62**	**47.98**	**4.98**	**1.35**	**2.87**	**1.93**
sd	0.34	1.08	2.00	1.28	8.45	1.06	0.22	0.80	0.83

7 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	5.05	15.1	44.4	8.6	57.38	3.2	1.37	1.84	1.03
2	5.27	15.7	45.9		46.45	4.4	1.08	2.57	1.66
3	4.49	13.7	41.2	6.8	46.45	4.2	1.32	1.96	2.02
4	4.59	13.8	40.2	6.2	39.89	5.5	1.34	3.04	2.46
5	4.90	15.4	46.4	7.5	46.45	4.7	1.16	2.74	1.77
6	4.78	15	43.7	8.1	60.66	3.9	1.74	1.88	0.61
Mean	**4.85**	**14.78**	**43.63**	**7.44**	**49.54**	**4.32**	**1.34**	**2.34**	**1.59**
sd	0.29	0.84	2.49	0.97	7.83	0.77	0.23	0.51	0.67

Appendix XXI: Haematological data for the PIO CTL group at each assessment during the intervention study.

Baseline									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.92	15.3	47.7	6.5	44.26	3.6	1.34	2.13	3.19
2	4.17	14.1	40.3	7.6	43.17				
3	5.09	15.2	46.7	7.5	48.63	4.3	1.11	2.75	2.31
4	4.98	15.7	45.8	7.7	51.91	2.9	1.37	1.5	0.86
5	4.81	13.6	41.6		56.28				
Mean	**4.79**	**14.78**	**44.42**	**7.33**	**48.85**	**3.60**	**1.27**	**2.13**	**2.12**
sd	0.36	0.89	3.27	0.56	5.43	0.70	0.14	0.63	1.18

3 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.66	14.7	43.7	6.2	43.17	3.6	1.48	1.89	2.25
2				8	39.89				
3	5.07	15.7	46.1	7.4	47.54	4.4	1.14	2.98	2.56
4	4.83	15.3	43.3	6.9	47.54	2.9	1.3	1.48	1.08
5	5.09	14.4	44.5	5.9	51.91	4.4	0.99	2.16	2.74
Mean	**4.91**	**15.03**	**44.40**	**6.88**	**46.01**	**3.83**	**1.23**	**2.13**	**2.16**
sd	0.21	0.59	1.24	0.86	4.61	0.72	0.21	0.63	0.75

7 Months									
	RBC	**Hb**	**Hct**	**FBG**	**HbA_{1c}**	**Cholesterol**	**HDL-c**	**LDL-c**	**Triglycerides**
ID	$10^{12}.L^{-1}$	$g.dL^{-1}$	%	$mmol.L^{-1}$	$mmol.mol^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$	$mmol.L^{-1}$
1	4.84	15.8	46.3	6.8					
2				6.7	39.89				
3	4.85	15	43.4	6.6		4.2	1.3	2.73	1.69
4	5.12	16.5	46.8	6.7	45.36	3	1.53	1.45	0.86
5	4.66	13.2	40.3	6.3	50.82	4.2	0.99	2.35	1.9
Mean	**4.87**	**15.13**	**44.20**	**6.62**	**45.36**	**3.80**	**1.27**	**2.18**	**1.48**
sd	0.19	1.42	3.00	0.19	5.46	0.69	0.27	0.66	0.55

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the ND EXS group at each assessment during the intervention study.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	190	18.0	160	2.62	31.68	1.63	19.67	87.36	160	100.63	1.09
2	220	21.0	190	3.50	39.14	3.03	33.64	110.62	179	101.13	1.13
3	160	15.0	130	2.10	27.31	1.21	15.68	90.32	130	86.09	1.08
4	190	16.0	160	2.33	23.95	1.50	15.45	105.63	159	101.27	1.15
5	190	16.0	160	2.52	25.77	1.44	14.74	103.15	170	102.41	1.16
6	190	18.0	160	2.58	28.64	2.32	25.72	85.90	173	106.13	1.00
7	220	21.0	190	3.21	28.35	2.63	23.24	105.90	167	105.70	1.03
8	250	24.0	220	3.72	33.03	3.21	28.57	96.79	161	95.83	1.04
9	160	14.0	130	2.55	23.77	1.70	15.86	105.05	162	103.85	1.11
10	190	18.0	160	3.11	40.95	2.67	28.55	110.93	165	104.43	1.07
Mean	**196.00**	**18.10**	**166.00**	**2.82**	**30.26**	**2.13**	**22.11**	**100.17**	**162.60**	**100.75**	**1.09**
sd	27.57	3.11	27.57	0.53	5.96	0.72	6.79	9.41	13.09	5.95	0.05

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	220	21.0	190	3.00	34.29	1.85	21.19	83.84	171	107.55	1.00
2	250	24.0	220	3.53	42.16	2.94	35.14	119.69	157	88.70	1.05
3	190	17.5	160	2.20	29.91	2.00	27.23	84.03	124	82.12	1.07
4	190	18.0	160	2.83	30.20	2.02	21.56	100.79	157	100.00	1.09
5	220	21.0	190	3.03	31.46	1.72	17.82	102.05	168	101.20	1.15
6	190	17.5	160	2.85	32.01	2.47	27.77	84.22	161	98.77	
7	250	24.0	220	3.80	35.02	2.65	24.44	110.75	165	104.43	1.00
8	280	27.0	250	4.21	38.40	3.66	33.34	120.08	176	104.76	1.01
9	190	18.0	130	2.78	26.00	2.25	20.93	95.46	160	102.56	1.03
10	220	21.0	190	3.33	45.36	2.58	35.07	119.39	169	106.96	1.10
Mean	**220.00**	**20.90**	**187.00**	**3.16**	**34.48**	**2.41**	**26.45**	**102.03**	**160.80**	**99.71**	**1.06**
sd	31.62	3.27	35.92	0.58	5.96	0.58	6.33	15.03	14.36	8.19	0.05

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	250	22.0	190	3.35	38.74	2.31	26.74	96.17	170	106.92	1.17
2	280	25.5	220	4.27	50.94	2.90	34.51	130.31	170	96.05	1.05
3	190	16.8	160	2.70	36.31	1.61	21.67	90.87	128	84.77	1.05
4	190	16.0	130	3.04	31.60	1.67	17.40	116.76	155	98.73	1.10
5	220	21.0	190	3.55	36.06	2.24	22.75	120.76	175	105.42	1.11
6	160	15.0	130	3.27	36.21	2.16	23.89	77.82	157	96.32	
7	280	24.0	190	4.06	37.07	3.50	32.01	116.59	169	106.96	0.99
8	280	24.0	250	4.19	38.37	3.15	28.83	107.91	169	100.60	0.99
9	220	21.0	190	3.32	31.11	2.15	20.21	124.33	156	100.00	0.99
10	220	21.0	190	3.27	43.74	2.43	33.06	108.64	172	108.86	1.04
Mean	**229.00**	**20.63**	**184.00**	**3.50**	**38.02**	**2.41**	**26.11**	**109.02**	**162.10**	**100.46**	**1.05**
sd	42.54	3.60	36.88	0.52	5.77	0.61	5.85	16.37	13.99	7.19	0.06

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the ND CTL group at each assessment during the intervention study.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	220	21.0	190	3.380	43.896	2.805	36.430	104.050	164	87.70	0.99
2	190	18.0	130	2.913	31.306	2.066	20.866	93.780			1.17
3	220	21.0	190	3.492	46.560	2.464	32.859	108.590	189	100.00	1.16
4	160	15.0	130	2.375	24.856	2.042	21.367	83.340	151	97.42	1.11
5	160	15.0	130	2.581	29.942	2.294	26.617	101.576	171	109.62	1.14
6	160	14.0	130	2.381	26.500	2.095	23.372	85.875	161	96.41	1.08
7	220	21.0	160	3.399	37.999	2.625	29.346	84.465	148	91.36	1.12
8	250	22.5	190	3.727	39.968	2.466	26.477	95.277	177	96.20	1.07
9	220	21.0	190	3.333	32.517	2.721	26.543	99.625	175	106.71	1.01
Mean	**200.00**	**18.72**	**160.00**	**3.06**	**34.84**	**2.40**	**27.10**	**95.18**	**167.00**	**98.18**	**1.09**
sd	33.54	3.27	30.00	0.51	7.64	0.29	5.15	9.10	13.76	7.28	0.06

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	220	21.0	190	3.266	42.416	2.501	32.482	99.550	167	89.30	1.10
2	190	18.0	130	2.745	29.500	1.955	21.007	80.970			1.06
3	220	21.0	190	3.255	43.342	2.174	28.990	104.340	184	97.35	1.13
4	190	16.5	160	2.633	27.484	1.844	19.247	104.790	148	95.48	1.13
5	160	15.0	130	2.182	25.491	2.053	23.984	90.039	177	113.46	1.20
6	160	13.5	130	2.339	26.595	1.756	19.966	89.790	159	95.21	1.13
7	250	22.5	190	3.052	33.392	2.245	24.562	87.759	135	83.33	1.10
8	250	22.5	190	3.469	36.944	2.934	31.246	112.648	164	89.13	1.13
9	250	24.0	190	3.249	31.574	2.396	23.285	109.845	173	105.49	1.11
Mean	**210.00**	**19.33**	**166.67**	**2.91**	**32.97**	**2.21**	**24.97**	**97.75**	**163.38**	**96.10**	**1.12**
sd	36.74	3.71	29.15	0.45	6.65	0.37	4.87	11.00	15.94	9.64	0.04

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	220	21.0	190	3.535	45.205	3.050	39.003	131.373	186	99.47	1.11
2	190	18.0	160	3.180	33.580	2.405	25.397	97.400			0.96
3	220	21.0	190	3.286	43.408	2.577	34.042	95.960	177	93.65	1.01
4	190	16.0	130	2.760	28.976	2.092	21.963	87.455	149	96.13	1.05
5	190	16.0	130	2.316	27.119	1.657	19.403	104.448	178	114.10	1.25
6	160	13.8	130	2.556	28.337	1.866	20.687	84.336	150	89.82	1.11
7	250	24.0	190	3.195	35.382	2.882	31.916	118.184	150	92.59	1.09
8	250	24.0	220	3.762	40.936	3.113	33.874	115.164	166	90.22	1.05
9	250	22.5	190	3.344	32.529	2.562	24.922	118.979	172	104.88	1.14
Mean	**213.33**	**19.58**	**170.00**	**3.10**	**35.05**	**2.47**	**27.91**	**105.92**	**166.00**	**97.61**	**1.09**
sd	32.79	3.77	33.54	0.47	6.72	0.51	6.96	15.95	14.65	8.34	0.08

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the T2D EXS group at each assessment during the intervention study.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	beats.min^{-1}	% Age-Predicted Max	a.u
1	130	12.0	100.0	2.29	26.39	1.50	17.31	86.73	138	88.46	1.09
2	190	18.0	160.0	2.92	29.76	2.25	22.99	114.72	159	100.00	1.14
3	160	15.0	130.0	2.54	28.60	1.57	17.74	68.99	163	107.24	1.06
4	160	15.0	130.0	2.85	26.87	1.80	16.93	99.39	133	81.60	1.06
5	130	12.0	130.0	2.13	22.91	1.81	19.46	73.94			1.12
6	130	12.5	100.0	2.11	26.22	1.54	19.12	81.97	161	99.38	1.10
7	160	13.0	100.0	2.03	23.55	1.46	16.91	74.70	170	107.59	1.17
8	160	15.0	130.0	2.40	28.49	1.82	21.60	111.03	163	101.24	1.15
9	220	19.5	160.0	2.99	33.08	2.20	24.35	102.05	177	113.46	1.13
10	190	18.0	130.0	2.56	32.39	2.03	25.71	98.95	173	99.43	1.16
11	160	15.0	130.0	2.44	29.79	1.99	24.33	91.00	148	90.80	1.07
Mean	**162.73**	**15.00**	**127.27**	**2.48**	**28.01**	**1.82**	**20.59**	**91.22**	**158.50**	**98.92**	**1.11**
sd	28.32	2.58	21.02	0.33	3.24	0.28	3.32	15.36	14.58	9.65	0.04

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	beats.min^{-1}	% Age-Predicted Max	a.u
1	160	15.0	130	2.23	25.22	1.81	20.42	108.31	147	94.23	1.16
2	250	22.5	190	3.05	31.81	2.51	26.19	102.82	175	110.06	1.06
3	190	18.0	160	2.74	30.74	2.43	27.38	84.17	170	111.84	0.99
4	220	21.0	190	3.16	29.81	2.59	24.42	100.83	147	90.18	1.01
5	160	13.5	130	2.29	24.91	1.82	19.79	93.48			1.15
6	160	15.0	130	2.32	27.98	1.85	22.34	89.26	161	99.38	1.04
7	160	15.0	130	2.39	27.41	1.72	19.77	86.78	165	104.43	1.01
8	190	18.0	160	2.77	32.44	2.35	27.59	101.34	164	101.86	1.16
9	250	23.0	190	3.06	33.46	2.58	28.33	102.50	179	114.74	1.05
10	190	18.0	160	2.70	34.07	2.35	29.68	102.94	152	87.36	1.15
11	220	19.0	160	2.87	35.18	2.39	29.25	80.47	145	88.96	1.04
Mean	**195.45**	**18.00**	**157.27**	**2.69**	**30.27**	**2.22**	**25.02**	**95.72**	**160.50**	**100.31**	**1.07**
sd	35.03	3.20	24.94	0.34	3.52	0.34	3.84	9.26	12.26	9.96	0.07

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	ml.kg^{-1}.min^{-1}	L.min^{-1}	beats.min^{-1}	% Age-Predicted Max	a.u
1	160	15.0	130	2.49	28.00	2.01	22.59	111.92	149	95.51	1.09
2	220	20.5	190	3.31	33.75	2.56	26.12	119.34	170	106.92	1.05
3	130	12.0	130	2.25	24.87	1.96	21.69	62.36			0.99
4	250	22.5	190	3.49	32.81	2.68	25.21	134.75	170	104.29	1.10
5	130	12.0	100	2.29	24.83	1.66	18.02	91.85			1.07
6	160	15.0	130	2.53	30.92	1.93	23.55	80.82	158	97.53	1.01
7	160	15.0	130	2.79	32.06	1.95	22.40	104.13	173	109.49	1.01
8	190	17.0	160	2.75	32.60	2.29	27.16	86.06	159	98.76	1.06
9	220	21.0	190	2.99	32.85	2.40	26.41	90.62	178	114.10	0.99
10	190	18.0	160	2.62	33.22	1.97	24.98	88.03	161	92.53	1.04
11	190	18.0	160	2.82	34.05	2.39	28.78	96.36	156	95.71	1.08
Mean	**181.82**	**16.91**	**151.82**	**2.76**	**30.90**	**2.16**	**24.26**	**96.93**	**163.78**	**101.65**	**1.04**
sd	38.16	3.50	30.27	0.39	3.42	0.32	3.01	19.82	9.40	7.36	0.04

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the T2D CTL group at each assessment during the intervention study.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	190	18.0	160	2.73	26.51	1.86	18.06	58.15	156	98.11	1.16
2	160	15.0	130	2.77	26.77	1.81	17.51	100.91	160	96.97	1.00
3	130	12.0	100	1.98	22.84	1.91	22.00	56.72	137	88.39	1.04
4	160	14.5	130	2.19	24.03	2.02	22.15	94.55	161	103.21	1.10
5	130	12.0	100	2.00	19.25	1.65	15.90	81.33	136	87.18	1.14
6	130	12.0	100	2.06	25.71	1.66	20.65	81.70			1.17
7	190	18.0	130	2.97	26.34	2.11	18.68	91.87	167	93.30	1.07
8	160	13.5	130	2.51	25.16	1.87	18.77	91.91	156	85.25	1.22
9	160	15.0	130	2.70	29.64	1.97	21.67	93.06			1.15
10	190	16.0	130	2.70	32.02	2.12	25.11	93.75	157	96.32	1.19
11	190	18.0	160	2.71	34.51	2.27	28.87	109.47	175	102.34	1.07
Mean	**162.73**	**14.91**	**127.27**	**2.48**	**26.62**	**1.93**	**20.85**	**86.67**	**156.11**	**94.56**	**1.12**
sd	24.94	2.40	21.02	0.36	4.23	0.19	3.72	16.41	12.67	6.50	0.07

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	190	17.0	130	2.39	23.01	2.15	20.87	81.08	159	100.00	1.05
2	190	16.0	130	2.72	26.76	2.06	20.36	98.92	156	94.55	
3	130	11.0	100	1.85	21.46	1.69	19.55	68.38	138	89.03	1.02
4	160	13.0	130	2.40	26.39	1.72	19.08	85.85	157	100.64	1.02
5	130	12.0	100	1.97	19.19	1.31	12.69	69.41	133	85.26	1.05
6	130	11.0	100	2.12	26.12	1.59	19.58	80.59			
7	220	19.5	160	3.92	33.57	2.81	24.07	121.53	188	105.03	1.02
8	160	15.0	130	2.40	24.10	2.02	20.22	102.46	164	89.62	1.18
9	160	15.0	130	2.25	24.71	1.78	19.62	95.95			1.16
10	190	16.0	160	3.08	36.53	2.39	28.42	89.48	159	97.55	
11	190	18.0	160	2.67	34.26	2.05	26.31	98.04	176	102.92	1.03
Mean	**168.18**	**14.86**	**130.00**	**2.53**	**26.92**	**1.96**	**20.98**	**90.15**	**158.89**	**96.07**	**1.07**
sd	30.27	2.83	23.24	0.58	5.56	0.41	4.15	15.51	16.90	6.86	0.07

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO_2peak	VO_2 @ VT	VO_2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$ml.kg^{-1}.min^{-1}$	$L.min^{-1}$	$beats.min^{-1}$	% Age-Predicted Max	a.u
1	190	18.0	160	2.66	25.12	2.33	22.05	73.29	154	96.86	0.98
2	160	15.0	130	2.78	27.06	2.06	20.07	102.49	159	96.36	0.99
3	130	11.0	100	1.90	22.36	1.48	17.44	74.31	138	89.03	1.04
4	160	13.0	130	2.44	26.42	1.74	18.88	85.78	160	102.56	1.11
5	130	10.0	100	1.74	16.71	1.46	13.96	74.96	122	78.21	1.06
6	130	11.0	100	2.05	25.35	1.55	19.20	83.53			1.10
7	220	19.5	160	3.66	30.36	2.55	21.16	128.57	178	99.44	1.02
8	160	15.0	130	2.64	27.14	2.11	21.65	88.69	159	86.89	1.09
9	160	15.0	130	2.53	27.95	2.22	24.56	84.04			1.08
10	190	16.0	160	2.55	31.24	2.38	29.20	89.48	161	98.77	1.15
11	190	18.0	160	2.83	35.92	1.95	24.89	101.98	173	101.17	1.05
Mean	**165.45**	**14.68**	**132.73**	**2.52**	**26.88**	**1.99**	**21.19**	**89.74**	**156.00**	**94.37**	**1.06**
sd	29.45	3.15	24.94	0.52	4.94	0.38	4.09	16.23	17.01	8.02	0.05

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the PIO EXS group at each assessment during the intervention study. Subject 1 was excluded from the analysis due to his failure to achieve maximum performance due to physical discomfort on the bike.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg.-1.min-1	L.min-1	ml.kg.-1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1											
2	190	16.5	160	2.73	26.68	2.29	22.37	72.07			1.02
3	220	21.0	160	3.18	35.55	2.49	27.87	114.91	168	105.00	1.09
4	160	14.3	130	2.32	29.16	1.81	22.77	104.37	168	102.44	1.05
5	160	15.0	130	2.32	26.23	1.71	19.31	99.61	154	95.06	1.16
6	190	16.3	160	2.89	34.76	2.26	27.17	97.45	150	87.72	1.05
Mean	**184.00**	**16.60**	**148.00**	**2.69**	**30.47**	**2.11**	**23.90**	**97.68**	**160.00**	**97.56**	**1.07**
sd	25.10	2.63	16.43	0.37	4.42	0.34	3.58	15.82	9.38	7.79	0.06

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg.-1.min-1	L.min-1	ml.kg.-1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1											
2	250	22.0	190	2.62	24.73	2.07	19.54	87.37			
3	250	23.0	190	3.24	36.28	2.59	28.95	121.99	172	107.50	1.04
4	190	17.0	160	2.49	31.10	2.00	24.87	105.56	155	94.51	1.15
5	190	18.0	160	2.97	33.19	2.24	25.06	103.91	158	97.53	1.07
6	220	19.0	190	2.72	32.20	2.21	26.12	98.02	145	84.80	1.10
Mean	**220.00**	**19.80**	**178.00**	**2.81**	**31.50**	**2.22**	**24.91**	**103.37**	**157.50**	**96.08**	**1.09**
sd	30.00	2.59	16.43	0.30	4.25	0.23	3.42	12.61	11.15	9.35	0.05

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg.-1.min-1	L.min-1	ml.kg.-1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1											
2	190	18.5	160	2.62	25.44	2.20	21.42	72.86			1.08
3	220	21.0	190	3.39	37.64	2.45	27.24	119.16	179	111.88	1.02
4	160	14.5	130	2.00	25.42	1.71	21.73	117.33	154	93.90	1.14
5	190	16.5	160	2.49	27.04	2.06	22.38	102.28	160	98.77	1.22
6	190	18.0	160	2.52	29.67	2.15	25.36	104.36	137	80.12	1.13
Mean	**190.00**	**17.70**	**160.00**	**2.60**	**29.04**	**2.12**	**23.63**	**103.20**	**157.50**	**96.16**	**1.12**
sd	21.21	2.41	21.21	0.50	5.11	0.27	2.56	18.55	17.33	13.12	0.08

Appendix XXII: Peak individual responses to graded cycle test to exhaustion in the PIO CTL group at each assessment during the intervention study.

Baseline											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg -1.min-1	L.min-1	ml.kg -1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1	220	19.5	160	3.66	30.36	2.55	21.16	128.57	178	99.44	1.02
2	160	15.0	130	2.53	27.95	2.22	24.56	84.04			1.08
3	130	11.0	100	1.90	22.36	1.48	17.44	74.31	138	90.20	1.04
4	190	16.0	160	2.55	31.24	2.38	29.20	89.48	161	99.38	1.15
5	160	15.0	130	2.43	22.63	1.88	17.55	84.47	163	103.16	1.03
Mean	**172.00**	**15.30**	**136.00**	**2.61**	**26.91**	**2.10**	**21.98**	**92.17**	**160.00**	**98.05**	**1.07**
sd	34.21	3.03	25.10	0.64	4.21	0.43	4.99	21.08	16.51	5.52	0.05

3 Months											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg -1.min-1	L.min-1	ml.kg -1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1	250	22.0	190	3.72	30.32	2.53	20.61	122.95	183	102.23	1.14
2	160	13.5	130	2.64	29.63	2.00	22.43	80.30			
3	130	11.5	100	2.17	25.00	1.70	20.06	76.18	142	92.81	
4	190	16.0	130	2.95	34.79	2.08	24.80	90.32	160	98.77	1.01
5	160	14.5	130	2.25	20.46	2.02	18.38	73.06	163	103.16	1.04
Mean	**178.00**	**15.50**	**136.00**	**2.75**	**28.04**	**2.07**	**21.28**	**88.56**	**162.00**	**99.24**	**1.06**
sd	45.50	3.98	32.86	0.63	5.48	0.30	2.45	20.29	16.79	4.69	0.07

7 Months											
	Peak Wkld	TTF	VT	VO2peak	VO2peak	VO2 @ VT	VO2 @ VT	Peak Ve	Peak HR	Peak HR	Peak RER
ID	W	Min	W	L.min-1	ml.kg -1.min-1	L.min-1	ml.kg -1.min-1	L.min-1	beats.min-1	% Age-Predicted Max	a.u
1	220	20.5	190	4.06	31.77	3.20	25.07	125.00	181	101.12	1.05
2	160	14.0	130	2.04	22.71	1.55	17.19	81.73			1.15
3	130	12.0	100	2.04	23.47	1.59	18.33	77.02	138	90.20	1.06
4	160	15.0	130	2.30	25.91	1.75	19.70	72.50	153	94.44	1.09
5	160	14.0	130	2.29	20.47	1.76	15.76	78.73	153	96.84	1.11
Mean	**166.00**	**15.10**	**136.00**	**2.55**	**24.87**	**1.97**	**19.21**	**87.00**	**156.25**	**95.65**	**1.09**
sd	32.86	3.21	32.86	0.85	4.32	0.69	3.58	21.51	17.95	4.56	0.04

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the ND EXS group at each assessment during the intervention study.

Baseline

ID	Workload W	a $L.min^{-1}$	A_1 $L.min^{-1}$	TD_1 s	Tau_1 s	A_2 $L.min^{-1}$	TD_2 s	Tau_2 s	End A $L.min^{-1}$	MRT s	VO_2 Gain $ml.min^{-1}.W^{-1}$
1	152	0.70	0.80	1.77	15.02	0.80	26.41	21.41	2.30	32.30	11.25
2	104	0.68	0.23	2.93	3.75	0.85	26.78	34.54	1.76	49.62	11.47
3	128	0.72	0.35	0.00	2.46	0.93	14.68	42.14	2.00	41.93	10.84
4	128	0.76	0.46	5.44	6.24	0.76	30.51	30.02	1.98	42.08	10.37
5	128	0.78	0.27	5.25	9.49	0.85	22.74	30.57	1.89	44.04	9.46
6	128	0.70	0.50	5.63	5.37	0.84	20.99	30.97	2.04	36.71	11.37
7	128	0.70	0.35	9.99	4.66	0.97	33.19	33.25	2.02	52.78	11.16
8	152	0.89	0.59	3.00	8.22	0.95	29.08	31.18	2.43	41.43	10.87
9	176	0.70	0.66	13.77	5.01	1.15	32.41	26.14	2.50	44.06	10.88
10	104	0.83	0.18	0.00	5.52	0.78	17.78	57.76	1.78	62.65	10.18
Mean	**132.80**	**0.75**	**0.44**	**4.78**	**6.57**	**0.89**	**25.46**	**33.80**	**2.07**	**44.76**	**10.78**
sd	22.05	0.07	0.20	4.36	3.59	0.12	6.25	9.98	0.26	8.53	0.62

3 Months

ID	Workload W	a $L.min^{-1}$	A_1 $L.min^{-1}$	TD_1 s	Tau_1 s	A_2 $L.min^{-1}$	TD_2 s	Tau_2 s	End A $L.min^{-1}$	MRT s	VO_2 Gain $ml.min^{-1}.W^{-1}$
1	176	0.79	0.18	0.02	20.57	1.60	20.05	33.00	2.57	49.78	10.72
2	128	0.55	0.38	5.99	12.22	0.95	36.03	41.04	1.87	60.10	11.25
3	128	0.80	0.76	7.00	29.00	0.63	35.30	27.06	2.18	47.94	11.73
4	152	0.69	0.73	6.99	29.42	1.20	41.28	45.39	2.61	67.68	13.53
5	128	0.73	0.56	8.99	26.00	0.83	32.33	41.71	2.12	58.30	11.76
6	152	0.77	0.50	12.00	6.65	1.00	31.26	32.73	2.27	48.85	10.55
7	152	0.77	0.84	7.11	19.12	0.85	32.15	46.46	2.46	52.52	11.88
8	176	1.13	0.50	12.00	6.62	1.48	28.99	29.79	3.12	48.62	11.94
9	200	0.73	0.10	4.82	2.95	1.62	16.89	31.32	2.46	45.76	9.09
10	104	0.94	0.31	10.60	9.46	0.69	32.93	35.80	1.94	53.49	10.69
Mean	**149.60**	**0.79**	**0.49**	**7.55**	**16.20**	**1.08**	**30.72**	**36.43**	**2.36**	**53.30**	**11.32**
sd	28.73	0.16	0.25	3.63	9.90	0.37	7.29	6.78	0.37	6.82	1.17

7 Months

ID	Workload W	a $L.min^{-1}$	A_1 $L.min^{-1}$	TD_1 s	Tau_1 s	A_2 $L.min^{-1}$	TD_2 s	Tau_2 s	End A $L.min^{-1}$	MRT s	VO_2 Gain $ml.min^{-1}.W^{-1}$
1	176	0.63	0.55	8.39	14.43	1.53	16.81	44.48	2.71	51.14	12.54
2	128	0.68	0.28	7.70	3.41	0.99	28.22	32.60	1.95	49.77	10.81
3	104	0.69	0.41	12.20	7.51	0.76	30.16	49.43	1.85	58.71	12.37
4	152	0.88	0.57	15.00	5.89	1.01	38.41	33.95	2.46	53.74	11.14
5	104	0.70	0.40	7.20	19.93	0.50	33.91	29.65	1.60	47.37	9.57
6	152	0.78	0.40	9.60	2.81	1.18	23.15	37.57	2.36	48.52	11.11
7	152	0.80	0.43	7.19	5.48	1.28	24.00	43.10	2.51	53.55	12.04
8	152	0.93	0.70	3.05	27.99	0.98	31.95	34.97	2.61	51.97	11.83
9	200	0.71	1.11	6.00	28.47	1.06	25.40	37.70	2.89	48.47	11.46
10	152	0.95	0.55	3.00	28.97	0.92	34.34	32.41	2.42	53.73	10.38
Mean	**147.20**	**0.78**	**0.54**	**7.93**	**14.49**	**1.02**	**28.63**	**37.58**	**2.34**	**51.70**	**11.32**
sd	29.50	0.11	0.23	3.71	10.96	0.28	6.42	6.26	0.41	3.40	0.92

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the ND CTL group at each assessment during the intervention study.

Baseline											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	104	0.64	0.32	4.43	11.52	0.78	22.50	41.98	1.75	50.34	11.77
2	152	0.68	0.40	0.13	29.99	1.05	30.99	24.93	2.13	48.79	10.21
3	152	0.87	0.80	0.01	14.41	0.69	27.35	19.85	2.36	29.60	10.53
4	104	0.80	0.35	15.50	14.68	0.64	39.87	37.68	1.79	60.79	10.55
5	104	0.73	0.18	0.00	10.59	0.86	30.46	41.40	1.77	61.02	11.05
6	104	0.78	0.37	1.93	12.79	0.78	32.18	44.92	1.93	57.18	12.25
7	128	0.86	0.57	1.19	29.99	0.70	33.65	10.22	2.13	38.16	10.79
8	152	0.90	0.40	10.36	6.04	1.15	27.85	37.85	2.45	52.98	10.92
9	152	0.71	0.74	7.59	14.53	1.03	26.00	48.34	2.48	52.50	12.48
Mean	**128.00**	**0.77**	**0.46**	**4.57**	**16.06**	**0.85**	**30.10**	**34.13**	**2.09**	**50.15**	**11.17**
sd	24.00	0.09	0.20	5.50	8.34	0.18	5.01	12.84	0.30	10.38	0.80

3 Months											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	104	0.64	0.50	10.97	9.70	0.64	34.02	43.42	1.78	52.56	12.14
2	152	0.64	0.46	2.89	29.97	1.18	32.49	25.76	2.29	51.09	11.58
3	152	0.92	0.55	4.00	16.46	0.97	28.51	31.10	2.43	45.44	10.67
4	128	0.86	0.52	14.18	19.23	0.87	38.04	96.90	2.23	96.88	11.56
5	104	0.75	0.45	0.19	22.01	0.56	30.89	56.57	1.76	58.37	10.71
6	104	1.05	0.42	12.88	16.26	0.56	41.71	16.82	2.03	45.83	10.41
7	152	0.78	0.52	1.20	19.12	0.83	25.33	22.17	2.13	36.97	9.53
8	152	0.81	0.56	4.50	9.55	0.89	31.25	30.03	2.25	43.04	10.18
9	152	0.69	0.50	15.00	3.57	1.03	37.59	21.77	2.23	46.01	10.83
Mean	**133.33**	**0.79**	**0.50**	**7.31**	**16.21**	**0.84**	**33.31**	**38.28**	**2.13**	**52.91**	**10.85**
sd	23.32	0.13	0.05	5.89	7.80	0.22	5.11	25.17	0.23	17.58	0.80

7 Months											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	128	0.73	0.37	8.56	5.70	0.93	29.40	52.83	2.02	63.00	10.94
2	152	0.62	0.33	5.13	17.38	1.29	25.85	29.13	2.24	48.33	11.43
3	152	0.77	0.66	5.95	21.63	0.78	29.31	32.21	2.21	46.06	10.15
4	104	0.76	0.30	12.20	7.83	0.85	31.31	48.75	1.90	64.45	12.14
5	104	0.72	0.28	9.95	5.00	0.69	37.67	31.37	1.69	53.51	10.28
6	104	0.96	0.26	11.60	8.29	0.66	36.00	37.22	1.87	58.24	9.69
7	152	0.80	0.71	13.30	12.52	0.67	26.99	33.50	2.18	42.72	9.74
8	152	0.83	0.80	6.33	12.35	0.67	43.13	40.08	2.30	48.18	10.32
9	176	0.75	0.90	8.25	14.57	1.15	27.01	35.08	2.80	44.84	12.35
Mean	**136.00**	**0.77**	**0.51**	**9.03**	**11.70**	**0.85**	**31.85**	**37.80**	**2.13**	**52.15**	**10.78**
sd	26.83	0.09	0.25	2.93	5.56	0.23	5.85	8.10	0.32	8.04	0.99

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the T2D EXS group at each assessment during the intervention study.

Baseline

ID	Workload W	a L.min^{-1}	A_1 L.min^{-1}	TD_1 s	Tau_1 s	A_2 L.min^{-1}	TD_2 s	Tau_2 s	End A L.min^{-1}	MRT s	VO_2 Gain ml.min^{-1}.W^{-1}
1	128	0.77	0.28	15.00	5.49	1.14	34.26	36.83	2.18	61.21	11.97
2	80	0.97	0.32	8.05	8.65	1.00	33.82	58.40	2.29	73.75	18.80
3	104	0.57	0.48	4.00	20.67	0.65	36.86	48.21	1.71	59.41	12.07
4	104	0.88	0.25	15.99	5.33	0.60	45.33	49.08	1.73	72.81	8.98
5	80	0.72	0.13	0.00	2.01	0.55	16.93	29.04	1.40	37.65	9.67
6	80	0.70	0.28	9.23	13.64	0.42	45.00	34.54	1.40	56.86	10.04
7	128	0.74	0.50	1.06	19.18	0.86	28.99	41.67	2.10	52.21	11.50
8	104	0.85	0.28	0.00	14.71	0.75	30.23	35.25	1.89	51.66	10.97
9	104	0.64	0.50	7.31	7.94	0.58	28.00	72.00	1.72	60.82	11.44
10	104	0.56	0.61	9.99	26.39	0.51	29.50	43.99	1.68	53.30	11.87
11	104										
Mean	**101.82**	**0.74**	**0.36**	**7.06**	**12.40**	**0.71**	**32.89**	**44.90**	**1.81**	**57.97**	**11.73**
sd	16.82	0.13	0.15	5.79	7.87	0.23	8.38	12.79	0.31	10.54	2.70

3 Months

ID	Workload W	a L.min^{-1}	A_1 L.min^{-1}	TD_1 s	Tau_1 s	A_2 L.min^{-1}	TD_2 s	Tau_2 s	End A L.min^{-1}	MRT s	VO_2 Gain ml.min^{-1}.W^{-1}
1	152	0.86	0.44	3.58	11.88	1.12	28.84	31.56	2.41	47.71	10.96
2	104	0.61	0.35	0.01	17.00	0.87	33.26	31.62	1.82	51.26	12.92
3	152	0.81	0.60	7.50	14.74	1.02	29.73	40.00	2.44	52.11	11.46
4	104	0.77	0.40	0.00	26.09	0.55	34.45	60.06	1.72	65.51	10.06
5	104	0.74	0.25	9.07	10.81	0.72	27.58	44.30	1.71	58.45	10.36
6	104	0.89	0.25	14.53	1.56	0.68	31.33	36.09	1.82	53.46	9.91
7	152	0.85	0.70	7.43	20.52	0.83	30.20	31.23	2.38	46.05	10.80
8	128	0.93	0.38	8.35	15.78	0.87	25.76	41.67	2.17	54.37	10.54
9	128	0.81	0.65	7.00	14.08	0.58	33.28	43.75	2.04	47.50	10.45
10	128	0.52	0.37	7.00	2.92	1.06	26.25	29.90	1.95	44.13	12.16
11	128	0.84	0.39	3.23	13.53	0.92	32.79	31.53	2.15	50.23	11.09
Mean	**125.82**	**0.78**	**0.44**	**6.15**	**13.54**	**0.84**	**30.32**	**38.34**	**2.06**	**51.89**	**10.97**
sd	19.95	0.12	0.15	4.23	7.01	0.19	2.98	9.03	0.27	6.10	0.91

7 Months

ID	Workload W	a L.min^{-1}	A_1 L.min^{-1}	TD_1 s	Tau_1 s	A_2 L.min^{-1}	TD_2 s	Tau_2 s	End A L.min^{-1}	MRT s	VO_2 Gain ml.min^{-1}.W^{-1}
1	152	0.74	0.82	4.80	21.64	0.77	35.95	38.08	2.33	49.50	11.21
2	104	0.77	0.31	7.89	5.37	0.84	25.19	37.52	1.92	49.26	12.22
3	152	0.80	0.58	10.50	11.64	1.03	32.77	34.08	2.41	50.67	11.38
4	80	0.70	0.13	8.68	3.00	0.59	25.86	62.91	1.41	74.99	10.14
5	104	0.75	0.39	8.80	19.00	0.69	22.81	62.32	1.83	64.57	11.44
6	104	0.87	0.35	9.48	15.46	0.58	31.80	33.21	1.80	49.94	9.94
7	152	0.83	0.70	4.99	29.80	0.78	29.34	38.82	2.30	52.38	10.41
8	128	0.79	0.42	6.41	22.15	0.88	25.00	52.29	2.09	61.58	11.02
9	128	0.72	0.51	10.16	7.63	0.62	32.83	37.67	1.85	46.85	9.57
10	128	0.73	0.23	0.01	20.61	0.96	23.62	47.04	1.92	60.82	10.08
11	104	0.72	0.31	7.81	3.94	0.86	29.30	54.98	1.89	65.12	12.40
Mean	**121.45**	**0.76**	**0.43**	**7.23**	**14.57**	**0.78**	**28.59**	**45.36**	**1.98**	**56.88**	**10.89**
sd	24.22	0.05	0.21	3.06	8.86	0.15	4.37	11.09	0.29	9.01	0.94

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the T2D CTL group at each assessment during the intervention study.

Baseline											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	L.min^{-1}	L.min^{-1}	s	s	L.min^{-1}	s	s	L.min^{-1}	s	ml.min^{-1}.W^{-1}
1	128	0.73	0.40	1.78	16.13	0.95	35.06	34.10	2.08	53.99	11.44
2	104	0.95	0.42	2.64	12.52	0.61	34.40	51.20	1.97	57.06	10.93
3	80	0.69	0.25	7.96	17.50	0.46	44.90	33.15	1.40	59.49	10.15
4	104	0.90	0.27	12.03	2.59	0.75	26.00	34.16	1.92	48.11	10.86
5	104	0.72	0.22	0.00	10.67	0.63	30.04	37.59	1.56	52.91	9.04
6	80	0.83	0.29	8.34	21.43	0.58	33.00	65.27	1.70	75.43	12.38
7	104	0.81	0.37	6.90	24.68	0.72	35.16	54.41	1.89	69.77	11.57
8	80	0.75	0.31	9.56	30.00	0.26	38.74	39.31	1.32	57.17	8.17
9	128	0.71	0.50	13.20	11.05	0.72	35.20	45.85	1.94	57.74	10.40
10	104	0.72	0.10	0.00	5.79	0.80	22.21	60.36	1.62	74.11	9.53
11	104	0.72	0.24	12.85	3.00	0.79	37.73	37.41	1.74	61.46	10.88
Mean	**101.82**	**0.77**	**0.31**	**6.84**	**14.12**	**0.66**	**33.86**	**44.80**	**1.74**	**60.66**	**10.49**
sd	16.82	0.09	0.11	5.01	8.85	0.19	6.15	11.38	0.25	8.83	1.21

3 Months											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	L.min^{-1}	L.min^{-1}	s	s	L.min^{-1}	s	s	L.min^{-1}	s	ml.min^{-1}.W^{-1}
1	104	0.64	0.36	9.81	2.98	0.74	32.30	30.43	1.74	46.30	11.70
2	104	0.80	0.29	8.76	1.81	1.04	25.38	70.06	2.12	76.92	14.06
3	80	0.73	0.33	2.93	25.22	0.43	38.00	62.67	1.49	68.95	10.89
4	128	0.99	0.36	12.55	1.68	1.03	30.51	44.53	2.37	59.40	11.75
5	104	0.76	0.18	6.82	9.60	0.77	30.95	44.08	1.70	64.08	10.02
6	80	0.64	0.47	5.26	16.79	0.41	46.87	62.71	1.52	63.17	12.54
7	128	0.82	0.40	9.20	20.28	1.10	28.28	80.16	2.31	87.38	12.59
8	80	0.68	0.33	4.48	26.71	0.31	44.01	33.57	1.32	53.66	9.20
9	128	0.60	0.62	6.50	12.59	0.80	35.64	54.76	2.02	59.29	12.06
10	104	0.77	0.20	7.99	15.92	0.69	20.21	30.14	1.66	44.40	9.49
11	104	0.72	0.45	2.71	29.99	0.53	43.03	49.26	1.70	64.95	10.43
Mean	**104.00**	**0.74**	**0.36**	**7.00**	**14.87**	**0.71**	**34.11**	**51.13**	**1.82**	**62.59**	**11.34**
sd	18.59	0.11	0.12	3.03	10.14	0.27	8.29	16.60	0.34	12.51	1.48

7 Months											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	L.min^{-1}	L.min^{-1}	s	s	L.min^{-1}	s	s	L.min^{-1}	s	ml.min^{-1}.W^{-1}
1	128	0.79	0.48	1.82	29.46	0.85	29.87	32.76	2.11	51.32	11.21
2	104	0.90	0.30	7.87	3.01	0.59	23.34	42.89	1.79	47.43	9.43
3	80	0.80	0.20	4.00	26.20	0.52	30.37	51.03	1.53	67.16	10.31
4	128	0.98	0.46	11.43	3.99	0.90	28.23	37.42	2.33	48.69	11.47
5	104	0.80	0.30	4.65	12.39	0.71	26.07	59.61	1.81	65.46	10.70
6	80	0.77	0.22	7.00	17.20	0.55	33.86	40.17	1.55	59.65	11.13
7	128	0.84	0.26	10.14	5.12	0.78	35.93	38.38	1.87	59.67	8.78
8	80	0.74	0.15	11.36	3.00	0.48	31.57	36.22	1.37	55.01	9.06
9	128	0.87	0.25	14.00	3.88	1.05	30.20	42.86	2.17	62.56	10.98
10	104	0.94	0.25	0.00	14.62	0.85	29.17	43.99	2.04	59.78	11.75
11	104	0.59	0.41	8.19	24.67	0.59	45.17	34.18	1.59	60.07	10.63
Mean	**106.18**	**0.82**	**0.30**	**7.31**	**13.05**	**0.71**	**31.25**	**41.77**	**1.83**	**57.89**	**10.50**
sd	19.95	0.11	0.11	4.35	10.14	0.18	5.74	7.84	0.31	6.52	1.00

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the PIO EXS group at each assessment during the intervention study.

Baseline											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	104	1.22	0.18	0.00	1.74	0.85	15.82	44.97	2.25	50.44	11.01
2	128	0.67	0.46	7.00	27.23	0.67	33.08	53.55	1.81	65.28	9.64
3	128	0.83	0.32	10.10	10.00	1.00	32.02	34.74	2.15	55.43	11.20
4	104	0.69	0.20	4.38	8.02	0.66	35.10	38.67	1.56	59.38	9.20
5	104	0.84	0.46	5.67	16.72	0.65	29.08	44.34	1.95	52.35	11.82
6	128	0.70	0.42	5.91	22.59	1.18	32.00	65.87	2.30	79.62	13.55
Mean	**116.00**	**0.83**	**0.34**	**5.51**	**14.38**	**0.84**	**29.52**	**47.02**	**2.00**	**60.42**	**11.07**
sd	13.15	0.20	0.13	3.32	9.56	0.22	6.99	11.22	0.29	10.80	1.57

3 Months											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	104	1.22	0.50	9.01	19.74	0.50	40.23	70.90	2.21	69.94	10.58
2	152	0.68	0.44	12.18	15.38	0.63	36.49	13.91	1.75	40.95	7.55
3	152	0.77	0.41	7.49	6.60	1.14	33.77	32.90	2.33	52.67	10.96
4	128	0.83	0.36	0.00	14.16	0.85	34.19	32.46	2.04	51.04	10.24
5	128	0.85	0.45	14.08	2.99	0.89	28.31	31.87	2.19	45.81	11.35
6	152	0.68	0.40	12.56	4.39	0.80	39.52	31.50	1.89	52.93	8.52
Mean	**136.00**	**0.84**	**0.43**	**9.22**	**10.54**	**0.80**	**35.42**	**35.59**	**2.07**	**52.22**	**9.87**
sd	19.60	0.20	0.05	5.13	6.80	0.22	4.38	18.79	0.22	9.84	1.50

7 Months											
	Workload	a	A_1	TD_1	Tau_1	A_2	TD_2	Tau_2	End A	MRT	VO_2 Gain
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	104	1.11	0.27	0.00	8.10	0.67	29.58	49.35	2.05	58.62	9.95
2	128	0.73	0.33	8.40	10.88	0.70	35.38	17.98	1.75	42.43	8.72
3	152	0.78	0.40	6.98	7.96	1.03	32.01	26.99	2.21	46.67	10.07
4	104	0.57	0.33	8.00	3.96	0.72	27.06	59.46	1.61	63.06	11.09
5	128	0.71	0.65	9.49	19.58	0.67	32.44	51.91	2.03	57.17	11.19
6	128	0.56	0.29	5.57	1.63	1.03	29.43	34.99	1.88	51.89	11.20
Mean	**124.00**	**0.74**	**0.38**	**6.41**	**8.68**	**0.80**	**30.98**	**40.12**	**1.92**	**53.31**	**10.37**
sd	18.07	0.20	0.14	3.41	6.27	0.18	2.91	16.04	0.22	7.78	0.99

Appendix XXIII: Individual model fits for $\dot{V}O_2$ kinetic responses to steady-state cycling at 80% VT in the PIO CTL group at each assessment during the intervention study.

Baseline											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	128	0.97	0.45	8.50	6.73	0.97	24.33	48.81	2.38	54.79	11.97
2	80	0.80	0.20	4.00	26.20	0.52	30.37	51.03	1.53	67.16	10.31
3	128	0.85	0.41	8.17	21.16	0.76	36.00	70.14	2.01	79.01	9.85
4	104	0.59	0.41	8.19	24.67	0.59	45.17	34.18	1.59	60.07	10.63
5	104	0.85	0.24	2.22	12.22	0.62	29.39	31.88	1.71	48.34	9.09
Mean	**108.80**	**0.81**	**0.34**	**6.22**	**18.20**	**0.69**	**33.05**	**47.21**	**1.84**	**61.87**	**10.37**
sd	20.08	0.14	0.11	2.91	8.40	0.18	7.94	15.39	0.35	11.81	1.06

3 Months											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	152	0.81	0.49	11.44	4.65	0.97	32.55	31.04	2.27	47.61	10.26
2	80	0.43	0.32	3.67	1.16	0.77	16.02	64.90	1.53	58.46	15.61
3	104	0.76	0.50	11.65	31.00	0.60	32.92	63.68	1.85	72.10	11.57
4	104	0.66	0.33	6.82	7.66	1.00	39.99	79.14	1.97	93.40	13.88
5	104	0.97	0.27	10.86	12.49	0.68	31.00	35.64	1.92	54.30	10.16
Mean	**108.80**	**0.73**	**0.38**	**8.89**	**11.39**	**0.80**	**30.50**	**54.88**	**1.91**	**65.18**	**12.30**
sd	26.29	0.20	0.10	3.52	11.72	0.18	8.81	20.65	0.27	18.14	2.38

7 Months											
	Workload	**a**	**A_1**	**TD_1**	**Tau_1**	**A_2**	**TD_2**	**Tau_2**	**End A**	**MRT**	**VO_2 Gain**
ID	W	$L.min^{-1}$	$L.min^{-1}$	s	s	$L.min^{-1}$	s	s	$L.min^{-1}$	s	$ml.min^{-1}.W^{-1}$
1	152	0.95	0.55	9.59	10.28	0.92	32.80	28.19	2.41	45.63	10.31
2	80	0.69	0.40	5.27	23.25	0.39	43.69	47.49	1.48	59.62	11.36
3	104	0.83	0.23	2.90	10.56	0.71	40.45	33.04	1.77	58.75	10.02
4	104	0.69	0.23	1.95	29.99	0.75	35.21	40.32	1.67	65.33	10.43
5	104	0.89	0.17	5.48	0.96	0.72	31.13	21.46	1.79	43.71	9.55
Mean	**108.80**	**0.81**	**0.32**	**5.04**	**15.01**	**0.70**	**36.66**	**34.10**	**1.83**	**54.61**	**10.33**
sd	26.29	0.12	0.15	2.96	11.53	0.19	5.27	10.17	0.35	9.44	0.66

Appendix XXIV: Goodness-of-fit data for model fits of the $\dot{V}O_2$ kinetic responses for baseline responses.

ND EXS		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	1.8005	7	344	1.0496	10	343	2.63	80.1212	Tri
	4	0.7057	7	344	0.5742	10	346	2.63	25.8877	Tri
	5	0.4135	7	341	0.2584	10	337	2.63	66.0262	Tri

ND CTL		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	0.9694	7	342	0.9390	10	340	2.63	3.5996	Tri
	8	0.6349	7	340	0.7464	10	336	2.63	-16.3774	Bi

T2D EXS		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	4	0.3734	7	336	0.3493	10	348	2.63	7.8452	Tri
	5	0.6821	7	341	0.5360	10	340	2.63	30.2437	Tri
	6	0.4583	7	336	0.4249	10	338	2.63	8.6725	Tri
	8	0.9779	7	345	0.7489	10	343	2.63	34.2455	Tri
	9	0.7253	7	332	0.7647	10	332	2.63	-5.5862	Bi

T2D CTL		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	0.5637	7	337	0.6130	10	340	2.63	-8.9304	Bi
	5	0.5921	7	345	0.4149	10	346	2.63	48.2441	Tri
	3	0.8124	7	341	0.5871	10	340	2.63	42.5835	Tri
	8	0.4473	7	346	0.4076	10	341	2.63	10.8232	Tri
	11	0.5513	7	341	0.4326	10	344	2.63	30.8087	Tri

PIO EXS		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	1.7744	7	341	1.5680	10	338	2.63	14.5240	Tri
	2	0.8622	7	350	0.3518	10	339	2.63	160.6048	Tri

PIO CTL		Bi-Phasic			Tri-Phasic					
Baseline	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	0.6386	7	345	0.6112	10	347	2.63	5.0768	Tri
	3	0.8269	7	349	0.6608	10	343	2.63	28.1652	Tri

Appendix XXIV: Goodness-of-fit data for model fits of the $\dot{V}O_2$ kinetic responses for responses at 3 months.

ND EXS		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	4	4.6481	7	339	4.9136	10	338	2.63	-5.9622	Bi
ND CTL		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	4	0.6346	7	342	0.3872	10	340	2.63	70.9064	Tri
	6	2.2889	7	349	1.6636	10	340	2.63	41.7201	Tri
	8	1.1686	7	339	0.9238	10	333	2.63	28.7978	Tri
	9	1.7249	7	343	1.6741	10	343	2.63	3.3948	Tri
T2D EXS		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	4	0.3536	7	343	0.2524	10	340	2.63	44.4924	Tri
	5	0.6055	7	338	0.5681	10	337	2.63	7.2570	Tri
	6	0.8650	7	342	0.5835	10	339	2.63	53.4032	Tri
T2D CTL		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	2	0.5822	7	347	0.6535	10	348	2.63	-12.4113	Bi
	5	0.5363	7	339	0.5189	10	343	2.63	3.7731	Tri
	6	0.4483	7	337	0.4483	10	339	2.63	-0.0035	Bi
	7	0.9895	7	337	0.9766	10	340	2.63	1.4662	Bi
	10	0.5698	7	335	0.4038	10	336	2.63	45.0627	Tri
	11	0.6310	7	342	0.6251	10	341	2.63	1.0461	Bi
PIO EXS		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	1.2821	7	343	1.2883	10	344	2.63	-0.5383	Bi
	4	0.8951	7	339	0.7371	10	340	2.63	23.8058	Tri
	6	1.9859	7	343	1.8281	10	348	2.63	9.8133	Tri
PIO CTL		Bi-Phasic			Tri-Phasic					
3 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	1.1820	7	344	0.7020	10	341	2.63	76.1218	Tri
	3	0.9065	7	342	0.8697	10	341	2.63	4.7130	Tri
	4	0.6313	7	344	0.6256	10	346	2.63	1.0357	Bi

Appendix XXIV: Goodness-of-fit data for model fits of the $\dot{V}O_2$ kinetic responses for responses at 7 months.

ND EXS		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	2	0.6968	7	337	0.5574	10	335	2.63	27.3347	Tri
	4	1.2273	7	340	1.0691	10	340	2.63	16.4218	Tri
	6	1.1472	7	341	1.0521	10	339	2.63	10.0064	Tri

ND CTL		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	0.9051	7	341	0.8741	10	345	2.63	3.9951	Tri
	5	0.2242	7	339	0.2098	10	343	2.63	7.6888	Tri

T2D EXS		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	4	0.4824	7	338	0.4616	10	338	2.63	4.9842	Tri
	5	0.9247	7	346	0.9187	10	347	2.63	0.7410	Bi
	6	0.4305	7	345	0.3533	10	345	2.63	24.6258	Tri
	9	0.5350	7	336	0.4532	10	335	2.63	19.7228	Tri

T2D CTL		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	0.6015	7	342	0.3826	10	341	2.63	63.7055	Tri
	2	0.7674	7	343	0.4643	10	340	2.63	72.4421	Tri
	4	0.6386	7	345	0.6112	10	347	2.63	5.0768	Tri
	5	1.0495	7	346	0.8276	10	346	2.63	30.3093	Tri
	6	0.8097	7	354	0.6091	10	347	2.63	37.3419	Tri
	7	0.8269	7	349	0.6608	10	343	2.63	28.1652	Tri
	8	0.3893	7	340	0.3350	10	336	2.63	17.7638	Tri

PIO EXS		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	2	0.7147	7	341	0.3107	10	339	2.63	143.9413	Tri
	3	1.3127	7	346	0.8977	10	352	2.63	53.1737	Tri

PIO CTL		Bi-Phasic			Tri-Phasic					
7 Mths	ID	RSS	p	N	RSS	p	N	Critical Value	F-value	Model
	1	1.6279	7	343	0.9651	10	334	2.63	74.8535	Tri
	2	0.6168	7	349	0.5964	10	347	2.63	3.8615	Tri

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the ND EXS group at each assessment during the intervention study.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1						
2	110.95	33.91	0.00	65.58	144.72	98.45
3	97.82	48.77	0.00	63.05	146.43	98.00
4	81.11	59.90	0.99	90.28	139.89	99.83
5	90.82	39.64	6.99	68.36	130.24	99.22
6	88.97	52.01	1.49	70.64	140.86	99.47
7	77.49	44.55	17.37	33.07	122.04	99.77
8	86.50	39.59	0.00	55.33	126.04	99.34
9	104.82	25.23	1.00	108.14	129.14	99.23
10	88.94	48.33	1.86	64.47	137.08	98.83
Mean	**91.94**	**43.55**	**3.30**	**68.77**	**135.14**	**99.13**
sd	10.82	10.31	5.70	21.01	8.59	0.60

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	58.82	36.74	20.00	44.11	95.54	98.49
2	84.80	47.07	9.56	52.38	131.81	99.36
3						
4	85.61	50.43	12.16	67.40	135.75	99.66
5	99.63	40.73	5.11	76.15	139.97	99.36
6	80.54	37.62	17.99	43.33	118.14	99.47
7	87.76	43.70	16.48	35.14	131.46	99.75
8	80.69	50.63	11.16	50.30	131.27	99.37
9	98.30	15.81	14.05	56.17	114.07	99.37
10	78.42	43.35	16.63	41.80	121.76	99.65
Mean	**83.84**	**40.67**	**13.68**	**51.87**	**124.42**	**99.39**
sd	12.01	10.58	4.67	13.07	13.73	0.37

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	61.69	40.07	14.61	62.74	101.60	99.47
2	86.12	36.10	12.23	62.11	122.09	99.11
3	94.45	48.80	15.63	42.79	143.23	99.36
4	93.19	54.20	12.74	86.99	146.39	99.82
5	96.43	46.63	0.00	75.81	142.65	98.44
6	78.89	32.44	11.67	54.58	111.28	99.21
7	83.60	50.05	12.59	47.17	133.62	99.49
8	89.78	41.34	11.17	57.77	131.02	99.43
9	99.44	28.00	3.69	74.28	127.21	99.63
10	82.82	31.14	12.48	34.04	113.96	99.23
Mean	**86.64**	**40.88**	**10.68**	**59.83**	**127.31**	**99.32**
sd	10.95	8.90	4.92	16.20	15.02	0.37

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the ND CTL group at each assessment during the intervention study. Subject 1 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1						
2	93.47	47.32	3.68	63.96	140.61	99.82
3	87.15	50.00	3.63	41.45	137.14	99.00
4	89.31	27.21	7.60	70.70	116.34	99.18
5	79.41	45.55	0.00	68.35	124.72	98.92
6	98.36	37.56	3.40	83.78	135.39	99.00
7	96.73	45.00	9.97	57.54	141.63	99.76
8	95.61	40.06	1.00	40.93	135.67	97.31
9						
Mean	**91.44**	**41.81**	**4.18**	**60.96**	**133.07**	**99.00**
sd	6.65	7.70	3.51	15.67	9.21	0.83

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1						
2	90.90	46.94	8.68	59.24	137.71	99.73
3	88.61	51.08	16.63	39.53	139.69	99.17
4	101.12	37.66	6.77	111.85	137.18	99.47
5	87.89	41.82	0.00	63.15	129.57	98.14
6	106.05	33.89	10.26	62.34	139.82	99.59
7	98.04	39.27	2.75	55.00	137.25	99.11
8	81.09	36.37	7.95	25.07	117.47	95.78
9	71.25	34.94	14.26	18.47	106.19	98.21
Mean	**90.62**	**40.25**	**8.41**	**54.33**	**130.61**	**98.65**
sd	11.22	6.05	5.49	28.88	12.39	1.30

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1						
2	99.65	48.78	13.91	60.78	148.26	99.17
3	89.02	59.26	3.96	63.41	148.06	98.82
4	86.52	27.10	12.88	62.65	113.52	99.22
5	91.24	48.01	3.75	68.57	138.99	99.06
6	103.55	40.40	2.84	94.62	143.02	99.37
7	102.38	39.36	5.97	59.55	141.63	99.36
8	92.71	47.00	12.67	38.07	139.71	98.94
9	64.36	34.61	21.49	25.80	98.97	99.33
Mean	**91.18**	**43.06**	**9.68**	**59.18**	**134.02**	**99.16**
sd	12.52	9.87	6.60	20.48	17.91	0.20

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the T2D EXS group at each assessment during the intervention study. Subject 5 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	84.97	26.94	12.99	54.77	111.86	97.25
2	88.06	41.98	1.00	100.04	128.88	98.08
3	84.27	42.18	11.05	64.62	126.26	99.74
4	98.78	32.03	1.00	124.70	129.01	95.55
5						
6	86.97	40.62	0.00	97.63	126.58	99.02
7	97.79	29.08	8.84	89.98	126.28	99.04
8	77.08	28.21	7.84	68.41	105.12	99.04
9	85.57	44.55	14.91	84.83	129.36	99.89
10	90.76	34.60	1.00	68.60	125.18	99.04
11	95.96	56.09	0.00	66.68	151.80	98.95
Mean	**89.02**	**37.63**	**5.86**	**82.03**	**126.03**	**98.56**
sd	6.85	9.15	5.89	21.36	12.16	1.30

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	88.90	39.02	12.00	58.00	127.83	99.09
2	83.64	37.17	15.98	51.95	120.75	98.90
3	74.75	44.05	10.78	44.03	118.79	99.59
4	113.91	32.12	13.70	79.45	145.61	99.64
5						
6	74.00	38.99	0.00	50.14	112.95	96.31
7	101.45	38.08	12.73	66.43	139.33	99.74
8	70.79	32.41	16.00	55.70	103.13	98.49
9	81.37	43.52	12.78	68.89	124.61	99.77
10	91.42	36.56	13.44	45.56	127.96	99.69
11	92.11	92.77	4.39	53.15	184.76	99.43
Mean	**87.23**	**43.47**	**11.18**	**57.33**	**130.57**	**99.07**
sd	13.36	17.76	5.11	11.17	22.58	1.06

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	81.20	39.42	14.30	57.68	120.53	99.64
2	84.52	40.67	14.38	66.20	124.97	98.28
3	88.59	48.81	2.36	69.80	137.11	99.45
4	97.60	37.51	13.06	58.76	135.01	99.73
5						
6	79.90	48.77	0.00	85.83	127.94	99.07
7	90.02	35.50	9.08	78.35	125.12	99.44
8	80.32	38.30	18.86	54.43	118.55	99.52
9	88.92	50.21	7.12	83.67	138.39	99.61
10	95.35	40.34	7.31	67.50	135.47	99.74
11						
Mean	**87.38**	**42.17**	**9.61**	**69.14**	**129.23**	**99.39**
sd	6.43	5.55	6.11	11.41	7.46	0.46

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the T2D CTL group at each assessment during the intervention study. Subjects 3 and 4 were excluded from analysis due to use of β-blockers to control hypertension.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	90.80	42.79	5.00	115.69	131.59	99.63
2	94.01	24.47	5.74	91.04	117.99	98.70
3						
4						
5	88.61	23.96	9.00	78.53	112.30	98.75
6	83.22	37.04	12.00	129.36	117.75	99.62
7	89.29	36.09	0.00	45.22	125.36	98.90
8	89.18	35.82	0.00	95.76	124.17	98.35
9	84.89	29.09	16.00	46.76	113.96	99.07
10	102.43	44.95	17.00	55.76	147.29	99.56
11	90.73	34.98	0.00	110.17	124.38	97.88
Mean	**90.35**	**34.36**	**7.19**	**85.36**	**123.87**	**98.94**
sd	5.54	7.33	6.73	30.89	10.69	0.60

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	105.26	33.27	20.43	65.55	138.34	99.31
2	90.34	34.36	1.59	111.65	123.31	99.03
3						
4						
5	86.93	23.71	19.25	54.34	110.59	98.94
6	84.18	27.09	20.50	69.75	111.06	99.69
7	86.12	27.70	16.71	33.71	113.82	98.47
8	86.65	32.63	18.52	87.15	118.63	99.63
9	89.97	41.07	15.57	44.46	131.02	99.56
10	118.47	41.42	21.00	59.01	159.76	99.61
11	84.75	33.89	10.72	97.30	117.70	99.66
Mean	**92.52**	**32.79**	**16.03**	**69.21**	**124.91**	**99.32**
sd	11.64	6.00	6.30	25.31	15.99	0.42

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	110.88	31.78	10.14	89.58	142.02	99.36
2	92.02	33.95	0.00	109.91	124.69	99.56
3						
4						
5	84.54	36.14	4.78	109.42	119.28	99.52
6	87.79	24.71	22.50	71.25	112.29	99.10
7	86.22	40.07	12.01	56.52	126.21	99.25
8	104.95	35.84	1.76	150.00	137.50	99.41
9	87.02	42.67	14.01	40.57	129.68	99.59
10	103.14	45.23	10.77	58.25	148.26	99.78
11	89.23	40.01	2.04	122.99	127.06	99.70
Mean	**93.96**	**36.71**	**8.67**	**89.83**	**129.66**	**99.47**
sd	9.70	6.20	7.24	36.03	11.25	0.22

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the PIO EXS group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	108.75	26.04	0.00	105.30	133.94	98.20
2						
3	93.57	28.06	13.04	41.87	121.63	99.20
4	96.20	37.13	6.98	129.59	130.89	99.23
5	95.9426	28.8987	0.00	42.62	124.84	98.51
6	98.04	29.99	0.00	64.68	127.92	96.44
Mean	**96.50**	**30.02**	**4.01**	**76.81**	**127.84**	**98.32**
sd	5.95	4.23	5.89	39.16	4.85	1.14

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	108.60	21.80	9.54	79.93	130.13	98.14
2						
3	101.59	34.33	16.40	78.65	135.49	99.58
4	103.01	34.98	0.00	75.17	137.70	99.57
5	95.31	34.16	2.02	39.52	129.47	98.37
6	100.18	33.88	1.65	79.39	133.69	98.79
Mean	**101.74**	**31.83**	**5.92**	**70.53**	**133.30**	**98.89**
sd	4.81	5.62	6.92	17.44	3.50	0.67

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	93.61	21.76	0.10	100.00	114.78	97.90
2						
3	95.53	35.99	8.31	68.53	131.31	99.08
4	106.62	32.86	1.04	85.37	138.99	98.15
5	98.56	34.91	9.77	50.37	133.43	99.71
6	92.66	27.68	5.80	65.05	120.22	98.19
Mean	**97.40**	**30.64**	**5.00**	**73.86**	**127.74**	**98.61**
sd	5.63	5.90	4.30	19.19	9.95	0.76

Appendix XXV: Individual monoexponential model fits for HR kinetic responses to steady-state cycling at 80% VT in the PIO CTL group at each assessment during the intervention study. Subject 4 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	87.02	42.67	14.01	40.57	129.68	99.59
2	87.79	24.71	22.50	71.25	112.29	99.10
3	92.02	33.95	0.00	109.91	124.69	99.58
4						
5	108.41	27.46	11.16	74.43	135.62	99.62
Mean	**93.81**	**32.20**	**11.92**	**74.04**	**125.57**	**99.47**
sd	9.98	7.99	9.29	28.37	9.92	0.25

3 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	101.07	42.03	5.95	56.08	143.03	99.21
2						
3	89.03	19.29	2.14	67.26	108.22	96.49
4						
5	112.57	29.97	4.63	67.62	142.38	98.97
Mean	**100.89**	**30.43**	**4.24**	**63.65**	**131.21**	**98.22**
sd	11.77	11.38	1.93	6.56	19.91	1.51

7 Months						
	a	A_1	TD_1	Tau_1	End A	Adj R^2
ID	beats.min^{-1}	beats.min^{-1}	s	s	beats.min^{-1}	%
1	114.38	41.56	8.79	60.95	155.81	99.48
2	85.15	30.82	3.89	99.27	115.12	99.48
3	92.98	22.93	0.00	71.13	115.76	95.66
4						
5	111.67	27.52	10.99	69.46	139.01	99.18
Mean	**101.04**	**30.71**	**5.92**	**75.20**	**131.42**	**98.45**
sd	14.24	7.93	4.94	16.65	19.69	1.87

Appendix XXVI: Individual CO responses and related parameters at rest for the ND EXS group at each assessment during the intervention study.

Baseline								
	CO	**HR**	**SV**	**SVI**	**CI**	**MAP**	**TPR**	**a-v O_2**
ID	L.min^{-1}	beats.min^{-1}	ml	ml.m^{-2}	L.min^{-1}.m^{-2}	mmHg	mmHg.L^{-1}.min^{-1}	ml O_2.100ml blood^{-1}
1	5.49	82	66.89	33.47	2.74			9.30
2	6.01	94	63.20	29.94	2.85			8.46
3	4.53	58	78.64	39.56	2.28	90.0	19.87	9.28
4	5.70	84	67.86	31.30	2.63			7.12
5	3.75	56	66.90	30.76	1.72	96.0	25.62	8.01
6	5.70	89	64.02	30.62	2.72			8.61
7	7.36	111	67.17	27.64	3.03	112.9	15.34	9.92
8	5.73	70	81.82	34.29	2.40	102.5	17.90	8.34
9	3.55	104	34.29	15.22	1.58	85.7	24.12	9.48
10	4.11	90	45.64	24.30	2.19	93.3	22.72	9.71
Mean	5.19	83.65	63.64	29.71	2.41	96.73	20.93	8.82
sd	1.19	18.04	14.11	6.50	0.48	9.75	3.92	0.87

3 Months								
	CO	**HR**	**SV**	**SVI**	**CI**	**MAP**	**TPR**	**a-v O_2**
ID	L.min^{-1}	beats.min^{-1}	ml	ml.m^{-2}	L.min^{-1}.m^{-2}	mmHg	mmHg.L^{-1}.min^{-1}	ml O_2.100ml blood^{-1}
1	6.31	73	86.94	42.38	3.08	91.7	14.52	5.93
2	7.66	56	136.75	66.81	3.74	98.3	12.84	5.07
3	3.13	51	61.40	31.55	1.61	83.3	26.62	10.19
4	5.37	69	78.64	37.19	2.54	100.0	18.61	5.83
5	4.95	77	64.28	29.81	2.29	92.3	18.66	5.83
6	4.96	70	71.00	34.20	2.39	103.3	20.82	7.15
7	5.39	86	63.52	26.77	2.27	103.3	19.17	10.29
8	5.18	67	77.35	33.01	2.21	95.8	18.48	6.44
9	4.43	88	50.34	22.47	1.98	94.7	21.38	8.24
10	5.41	91	59.49	32.06	2.92	95.0	17.55	7.72
Mean	5.28	72.65	74.97	35.63	2.50	95.78	18.87	7.27
sd	1.17	13.16	24.20	12.22	0.61	6.00	3.76	1.83

7 Months								
	CO	**HR**	**SV**	**SVI**	**CI**	**MAP**	**TPR**	**a-v O_2**
ID	L.min^{-1}	beats.min^{-1}	ml	ml.m^{-2}	L.min^{-1}.m^{-2}	mmHg	mmHg.L^{-1}.min^{-1}	ml O_2.100ml blood^{-1}
1	4.65	85	54.72	26.85	2.28	102.3	22.01	6.50
2	6.39	94	69.49	33.97	3.13	100.8	15.77	6.27
3	4.59	58	79.21	40.52	2.35	83.8	18.24	5.89
4	4.27	67	63.74	29.64	1.99	104.0	24.36	7.38
5	4.55	87	52.35	24.01	2.09	102.2	22.45	7.82
6	5.00	77	64.90	31.13	2.40	105.0	21.01	5.20
7	4.55	84	54.18	22.80	1.91	113.3	24.90	8.97
8	6.14	63	97.51	41.45	2.61	101.7	16.56	5.11
9	4.19	93	45.02	20.04	1.86	93.3	22.28	11.97
10	4.15	94	44.14	23.71	2.23	94.0	22.67	8.20
Mean	4.85	80.15	62.53	29.41	2.28	100.04	21.02	7.33
sd	0.79	13.28	16.45	7.39	0.38	7.99	3.14	2.07

Appendix XXVI: Individual CO responses and related parameters at rest for the ND CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	8.57	72	114.10	57.85	4.34	100.0	11.67	7.29
2								
3	7.05	87	81.46	42.45	3.67	99.0	14.04	5.54
4	4.94	76	65.01	29.37	2.23	84.7	17.15	7.30
5	4.29	79	54.32	26.90	2.13	96.7	22.54	4.15
6	6.29	91	69.15	35.43	3.22	98.3	15.62	6.66
7	3.75	60	62.51	30.29	1.82	103.3	27.56	4.69
8	5.61	91	61.56	29.73	2.71	99.2	17.69	5.10
9	7.29	92	79.20	36.67	3.37	115.0	15.78	4.97
Mean	5.97	80.94	73.41	36.09	2.94	99.52	17.76	5.71
sd	1.63	11.35	18.77	10.13	0.87	8.32	5.05	1.22

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	5.96	82	72.70	36.72	3.01	102	17.11	6.22
2								
3	5.75	84	68.86	35.94	3.00	105.8	18.40	5.34
4	4.97	85	58.53	26.31	2.24	83.0	16.69	7.47
5	4.92	72	68.18	33.87	2.45	91.3	18.55	5.40
6	4.35	79	55.10	28.49	2.25	108.3	24.89	3.19
7	4.24	65	65.19	31.30	2.03	98.7	23.29	8.80
8	5.07	79	64.17	30.90	2.44	90.0	17.75	6.35
9	5.89	92	64.00	29.58	2.72	107.3	18.23	5.46
Mean	5.14	79.69	64.59	31.64	2.52	98.31	19.36	6.03
sd	0.67	8.24	5.66	3.63	0.36	9.29	3.01	1.65

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	5.44	67	81.18	40.73	2.73	103.3	19.00	5.81
2								
3	6.55	92	71.15	36.93	3.40	103.3	15.78	5.79
4	4.58	82	55.83	25.26	2.07	88.3	19.29	7.75
5	5.95	77	77.23	38.40	2.96	96.0	16.14	3.04
6	6.47	88	73.47	37.58	3.31	103.3	15.98	4.92
7	5.74	60	95.63	46.15	2.77	102.7	17.89	4.62
8	5.38	77	69.59	33.82	2.62	102.7	19.07	8.75
9	4.40	99	44.44	20.55	2.03	112.7	25.61	4.64
Mean	5.56	80.25	71.07	34.93	2.74	101.53	18.59	5.67
sd	0.79	12.87	15.55	8.32	0.50	6.99	3.19	1.83

Appendix XXVI: Individual CO responses and related parameters at rest for the T2D EXS group at each assessment during the intervention study. Subject 5 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline									
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	4.15	71	76.77	38.46	2.08	105.0	25.31	7.05
2	4.85	90	53.94	24.99	2.25	100.0	20.60	6.37
3	4.99	84	59.45	29.52	2.48	105.0	21.03	6.24
4	5.53	75	73.49	32.21	2.43	95.5	17.26	5.60
5								
6	5.40	87	62.02	32.57	2.83	105.0	19.46	5.97
7	4.20	87	48.57	23.56	2.04	100.0	23.81	6.76
8	6.03	82	74.05	37.67	3.07	103.3	17.13	5.44
9	4.32	85	50.80	23.97	2.04	98.3	22.78	8.31
10	5.03	79	63.70	33.43	2.64	104.2	20.71	5.24
11	4.36	70	62.23	32.47	2.27	100.0	22.96	7.32
Mean	4.89	80.90	62.50	30.88	2.41	101.64	21.10	6.43
sd	0.64	6.94	9.85	5.32	0.35	3.33	2.69	0.95

3 Months									
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	4.10	68	60.50	30.26	2.05	106.7	26.01	5.10
2	4.18	86	48.57	22.81	1.96	105.8	25.33	5.71
3	4.39	80	54.89	27.23	2.18	100.8	22.96	6.22
4	5.91	77	77.31	34.00	2.60	103.3	17.47	7.02
5								
6	5.91	87	68.33	35.54	3.07	94.7	16.02	4.81
7	4.37	97	45.00	21.75	2.11	102.8	23.55	6.93
8	4.82	73	65.96	33.42	2.44	105.7	21.95	7.00
9	5.36	63	85.02	39.92	2.51	100.0	18.67	7.06
10	4.81	70	68.72	36.03	2.52	106.7	22.18	3.80
11	4.26	60	71.05	37.08	2.22	102.0	23.93	6.52
Mean	4.81	76.00	64.54	31.80	2.37	102.85	21.81	6.02
sd	0.69	11.55	12.49	6.13	0.33	3.74	3.35	1.13

7 Months									
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	4.09	83	49.29	24.50	2.03	100.0	24.44	8.31
2	4.49	92	48.81	22.69	2.09	110.0	24.49	6.44
3	5.30	85	62.87	31.01	2.61	113.3	21.39	4.62
4	5.61	76	74.28	32.69	2.47	97.5	17.39	6.97
5								
6	5.57	79	69.61	36.45	2.92	90.0	16.16	4.04
7	4.85	82	59.43	28.83	2.35	100.0	20.64	8.28
8	5.14	81	63.84	32.64	2.63	108.3	21.07	6.04
9	3.97	87	45.58	21.44	1.87	105.0	26.48	9.48
10	5.55	82	66.90	35.15	2.92	104.7	18.85	5.73
11	5.35	55	96.61	50.11	2.78	93.3	17.44	6.10
Mean	4.99	80.05	63.72	31.55	2.47	102.21	20.83	6.60
sd	0.62	9.89	14.97	8.31	0.37	7.41	3.46	1.70

Appendix XXVI: Individual CO responses and related parameters at rest for the T2D CTL group at each assessment during the intervention study. Subjects 6 and 9 were excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	5.35	82	66.07	29.24	2.37	106.2	19.83	6.39
2	5.34	75	71.22	33.28	2.50	99.2	18.57	7.56
3	4.51	77	58.93	29.01	2.22	82.5	18.30	7.02
4	5.10	87	58.69	28.33	2.46	95.8	18.79	7.31
5	5.58	77	72.45	34.06	2.62	90.8	16.28	6.31
6								
7	6.09	69	88.84	37.69	2.58	116.7	19.17	5.54
8	5.05	83	61.12	28.87	2.39	93.5	18.50	4.36
9								
10	6.15	84	73.23	36.47	3.06	107.5	17.48	3.85
11	4.19	97	43.36	22.03	2.13	102.5	24.48	7.31
Mean	5.26	81.06	65.99	31.00	2.48	99.40	19.04	6.18
sd	0.65	7.95	12.66	4.85	0.27	10.16	2.28	1.34

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	5.17	71	72.86	32.30	2.29	103.7	20.04	5.68
2	4.90	74	58.12	27.34	2.31	93.3	19.03	5.87
3	4.99	80	62.37	30.73	2.46	93.3	18.70	7.02
4	6.02	97	62.09	29.98	2.91	95.0	15.77	6.38
5	4.55	60	75.85	35.76	2.15	90.0	19.78	3.16
6								
7	6.12	77	79.50	33.24	2.56	109.2	17.84	7.33
8	6.45	73	88.30	41.98	3.07	91.7	14.22	4.91
9								
10	5.26	88	60.14	30.01	2.63	101.8	19.35	5.94
11	3.43	113	30.33	15.48	1.75	87.2	25.44	6.13
Mean	5.21	81.39	65.51	30.76	2.46	96.13	18.91	5.82
sd	0.92	15.78	16.66	7.12	0.40	7.21	3.12	1.23

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	5.35	76	70.36	30.92	2.35	103.3	19.32	6.69
2	4.13	94	43.98	20.56	1.93	101.7	24.59	6.87
3	5.35	81	66.09	32.96	2.67	83.3	15.57	7.08
4	5.33	86	61.95	29.70	2.56	90.0	16.88	6.66
5	4.60	71	64.72	30.44	2.16	93.3	20.31	4.29
6								
7	6.86	75	91.45	37.42	2.81	113.3	16.52	7.33
8	5.18	67	77.31	37.14	2.49	96.7	18.66	4.81
9								
10	5.77	84	68.70	34.82	2.93	106.7	18.48	5.13
11	4.15	79	52.56	26.73	2.11	100.0	24.08	5.49
Mean	5.19	79.22	66.35	31.19	2.44	98.70	19.38	6.04
sd	0.85	8.18	13.62	5.32	0.33	9.04	3.17	1.12

Appendix XXVI: Individual CO responses and related parameters at rest for the PIO EXS group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	6.33	78	81.18	34.03	2.65	91.7	14.48	7.49
2								
3	4.54	91	49.87	23.63	2.15	95.3	21.01	8.35
4	4.45	83	53.59	27.93	2.32	99.3	22.32	4.86
5	6.00	78	76.88	39.64	3.09	96.7	16.13	5.88
6	4.46	85	52.44	26.70	2.27	90.0	20.19	4.91
Mean	5.15	83.00	62.79	30.39	2.50	94.61	18.83	6.30
sd	0.93	5.43	14.96	6.41	0.38	3.76	3.36	1.57

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	7.17	94	76.28	31.76	2.99	93.3	13.01	6.11
2								
3	4.77	100	47.65	22.60	2.26	93.3	19.58	6.88
4	4.95	99	50.04	25.99	2.57	98.3	19.85	4.93
5	6.50	71	92.85	47.66	3.34	96.7	14.88	4.59
6	4.71	74	63.62	32.16	2.38	90.0	19.12	6.10
Mean	5.62	87.50	66.09	32.03	2.71	94.32	17.29	5.72
sd	1.14	14.16	18.87	9.61	0.45	3.25	3.13	0.94

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	7.15	80	90.07	37.00	2.94	93.3	13.05	6.34
2								
3	5.62	91	61.77	29.25	2.66	96.7	17.20	7.97
4	4.91	99	49.58	25.94	2.57	111.3	22.68	4.63
5	5.71	69	82.79	42.01	2.90	94.3	16.51	4.32
6	5.75	92	62.52	31.56	2.90	90.7	15.76	4.35
Mean	5.83	86.10	69.35	33.15	2.79	97.27	17.04	5.52
sd	0.82	11.84	16.62	6.38	0.17	8.15	3.52	1.60

Appendix XXVI: Individual CO responses and related parameters at rest for the PIO CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	6.86	75	91.45	37.42	2.81	113.3	16.52	7.33
2								
3	5.35	81	66.09	32.96	2.67	83.3	15.58	7.08
4	5.77	84	68.70	34.82	2.93	106.7	18.49	5.13
5	6.99	95	73.79	33.72	3.20	95.8	13.70	3.57
Mean	6.24	83.75	75.01	34.73	2.90	99.78	16.07	5.78
sd	0.81	8.38	11.42	1.95	0.22	13.14	1.99	1.77

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	6.71	83	81.80	33.50	2.75	120.0	17.88	5.45
2								
3	5.03	82	61.37	30.30	2.48	90.0	17.89	4.87
4	5.30	59	89.82	44.77	2.64	113.3	21.38	6.11
5	6.84	98	70.38	31.83	3.10	100.7	14.71	4.76
Mean	5.97	80.25	75.84	35.10	2.74	105.99	17.96	5.30
sd	0.94	15.89	12.52	6.58	0.26	13.34	2.73	0.62

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	6.71	83	80.83	32.54	2.70	112.7	16.79	5.81
2								
3	5.50	71	76.76	37.91	2.71	88.7	16.14	5.27
4	7.71	84	91.73	44.84	3.77	110.0	14.28	4.50
5	8.72	94	92.79	41.66	3.92	103.3	11.85	4.14
Mean	7.16	82.88	85.53	39.24	3.27	103.67	14.76	4.93
sd	1.38	9.63	7.96	5.29	0.66	10.74	2.22	0.75

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the ND EXS group at each assessment during the intervention study.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	12.67	95	133.49	68.79	6.34			11.61
2	13.62	114	119.90	58.80	6.45			10.25
3	7.89	78	101.80	51.21	3.97	105.0	13.31	12.53
4	11.94	114	104.71	48.30	5.51			10.18
5	13.03	109	119.56	54.97	5.99			9.28
6	10.97	115	95.39	45.63	5.25			10.45
7	13.07	126	104.13	42.85	5.38	122.8	9.40	10.99
8	13.62	107	127.24	53.32	5.71	114.2	8.38	9.40
9	8.11	113	71.78	31.85	3.60	105.0	12.94	14.09
10	10.23	114	89.77	47.80	5.45	108.3	10.59	10.32
Mean	11.51	108.35	106.78	49.95	5.36	111.07	10.92	10.91
sd	2.15	13.26	18.70	9.27	0.93	7.57	2.16	1.48

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	12.35	107	115.94	56.51	6.02	117.5	9.52	9.22
2	13.33	96	139.68	68.24	6.51	111.7	8.38	9.85
3	7.85	69	113.81	58.49	4.04	100.8	12.84	11.12
4	11.28	97	116.99	55.33	5.33	123.3	10.94	11.06
5	11.48	106	108.35	50.25	5.32	112.5	9.80	9.91
6	9.82	100	98.90	47.64	4.73	121.7	12.38	10.71
7	12.85	117	109.86	46.30	5.42	119.2	9.27	12.36
8	12.73	118	107.87	46.03	5.43	118.3	9.29	10.80
9	8.79	96	91.54	40.87	3.92	110.0	12.52	13.81
10	10.88	114	95.83	51.65	5.86	114.2	10.50	12.00
Mean	11.14	101.75	109.88	52.13	5.26	114.92	10.54	11.09
sd	1.82	14.35	13.55	7.83	0.83	6.60	1.57	1.35

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	11.28	106	106.51	52.26	5.54	113.3	10.04	12.30
2	14.17	118	120.64	58.98	6.93	116.7	8.23	10.08
3	9.00	79	113.93	58.28	4.60	103.3	11.48	11.12
4	8.82	98	90.70	42.18	4.10	135.8	15.40	11.18
5	10.17	116	88.10	40.41	4.67	111.7	10.98	13.95
6	10.09	100	101.39	48.63	4.84	118.3	11.73	9.95
7	11.18	113	98.95	41.63	4.70	130.0	11.63	12.39
8	14.44	114	126.66	53.85	6.14	121.7	8.43	10.31
9	10.43	103	101.22	45.07	4.64	108.3	10.39	12.49
10	10.89	119	91.52	49.17	5.85	108.3	9.94	13.22
Mean	11.05	106.40	103.96	49.05	5.20	116.74	10.82	11.70
sd	1.90	12.27	13.00	6.75	0.88	10.15	2.02	1.38

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the ND CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.87	110	135.83	68.87	7.54	116.7	7.85	11.19
2								
3	11.40	107	107.07	55.80	5.94	103.3	9.06	8.13
4	6.53	96	67.99	30.72	2.95	120.0	18.39	16.68
5	9.10	100	91.03	45.08	4.51	113.3	12.45	9.08
6	10.67	111	96.13	49.25	5.47	111.7	10.47	9.97
7	8.12	78	104.16	50.47	3.94	116.7	14.36	14.59
8	13.66	116	117.79	56.89	6.60	106.7	7.81	11.75
9	11.25	117	96.17	44.53	5.21	125.0	11.11	9.30
Mean	10.70	104.25	102.02	50.20	5.27	114.17	11.44	11.34
sd	2.77	12.84	19.89	11.11	1.47	7.01	3.60	2.94

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.89	105	113.28	57.21	6.01	113.3	9.53	11.13
2								
3	11.44	113	101.29	52.86	5.97	113.3	9.90	8.39
4	10.13	101	101.32	45.55	4.55	117.5	11.60	11.49
5	9.18	106	85.51	42.47	4.56	106.7	11.62	11.29
6	8.97	109	82.32	42.56	4.64	116.7	13.00	14.72
7	11.77	85	138.50	66.49	5.65	121.7	10.34	11.59
8	13.10	102	128.42	61.84	6.31	98.3	7.51	11.73
9	11.25	116	96.98	44.83	5.20	126.7	11.26	8.83
Mean	10.97	104.56	105.95	51.73	5.36	114.27	10.59	11.14
sd	1.43	9.48	19.70	9.31	0.72	8.77	1.67	1.94

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	13.40	110	134.04	67.25	6.72	113.3	8.46	8.56
2								
3	11.92	118	101.02	52.44	6.19	110.0	9.23	8.97
4	9.57	94	101.77	46.04	4.33	122.5	12.80	10.45
5	10.12	107	94.56	47.02	5.03	116.7	11.53	9.82
6	10.57	112	94.25	48.20	5.40	110.0	10.41	11.50
7	12.20	79	154.44	74.53	5.89	120.0	9.84	10.63
8	15.77	115	137.16	66.66	7.67	116.7	7.40	11.06
9	13.30	118	112.71	52.12	6.15	128.3	9.65	9.43
Mean	12.11	106.63	116.24	56.78	5.92	117.19	9.91	10.05
sd	2.05	13.59	22.74	11.00	1.03	6.31	1.70	1.03

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the T2D EXS group at each assessment during the intervention study. Subject 5 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	9.37	92	101.86	51.03	4.69	110.0	11.74	14.84
2	10.77	100	107.72	49.91	4.99	110.0	10.21	9.80
3	9.66	103	94.25	46.79	4.80	123.3	12.77	10.72
4	9.87	100	98.73	43.27	4.33	119.2	12.07	10.17
5								
6	8.34	106	79.05	41.51	4.38	115.0	13.79	12.66
7	8.92	106	84.15	40.82	4.33	118.3	13.27	11.01
8	10.96	98	111.88	56.91	5.57	130.0	11.87	9.87
9	10.74	103	104.24	49.18	5.07	116.7	10.86	10.67
10	8.88	103	86.18	45.23	4.66	117.5	13.24	10.23
11	9.60	97	98.97	51.64	5.01	116.7	12.16	10.67
Mean	9.71	100.70	96.70	47.63	4.78	117.67	12.20	11.06
sd	0.89	4.25	10.70	5.06	0.39	5.90	1.11	1.55

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	8.94	83	107.57	53.80	4.47	112.5	12.58	10.99
2	10.87	109	99.68	46.81	5.10	118.3	10.89	12.33
3	10.87	105	103.55	51.37	5.39	125.8	11.57	10.43
4	12.13	104	116.63	51.29	5.33	125.0	10.31	10.53
5								
6	10.98	112	98.01	50.97	5.71	120.8	11.00	9.17
7	10.09	119	85.14	41.15	4.88	117.5	11.65	10.96
8	11.05	91	122.28	61.96	5.60	135.0	12.21	11.26
9	10.35	95	108.99	51.17	4.86	122.5	11.83	12.83
10	10.23	97	105.43	55.28	5.36	110.0	10.76	9.50
11	10.43	90	115.93	60.50	5.45	111.7	10.71	13.05
Mean	10.59	100.40	106.32	52.43	5.22	119.91	11.35	11.10
sd	0.82	11.15	10.71	6.06	0.38	7.64	0.73	1.31

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	8.09	92	87.91	43.71	4.02	116.7	14.43	15.03
2	11.79	116	101.65	47.26	5.48	120.0	10.18	11.45
3	10.64	115	92.49	45.62	5.25	130.0	12.22	9.61
4	11.03	96	115.86	51.00	4.85	108.3	9.82	11.14
5								
6	10.55	101	104.47	54.70	5.53	113.3	10.74	9.87
7	9.20	108	85.21	41.33	4.46	120.0	13.04	12.12
8	10.66	98	108.77	55.61	5.45	128.3	12.04	10.41
9	10.46	110	95.04	44.71	4.92	123.3	11.79	11.75
10	11.17	108	103.45	54.35	5.87	111.7	10.00	9.33
11	9.33	90	103.67	53.77	4.84	123.3	13.22	12.54
Mean	10.29	103.35	99.85	49.21	5.07	119.49	11.75	11.32
sd	1.10	9.35	9.56	5.28	0.56	7.07	1.55	1.70

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the T2D CTL group at each assessment during the intervention study. Subjects 6 and 9 were excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.66	108	108.48	48.00	5.16	123.3	10.58	9.13
2	8.69	100	87.31	40.80	4.06	122.5	14.10	15.83
3	7.85	92	85.75	42.21	3.86	112.5	14.34	9.72
4	9.31	107	83.41	40.27	4.49	96.7	10.39	10.14
5	9.32	96	97.21	45.70	4.38	111.7	11.98	10.00
6								
7	11.48	96	120.16	50.97	4.87	130.8	11.40	10.23
8	10.95	98	112.46	53.12	5.17	113.3	10.35	8.09
9								
10	11.37	102	111.96	55.76	5.66	125.8	11.07	8.99
11	9.42	121	78.19	39.72	4.79	121.7	12.92	12.00
Mean	10.00	101.83	98.33	46.28	4.72	117.60	11.90	10.46
sd	1.39	8.75	15.30	6.00	0.58	10.20	1.55	2.28

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	10.83	91	118.99	52.74	4.80	122.5	11.31	9.23
2	8.91	98	90.95	42.78	4.19	123.3	13.83	12.35
3	8.68	90	96.44	47.52	4.28	108.3	12.48	11.73
4	10.61	115	92.22	44.52	5.12	106.7	10.06	10.88
5	9.14	96	95.95	45.23	4.31	111.7	12.22	9.65
6								
7	12.77	112	114.01	47.67	5.34	141.7	11.10	9.71
8	10.22	93	109.86	52.23	4.86	114.2	11.17	8.80
9								
10	11.62	104	111.74	55.76	5.80	128.3	11.04	8.98
11	9.23	132	69.94	35.70	4.71	121.7	13.18	12.54
Mean	10.22	103.39	100.01	47.13	4.82	119.82	11.82	10.43
sd	1.38	13.96	15.28	6.05	0.53	11.05	1.19	1.47

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.99	103	116.37	51.13	5.27	128.3	10.71	8.99
2	9.00	114	78.98	36.92	4.21	136.7	15.18	14.94
3	8.01	90	89.03	44.40	4.00	106.7	13.31	11.18
4	10.33	114	90.64	43.46	4.95	103.3	10.00	10.60
5	9.30	96	96.90	45.58	4.38	120.8	12.99	9.42
6								
7	12.76	105	121.55	49.74	5.22	130.0	10.19	11.61
8	10.45	102	102.42	49.20	5.02	118.3	11.33	10.32
9								
10	11.15	105	106.16	53.81	5.65	130.0	11.66	9.96
11	9.25	119	77.72	39.53	4.70	125.0	13.51	11.83
Mean	10.25	105.33	97.75	45.97	4.82	122.13	12.10	10.98
sd	1.53	9.19	15.36	5.54	0.54	11.13	1.75	1.76

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the PIO EXS group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.52	118	97.62	40.92	4.83	103.3	8.97	14.01
2								
3	9.22	105	87.78	41.60	4.37	107.5	11.66	11.84
4	9.51	106	89.73	46.77	4.96	115.0	12.09	8.69
5	11.40	109	104.58	53.92	5.88	123.3	10.82	10.01
6	9.77	114	85.72	43.64	4.97	105.0	10.74	10.29
Mean	10.28	110.40	93.09	45.37	5.00	110.82	10.86	10.97
sd	1.09	5.50	7.85	5.29	0.55	8.29	1.20	2.04

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.49	118	97.36	40.53	4.78	106.7	9.29	13.36
2								
3	9.96	119	83.71	39.71	4.73	110.0	11.04	10.86
4	10.37	115	90.19	46.83	5.39	121.7	11.73	11.23
5	10.98	104	105.59	54.20	5.64	123.3	11.23	12.20
6	11.14	113	98.55	49.82	5.63	101.7	9.13	9.57
Mean	10.79	113.80	95.08	46.22	5.23	112.67	10.48	11.44
sd	0.61	5.97	8.38	6.16	0.45	9.47	1.19	1.42

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	11.36	101	112.44	46.18	4.66	103.3	9.10	12.01
2								
3	8.96	113	79.29	37.55	4.24	110.0	12.28	12.67
4	10.74	114	94.17	49.27	5.62	118.3	11.02	8.36
5	15.41	116	133.49	67.74	7.82	123.3	8.00	9.76
6	10.09	109	92.56	46.72	5.09	103.3	10.24	8.07
Mean	11.31	110.50	102.39	49.49	5.49	111.67	10.13	10.17
sd	2.46	5.83	21.01	11.12	1.40	8.98	1.66	2.09

Appendix XXVII: Individual CO responses and related parameters after 30s steady-state cycling at 80% VT in the PIO CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	12.76	105	121.55	49.74	5.22	130.0	10.19	11.61
2								
3	8.01	90	89.03	44.40	4.00	106.7	13.32	11.18
4	11.15	105	106.16	53.81	5.65	130.0	11.66	9.96
5	10.02	122	82.30	37.60	4.58	123.3	12.31	10.75
Mean	10.48	105.50	99.76	46.39	4.86	122.50	11.87	10.88
sd	2.00	13.08	17.66	7.01	0.73	11.00	1.31	0.70

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	13.54	117	115.83	47.44	5.55	138.3	10.21	8.47
2								
3	8.22	92	89.30	44.10	4.06	105.0	12.78	9.90
4	10.64	100	106.41	53.04	5.30	133.3	12.53	9.38
5	8.59	124	69.28	31.34	3.89	124.2	14.45	13.73
Mean	10.25	108.25	95.20	43.98	4.70	125.19	12.49	10.37
sd	2.44	14.80	20.48	9.20	0.85	14.68	1.74	2.32

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.40	130	111.20	44.77	5.80	138.3	9.61	9.86
2								
3	9.36	88	106.40	52.55	4.62	110.0	11.75	10.07
4	12.02	108	111.32	54.41	5.88	126.7	10.54	8.27
5	12.86	121	106.31	47.73	5.78	121.7	9.46	7.90
Mean	12.16	111.63	108.81	49.87	5.52	124.17	10.34	9.03
sd	2.11	18.06	2.84	4.41	0.60	11.75	1.05	1.10

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the ND EXS group at each assessment during the intervention study.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	16.10	123	131.34	65.71	8.06			12.99
2	15.79	145	108.92	51.60	7.48			15.80
3	10.13	99	102.39	51.50	5.10			18.05
4	13.86	140	99.01	45.67	6.39			14.97
5	14.08	146	96.47	44.36	6.48			15.61
6	12.29	140	87.72	41.96	5.88			15.99
7	14.29	143	100.30	41.28	5.88	123.3	8.63	16.56
8	15.80	126	125.41	52.56	6.62	115.8	7.33	16.15
9	10.05	131	76.71	34.04	4.46	113.3	11.28	16.81
10	11.47	133	86.19	45.89	6.11	110.8	9.66	18.59
Mean	13.39	132.50	101.45	47.46	6.25	115.83	9.23	16.15
sd	2.29	14.21	16.97	8.54	1.04	5.40	1.67	1.56

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.50	131	110.71	53.96	7.07	125.8	8.68	15.59
2	14.42	126	114.43	55.90	7.04	115.0	7.98	16.81
3	9.01	99	90.98	46.76	4.63	112.5	12.49	21.89
4	12.59	119	106.24	50.24	5.95	131.7	10.46	17.67
5	13.49	137	98.84	45.84	6.26	112.5	8.34	19.62
6	13.32	122	108.94	52.48	6.41	135.0	10.14	15.80
7	14.12	134	105.78	44.58	5.95	123.3	8.73	21.53
8	14.69	136	108.03	46.11	6.27	120.8	8.22	16.51
9	9.52	109	87.30	38.97	4.25	115.8	12.17	20.29
10	12.96	137	94.60	50.98	6.98	123.3	9.51	20.07
Mean	12.86	124.85	102.58	48.58	6.08	121.57	9.67	18.58
sd	2.02	12.86	9.10	5.07	0.97	7.80	1.62	2.37

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.11	131	108.12	53.06	6.92	120.8	8.56	16.27
2	17.35	141	123.92	60.58	8.48	123.3	7.11	15.68
3	10.06	100	100.56	51.44	5.14	111.7	11.11	19.90
4	9.80	115	85.61	39.82	4.56	143.3	14.62	18.61
5	14.20	146	77.44	35.52	6.51	118.3	8.33	18.52
6	11.47	114	100.80	48.25	5.50	125.0	10.90	13.51
7	13.34	127	105.01	44.18	5.61	128.3	9.62	20.42
8	15.32	142	107.89	45.87	6.51	128.3	8.37	18.00
9	11.28	124	90.97	40.50	5.02	113.3	10.04	21.42
10	13.56	143	94.85	50.95	7.29	130.0	9.58	18.06
Mean	13.05	128.15	99.50	47.02	6.16	124.23	9.82	18.04
sd	2.39	15.23	13.11	7.40	1.21	9.16	2.09	2.37

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the ND CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	17.47	138	127.10	64.44	8.86	120.8	6.92	14.01
2								
3	15.06	141	106.83	55.67	7.85	111.7	7.41	15.48
4	12.00	113	102.00	46.08	5.42	121.7	10.14	14.46
5	11.48	126	91.14	45.14	5.69	115.0	10.01	14.33
6	11.49	137	83.84	42.95	5.88	113.3	9.86	16.76
7	11.29	94	120.14	58.21	5.47	118.3	10.48	17.56
8	17.79	134	132.78	64.13	8.59	111.7	6.28	16.70
9	15.12	141	107.21	49.64	7.00	130.0	8.60	14.74
Mean	13.96	127.88	108.88	53.28	6.85	117.81	8.71	15.50
sd	2.75	16.67	17.01	8.54	1.43	6.29	1.65	1.34

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	15.70	146	107.52	54.30	7.93	120.0	7.64	15.00
2								
3	13.55	140	97.13	50.69	7.07	118.3	8.74	17.07
4	12.56	125	100.49	45.17	5.65	135.8	10.81	18.03
5	12.14	132	91.94	45.67	6.03	113.3	9.34	14.57
6	13.84	135	102.49	52.98	7.15	121.7	8.79	14.98
7	13.99	104	134.50	64.57	6.72	121.7	8.70	13.51
8	14.77	119	127.30	61.30	7.11	106.7	7.22	14.90
9	14.63	135	108.40	50.10	6.76	131.7	9.00	15.38
Mean	13.90	129.44	108.72	53.10	6.80	121.14	8.78	15.43
sd	1.17	13.20	14.80	6.90	0.71	9.30	1.08	1.44

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	15.22	152	100.14	50.24	7.64	118.3	7.77	15.14
2								
3	15.46	148	104.44	54.21	8.02	120.0	7.76	14.65
4	11.49	110	104.42	47.24	5.20	133.3	11.61	16.10
5	12.54	137	90.89	45.19	6.24	125.0	9.97	13.86
6	14.28	140	102.03	52.18	7.31	115.0	8.05	13.33
7	13.25	94	139.42	67.28	6.39	126.7	9.56	16.07
8	18.42	137	134.47	65.35	8.95	121.7	6.60	14.69
9	15.20	143	106.29	49.15	7.03	135.0	8.88	14.74
Mean	14.48	132.63	110.26	53.86	7.10	124.37	8.78	14.82
sd	2.13	20.06	17.18	8.19	1.16	7.06	1.57	0.96

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the T2D EXS group at each assessment during the intervention study. Subject 5 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	10.92	111	98.42	49.31	5.47	113.3	10.38	18.74
2	13.24	133	99.91	46.29	6.14	116.7	8.81	18.28
3	11.56	116	99.68	49.49	5.74	125.0	10.81	12.13
4	11.52	114	101.52	44.49	5.05	120.8	10.49	14.08
5								
6	10.10	124	81.48	42.79	5.31	128.3	12.70	14.55
7	9.73	123	79.09	38.37	4.72	125.0	12.85	14.56
8	12.63	117	107.98	54.93	6.43	136.7	10.82	16.60
9	12.50	125	100.41	47.38	5.90	120.0	9.60	17.21
10	11.66	137	85.10	44.66	6.12	118.3	10.15	14.29
11	11.35	115	98.73	51.51	5.92	128.3	11.30	13.55
Mean	11.52	121.35	95.23	46.92	5.68	123.24	10.79	15.40
sd	1.10	8.47	9.69	4.71	0.53	6.83	1.25	2.18

3 months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	10.24	97	106.93	53.48	5.12	122.5	11.96	18.75
2	13.05	126	103.53	48.62	6.13	124.2	9.52	19.32
3	12.29	120	102.44	50.82	6.10	125.0	10.17	17.84
4	14.43	125	115.86	50.95	6.34	134.2	9.30	16.94
5								
6	12.56	136	92.39	48.05	6.53	133.3	10.61	14.53
7	11.94	140	85.25	41.20	5.77	127.5	10.68	16.08
8	12.50	114	109.62	55.55	6.33	138.3	11.07	17.40
9	12.65	119	106.76	50.13	5.94	124.2	9.82	18.67
10	11.61	120	96.77	50.74	6.09	113.3	9.76	17.11
11	12.10	110	110.04	57.43	6.32	116.7	9.64	16.97
Mean	12.34	120.55	102.96	50.70	6.07	125.92	10.25	17.36
sd	1.06	12.47	9.16	4.45	0.40	7.76	0.83	1.40

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	ml O_2.100ml $blood^{-1}$
1	10.03	118	84.96	42.24	4.98	121.7	12.14	18.61
2	14.57	140	104.06	48.38	6.77	128.3	8.81	15.78
3	11.18	124	90.14	44.46	5.51	136.7	12.23	17.69
4	12.65	120	105.44	46.41	5.57	125.8	9.94	18.84
5								
6	12.69	122	103.99	54.45	6.64	123.3	9.72	14.18
7	11.36	129	88.04	42.70	5.51	126.7	11.16	16.93
8	13.96	126	111.24	56.88	7.14	136.7	9.79	15.10
9	12.17	137	88.22	41.50	5.73	126.7	10.41	19.03
10	12.81	138	92.85	48.79	6.73	118.3	9.24	15.74
11	12.18	128	95.15	49.35	6.32	123.3	10.13	15.32
Mean	12.36	128.15	96.41	47.51	6.09	126.76	10.36	16.72
sd	1.32	7.80	9.06	5.16	0.72	5.97	1.15	1.74

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the T2D CTL group at each assessment during the intervention study. Subjects 6 and 9 were excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.25	123	115.85	51.26	6.30	133.3	9.36	14.58
2	10.30	127	81.08	37.89	4.81	135.0	13.11	18.44
3	10.97	110	99.73	49.09	5.40	121.7	11.09	12.48
4	10.28	134	76.68	37.02	4.96	109.2	10.63	16.01
5	10.10	108	93.61	44.00	4.75	117.5	11.63	13.53
6								
7	13.32	116	114.84	48.72	5.65	135.0	10.13	14.01
8	12.59	124	101.34	47.87	5.95	126.7	10.06	13.69
9								
10	12.06	116	103.98	51.79	6.01	130.8	10.85	15.53
11	11.40	147	77.76	39.50	5.79	126.7	11.12	17.10
Mean	11.70	122.72	96.10	45.24	5.51	126.21	10.89	15.04
sd	1.47	12.15	14.95	5.80	0.56	8.75	1.07	1.90

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	10.91	110	99.21	43.97	4.84	123.3	11.30	15.97
2	10.73	124	86.51	40.69	5.05	131.7	12.28	19.18
3	9.58	112	85.57	42.16	4.72	116.7	12.18	15.85
4	13.07	140	93.32	45.05	6.31	113.3	8.67	14.18
5	10.64	110	96.70	45.59	5.01	117.5	11.05	12.67
6								
7	15.11	140	107.95	45.14	6.32	148.3	9.81	15.29
8	12.72	118	107.82	51.26	6.05	124.2	9.76	14.80
9								
10	13.05	125	104.38	52.09	6.51	136.7	10.48	17.26
11	10.93	158	69.38	35.41	5.58	125.8	11.51	17.99
Mean	11.86	126.28	94.54	44.60	5.60	126.39	10.78	15.91
sd	1.73	16.43	12.54	5.10	0.71	11.03	1.20	2.00

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.52	124	117.11	51.46	6.38	130.0	8.95	15.40
2	11.87	139	85.43	39.94	5.55	142.5	12.00	16.77
3	10.38	107	96.99	48.37	5.18	116.7	11.24	14.35
4	11.57	139	83.24	39.91	5.55	106.7	9.22	17.25
5	11.64	120	96.98	45.61	5.47	125.0	10.74	12.18
6								
7	14.66	134	109.38	44.76	6.00	143.3	9.78	16.70
8	14.60	132	110.61	53.14	7.01	133.3	9.13	13.28
9								
10	14.04	130	107.98	54.74	7.12	140.0	9.97	14.29
11	12.27	148	82.92	42.17	6.24	133.3	10.86	17.70
Mean	12.84	130.33	98.96	46.68	6.06	130.09	10.21	15.32
sd	1.62	12.09	12.99	5.58	0.69	12.26	1.06	1.92

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the PIO EXS group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	13.81	131	105.44	44.20	5.79	110.0	7.96	18.03
2								
3	11.36	126	90.12	42.71	5.38	118.3	10.42	19.38
4	11.03	135	81.67	42.57	5.75	120.0	10.88	15.60
5	14.16	125	113.29	58.42	7.30	126.7	8.95	13.58
6	12.07	134	90.08	45.86	6.15	115.0	9.53	19.46
Mean	12.49	130.20	96.12	46.75	6.07	118.01	9.55	17.21
sd	1.43	4.55	12.88	6.66	0.74	6.18	1.16	2.56

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2\ 100ml\ blood^{-1}$
1	15.32	125	122.56	51.03	6.38	116.7	7.62	14.32
2								
3	11.62	139	83.56	39.64	5.51	121.7	10.48	20.21
4	12.62	139	90.77	47.14	6.55	126.7	10.04	16.29
5	15.47	130	118.98	61.07	7.94	130.0	8.40	14.11
6	13.27	137	96.84	48.95	6.71	111.7	8.42	15.63
Mean	13.66	134.00	102.54	49.57	6.62	121.35	8.99	16.11
sd	1.69	6.24	17.34	7.73	0.87	7.39	1.21	2.46

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	12.89	114	113.10	46.45	5.30	110.0	8.53	14.85
2								
3	11.19	135	82.91	39.26	5.30	126.7	11.32	20.18
4	12.85	138	93.14	48.73	6.72	126.7	9.86	12.15
5	16.82	140	120.17	60.98	8.54	138.3	8.22	11.62
6	12.45	122	102.06	51.51	6.28	108.3	8.70	15.00
Mean	13.24	129.80	102.27	49.39	6.43	122.01	9.33	14.76
sd	2.12	11.28	14.97	7.91	1.33	12.66	1.27	3.40

Appendix XXVIII: Individual CO responses and related parameters after 240s steady-state cycling at 80% VT in the PIO CTL group at each assessment during the intervention study. Subject 2 was excluded from analysis due to use of β-blockers to control hypertension.

Baseline								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	14.66	134	109.38	44.76	6.00	143.3	9.78	16.70
2								
3	10.38	107	96.99	48.37	5.18	116.7	11.24	14.35
4	14.04	130	107.98	54.74	7.12	140.0	9.97	14.29
5	14.75	144	102.81	46.98	6.74	126.7	8.59	11.56
Mean	13.46	128.63	104.29	48.71	6.26	131.68	9.90	14.22
sd	2.08	15.49	5.63	4.29	0.86	12.29	1.09	2.10

3 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	19.17	153	125.30	51.32	7.85	142.5	7.43	13.13
2								
3	12.00	117	102.53	50.63	5.92	116.7	9.73	11.67
4	13.19	107	123.25	61.43	6.57	140.0	10.62	14.62
5	13.09	143	91.53	41.40	5.92	128.3	9.80	13.94
Mean	14.36	130.00	110.65	51.20	6.57	131.88	9.39	13.34
sd	3.25	21.57	16.38	8.19	0.91	11.87	1.37	1.27

7 Months								
	CO	HR	SV	SVI	CI	MAP	TPR	a-v O_2
ID	$L.min^{-1}$	$beats.min^{-1}$	ml	$ml.m^{-2}$	$L.min^{-1}.m^{-2}$	mmHg	$mmHg.L^{-1}.min^{-1}$	$ml\ O_2.100ml\ blood^{-1}$
1	20.91	158	132.35	53.28	8.42	141.7	6.77	12.68
2								
3	13.71	112	122.41	60.46	6.77	123.3	9.00	11.39
4	14.56	123	118.38	57.87	7.12	140.0	9.61	12.29
5	15.07	143	105.41	47.33	6.77	125.0	8.29	11.78
Mean	16.06	134.00	119.64	54.73	7.27	132.50	8.42	12.04
sd	3.28	20.51	11.16	5.76	0.78	9.67	1.22	0.57

Appendix XXIX: Reliability Data comparing blood pressure measurements between the COLIN and Finapres measurement systems. The inter-correlation coefficient (ICC) value of 0.82 indicates high reliability of the readings from the two systems. The reliability study was performed by Ms. Heather Reilly.

Subject		Colin	Finopres	diff	$(diff)^2$
1	REST	83.45	78.85	4.60	21.14
1	Onset	82.64	81.67	0.97	0.94
1	Min 1	90.51	88.46	2.05	4.21
1	Min 2	92.12	89.47	2.65	7.02
1	Min 3	97.25	94.98	2.27	5.17
1	Min 4	79.39	80.88	-1.49	2.22
1	Min 5	67.53	68.97	-1.44	2.07
1	Min 6	90.05	84.98	5.08	25.78
1	Min 7	93.53	93.31	0.22	0.05
1	Min 8	88.06	88.65	-0.58	0.34
1	Min 9	98.27	100.98	-2.71	7.33
1	Min 10	100.53	102.31	-1.78	3.16
1	Min 11	96.79	91.31	5.48	30.02
1	Min 12	98.37	93.98	4.40	19.34
2	REST	73.52	76.54	-3.02	9.12
2	Onset	99.58	95.89	3.69	13.61
2	Min 1	94.61	90.54	4.07	16.55
2	Min 2	97.82	95.65	2.18	4.74
2	Min 3	101.00	97.06	3.94	15.51
2	Min 4	103.84	99.52	4.32	18.67
2	Min 5	94.62	93.21	1.40	1.97
2	Min 6	100.33	94.80	5.53	30.59
2	Min 7	99.99	95.80	4.19	17.54
2	Min 8	100.21	95.80	4.41	19.41
2	Min 9	99.90	96.34	3.55	12.62
2	Min 10	98.99	98.69	0.30	0.09
2	Min 11	99.80	98.64	1.16	1.34
2	Min 12	100.55	99.98	0.58	0.33
3	REST	82.76	85.27	-2.51	6.29
3	Onset	98.74	96.91	1.83	3.34
3	Min 1	109.14	95.68	13.46	181.22
3	Min 2	104.00	87.93	16.07	258.14
3	Min 3	111.87	103.52	8.35	69.70
3	Min 4	108.72	100.42	8.30	68.88
3	Min 5	112.81	99.38	13.42	180.13
3	Min 6	105.36	95.19	10.17	103.52
3	Min 7	110.39	104.00	6.39	40.85
3	Min 8	105.36	97.51	7.85	61.63
3	Min 9	107.31	102.84	4.47	19.98
3	Min 10	104.98	99.79	5.18	26.84
3	Min 11	104.64	111.39	-6.75	45.52
3	Min 12	105.64	109.75	-4.11	16.85
4	REST	86.41	83.46	2.95	8.69
4	Onset	89.64	91.97	-2.33	5.43
4	Min 1	89.31	91.31	-2.00	3.99
4	Min 2	91.64	91.31	0.33	0.11
4	Min 3	86.74	92.31	-5.56	30.94
4	Min 4	95.64	90.31	5.34	28.47
4	Min 5	97.97	97.97	0.00	0.00
4	Min 6	95.64	98.64	-3.00	8.98
4	Min 7	95.30	96.30	-1.00	1.00
4	Min 8	96.30	97.97	-1.66	2.77
4	Min 9	99.30	97.64	1.66	2.77
4	Min 10	107.26	104.93	2.33	5.43
4	Min 11	108.18	108.84	-0.67	0.44
4	Min 12	110.97	112.64	-1.67	2.77
5	REST	82.76	85.27	-2.51	6.29
5	Onset	98.74	96.91	1.83	3.34
5	Min 1	109.14	95.68	13.46	181.23
5	Min 2	104.00	87.93	16.07	258.14
5	Min 3	111.87	103.52	8.35	69.70
5	Min 4	101.00	100.42	0.58	0.34
5	Min 5	112.81	99.39	13.42	180.13
5	Min 6	105.36	104.00	1.36	1.85
5	Min 7	97.00	95.82	1.18	1.39
5	Min 8	105.36	102.00	3.36	11.29
5	Min 9	107.31	106.00	1.31	1.71
5	Min 10	104.98	99.79	5.18	26.84
5	Min 11	112.00	111.39	0.61	0.37
5	Min 12	105.64	109.75	-4.11	16.85
	MEAN	**98.62**	**95.86**	**2.76**	**2235.05**
	SD	9.50	8.36	4.97	15.96
					4.0
			%TEM		4.1
			MEAN + 1.9	12.49	
			MEAN - 1.9	-6.98	
			ICC	0.82	

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the ND EXS group at each assessment during the intervention study.

Baseline				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	937	6.9	700	74.71
2	1035	10.5	900	86.96
3	1190	10.0	900	75.63
4	936	6.0	500	53.42
5	842	8.0	700	83.14
6	1033	8.0	700	67.76
7	1657	10.0	900	54.32
8	1730	12.0	1100	63.58
9	924	4.0	300	32.47
10	807	8.0	700	86.74
Mean	**1109.10**	**8.34**	**740.00**	**67.87**
sd	326.60	2.36	227.06	17.40

3 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1661	10.1	900	54.18
2	1542	10.0	900	58.37
3	1530	10.0	900	58.82
4	1300	8.7	700	53.85
5	1675	12.0	1100	65.67
6	1130	10.0	900	79.65
7	1621	12.8	1300	80.20
8	1849	14.0	1300	70.31
9	1169	6.0	500	42.77
10	1100	8.0	700	63.64
Mean	**1457.70**	**10.16**	**920.00**	**62.75**
sd	263.36	2.34	257.34	11.75

7 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1629	12.0	1100	67.53
2	1865	12.0	1100	58.98
3	1521	12.0	1100	72.32
4	1247	8.0	700	56.13
5	1580	10.0	900	56.96
6	1105	8.0	700	63.35
7	1606	12.0	1100	68.49
8	1657	14.0	1300	78.46
9	1108	8.0	700	63.18
10	1033	8.0	700	67.76
Mean	**1435.10**	**10.40**	**940.00**	**65.32**
sd	287.18	2.27	227.06	7.03

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the ND CTL group at each assessment during the intervention study.

Baseline				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1024	8.0	700	68.36
2	646	6.0	500	77.40
3	852	6.3	500	58.69
4	947	6.0	500	52.80
5	1085	8.0	700	64.52
6	1312	10.0	900	68.60
7	1042	7.5	700	67.18
8	1426	10.0	900	63.11
9	1039	10.0	900	86.62
Mean	**1041.44**	**7.98**	**700.00**	**67.47**
sd	230.35	1.70	173.21	9.92

3 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1369	12.0	1100	80.35
2	860	8.0	700	81.40
3	1168	6.0	500	42.81
4	900	6.0	500	55.56
5	1165	10.0	900	77.25
6	1380	10.0	900	65.22
7	914	6.0	500	54.70
8	1405	9.0	900	64.06
9	1025	7.0	700	68.29
Mean	**1131.78**	**8.22**	**744.44**	**65.51**
sd	218.34	2.17	218.58	13.00

7 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1345	10.0	900	66.91
2	1069	10.0	900	84.19
3	1230	10.0	900	73.17
4	1001	7.0	700	69.93
5	1307	10.0	900	68.86
6	1230	9.0	900	73.17
7	833	8.0	700	84.03
8	1564	10.0	900	57.54
9	994	8.0	700	70.42
Mean	**1174.78**	**9.11**	**833.33**	**72.03**
sd	222.19	1.17	100.00	8.27

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the T2D EXS group at each assessment during the intervention study.

Baseline				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	933	6.0	500	53.59
2	1133	7.2	700	61.78
3	1080	8.0	700	64.81
4	1586	7.0	700	44.14
5	861	5.3	500	58.07
6	870	6.0	500	57.47
7	1160	6.0	500	43.10
8	1184	10.0	900	76.01
9	1317	6.0	500	37.97
10	1470	10.0	900	61.22
11	1125	8.0	700	62.22
Mean	**1156.27**	**7.23**	**645.45**	**56.40**
sd	231.52	1.62	157.25	11.05

3 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1034	8.0	700	67.70
2	1216	8.0	700	57.57
3	1299	10.0	900	69.28
4	1554	10.0	900	57.92
5	1136	8.0	700	61.62
6	936	8.0	700	74.79
7	941	8.0	700	74.39
8	1326	8.0	700	52.79
9	1551	10.0	900	58.03
10	1315	8.0	700	53.23
11	1386	10.0	900	64.94
Mean	**1244.91**	**8.73**	**772.73**	**62.93**
sd	216.45	1.01	100.90	7.85

7 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1380	10.0	900	65.22
2	1426	9.6	900	63.11
3	1202	8.0	700	58.24
4	1836	14.0	1300	70.81
5	1038	6.0	500	48.17
6	986	8.0	700	70.99
7	1357	12.0	1100	81.06
8	1392	10.0	900	64.66
9	1452	10.0	900	61.98
10	1286	10.0	900	69.98
11	1280	8.0	700	54.69
Mean	**1330.45**	**9.60**	**863.64**	**64.45**
sd	226.19	2.15	215.74	8.93

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the T2D CTL group at each assessment during the intervention study.

Baseline				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1612	12.0	1100	68.24
2	1050	6.0	500	47.62
3	1231	8.0	700	56.86
4	1002	8.0	700	69.86
5	1294	8.0	700	54.10
6	1339	8.0	700	52.28
7	1759	12.0	1100	62.54
8	1048	8.0	700	66.79
9	1390	6.0	500	35.97
10	1078	4.0	300	27.83
11	1504	10.0	900	59.84
Mean	**1300.64**	**8.18**	**718.18**	**54.72**
sd	250.63	2.44	244.21	13.35

3 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1585	12.0	1100	69.40
2	991	4.0	300	30.27
3	1082	6.0	500	46.21
4	830	6.0	500	60.24
5	1133	8.0	700	61.78
6	1254	10.0	900	71.77
7	1680	12.0	1100	65.48
8	1016	6.0	500	49.21
9	1229	8.0	700	56.96
10	1152	6.0	500	43.40
11	1580	12.0	1100	69.62
Mean	**1230.18**	**8.18**	**718.18**	**56.76**
sd	274.21	2.89	289.20	13.08

7 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1576	13.0	1300	82.49
2	1048	8.0	700	66.79
3	987	6.0	500	50.66
4	939	8.0	700	74.55
5	1114	8.0	700	62.84
6	1148	8.0	700	60.98
7	1764	12.0	1100	62.36
8	1102	8.0	700	63.52
9	1094	8.0	700	63.99
10	987	6.0	500	50.66
11	1520	12.0	1100	72.37
Mean	**1207.18**	**8.82**	**790.91**	**64.65**
sd	278.25	2.40	258.67	9.46

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the PIO EXS group at each assessment during the intervention study.

Baseline				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1316	8.0	700	53.19
2	1298	10.0	900	69.34
3	1506	10.0	900	59.76
4	1255	9.2	900	71.71
5	788	6.0	500	63.45
6	1432	10.0	900	62.85
Mean	**1265.83**	**8.87**	**800.00**	**63.38**
sd	251.91	1.61	167.33	6.67

3 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1271	8.0	700	55.07
2	1309	10.0	900	68.75
3	1515	12.0	1100	72.61
4	1469	11.0	1100	74.88
5	952	8.0	700	73.53
6	1353	9.0	900	66.52
Mean	**1311.50**	**9.67**	**900.00**	**68.56**
sd	199.41	1.63	178.89	7.31

7 Months				
	MVC	TTF	Peak Force	Peak Force
ID	N	min	N	% MVC
1	1161	7.0	700	60.29
2	1273	9.0	900	70.70
3	1497	11.0	1100	73.48
4	1334	10.0	900	67.47
5	999	7.5	700	70.07
6	1217	8.0	700	57.52
Mean	**1246.83**	**8.75**	**833.33**	**66.59**
sd	167.66	1.54	163.30	6.31

Appendix XXX: Individual MVC responses and peak parameters from the incremental plantar-flexion test in the PIO CTL group at each assessment during the intervention study.

Baseline				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1764	12.0	1100	62.36
2	1094	8.0	700	63.99
3	987	6.0	500	50.66
4	987	6.0	500	50.66
5	744	6.0	500	67.20
Mean	**1115.20**	**7.60**	**660.00**	**58.97**
sd	384.72	2.61	260.77	7.79

3 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1597	12.0	1100	68.88
2	1131	8.0	700	61.89
3	1123	8.0	700	62.33
4	1169	8.0	700	59.88
5	800	7.5	700	87.50
Mean	**1164.00**	**8.70**	**780.00**	**68.10**
sd	284.07	1.86	178.89	11.36

7 Months				
	MVC	**TTF**	**Peak Force**	**Peak Force**
ID	N	min	N	% MVC
1	1783	12.0	1100	61.69
2	1107	8.0	700	63.23
3	1111	8.0	700	63.01
4	1043	8.0	700	67.11
5	651	6.0	500	76.80
Mean	**1139.00**	**8.40**	**740.00**	**66.37**
sd	407.40	2.19	219.09	6.17

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the ND EXS group at each assessment during the intervention study.

Baseline									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.18	9.21	0.93	2.27	4.80	6.64	9.21		
2	0.57	5.66	1.01	1.40	2.89	4.70	5.66		
3	0.94	6.04	1.77	2.29	4.45	6.04			
4	1.17	4.91	1.97	2.56	4.43	4.91			
5	0.37	2.93	0.94	1.61	2.65	2.93			
6	1.26	7.32	3.52	5.26	6.90	6.53	7.32		
7	1.31	17.65	2.62	5.08	7.80	12.24	16.09	17.65	
8	1.13	4.36	1.43	1.96	2.63	4.36			
9	0.52	2.15	0.80	2.15					
10	0.36	3.83	1.10	2.10	3.13	3.83			
Mean	**0.88**	**6.41**	**1.61**	**2.67**	**4.41**	**5.80**	**9.57**	**17.65**	
sd	0.38	4.46	0.89	1.36	1.87	2.71	4.58		

3 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.01	8.76	1.41	3.33	5.59	5.49	8.76		
2	0.80	7.76	0.53	2.22	4.30	6.26	7.76		
3	0.58	7.10	0.86	2.32	4.34	7.10			
4	0.70	6.26	1.75	3.16	4.37	6.26	5.75		
5	0.40	2.74	0.68	1.61	2.35	2.74	1.99		
6	0.81	10.68	0.84	2.38	3.57	5.19	7.57	9.20	10.68
7	1.44	15.33	1.64	3.86	6.17	8.24	10.97	13.03	15.33
8	0.83	5.46	0.88	1.63	2.79	3.62	4.89	5.46	
9	1.07	4.88	1.44	3.14	4.88				
10	0.60	4.90	1.16	2.11	3.55	4.90			
Mean	**0.82**	**7.39**	**1.12**	**2.58**	**4.19**	**5.53**	**6.81**	**9.23**	**13.01**
sd	0.29	3.58	0.42	0.76	1.18	1.69	2.91	3.78	3.29

7 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.63	4.24	0.66	1.24	1.76	2.43	3.35	4.24	
2	1.16	7.16	0.94	2.99	4.09	5.88	7.16	7.10	
3	0.69	5.72	0.61	2.18	3.98	5.72			
4	0.90	9.10	1.57	4.56	5.59	9.10	9.09	8.22	
5	0.38	2.06	0.56	1.54	1.80	2.06			
6	1.82	9.58	1.28	3.44	4.22	5.30	7.45	9.58	
7	2.63	25.16	3.44	7.06	10.43	13.95	21.05	25.16	21.24
8	0.90	5.11	1.13	1.77	2.99	4.25	5.11		
9	0.71	4.57	1.35	2.40	3.81	4.57			
10	0.70	5.53	0.95	3.23	5.50	5.53			
Mean	**1.05**	**7.82**	**1.25**	**3.04**	**4.42**	**5.88**	**8.87**	**10.86**	**21.24**
sd	0.68	6.49	0.84	1.73	2.48	3.45	6.29	8.23	

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the ND CTL group at each assessment during the intervention study.

Baseline									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.12	7.17	1.48	3.58	4.32	7.17			
2	1.02	5.80	1.37	3.00	5.80				
3	0.54	5.39	1.48	3.53	5.39				
4	0.76	2.06	0.94	1.43	2.06				
5	1.35	6.56	1.23	3.98	6.56	5.91			
6	0.82	7.10	1.26	5.68	6.89	6.87	7.10		
7	1.17	5.67	2.75	3.56	4.92	5.67			
8	0.64	5.15	0.67	1.78	2.85	3.24	5.15		
9	0.98	8.22	1.60	3.69	5.33	6.62	8.22		
Mean	**0.93**	**5.90**	**1.42**	**3.36**	**4.90**	**5.91**	**6.82**		
sd	0.27	1.75	0.58	1.24	1.60	1.43	1.55		

3 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.74	7.13	1.34	2.96	5.15	5.32	7.11	7.13	
2	0.76	3.88	1.76	2.57	3.88				
3	0.59	3.24	0.87	1.62	2.36	3.24			
4	0.57	3.34	1.60	2.16	3.34				
5	1.11	8.24	1.23	3.13	5.85	7.36	8.24		
6	0.63	7.84	0.92	3.62	5.17	7.08	7.84		
7	1.00	6.17	2.93	4.06	6.17				
8	1.51	4.89	0.80	2.63	3.17	4.89			
9	1.05	16.36	2.27	4.51	7.81	11.27	16.36		
Mean	**0.88**	**6.79**	**1.53**	**3.03**	**4.77**	**6.53**	**9.89**	**7.13**	
sd	0.31	4.06	0.71	0.92	1.73	2.77	4.34		

7 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.97	7.93	1.11	3.68	6.29	6.18	7.93		
2	0.73	8.67	1.42	2.72	4.36	6.24	8.67		
3	0.54	4.97	0.79	2.83	3.43	4.51	4.97		
4	0.67	4.80	1.13	2.21	3.31	4.80			
5	1.06	10.03	1.63	4.05	6.73	8.00	10.03		
6	0.70	7.24	0.69	2.96	6.01	6.63	7.24		
7	1.49	9.46	2.90	4.11	4.77	9.46			
8	1.14	6.42	0.98	3.18	4.77	6.42			
9	0.89	11.29	1.21	3.24	4.94	6.83	11.29		
Mean	**0.91**	**7.87**	**1.32**	**3.22**	**4.95**	**6.56**	**8.36**		
sd	0.29	2.23	0.66	0.63	1.20	1.51	2.21		

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the T2D EXS group at each assessment during the intervention study.

Baseline									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.35	2.68	0.44	1.54	1.62	2.68			
2	0.99	7.40	0.89	4.21	7.40				
3	0.79	6.80	1.64	2.59	4.74	6.80			
4	1.21	3.66	0.71	0.89	2.10	3.66			
5	0.50	5.36	1.11	1.82	2.33	4.86	5.36		
6	0.75	4.77	1.53	2.94	4.77				
7	0.73	2.47	1.16	1.81	2.47				
8	0.60	3.44	0.51	0.90	3.44				
9	0.63	4.77	1.33	3.04	4.77				
10	0.92	3.77	2.09	2.83	2.82	2.47	3.77		
11	0.63	6.75	1.31	2.90	4.88	6.75			
Mean	**0.74**	**4.71**	**1.16**	**2.32**	**3.76**	**4.54**	**4.56**		
sd	0.24	1.70	0.50	1.02	1.72	1.93	1.12		

3 Months									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.59	2.99	0.52	1.14	1.68	2.99	2.90		
2	0.77	8.69	1.36	4.73	7.13	8.69			
3	0.48	5.60	1.46	1.74	3.05	5.60			
4	0.74	5.55	0.94	1.56	2.45	3.41	5.55		
5	0.84	7.06	1.64	3.27	5.44	7.06			
6	1.04	7.72	1.43	3.33	5.28	6.27	7.72		
7	0.62	3.46	0.63	2.25	3.16	3.46			
8	0.57	5.21	1.16	1.86	3.72	5.21			
9	0.81	6.93	1.23	3.00	5.02	6.93			
10	0.99	7.11	1.44	4.18	5.18	7.11			
11	1.17	8.02	2.05	3.89	4.79	6.71	8.02		
Mean	**0.78**	**6.21**	**1.26**	**2.81**	**4.26**	**5.77**	**6.05**		
sd	0.22	1.83	0.44	1.18	1.59	1.83	2.37		

7 Months									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.46	3.00	0.55	1.13	2.23	3.00			
2	0.75	8.99	1.07	3.54	6.20	8.80	8.99		
3	0.80	8.13	0.64	1.59	3.53	5.50	8.13		
4	1.44	7.13	0.86	1.89	2.78	3.99	5.02	5.58	7.13
5	1.13	6.75	2.22	2.41	4.13	5.72	6.75		
6	1.04	8.38	2.03	3.60	4.49	7.20	8.38		
7	0.90	5.30	0.79	1.33	2.69	3.24	4.84	5.30	
8	0.47	5.78	1.08	3.21	5.78				
9	1.08	8.73	1.49	3.34	5.43	8.73			
10	0.84	5.80	1.13	2.68	4.06	4.51	5.80		
11	0.69	7.18	1.20	3.00	4.64	7.18			
Mean	**0.87**	**6.83**	**1.19**	**2.52**	**4.18**	**5.79**	**6.84**	**5.44**	**7.13**
sd	0.29	1.78	0.54	0.91	1.30	2.13	1.69	0.20	

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the T2D CTL group at each assessment during the intervention study.

Baseline									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.19	6.29	1.25	2.66	4.52	6.29			
2	0.42	1.21	1.07	1.21					
3	0.69	3.45	0.70	1.21	3.04	3.45			
4	0.46	2.78	1.21	1.79	2.78				
5	0.81	6.67	1.14	2.88	4.48	6.67			
6	0.67	4.31	1.71	2.76	3.27	4.31			
7	1.22	8.76	1.00	3.53	4.47	6.08	6.41	8.76	
8	0.49	5.89	1.12	2.90	5.89				
9	0.38	5.96	0.72	1.10	2.48	3.85	5.29	5.96	
10	0.61	10.78	0.94	3.06	4.36	6.47	10.78		
11	1.35	5.74	0.84	2.61	3.88	5.74			
Mean	**0.75**	**5.62**	**1.06**	**2.34**	**3.92**	**5.36**	**7.49**	**7.36**	
sd	0.35	2.69	0.29	0.85	1.03	1.28	2.90	1.98	

3 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	1.79	6.21	2.26	5.14	6.21				
2	0.41	2.66	0.58	1.37	2.66				
3	0.89	4.72	0.57	1.05	2.38	4.72	4.70		
4	0.56	5.34	0.88	2.34	4.10	5.34			
5	0.68	6.32	1.18	2.33	3.75	6.32			
6	0.64	2.93	1.26	2.79	2.93				
7	0.74	6.72	0.78	2.31	2.96	5.07	4.85	6.72	
8	0.74	3.61	1.32	3.61					
9	0.52	6.28	0.50	0.98	1.86	3.38	6.28	6.19	
10	1.00	9.17	1.37	3.90	4.75	6.52	7.89	9.17	
11	1.09	3.44	1.16	1.90	3.44				
Mean	**0.82**	**5.22**	**1.08**	**2.52**	**3.50**	**5.22**	**5.93**	**7.36**	
sd	0.38	1.97	0.51	1.28	1.27	1.15	1.49	1.59	

7 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	2.02	9.55	1.99	3.69	6.44	9.55			
2	0.49	3.09	0.68	1.61	3.09				
3	1.27	4.69	1.08	2.35	4.23	4.69			
4	0.59	3.59	1.16	2.08	3.01	3.59			
5	1.10	8.02	1.41	3.02	4.94	8.02			
6	1.26	6.28	2.29	5.01	6.28				
7	2.06	10.55	1.58	3.78	5.25	7.02	5.97	10.55	8.53
8	1.05	8.26	1.97	4.76	6.72	8.26			
9	0.71	6.28	0.85	1.73	3.47	5.01	5.81	6.28	
10	1.35	10.19	1.21	3.26	5.76	6.80	9.31	10.19	
11	0.92	5.66	1.10	2.43	4.40	5.66			
Mean	**1.17**	**6.92**	**1.39**	**3.06**	**4.87**	**6.51**	**7.03**	**9.01**	**8.53**
sd	0.51	2.59	0.51	1.16	1.34	1.93	1.98	2.37	

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the PIO EXS group at each assessment during the intervention study.

Baseline									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.66	7.60	0.57	2.36	4.82	6.39	7.60		
2	1.77	9.56	2.04	5.35	7.87	9.56			
3	0.79	8.33	1.39	3.66	7.88	7.94	8.33		
4	0.74	4.52	1.77	3.29	4.06	4.52	4.09		
5	1.07	4.62	1.61	3.49	4.62				
6	1.25	8.85	1.19	4.56	6.59	7.78	8.85		
Mean	**1.05**	**7.25**	**1.43**	**3.79**	**5.97**	**7.24**	**7.22**		
sd	0.42	2.17	0.52	1.04	1.70	1.89	2.15		

3 Months									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.57	7.62	0.68	1.81	3.80	5.26	6.51	7.62	
2	1.54	7.15	2.34	5.12	6.71	7.15			
3	1.24	8.24	0.94	3.02	5.17	7.02	8.24		
4	0.67	5.52	1.04	3.49	5.41	5.38	5.52	5.41	
5	0.54	4.32	0.62	2.26	2.87	4.32			
6	1.16	7.89	1.50	5.12	6.84	6.66	7.89		
Mean	**0.95**	**6.79**	**1.19**	**3.47**	**5.13**	**5.96**	**7.04**	**6.52**	
sd	0.42	1.54	0.64	1.41	1.57	1.14	1.26	1.56	

7 Months									
	Rest	**Peak**	**100N**	**300N**	**500N**	**700N**	**900N**	**1100N**	**1300N**
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.78	7.90	0.87	2.27	4.90	5.02	6.91	7.90	
2	1.02	5.84	1.55	4.60	5.84	5.76			
3	0.98	8.58	1.54	3.88	6.09	7.79	8.58		
4	1.10	8.33	1.84	5.82	8.33	7.25	6.43		
5	1.09	5.42	1.14	3.30	3.80	5.42			
6	0.56	6.75	1.03	3.42	5.66	6.75			
Mean	**0.92**	**7.14**	**1.33**	**3.88**	**5.77**	**6.33**	**7.30**	**7.90**	
sd	0.21	1.33	0.37	1.22	1.50	1.10	1.13		

Appendix XXXI: Individual LVC responses to the incremental plantar-flexion test in the PIO CTL group at each assessment during the intervention study.

Baseline									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.49	3.09	0.68	1.69	3.09				
2	0.59	3.59	1.16	2.08	3.01	3.59			
3	1.26	3.86	1.44	2.84	3.86				
4	0.71	6.28	0.85	1.73	3.47	5.01	5.81	6.28	
5	0.76	3.09	0.99	1.71	3.09				
Mean	**0.76**	**3.98**	**1.02**	**2.01**	**3.30**	**4.30**	**5.81**	**6.28**	
sd	0.30	1.33	0.29	0.49	0.36	1.01			

3 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.60	5.51	0.43	2.07	3.22	5.51			
2	0.48	4.23	0.98	2.36	3.56	4.23			
3	0.88	5.25	1.49	3.19	4.31	5.25			
4	0.71	6.60	1.07	2.22	2.74	4.79	5.63	6.60	
5	1.16	5.03	1.01	2.08	2.88	5.03			
Mean	**0.77**	**5.32**	**1.00**	**2.38**	**3.34**	**4.96**	**5.63**	**6.60**	
sd	0.26	0.86	0.38	0.47	0.63	0.49			

7 Months									
	Rest	Peak	100N	300N	500N	700N	900N	1100N	1300N
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}
1	0.71	6.36	0.85	2.32	4.27	6.36			
2	0.83	5.76	1.09	1.93	4.53	5.76			
3	0.90	5.50	1.39	3.22	4.72	5.50			
4	0.51	5.29	0.71	2.57	3.11	4.09	5.29	5.00	
5	0.74	1.72	0.73	1.56	1.72				
Mean	**0.74**	**4.93**	**0.95**	**2.32**	**3.87**	**5.43**	**5.29**	**5.00**	
sd	0.15	1.84	0.29	0.63	1.25	0.96			

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the ND EXS group at each assessment during the intervention study.

Baseline

ID	a ml.min^{-1}.mmHg^{-1}	A_1 ml.min^{-1}.mmHg^{-1}	TD_1 s	Tau_1 s	A_2 (decay) ml.min^{-1}.mmHg^{-1}	TD_2 (decay) s	Tau_2 (decay) s	A_3 ml.min^{-1}.mmHg^{-1}	TD_3 s	Tau_3 s	A_4 (decay) ml.min^{-1}.mmHg^{-1}	TD_4 (decay) s	Tau_4 (decay) s	End A ml.min^{-1}.mmHg^{-1}	Adj R^2 %
1	1.15	0.92	3.06	3.26	0.88	11.68	2.05	2.05	18.00	46.30	0.74	87.78	22.57	2.46	62.64
2	1.01	1.49	1.02	4.03	1.22	10.53	3.71	0.98	13.02	6.93	0.25	111.78	25.87	2.01	57.92
3	0.85	2.69	0.00	2.42	0.70	5.16	3.99	1.05	24.65	24.48	0.64	85.93	117.33	3.32	77.81
4	0.83	0.46	0.00	2.44	0.57	6.07	13.20	1.44	17.81	41.18	0.61	71.00	8.00	1.55	15.87
5	0.34	2.03	1.76	3.66	0.43	13.17	13.71	0.55	29.81	30.60	0.35	95.81	5.00	2.13	51.05
6	0.98	1.98	0.01	3.17	2.14	6.51	16.00	4.95	11.93	41.42	1.03	113.73	7.81	4.73	61.27
7	0.40	2.35	0.99	3.00	1.10	6.00	7.07	0.70	28.70	12.59	0.38	64.42	19.00	1.97	68.18
8	1.19	2.06	0.00	2.43	0.45	11.99	2.04	2.49	20.54	37.45	1.31	66.00	31.00	3.99	78.56
9	0.94	8.71	0.00	0.31	3.95	12.49	5.36	2.92	17.37	5.25	0.87	79.55	32.35	7.74	87.53
10	0.43	1.44	1.64	3.10	1.00	12.13	7.62	2.07	18.00	21.31	1.13	45.82	19.74	1.81	44.79
Mean	**0.81**	**2.41**	**0.85**	**2.83**	**1.24**	**9.57**	**7.47**	**1.92**	**19.98**	**26.75**	**0.73**	**80.18**	**28.97**	**3.17**	**60.54**
sd	0.31	2.31	1.05	0.89	1.07	3.22	5.10	1.32	6.03	15.00	0.35	21.97	32.52	1.91	20.41

3 Months

ID	a ml.min^{-1}.mmHg^{-1}	A_1 ml.min^{-1}.mmHg^{-1}	TD_1 s	Tau_1 s	A_2 (decay) ml.min^{-1}.mmHg^{-1}	TD_2 (decay) s	Tau_2 (decay) s	A_3 ml.min^{-1}.mmHg^{-1}	TD_3 s	Tau_3 s	A_4 (decay) ml.min^{-1}.mmHg^{-1}	TD_4 (decay) s	Tau_4 (decay) s	End A ml.min^{-1}.mmHg^{-1}	Adj R^2 %
1	1.20	1.79	1.35	2.35	0.55	14.20	4.51	2.26	18.57	31.28	0.35	78.07	0.59	4.35	86.10
2	1.07	2.57	1.18	2.66	0.54	12.00	2.50	2.30	24.33	32.35	1.55	55.24	23.40	3.85	83.60
3	0.53	3.56	1.58	3.25	0.64	11.93	23.96	0.84	28.08	2.85	0.68	52.92	5.00	3.61	78.41
4	0.99	1.85	0.96	2.43	1.26	8.92	1.46	1.43	14.66	65.95	0.36	102.00	130.83	2.67	54.60
5	0.47	1.20	1.27	2.97	0.61	5.99	4.00	1.04	10.43	41.27	0.32	80.72	14.82	1.78	74.39
6	0.75	3.24	0.00	3.78	0.58	20.79	1.07	1.92	26.53	25.67	0.51	90.00	2.84	4.82	78.66
7	0.45	2.69	1.39	3.94	1.06	11.71	6.49	1.01	27.55	7.96	0.53	80.75	28.57	2.57	92.40
8	0.97	3.63	0.00	4.07	1.26	11.66	7.05	1.40	17.99	35.69	0.95	77.40	33.97	3.79	78.87
9	1.19	6.96	1.00	2.20	1.55	6.03	5.21	1.87	22.74	19.31	1.68	48.47	33.06	6.80	90.06
10	0.60	2.72	1.66	3.06	0.57	11.97	6.00	0.71	19.86	21.80	0.59	148.17	64.69	2.90	71.53
Mean	**0.82**	**3.02**	**1.04**	**3.12**	**0.86**	**11.52**	**6.23**	**1.48**	**21.07**	**30.41**	**0.75**	**81.37**	**33.76**	**3.71**	**78.86**
sd	0.30	1.59	0.59	0.64	0.38	4.23	6.56	0.58	5.83	22.90	0.49	28.99	39.04	1.41	10.78

7 Months

ID	a ml.min^{-1}.mmHg^{-1}	A_1 ml.min^{-1}.mmHg^{-1}	TD_1 s	Tau_1 s	A_2 (decay) ml.min^{-1}.mmHg^{-1}	TD_2 (decay) s	Tau_2 (decay) s	A_3 ml.min^{-1}.mmHg^{-1}	TD_3 s	Tau_3 s	A_4 (decay) ml.min^{-1}.mmHg^{-1}	TD_4 (decay) s	Tau_4 (decay) s	End A ml.min^{-1}.mmHg^{-1}	Adj R^2 %
1	0.65	1.33	0.17	3.49	0.70	11.97	8.64	1.00	17.99	19.61	0.08	59.22	10.01	2.18	74.31
2	1.16	3.01	1.99	2.42	0.89	6.94	3.11	1.97	21.83	38.86	0.45	96.00	41.30	4.81	92.72
3	0.71	2.94	1.41	3.82	1.09	14.75	5.58	1.17	21.97	13.42	1.05	60.00	67.95	2.69	85.50
4	0.81	2.19	0.75	3.86	2.31	13.14	14.52	4.24	23.37	48.07	0.10	97.46	23.01	4.84	81.24
5	0.35	1.80	1.00	3.00	1.12	3.02	5.33	1.70	27.44	42.05	0.67	86.00	47.29	1.86	89.98
6	0.77	4.48	1.00	3.02	1.38	11.68	5.09	2.75	22.45	15.45	0.69	82.23	15.00	5.93	83.42
7	0.61	3.10	0.01	2.47	2.30	4.69	19.63	2.71	15.76	28.35	0.67	86.04	43.51	3.25	86.76
8	0.93	3.53	1.46	3.09	1.35	6.64	3.11	1.49	17.00	25.56	0.50	130.06	39.60	4.10	73.89
9	1.31	7.85	0.96	2.77	2.38	5.99	4.19	1.78	23.44	8.08	1.29	84.00	103.05	7.35	93.89
10	0.74	2.42	0.74	1.89	0.91	16.09	23.27	1.38	23.93	24.82	0.70	102.00	20.31	2.93	78.34
Mean	**0.81**	**3.26**	**0.95**	**2.99**	**1.44**	**9.49**	**8.95**	**2.02**	**21.52**	**26.43**	**0.66**	**82.30**	**40.80**	**3.99**	**82.31**
sd	0.27	1.84	0.59	0.64	0.64	4.56	7.19	0.98	3.58	13.11	0.39	23.70	28.14	1.75	9.82

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the ND CTL group at each assessment during the intervention study.

Baseline

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.63	2.00	0.97	2.98	0.24	5.46	19.27	1.49	14.90	55.49	1.12	42.00	41.13	2.75	56.89
2	0.86	2.03	0.00	3.97	0.73	16.98	13.70	0.84	35.73	6.66	0.10	87.21	20.00	2.90	69.73
3	0.87	4.94	1.27	3.11	2.88	15.26	9.55	4.29	22.05	19.72	0.82	60.40	1.53	6.40	65.76
4	0.98	1.01	0.51	3.52	0.82	5.33	8.63	0.59	10.43	7.39	0.25	96.01	264.88	1.61	26.15
5	0.75	1.67	0.00	1.39	0.13	9.67	18.58	1.90	29.94	20.14	0.25	77.62	5.00	3.93	82.00
6	1.51	3.10	0.00	1.36	1.10	11.08	5.09	0.92	19.80	11.09	0.90	70.62	13.05	3.51	87.40
7	1.19	4.50	3.24	2.50	0.87	11.96	1.20	0.75	17.24	7.61	1.30	43.37	51.05	4.27	93.93
8	0.47	1.95	0.00	1.55	0.45	7.44	8.28	0.89	18.42	6.42	0.91	35.00	49.59	1.95	22.67
9	0.76	2.40	1.00	1.00	0.91	11.68	1.65	2.58	19.48	102.00	0.51	57.98	69.42	4.23	87.79
Mean	**0.89**	**2.62**	**0.78**	**2.38**	**0.90**	**10.54**	**9.55**	**1.58**	**20.89**	**26.28**	**0.89**	**63.36**	**57.29**	**3.51**	**65.81**
sd	0.31	1.32	1.06	1.08	0.81	4.04	6.59	1.20	7.67	32.37	0.42	21.18	81.23	1.44	26.29

3 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.72	1.89	1.16	2.56	0.35	14.36	5.12	0.21	28.47	10.38	0.49	68.76	52.02	1.97	77.55
2	0.64	1.92	2.64	2.19	0.37	14.49	3.71	1.20	17.90	25.33	0.50	70.00	59.83	2.89	81.72
3	0.72	3.74	1.02	2.21	1.53	14.23	8.20	1.44	28.62	9.54	0.54	80.02	1.52	3.82	49.05
4	1.21	1.96	1.34	2.68	1.81	6.50	10.85	1.74	17.30	46.68	0.35	114.00	8.19	2.75	54.23
5	0.61	2.79	2.06	1.11	0.31	6.97	3.97	2.21	19.37	72.66	0.54	108.00	14.65	4.74	88.88
6	1.02	3.29	1.06	2.74	1.89	5.60	12.55	1.94	31.93	22.49	0.78	72.00	6.32	3.57	82.18
7	1.54	4.80	1.25	3.50	1.50	11.70	5.14	0.84	16.23	6.00	1.59	51.28	53.95	4.09	90.88
8	0.95	1.98	0.22	1.29	0.25	5.82	29.50	0.65	12.02	10.07	1.15	41.91	39.95	2.18	82.92
9	1.21	4.85	2.70	2.22	2.57	16.02	7.22	2.79	23.00	7.24	0.14	65.34	8.00	6.15	78.89
Mean	**0.96**	**3.02**	**1.49**	**2.28**	**1.16**	**10.63**	**9.58**	**1.45**	**21.65**	**23.38**	**0.68**	**74.59**	**27.16**	**3.57**	**76.26**
sd	0.32	1.22	0.82	0.73	0.87	4.34	8.06	0.81	6.74	22.57	0.44	23.61	23.83	1.32	14.65

7 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.78	2.15	0.50	1.70	0.34	8.49	11.54	0.84	17.50	30.97	0.91	70.55	31.87	2.50	89.06
2	0.42	2.39	1.18	3.57	0.59	14.65	7.77	1.73	18.18	31.82	0.70	66.00	24.41	3.26	92.73
3	0.78	5.00	1.50	4.00	1.29	13.70	43.48	0.14	19.98	17.17	0.56	28.90	41.57	4.07	58.55
4	1.10	1.23	0.00	1.26	0.83	2.03	11.01	0.62	29.11	28.34	0.32	151.00	159.79	2.09	75.39
5	0.63	2.79	2.06	1.11	1.20	30.26	8.34	2.80	35.88	17.96	0.25	130.00	30.34	4.77	81.52
6	1.02	3.45	1.31	1.93	1.10	5.83	1.54	1.84	28.00	31.51	0.24	71.12	5.00	4.87	94.86
7	1.26	5.00	1.25	2.50	1.52	12.74	2.89	1.28	17.75	2.87	1.75	58.19	88.84	4.34	91.62
8	0.77	2.44	1.04	3.03	1.09	12.19	3.38	0.52	17.88	4.95	0.39	108.00	15.07	2.28	84.60
9	0.93	3.38	0.83	2.14	1.10	6.52	3.09	2.71	22.48	79.21	0.12	88.21	5.01	5.75	84.15
Mean	**0.85**	**3.09**	**1.07**	**2.36**	**0.98**	**11.82**	**10.34**	**1.39**	**22.97**	**27.20**	**0.58**	**85.77**	**44.43**	**3.78**	**81.39**
sd	0.26	1.27	0.59	1.00	0.38	8.09	12.96	0.95	6.57	22.46	0.50	37.99	49.80	1.31	11.95

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the T2D EXS group at each assessment during the intervention study.

Baseline

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.53	3.08	1.70	6.47	0.48	30.01	5.56	0.63	41.08	3.54	0.53	140.06	26.22	3.22	88.66
2	0.99	2.24	1.99	1.58	1.02	21.08	4.86	2.60	23.98	30.37	0.84	90.46	17.55	3.97	83.26
3	0.69	2.01	0.00	5.04	1.07	11.89	6.65	0.99	37.94	29.11	0.41	71.00	39.38	2.21	72.21
4	0.74	0.50	1.52	3.01	0.41	10.42	29.98	0.61	15.61	40.50	0.05	144.00	96.23	1.39	60.02
5	0.56	2.50	0.00	1.25	0.81	4.78	7.48	0.98	23.43	25.01	0.41	78.07	9.00	2.82	77.28
6	0.58	1.34	0.00	2.64	1.32	4.23	8.76	1.24	4.03	15.53	0.14	128.25	20.00	1.71	9.33
7	0.82	3.54	1.48	2.89	0.42	12.50	6.39	0.49	23.44	2.00	0.74	104.41	28.92	3.69	77.70
8	0.51	1.79	1.98	1.71	0.90	15.42	6.98	0.97	16.36	46.00	0.51	66.00	11.90	1.86	71.82
9	0.50	0.37	0.00	13.54	0.24	12.87	9.30	0.82	18.00	43.76	0.30	99.65	46.14	1.14	86.69
10	0.74	2.12	0.00	1.94	1.10	8.47	7.00	1.15	17.93	15.20	0.14	84.00	15.01	2.78	72.84
11	1.00	3.78	0.00	0.83	1.21	5.65	7.03	0.44	12.00	5.05	0.38	68.04	147.95	3.69	87.04
Mean	**0.70**	**2.12**	**0.79**	**3.72**	**0.82**	**12.48**	**9.09**	**0.99**	**21.26**	**23.28**	**0.41**	**97.63**	**41.66**	**2.89**	**71.53**
sd	0.18	1.11	0.92	3.66	0.37	7.65	7.04	0.60	10.71	16.19	0.25	28.54	42.91	0.99	22.31

3 Months

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.60	2.19	1.42	5.48	0.63	18.58	11.34	0.87	23.52	7.00	0.69	70.29	52.28	2.33	82.33
2	0.70	3.21	1.70	5.24	1.04	12.00	6.91	1.80	33.85	12.03	0.20	65.30	6.00	4.46	88.17
3	0.73	1.76	1.33	1.61	0.52	12.23	2.52	1.57	24.00	73.29	0.82	75.44	46.42	2.69	76.25
4	0.60	0.85	0.73	1.90	0.19	19.64	30.00	0.88	24.06	90.42	0.01	72.45	31.70	2.10	66.15
5	0.76	2.14	0.98	2.02	0.78	6.01	1.00	1.17	3.43	50.67	0.43	86.42	29.31	2.85	83.22
6	1.01	1.41	2.00	6.74	0.55	12.39	13.34	1.02	22.62	46.95	0.51	75.00	266.09	2.55	60.19
7	1.07	4.23	0.84	2.77	0.59	11.99	7.28	0.90	24.53	38.50	1.54	75.91	150.00	4.30	81.21
8	0.80	3.89	0.98	3.99	1.39	12.58	11.37	1.56	43.52	32.43	0.39	76.48	10.00	4.47	69.17
9	0.58	0.99	1.98	4.36	0.45	12.16	9.31	1.12	17.91	20.16	0.16	74.57	14.00	2.08	79.60
10	1.50	2.59	1.99	1.79	0.48	12.00	6.74	2.02	20.01	29.71	0.85	62.60	38.21	4.78	91.17
11	0.99	4.04	0.87	1.46	0.66	11.58	5.96	1.02	17.60	85.33	0.45	150.00	12.69	4.91	88.88
Mean	**0.85**	**2.48**	**1.36**	**3.40**	**0.66**	**12.83**	**9.56**	**1.27**	**23.19**	**44.23**	**0.55**	**80.41**	**59.70**	**3.41**	**78.76**
sd	0.28	1.22	0.49	1.85	0.32	3.61	7.73	0.40	9.96	28.46	0.42	23.90	79.25	1.16	9.93

7 Months

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.69	2.28	0.88	3.42	1.20	11.98	0.29	1.57	11.35	12.04	0.66	69.85	17.41	2.69	79.40
2	0.47	3.30	0.00	1.23	1.63	5.72	2.68	1.94	30.24	20.24	0.41	75.00	16.60	3.68	91.13
3	1.06	3.09	1.91	4.18	1.27	10.06	1.18	2.77	18.15	120.00	1.48	86.55	63.47	4.03	88.54
4	0.58	0.51	1.28	4.17	0.45	5.99	11.05	1.51	12.98	33.61	0.10	131.59	4.00	2.05	59.00
5	0.93	2.40	0.00	2.24	1.83	6.72	20.92	2.99	15.89	39.03	1.06	71.41	61.14	3.44	89.79
6	0.76	1.00	0.99	3.17	0.95	11.60	15.30	1.83	18.00	51.09	0.64	93.07	7.61	2.00	57.55
7	0.82	5.00	2.00	1.52	2.13	8.28	9.63	0.99	19.42	22.64	0.94	49.61	299.97	4.07	83.34
8	0.80	3.98	0.82	2.01	2.08	5.88	7.06	1.38	18.78	25.32	0.24	91.66	10.00	3.81	69.59
9	0.37	0.78	1.18	2.01	0.31	19.52	8.85	1.00	23.51	45.32	0.35	88.00	38.53	1.48	75.52
10	0.68	2.10	1.22	1.96	0.40	13.51	12.20	1.80	17.94	24.99	0.30	89.77	19.48	3.87	85.96
11	0.82	3.95	0.12	0.55	2.16	7.47	11.08	1.20	17.96	15.16	0.30	59.00	17.93	3.49	69.97
Mean	**0.73**	**2.58**	**0.95**	**2.41**	**1.31**	**9.70**	**9.11**	**1.72**	**18.57**	**37.22**	**0.59**	**82.32**	**50.56**	**3.15**	**77.25**
sd	0.20	1.44	0.69	1.18	0.71	4.24	6.18	0.66	5.03	30.07	0.42	21.63	85.19	0.93	11.96

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the T2D CTL group at each assessment during the intervention study.

Baseline

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	1.07	2.10	1.29	2.92	0.74	15.08	4.52	4.56	17.45	64.03	2.34	54.00	55.23	4.63	85.79
2	0.50	3.94	0.61	1.88	0.76	5.79	8.61	0.60	12.15	6.16	0.20	52.00	15.97	4.08	73.66
3	0.51	2.41	0.00	1.89	0.94	5.81	1.01	1.92	21.19	71.30	0.35	198.00	15.16	3.54	92.08
4	0.44	1.84	0.00	3.77	0.55	5.73	7.00	1.26	19.97	55.51	0.60	106.71	6.60	2.38	79.79
5	1.06	0.50	0.00	0.82	0.31	12.07	8.55	2.61	20.48	72.24	1.30	48.00	47.33	2.53	86.53
6	1.05	1.40	1.00	2.00	0.23	15.00	24.58	0.80	29.71	6.66	0.80	113.63	60.06	2.23	87.13
7	0.51	0.96	0.92	1.97	0.24	6.75	6.50	0.40	17.20	82.49	0.12	98.84	15.00	1.52	50.07
8	0.65	4.16	0.00	4.67	0.64	32.27	7.70	0.51	41.36	3.00	0.65	96.32	13.33	4.02	76.34
9	0.80	2.12	0.00	5.33	1.06	11.71	11.73	1.79	23.38	20.39	1.15	90.00	146.21	2.68	72.40
10	1.48	1.84	0.00	1.83	0.95	6.50	18.36	1.35	24.16	40.44	0.80	77.24	40.51	2.92	54.48
11	0.44	2.90	0.30	3.88	0.54	11.06	29.99	1.56	17.64	44.22	0.86	73.13	158.00	3.03	91.03
Mean	**0.77**	**2.14**	**0.37**	**2.81**	**0.63**	**11.62**	**11.69**	**1.58**	**22.25**	**42.40**	**0.83**	**91.62**	**52.13**	**3.05**	**77.21**
sd	0.35	1.11	0.49	1.41	0.29	7.76	8.92	1.20	7.79	29.34	0.62	41.88	52.84	0.93	14.03

3 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.91	2.58	2.00	9.65	0.46	27.29	30.00	1.24	29.32	30.01	0.41	65.54	32.33	3.85	79.39
2	0.57	2.53	1.99	1.40	0.52	11.77	10.38	1.28	28.05	120.00	0.70	103.83	26.50	3.08	76.22
3	0.79	2.43	1.45	2.52	1.55	5.94	3.43	1.77	9.35	46.96	0.35	84.00	7.91	3.09	78.46
4	0.32	1.80	2.00	1.40	0.15	8.55	14.73	0.72	34.20	14.21	0.20	55.84	10.00	2.48	78.28
5	1.05	0.87	0.75	1.95	0.51	5.85	3.00	0.75	29.89	38.98	0.24	102.01	16.91	1.93	69.62
6	0.92	0.79	0.93	2.16	0.41	24.00	9.78	0.73	30.00	8.52	0.29	58.09	145.70	1.78	70.76
7	0.46	1.30	1.63	2.70	0.66	12.99	9.52	0.67	27.25	9.16	0.12	72.00	36.94	1.64	64.08
8	0.66	2.79	1.44	2.57	0.45	11.97	3.13	0.70	22.83	19.73	0.40	53.00	23.86	3.31	75.30
9	0.88	2.08	1.62	2.67	0.33	11.91	3.08	1.31	34.33	9.10	0.55	95.48	22.27	3.40	89.24
10	1.36	2.65	1.52	3.53	1.12	11.88	2.00	0.82	29.13	22.58	0.20	89.00	13.70	3.51	63.38
11	0.62	2.15	2.00	4.64	0.62	13.33	17.25	1.86	29.97	37.67	0.85	69.27	47.15	3.16	91.10
Mean	**0.77**	**2.00**	**1.57**	**3.20**	**0.62**	**13.23**	**9.66**	**1.08**	**27.67**	**32.45**	**0.39**	**77.09**	**34.84**	**2.84**	**77.80**
sd	0.29	0.72	0.43	2.33	0.39	6.71	8.52	0.44	6.83	31.94	0.23	18.64	38.61	0.76	8.27

7 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	1.18	1.70	0.42	1.83	1.07	11.92	3.50	2.73	18.69	32.45	0.67	63.79	12.15	3.86	71.51
2	0.77	2.70	0.00	1.00	0.61	17.46	13.59	0.64	38.61	85.69	0.10	130.27	0.14	3.38	45.15
3	0.84	2.15	0.62	4.92	1.23	5.99	3.25	1.78	9.16	81.72	0.36	94.56	16.42	3.15	75.94
4	0.76	3.64	1.28	3.83	2.57	6.00	5.52	2.00	18.09	37.62	0.84	72.00	16.42	3.00	58.44
5	0.76	1.40	1.50	3.06	0.91	3.10	16.45	1.15	26.71	10.16	0.03	97.70	38.74	2.38	79.36
6	1.12	0.80	2.00	3.55	0.89	11.45	16.31	3.00	18.00	58.32	1.87	78.27	60.42	2.37	74.41
7	0.37	1.26	0.93	1.97	0.32	7.00	10.01	0.99	11.93	30.68	0.76	66.05	38.36	1.55	74.94
8	0.73	2.71	1.18	1.02	1.70	6.62	19.30	1.10	42.01	18.01	0.15	101.95	9.00	2.69	79.60
9	1.05	1.26	0.93	1.97	2.02	5.61	20.02	2.50	29.77	20.01	0.85	101.81	9.00	1.94	52.61
10	1.53	1.50	1.47	2.98	0.41	12.05	2.15	1.13	16.58	111.60	0.80	71.92	43.24	3.08	66.28
11	0.52	1.69	0.79	1.75	0.61	13.09	6.84	1.40	17.93	19.98	0.25	107.59	18.00	2.75	64.53
Mean	**0.88**	**1.89**	**1.01**	**2.54**	**1.12**	**9.12**	**10.63**	**1.67**	**22.50**	**46.02**	**0.57**	**89.63**	**23.61**	**2.74**	**67.52**
sd	0.32	0.63	0.56	1.24	0.71	4.31	6.76	0.78	10.54	33.49	0.47	20.84	18.52	0.66	11.36

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the PIO EXS group at each assessment during the intervention study.

Baseline

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.64	2.15	0.73	1.53	0.76	6.21	6.30	1.52	11.00	46.49	0.13	90.00	1.11	3.41	79.53
2	0.67	1.73	0.00	1.34	0.13	11.16	6.19	0.98	25.87	20.21	0.74	68.94	9.53	2.51	63.31
3	0.73	2.29	0.88	1.62	0.62	19.33	9.00	2.18	19.98	53.76	1.51	189.32	60.78	3.12	67.72
4	1.24	5.94	0.95	1.24	3.24	7.27	15.44	2.22	23.95	11.91	0.69	100.05	20.00	5.47	64.29
5	0.87	3.25	0.99	1.25	0.94	13.38	13.27	0.95	16.63	6.00	0.84	101.07	42.48	3.28	68.44
6	0.78	2.50	1.37	2.21	0.85	6.02	3.19	3.00	12.04	50.10	0.65	66.00	29.56	4.77	82.88
Mean	**0.82**	**2.98**	**0.82**	**1.53**	**1.09**	**10.56**	**8.90**	**1.81**	**18.25**	**31.41**	**0.76**	**99.23**	**27.24**	**3.76**	**70.53**
sd	0.22	1.53	0.45	0.37	1.09	5.21	4.66	0.80	6.12	21.11	0.44	37.48	21.96	1.12	7.64

3 Months

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.63	2.44	0.93	1.97	0.70	5.92	5.57	1.01	17.00	45.37	0.23	75.68	4.78	3.15	74.47
2	0.71	1.32	1.24	4.75	1.49	14.45	20.49	4.00	19.82	90.71	2.31	86.56	81.19	2.22	75.78
3	0.91	2.46	1.14	1.50	1.15	7.41	8.99	2.59	29.13	25.98	0.51	58.79	10.00	4.31	75.30
4	0.99	4.60	1.00	1.50	1.23	6.30	6.18	0.87	17.60	4.00	0.48	80.49	6.44	4.75	58.92
5	0.67	3.99	1.06	2.03	0.66	18.50	3.03	1.89	22.57	11.18	0.95	53.33	14.59	4.73	85.65
6	0.79	3.23	1.21	2.94	0.53	14.26	2.70	2.99	16.50	30.62	0.27	140.31	18.95	6.20	87.58
Mean	**0.78**	**3.01**	**1.10**	**2.45**	**0.96**	**11.14**	**7.83**	**2.19**	**20.44**	**34.64**	**0.79**	**82.53**	**22.66**	**4.23**	**75.95**
sd	0.14	1.19	0.12	1.24	0.38	5.28	6.62	1.22	4.81	31.11	0.79	31.05	29.15	1.39	10.90

7 Months

ID	a (ml.min^{-1}.mmHg^{-1})	A_1 (ml.min^{-1}.mmHg^{-1})	TD_1 (s)	Tau_1 (s)	A_2 (decay) (ml.min^{-1}.mmHg^{-1})	TD_2 (decay) (s)	Tau_2 (decay) (s)	A_3 (ml.min^{-1}.mmHg^{-1})	TD_3 (s)	Tau_3 (s)	A_4 (decay) (ml.min^{-1}.mmHg^{-1})	TD_4 (decay) (s)	Tau_4 (decay) (s)	End A (ml.min^{-1}.mmHg^{-1})	Adj R^2 (%)
1	0.64	2.53	0.68	2.33	1.24	7.00	2.68	2.76	9.87	35.37	1.14	104.55	32.33	3.53	87.75
2	1.14	2.54	1.67	3.25	1.19	17.07	0.90	1.27	27.49	47.18	0.56	92.08	5.*0	3.20	73.78
3	1.29	3.61	1.03	3.03	1.68	5.93	4.88	2.41	31.24	71.03	0.39	106.36	10.00	5.22	71.99
4	0.87	5.08	0.66	1.41	1.07	13.09	7.00	1.50	30.12	50.00	0.75	144.00	35.00	5.63	73.66
5	0.65	4.50	1.64	3.30	0.65	9.88	3.01	2.23	18.85	15.38	0.97	80.87	18.61	5.77	93.17
6	0.71	3.00	0.62	1.50	0.65	6.18	3.27	2.00	11.50	29.61	0.36	105.12	15.34	4.70	88.92
Mean	**0.88**	**3.54**	**1.05**	**2.47**	**1.08**	**9.83**	**3.82**	**2.03**	**21.51**	**41.43**	**0.70**	**105.49**	**19.57**	**4.67**	**81.55**
sd	0.27	1.06	0.49	0.86	0.39	4.46	2.09	0.56	9.46	19.17	0.32	21.31	11.94	1.08	9.40

Appendix XXXII: Individual LVC quad-phasic model fits to the steady-state exercise at 30% MVC in the PIO CTL group at each assessment during the intervention study.

Baseline

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.37	1.26	0.93	1.97	0.32	7.00	10.01	0.99	11.93	30.68	0.76	66.05	38.36	1.55	74.94
2	0.52	1.69	0.79	1.75	0.61	13.09	6.84	1.40	17.93	19.98	0.25	107.59	18.00	2.75	64.53
3	0.80	1.03	1.40	2.02	0.84	5.95	9.65	1.38	24.28	19.20	0.43	52.00	49.68	1.95	30.18
4	0.84	2.15	0.62	4.92	1.23	5.99	3.25	1.78	9.16	81.72	0.36	94.56	15.42	3.15	75.94
5	0.76	3.64	1.28	3.83	2.57	6.00	5.52	2.00	18.09	37.62	0.84	72.00	15.42	3.00	58.44
Mean	**0.66**	**1.96**	**1.00**	**2.90**	**1.11**	**7.60**	**7.05**	**1.51**	**16.28**	**37.84**	**0.53**	**78.44**	**27.78**	**2.48**	**60.81**
sd	0.20	1.03	0.33	1.41	0.88	3.10	2.84	0.39	5.91	25.70	0.26	22.38	15.37	0.70	18.62

3 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.59	1.80	0.79	1.92	0.28	11.91	2.32	0.50	25.00	21.98	0.22	93.97	21.69	2.39	88.12
2	0.53	2.09	1.96	5.09	0.41	12.20	4.17	0.64	16.94	20.17	0.51	91.74	92.41	2.38	93.90
3	0.85	1.10	0.00	1.99	1.25	5.59	6.31	1.45	11.23	28.00	0.10	99.79	3.00	2.05	42.01
4	0.94	2.71	1.56	1.33	1.33	12.11	7.80	1.74	18.46	20.93	0.47	94.00	98.04	3.62	85.70
5	0.73	2.74	2.00	1.90	1.55	7.03	11.63	2.05	30.00	59.97	0.84	78.00	18.50	3.12	77.77
Mean	**0.73**	**2.09**	**1.26**	**2.45**	**0.96**	**9.77**	**6.45**	**1.28**	**20.33**	**30.21**	**0.43**	**91.50**	**46.73**	**2.71**	**77.50**
sd	0.17	0.68	0.86	1.50	0.58	3.20	3.57	0.68	7.30	16.92	0.29	6.11	44.88	0.64	20.67

7 Months

ID	a	A_1	TD_1	Tau_1	A_2 (decay)	TD_2 (decay)	Tau_2 (decay)	A_3	TD_3	Tau_3	A_4 (decay)	TD_4 (decay)	Tau_4 (decay)	End A	Adj R^2
	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	s	s	ml.min^{-1}.mmHg^{-1}	%
1	0.65	1.74	0.01	2.11	0.24	13.98	3.50	1.01	21.93	17.90	0.34	73.67	64.51	2.32	90.87
2	0.77	1.18	1.06	1.79	0.40	17.72	7.79	1.92	23.38	35.29	0.37	89.49	10.00	3.10	87.17
3	0.73	0.59	0.01	1.41	0.51	5.78	12.55	1.10	29.45	36.65	0.19	99.21	20.00	1.72	63.04
4	0.70	2.70	1.12	2.62	1.70	5.79	11.51	2.75	16.96	30.24	0.15	76.81	5.00	4.30	83.37
5	0.50	2.66	0.86	2.44	0.68	11.86	9.81	1.66	18.88	15.59	0.64	59.71	15.00	3.50	85.58
Mean	**0.67**	**1.57**	**0.61**	**2.07**	**0.71**	**11.02**	**9.03**	**1.69**	**22.12**	**27.13**	**0.34**	**79.78**	**22.90**	**2.99**	**82.01**
sd	0.10	0.95	0.58	0.49	0.58	5.22	3.58	0.71	4.81	9.81	0.19	15.17	23.92	1.01	10.95

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the ND EXS group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.53	15.42	0.013	17.95	2521.34	18.13	93.90
2	2.87	16.65	0.029	19.52	1951.66	19.68	97.18
3	1.20	14.27	0.067	15.47	935.72	15.30	97.44
4	5.33	9.49	0.025	14.82	3579.65	13.33	75.61
5	3.74	28.99	0.058	32.73	2516.99	32.99	96.11
6	4.20	9.75	0.034	13.95	2028.26	15.36	62.37
7	3.02	22.33	0.123	25.35	1992.05	25.34	98.16
8	3.72	17.76	0.034	21.48	2762.13	22.65	92.48
9	2.36	20.65	0.023	23.01	2444.83	23.07	98.77
10	4.38	14.22	0.037	18.60	3012.70	19.09	88.30
Mean	3.33	16.95	0.04	20.29	2374.53	20.49	90.03
sd	1.18	5.89	0.03	5.69	712.72	5.81	11.94

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	3.83	21.48	0.019	25.32	3182.42	23.94	94.57
2	1.70	16.03	0.047	17.73	1361.98	17.34	96.46
3	1.15	6.63	0.041	7.78	782.08	7.70	96.00
4	5.31	15.82	0.041	21.13	3570.80	21.36	91.88
5	2.36	27.38	0.070	29.75	1807.53	29.86	98.30
6	1.89	15.94	0.091	17.83	1311.4	17.92	95.65
7	2.85	26.70	0.091	29.55	2000.22	29.30	97.72
8	3.45	25.12	0.078	28.57	2390.54	29.10	97.19
9	1.77	23.04	0.073	24.81	1377.75	24.76	98.79
10	2.34	18.72	0.138	21.06	1540.08	21.02	96.72
Mean	2.66	19.69	0.07	22.35	1932.48	22.23	96.33
sd	1.24	6.38	0.03	6.81	880.20	6.84	2.01

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	1.77	16.42	0.035	18.19	1318.13	18.66	96.69
2	1.67	9.37	0.039	11.03	1241.27	11.42	95.19
3	1.11	7.64	0.067	8.76	716.10	8.76	95.38
4	2.91	21.06	0.043	23.97	2235.47	23.99	98.25
5	1.66	18.71	0.032	20.37	1586.71	20.66	96.98
6	3.07	16.80	0.052	19.88	1797.38	20.54	84.10
7	3.08	24.11	0.095	27.18	1914.44	27.12	97.99
8	3.95	25.38	0.022	29.32	3519.63	33.79	93.14
9	1.59	21.27	0.040	22.86	1386.98	23.46	98.55
10	1.90	19.47	0.111	21.37	1200.35	21.33	98.10
Mean	2.27	18.02	0.05	20.29	1691.65	20.97	95.44
sd	0.91	5.77	0.03	6.44	770.09	7.18	4.33

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the ND CTL group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	3.50	19.92	0.033	23.42	2489.89	24.67	96.05
2	4.32	26.97	0.085	31.28	2905.11	30.70	96.77
3	1.52	22.67	0.048	24.19	787.16	24.32	96.70
4	1.66	15.32	0.012	16.97	2358.69	17.07	96.69
5	4.61	20.00	0.046	24.61	3198.47	26.26	87.90
6	7.75	20.26	0.017	28.00	5373.65	27.22	90.39
7	2.68	19.84	0.020	22.52	2597.37	20.55	94.84
8	3.10	19.48	0.034	22.59	2443.07	23.31	96.12
9	3.45	25.18	0.015	28.63	3791.43	29.53	94.83
Mean	3.62	21.07	0.03	24.69	2882.76	24.85	94.48
sd	1.87	3.44	0.02	4.19	1236.21	4.27	3.17

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	1.76	14.32	0.068	16.08	1264.33	16.27	95.33
2	2.60	10.89	0.052	13.49	1769.70	13.50	94.98
3	10.00	59.17	0.018	69.17	9235.98	66.86	92.90
4	1.35	18.02	0.101	19.38	989.67	19.38	98.88
5	2.84	23.01	0.054	25.84	2128.6	27.00	94.33
6	5.15	17.35	0.015	22.50	4227.05	19.93	88.91
7	2.84	19.59	0.057	22.43	2048.24	22.57	97.34
8	2.56	27.05	0.042	29.62	2174.17	30.60	95.15
9	5.62	22.29	0.016	27.91	4773.28	26.25	89.23
Mean	3.86	23.52	0.05	27.38	3179.00	26.93	94.12
sd	2.70	14.20	0.03	16.53	2602.02	15.92	3.33

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	1.37	13.16	0.042	14.53	722.87	14.44	97.62
2	1.63	12.26	0.056	13.89	1294.58	13.87	97.83
3	3.13	45.16	0.035	48.29	2980.32	51.31	96.62
4	1.11	11.17	0.055	12.27	733.75	12.12	96.99
5	3.43	21.34	0.037	24.77	2630.29	25.68	91.48
6	3.61	22.59	0.042	26.20	2698.63	26.34	94.98
7	2.11	20.92	0.030	23.03	1965.74	23.43	98.04
8	2.85	34.18	0.045	37.03	2635.91	36.72	95.65
9	4.79	20.37	0.046	25.16	3027.35	26.59	91.93
Mean	2.67	22.35	0.04	25.02	2076.60	25.61	95.68
sd	1.21	11.06	0.01	11.66	934.52	12.42	2.47

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the T2D EXS group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	unit	ml.min^{-1}.mmHg^{-1}	a.u.	ml.min^{-1}.mmHg^{-1}	%
1	3.28	16.51	0.032	19.79	2478.40	20.33	90.00
2	2.77	14.10	0.019	16.88	2423.86	17.77	89.00
3	2.59	20.31	0.030	22.90	2224.06	23.20	98.46
4	4.34	21.41	0.026	25.75	3418.19	26.41	95.78
5	2.94	19.92	0.052	22.86	2142.36	23.65	96.84
6	1.67	15.09	0.054	16.76	1282.95	16.81	96.97
7	6.20	19.77	0.015	25.97	5043.04	26.15	92.94
8	4.53	13.24	0.019	17.77	3686.97	17.87	92.67
9	3.47	11.73	0.021	15.20	2653.86	16.45	94.96
10	3.90	22.54	0.040	26.44	2899.32	25.90	95.72
11	2.83	21.70	0.124	24.52	1870.66	24.63	96.96
Mean	3.50	17.85	0.04	21.35	2738.51	21.74	94.57
sd	1.22	3.82	0.03	4.19	1018.04	3.97	3.05

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	unit	ml.min^{-1}.mmHg^{-1}	a.u.	ml.min^{-1}.mmHg^{-1}	%
1	3.25	15.11	0.072	18.37	2160.46	18.76	90.72
2	2.74	16.41	0.033	19.15	2141.57	20.51	94.74
3	3.04	23.78	0.050	26.82	2481.54	26.91	97.23
4	2.23	13.58	0.035	15.81	1727.53	15.44	95.44
5	1.09	13.52	0.062	14.61	867.86	14.66	97.94
6	3.39	17.32	0.055	20.70	2144.02	20.73	93.83
7	3.38	17.90	0.030	21.28	2631.94	24.59	89.34
8	3.30	8.78	0.013	12.08	2669.49	12.23	93.21
9	5.32	20.18	0.034	25.50	3472.02	25.48	94.23
10	2.08	20.40	0.091	22.48	1474.01	22.53	97.42
11	1.92	18.02	0.082	19.94	1371.58	20.05	96.89
Mean	2.88	16.82	0.05	19.70	2103.82	20.17	94.64
sd	1.10	4.06	0.02	4.44	722.62	4.66	2.78

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	ml.min^{-1}.mmHg^{-1}	ml.min^{-1}.mmHg^{-1}	unit	ml.min^{-1}.mmHg^{-1}	a.u.	ml.min^{-1}.mmHg^{-1}	%
1	2.91	17.52	0.033	20.43	2270.41	22.37	91.49
2	2.41	18.24	0.022	20.65	2128.29	20.46	95.94
3	10.12	26.08	0.022	36.21	6632.40	37.16	94.84
4	5.86	24.56	0.024	30.42	4181.53	29.72	89.04
5	3.34	19.24	0.077	22.58	2254.24	22.38	96.69
6	2.31	19.93	0.073	22.24	1661.58	22.26	98.57
7	5.01	16.98	0.021	21.98	1394.83	25.29	79.39
8	5.33	23.09	0.039	28.42	3796.25	28.89	94.75
9	4.35	17.45	0.021	21.80	3443.74	22.43	96.29
10	4.98	19.42	0.041	24.40	2867.53	24.71	93.41
11	3.02	14.50	0.037	17.52	2023.22	18.20	96.00
Mean	4.51	19.73	0.04	24.24	2968.55	24.90	93.31
sd	2.23	3.50	0.02	5.38	1503.27	5.29	5.31

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the T2D CTL group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	4.98	29.09	0.071	34.07	3098.77	34.00	99.29
2	3.05	18.70	0.042	21.76	2273.59	22.03	93.13
3	2.02	14.51	0.053	16.53	1604.52	16.65	96.25
4	1.96	12.81	0.035	14.77	1540.36	15.67	93.72
5	3.43	13.78	0.038	17.21	2628.55	18.01	92.35
6	4.09	17.39	0.025	21.48	3155.75	21.96	95.55
7	3.13	19.39	0.047	22.52	2474.60	23.05	95.23
8	4.75	21.29	0.031	26.04	3817.87	27.69	95.28
9	3.34	8.63	0.029	11.97	2303.60	11.80	88.54
10	2.47	23.22	0.039	25.69	2077.59	26.14	95.75
11	10.78	23.48	0.025	34.25	6763.80	20.88	72.92
Mean	4.00	18.39	0.04	22.39	2885.36	21.63	92.55
sd	2.46	5.80	0.01	7.28	1451.89	6.19	7.04

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	1.80	19.11	0.049	20.91	1253.90	20.99	97.18
2	2.32	23.03	0.142	25.35	1555.57	25.52	94.78
3	1.47	16.12	0.101	17.59	1043.34	17.78	97.11
4	1.86	18.17	0.048	20.03	1498.54	19.77	92.44
5	1.47	9.29	0.061	10.76	1033.20	10.81	90.16
6	4.27	15.23	0.027	19.50	2872.44	19.78	86.51
7	1.38	11.59	0.071	12.97	907.42	12.93	92.13
8	2.37	14.96	0.095	17.32	866.69	17.68	93.58
9	1.98	9.88	0.016	11.86	1785.64	10.54	91.86
10	2.00	16.30	0.047	18.30	1424.57	18.45	97.34
11	2.82	16.49	0.048	19.31	2038.60	19.96	96.05
Mean	2.16	15.47	0.06	17.63	1479.99	17.66	93.56
sd	0.83	4.06	0.04	4.30	591.51	4.56	3.39

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	4.24	35.86	0.057	40.09	3167.79	40.22	99.54
2	3.22	15.46	0.041	18.68	2117.47	18.26	96.15
3	3.32	17.64	0.054	20.96	2115.07	21.40	97.99
4	3.03	20.29	0.057	23.31	1988.04	23.37	96.71
5	5.23	21.16	0.045	26.39	3295.39	26.02	91.25
6	4.73	15.60	0.061	20.33	2811.81	20.18	93.59
7	1.98	24.09	0.098	26.08	1315.32	26.17	97.88
8	3.69	30.04	0.063	33.72	2471.33	33.71	97.54
9	2.81	15.24	0.035	18.05	1617.63	18.41	96.33
10	3.26	22.23	0.069	25.49	2085.72	25.18	97.81
11	2.77	18.56	0.075	21.33	1746.11	22.08	92.44
Mean	3.48	21.47	0.06	24.95	2248.34	25.00	96.11
sd	0.94	6.50	0.02	6.71	629.17	6.68	2.59

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the PIO EXS group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	3.40	26.43	0.020	29.83	3138.33	29.51	95.71
2	4.01	26.85	0.069	30.85	2550.69	30.96	98.03
3	2.32	15.57	0.016	17.89	2496.05	16.49	94.44
4	5.43	14.93	0.013	20.36	4051.25	21.35	84.19
5	7.37	22.89	0.025	30.26	5342.25	31.56	96.62
6	9.67	20.31	0.022	29.97	6723.10	30.16	97.40
Mean	5.37	21.16	0.03	26.53	4050.28	26.67	94.40
sd	2.74	5.17	0.02	5.80	1691.70	6.24	5.16

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.65	6.77	0.022	9.41	1896.7	9.48	86.79
2	4.05	19.05	0.041	23.11	2651.44	24.38	96.08
3	1.93	20.55	0.037	22.48	1717.32	22.57	97.68
4	3.56	24.00	0.019	27.56	3397.46	28.30	96.33
5	3.88	27.36	0.078	31.24	2677.59	31.33	97.45
6	2.64	25.36	0.041	28.00	2194.83	27.86	97.29
Mean	3.12	20.52	0.04	23.63	2422.56	23.99	95.27
sd	0.84	7.40	0.02	7.69	615.40	7.75	4.20

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.09	31.27	0.027	33.37	2400.04	33.50	98.85
2	3.17	12.68	0.024	15.85	2435.78	17.17	91.23
3	5.43	27.26	0.018	32.69	4485.63	35.32	87.92
4	2.96	25.32	0.042	28.28	2386.46	28.72	93.89
5	3.66	30.27	0.070	33.93	2631.80	34.60	96.19
6	2.75	33.70	0.044	36.46	2411.47	36.57	98.92
Mean	3.35	26.75	0.04	30.10	2791.86	30.98	94.50
sd	1.14	7.50	0.02	7.47	834.73	7.28	4.37

Appendix XXXIII: Individual LVC mono-phasic model fits to RH in the PIO CTL group at each assessment during the intervention study.

Baseline							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	3.22	15.46	0.041	18.68	2117.47	18.26	96.15
2	3.03	20.29	0.057	23.31	1988.04	23.37	96.71
3	4.73	15.60	0.061	20.33	2811.81	20.18	93.59
4	2.81	15.24	0.035	18.05	1617.63	18.41	96.33
5	4.59	12.91	0.022	17.51	3057.8	16.17	77.11
Mean	3.68	15.90	0.04	19.58	2318.55	19.28	91.98
sd	0.91	2.69	0.02	2.34	598.07	2.69	8.40

3 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.75	19.51	0.034	22.26	2228.27	22.42	96.61
2	0.87	12.85	0.055	13.72	756.29	13.71	98.43
3	4.11	20.48	0.049	24.59	2878.56	23.65	95.81
4	4.37	10.26	0.015	14.62	3303.50	15.78	86.56
5	2.74	17.81	0.026	20.55	2334.7	22.14	94.69
Mean	2.97	16.18	0.04	19.15	2300.27	19.54	94.42
sd	1.39	4.43	0.02	4.77	965.81	4.47	4.60

7 Months							
	a	b	c	a + b	AUC	Peak VC	Adj R^2
ID	$ml.min^{-1}.mmHg^{-1}$	$ml.min^{-1}.mmHg^{-1}$	unit	$ml.min^{-1}.mmHg^{-1}$	a.u.	$ml.min^{-1}.mmHg^{-1}$	%
1	2.53	11.70	0.025	14.23	1996.41	12.77	94.24
2	4.40	20.64	0.035	25.04	3236.29	25.26	95.12
3	5.15	15.41	0.032	20.56	3569.54	20.69	94.44
4	4.73	14.58	0.023	19.31	3463.99	19.77	90.23
5	2.38	23.34	0.061	25.72	1814.79	25.25	97.81
Mean	3.84	17.14	0.04	20.97	2816.20	20.75	94.37
sd	1.29	4.74	0.02	4.68	842.39	5.13	2.72

Appendix XXXIV: Individual RNA levels and purity from the whole blood samples collected throughout the study.

Sample	RNA (ng/ul)	$OD_{260}:OD_{280}$	Sample	RNA (ng/ul)	$OD_{260}:OD_{280}$	Sample	RNA (ng/ul)	$OD_{260}:OD_{280}$
1	145.9	2.08	55	65.6	2.12	109	71.3	2.15
2	176.7	2.09	56	67.6	2.06	110	45	2.26
3	21.8	2.08	57	65.7	2.15	111	12.5	2.32
4	49.2	2.13	58	107.5	2.08	112	32.8	2.31
5	49	2.08	59	38.3	2.02	113	64.9	2.18
6	91.6	2.12	60	96.3	2.07	114	140.7	2.16
7	52.8	2.07	61	18	2.11	115	176.9	2.16
8	61	2.06	62	100.6	2.06	116	9.6	2.49
9	54.4	2.09	63	124.5	2.11	117	63	2.15
10	87.8	2.1	64	135.7	2.1	118	109.4	2.13
11	14.9	2.14	65	35.5	2.2	119	27.8	2.34
12	108.2	2.09	66	39	2.02	120	70.3	2.21
13	53.6	2.06	67	56.9	2.09	121	22.9	2.21
14	65.7	2.12	68	45.7	2.04	122	31.9	2.22
15	53.7	2.06	69	122.4	2.1	123	95.4	2.14
16	83.6	2.07	70	102	2.11	124	21.1	2.24
17	41.6	2.05	71	93.8	2.08	125	100	2.12
18	97.2	2.09	72	31.5	2.19	126	54.9	2.2
19	119.5	2.08	73	93.7	2.11	127	70.1	2.1
20	88	2.07	74	118.1	2.14	128	6.3	1.71
21	184	2.1	75	71.6	2.12	129	38.8	2.05
22	146.7	2.08	76	77.3	2.08	130	46.8	2.12
23	34.5	2.09	77	27.7	2.19	131	76.9	2.12
24	90.8	2.12	78	61.2	2.11	132	85.1	2.11
25	27	2.12	79	75.4	2.09	133	98.6	2.1
26	144.5	2.11	80	18	1.93	134	99.8	2.11
27	30.1	2.01	81	100.9	2.09	135	108.9	2.09
28	23.8	2.16	82	20.5	2.08	136	114.5	2.1
29	14.8	1.83	83	82.7	2.08	137	181.5	2.11
30	68.1	2.04	84	164.7	2.1	138	140.5	2.1
31	50.9	2.01	85	97.3	2.09	139	74.2	2.14
32	30.2	2.16	86	114.4	2.13	140	142.4	2.12
33	32.5	2.03	87	114.4	2.26	141	91.1	2.13
34	176	2.08	88	86.8	2.16	142	108.8	2.12
35	22.5	2.06	89	68.3	2.23	143	106.4	2.09
36	102	2.07	90	74	2.13	144	103.4	2.05
37	106.3	2.12	91	114.2	2.12	145	86.8	2.09
38	215.5	2.15	92	24.7	2.17	146	147	2.12
39	180.7	2.12	93	109.6	2.19	147	54.8	2.08
40	73.9	2.08	94	34.3	2.19	148	27.6	2.2
41	111.1	2.08	95	148.4	2.1	149	102.4	2.09
42	31.2	2.03	96	19.5	2.15	150	45.6	2.18
43	103.4	2.1	97	64.4	2.1	151	62.4	2.17
44	89.2	2.07	98	61.1	2.13	152	56.1	2.08
45	38.8	2.26	99	35	2.18	153	26.7	2.27
46	12.5	2.17	100	99.8	2.16	154	171	2.11
47	75.2	2.1	101	113.3	2.08	155	301.8	2.09
48	214.7	2.1	102	27.5	2	156	68.9	2.11
49	113.3	2.11	103	19.8	2.21	157	65.1	2.16
50	139.2	2.12	104	65.3	2.12	158	38.4	2.04
51	58.3	2.09	105	50.2	2.06	159	55.8	2.16
52	121.6	2.07	106	91.5	2.14	160	202.1	2.1
53	84.4	2.14	107	47.9	2.17	161	75.6	2.08
54	30.9	2.06	108	66.8	2.11	162	78.4	2.17